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Greater Somatosensory Afference with Acupuncture Increases Primary Somatosensory Connectivity and Alleviates Fibromyalgia Pain via Insular GABA: A Randomized Neuroimaging Trial

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Conflicts of Interest
All authors have no conflicts of interest with this manuscript.

Abstract

Objective: Acupuncture is a complex multi-component treatment that has shown promise for the treatment of Fibromyalgia (FM), however, clinical trials have shown mixed results, possibly due to heterogeneous methodology and lack of understanding of the underlying mechanism of action. We sought to understand the specific contribution

of somatosensory afference to improvements in clinical pain, and the specific brain circuits involved.

Methods: 76 FM patients were randomized to receive 8 weeks (2 treatments/week) of electroacupuncture (EA, with somatosensory afference) or mock laser acupuncture (ML, with no somatosensory afference). Brief Pain Inventory (BPI) Severity, resting state functional MRI (rs-fMRI), and proton magnetic resonance spectroscopy (^{1}H-MRS) in the right anterior insula (aINS) were collected at pre- and post-treatment.

Results: FM patients receiving EA experienced a greater reduction in pain severity compared to ML (mean difference, EA=-1.14, ML=-0.46, Group x Time interaction, p=0.036). Participants receiving EA, as compared to ML, also displayed increased resting functional connectivity between the primary somatosensory cortical representation of the leg ($S1_{leg}$; i.e. S1 subregion activated by EA) and aINS. Increase in $S1_{leg}$-aINS connectivity was associated with reductions in BPI severity (r=-0.44, p=0.01) and increases in aINS gamma-aminobutyric acid (GABA+) (r=-0.48, p=0.046) following EA. Moreover, increases in aINS GABA+ was associated with reductions in BPI severity (r=-0.59, p=0.01). Finally, post-EA changes in aINS GABA+ mediated the relationship between changes in $S1_{leg}$-aINS and BPI severity, bootstrapped CI=[-0.533, -0.037].

Conclusion: The somatosensory component of acupuncture modulates primary somatosensory functional connectivity in association with insular neurochemistry to reduce pain severity in FM.

Introduction

Fibromyalgia (FM) is a common chronic pain condition afflicting 2-8% of the population, and is characterized by widespread somatic pain, fatigue, poor sleep, negative mood, and cognitive disturbances[1]. While peripheral factors (e.g., small fiber neuropathy[2], immune system[3]) may play some role in FM, the disorder is thought to be caused primarily by aberrant central nervous system (CNS) physiology which amplifies

the perception of pain (also known as *centralized* or *nociplastic* pain[4]). Notably, neuroimaging research has shown that FM patients demonstrate increased levels of the excitatory neurotransmitter glutamate[5], decreased levels of the inhibitory neurotransmitter gamma aminobutyric acid (GABA)[6], and upregulated $GABA_A$ receptor concentration[7], within the insula. Moreover, increased functional brain network connectivity to pro-nociceptive brain areas and decreased connectivity to anti-nociceptive brain areas have been found in FM[8-10]. These results suggest that the CNS is a prime target for therapeutic interventions for FM.

Due to the ongoing opioid public health crisis[11], non-pharmacologic interventions for FM such as acupuncture have been gaining attention. However, meta-analyses of acupuncture trials have shown mixed results, with some showing that verum (active) acupuncture is no more effective than sham controls[12,13], while others have shown that acupuncture is superior to both sham and no-acupuncture controls in reducing pain[14]. One reason for the mixed meta-analytic results may be the inclusion of heterogenous treatment paradigms and sham controls across different trials. Acupuncture is a complex procedure that consists of multiple methodological (e.g., needling sensation, location, depth, etc), and contextual components (e.g., expectancy, patient-practitioner rapport, treatment ritual, etc.)[15]. Importantly, sham controls used in previous acupuncture trials may not have properly accounted for all of these different components of acupuncture.

In this study, we specifically evaluated CNS mechanism(s) of action underlying the somatosensory afferent component of acupuncture, and how such mechanisms may engender an analgesic response in FM. Since verum acupuncture produces somatosensory sensation through needling and palpation, we designed a comparator sham control procedure to lack all aspects of tactile sensation. Many previous trials of acupuncture used sham controls with acupoint palpation and tactile stimulation, mimicking real needle insertion and manipulation, thus confounding verum and sham acupuncture in terms of somatosensory afference[12-14]. We randomized FM patients into two separate acupuncture therapy groups: electroacupuncture (EA, i.e. *with* somatosensation) and mock laser acupuncture (ML, *without* somatosensation). EA has been demonstrated to be clinically effective at reducing pain for FM[13]. We hypothesized

that EA would specifically recruit somatosensory pathways in the CNS in order to produce greater analgesia compared to ML.

Methods

Overall Protocol

This study was a single-center, blinded, sham controlled, randomized non-crossover longitudinal neuroimaging study, pre-registered with ClinicalTrials.gov (NCT02064296). The study took place at the University of Michigan, Ann Arbor, MI from December 2014 to November 2019. All study protocols were approved by the University of Michigan Institutional Review board, and all study participants provided written informed consent in accordance with the Declaration of Helsinki.

