

Dynamic Levels of Glutamate Within the Insula Are Associated With Improvements in Multiple Pain Domains in Fibromyalgia

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Objective. Fibromyalgia (FM) is a chronic widespread pain condition that is thought to arise from augmentation of central neural activity. Glutamate (Glu) is an excitatory neurotransmitter that functions in pain-processing pathways. This study was carried out to investigate the relationship between changing levels of Glu within the insula and changes in multiple pain domains in patients with FM.

Methods. Ten patients with FM underwent 2 sessions of proton magnetic resonance spectroscopy (H-MRS) and 2 sessions of functional magnetic resonance imaging (fMRI), each conducted before and after a nonpharmacologic intervention to reduce pain. During H-MRS, the anterior and posterior insular regions were examined separately using single-voxel spectroscopy. The levels of Glu and other metabolites were estimated relative to levels of creatine (Cr) (e.g., the Glu/Cr ratio). During fMRI, painful pressures were applied to the thumbnail to elicit neuronal activation.

Experimental pressure-evoked pain thresholds and clinical pain ratings (on the Short Form of the McGill Pain Questionnaire [SF-MPQ]) were also assessed prior to each imaging session

Results. Both experimental pain ($P = 0.047$ versus pretreatment) and SF-MPQ-rated clinical pain ($P = 0.043$ versus pretreatment) were reduced following treatment. Changes from pre- to posttreatment in Glu/Cr were negatively correlated with changes in experimental pain thresholds ($r = -0.95$, $P < 0.001$) and positively correlated with changes in clinical pain ($r = 0.85$, $P = 0.002$). Changes in the fMRI-determined blood oxygenation level-dependent effect (a measure of neural activation) were positively correlated with changes in Glu/Cr within the contralateral insula ($r = 0.81$, $P = 0.002$).

Conclusion. Changes in Glu levels within the insula are associated with changes in multiple pain domains in patients with FM. Thus, H-MRS data may serve as a useful biomarker and surrogate end point for clinical trials of FM.

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Fibromyalgia (FM) is a chronic widespread pain disorder that affects ~2–4% of individuals in industrialized countries (1). Although the underlying etiology of this condition is unknown, dysfunction within the central nervous system has been implicated. Results from functional magnetic resonance imaging (fMRI) (2,3), single-photon emission tomography (4), and positron emission tomography (5) support this hypothesis.

One structure that is consistently found to be associated with augmented evoked pain activity in FM is the insula (2,3). In addition to its function in speech, taste, and auditory systems, the insula is also intimately involved in somatosensory and visceral pain processing (6). It is strategically located in a bidirectional pathway between the secondary somatosensory cortex and the amygdala (6). This anatomic position may give the insula

a unique regulatory function within the “pain matrix.” Topographically, the posterior insula is thought to be involved in discriminative activities of sensory pain (7), whereas the anterior insula may play a greater role in processing the affective dimension of pain (8).

Glutamate (Glu) is a major excitatory neurotransmitter within the nervous system and is known to function in pain neuropathways. The binding of Glu to ionotropic receptors increases the sodium permeability of neuronal membranes and results in cell activation (i.e., membrane depolarization). Since elevated Glu levels have been reported in the cerebrospinal fluid of patients with FM (9), it is reasonable to suspect that this molecule may be responsible for the augmented pain transmission observed in FM (2,3).

We performed a longitudinal proton magnetic resonance spectroscopy (H-MRS) study to investigate the role of Glu within the insula of patients with FM. H-MRS is a noninvasive procedure that can be used to determine the relative concentration of specific brain metabolites *in vivo*. We focused our investigation on the changing levels of Glu following a nonpharmacologic treatment, within both the anterior and posterior insula of patients with FM. We hypothesized that changes in the levels of Glu should be positively correlated with changes in clinical pain. Conversely, changes in Glu levels should be negatively correlated with changes in pressure-evoked pain thresholds, since lower thresholds are indicative of greater pain sensitivity. In addition, we used FMRI in these same subjects to determine whether changes in Glu levels were related to changes in pain-related neural activity.

PATIENTS AND METHODS

Participants. As part of an ongoing study investigating the impact of acupuncture treatment in FM, 10 female patients (mean \pm SD age 48 ± 15 years) were examined in 2 sessions of H-MRS and 2 sessions of FMRI, with the sessions spaced 1 month apart (Figure 1). Participants were randomized to receive either 9 traditional acupuncture treatments or 9 non-skin-penetrating sham acupuncture treatments, administered between imaging sessions. All analyses described herein were carried out in a blinded manner with the treatment assignment masked, since we were not interested in the potential differential effects between treatment with acupuncture and sham treatment, but rather in whether changes in the levels of Glu correspond to changes in pain. All participants gave their written informed consent, and all study protocols were approved by the University of Michigan Institutional Review Board.

