Pregabalin Rectifies Aberrant Brain Chemistry, Connectivity, and Functional Response in Chronic Pain Patients

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ABSTRACT

Background: Chronic pain remains a significant challenge for modern health care as its pathologic mechanisms are largely unknown and preclinical animal models suffer from limitations in assessing this complex subjective experience. However, human brain neuroimaging techniques enable the assessment of functional and neurochemical alterations in patients experiencing chronic pain and how these factors may dynamically change with pharmacologic treatment.

Methods: To identify the clinical action of pregabalin, a proven analgesic, the authors performed three complementary brain neuroimaging procedures: (proton magnetic resonance spectroscopy, functional magnetic resonance imaging, and functional connectivity magnetic resonance imaging) in 17 chronic pain patients diagnosed with fibromyalgia.

Results: The authors found that pregabalin but not placebo reduces combined glutamate + glutamine levels within the posterior insula (pregabalin $P = 0.016$; placebo $P = 0.71$). Interestingly, reductions in clinical pain were associated with reductions in brain connectivity of this structure to brain regions within the default mode network during pregabalin ($r = 0.82$; $P = 0.001$) but not placebo ($r = -0.13$; $P = 0.63$). Response of default mode network regions to experimental pain was also reduced with pregabalin ($P = 0.018$) but not placebo ($P = 0.182$). Perhaps most importantly, baseline values for all three neuroimaging markers predicted subsequent analgesic response to pregabalin but not placebo.

Conclusions: The results of this study suggest that pregabalin works in part by reducing insular glutamatergic activity, leading to a reduction of the increased functional connectivity seen between brain regions in chronic pain states. The study also supports a role for human brain imaging in the development, assessment, and personalized use of central-acting analgesics.

What We Already Know about This Topic

- Both brain insular glutamate and insular connectivity to the default mode network (a constellation of brain regions to which is attributed self-referential thinking and autobiographical memory) have been implicated as pathologic factors in chronic pain

What This Article Tells Us That Is New

- Using three complementary imaging techniques (proton magnetic resonance spectroscopy, functional magnetic resonance imaging, and functional connectivity magnetic resonance imaging) in chronic pain patients with fibromyalgia, it was shown that pregabalin treatment reduced brain insula glutamate levels and was associated with decreased connectivity of this structure to the default mode network
- These factors were associated with the clinical analgesic response to this drug

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CHRONIC pain is inherently a subjective disease state whose intensity and frequency depend critically on central neural activity.[1–3] Although the processes involved in nociception and the initial steps of pain neurotransmission in the periphery are generally known,[4] our understanding of central constituents of persistent chronic pain in humans is only recently emerging. Alterations in brain structure,[5,6] function,[7,8] and neurochemistry[9,10] have been reported; however, it remains to be seen which, if any, of these neuroimaging outcomes are actually responsible for chronic pain. One approach to address this question is to use an efficacious pharmacologic agent as a "probe" of analgesic brain response in humans.

Pregabalin, originally developed as an antiepileptic drug,[11] has subsequently been demonstrated to be efficacious in treating neuropathic pain[12] and fibromyalgia,[13,14] both of which are accompanied by aberrant brain pathophysiology.[7,8,15] Although pregabalin's clinical mechanism of action in humans is unknown, both *in vitro* and *in vivo* studies indicate that pregabalin, and its structurally similar compound gabapentin, bind to the α2δ-subunit of voltage-gated calcium channels,[16,17] which is thought to reduce the influx of calcium into the presynaptic cell and thereby decrease the release of glutamate into the synapse.[18,19]

Both pregabalin and the similarly acting gabapentin have also gained wide use as preemptive analgesics in the perioperative environment. Both drugs have been generally shown to reduce opioid consumption in the perioperative period, as well as the development of chronic postsurgical pain.[20,21] One of the reasons that preemptive analgesia with these compounds is not more broadly used is that they need to be given to all surgical patients to benefit a few; it would be helpful to identify the underlying mechanisms of action of these compounds so that eventually only those who would benefit from these drugs would need to be treated with them during the perioperative period.

Recently we have reported increased levels of resting glutamate and combined glutamate and glutamine (Glx) within the posterior insula, a key sensory processing brain region,[22] in fibromyalgia patients.[9] Moreover, we demonstrated that longitudinal decreases in pain were associated with concomitant reductions in resting Glx within this structure.[23] Although brain chemical alterations may play a role in chronic pain, a separate line of investigation has focused on resting, or intrinsic, brain connectivity as an indicator or marker of clinical pain. Recently we found that resting insula connectivity to the brain's default mode network (DMN), a constellation of brain regions to which is attributed self-referential thinking and autobiographical memory,[24,25] is augmented in fibromyalgia.[26] Patients reporting greater clinical pain also demonstrate greater DMN–insula connectivity. Moreover, we have shown that longitudinal decreases in chronic pain were also accompanied by reduced DMN–insula connectivity.[27] Interestingly, mitigated DMN deactivation during an external task has also been noted in another chronic pain state.[28]

These data implicate brain insular glutamate and its connectivity to the DMN as pathologic factors in chronic pain. Here we employ three complementary brain neuroimaging methods (proton magnetic resonance spectroscopy [^{1}H-MRS], functional magnetic resonance imaging [fMRI], and functional connectivity magnetic resonance imaging [fcMRI]) to assess their sensitivity as targets and predictors of successful treatment of chronic pain patients with pregabalin.

Materials and Methods

Participants

Twenty-seven female fibromyalgia patients were enrolled in the study. Nine were excluded from all analysis for the following reasons: six did not complete all imaging sessions; two missed study medication doses within 48 h of imaging; and one developed a new chronic pain condition midway through the study, confounding effects of pregabalin on fibromyalgia pain. One participant had dental work that resulted in poor ^{1}H-MRS data and she had to be excluded from spectroscopy analyses. In addition, three were found to have head motion exceeding 3 mm during fMRI and for one patient clinical pain data were missing, excluding them from fcMRI and fMRI analyses.