Participants and Study Timeline

Participants suffering from FM were recruited for the study. Full details of inclusion and exclusion criteria are provided in **Supplementary Methods A**. Following screening, participants were invited to complete a baseline behavioral (day 0) and baseline MRI assessment (occurred anywhere between day 1 and 3), and eligible subjects were randomly assigned to one of two parallel study arms (**Figure 1A**). We used computer generated permuted block randomization (blocks of 4, 6, or 8). An acupuncturist was informed of group allocation of each participant through a sealed envelope, which was not accessible by the principal investigators, study staff, or data analysts. The two intervention arms were (i) Electroacupuncture (EA), *with* somatosensory afference, and (ii) Mock Laser acupuncture (ML), *without* somatosensory afference. After treatment, a second behavioral (day 33-40) and MRI assessment (day 34-43) were collected. Patient-reported outcomes were collected before and after therapy during the behavioral session. Whole brain resting state functional MRI (rs-fMRI) and right anterior insula (aINS) proton magnetic resonance spectroscopy (^{1}H-MRS) were collected during the MRI sessions before and after therapy.

Acupuncture Treatment

FM participants received 8 EA or ML treatments over four weeks (twice a week). During all treatment sessions, participants were positioned supine on an exam table and blindfolded. Blindfolding ensured masking of the treatments in order to avoid any visual afference, as visual afference can also influence acupuncture-induced analgesia[16]. All treatments were performed by three (H.B., M.D.B., and H.S.) trained acupuncturists with board certification from the National Certification Commission for Acupuncture and Oriental Medicine.

The EA group received low frequency EA at 3 pairs of acupoints: right side LI-11 to LI-4, left side GB-34 to SP-6, and bilateral ST-36. Needles were also inserted in Du-20, right ear shenmen, and left LV-3 (**Figure 1B**) but with no electrical current delivered. EA needles were stimulated with low intensity and frequency using a constant-current electro-acupuncture device (AS SUPER 4 Digital Needle Stimulator) which allowed for flexible setting of pulse width (1 ms), frequency (2 Hz), and shape (biphasic rectangular) parameters. The current intensity was set at each session for each patient individually at the midpoint between sensory and pain thresholds, based on common clinical practice and our previous EA study with chronic pain patients[17], and stimulation lasted 25 minutes per session. The duration and frequency of treatment is based on common clinical practice and is within the bounds of previous acupuncture trials[18]. The selection of acupuncture sites was based on predominant FM symptoms including multisite pain; headache, gastrointestinal pain and dysfunction; disrupted sleep; and chronic fatigue.

For the ML acupuncture therapy group, a laser acupuncture device (VitaLaser 650, Lhasa OMS) was manually positioned approximately 1-2 cm over all of the same acupoints used in EA. There was no palpation prior to positioning the device, and there was no physical contact between the device and skin. The laser light was demonstrated to the participants at the first visit to enhance credibility of the intervention; however, the laser was turned off during the actual treatment, thus removing any potential optically induced or thermal sensation, whilst maintaining all treatment rituals, as previously described[19,20] (**Figure 1B**). ML treatments also lasted 25 minutes.

Participants were not informed about a sham or placebo at consent, so all participants were led to believe that both EA and ML are viable treatments for FM.

These procedures constituted as IRB authorized deception, and all participants were fully debriefed after the final MRI visit.

The verbal instructions used by acupuncturists were standardized across all treatments (**Supplementary Methods B and C)**. After each treatment, the MGH Acupuncture Sensation Scale (MASS)[21] was administered to evaluate *de qi* and perceived somatosensory afference. The 13-item questionnaire included sensations such as Soreness, Aching, Deep Pressure, Tingling, etc. (0=none; 10=unbearable scale) and weighted summation of these sensations constituted the MASS Index. This measure served as a fidelity check to assess whether FM patients consistently reported increased levels of sensation in response to EA compared to ML. In addition, after the first treatment and after the last treatment, a Credibility Questionnaire (**Supplementary Methods D)** was administered which assessed the perception of validity and credibility of the treatments. This ensured that any differences in clinical or neuroimaging outcomes were not due to differences in perception of credibility.

Clinical outcomes

The Severity subscale of the Short Form Brief Pain Inventory (BPI) was the primary clinical outcome. BPI-Severity measures worst pain in 24 hours, least pain in 24 hours, pain on average, and pain right now. BPI-Severity was measured at pre- and post-therapy. PROMIS (https://www.healthmeasures.net/explore-measurement-systems/promis) Anxiety and Depression scales were used as secondary clinical outcomes and to assess whether neuroimaging outcomes were influenced by these factors. Furthermore, we collected a series of exploratory outcome measures: BPI Pain Interference, American College of Rheumatology 2011 FM Survey Criteria[22], Pain Catastrophizing, and PROMIS (Physical Function, Fatigue, Sleep). A detailed analysis of these exploratory outcomes is outside the scope of the present manuscript; however, descriptive statistics for each are reported in **Supplementary Results B**.

Mechanistic outcome: rs-fMRI of the primary somatosensory cortex

Resting state functional MRI (rs-fMRI) in an awake, eyes-open state, and anatomical T1-weighted (T1w) MRI were acquired with a 15-channel head coil in a 3.0T

MRI system (Philips Ingenia, Best, Netherlands). Minimal preprocessing of rs-fMRI and T1w images were performed using fMRIprep 1.1.8[23]. Full details of MRI acquisition parameters and preprocessing steps are provided in **Supplementary Methods E**.