Participant inclusion and exclusion criteria have been reported previously (5). All participants met the American College of Rheumatology 1990 criteria for the diagnosis of FM

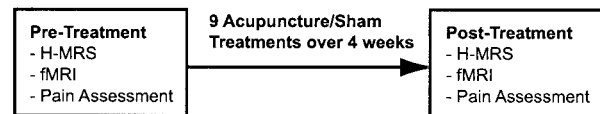


Figure 1. Experimental design. Patients with fibromyalgia underwent pretreatment assessments with both proton magnetic resonance spectroscopy (H-MRS) and functional magnetic resonance imaging (FMRI). During H-MRS testing, resting levels of glutamate were obtained in the insula. During FMRI, neural activations (i.e., BOLD [blood oxygenation level–dependent] effects) were elicited with painful pressure stimuli applied to the left thumbnail bed. Pretreatment assessments of clinical pain using the Short Form of the McGill Pain Questionnaire, and experimental pressure-evoked pain sensitivity thresholds were also obtained. Following the baseline assessment, participants received either 9 acupuncture treatments or 9 sham acupuncture treatments over 4 weeks. H-MRS, FMRI, and pain outcomes were then measured again, following the last treatment.

(10) and had a disease duration of at least 1 year. In addition, all patients reported experiencing pain for more than 50% of the days prior to the trial period.

H-MRS. All participants underwent conventional MR imaging of the brain on a General Electric 3.0T MR scanner (GE, Milwaukee, WI). Single-voxel spectroscopy (SVS) was performed using point-resolved spectroscopy, with a repetition time (TR) of 3,000 msec, echo time (TE) of 30 msec, 90° flip angle, number of excitations 8, field of view (FOV) 16 cm, and volume of interest (VOI) of $2 \times 2 \times 3$ cm. During each session, 2 separate SVS sequences were performed, once with the VOI placed in the right anterior insula and once in the right posterior insula (Figure 2A). The right insula was chosen because it is contralateral to the pressure-evoked pain stimulus applied during FMRI. Patients were at rest during both H-MRS sessions.

The raw data from each SVS sequence were subjected to manual postprocessing using H-MRS software (LCModel; Oakville, Ontario, Canada) (Figure 2B). Values for Glu, glutamine (Gln), and the combination of Glu and Gln (Glx) were calculated as ratios to an internal standard, the creatine (Cr) level (e.g., Glu/Cr). Similar calculations were done for other major metabolites, including *N*-acetylaspartate (NAA), choline (Cho) compounds, and myoinositol (MI). Although these other metabolites are not known to play a role in neuronal activity, they were measured to assess the relative specificity of Glu and Gln in our analyses.

Functional MRI. To assess the relationship between changes in Glu and changes in neural activity, all participants also underwent 2 FMRI sessions, once prior to treatment and once posttreatment. Functional MRI scans were acquired on the same 3.0T scanner as used for H-MRS. On each scanning day, subjects completed 2 FMRI runs, acquired with a spiral gradient-echo sequence (TR 2,500 msec, TE 30 msec, 90° flip angle, FOV 22 cm). Slices were 3-mm thick, with an in-plane resolution of 3.125×3.125 , acquired at 48 locations parallel to the anterior-posterior commissure plane. Preprocessing was performed using statistical parametric mapping 2 (SPM2; Wellcome Department of Cognitive Neurology, London, UK) and included correction for slice-acquisition time to the middle slice, realignment to the first volume of each run to correct for

