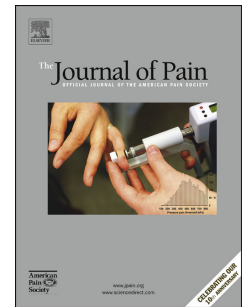


# Accepted Manuscript

Painful After-Sensations in Fibromyalgia are Linked to Catastrophizing and Differences in Brain Response in the Medial Temporal Lobe

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PII: S1526-5900(17)30492-3

DOI: [10.1016/j.jpain.2017.02.437](https://doi.org/10.1016/j.jpain.2017.02.437)

Reference: YJPAI 3393

To appear in: *Journal of Pain*

Received Date: 8 October 2016

Revised Date: 17 February 2017

Accepted Date: 27 February 2017

Please cite this article as: Schreiber KL, Loggia ML, Kim J, Cahalan CM, Napadow V, Edwards RR, Painful After-Sensations in Fibromyalgia are Linked to Catastrophizing and Differences in Brain Response in the Medial Temporal Lobe, *Journal of Pain* (2017), doi: 10.1016/j.jpain.2017.02.437.

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**Title:** Painful After-Sensations in Fibromyalgia are Linked to Catastrophizing and Differences in Brain Response in the Medial Temporal Lobe

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**Disclosures/ COI/Research funding sources:** There are no conflicts of interest for any authors.

This work was supported by the National Institutes of Health, National Center for Complementary and Integrative Health [R01-AT007550, P01-AT006663]; National Institute of Arthritis and Musculoskeletal and Skin Diseases [R01-AR064367]; and the National Center for Research Resources [P41RR14075, S10RR021110, S10RR023043].

**Acknowledgements:** The authors would like to acknowledge the subjects for their willingness and time to participate in the study.

**Short running title:** Painful After-Sensations in Fibromyalgia

## **Abstract**

Fibromyalgia (FM) is a complex syndrome characterized by chronic widespread pain, hyperalgesia, and other disabling symptoms. While the brain response to experimental pain in FM patients has been the object of intense investigation, the biological underpinnings of painful after-sensations (PAS), and their relation to negative affect have received little attention. In this cross-sectional cohort study, subjects with FM (n=53) and healthy controls (n=17) were assessed for PAS by exposure to a sustained, moderately painful cuff stimulus to the leg, individually calibrated to a target pain intensity of 40/100. Despite requiring lower cuff pressures to achieve the target pain level, FM patients reported more pronounced PAS 15s following end of cuff stimulation, which correlated positively with clinical pain scores. Functional magnetic resonance imaging (fMRI) revealed reduced deactivation of the medial temporal lobe (MTL; amygdala, hippocampus, parahippocampal gyrus) in FM patients, during both pain stimulation, and in the ensuing post-stimulation period, when PAS are experienced. Moreover, the fMRI signal measured during the post-stimulation period in MTL, as well as in the insular and anterior middle cingulate and medial prefrontal cortices, correlated with the severity of reported PAS by FM patients. These results suggest that the MTL plays a role in PAS in FM patients.

Perspective: PAS are more common and severe in FM, and are associated with clinical pain and catastrophizing. PAS severity is also associated with less MTL deactivation, suggesting that the MTL, a core node of the default mode network, may be important in the prolongation of pain sensation in FM.

Key words: human, psychophysics, neuroimaging, psychosocial, temporal summation, sensitization, default mode network

## Introduction:

Historically, many in the medical community have viewed fibromyalgia (FM) with skepticism. Clear peripheral signs in FM are difficult to discern, and an unfortunate consequence which many patients anecdotally report is a lingering tendency of some medical practitioners to underestimate their pain. Over the last two decades, an improvement in the specificity of diagnostic criteria for FM,<sup>81</sup> along with the accumulation of evidence of objective anatomical, functional, and neurochemical alterations in the central nervous system (e.g., changes in brain morphometry, functional connectivity, and concentration of various neurotransmitters and metabolites),<sup>19,20,31,36,41,45,49,50,56,79</sup> as well as more recent recognition of peripheral changes in at least a subgroup of FM patients,<sup>59,77</sup> has helped to increase the acceptance of FM as a clinically recognized pain disorder with a neurobiological basis. Formal quantitative sensory testing (QST) studies have also shown that individuals with FM have greater sensitivity (compared to pain-free controls) to a broad variety of standardized noxious stimuli, and importantly, have implicated alterations in the central nervous system as a possible substrate for differences in pain sensitivity between FM and non-FM. Specifically, FM patients exhibit a tendency toward greater central sensitization-like processes, such as temporal summation of pain (TSP).<sup>62,70,72</sup> Amplified TSP in FM patients is a phenomenon that has been well-studied using a variety of stimulus modalities, including repetitive or prolonged<sup>75</sup> heat, pressure or pinprick mechanical stimuli.<sup>5,69,72</sup>

Painful after-sensations (PAS), defined as painful sensations persisting beyond the offset of a noxious stimulus, represent a clinically relevant, but less well-studied, example of the sensitization-related processes that appear to be enhanced in FM. These PAS, which some authors have also called ‘windup after-sensations’ or ‘prolonged pain after-sensations’,<sup>2,30,64,65,66,69,71,73</sup> are typically experimentally measured 15 or 30 seconds after the

removal of a nociceptive stimulus (or train of stimuli) of relatively long duration. PAS are enhanced in individuals with neuropathic pain compared to controls,<sup>30</sup> and are more frequent and pronounced in post-mastectomy patients with persistent postsurgical pain than those without it.<sup>66</sup> Importantly, a handful of studies have shown that PAS are more pronounced in FM patients than controls.<sup>12,62,69,71</sup> Additionally, PAS appear to be more closely correlated to pain severity than other QST measures, including indices of temporal summation,<sup>2,65,73</sup> underlining their potential clinical relevance. While PAS have been studied by several groups psychophysically, their associated brain activity has not been investigated. Furthermore, while negative cognitive and affective factors such as catastrophizing, anxiety and fear are known to contribute to pain-facilitatory processes,<sup>24,26,63,67,80</sup> it is unclear from previous work how negative affect relates to the experience of PAS.

In the current study, we evaluated the incidence and severity of painful after-sensations in FM, hypothesizing that PAS would be associated with clinical pain and catastrophizing, as well as differential brain activation, as assessed using fMRI, compared to control subjects.

## Methods:

This cross-sectional cohort study included patients suffering from Fibromyalgia (FM), as well as healthy control (HC) volunteers. Study design and procedures were reviewed and approved by the institutional IRB. Subjects were recruited through online advertising and flyers posted in the Boston community. Patients were eligible if they held a diagnosis of Fibromyalgia meeting criteria outlined by Wolfe, 2010<sup>81</sup>. Exclusion criteria included: 1) history of significant neurologic disorder, 2) history of anxiety disorders or significant anxiety symptoms interfering with MRI procedures, 3) history of significant cardiac events, 4) history of significant head

injury 5) current treatment with opioids, 6) current/recent use of recreational drugs, and 7) implanted metallic objects and other typical contraindications for MRI, 8) pregnancy. Patients' medication regimens included gabapentin, a variety of antidepressants, NSAIDs and acetaminophen, and were not altered during the course of this study. Healthy controls were recruited so as to achieve a balance of age and sex between FM and control groups. Subjects attended two study visits. The first visit consisted of a behavioral assessment, performed in a single, large, urban, university-based pain management center (Brigham and Women's Hospital). The second was a neuroimaging visit, performed at the A. A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital, an average of two weeks later (mean duration between visits  $\pm$  SD: 15.1 $\pm$ 16.2 days).

*Behavioral visit:* At the beginning of the visit, all participants provided informed consent. FM patients (n=53) and healthy controls (n=17) then rated the severity and extent of their clinical pain by using a Numeric Rating Scale (NRS) (0-100, with anchors 0= no pain, 100=most intense pain imaginable; numbers indicated verbally), the Brief Pain Inventory,<sup>76</sup> the neuropathic pain questionnaire,<sup>13</sup> as well as the Widespread Pain Inventory and the Symptom Severity index, which are used in the diagnostic criteria for FM.<sup>81</sup> Negative affect and cognitions were assessed using the Beck Depression Inventory,<sup>10</sup> Pain Catastrophizing Scale,<sup>74</sup> and an anxiety NRS. Subjects also completed a questionnaire at the end of the QST session, the Situational Pain Catastrophizing Scale (SPCS)<sup>16</sup>, which assessed catastrophizing during the painful events of the testing session itself, and is distinct from the PCS.<sup>17</sup> Clinical pain NRS rating was also assessed at several points between Quantitative Sensory Testing (QST). Pain scores in response to QST were also rated using a NRS (0-100). QST was performed by a single practitioner during the

study visit for consistency, and the order of tests was kept constant across participants, as follows:

*Mechanical pain* thresholds were assessed using a digital pressure algometer (Somedic) bilaterally at the trapezius muscle and the metacarpophalangeal joint of the thumb.