Since somatosensory afferent input is encoded in the primary somatosensory cortex (S1), we chose the S1 cortical representation of the legs as the seed region to examine somatosensory circuits (i.e. communication between $S1_{leg}$ and other brain regions). $S1_{leg}$ was the chosen seed as most EA needles were placed on the leg (**Figure 1B**), and our group has previously localized this $S1_{leg}$ region in FM patients (centroid MNI coordinates x=± 8, y=−38, z=68)[24]. Bilateral spherical seeds (4 mm radius) were used to extract fMRI timeseries and seed-to-voxel correlation analysis was used to evaluate whole-brain connectivity maps for $S1_{leg}$. Timeseries from the $S1_{leg}$ seed (fslmeants) were used as a GLM regressor (fsl_glm) to obtain whole-brain parameter estimates and associated variances, for each participant. These parameter estimates and variances were then passed on to group level analysis, conducted on FMRIB's Local Analysis of Mixed Effects (FLAME 1+2)[25] to improve mixed-effects variance estimation. $S1_{leg}$ connectivity was then contrasted between pre- and post-treatment periods using paired sample *t*-tests for EA and ML separately. Interaction between EA and ML was conducted using an independent samples *t*-test of the paired post-pre difference images. As age influences neuroimaging outcomes, it was included as a regressor of no-interest in all analyses. Multiple comparisons family-wise error correction was conducted using gaussian random field (GRF) cluster threshold ($Z > 2.3$) and significance at corrected $p < 0.05$.

Mechanistic outcome: ^{1}H-MRS measurement of Glx and GABA+ in the right anterior insula

^{1}H-MRS spectra were acquired from automated voxel placement covering the right aINS, as our previous study showed differences between FM and pain-free controls in this region[6]. The ^{1}H-MRS voxel dimensions were based on our previous study[6]. Single-voxel point resolved spectroscopy (PRESS) was used to measure Glx. A separate GABA+ edited Mescher-Garwood-PRESS (MEGA-PRESS), which co-edits signal from macromolecules and homocarnosine, was conducted to estimate GABA+

levels[26]. Conventional PRESS spectroscopy data was analyzed with LCModel[27]. MEGA-PRESS spectra were processed in Gannet 3.1.5[28], a MATLAB-based toolbox specifically developed for edited MRS. Full details of PRESS and MEGA-PRESS acquisition parameters, preprocessing and analysis details are provided in **Supplementary Methods F**. The final GABA+ estimates were reported in institutional units (GABA+ (i.u.), approximating millimolar concentrations) and also as an integral ratio with respect to the creatine signal (GABA+/Cr). Treatment-related change in Glx and GABA+ was computed as the difference between pre- and post-therapy values.

Statistical analyses

Besides the aforementioned image-based statistics, statistical analyses were performed in IBM SPSS Statistics 26 (IBM, Armonk, NY). For the assessment of changes in the primary clinical outcome (BPI-Severity) and secondary outcomes (**Supplementary Results B**), a 2 (Group: EA, ML) by 2 (Time: Pre, Post) mixed-design ANOVA was conducted. For the assessment of MASS Index, a 2 (Group: EA, ML) by 8 (Time: Pre, Post) mixed-design ANOVA was conducted. Greenhouse-Geisser correction was used to adjust for sphericity assumptions in the repeated measures ANOVA. Mean credibility scores were assessed for group differences using an independent samples *t*-test. Associations between changes in extracted values from $S1_{leg}$ connectivity, GABA, and BPI-severity were conducted using Pearson's correlation adjusted for age. To determine whether relationships assessed with Pearson's *r* were directionally different for EA compared to ML, the single-tailed Fisher's *z* cocor algorithm[29] was used. For mediation analyses, bias-corrected bootstrapped (10,000x) mediation was conducted using the Process Macro on SPSS[30], and estimates of indirect effects were computed at the 95% confidence level (adjusting for age).

Charts and Figures

All charts were created on GraphPad PRISM Version 8.2.1 (GraphPad Software, San Diego, California USA, www.graphpad.com). **Figure 1 and 5B** were created with BioRender.com

Results

Clinical characteristics and demographics

Flow of participants in the protocol is described in **Supplementary Results A**. Full demographic and clinical characteristics are listed in **Supplementary Results B** and medication usage for each participant is listed in **Supplementary Results C**.

Post-therapy reduction in BPI Severity is greater in EA versus ML

For BPI Severity, the two-way Group x Time mixed-design ANOVA demonstrated a significant main effect of Time ($F(1, 70)=25.09$, $p<0.001$) and no main effect of Group ($F(1, 70)=0.03$, $p=0.861$). However, there was a significant Group x Time interaction ($F(1, 70)=4.56$, $p=0.036$), such that EA reduced BPI Severity to a greater extent compared to ML (**Figure 2A**). There was no baseline difference in BPI Severity between EA and ML ($t(70)=0.85$, $p=0.396$). Changes in BPI Severity were not related to changes in Depression (EA: $r(33)=0.24$, $p=0.165$; ML: $r(35)=-0.08$, $p=0.65$) or Anxiety (EA: $r(33)=0.07$, $p=0.71$; ML: $r(35)=0.17$, $p=0.31$).

EA elicits greater somatosensory afference compared to ML

For MASS Index scores, the 2 (Group) x 8 (Time) mixed-design ANOVA demonstrated a significant main effect of Time ($F(4.0, 224.9)=2.85$, $p=0.025$), a significant main effect of Group ($F(1, 56)=31.01$, $p<0.001$), but no Group x Time interaction effect ($F(4.0, 224.9)=0.35$, $p=0.84$) (**Figure 2B**). Treatment credibility was equal across both groups (**Supplementary Results D**).