All patients were randomized in a double-blind, two-period, crossover study of pregabalin *versus* placebo (fig. 1). Major inclusion criteria were: (1) meeting 1990 American College of Rheumatology criteria for fibromyalgia with chronic widespread pain for at least 6 months; (2) 18–70 yr of age; (3) nonlactating and not pregnant; (4) body mass index of less than 36; (5) right handed; and (6) score of ≥40 mm on a 10-cm pain Visual Analog Scale (VAS). Major exclusion criteria were: (1) pain due to other conditions that could confound fibromyalgia pain; (2) widespread inflammatory musculoskeletal disorder, rheumatic disease other than fibromyalgia, active infections, or untreated endocrine disorder; (3) severe depression; (4) any other severe, acute, or chronic medical or psychiatric conditions that could increase risk or interfere with trial results; (5) contraindications with magnetic resonance imaging procedures; (6) unresponsiveness to previous pregabalin treatment of 300 mg/day or greater; (7) positive urine drug screen for drugs of abuse; (8) use of opiates, sedatives, or hypnotics; (9) unstable doses of antidepressants, nonsteroidal antiinflammatory drugs, or muscle relaxants; and (10) treatment with an investigational drug within 30 days of randomization.

All study participants gave written informed consent, and the study protocol and informed consent documents were approved by the University of Michigan Institutional Review Board (Ann Arbor, Michigan) and Pfizer (Groton, CT). All imaging data were stored, validated, analyzed, and assessed for quality at the University of Michigan and Massachusetts General Hospital independently of Pfizer personnel. All clinical data were double-entered, quality checked, and all databases were locked before analysis. Patient demographics,

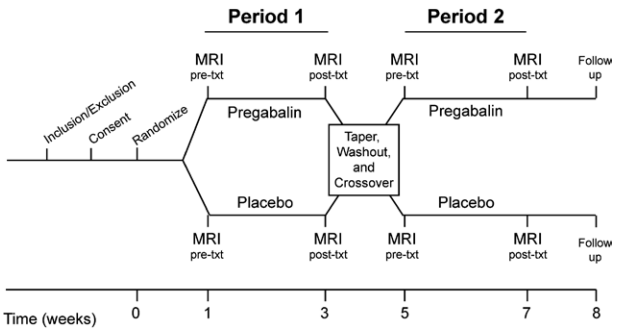

Fig. 1. Overview of study design. Subjects underwent a baseline visit, before the first imaging session, wherein they were evaluated for study criteria. Subjects meeting inclusion/exclusion criteria were randomized and underwent their first magnetic resonance imaging (MRI) session (pretreatment Period 1) 7 days later. After the first neuroimaging session, those randomized to pregabalin (PG) for Period 1 underwent dose escalation of PG to 450 mg/day over the course of 14 days, with a maintained fixed dose of 450 mg/day for the last 3 days, after which they had a repeat neuroimaging session (posttreatment Period 1). Those randomized to placebo for Period 1 took matching placebo pills over the course of 14 days and also had a posttreatment neuroimaging session (posttreatment Period 1). After Period 1, all participants underwent a 7-day taper and 8 days of placebo treatment for washout. After washout, participants crossed over to the other study drug for Period 2 (*i.e.*, those who had PG for Period 1, took placebo for Period 2 and *vice versa*). Neuroimaging sessions in Period 2 were identical to those of Period 1. Subjects were informed that they would be dosed with placebo or PG at various times during the study but they were not told when they were transferred from one treatment to the other. All investigators and members of the research team were blinded to study drug timing.

medications, and identification of inclusion in each imaging analysis are listed in table 1. Adverse events from this trial were generally consistent with findings from other studies of pregabalin in fibromyalgia.

Proton Magnetic Resonance Spectroscopy

Patients underwent ^{1}H-MRS imaging on a General Electric 3.0 Tesla magnetic resonance scanner (GE, Milwaukee, WI) at rest before and after placebo and pregabalin treatments. Seventeen patients, all having complete ^{1}H-MRS data, were included in this analysis. Single voxels were placed in the right anterior insula and the right posterior insula as described previously.[9,23] The insula cortex was chosen based on our previous findings of increased level of glutamate and Glx in this structure.[9] In brief, single-voxel point resolved spectroscopy spectra were acquired from each region of interest with the following parameters: TR 3000ms/TE 30ms, 90-degree flip angle, NEX 8, FOV 16, with a voxel size of 2 × 2 × 3 cm. Spectra were analyzed offline with LCModel (Stephen Provencher, Oakville, Ontario, Canada).[29] All spectra were of good quality. Values for Glx and glutamate were calculated as ratios to the internal standard creatine (*e.g.*, glutamate/creatine), our *a priori* defined outcomes. Because the study was a within-subject design and used ratios to creatine, correction for cerebrospinal fluid within the voxels was not required. Ratios of Glx and glutamate and other metabolites (*N*-acetyl aspartate; choline; myoInositol; and glutamine) to creatine were entered into SPSS v.19 IBM (Armonk, NY) for statistical analyses. Comparisons of change in metabolite ratios, pre- and posttreatment, were made separately for pregabalin and placebo by using paired sample *t* tests. Pearson correlations were made within SPSS between pretreatment Glx/creatine levels, adjusted for age (by regressing age on metabolite levels), and changes in pressure pain and clinical ratings. Significance was set at a *P* value less than 0.05, with no adjustment for multiple comparisons.

Functional Connectivity Magnetic Resonance Imaging

Resting state fMRI data of 6 min were collected as the first functional scan run in the session as described previously.[26,27] We used a spiral in–out gradient echo T2*-weighted blood oxygenation-level dependent (BOLD) pulse sequence (TR 2000 ms/TE30 ms, 180 volumes, 43 AC-PC aligned slices, voxel size = 3.13 × 3.13 × 4.0 mm) running on the same 3.0 Tesla magnetic resonance scanner as for ^{1}H-MRS, equipped with a eight-channel head coil. Subjects were instructed to keep their eyes open and to rest comfortably during the functional scan without moving or falling asleep. Structural data were also collected using a spoiled gradient echo pulse sequence (TR/TE/TI = 14/5.5/300 ms, 20-degree flip angle, 124 contiguous axial slices, voxel size = 1 × 1 × 1.5 mm).

Physiological data were collected simultaneously to fMRI data, as cardiorespiratory fluctuations are known to influence fMRI intrinsic connectivity estimation within several brain networks. Cardiac data were acquired using an infrared pulse oximeter (GE) attached to the right middle finger. Respiratory volume data were acquired using a magnetic resonance–compatible belt (GE) placed around the subject's ribcage.

Functional MRI data were preprocessed and analyzed using the validated FSL (FMRIB's Software Library) software package,## and FreeSurfer.*** Data were corrected for cardiorespiratory artifacts by using RETROICOR,[30] and for head motion by using FSL-MCFLIRT.[31] Brain extraction was performed by using FSL-BET.[32] Cortical surface reconstruction was completed to perform improved structural–functional coregistration by using FreeSurfer's bbregister tool.[33] Functional data were then registered to standard Montreal Neurological Institute space by using FMRIB's nonlinear coregistration tool (FNIRT). Functional data were smoothed using a Gaussian kernel of FWHM 6 mm and high-pass temporal filtering (f = 0.008 Hz) was performed.