*Cuff pain, temporal summation, and painful after-sensations* were assessed using a 13.5cm-wide velcro-adjusted blood pressure cuff placed around the left leg (gastrocnemius muscle belly) and connected to a rapid cuff inflator (Hokanson Inc, Bellevue, WA, USA). The cuff was inflated to a pressure corresponding to ~40/100 pain intensity for each patient. After determination of this 40/100 pressure, the cuff was deflated for a rest period and then again inflated to this value and held for a longer duration (2 minutes), after which it was rapidly deflated. Patients were asked to rate the pain experienced from this stimulus upon initial inflation, at 60 s, and at 120 s. Temporal summation was measured as the difference between pain rating at 120s and initial pain rating. Additionally, the patients were asked to rate any ongoing pain at the cuff site 15 seconds following cuff deflation (painful after-sensations).

*MRI visit:* FM patients (n=43) and healthy controls (n=15) also participated in the imaging visit. Out of these, five subjects with FM were excluded from the analyses: three because of differences in the fMRI scanning parameters used, one because of technical difficulties with the scanner, and one because of falling asleep during the scan. These exclusions did not substantially alter the clinical and demographic profile of participants. In addition, three of the participants completed only 3 out of 4 runs, because either they fell asleep during the scanning procedures (n=1), or their clinical pain was excessive and prevented them from proceeding further with the scan (n=2).



FMRI data were acquired using a 3T Siemens Tim Trio MRI System, equipped for echoplanar imaging with a 32-channel head coil. During 4 BOLD fMRI runs (TR/TE=2sec/30ms, 37 slices, voxel size = 3.1x3.1x3.6mm), patients received a total of eight 75-105s (average  $\pm$  SD: 90  $\pm$  10s) cuff pain stimuli (2 per run) individually calibrated to elicit target intensity ratings of ~40/100, on the left calf. In order to evaluate the impact of attentional focus on pain processing (an ancillary aim of the original study), we varied subjects' focus of attention across the 4 runs; in 2 runs subjects were instructed to keep their eyes open and to look at a fixation cross and in 2 runs subjects were instructed to keep their eyes closed and to focus on a pleasant visual image in a pseudorandomized order. In an effort to avoid disruption of the imagery task during the imaging runs, subjects were asked to keep their eyes closed, and only expressed 'average' pain intensity (0=no pain; 100=the most intense pain imaginable) and unpleasantness ratings (0=not unpleasant at all; 100=extremely unpleasant) at the end of each run. Since mixed factorial ANOVA (group X focus condition X time) revealed that neither the effect of time on these pain ratings was significant ( $F(1,54)=0.357$ ,  $p=.553$ ), nor was there a significant group\*time interaction ( $F(1,52)=0.392$ ,  $p=0.534$ ), and focus condition did not have any significant differences across groups (Group\*focus condition interaction:  $F(1,52)=0.395$ ,  $p=0.533$ ), all four imaging runs were collapsed into one average for further analyses, and the effect of attentional focus will not be discussed further. We also collected anatomical MRI data, using a multi-echo MPRAGE pulse sequence (TR/TE1/TE2/TE3/T4=2530/1.64/3.5/5.36/7.22 ms, flip angle=7°, voxel size=1mm isotropic).

*Statistics:* Analysis of behavioral data was performed using SPSS (V 22, Chicago, IL). Data for continuous variables are presented as means and standard deviations (SDs), and data for categorical variables are presented as percentages. A temporal summation score was computed

by subtracting a patient's end pressure pain rating from their initial pressure pain rating during the prolonged painful cuff stimulus. To determine significant group differences in reported clinical symptoms, psychosocial and psychophysical variables, independent samples t-test or Fisher/Chi-square tests were performed. In order to examine whether pressure pain ratings varied as a function of cuff time, or subject group, mixed-model analysis of variance (ANOVA) was conducted. Additionally, a paired samples t-test between initial and final pain ratings was conducted to assess temporal summation. To compare PAS ratings between groups, an independent samples t-test was used. In order to investigate the inter-relationships between PAS and other variables amongst the subjects with FM, Pearson correlation coefficients were calculated. PAS from the behavioral visit were also used in the imaging regression analysis. Significance for all tests was set at  $\alpha = 0.05$ .

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)). Data were first corrected for slice timing (slicetimer) and motion (MCFLIRT), and were then skull stripped (BET), realigned to a single fMRI volume within-session (FLIRT), grand-mean intensity normalized by a single multiplicative factor, high-pass temporal filtered (Gaussian-weighted least-squares straight line fitting, with  $\sigma=136-164s$  depending on the run, and estimated using FSL's `cutoffcalc` tool) and spatially smoothed (FWHM=5mm). Time-series statistical analysis was carried out using FILM with local autocorrelation correction. Cortical surface reconstruction was performed using FreeSurfer (<http://surfer.nmr.mgh.harvard.edu/>) for improved structural-functional co-registration, which was carried out using FreeSurfer's `bbregister` tool.<sup>33</sup> In order to ensure that any effect observed in the fMRI data would not be confounded by differences in head motion, we computed for each subject the maximum rotation

(in radians, rad) and the maximum translation (in millimeters, mm) along the x, y and z axes, and compared them across groups. Maximum rotation values (mean $\pm$ stdev) were 0.023 $\pm$ 0.012, 0.011 $\pm$ 0.009 and 0.014 $\pm$ 0.012 rad for controls and 0.028 $\pm$ 0.027, 0.010 $\pm$ 0.009 and 0.013 $\pm$ 0.011 rad for FM patients. Maximum translation values were 0.649 $\pm$ 0.489, 0.353 $\pm$ 0.160 and 1.332 $\pm$ 0.581 for the controls and 0.704 $\pm$ 0.634, 0.682 $\pm$ 0.715 and 1.504 $\pm$ 1.827 for FM patients. None of the group differences for each the 6 motion parameters were statistically significant (p's=0.45, 0.93 and 0.53 for the rotations; 0.76, 0.09, 0.72 for the translations).

A first level within-subject general linear model (GLM) analysis was performed by modeling the sustained tonic response as a boxcar function (beginning from cuff inflation to cuff deflation), and the 15s period after stimulus offset. The latter regressor was used in our design matrix because our behavioral data (see results) in this as well as other studies<sup>66</sup> indicated the presence of PAS during this period. In addition, the stimulus onset and offset transients were modeled using stick functions (1 TR each), similar to previous investigations.<sup>11,44,78</sup> These regressors were included to improve the overall modeling of brain activity, as previous work has demonstrated that task/stimulus transitions give rise to transient BOLD responses which are, at least in part, spatially separable from the tonic/sustained brain responses observed throughout the presentation of the task or stimulus.<sup>23,29,44,60,78</sup> Such modeling is arguably more important in the presence of relatively long stimulus blocks, as was the case in our study. The inclusion of the offset regressor, in particular, improved the separation between brain responses to the discrete act of cuff deflation versus the more prolonged 15s post-stimulus period with noted PAS. A canonical double-gamma hemodynamic response function was convolved with all of the above regressors. Finally, six head motion parameters (3 translations and 3 rotations), as well as a regressor of no interest for each volume deemed to be an outlier in terms of motion (as computed

using `fsl_motions_outliers`) were also included in the design matrix to minimize the effect of motion in the estimation of our brain responses to our variables of interest. The first-level parameter estimate and corresponding variance maps were registered to the MNI152 standard space using the FMRIB's Nonlinear Image Registration Tool (FNIRT) for group analyses. Group differences in the 15s post-stimulus period, as well as the association between this activity and the behavioral ratings of PAS (for subjects with FM), were then assessed in whole-brain voxelwise GLMs, using FLAME (FMRIB's Local Analysis of Mixed Effects) 1+2, with automatic outlier detection enabled. The resulting statistical map was cluster corrected for multiple comparisons using the FSL default cluster-forming voxel-wise threshold of  $Z > 2.3$ , and a (corrected) cluster significance threshold of  $P < 0.05$ . For illustrative purposes, the average percent signal change was extracted from the statistically significant clusters, masked by anatomical labels obtained from the Harvard Oxford Atlas (thresholded at the arbitrary value of 30). The medial temporal lobe (MTL) label was obtained by fusing the Amygdala, Hippocampus and Parahippocampal Gyrus labels from the Atlas.

## Results:

### *Demographic, psychosocial, psychophysical and clinical pain characteristics of subjects*

Subjects with FM and healthy controls were comparable in age, sex, ethnicity and education (**Table 1**). However, as expected, subjects with FM differed from healthy control subjects in their degree of pain and physical symptoms, including pain severity, pain interference, fatigue, widespread pain, and symptom severity, according to commonly used clinical assessment tools for FM. Additionally, subjects with FM reported a greater degree of depressive symptoms, and higher ‘trait’ pain catastrophizing (although not different ‘situational’ catastrophizing during QST) than controls (Table 1). Upon psychophysical testing, subjects with FM reported greater pain sensitivity overall, with lower pressure pain thresholds at multiple body locations, and higher PAS ratings (**Table 2**).