$S1_{leg}$ connectivity increases post-therapy in EA versus ML

A whole-brain seed connectivity analysis of $S1_{leg}$ showed significant post-therapy increases in connectivity for the EA group, notably to the bilateral anterior insula (aINS), posterior insular (pINS), and right non-leg S1 subregions. Conversely, the ML group showed reductions in $S1_{leg}$ connectivity to the left anterior/mid insula (a/mINS). The whole-brain Group x Time interaction effect showed that the magnitude of increase in $S1_{leg}$ connectivity for EA was greater than that of ML, notably showing regions such as the bilateral aINS, pINS, and right non-leg S1. **Figure 3A** shows relevant contrasts, and

full detail of clusters are in **Supplementary Results E**. We also confirmed that our rs-fMRI results were not confounded by head motion (**Supplementary Results F**).

Increases in $S1_{leg}$ connectivity were related to improvements in BPI Severity in EA

For EA, there was a significant relationship between change in $S1_{leg}$-aINS connectivity and change in BPI severity ($r(30)=-0.44$, $p=0.01$), such that the greater the increase in $S1_{leg}$-aINS connectivity, the greater the reduction in BPI-Severity post-therapy (**Figure 3B**). Change in $S1_{leg}$-aINS connectivity was not related to change in BPI severity for ML ($r(35)=-0.02$, $p=0.91$). The correlation for EA was significantly stronger than that of ML (Fisher's $z=-1.78$, $p=0.04$). Changes in $S1_{leg}$-aINS connectivity were not related to post-therapy changes in depression (EA: $r(30)=0.02$, $p=0.93$; ML: $r(35)=-0.14$, $p=0.41$) or anxiety (EA: $r(30)=-0.12$, $p=0.51$; ML: $r(35)=0.11$, $p=0.50$).

Similarly, we found that for EA, there was a significant relationship between change in $S1_{leg}$-pINS connectivity and change in BPI severity ($r(30)=-0.43$, $p=0.01$), such that the greater the increase in $S1_{leg}$-pINS connectivity, the greater the reduction in BPI severity post-therapy (**Figure 3B**). Change in $S1_{leg}$-pINS connectivity was not related to change in BPI severity for ML ($r(30)=-0.04$, $p=0.84$). The correlation for EA was significantly stronger than that of ML (Fisher's $z=-1.70$, $p=0.04$). Changes in $S1_{leg}$-pINS connectivity were not related to post-therapy changes in depression (EA: $r(30)=-0.19$, $p=0.29$; ML: $r(35)=0.18$, $p=0.29$) or anxiety (EA: $r(30)=-0.24$, $p=0.18$; ML: $r(35)=0.13$, $p=0.45$).

Changes in aINS GABA+ is linked with changes in $S1_{leg}$-aINS connectivity in EA

Figure 4A shows the average MEGA-PRESS spectrum across all subjects, respectively**.** We found that the right aINS cluster from the post-pre $S1_{leg}$ connectivity group map in EA overlapped with the MNI-transformed aINS ^{1}H-MRS voxel placement (**Figure 4B**). There was no main effect of treatment on GABA+ (**Supplementary Results G**). However, we found that greater increase in $S1_{leg}$-aINS connectivity was associated with greater increase in aINS GABA+ post-therapy (GABA+(i.u.): $r(16)=0.48$, $p=0.046$ (**Figure 4C**); GABA+/Cr: $r(16)=0.46$, trending $p=0.052$). This $S1_{leg}$-aINS connectivity and aINS GABA+ relationship was not present for ML (GABA+(i.u.): $r(23)=-$

0.17, p=0.43; GABA+/Cr: r(23)=-0.15, p=0.47), and the correlation for EA was significantly stronger than that of ML (GABA+(i.u.): Fisher's z=2.08, p=0.02; GABA+/Cr: Fisher's z=1.94, p=0.03). Furthermore, we confirmed that this relationship was specific to inhibitory and not excitatory neurotransmitter changes (**Supplementary Results H**).

Changes in aINS GABA+ is linked with improvements in BPI Severity in EA

We found that greater increases in aINS GABA were associated with greater reductions in BPI severity (GABA+(i.u.): r(16)=-0.59, p=0.01 (**Figure 4D**); GABA+/Cr: r(16)=-0.65, p=0.004). This relationship was not found for ML (GABA+(i.u.): r(16)=-0.16, p=0.44; GABA+/Cr: r(23)=-0.13, p=0.53), and the correlation for EA was stronger than that of ML (GABA+(i.u.): Fisher's z=-1.54, trending p=0.06; GABA+/Cr: Fisher's z=-1.92, p=0.03). Changes in aINS GABA+ in EA and ML were not related to post-therapy changes in depression (GABA+(i.u.): EA: r(16)=0.12, p=0.63 and ML: r(23)=0.07, p=0.74; GABA+/Cr: EA: r(16)=0.23, p=0.36 and ML: r(23)=0.03, p=0.89) or anxiety (GABA+(i.u.): EA: r(16)=-0.21, p=0.40 and ML: r(23)=0.10, p=0.65; GABA+/Cr: EA: r(16)=-0.06, p=0.82 and ML: r(23)=0.08, p=0.72). Furthermore, we confirmed that this relationship was specific to inhibitory and not excitatory neurotransmitter changes (**Supplementary Results H**).