Seed-based resting connectivity analyses were performed by using the same regions evaluated with ^{1}H-MRS—the

Available at: http://www.fmrib.ox.ac.kr/fsl. Accessed January 2, 2013.

*** Available at: http://surfer.nmr.mgh.harvard.edu/. Accessed January 2, 2013.

Table 1. Patients Included in Imaging Analyses, Demographics, and Medications/Supplements

Patient	^{1}H-MRS	fcMRI and fMRI	Age	Race	BMI	Medications and Supplements
1	Yes	No	49	White	30	Calcium/Effexor/Fluticason Propionate/Restasis/Tylenol PM
2	Yes	Yes	44	White	25	Augmentin/Motrin/Multivitamin
3	Yes	Yes	29	White	21	Albuterol/Erythromycin Eye lotion/Extra Strength Tylenol/ Ibuprofen/Ortho Tri-Cyclen/Zantac/Zyrtec
4	Yes	Yes	25	White	23	Children's Tylenol Plus Cough and Running Nose/Motrin
5	Yes	Yes	41	White	31	Albuterol/Estradiol/Ibuprofen
6	Yes	No	43	White	25	Triamicinolone acetonide 0.5%
7	Yes	No	47	White	34	Tylenol Extra Strength/Vitamin D
8	Yes	Yes	25	White	34	Prolosec
9	Yes	Yes	36	White	21	Amoxicillin/Augmentin/Motrin/Synthroid/Tylenol
10	Yes	Yes	42	White	27	Calcium/Cinnamon supplement/Fish oil/Multi-Vitamin/ Sudafed/Tylenol/Zyrtec
11	Yes	Yes	42	White	26	Advil/CVS Sinus Allergy/Effexor/Nyquil/Tylenol
12	Yes	Yes	39	White	25	Claritin/Melatonin/Nubaring/Propionate Fluticasone/Tylenol
13	Yes	Yes	44	White	30	Amoxicillin/Nyquil/Prednisone/Proventil/Rocephin/Tylenol
14	Yes	Yes	59	White	29	Calcium/Colchicine/Flexeril/Hydrochlorrothiazide/Melatomin/ Nabumetone/Omacor/Prilosec Trazadone/Vitamin D
15	Yes	Yes	19	White	23	Bupropion/Calcium/Concerta/Fish Oil/Iron/Loestrin/Vitamin B12
16	Yes	Yes	19	White	26	Claritin/Concerta/Loestrin
17	Yes	Yes	39	White	25	Effexor/Excedrin ES/Fluticasone Propionate Nasal Spray/ Ibuprofen/Magnesium Glycinate/Maxalt/Proventil HFA/Pseu-doephedrine/Seasonale/Topomax/Vitamin C/Zonisamide

BMI = body mass index; fcMRI = functional connectivity magnetic resonance imaging; fMRI = functional magnetic resonance imaging; ^{1}H-MRS = proton magnetic resonance spectroscopy.

right anterior and posterior insulae. The exact seeds were first eroded to include only gray matter voxels, using the Johns Hopkins University –International Consortium of Brain Mapping white-matter atlas.[34] The seed time series were produced by extracting the time series within anterior or posterior insula gray matter voxels from the preprocessed resting fcMRI data. This extracted time series was used as a regressor in a general linear model. Nuisance regressors in this model included fMRI time series from white matter and ventricular regions, motion correction time series for the six translation/rotation correction parameters reflecting rigid body head motion correction, and cardiorespiratory artifacts defined by convolving the heart rate and respiratory variation time series with appropriate cardiac and respiratory transfer functions, as defined by Chang *et al.*[35] and Birn *et al.*,[36] respectively. No global signal regression was used in this analysis.

To investigate the link between baseline resting insula connectivity and individual differences in pain sensitivity at baseline, we performed a linear regression with fcMRI data as the dependent variable, and baseline pain levels (VAS, 0–100) as the independent variable. In addition, because pain was reduced after pregabalin but not placebo, we also used a linear regression model to explore the association between pregabalin-modulated clinical pain and the change in both anterior and posterior insula resting brain connectivity. For comparison we extracted all regions of interest identified in the pregabalin analyses, from the placebo period. All group analyses used FLAME (FMRIB's local analysis of mixed effects). Group brain maps were thresholded using cluster correction for multiple comparisons with a cluster-forming threshold of Z-score greater than 2.3 and a cluster-size threshold of P value less than 0.05. Finally, we used Pearson correlations to investigate the predictive ability of baseline resting insula connectivity, extracted as Z-statistics from imaging data, to predict postpregabalin (or postplacebo) change in pain levels in IBM SPSS v.19.

Functional Magnetic Resonance Imaging

During fMRI sessions, two separate runs were performed wherein pressure pain was administered to the thumbnail bed as described previously.[23] fMRI scans were acquired on the same 3.0 Tesla scanner as used for ^{1}H-MRS and included data from the 14 participants analyzed in fcMRI. fMRI data were acquired with a spiral gradient echo sequence (TR 2500ms/TE 30ms, 90-degree flip angle, FOV 22 cm). Slices were 3-mm thick, with an in-plane resolution of 3.125 × 3.125 mm, acquired at 48 locations parallel to the anterior–posterior commissure plane. Preprocessing was performed by using statistical parametric mapping 2 (SPM2; Wellcome Department of Cognitive Neurology, London, United Kingdom) and included correction for slice-acquisition time to the middle slice, realignment to the first volume of each run to correct for intrascan movement, and smoothing with a Gaussian kernel of 8-mm full width at half maximum. 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 in each block. During each session two pressures were applied (each six times), a 2 kg/cm^2 stimulus and either a "mild" pain stimulus (7.5 on the Gracely Box Scale) or a "slightly intense" pressure (13.5 Gracely Box Scale), whose pressure values were determined before period 1 as reported previously by using the multiple random staircase method.[37] Individual participant pressure levels remained constant for all fMRI sessions. Blocks were 25s in duration and presented according to a fixed pseudo-random paradigm in which every other block consisted of the "no touch" condition. Parameter estimates of block-related activity, within the first 5 s of pressure, were established for each voxel, and contrast images were calculated by applying a linear contrast of the parameter estimates of the painful pressure *versus* the "off" 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 BOLD effects from pretreatment and posttreatment were calculated, and individual BOLD activation responses were extracted from the inferior parietal lobule (IPL) and posterior cingulate cortex (PCC) DMN regions (identified as being correlated with clinical pain in the fcMRI analysis outlined above) by using the Marsbar Region of Interest Toolbox.††† Paired sample *t* tests were performed with SPSS for each region of interest pre- and posttreatment, with significance set at *P* value less than 0.05 with no adjustment for multiple comparisons. We used Pearson correlations to investigate the predictive ability of baseline DMN regions (IPL and PCC) activations to predict postpregabalin (and postplacebo) change in clinical pain levels in SPSS v.19 IBM.