### *Temporal Summation of prolonged cuff Pain in FM and control subjects*

To assess the degree of temporal summation of pain (TSP) in subjects, a prolonged, moderately painful pressure of inflatable cuff was applied to the left leg, using an individually-determined pressure to produce a 40/100 NRS rating for each subject at cuff onset. Consistent with other QST studies, significantly less pressure was needed to produce 40/100 level of pain in FM subjects than controls (**Table 2**). This pressure was then used in a 2-minute long sustained cuff inflation, with patients rating pain at 0, 60 and 120 seconds post-stimulation onset. Mixed model ANOVA revealed that pain scores significantly increased over time ( $F(66,2)=9.135$ ,  $p<0.001$ ), and that there was a significant effect of group on pain scores ( $F(66,1)=5.303$ ,  $p=0.024$ ), indicating that those with FM had significantly higher pain scores. A paired sample t-test showed a significant increase in pain scores from baseline to 120 seconds in the FM group (t

(51)=4.9,  $p < .001$ ), but not in the control group ( $t(16)=1.1$ ,  $p=.30$ ) (**Figure 1A**). Notably, there was some variability among individuals within both groups, in terms of whether temporal summation of pain occurred (i.e. last score higher than initial score), pain remained the same throughout the duration of the cuff stimulus, or habituation occurred (i.e. last score lower than initial score). Among the FM patients, 69% had temporal summation, 13% had no change, and 17% habituated. Among controls, 53% had temporal summation, 12% reported no change, and 35% habituated. However, these proportions were not statistically different between groups ( $\chi^2=2.46$ ,  $p=0.293$ ) (**Figure 1B**).

#### *Painful After-sensations (PAS) in FM and controls after prolonged cuff stimulus*

Subjects were also asked to rate the degree of pain they experienced 15 seconds after the deflation of the painful cuff stimulus (painful after-sensations, PAS). Interestingly, despite experiencing a significantly lower cuff pressure over these 2 minutes (mean cuff inflation = ~100 mmHg for FM subjects, mean cuff inflation = ~160 mmHg for controls), subjects with fibromyalgia more frequently reported PAS at 15 seconds (50% of FM v 12% of controls,  $\chi^2=7.77$ ,  $p=0.005$ ) (Figure 2B). The average severity of PAS was also significantly higher in subjects with FM ( $t(67)=3.5$ ,  $p=0.001$ ) (**Figure 2A**). We then examined the relation of PAS severity with other relevant QST parameters including TSP, general and more specific measures of clinical pain, and measures of pain catastrophizing, in FM patients. PAS severity was correlated with overall cuff pain ratings but not to the degree of TSP. PAS severity was also significantly correlated to clinical pain as rated on the BPI, but not to measures of pain catastrophizing (**Table 3**).

### *FMRI response to pain and painful after-sensations (PAS)*

The group difference maps for brain responses to the prolonged painful cuff stimulus, as well as to the 15s post-stimulus offset, are shown in **Figure 3** (see **Table 4** for cluster information). Group maps for the various regressors modeled in the GLM are presented in **Supplementary Figure 1**.

Whole-brain voxelwise group comparisons revealed that FM patients demonstrated significantly dampened deactivation (less negative BOLD signal) of the medial temporal lobe (MTL; amygdala, hippocampus, parahippocampal gyrus, entorhinal cortex) during both cuff pain stimulation (**Figure 3A**), as well as during the 15-second period following cuff deflation (post-offset period) which corresponds to timing of PAS (**Figure 3B**). This group difference reached statistical significance for the right MTL during the pain stimulation, and bilaterally during the post-offset period. Additional regions showing higher BOLD signal in FM patients during the post-offset period included the anterior middle cingulate cortex (aMCC), the medial prefrontal cortex (MPFC), the supplementary and pre-supplementary motor cortices, the ventral striatum/nucleus accumbens, the frontal pole, the posterior parietal cortex, and the cerebellum (**Figure 3B; Table 5**).

In FM patients, the magnitude of PAS measured at the behavioral visit was correlated with brain activity during the corresponding 15-second period following cuff deflation in several regions, including the left MTL, the left middle insular and frontoinsula cortex, dorsal ACC/MPFC, frontal pole, the putamen, and the occipital cortex (**Figure 3C, Table 6**). Among these regions, the MTL and dACC/MPFC clusters overlapped (shown in green) with those observed when comparing groups during the post-offset period.

## Discussion:

We found that painful after-sensations (PAS) following a perceptually matched, moderately painful mechanical stimulus were more intense and of higher incidence in subjects with FM than in healthy controls. Similar to previous investigators,<sup>2,24,65</sup> we also found that PAS were significantly correlated with clinical pain severity in FM (i.e., participants with higher ratings of the intensity of PAS also reported more severe daily pain). Although PAS and catastrophizing scores were higher in subjects with FM, amongst FM subjects PAS severity was not correlated to trait (PCS) and state (SPCS) catastrophizing scores. Interestingly, brain activity during the time period when PAS were measured (i.e. 15 seconds after prolonged moderately painful cuff stimuli) differed between FM and healthy control subjects. Specifically, healthy controls, who reported lower incidence and severity of PAS and lower catastrophizing, showed greater deactivation in the medial temporal lobe, including amygdala, hippocampus and parahippocampal gyrus. Moreover, fMRI signal in MTL and dACC/MPFC correlated with PAS severity. These findings may suggest that a relative lack of deactivation in these areas may be important to the experience of PAS, and possibly to pain sensitization in the temporal dimension (pain prolongation) in FM.

Although the majority of studies of temporal summation in FM have employed a cutaneous heat stimulus, repeated or sustained pressure stimuli also produce temporal summation of pain in FM.<sup>43,69,70</sup> Furthermore, both phasic and tonic application of a painful stimulus can produce temporal summation,<sup>3</sup> and both modes of application show a close correlation with psychosocial factors such as catastrophizing.<sup>32</sup> As in the current study, lower pressures are typically required to produce pain in FM patients compared to controls.<sup>40,51,70</sup> Thus, given similar basal mean arterial pressures between FM and controls, one would expect that the degree of



ischemia from cuff inflation would be more pronounced in controls (average SBP 120.31 vs 120.24,  $t(66) = -0.019$   $p = 0.985$ ). However, we cannot exclude differences in peripheral pressure sensitivity and perfusion dynamics as accounting for group differences in this paradigm. Our moderately painful (40/100) prolonged cuff stimulus produced temporal summation in most patients with FM, consistent with previous studies which observe a modest increase in temporal summation in FM as a group over controls with stimulus matching.<sup>43,69,70</sup> The duration of the painful cuff stimulus we employed was relatively long compared to some other measures of temporal summation<sup>5</sup>, for the purpose of increasing the likelihood of detecting both temporal summation and PAS. Although there was some variability in the response to prolonged cuff stimulus in both groups, FM patients reported increased incidence and severity of PAS compared to controls, similar to previous reports.<sup>70</sup> TSP and PAS, although collected in tandem in a combined psychophysical test, may represent separable psychophysical phenomena,<sup>61</sup> and, importantly, this study noted no correlation between measurements of an individual's TSP and PAS (**Table 3**). While TSP measures augmentation of pain in terms of increased *amplitude* of pain with a prolonged or repeated stimulus, PAS measures increased *duration* of pain beyond the end of the pain stimulus. To the extent that both tests attempt to assess pain plasticity at a central level, it is not surprising that they are both amplified in FM patients, as FM is increasingly recognized as a pain disorder with a prominent component of central sensitization. Importantly, variability in the degree of TSP and PAS as measured by QST can be observed generally among individuals, as well as between groups such as men/women<sup>28</sup> or older/younger adults.<sup>35</sup> This individual variability has been shown to distinguish those who develop post-surgical pain from those who do not,<sup>66</sup> and to predict risk for opiate misuse in individuals with chronic pain.<sup>25</sup>

The degree to which psychosocial processes such as catastrophizing influence an individual's experience of pain also varies amongst individuals. These processes likely exert their influence on pain-related outcomes at a variety of central nervous system sites.<sup>21</sup> Interestingly, previous studies have also found a relationship between measures of pain augmentation (such as TSP and PAS), and indices of negative affect, anxiety, and fear of pain.<sup>24,63,67,80</sup> In the present study, we similarly found a significantly higher report of both pain-related catastrophizing and PAS among subjects with FM, whether measured as a stable trait with the PCS, or measured "situationally" in reference to experimental pain stimulation (SPCS). However, within the FM subject cohort, there was not a significant correlation between PAS and measures of catastrophizing (Table 3). Given the cross-sectional nature of this study, we are unable to determine the temporal association between amplified PAS, and fibromyalgia-relevant clinical outcomes such as the spatial extent of clinical pain and FM symptom severity. However, the incidence of both higher PAS and catastrophizing in subjects with FM, together with a concurrent decreased activation in MTL during the period immediately following offset of a noxious stimulus, suggests at least the possibility that altered brain processing in these regions may represent an underlying pathophysiological process that contributes to a variety of clinical manifestations of chronic pain (e.g., amplified pain sensitivity, perceptions of pain that outlast the stimulus, spreading pain, elevated pain-related distress, etc.).