aINS GABA+ mediated the effect of $S1_{leg}$-aINS connectivity on BPI severity in EA

Finally, we conducted a mediation analysis to link $S1_{leg}$-aINS connectivity (X), BPI severity (Y), and aINS GABA+(i.u.) (Mediator) in one statistical model. Results showed that greater increase in $S1_{leg}$-aINS connectivity was associated with greater reduction in BPI severity post-therapy indirectly through greater increase in aINS GABA+(i.u.) (β=-0.187, BootSE=0.130, BootLLCI=-0.533, BootULCI=-0.037, **Figure 5A**). The direct effect of increase in $S1_{leg}$-aINS connectivity on reduction in BPI severity post-therapy was not significant (Effect=-0.184, SE=0.186, LLCI=-0.581, ULCI=0.212), suggesting that the effect of $S1_{leg}$-aINS connectivity on BPI severity is transmitted through aINS GABA+(i.u.). The R^2 value for BPI Severity in this model was 0.39. This effect was also present when GABA+/Cr estimates were used as the mediator (**Supplementary Results I**).

Discussion

Our randomized neuroimaging trial evaluated the role of somatosensory afference in acupuncture to the reduction of clinical pain in FM. We found that EA (designed to generate sustained somatosensory afferent activity) was more effective than ML acupuncture (designed to generate no somatosensory afference) in reducing clinical pain. As the EA intervention was heavily directed to the patient's legs, we examined brain connectivity with the primary somatosensory cortical representation of the leg ($S1_{leg}$). We found that following EA therapy, FM patients demonstrated increased communication of this $S1_{leg}$ region with the anterior and posterior insula (aINS, pINS), as well as non-leg S1 subregions. Greater post-therapy increases in $S1_{leg}$-aINS and $S1_{leg}$-pINS connectivity were associated with greater reduction in clinical pain. Moreover, we measured the concentration of the inhibitory neurotransmitter GABA in the insula and found that greater post-therapy increase in $S1_{leg}$-aINS connectivity was associated with greater increase in aINS GABA+, suggesting that $S1_{leg}$ signaling may increase GABAergic inhibition in the aINS. Furthermore, we found that greater increases in aINS GABA+ were associated with greater reduction in clinical pain. Finally, increased aINS GABA+ mediated the effect of increased $S1_{leg}$-aINS connectivity on reduced clinical pain in EA. Cumulatively, these results allow us to establish a mechanistic model for the role of somatic sensation in acupuncture therapy: somatosensory afference leads to increased $S1_{leg}$-aINS signaling, resulting in increased GABAergic inhibition in the aINS, ultimately reducing clinical pain (**Figure 5B**).

Our research extends previous work demonstrating somatotopically-specific involvement of S1 in acupuncture. Early research found that ST-36 EA produced stimulus-evoked BOLD activation in the contralateral $S1_{leg}$ region[31]. Later work examined somatotopic specificity of S1 morphology and functional involvement in clinical populations, linking S1-metrics with therapeutic outcomes. Specifically, in Carpal Tunnel Syndrome (CTS), longitudinal electroacupuncture therapy targeting the median nerve at the wrist increased the S1 separation distance between median-nerve innervated digits 2 and 3, and this increase in S1 digit separation predicted long-term clinical improvements[17]. Another recent study using manual acupuncture in chronic low back pain showed increases in gray matter volume and white matter integrity in back-

specific S1[20]. However, these studies were limited to local changes within S1 and did not explore cross-network signaling.

There is some evidence of increased cross-network communication in response to acute EA stimulation. In healthy individuals, acute EA stimulation produced increased connectivity of the Default Mode and sensorimotor network to the anterior cingulate (a key node of the salience network)[32]. In the current study, we found evidence for increased connectivity between $S1_{leg}$ and right aINS, and the degree of this connectivity increase was linked to improvements in clinical pain. This result may seem counterintuitive as chronic nociplastic pain is often characterized by heightened resting functional connectivity of S1 and the aINS relative to pain-free controls[33,34]. However, those studies assessed pathology-specific S1 subregions (e.g., $S1_{back}$ for lower back pain). In our study, analyses evaluated connectivity of S1 subregions specifically targeted by EA – i.e. $S1_{leg}$. Furthermore, recent work has causally shown that GABAergic inhibition is recruited in the aINS to reduce nocifensive behavior[35]. Therefore, our results suggest that $S1_{leg}$ may be signaling the aINS to reduce clinical pain via GABAergic inhibition. Alternately, acupuncture may temporarily upregulate pronociceptive signaling between $S1_{leg}$ and aINS, which may trigger endogenous descending inhibitory systems to counteract through GABAergic inhibition of the aINS (i.e. healing processes initiated by temporary injury)[36]. These frameworks need further validation through reverse translational studies.

In FM patients reduced levels of GABA in the aINS[6], and a compensatory upregulation of $GABA_A$ receptors[7] have been reported. Pharmacologic interventions that enhance GABAergic neurotransmission have been found efficacious for FM–a phase-3 randomized trial of sodium oxybate (a GABA agonist) showed improvements in FM symptoms[37]. Based on these observations, reverse translational research has shown a causal link between aINS GABA and nocifensive behaviors in rats–decreasing endogenous levels of GABA in the agranular insula (rat homolog of the aINS) increased thermal and mechanical sensitivity[38]. Our study extends this literature by showing that increases in aINS GABA+ were associated with improvements in clinical pain following EA treatment, suggesting that somatosensory afference may modulate GABAergic inhibition to produce analgesia. The aINS is a hyper-reactive locus in FM patients[39], and

patients that have a post-therapy increase in aINS GABA+ may reduce such hyper-reactivity or hyperactivity, resulting in analgesia. Interestingly, although GABA is a molecular product of glutamate, our study did not note any association between clinical outcomes and Glx, suggesting that specific GABAergic pathways may be involved in somatosensation-enhanced acupuncture analgesia.