Pain Assessment (Experimental and Clinical)

In this study, we assessed treatment response for both evoked experimental pain as well as spontaneous clinical pain. For evoked pain, participants were asked to rate their maximum pain level on the Gracely Box Scale during each of the two evoked-pain fMRI runs. Pain ratings were then averaged within each pre- and posttreatment session, and these pain ratings were used as a measure of treatment response for evoked pain. As a measure of treatment response for spontaneous clinical pain, we administered a 100-mm VAS bounded by the words "no pain" and "worst pain imaginable," immediately before each neuroimaging session. Differences in pain ratings, pre- and posttreatment, were assessed with paired sample *t* tests.

Analysis of Ability of Neuroimaging Modalities to Predict Analgesic Effect of Pregabalin

Two separate linear regression models were constructed with either postpregabalin experimental pain or postpregabalin clinical pain as dependent variables. Prepregabalin neuroimaging metrics (posterior insula Glx, posterior insula to PCC connectivity, and IPL pain-evoked activation) were entered as independent variables along with prepregabalin pain and age. Neuroimaging variables were retained in the model if they were related to postpregabalin pain. Significance was set at a *P* value less than 0.10.

Statistical Considerations

For all of our imaging analyses we specifically were interested in identifying pregabalin effects in isolation from placebo effects. Previous neuroimaging work indicates that placebo brain mechanisms may not be "additive" with drug effects,[38] thereby complicating the interpretation of findings when the drug period is statistically contrasted with the placebo period. This can result in situations (*i.e.*, brain regions) wherein the placebo period is more influential than the drug period, thus providing less information about the drug. Moreover, the placebo mechanisms may not be "additive" or "imbedded" within the drug period, thereby complicating the identification of a drug mechanism when the drug is analyzed in conjunction with placebo. For these reasons we examined the pregabalin treatment period in isolation from the placebo period for all three of our imaging modalities. For reference all placebo-adjusted results are reported at clinicaltrials.gov.

Our *a priori* primary study objectives were to explore the effect of pregabalin compared with that of placebo on neuronal activity during blunt pressure pain in fibromyalgia subjects by using fMRI brain imaging, and to explore the effect of pregabalin compared with placebo on changes in glutamate concentrations within the anterior and posterior insula in fibromyalgia subjects by using ^{1}H-MRS. One of our secondary objectives was to explore the effect of pregabalin compared with placebo on resting brain activity measurements in fibromyalgia. Correlations between imaging outcomes and clinical pain, as well as the focus on DMN activity as a response to evoked pressure pain during fMRI, were *post hoc* analyses.

Results

Insular Glx Is a Target and Predictor of Successful Pregabalin Treatment

Participants underwent ^{1}H-MRS of the right anterior and posterior insula during rest (fig. 2, A and B). Patients displayed a significant reduction in the Glx to creatine (an internal standard) ratio (*i.e.*, Glx/creatine) within the right posterior insula after pregabalin treatment (fig. 2C; mean difference ± SD post minus pre: −0.116 ± 0.177; 95% CI, −0.02 to −0.21; $P = 0.016$), but not after placebo (mean difference ± SD post minus pre: 0.029 ± 0.308; 95% CI, 0.19 to −0.13; $P = 0.71$). No significant changes after either pregabalin ($P = 0.90$) or placebo ($P = 0.49$) were detected for Glx/creatine in the anterior insula (fig. 2C). No significant changes were detected for any of the other ^{1}H-MRS detected metabolites within either the anterior or posterior insula for either pregabalin or placebo (all $P > 0.10$).

††† Version 0.38. Available at: http://marsbar.sourceforge.net. Accessed January 2, 2013.