While PAS have been reported in several chronic pain disorders,<sup>27,30,58,70</sup> the neural correlates of PAS have not previously been elucidated in the brain, although a recent study showed higher dorsal horn spinal activation in FM, who also had higher PAS<sup>12</sup>. The present study found that PAS, which were prominent in subjects with FM, and more rare in control subjects, were associated with differences in medial temporal lobe (MTL) response (specifically,

deactivation). Of note, MTL alterations have been previously reported in FM. For instance, the parahippocampus was shown to have significantly reduced gray matter density,<sup>46,83</sup> reduced cerebral blood flow,<sup>34</sup> as well as reduced binding potential to dopamine tracers,<sup>82</sup> which correlated with self-reported pain<sup>1</sup> in FM patients. Anatomic-functional changes have also been reported in patients with chronic pain in the amygdala,<sup>22,42</sup> including decreases in gray matter volume,<sup>15,53</sup> increases in fractional anisotropy,<sup>53</sup> and alterations of power spectral density of low-frequency.<sup>42</sup> Finally, studies have also reported FM-related changes in the hippocampus, including hippocampal atrophy,<sup>55</sup> reduced activation during a cognitive performance task,<sup>54</sup> reduced levels of N-acetyl aspartate, a measure of neuronal integrity,<sup>4</sup> as well as reduced binding potential for mu opioid receptor ligands.<sup>36</sup> Our study further uncovers a link between altered functional response in the MTL in FM patients and their experience of PAS. In addition, post-stimulus brain activity in the insula, anterior middle cingulate cortex and medial prefrontal cortex correlated with severity of PAS. While these brain regions have been associated with hyperalgesia in studies evaluating brain response to experimental pain administration, to our knowledge their role in PAS has not previously been evaluated.

Interestingly, MTL regions and medial prefrontal cortex are ‘core’ structures of the default-mode network (DMN),<sup>14</sup> while anterior insula and anterior middle cingulate cortex are key nodes of the salience network (SN)<sup>68</sup>. The DMN and SN networks are key subsystems of what has been recently labeled the ‘pain connectome’,<sup>47</sup> and are thought to dynamically contribute to the experience of experimental pain by modulating one’s propensity to attend to a noxious stimulus. For instance, when attention is maintained on a nociceptive stimulus, SN regions show increased activity and DMN regions show decreased activity. Conversely, when attention fluctuates away from a nociceptive stimulus, SN regions show relatively reduced

activation, and DMN regions show reduced deactivation.<sup>46,48</sup> While this often antagonistic interplay between SN and DMN is commonly observed in healthy volunteers, it becomes disrupted in the context of chronic pain. For instance, Napadow and colleagues<sup>56</sup> have demonstrated that the insula, a core SN region, becomes abnormally connected to the DMN in FM patients, and that the stronger the functional connectivity between insula and DMN, the greater the severity of clinical pain reported. Aberrantly increased connectivity between DMN regions and the insula has been replicated in patients with other disorders, including chronic low back pain, complex regional pain syndromes and osteoarthritis.<sup>9,51</sup> In the present study, we show that alterations in the response of DMN and SN regions are also associated with PAS. While additional studies are needed to further characterize this association, these findings add to the body of evidence implicating these networks in chronic pain disorders, including FM<sup>18,56,57</sup> and others.<sup>6-8,38,51,75</sup>

#### Limitations:

While the presence of PAS and related brain activity may be interpreted as a manifestation of central sensitization, it is possible that other factors may contribute the group differences in psychophysical and imaging data here reported. For instance, it is possible that local changes potentially associated with cuff algometry (e.g., group differences in the latency and magnitude of muscle ischemia) may have a role. However, while we were not able to directly measure tissue ischemia, it should be noted that systolic blood pressure was similar between groups. Furthermore, due to our percept-matching design, patients with FM received significantly *lower* cuff pressures than controls. Nonetheless, future studies should assess the role of peripheral mechanisms mediating PAS in FM. Another potential limitation of the present

study is that the neurobiological substrates of TSP obtained in our study are more uncertain than if we had adopted a more conventional wind-up-like behavioral paradigm (which is more clearly known to be mediated by c-fiber-induced wind-up of dorsal horn neurons). Nonetheless, while the neurobiological correlates of TSP induced by tonic stimuli may not have been as well-studied as in the context of wind-up paradigms, previous neurophysiological investigations have indeed demonstrated a contribution of c-fibers to sensitization in the context of sustained mechanical stimulation.<sup>3</sup> It is also important to note that control subjects, despite being exposed to a higher pressure cuff stimulus to produce moderate pain (40/100), did not develop the same degree of TSP over the course of the stimulus as did FM subjects as a group, thus potentially confounding the interpretation of differences in PAS. Viewing this difference in the propensity to develop TSP as a confound, however, rests on the supposition that TSP is necessary to produce the experience of PAS, which a recent study suggests.<sup>39</sup> Interestingly, however, we did not observe a significant correlation between the degree of TSP and PAS within individual subjects, thus suggesting that these are at least partially separable phenomena. It should also be recognized that, although TSP and PAS have been observed with a variety of stimulus modalities (i.e. thermal or mechanical), it is as yet unclear whether the same mechanisms underlie TSP and PAS in these different behavioral paradigms. Finally, another limitation of our study is that the PAS and brain imaging data were collected in separate visits. While collecting ratings in real-time would have allowed a more direct assessment of the association between these variables, we decided against this option in order to reduce interference of real time pain rating with fMRI data collection. Lastly, while we observed differences between FM and controls in this study, we cannot claim that the effects observed are specific to FM. Future studies including an additional

group of patients with a different etiology of chronic pain could potentially address the question of whether the observed differences are unique to FM.

In summary, we observed that PAS after a prolonged pressure pain stimulus were both more prevalent and more severe in subjects with FM. Importantly, we found that these PAS were correlated with the degree of clinical symptoms reported in these subjects, consistent with previous findings that PAS were the most robust psychophysical predictor of clinical pain in FM.<sup>25</sup> As with TSP, the severity of PAS varied between subjects, and higher PAS severity was correlated with higher pain scores. This first study of the neural correlates of PAS found that PAS severity corresponded to less deactivation in the MTL, suggesting that altered brain processing in these regions may represent an underlying pathophysiological process that contributes, at least in part, to pain amplification and prolongation in FM.

## References:

1. Albrecht DS, MacKie PJ, Kareken DA, Hutchins GD, Chumin EJ, Christian BT, Yoder KK: Differential dopamine function in fibromyalgia. *Brain Imaging Behav.* 10:829-39, 2016
2. Anderson RJ, McCrae CS, Staud R, Berry RB, Robinson ME: Predictors of clinical pain in fibromyalgia: examining the role of sleep. *J Pain.* 13:350-8, 2012
3. Andrew D, Greenspan JD. Peripheral coding of tonic mechanical cutaneous pain: comparison of nociceptor activity in rat and human psychophysics. *J Neurophysiol.* 82:2641-8, 1999
4. Aoki Y, Inokuchi R, Suwa H: Reduced N-acetylaspartate in the hippocampus in patients with fibromyalgia: a meta-analysis. *Psychiatry Res.* 213:242-8, 2013
5. Arendt-Nielsen L: Central sensitization in humans: assessment and pharmacology. *Handb Exp Pharmacol.* 227:79-102, 2015
6. Baliki MN, Chialvo DR, Geha PY, Levy RM, Harden RN, Parrish TB, Apkarian AV: Chronic pain and the emotional brain: specific brain activity associated with spontaneous fluctuations of intensity of chronic back pain. *J Neurosci* 26:12165–73, 2006
7. Baliki MN, Geha PY, Apkarian AV, Chialvo DR: Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J Neurosci.* 28:1398–403, 2008
8. Baliki MN, Baria AT, Apkarian AV: The cortical rhythms of chronic back pain. *J Neurosci.* 31:13981–90, 2011
9. Baliki MN, Mansour AR, Baria AT, Apkarian AV: Functional reorganization of the default mode network across chronic pain conditions. *PLoS One.* 9:e106133, 2014
10. Beck AT, Steer RA, Garbin MG: Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clinical Psychology Reviews.* 8:77-100, 1988
11. Becerra L, Navratilova E, Porreca F, Borsook D: Analogous responses in the nucleus accumbens and cingulate cortex to pain onset (aversion) and offset (relief) in rats and humans. *J Neurophysiol.* 110:1221-6, 2013
12. Bosma RL, Mojarad EA, Leung L, Pukall C, Staud R, Stroman PW: FMRI of spinal and supra-spinal correlates of temporal pain summation in fibromyalgia patients. *Hum Brain Mapp.* 37:1349-60, 2016
13. Bouhassira D, Attal N, Alchaar H, Boureau F, Brochet B, Bruxelle J, Cunin G, Fermanian J, Ginies P, Grun-Overdyking A, Jafari-Schluep H, Lantéri-Minet M, Laurent B, Mick G, Serrie A, Valade D, Vicaute E.: Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4). *Pain.* 114:29–36, 2005
14. Buckner RL, Andrews-Hanna JR, Schacter DL: The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci.* 1124:1-38. Review, 2008
15. Burgmer M, Gaubitz M, Konrad C, Wrenger M, Hilgart S, Heuft G, Pfeleiderer B: Decreased gray matter volumes in the cingulo-frontal cortex and the amygdala in patients with fibromyalgia. *Psychosom Med.* 71:566-73, 2009
16. Campbell CM, Kronfli T, Buenaver LF, Smith MT, Berna C, Haythornthwaite JA, Edwards RR: Situational versus dispositional measurement of catastrophizing: Associations with pain responses in multiple samples. *J Pain* 11: 443–53, 2010