Another notable link established in our study was that increased long-range cortico-cortico communication post-therapy may lead to increased GABAergic inhibition. Although GABAergic neurons contribute significantly to local energy consumption[40], the relationship between BOLD activity and GABA derived from ^{1}H-MRS is complex–some studies in healthy individuals have shown that greater GABA is related to greater task-based negative BOLD responses[41,42] while other research across multiple cortical regions have shown no such relationships[43]. With regards to BOLD functional connectivity, both positive and negative correlations with GABA have been noted–greater within-primary motor (M1) connectivity has been shown to be negatively correlated with M1 GABA[44], whereas dorsal anterior cingulate GABA was not related to salience network GABA[45]. One recent study in healthy individuals measured GABA in two nodes of traditionally anti-correlated networks, the medial prefrontal cortex (mPFC) and the dorsolateral prefrontal cortex (dlPFC), and showed that mPFC-dlPFC functional connectivity at rest was positively correlated with dlPFC GABA and negatively correlated with mPFC GABA[46], suggesting that intrinsic functional connectivity architecture may be associated with varying GABAergic tone across the cortex. Few studies have noted treatment-related changes in GABA and functional connectivity; one study noted that administration of Gamma-hydroxybutyrate (a GABA agonist) increased right aINS functional connectivity[47]. Due to the complex relationship between GABA and BOLD functional connectivity across previous studies, our results need further validation. Nevertheless, our longitudinally-informed model (**Figure 5B**) proposes that increased $S1_{leg}$-aINS connectivity influenced GABA+ in the aINS to reduce clinical pain. The downstream effects of this $S1_{leg}$-aINS pathway needs further investigation; one possibility is that S1 taps into aINS regulation of sympathetic outflow as the aINS is part of the central autonomic network[48]. In fact, our previous study has shown that during experimental pressure pain in FM patients, $S1_{leg}$-aINS connectivity was associated with

reduced cardiovagal modulation[24]. Additionally, GABA is not the only neurotransmitter regulating aINS function; in a subsample of FM participants from this study, we found that elevated Choline (often involved in neuroinflammation) in FM was related to pain interference via aINS-Putamen functional connectivity[49]. Future studies should more explicitly examine the role of the autonomic nervous system and/or other neurotransmitters involved in somatosensation-induced acupuncture analgesia.

While our study demonstrates mechanistic links of acupuncture treatment via $S1_{leg}$-aINS connectivity and aINS GABA, the clinical translation of these brain markers warrants further evaluation. For instance, a possible hypothesis is that aINS GABA and $S1_{leg}$-aINS connectivity at baseline is predictive of the therapeutic trajectory of acupuncture, which would increase its clinical utility. Future studies should be focused on using neuroimaging markers at baseline to predict acupuncture treatment outcomes.

Our study was designed to specifically examine somatosensory afference, but other contextual factors (patient-clinician rapport, expectations, etc) may have contributed to analgesia as well, particularly in the ML comparator group. Thus, our results highlight the importance of carefully designed controls in acupuncture trials, as various specific and non-specific components contribute towards treatment outcomes. Researchers need a thorough understanding of the various factors that might be contributing towards analgesia while designing an acupuncture trial.

Limitations of our results should be noted. Despite a strong relationship between changes in aINS GABA+ and changes in clinical pain/$S1_{leg}$-aINS connectivity, we did not observe a main effect of post-therapy GABA+ increase. We reason that aINS may be downstream of our proposed pathway (**Figure 5B**) and 4-weeks of treatment may not be sufficient to increase aINS GABA+. Future studies should be designed with a longer treatment schedule, including a post-therapy assessment period to examine long-term effects.

In summary, our study found that the somatosensory component of acupuncture specifically modulated functional communication and inhibitory neurochemistry in the somatosensory-insular circuit in order to reduce clinical pain in FM patients. With future rigorous mechanistic studies of acupuncture, we may be able to discover novel CNS

pathways involved in non-pharmacologically induced analgesia and design new treatments that modulate CNS pathways in chronic pain pathologies.