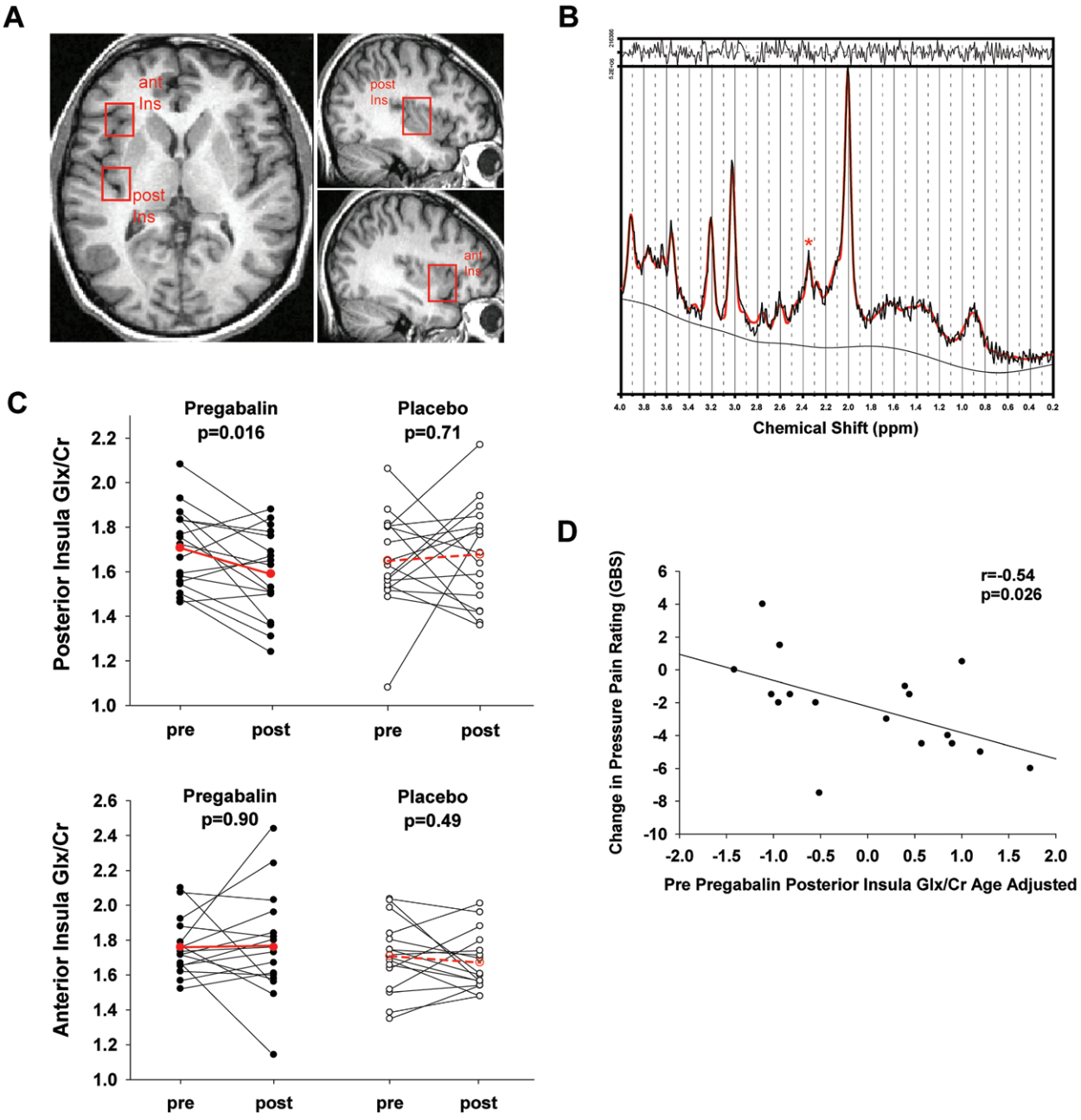

Fig. 2. Posterior insula Glx is a target and predictor of successful PG (pregabalin) treatment. (*A*) Axial and sagittal T1-weighted images showing single-voxel placement for right anterior (ant Ins) and right posterior (post-Ins) insula. (*B*) Representative proton magnetic resonance spectroscopy spectrum from the posterior insula fit with LCModel (*red trace*; *resonance from two glutamate γ proton resonances at 2.35 ppm). (*C*) PG reduces Glx/Cr within the posterior insula. Pre- and posttreatment (*solid circles* PG; *open circles* placebo) Glx/Cr values are plotted separately for the posterior and anterior insula. *Red lines* and *circles* represent group means. (*D*) Pre-PG treatment levels of Glx/Cr in the posterior insula are associated with greater reductions in pressure pain sensitivity after PG. Greater pre-PG levels of Glx/Cr are associated with greater subsequent reductions in evoked pressure pain ratings after PG. Cr = creatine; Glx = combined glutamate + glutamine.

For the 17 patients analyzed with ^{1}H-MRS, mechanical pressure pain sensitivity was significantly improved after pregabalin (mean difference ± SD for maximum pain post- minus pretreatment: −2.24 ± 2.27; 95% CI, −0.75 to −3.71; P = 0.005), but not after placebo (mean difference ± SD post minus pre; −0.15 ± 2.62; 95% CI, 1.20 to −1.49; P = 0.82). Clinical pain ratings were not reduced after either pregabalin (mean difference ± SD post minus pre; VAS: −10.25 ± 26.95; 95% CI, 4.11 to −24.61; P = 0.15) or placebo (mean difference ± SD post minus pre; VAS: −5.71 ± 24.81; 95% CI, 7.05 to −18.47; P = 0.36).

Table 2. Resting Insula Connectivity Tracks with PG Modulated Clinical Pain

	Side	Size (mm²)	Location (MNI) X	Y	Z	Z-score (PG)	Z-score (placebo)
Pre-PG posterior insula *vs.* clinical pain							
IPL	L	507	−24	−56	36	3.78	−0.14
PCC	L	507	−12	−54	38	3.12	0.48
Cerebellum	L	934	−4	−60	−28	−3.45	−0.55
Change in posterior insula connectivity *vs.* change in clinical pain after PG							
IPL	L	1,112	−62	−50	32	4.02	−0.50
	R	791	52	−54	52	3.96	0.67
M1	L	442	−48	−2	14	4.02	0.28
Cuneus	R	345	36	−82	16	−3.69	−0.72
Pre-PG anterior insula *vs.* clinical pain							
IPL	R	464	50	−48	42	3.39	0.62
Cuneus	L	3,105	−20	−76	0	−4.06	0.37
Change anterior insula connectivity *vs.* change in clinical pain after PG							
IPL	L	429	−50	−44	36	3.94	0.71
S2	L	363	−50	−16	26	3.71	1.09

Resting-state insula seeds (anterior and posterior) were used to assess connectivity of each seed to the rest of the brain with respect to clinical pain. Posterior and anterior insula connectivity to the DMN (IPL and PCC) was related to clinical pain intensity: greater connectivity was associated with greater clinical pain. Negative correlations were seen with the cerebellum and the cuneus. No significant correlations were seen for the same regions during placebo. Similar analyses were performed for change in insula connectivity with change in clinical pain after PG and placebo. Reductions in clinical pain were associated with reductions in insular to DMN connectivity during PG but not placebo.

DMN = default mode network; IPL = inferior parietal lobule; L = left; MNI = Montreal Neurological Institute; M1 = motor cortex; PCC = posterior cingulate cortex; PG = pregabalin; R = right; S2 = secondary somatosensory cortex.

Higher pretreatment levels of Glx/creatine within the posterior insula were associated with greater improvements in pressure pain sensitivity after pregabalin administration (fig. 2D; $r = -0.54$; $P = 0.026$) but not postplacebo administration ($r = 0.44$; $P = 0.07$). This relationship was not seen for the anterior insula after either pregabalin ($r = 0.01$; $P = 1.0$) or placebo ($r = -0.37$; $P = 0.14$). Prepregabalin Glx/creatine within the posterior insula did not predict subsequent change in clinical pain ($r = 0.33$; $P = 0.22$).

DMN–Insula Connectivity Is a Target and Predictor of Successful Pregabalin Treatment

On the basis of our previous findings of resting state connectivity and clinical pain in FM,[26,27] we sought to explore the relationship between clinical pain response to pregabalin and insula connectivity. For the 14 patients who had complete fcMRI and clinical data, clinical pain was reduced after pregabalin (mean difference ± SD post minus pre; VAS: −15.4 ± 24.1; 95% CI, −1.51 to −29.35; $P = 0.03$) but not after placebo (mean difference ± SD; VAS: −8.43 ± 25.51; 95% CI, 6.30 to −23.16; $P = 0.24$). Resting brain connectivity between the anterior insula and IPL, a region within the DMN, was positively correlated with clinical pain at baseline, before pregabalin administration (table 2). Similarly, resting connectivity between the posterior insula and both IPL and PCC, another DMN region, was also correlated with clinical pain at baseline (fig. 3A; table 2). These data indicate that patients with greater pretreatment clinical pain displayed greater resting connectivity between the insula and brain regions comprising the DMN (*i.e.*, PCC and IPL). Reductions in clinical pain after pregabalin were also associated with reduced resting connectivity between both anterior and posterior insula and IPL (fig. 3B; table 2). These relationships were not seen when extracting the same regions of interest from the placebo period (all $P > 0.20$).

