Baseline Resting-State Functional Connectivity Determines Individual Pain Ratings to an Ecologically Valid Model of Orofacial Pain

Abbreviated title: Baseline rsFC Determines Individual Pain Ratings

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ABSTRACT

Pain is a subjective experience with significant individual differences. Laboratory studies investigating pain thresholds and acute pain have identified structural and functional neural correlates. However, these types of pain stimuli have limited ecological validity to real-life pain experience. Here, we use an orthodontic procedure—the insertion of an elastomeric separator between teeth—which typically induces pain that peaks within 2 days and lasts several days. We aimed to determine whether the baseline structure and resting-state functional connectivity (rsFC) of key regions along the trigeminal nociceptive and pain modulatory pathways correlate with subsequent peak pain ratings. Twenty-six healthy individuals underwent structural (T1, diffusion-weighted MRI) and resting-state functional (rs-fMRI) scanning prior to the separator placement for five days. Participants recorded pain ratings three times, daily. Peak pain was not correlated with diffusion metrics of the trigeminal nerve, or grey matter volume of any brain region. However, peak pain correlated with baseline rsFC between the contralateral thalamus and bilateral insula, and negatively correlated with connectivity between the periaqueductal gray (PAG) and core nodes of the default mode network (medial prefrontal and posterior cingulate cortices). Collectively, the ascending (thalamic) nociceptive and the descending (PAG) pain modulatory pathways at baseline accounted for 78% of the variance in peak pain intensity ratings in a combined model. We further cross-validate our results, and show strong reliability between predicted and actual peak pain ratings ($r = 0.86$). In sum, baseline functional neural architecture of both systems determines the individual pain experience to a subsequent ecologically valid pain stimulus.

INTRODUCTION

Pain perception can vary dramatically between individuals: what feels like a light noxious stimulus to one person may feel excruciating to someone else. This experience is shaped by various factors including biological (e.g. genetics), psychological (e.g. anxiety, catastrophizing) and sociocultural factors (e.g. socioeconomic status) [20,21,44,55,57,66,71]. Greater insight into these individual differences has been provided by brain imaging studies using structural and functional magnetic resonance imaging (sMRI, fMRI) [21,28,36,38,51-53]. Notably, resting-state functional connectivity (rsFC) has been shown to be related to individual differences in pain sensitivity in laboratory-based studies [19,73]. However, laboratory-based studies of experimental pain are limited in their ecological validity in evaluating a realistic painful experience. Recent studies of brain-based predictors of pain have relied on multivariate machine learning approaches, which provide limited insight into neural mechanisms [73,75,79]. Here, we investigate whether baseline brain structure and function can predict pain induced by an ecologically valid and clinically relevant tonic orofacial pain stimulus—orthodontic elastomeric separator insertion.

Orthodontic treatments cause pain in 72-95% of individuals [7,41]. The placement of an elastomeric separator between molars is an orthodontic procedure that creates space between teeth before brace bonding [60]. Orofacial pain induced by the separators in healthy adolescents and adults peaks within 48 hours after insertion, then resolves within 5-7 days [1,13,20]. The separator compresses the periodontal ligament of the alveolar bone, which induces pressure, inflammation and tooth pain [42]. Pain induced by the separator is shaped by cognitive-affective factors such as somatosensory amplification and trait anxiety [20].

Pain in the orofacial region is mediated by the trigeminal nociceptive system. The trigeminal nerve carries orofacial nociceptive signals to the brainstem [68], and further to the thalamus and cortex for processing [26]. The thalamus is a site of nociceptive relay [4,22,74] and one of the most consistently activated brain regions in response to an orofacial noxious stimulus [10]. The experience of pain is

modulated by descending circuits. Notably, the periaqueductal grey (PAG), an opiate-rich region, is a key node of these descending circuits [49].