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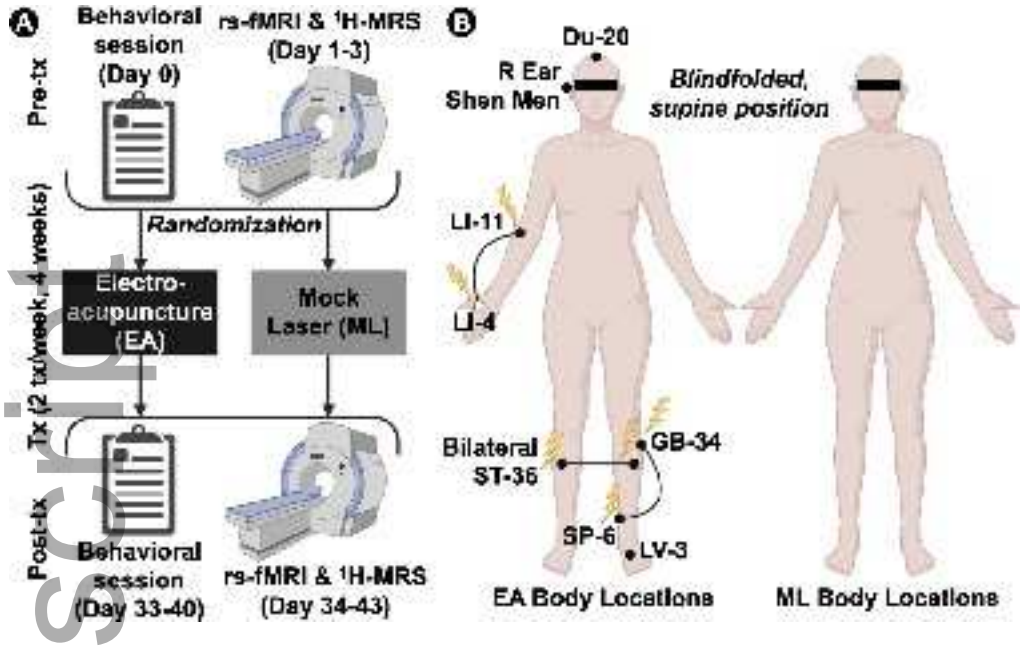

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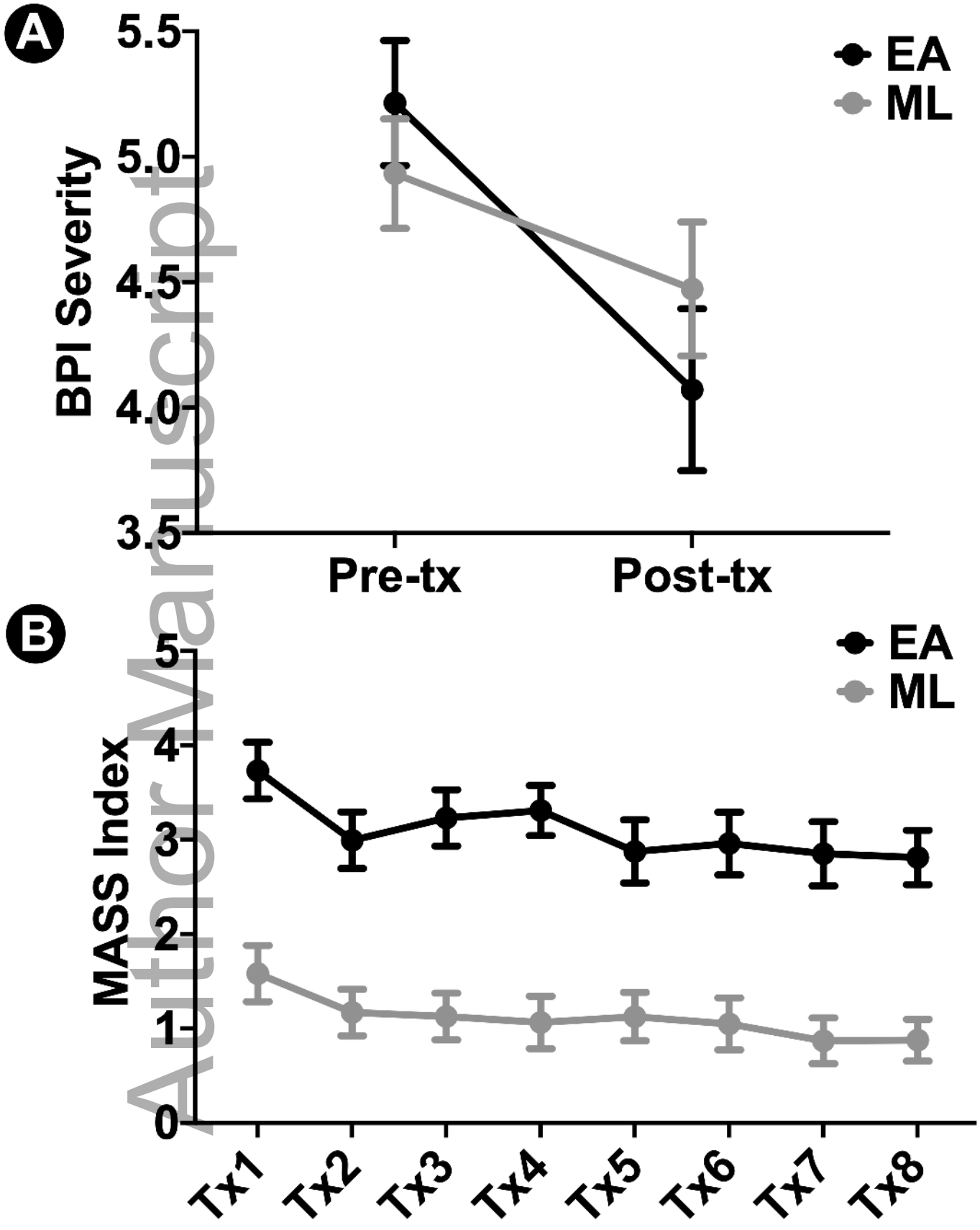