We then explored the potential ability of baseline insula connectivity to predict subsequent reduction in clinical pain after pregabalin treatment. We focused our analysis on regions demonstrating significant baseline correlation to clinical pain and found that baseline posterior insula connectivity to both PCC ($r = -0.72$; $P = 0.004$) and IPL ($r = -0.65$; $P = 0.01$) were associated with reduced clinical pain during pregabalin (fig. 3C). A somewhat less robust predictive correlation was found for baseline anterior insula connectivity to the IPL ($r = -0.55$; $P = 0.04$). These results were not obtained when we extracted Z-scores for the same regions before placebo treatment (all $P > 0.15$). Hence, prepregabalin anterior and posterior insula connectivity to DMN regions was associated with greater subsequent reductions in clinical pain after pregabalin but not after placebo. This was not observed for reductions in experimental pain sensitivity for either pregabalin or placebo (all $P > 0.05$).

Pain-evoked fMRI Activity within the DMN Is a Target and Predictor of Successful Pregabalin Treatment

As a final test of our neuroimaging assessment of pregabalin action, we chose to investigate further the nature of DMN activity by measuring its response to evoked pressure

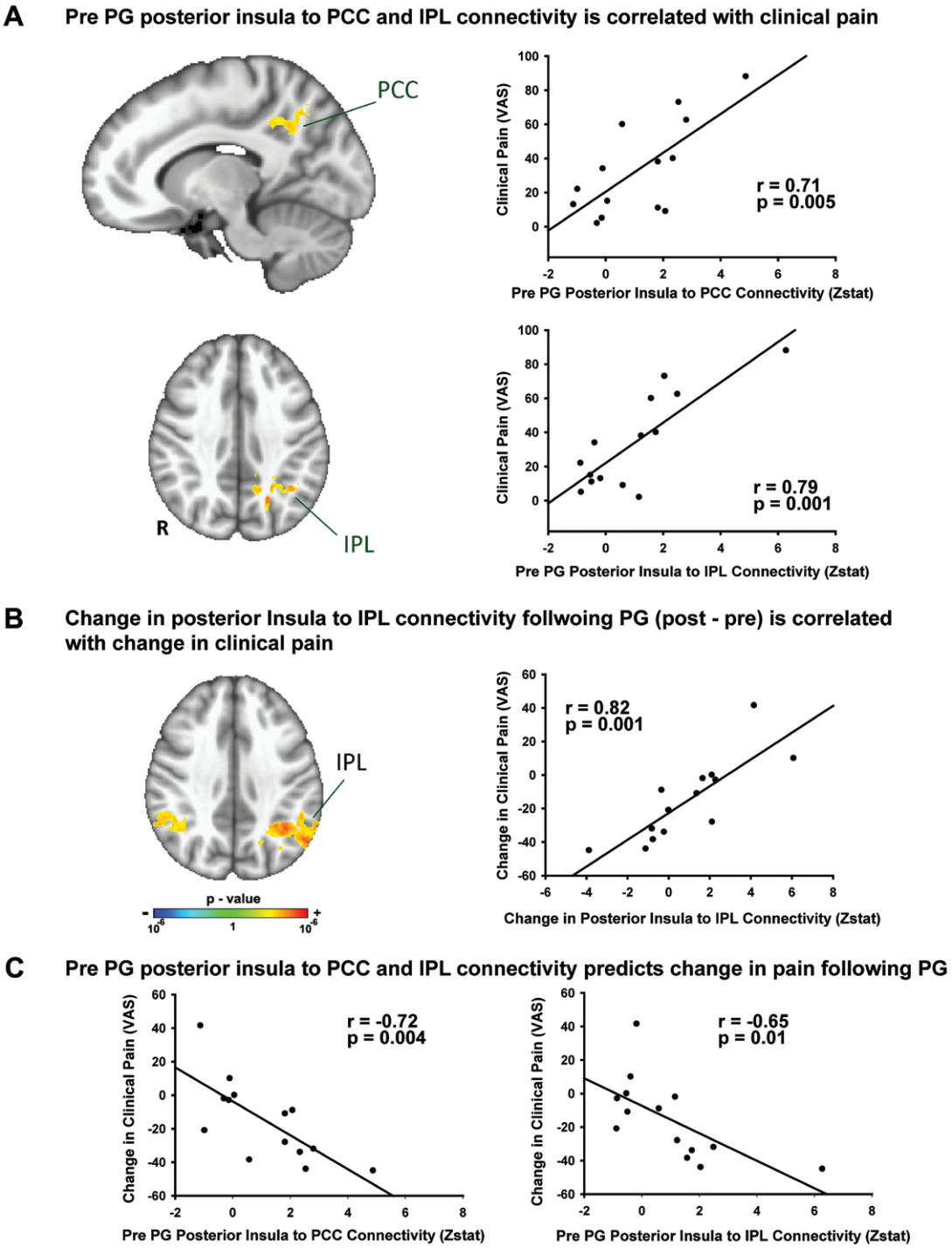

Fig. 3. Resting-state connectivity between the insula and the default mode network (DMN) is a target and predictor of successful PG (pregabalin) therapy. (*A*) Pre-PG right posterior insula connectivity to left inferior parietal lobule (IPL) and posterior cingulate (PCC) is associated with greater clinical pain. (*B*) Reduction in right posterior insula connectivity to IPL post minus pre-PG is associated with greater decreases in clinical pain. (*C*) Pre-PG levels of connectivity for right posterior insula–PCC and IPL connectivity are associated with greater reductions in clinical pain after PG. VAS = Visual Analog Scale.

pain during fMRI. We narrowed our analysis to two DMN regions that displayed enhanced connectivity to the insula during higher clinical pain: namely the PCC and the IPL. These analyses are from the same subjects as in our fcMRI analyses above. Both the IPL and the PCC showed greater deactivations in response to evoked pressure pain after pregabalin (fig. 4, A and B: BOLD mean difference ± SD post minus pre: IPL −0.089 ± 0.122; 95% CI, −0.02 to −0.15; P = 0.018; PCC −0.098 ± 0.156; 95% CI, −0.002 to −0.17; P = 0.035) but not placebo (IPL 0.047 ± 0.123; 95% CI,