17. Campbell CM, McCauley L, Bounds SC, Mathur VA, Conn L, Simango M, Edwards RR, Fontaine KR: Changes in pain catastrophizing predict later changes in fibromyalgia clinical and experimental pain report: cross-lagged panel analyses of dispositional and situational catastrophizing. *Arthritis Res Ther*. 14:R231, 2012
18. Cifre I, Sitges C, Fraiman D, Munoz MA, Balenzuela P, Gonzalez-Roldan A, Martinez-Jauand M, Birbaumer N, Chialvo DR, Montoya P: Disrupted functional connectivity of the pain network in fibromyalgia. *Psychosom Med* 74: 55–62, 2012
19. Clauw DJ: Fibromyalgia: a clinical review. *JAMA*. 311:1547-55, 2014
20. Cook DB, Stegner AJ, McLoughlin MJ: Imaging pain of fibromyalgia. *Curr Pain Headache Rep*. 11:190-200. Review, 2007
21. Darnall BD: Pain Psychology and Pain Catastrophizing in the Perioperative Setting: A Review of Impacts, Interventions, and Unmet Needs. *Hand Clin*. 32:33-9. Review, 2016
22. Dehghan M, Schmidt-Wilcke T, Pfliegerer B, Eickhoff SB, Petzke F, Harris RE, Montoya P, Burgmer M: Coordinate-based (ALE) meta-analysis of brain activation in patients with fibromyalgia. *Hum Brain Mapp*. 37:1749-58, 2016
23. Downar J, Mikulis DJ, Davis KD: Neural correlates of the prolonged salience of painful stimulation. *Neuroimage*. 20:1540-51, 2003
24. Edwards RR, Smith MT, Stonerock G, Haythornthwaite JA: Pain-related catastrophizing in healthy women is associated with greater temporal summation of and reduced habituation to thermal pain. *Clin J Pain*. 22:730-7, 2006
25. Edwards RR, Wasan AD, Michna E, Greenbaum S, Ross E, Jamison RN: Elevated pain sensitivity in chronic pain patients at risk for opioid misuse. *J Pain*. 12:953-63, 2011
26. Edwards RR, Mensing G, Cahalan C, Greenbaum S, Narang S, Belfer I, Schreiber KL, Campbell C, Wasan AD, Jamison R.: Alteration in pain modulation in women with persistent pain after lumpectomy: influence of catastrophizing. *J Pain Symptom Manage*. 46:30-42, 2013
27. Eide PK, Rabben T: Trigeminal neuropathic pain: pathophysiological mechanisms examined by quantitative assessment of abnormal pain and sensory perception. *Neurosurgery*. 43:1103-10, 1998
28. Fillingim RB, Maixner W, Kincaid S, Silva S: Sex differences in temporal summation but not sensory-discriminative processing of thermal pain. *Pain* 75: 121–127, 1998
29. Fox MD, Snyder AZ, Barch DM, Gusnard DA, Raichle ME: Transient BOLD responses at block transitions. *Neuroimage*. 28:956-66, 2005
30. Gottrup H1, Kristensen AD, Bach FW, Jensen TS: Aftersensations in experimental and clinical hypersensitivity. *Pain*. 103:57-64, 2003
31. Gracely RH, Petzke F, Wolf JM, Clauw DJ: Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum*. 46:1333-43, 2002
32. Granot M, Granovsky Y, Sprecher E, Nir RR, Yarnitsky D: Contact heat-evoked temporal summation: tonic versus repetitive-phasic stimulation. *Pain*. 122:295-305, 2006
33. Greve DN, Fischl B: Accurate and robust brain image alignment using boundary- based registration. *Neuroimage*. 48:63-72, 2009
34. Guedj E, Taieb D, Cammilleri S, Lussato D, de Laforte C, Niboyet J, Mundler O: 99mTc-ECD brain perfusion SPECT in hyperalgesic fibromyalgia. *Eur J Nucl Med Mol Imaging*. 34:130-4, 2007



35. Harkins SW, Davis MD, Bush FM, Kasberger J: Suppression of first pain and slow temporal summation of second pain in relation to age. *J Gerontol* 51: 260–265, 1996
36. Harris RE, Clauw DJ, Scott DJ, McLean SA, Gracely RH, Zubieta JK: Decreased central mu-opioid receptor availability in fibromyalgia. *J Neurosci.* 27:10000-6, 2007
37. Harris RE, Sundgren PC, Craig AD, Kirshenbaum E, Sen A, Napadow V, Clauw DJ: Elevated insular glutamate in fibromyalgia is associated with experimental pain. *Arthritis Rheum.* 60:3146-52, 2009
38. Hemington KS, Wu Q, Kucyi A, Inman RD, Davis KD: Abnormal cross-network functional connectivity in chronic pain and its association with clinical symptoms. *Brain Struct Funct.* 221:4203-4219, 2016
39. Janal MN, Raphael KG, Cook DB, Sirois DA, Nemelivsky L, Staud R: Thermal temporal summation and decay of after-sensations in temporomandibular myofascial pain patients with and without comorbid fibromyalgia. *J Pain Res.* 9:641-52, 2016
40. Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Giesecke T, Mainguy Y, Gracely R, Ingvar M: Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. *Pain.* 144:95-100, 2009
41. Jensen KB, Srinivasan P, Spaeth R, Tan Y, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Vitton O, Gracely R, Ingvar M, Kong J: Overlapping structural and functional brain changes in patients with long-term exposure to fibromyalgia pain. *Arthritis Rheum.* 65:3293-303, 2013
42. Kim JY, Kim SH, Seo J, Kim SH, Han SW, Nam EJ, Kim SK, Lee HJ, Lee SJ, Kim YT, Chang Y: Increased power spectral density in resting-state pain-related brain networks in fibromyalgia *Pain.* 154:1792-7, 2013
43. Kim J, Loggia ML, Cahalan CM, Harris RE, Beissner F, Garcia RG, Kim H, Barbieri R, Wasan AD, Edwards RR, Napadow V: The somatosensory link in fibromyalgia: functional connectivity of the primary somatosensory cortex is altered by sustained pain and is associated with clinical/autonomic dysfunction. *Arthritis Rheumatol.* 67:1395-405, 2015
44. Konishi S, Donaldson DI, Buckner RL: Transient activation during block transition. *Neuroimage.* 13:364-74, 2001
45. Kuchinad A1, Schweinhardt P, Seminowicz DA, Wood PB, Chizh BA, Bushnell MC: Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? *J Neurosci.* 11;27:4004-7, 2007
46. Kucyi A, Davis KD. Dynamic functional connectivity of the default mode network tracks daydreaming. *Neuroimage.* 2014 Oct 15;100:471-80
47. Kucyi A, Davis KD. The dynamic pain connectome. *Trends Neurosci.* 38:86-95, Review, 2015
48. Kucyi A, Salomons TV, Davis KD. Mind wandering away from pain dynamically engages antinociceptive and default mode brain networks. *Proc Natl Acad Sci U S A.* 110:18692-7, 2013
49. Larson AA, Giovengo SL, Russell IJ, Michalek JE: Changes in the concentrations of amino acids in the cerebrospinal fluid that correlate with pain in patients with fibromyalgia: implications for nitric oxide pathways. *Pain.* 87:201-11, 2000
50. Legangneux E, Mora JJ, Spreux-Varoquaux O, Thorin I, Herrou M, Alvado G, Gomeni C: Cerebrospinal fluid biogenic amine metabolites, plasma-rich platelet serotonin and [3H]imipramine reuptake in the primary fibromyalgia syndrome. *Rheumatology.* 40:290-6, 2001