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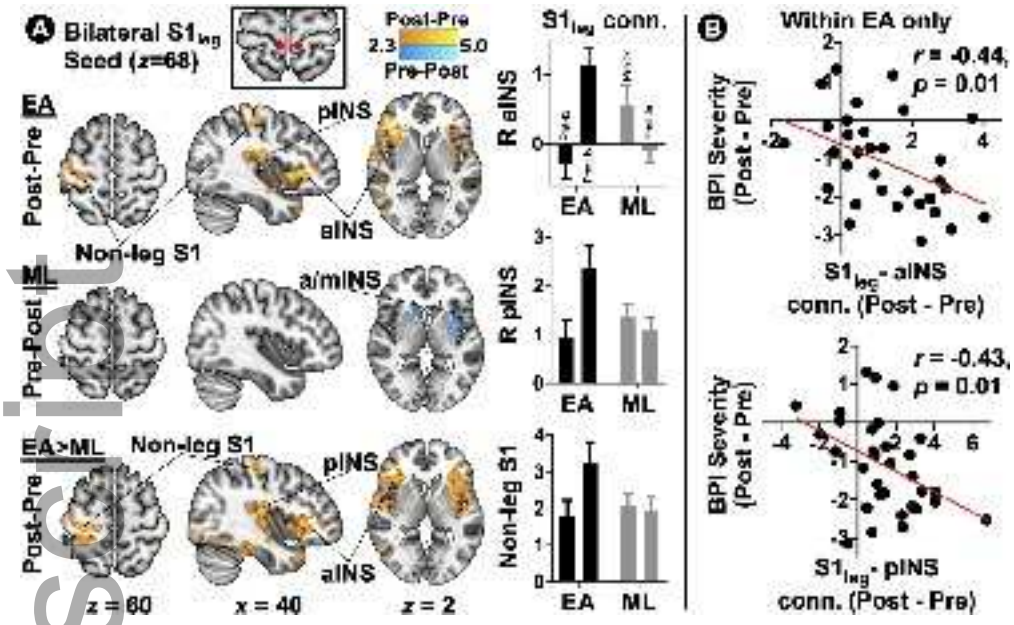

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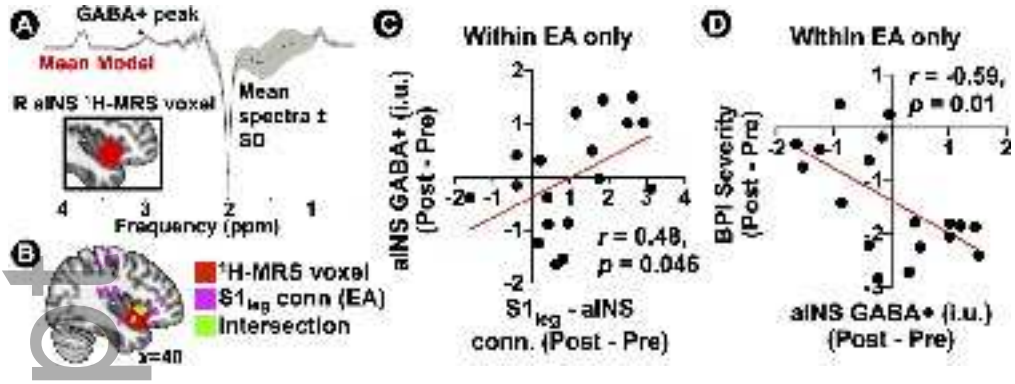

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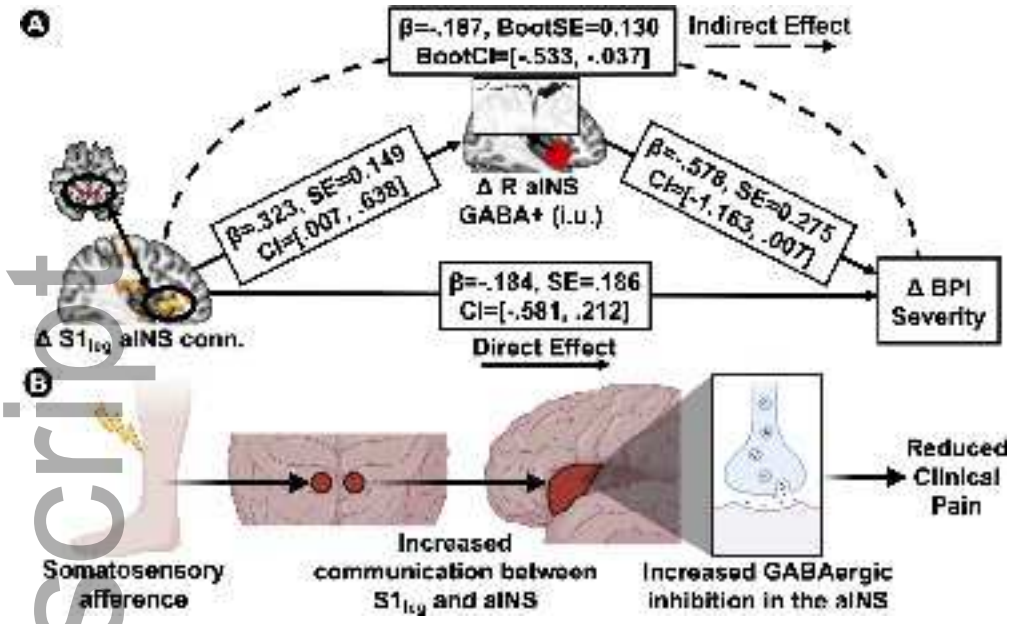

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