Here, we aim to determine whether key structures of the ascending trigeminal nociceptive system and descending pain modulatory pathways correlate with peak pain intensity induced by an orthodontic separator placement. We hypothesize that pre-existing structure and functional connectivity of regions involved in the trigeminal nociceptive system will determine an individual's pain severity to this ecologically valid pain model. Specifically, we expect that the structural integrity of the trigeminal nerve and grey matter volume of nociceptive processing and pain modulatory brain regions at baseline will correlate with peak pain intensity. We further expect that stronger baseline thalamic and weaker PAG functional connectivity with pain-related brain regions will correlate with peak pain intensity. Finally, we expect that the combined baseline neural correlates of peak pain will better explain individual differences in pain ratings than any measure alone.

MATERIALS AND METHODS

Participants

Twenty-seven healthy individuals consented to a study approval by University of Toronto Human Research Ethics Board (#32797). One participant was unable to complete the study, and thus is not included in the analysis. The final cohort comprised 26 participants (11 women, 16 men; 25.7 ± 4.4 years). Given that the placement of orthodontic separators is a dental intervention, in Canada, a medical screening by a licensed Orthodontist (IC) is required. As such, participants were screened, and excluded based on the following criteria: current pain, history of chronic pain, chronic illness, psychiatric disorder, pregnancy, metal implants, fixed orthodontic retainers, porcelain-fused-to-metal or full metal crowns, severe dental malocclusions, spaces in dental quadrant 4 (lower right mandible; absence of interproximal tooth contacts), undergoing current orthodontic treatment, use of habitual analgesic medication, and presence of dentures. A clinical examination was performed to ensure they did not have

temporomandibular disorders (TMD) using the Diagnostic Criteria for Temporomandibular Disorder (DC/TMD) [67]. Participants were financially compensated for their time.

Pain Model

An elastomeric separator (American Orthodontics, X-Ring Separators, Sheboygan, USA) was placed at the mesial interproximal contact of the right permanent mandibular first molar by a licensed orthodontist (IC). The separator was kept in place for five days, as previously done [20]. Participants were instructed not to take any analgesics, such as ibuprofen or acetaminophen to reduce pain.

Questionnaires

Pain Diary

We provided a pain diary to participants to record their pain intensity three times per day (10:00, 16:00, 22:00) on a 100 mm visual analogue scale (VAS) over the course of five days following separator placement, as previously done [20]. Specifically, participants reported their tooth pain, in response to the following question: "How severe is your tooth pain now? Place a vertical mark on the line below to indicate how bad you feel your tooth pain is now." The VAS scale had two anchors: left anchor "no pain" and right anchor "worst pain imaginable" [59].

Cognitive affective measures

Given that we have previously shown that trait anxiety and somatosensory amplification were related to the pain experience induced by the insertion of orthodontic separators [20], we asked participants to complete the following questionnaires before the procedure.

The state-trait anxiety inventory (STAI)-trait anxiety (Y2) is a twenty-item questionnaire, with the scale for each item ranging from 0 to 4 points [72]. It is a self-report questionnaire designed to evaluate trait levels of anxiety. The STAI-state anxiety (Y1) was not used.

The somatosensory amplification scale (SSAS) questionnaire is a ten-item questionnaire, scale for each item ranging from 1 to 5 points [11]. The self-report questionnaire detects whether an individual has a tendency to amplify uncomfortable somatic or visceral sensations.

Pain rating analyses

To determine pain ratings from the pain diaries, we measured each marking on the pain VAS scale in millimetres and took the average of the three daily pain ratings. As such, each participant had one pain rating per day. The highest average daily pain rating was considered peak pain intensity score for each individual. These pain ratings were used in all neuroimaging analyses.

Daily pain intensity ratings (five per individual) were tested for normality using Shapiro-Wilk's test. Given that these were not normally distributed, differences between daily ratings were assessed using Friedman's test, a non-parametric repeated measures ANOVA. Significance was set at $P < 0.05$. Multiple correction of post-hoc tests was performed using Dunn's test. All statistical analyses on pain ratings were performed using GraphPad v8.4 (https://graphpad.com).