51. Loggia ML, Kim J, Gollub RL, Vangel MG, Kirsch I, Kong J, Wasan AD, Napadow V: Default mode network connectivity encodes clinical pain: an arterial spin labeling study. *Pain*.154:24-33, 2013
52. Loggia ML, Berna C,J, Cahalan CM, Gollub RL, Wasan AD, Harris RE, Edwards RR, Napadow V: Disrupted brain circuitry for pain-related reward/punishment in fibromyalgia. *Arthritis Rheumatol*. 66:203-12, 2014
53. Lutz J, Jäger L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G: White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. *Arthritis Rheum*. 58:3960-9, 2008
54. Martinsen S, Flodin P, Berrebi J, Löfgren M, Bileviciute-Ljungar I, Ingvar M, Fransson P, Kosek E: Fibromyalgia patients had normal distraction related pain inhibition but cognitive impairment reflected in caudate nucleus and hippocampus during the Stroop Color Word Test. *PLoS One*. 9:e108637, 2014
55. McCrae CS, O'Shea AM, Boissoneault J, Vathauer KE, Robinson ME, Staud R, Perlstein WM, Craggs JG: Fibromyalgia patients have reduced hippocampal volume compared with healthy controls. *J Pain Res*. 8:47-52, 2015
56. Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE: Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum*. 62:2545-55, 2010
57. Napadow V, Kim J, Clauw DJ, Harris RE: Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. *Arthritis Rheum*. 64:2398-403, 2012
58. Noordenbos W: *Pain*. Amsterdam: Elsevier, 1959
59. Oaklander AL, Herzog ZD, Downs HM, Klein MM: Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *Pain*.154:2310-6, 2013
60. Paret C, Kluetsch R, Ruf M, Demirakca T, Kalisch R, Schmahl C, Ende G: Transient and sustained BOLD signal time courses affect the detection of emotion-related brain activation in fMRI. *Neuroimage*. 103:522-32, 2014
61. Price DD, Hayes RL, Ruda M, Dubner R: Neural representation of cutaneous aftersensations by spinothalamic tract neurons. *Fed Proc*. 37:2237-9, 1978
62. Price, D.D., R. Staud, M.E. Robinson, A.P. Mauderli, R. Cannon, C.J. Vierck: Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. *Pain*, 99:49–59, 2002
63. Robinson ME, Bialosky JE, Bishop MD, Price DD, George SZ: Supra-threshold scaling, temporal summation, and after-sensation: relationships to each other and anxiety/fear. *J Pain Res*. 3:25-32, 2010
64. Sarlani E, Grace EG, Reynolds MA, Greenspan JD: Sex differences in temporal summation of pain and aftersensations following repetitive noxious mechanical stimulation. *Pain*. 109:115-23, 2004
65. Sato H, Saisu H, Muraoka W, Nakagawa T, Svensson P, Wajima K: Lack of temporal summation but distinct aftersensations to thermal stimulation in patients with combined tension-type headache and myofascial temporomandibular disorder. *J Orofac Pain*. 26:288-95, 2012
66. Schreiber KL, Martel MO, Shnol H, Shaffer JR, Greco C, Viray N, Taylor LN, McLaughlin M, Brufsky A, Ahrendt G, Bovbjerg D, Edwards RR, Belfer I: Persistent pain in postmastectomy patients: comparison of psychophysical, medical, surgical, and psychosocial characteristics between patients with and without pain. *Pain*. 154:660-8, 2013
67. Schreiber KL, Campbell C, Martel MO, Greenbaum S, Wasan AD, Borsook D, Jamison RN, Edwards RR: Distraction analgesia in chronic pain patients: the impact of catastrophizing. *Anesthesiology*.121:1292-301, 2014

68. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL and Greicius MD: Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 27: 2349-2356, 2007
69. Staud R, Vierck C.J, Cannon R.L, Mauderli A.P, Price D.D: Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain*. 91:165–175, 2001
70. Staud RC, Cannon RL, Mauderli AP, ME, Robinson, Price DD, Vierck Jr CJ: Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome. *Pain*, 102:87–95, 2003
71. Staud R, Price DD, Robinson, ME, Vierck Jr. CJ: Body pain area and pain-related negative affect predict clinical pain intensity in patients with fibromyalgia. *J Pain*. 5:338–343, 2004
72. Staud R, Robinson ME, Price DD: Temporal summation of second pain and its maintenance are useful for characterizing widespread central sensitization of fibromyalgia patients. *J Pain*. 8:893-901, 2007
73. Staud R, Weyl EE, Riley JL 3rd, Fillingim RB: Slow temporal summation of pain for assessment of central pain sensitivity and clinical pain of fibromyalgia patients. *PLoS One*. 18;9(2):e89086, 2014
74. Sullivan MJ, Bishop SR, Pivik J: The Pain Catastrophizing Scale: Development and Validation. *Psychol Assess*. 7:524-32, 1995
75. Tagliazucchi E, Balenzuela P, Fraiman D, Chialvo DR: Brain resting state is disrupted in chronic back pain patients. *Neurosci Lett*. 485:26–31, 2010
76. Tan G, Jensen MP, Thornby JJ, Shanti BF: Validation of the Brief Pain Inventory for chronic nonmalignant pain. *J Pain*. 5:133-137, 2004
77. Üçeyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C: Small fibre pathology in patients with fibromyalgia syndrome. *Brain*. 136:1857-67, 2013
78. Uludağ K: Transient and sustained BOLD responses to sustained visual stimulation. *Magn Reson Imaging*. 26(7):863-9, 2008
79. Vaerøy H, Helle R, Førre O, Kåss E, Terenius L: Elevated CSF levels of substance P and high incidence of Raynaud phenomenon in patients with fibromyalgia: new features for diagnosis. *Pain*. 32:21-6, 1988
80. Wideman TH, Finan PH, Edwards RR, Quartana PJ, Buenaver LF, Haythornthwaite JA, Smith MT: Increased sensitivity to physical activity among individuals with knee osteoarthritis: relation to pain outcomes, psychological factors, and responses to quantitative sensory testing. *Pain*. 155:703-11, 2014
81. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB: The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. *Arthritis Care Res* 62:600-10, 2010
82. Wood PB, Patterson JC 2nd, Sunderland JJ, Tainter KH, Glabus MF, Lilien DL: Reduced presynaptic dopamine activity in fibromyalgia syndrome demonstrated with positron emission tomography: a pilot study. *J Pain*. 8:51-8, 2007
83. Wood PB, Glabus MF, Simpson R, Patterson JC 2nd: Changes in gray matter density in fibromyalgia: correlation with dopamine metabolism. *J Pain*. 10:609-18, 2009

Figure legends:

**Figure 1: Temporal summation to prolonged moderately painful leg cuff in subjects with FM and controls**

A cuff was applied to the left leg and inflated to a pressure corresponding to 40/100 pain for each subject. This pressure was then held for a 2 minute period and subjects asked to rate the pain on a numeric rating scale (NRS) (0-100). A) Pain ratings at 0, 60, and 120 seconds, showing significant increase in cuff scores in FM, but not controls (means and standard error shown). B) Variability of response to sustained painful cuff, showing the proportion of subjects with increase in score between initiation and end of cuff stimulus (temporal summation of pain, TSP), no change (NC), and with decrease in score between initiation and end of cuff stimulus (habituation, HAB), although no difference in these groupings between FM and controls (Chi 2.46,  $p=0.293$ ).

**Figure 2: Painful After-sensations (PAS) in subjects with FM and controls**

15 seconds after deflation of the painful leg cuff, subjects were asked to rate any ongoing pain in their leg. A) Subjects with FM had a significantly rating of painful after sensations than controls (means and standard error shown). B) Variability of PAS ratings in subjects with FM (closed triangles) and control subjects (open triangles).

**Figure 3: Group differences in brain responses during and after painful cuff stimulus.**

A) Patients demonstrated markedly dampened deactivations during pain stimulation, and (B) in the 15s period after stimulus offset. (C) Brain activity during the post-offset period was statistically associated with the reports of painful after sensations measured in the behavioral

visit, including in regions demonstrating group differences (green). Scatterplots are presented for illustrative purposes. MTL=medial temporal lobe; aMCC=anterior middle cingulate cortex, MPFC=medial prefrontal cortex.

**Supplementary Figure 1:** Brain responses to the various components of the stimulation paradigm: onset, block, offset and post-offset period, averaged among all participants. S1/M1 = primary somatosensory / motor cortex; SMA=supplementary motor area, aMCC=anterior middle cingulate cortex; MPFC=medial prefrontal cortex, pgACC=pregenual anterior cingulate cortex, MOC=medial occipital cortex, PCC=posterior cingulate cortex; IPL=inferior parietal lobule; S2=secondary somatosensory cortex; operc.=operculum; LTC=lateral temporal cortex

## Tables:

Table 1: Demographic, clinical, and psychosocial characteristics

| Factor  | Control            | Fibromyalgia       |                  |
|---|--------------------|--------------------|------------------|
|   | mean±SD or %       | mean±SD or %       | P value          |
| <b><i>Demographic</i></b>   |                    |                    |                  |
| Age   | <b>44.1 ± 14.8</b> | <b>46.3 ± 11.4</b> | <b>.52</b>       |
| Female gender   | <b>71%</b>         | <b>87%</b>         | <b>.15</b>       |
| Ethnicity (% non-caucasian)                                       | <b>11.7%</b>       | <b>20.7%</b>       | <b>.50</b>       |
| Education (7 pt scale, range some high school to doctoral degree) | <b>5.0 ± .5</b>    | <b>4.4 ± 1.3</b>   | <b>.10</b>       |
| <b><i>Clinical Pain characteristics</i></b>                       |                    |                    |                  |
| Pain severity (BPI)(NRS 0-10)                                     | <b>.29 ± .51</b>   | <b>5.4 ± 2.1</b>   | <b>&lt; .001</b> |
| Pain interference (BPI)(NRS 0-10)                                 | <b>.01 ± .05</b>   | <b>5.8 ± 2.1</b>   | <b>&lt; .001</b> |
| Fatigue (NRS)(0-100)  | <b>15.8 ± 18.4</b> | <b>66.6 ± 21.4</b> | <b>&lt; .001</b> |
| Widespread pain index (FM diag ≥ 7)                               | <b>.4 ± .8</b>     | <b>10.9 ± 2.6</b>  | <b>&lt; .001</b> |
| Symptom severity sum (FM diag ≥ 5)                                | <b>1.5 ± 1.7</b>   | <b>9.3 ± 1.9</b>   | <b>&lt; .001</b> |
| Neuropathic Pain Questionnaire (NPQ)(0-10)                        | <b>.09 ± 0.18</b>  | <b>4.2 ± 1.8</b>   | <b>&lt; .001</b> |
| <b><i>Psychosocial characteristics</i></b>                        |                    |                    |                  |
| Depression (BDI)(0-63)  | <b>3.1 ± 3.8</b>   | <b>15.2 ± 8.2</b>  | <b>&lt; .001</b> |
| Catastrophizing (PCS)(0-56)                                       | <b>5.6 ± 5.8</b>   | <b>23.3 ± 13.0</b> | <b>&lt; .001</b> |
| Situational catastrophizing score (0-10)                          | <b>3.3 ± 4.1</b>   | <b>4.7 ± 4.9</b>   | <b>.28</b>       |