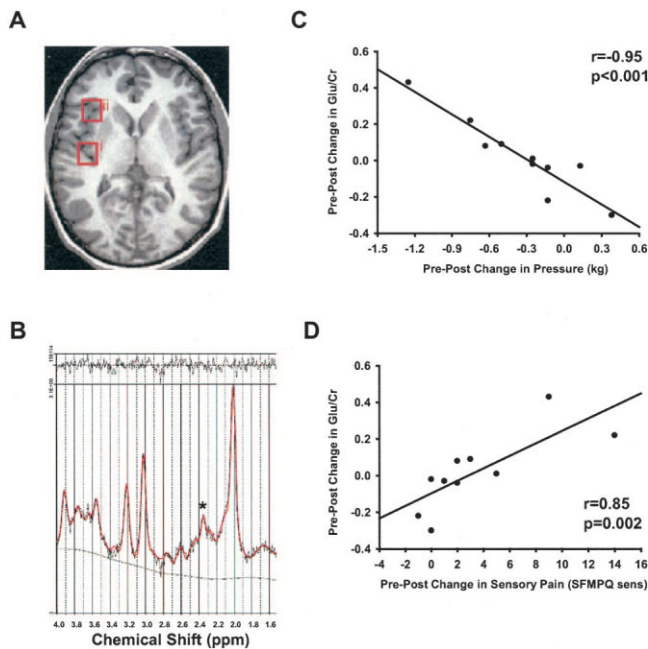


Figure 2. Association of changing levels of glutamate (Glu) with improvements in pain outcomes in patients with fibromyalgia (FM). **A**, Axial T1-weighted image showing single-voxel placement for both the posterior (i) and anterior (ii) right insula in proton magnetic resonance spectroscopy (H-MRS). **B**, Representative H-MRS spectrum from the posterior insula of a patient with FM, analyzed by fitting data with the LCModel, which uses a linear combination of individual spectra obtained from pure molecular species to fit the experimental spectra (red trace); * = resonance from 2 Glu γ -proton resonances at 2.35 ppm. Values for Glu were calculated as a ratio to the creatine (Cr) internal standard (Glu/Cr). **C**, Negative correlation of pre- to post-treatment changes in Glu/Cr within the posterior insula with pre- to post-treatment changes in mildly painful pressure-evoked pain thresholds. **D**, Positive correlation of pre- to post-treatment changes in Glu/Cr within the posterior insula with pre- to post-treatment changes in clinical pain (assessed as the sensory pain score on the Short Form of the McGill Pain Questionnaire [SF-MPQ]).

intrascan movement, and smoothing with a Gaussian kernel of 8 mm full width at half maximum to compensate for small residual anatomic variations across subjects. Smoothed images were then band pass-filtered to eliminate low-frequency signals.

A general linear model was constructed with parameters corresponding to the type of pressure stimulus applied (either no touch or innocuous touch) in each block, with Gracely Box Scale (GBS) scores of 0.5 for low pain, 7.5 for mild pain, and 13.5 for moderate pain; modeling of each run was carried out separately. Blocks were 25 seconds in duration and presented according to a fixed pseudorandom paradigm in which every other block consisted of the “no touch” condition. To allow for comparison across individuals, 1 of the 3 painful pressure blocks was set to 2 kg/cm². Each stimulus block was convolved with a canonical hemodynamic response function. Parameter estimates of block-related activity were established

for each voxel, and contrast images were calculated by applying a linear contrast of the parameter estimates of the 2 kg/cm² pressure versus the “no touch” condition for each participant. The resulting statistical images obtained for each subject were then spatially normalized into International Consortium for Brain Mapping space by applying T1-weighted spoiled gradient echo transformation parameters to the SPM2 contrast image.

Differences in the blood oxygenation level-dependent (BOLD) effects (a measure of neural activation) from pre-treatment to post-treatment were calculated (as a percentage) for the entire volume in each individual, and were assessed for correlations with Glu/Cr change scores (pretreatment to post-treatment) in the posterior insula. Our a priori hypothesis was that changes in BOLD activation within the insula would be correlated with changes in Glu/Cr values. Therefore, we used an uncorrected significance threshold of $P < 0.001$, with a minimum cluster size of 10 voxels for clusters identified within the insula. Individual BOLD activation responses were extracted using the Marsbar Region of Interest Toolbox (version 0.38; <http://marsbar.sourceforge.net>).

Clinical pain. Assessment of clinical pain was performed prior to each imaging session using ratings on the Short Form of the McGill Pain Questionnaire (SF-MPQ) (11). Our analysis focused on the sensory dimension of pain assessed by this questionnaire, since the magnitude of sensory pain was reduced with treatment.

Experimental pain. Pressure-evoked pain tenderness was assessed prior to each imaging session (12,13). Briefly, discrete pressure stimuli were applied to the subject’s left thumbnail using a stimulation device that eliminates any direct examiner–subject interaction. Pain intensity ratings were recorded on a GBS questionnaire using a random presentation paradigm. During the testing, stimulus pressures were determined interactively; a computer program continuously adjusted the stimulus pressures at 3 levels to produce the same response distribution (i.e., GBS scores of 0.5, 7.5, and 13.5) in each subject. We assessed correlations of changes in metabolite levels with changes in the pressure-evoked pain thresholds at the mildly painful pressure level (GBS score of 7.5), since this threshold increased following treatment.