Relationship between psychological traits and peak pain ratings

Given our previous findings that STAI-trait anxiety and SSAS scores affected the experience of pain induced by elastomeric separators [20], we correlated these psychological measures with peak pain intensity ratings. We tested the STAI-trait and SSAS scores for normality using Shapiro-Wilk's test. In cases where the data were not normally distributed, we used Spearman's correlation test to measure the degree of association between each of these scores and peak pain ratings over five days. Significance was set at $P < 0.05$.

Structural and Functional Neuroimaging

All participants underwent magnetic resonance imaging (MRI) before the insertion of the separator. All scans were acquired using a 3T Siemens Prisma-fit MRI scanner equipped with a 32-channel head coil at the Hospital for Sick Children in Toronto, Canada. Participants were asked to "relax and fixate on the crosshair at the center of the display in the scanner, and not to think about anything in particular."

Structural imaging scan

Structural T1-weighted MRI scans were acquired with a magnetization prepared rapid gradient echo (MPRAGE) using the following sequence: echo time (TE) = 2.96ms; repetition time (TR) = 2300ms; inversion time (TI) = 900ms; 256 sagittal slices; flip angle = 9°; in-plane matrix resolution = 256 × 256 and field-of-view = 256 x 256 mm, resulting in a voxel size = 1 x 1 x 1 mm^3; with a GRAPPA acceleration factor = 2.

Functional imaging scan

Rs-fMRI scans were collected using T2*-weighted echo-planar pulse imaging (EPI) sequence, with the following parameters: TE = 30ms, TR = 1500ms, 50 axial slices; flip angle = 70°; in-plane matrix resolution = 74 × 74 and field-of-view = 222 x 222 mm, resulting in a voxel size = 3 x 3 x 3 mm^3; 200 volumes, with a multiband slice acceleration factor = 2, total scan time = 5 minutes.

Diffusion weighted imaging scan

Two sets of diffusion-weighted images were acquired with reverse phase-encode blips (anterior-posterior and posterior-anterior) resulting in images with distortions in opposite directions with the following parameters: 60 non-collinear diffusion encoding directions with b = 1000 s/mm^2, 7 non-diffusion encoding volumes (B0). DWI acquisition used the following sequence: TE = 73ms, TR = 4400ms; 80 slices; flip angle = 90°; in-plane matrix resolution = 122 × 122, field-of-view = 244 x 244 mm, resulting in a 2 mm isotropic voxel size; with a multiband slice acceleration factor = 2.

<u>Neuroimaging analysis</u>

Voxel-Based Morphometry

We sought to determine whether brain grey matter volume correlated with peak pain ratings. To do so, we performed a voxel-based morphometry (VBM) analysis on all anatomical T1 scans [6]. VBM was performed in SPM v12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12) running on MATLAB (R2016b v.9.1; Mathworks, Nantick, MA). First, all scans were centered at the anterior commissure, and were segmented into gray matter, white matter and CSF tissues. Next, the DARTEL toolbox was used to iteratively align grey and white matter images to a study specific template aligned to the MNI template space [5]. Images were smoothed using an 8 mm at full-width at half-maximum (FWHM) Gaussian kernel. We performed a voxelwise general linear model to determine whether grey matter volume correlates with peak pain intensity. Significance was set a cluster-corrected $P_{FWE} < 0.05$ (with a cluster-forming height threshold of $P < 0.001$). Total intracranial volume was included in the general linear model as a nuisance covariate. Furthermore, a secondary analysis was run based on rsFC results, restricted to voxels within the seeds and rsFC resulting clusters (see below). Significance was set a cluster mass-corrected $P_{FWE} < 0.05$ (with a cluster-forming height threshold of $P < 0.001$).