Fibromyalgia patients had more widespread pain, fatigue, and higher trait, but not situational, catastrophizing or depression scores than control subjects; Fisher's Exact test, Pearson Chi-Square, or independent samples t-test used for group comparisons, as appropriate. Abbreviations: BPI= Brief Pain Inventory, FM= Fibromyalgia, NRS= numerical rating scale, SD= standard deviation, VAS= Visual analog scale

Table 2: Psychophysical Characteristics

| Quantitative Sensory Test  | Control        | Fibromyalgia     |             |
|--|----------------|------------------|-------------|
|  | mean±SD        | mean±SD          | p-value     |
| Cuff pressure (mmHg) producing 40/100 pain intensity                 | <b>160±74</b>  | <b>100±43</b>    | <b>.005</b> |
| Pressure pain threshold thumb (mmHg)                                 | <b>337±92</b>  | <b>258±87</b>    | <b>.004</b> |
| Pressure pain threshold trapezius (mmHg)                             | <b>361±131</b> | <b>250±131</b>   | <b>.005</b> |
| Painful after sensation rating 15 s after cuff deflation (NRS 0-100) | <b>2.1±7.3</b> | <b>13.6±20.4</b> | <b>.001</b> |

Fibromyalgia patients had lower pain thresholds and higher pain ratings than their age- and gender- matched controls; independent samples t-test used for group comparisons. Abbreviations: mmHg: millimeters of mercury, NRS=numerical rating scale

Table 3: Correlation of Painful After-Sensations with psychophysical, clinical and psychosocial characteristics

| Variable   | Correlation with PAS            |             |
|--|---------------------------------|-------------|
|  | Pearson correlation coefficient | p-value     |
| <i><b>Psychophysical characteristics</b></i>         |                                 |             |
| Cuff pressure (mmHg) producing 40/100 pain intensity | <b>-.365</b>                    | <b>.008</b> |
| Average cuff pain over 2 min inflation               | <b>.330</b>                     | <b>.017</b> |
| Temporal summation of pain                           | <b>.187</b>                     | <b>.185</b> |
| <i><b>Clinical pain characteristics</b></i>          |                                 |             |
| BPI Severity   | <b>.380</b>                     | <b>.005</b> |
| BPI Interference                                     | <b>.303</b>                     | <b>.029</b> |
| Widespread pain index                                | <b>.199</b>                     | <b>.171</b> |
| Symptom Severity Sum                                 | <b>-.064</b>                    | <b>.664</b> |
| <i><b>Psychosocial characteristics</b></i>           |                                 |             |
| Pain Catastrophizing (PCS)                           | <b>.202</b>                     | <b>.151</b> |
| Situational pain catastrophizing (SPCS)              | <b>.204</b>                     | <b>.146</b> |

Painful aftersensations were correlated with other psychosocial measures of pain sensitivity, clinical pain and FM symptom measures, as well as a measure of trait and state catastrophizing in FM subjects. Abbreviations: BPI: Brief Pain Inventory, mmHg: millimeters of mercury



Table 4. Group differences in brain responses to cuff stimulation blocks

| Cluster size (# voxels) | Cluster P value | Local maxima |            |            |            |                           |
|-------------------------|-----------------|--------------|------------|------------|------------|---------------------------|
|                         |                 | Z            | MNI x (mm) | MNI y (mm) | MNI z (mm) | Label                     |
| <i>FM&gt;CTRL</i>       |                 |              |            |            |            |                           |
| 1329                    | 0.000757        | 4.33         | 20         | 2          | -24        | R Entorhinal cortex       |
|                         |                 | 4.2          | 20         | -2         | -22        | R amygdala                |
|                         |                 | 3.98         | 38         | 18         | -42        | R temporal pole           |
|                         |                 | 3.84         | 20         | -2         | -34        | R parahippocampal gyrus   |
|                         |                 | 3.56         | 26         | -10        | -22        | R hippocampus             |
|                         |                 | 3.05         | 40         | 0          | -42        | R inferior temporal gyrus |
| <i>CTRL&gt;FM</i>       |                 |              |            |            |            |                           |
| n.s.                    |                 |              |            |            |            |                           |

Abbreviations: FM: fibromyalgia, CTRL: control, MNI: Montreal Neurological Institute

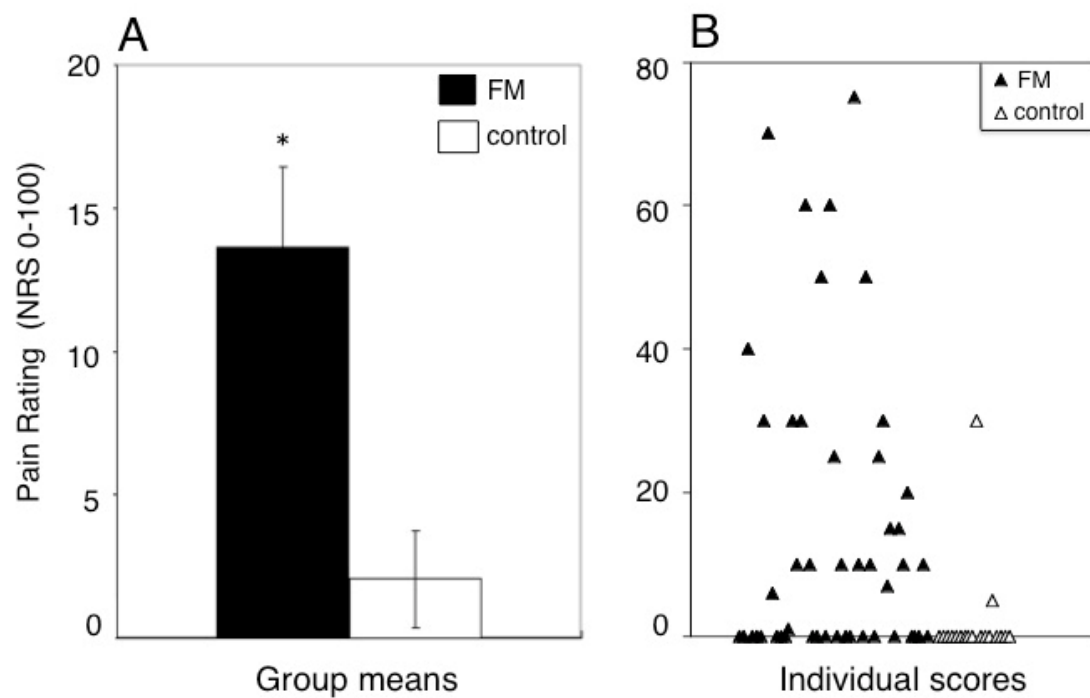
Table 5. Group differences in brain responses to post-offset period