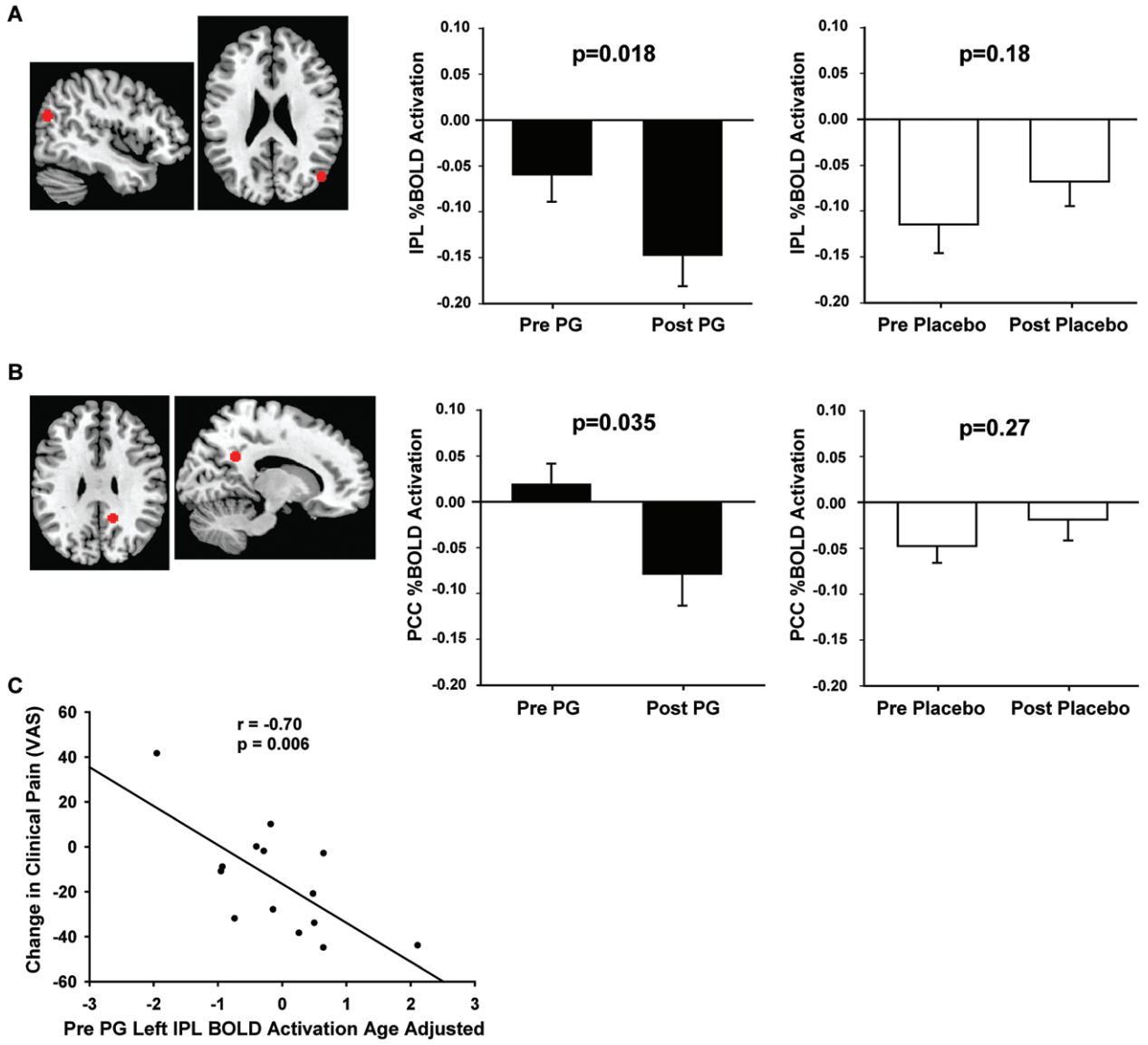

Fig. 4. Evoked-pain default mode network activity is a target and predictor of successful pregabalin (PG) treatment. (*A*) Left inferior parietal lobule (IPL) region of interest and (*B*) left posterior cingulate region of interest (both identified from resting connectivity association with baseline clinical pain) display greater evoked-pain deactivation after PG but not after placebo. Percent blood oxygenation level dependent (BOLD) activations are plotted for both pre- and post-PG as well as pre- and postplacebo. *Error bars* are standard error. (*C*) Pre-PG IPL evoked-pain BOLD activation is associated with greater clinical pain response to PG. PCC = posterior cingulate cortex; VAS = Visual Analog Scale.

0.11 to −0.03; $P = 0.182$; PCC 0.029 ± 0.92; 95% CI, 0.09 to −0.02; $P = 0.265$). Similar to our fcMRI findings, prepregabalin levels of IPL activation were associated with greater reductions in clinical pain after pregabalin (fig. 4C; $r = -0.70$; $P = 0.006$). This relationship was not seen for placebo ($r = -0.34$; $P = 0.23$), nor for the PCC for either pregabalin ($r = -0.44$; $P = 0.13$) or placebo ($r = 0.39$; $P = 0.16$).

Neuroimaging Metrics Independently Predict Analgesia of Clinical and Experimental Pain after Pregabalin Treatment

All three of our imaging modalities were able to predict subsequent changes in pain ratings after pregabalin. However, these initial analyses focused on correlating baseline imaging outcomes with change scores (*i.e.*, post- minus pretreatment pain levels). One limitation of these analyses is that they do not control for regression to the mean nor the amount of pretreatment pain. A more rigorous approach to predicting treatment response would be to use posttreatment pain ratings as a dependent variable while covarying pretreatment pain levels, thereby controlling for the above issues. Table 3 shows that prepregabalin levels of Glx/creatine within the posterior insula predict postpregabalin-evoked pressure pain levels while controlling for prepregabalin-evoked pressure pain as well as age. Similarly, prepregabalin pain activations

Table 3. Pretreatment Neuroimaging Outcomes Predict Subsequent-evoked Pain and Clinical Pain Response to Pregabalin

Dependent Variable	Predictor	Standardized β	S.E. Unstandardized	*P* Value	R Square Full Model
Evoked pain: post-PG (GBS)					0.55
	Age	–0.17	0.07	0.44	
	Evoked pain: pre-PG (GBS)	0.71	0.24	0.003	
	Posterior insula Glx/Cr: pre-PG	–0.45	4.20	0.05	
Clinical pain: Post-PG (VAS)					0.69
	Age	–0.51	0.38	0.048	
	Clinical pain: pre-PG (VAS)	1.25	0.20	0.002	
	IPL BOLD response to pressure pain: pre-PG	–0.69	42.8	0.02	
	Posterior insula–PCC connectivity: pre-PG	–0.55	2.93	0.07	

Two separate linear regression models, one for postpregabalin-evoked pain and one for postpregabalin clinical pain, were constructed with prepregabalin neuroimaging metrics, prepregabalin pain levels, and age as predictors. The models explain 55–69% of the variance in postpregabalin pain.