Statistical analysis. Ratios of the different metabolites to Cr, percent changes in BOLD activation, and pain ratings were analyzed using SPSS version 14 (SPSS, Chicago, IL). Due to our small sample size, we performed nonparametric Spearman’s correlation tests to determine significant relationships between Glu/Cr and changes in pain outcomes. For these correlation analyses, a Bonferroni-corrected P value of less than 0.0042 (calculated as 0.05 divided by 12) was applied as the level of significance for correlations between changes in metabolite ratios (Glu/Cr, Gln/Cr, and Glx/Cr) and changes in pain domains (i.e., 2 brain regions [anterior and posterior insula], 2 pain domains [clinical and experimental], and 3 metabolites). A similar correction (corrected $P < 0.0042$) was performed for the analysis of baseline and post-treatment metabolite ratios within the posterior insula and changes in pain (i.e., 3 metabolites, 2 time points, and 2 pain domains). Nonparametric Wilcoxon’s signed rank tests were performed to determine changes from pretreatment to post-treatment in clinical and experimental evoked pain.

RESULTS

Following acupuncture or sham treatments in this population of patients with FM, the pressure-evoked pain sensitivity after application of mildly painful pressures was significantly reduced (mean difference in experimental pain thresholds -0.34 kg [SD 0.46 kg]; $P = 0.047$). Moreover, clinical pain improved from pre- to posttreatment according to SF-MPQ ratings of the sensory dimension of pain (mean difference in clinical pain ratings 3.50 [SD 4.70]; $P = 0.043$).

Figure 2B depicts a representative spectrum obtained from the posterior insula of a patient prior to treatment. A significant negative correlation was detected between change scores for Glu/Cr in the posterior insula from pre- to posttreatment and changes in the pressures required to elicit mild pain from pre- to posttreatment ($r = -0.95$, $P < 0.001$) (Figure 2C). Similarly, a positive correlation was detected between changes in Glu/Cr in the posterior insula and changes in SF-MPQ (sensory) clinical pain ratings ($r = 0.85$, $P = 0.002$) (Figure 2D). Furthermore, higher levels of Glu/Cr in the posterior insula were also associated with greater reductions in clinical pain posttreatment ($r = 0.81$, $P = 0.004$).

No significant correlations were detected between change scores of any other metabolite ratios (i.e., NAA/Cr, Cho/Cr, or MI/Cr) in the posterior insula and changes in either clinical pain ratings or experimental evoked pain thresholds (all $P > 0.10$). In addition, no significant changes in Cr concentrations were detected within the posterior insula ($P = 0.98$).

Since there is debate as to whether H-MRS can accurately measure Glu separately from Gln within humans at 3T, we also assessed the combination of Glu and Gln as a ratio (i.e., Glx/Cr). Changes in Glx/Cr within the anterior insula ($r = -0.63$, $P = 0.049$) and posterior insula ($r = -0.62$, $P = 0.058$) were both negatively correlated with changes in pressure-evoked pain, albeit at the level of trend toward significance. Overall, these data are consistent with the idea that either insular Glu or insular Gln or both are associated with changes in multiple pain domains in FM.

Since Glu functions in pain neurotransmission, we next investigated whether pre- to posttreatment changes in Glu/Cr within the right posterior insula were associated with changes in the BOLD responses elicited by painful pressure applied to the thumbnail bed. Changes in Glu/Cr within the right posterior insula were positively correlated with changes in BOLD activation within the left posterior insula (T score 6.6, uncorrected