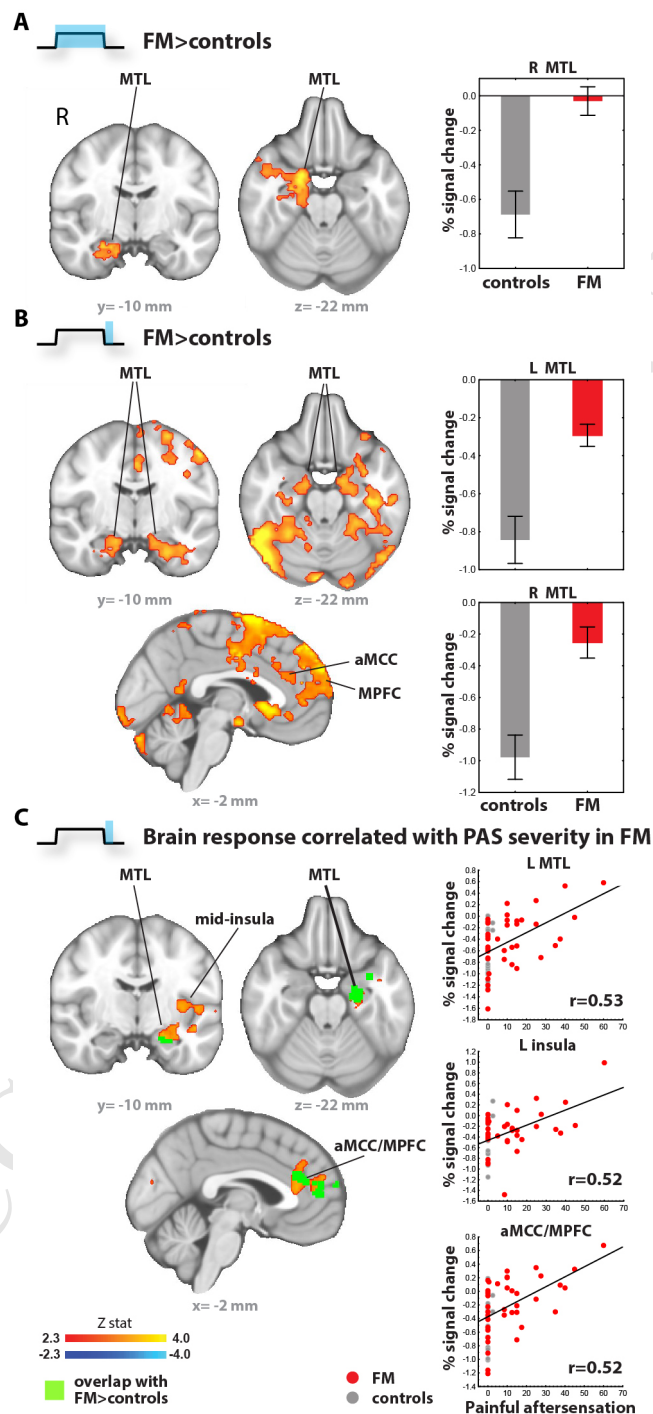
| Cluster size (# voxels) | Cluster P value | Local maxima |     |     |     |                                       |
|-------------------------|-----------------|--------------|-----|-----|-----|---------------------------------------|
|                         |                 | Z            | x   | y   | z   | Label                                 |
| <i>FM&gt;CTRL</i>       |                 |              |     |     |     |                                       |
| 17473                   | 7.99E-26        | 6.18         | 54  | -56 | -20 | R inferior temporal gyrus             |
|                         |                 | 5.33         | -16 | 62  | 20  | L frontal pole                        |
|                         |                 | 4.77         | 48  | -74 | -32 | R cerebellum (hemisphere)             |
|                         |                 | 4.7          | 8   | 36  | -8  | R subgenual cingulate cortex          |
|                         |                 | 4.64         | -2  | 2   | 72  | L supplementary motor area            |
|                         |                 | 4.55         | -22 | 40  | 50  | L superior frontal gyrus              |
|                         |                 | 4.38         | 20  | 64  | 24  | R frontal pole                        |
|                         |                 | 4.23         | -4  | -6  | 42  | L posterior middle cingulate cortex   |
|                         |                 | 4.2          | -28 | -8  | 66  | L premotor cortex                     |
|                         |                 | 4.18         | -52 | -6  | 50  | L precentral gyrus                    |
|                         |                 | 4.09         | -36 | -22 | -22 | L fusiform cortex                     |
|                         |                 | 4.07         | -46 | -72 | -24 | L cerebellum (hemisphere)             |
|                         |                 | 4.06         | -28 | 12  | -16 | L frontoinsular cortex                |
|                         |                 | 4.02         | -6  | 44  | 8   | L pregenual anterior cingulate cortex |
|                         |                 | 3.97         | 16  | 44  | 50  | R superior frontal gyrus              |
|                         |                 | 3.89         | -32 | -20 | 42  | L postgenual gyrus                    |
|                         |                 | 3.88         | 20  | -14 | -26 | R entorhinal cortex                   |
|                         |                 | 3.68         | -10 | 28  | 28  | L anterior middle cingulate cortex    |
|                         |                 | 3.64         | 22  | -20 | -16 | R hippocampus                         |
|                         |                 | 3.59         | -14 | -8  | -20 | L amygdala                            |
|                         |                 | 3.43         | 30  | -24 | -24 | R parahippocampal gyrus               |
|                         |                 | 3.32         | -16 | -12 | -18 | L hippocampus                         |
|                         |                 | 3.32         | 8   | 8   | -10 | R nucleus accumbens                   |
|                         |                 | 3.27         | -6  | 14  | -4  | L nucleus accumbens                   |
|                         |                 | 2.95         | -18 | -6  | -32 | L entorhinal cortex                   |
|                         |                 | 2.82         | 16  | -6  | -20 | R amygdala                            |
| 702                     | 0.0305          | 4            | 0   | -60 | 62  | precuneus                             |
|                         |                 | 3.56         | 8   | -66 | 66  | R superior parietal lobule            |
| <i>CTRL&gt;FM</i>       |                 |              |     |     |     |                                       |
| n.s.                    |                 |              |     |     |     |                                       |

Abbreviations:FM:fibromyalgia, CTRL:control

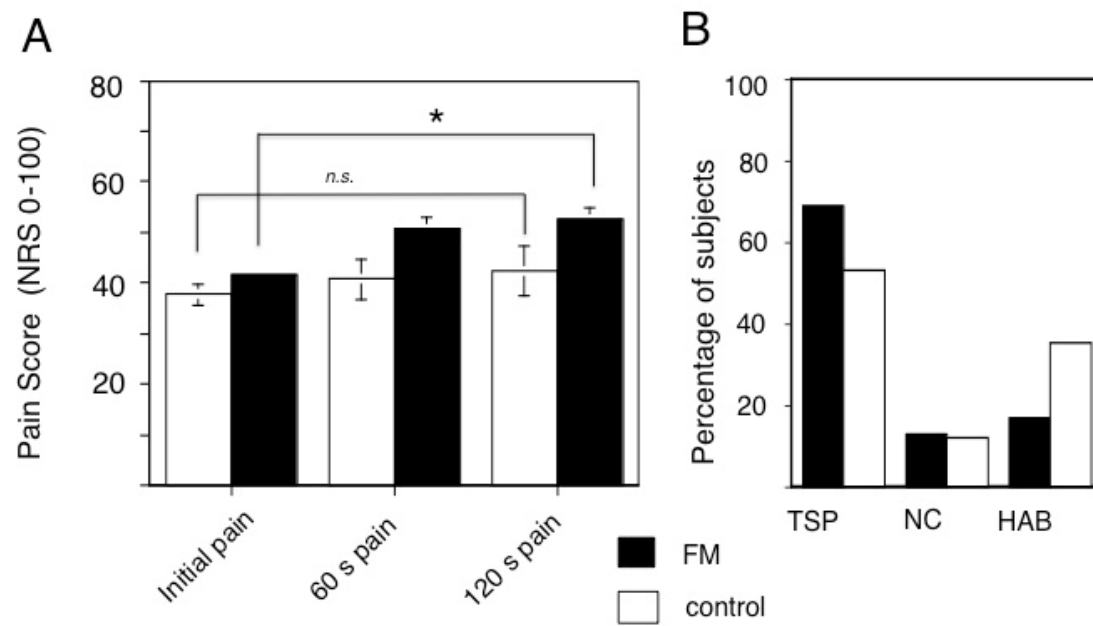
Table 6. Correlations between PAS and brain responses to post-offset period

|                         |                 | Local maxima |     |     |     |   |
|-------------------------|-----------------|--------------|-----|-----|-----|---|
| Cluster size (# voxels) | Cluster P value | Z            | x   | y   | z   | Label   |
| positive correlations   |                 |              |     |     |     |   |
| 1485                    | 0.000269        | 3.69         | -24 | 16  | -8  | L putamen                                     |
|                         |                 | 3.57         | -56 | 36  | 0   | L ventrolateral prefrontal cortex             |
|                         |                 | 3.48         | -20 | -4  | -16 | L amygdala                                    |
|                         |                 | 3.35         | -12 | 20  | 2   | L head of the caudate                         |
|                         |                 | 3.19         | -42 | -6  | -12 | L planum polare                               |
|                         |                 | 3.15         | -22 | -16 | -20 | L hippocampus                                 |
|                         |                 | 3.03         | -36 | -10 | 6   | L posterior insula                            |
| 1127                    | 0.00201         | 3.74         | 14  | -98 | 14  | R occipital pole                              |
|                         |                 | 3.54         | -8  | -94 | 4   | L occipital pole                              |
| 855                     | 0.0107          | 3.79         | -4  | 52  | 24  | L medial frontal gyrus                        |
|                         |                 | 3.74         | 0   | 48  | 14  | anterior middle cingulate/paracingulate gyrus |
| negative correlations   |                 |              |     |     |     |   |
| n.s.                    |                 |              |     |     |     |   |

**Figure 2: Painful After-sensations (PAS) in subjects with FM and controls**



**Figure 1: Pain ratings during prolonged cuff stimulus in subjects with FM and controls**



Highlights:

- Painful After-sensations (PAS) after a prolonged mechanical stimulus are more common and severe in individuals with Fibromyalgia (FM)
- PAS and catastrophizing are associated with clinical pain severity in FM
- fMRI showed reduced deactivation of the medial temporal lobe (MTL) in FM patients in the post-stimulation period
- Greater PAS severity is associated with less MTL deactivation