BOLD = blood oxygenation level dependent effect; Cr = creatine; GBS = Gracely box scale; Glx = glutamate + glutamine; IPL = inferior parietal lobule; PCC = posterior cingulate cortex; PG = pregabalin; S.E. = standard error; VAS = Visual Analog Scale.

within the IPL, and posterior insula connectivity to the PCC, both independently predict subsequent clinical pain after pregabalin while controlling for prepregabalin clinical pain and age. In aggregate, ^{1}H-MRS is sensitive to predicting changes in evoked pain whereas resting connectivity and evoked neural activity in the DMN are more sensitive to predicting changes in clinical pain. These findings have implications for drug development and potentially "personalized" analgesic treatment.[39]

Discussion

Here we provide direct evidence for the first time that pregabalin, an approved analgesic medication for the treatment of fibromyalgia and neuropathic pain, modulates multiple brain outcomes that have been previously associated with chronic pain.[7,9,23,26,27] Perhaps more importantly, baseline values of all three imaging modalities were associated with subsequent reductions in pain after pregabalin. These results are likely to be due to the medication, as these findings were not obtained after placebo treatment of the same individuals.

Our group and others have shown that the posterior insula is intimately involved with evoked and clinical pain processing and modulation in fibromyalgia. Increased levels of Glx within this region are present in these patients, and changes in these levels, after nonpharmacological treatment, are directly related to changes in evoked and clinical pain.[9,23] Here we expand these findings by demonstrating that pregabalin treatment can also decrease Glx specifically within the posterior insula, thereby identifying this neurotransmitter, and this brain region, as a potential target for pregabalin pharmacotherapy. Of note, a recent ^{1}H-MRS study in pain-free controls failed to see a reduction in Glx after gabapentin administration; however, this was an acute administration of the drug and the researchers did not investigate the insula.[40]

Our ^{1}H-MRS data are entirely consistent with preclinical *in vitro* and *in vivo* studies of pregabalin action. Pregabalin has been shown to reduce glutamate release from neuronal slices[18,41,42] and also inhibit glutamatergic neurotransmission within the spinal cord.[43] The mechanism by which pregabalin acts in humans with fibromyalgia may involve the binding of pregabalin to the α2δ subunit of calcium channels, within the posterior insula. Inhibition of glutamate release in this region may result in pain reduction, however, how this results in more lasting reductions in Glx and glutamate levels in ^{1}H-MRS is unknown. We speculate that this action may be related to pregabalin's effects on neuronal plasticity and perhaps neuronal balance of synapse formation and elimination.[44]

Interestingly, we also found that higher pretreatment levels of Glx within the posterior insula were associated with greater decreases in pressure pain sensitivity after pregabalin but not after placebo. These results have significance for "personalized" analgesia as pretreatment ^{1}H-MRS assessment might help guide physicians in choosing *a priori* which patients may be more likely to respond to pregabalin. Pretreatment identification of responders has recently been suggested as an area for improvement in chronic pain management.[39] Currently there are no guidelines that physicians can use to aid in the choice of specific pharmacologic therapies for individuals with chronic pain. Although no significant results were found in the anterior insula, we suspect it unlikely that the posterior insula is the only location of pregabalin action on Glx in the brain.

We also found that patients with greater pretreatment clinical pain also displayed greater resting connectivity between the anterior and posterior insula and brain regions

comprising the DMN (*i.e.*, PCC and IPL). Moreover, reductions in clinical pain after pregabalin were associated with reduced resting connectivity between both anterior and posterior insula and the IPL. These results corroborate our previous findings in both baseline fibromyalgia patients[26] and in patients successfully treated by nonpharmacological therapy.[27] Accumulating evidence suggests that insula–DMN connectivity may be useful as an objective biomarker for clinical pain in chronic pain patients.

Of significance is the fact that baseline insula connectivity to DMN structures were correlated with the extent of clinical pain reduction after pregabalin. Insula connectivity has been previously used to predict pain report in healthy adults. Ploner *et al.*[45] found that functional connectivity between the insula and periaqueductal gray just before a cutaneous stimulus predicts whether or not that stimulus is rated as painful. Our results suggest that resting insula connectivity in pain patients can also be used to predict pain reduction on a much longer time scale, after weeks of pregabalin treatment. Similar to our spectroscopy findings, such prediction has potential as a prognostic marker for improvement to therapy, as physicians could predict which pain patients may benefit most from pregabalin therapy as a step toward "personalized" analgesic treatment.

Finally in our assessment of brain response to evoked pressure pain, we find that the same IPL and PCC regions that displayed greater connectivity to the insula during higher chronic pain, also showed enhanced pain-evoked deactivation following pregabalin treatment. Mitigated task-evoked deactivation of the DMN in untreated chronic low back pain patients has been reported previously.[28] Our data are consistent with these results and further suggest that pregabalin may restore task-evoked DMN deactivations in fibromyalgia patients. We also find, similar to ^{1}H-MRS and fcMRI, that patients with less pain-evoked deactivation of the IPL pretreatment, had a greater subsequent response to pregabalin, further linking DMN deactivation to external tasks as a prognostic marker for pregabalin treatment of fibromyalgia.

Our findings indicate multiple clinical mechanisms of action of pregabalin, a centrally acting agent, in humans suffering from chronic fibromyalgia pain. Although these data originate from a small sample and there is a need to replicate these findings, this study adds to our understanding of how clinical pain is processed and successfully relieved in these complex patients. Moreover, these results point toward a future in which more targeted approaches can be implemented for pharmacological treatment for chronic widespread pain, rather than the current "trial-and-error" approach. Future work is needed to determine whether these findings can be extrapolated to other pain syndromes and other disorders with increased brain glutamate such as neuropathic pain.[46]

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