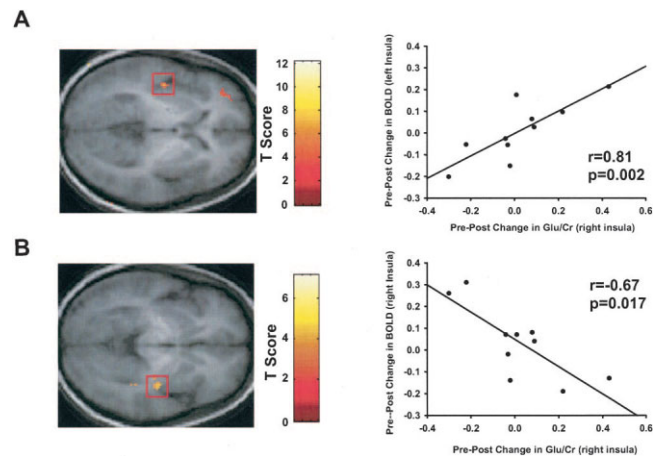


Figure 3. Association of dynamic levels of glutamate:creatine (Glu/Cr) within the posterior insula with changes in neural activity. **A**, Positive correlation of pre- to posttreatment changes in functional magnetic resonance imaging (fMRI)-determined blood oxygenation level-dependent (BOLD) activation within the left posterior insula with pre- to posttreatment changes in Glu/Cr within the right posterior insula (left). Scatterplot (right) depicts individual BOLD and Glu/Cr changes. **B**, Negative correlation of pre- to posttreatment changes in fMRI-determined BOLD activation within the right posterior insula with pre- to posttreatment changes in Glu/Cr within the right posterior insula (left). Scatterplot (right) depicts individual BOLD and Glu/Cr changes.

$P < 0.001$; Montreal Neurological Institute (MNI) coordinates $x = -42$, $y = -12$, $z = 0$) (Figure 3A). In contrast, a negative correlation, at the level of trend toward significance, was detected for changes in Glu/Cr and changes in BOLD activation in the right posterior insula (T score 4.1, uncorrected $P = 0.0018$; MNI coordinates $x = 38$, $y = -14$, $z = -6$) (Figure 3B).

DISCUSSION

These data are the first evidence of a correlation between changing levels of insular Glu and changes in pain in patients with FM. Since Glu is a major excitatory neurotransmitter involved in pain transmission, these observations are not unexpected. Our data are also consistent with findings from a recent H-MRS study in which increases in Glu/Cr within the anterior cingulate were observed in response to cold pain in healthy pain-free controls (14). However, the present data show primarily the converse of this relationship, namely, reductions in pain in association with lower Glu/Cr values.

Since detecting Glu-specific concentrations accurately at 3T in humans is difficult because of the

overlapping proton resonances between Gln and Glu, we also investigated the combinations of Glu/Cr and Gln/Cr (i.e., Glx/Cr), which may be less controversial (14). Similar to the above-described results, we found that pre- to posttreatment changes in Glx/Cr within the insula were negatively correlated with pre- to posttreatment changes in pressure-evoked pain thresholds. Since we did not detect a significant relationship between changes in any other major metabolites and improvements in pain outcomes, our findings are likely to be specific for Glu and/or Gln.

It is unlikely that our Glu measurements reflect solely synaptic levels of this neurotransmitter, since the volume of brain tissue sampled also included cell bodies and processes of nonneuronal cells. Our measurements probably reflect an average of combined intra- and extracellular Glu levels arising from both neuronal and nonneuronal cells. A growing body of research over the last decade suggests that the Glu–Gln cycle between astrocytes and neurons may regulate synaptic activity (15). Interestingly, individuals with the greatest pain reduction also showed higher levels of Gln/Cr posttreatment, suggesting that our treatment intervention may have altered the Glu–Gln cycle.

Consistent with our observed changes in Glu functioning in evoked pain activity, we also detected changes in fMRI-determined BOLD activation that occurred in parallel to the dynamic Glu/Cr levels in the posterior insula. These data are consistent with the idea that neural activity is augmented within this region in FM (2,3). However, we found a differential relationship between Glu/Cr within the right posterior insula and changes in BOLD activity within the left insula compared with the right insula. This observation was unexpected, and may reflect the possibility that our intervention influenced the left and right insula in a differential manner. Alternatively, Glu levels may influence activation of the BOLD effect differentially during task conditions compared with resting conditions. Additional research will be required to further explore this finding.

Due to the small sample size used in our trial, these findings should be interpreted carefully. However, our data suggest that Glu may be a useful biomarker for disease severity in FM. Thus, future investigations of Glu within FM patient populations are warranted.

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AUTHOR CONTRIBUTIONS

Dr. Harris had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Harris, Sundgren, Petrou, Gracely, Clauw.

Acquisition of data. Harris, Sundgren.

Analysis and interpretation of data. Harris, Pang, Hsu, Kim, McLean, Gracely, Clauw.

Manuscript preparation. Harris, Sundgren, Petrou, McLean, Gracely, Clauw.

Statistical analysis. Harris.

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