Diffusion Tensor Imaging

We sought to determine whether microstructure of the cisternal segment of the trigeminal nerve, just outside the pontine trigeminal root-entry zone, is correlated to peak pain ratings. To do so, we performed a diffusion tensor imaging (DTI) analysis using FDT toolbox (FMRIB's Diffusion Toolbox) in FSL 5.0.11 (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT). Volumes with no diffusion weighting (B0 volumes) underwent topup, which estimates the susceptibility-induced off-resonance field, i.e. distortion in the subject's head [2,70]. Next, we used the eddy tool to correct for eddy current distortions, susceptibility-induced distortion, and subject movement [3]. We then fit a tensor model using DTIFIT on the eddy corrected scans. We identified the cisternal segment of the trigeminal nerve, as previously done [18,52], by overlaying the principal eigenvector (V1) map, colored in RGB, onto and modulated to the FA map

for each subject in FSLeyes (https://git.fmrib.ox.ac.uk/fsl/fsleyes/fsleyes/). Individual masks of the right cisternal segment of the trigeminal nerve were used to extract FA, MD, AD and RD. We tested all DTI metrics for normality using Shapiro-Wilk's test. Then, we tested whether these values were correlated with peak pain ratings using Spearman's correlation. Significance was set at $P < 0.05$.

Seed-to-voxel rsFC analysis

We performed a seed-to-voxel rsFC analysis using CONN v17.f toolbox (http://www.conn-toolbox.org) running on MATLAB (R2016b v.9.1; Mathworks, Nantick, MA). We preprocessed anatomical T1 scans and rs-fMRI scans using tools from the Statistical Parametric Mapping software package (SPM v.12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12) incorporated within CONN. First, we removed five initial scans to allow for field homogeneity. All functional scans underwent realignment, which estimates six parameters of motion and corrects it in the x, y and z planes as well as their rotations, roll, pitch, yaw. Next, we used the ART toolbox, implemented in CONN, to detect outlier scans based on conservative settings (global signal threshold at Z=3; subject motion threshold at 0.5 mm) [58]. Following this step, structural and functional scans underwent grey, white and cerebrospinal fluid (CSF) segmentation, and were normalized to the Montreal Neurological Institute (MNI) standard space template. Scans were resliced using default Tissue Probability Maps (structural and functional target resolution at 2 mm). Functional scans were smoothed using an 8-mm FWHM Gaussian kernel. Preprocessed scans underwent denoising, where aCompCor regressed blood-oxygen-level-dependent (BOLD) signal of non-neuronal origin [12], and five principal components were derived for each tissue type (white matter and CSF). Additionally, the following confounding variables were regressed from the model using the indicated number of vectors: white matter (5), CSF (5), motion parameters and their temporal derivatives (12), scrubbing (22) and a regressor to model the first 15 frames and its temporal derivative. This last regressor was added to baseline correct after denoising as normally done in CONN [77]. To identify low-frequency fluctuations characterizing resting state connectivity, we applied a band-pass filter of 0.008-

0.09Hz to the data. Then, we performed a whole brain seed-to-voxel rsFC with two seeds: the left thalamus, contralateral to the site of separator placement, and the PAG. The left thalamus region-of-interest (ROI) was anatomically defined based on the AAL atlas, included within CONN. Given the absence of a PAG ROI in any standard neuroimaging atlas, and because of the significant functional heterogeneity of this region, we defined the PAG ROI based on a functional localizer. Specifically, we performed a meta-analytic search on Neurosynth (https://neurosynth.org) with the term "pain". We extracted the map at a threshold of Z = 17.5 of all the brain regions that showed activation and selected the PAG cluster (86 voxels with a centre-of-gravity at MNI (X,Y,Z): (-1.6, -27.7, -9.88). For the first-level rsFC analysis, we used bivariate correlations to quantify connectivity between each seed to every other voxel in the brain within each subject. At second-level analysis, we evaluated the effect of peak pain intensity, accounting for sex in the model, using a non-parametric cluster-mass $P_{FDR} < 0.05$ (cluster-mass-forming height threshold $P_{uncorrected} < 0.001$, 1000 permutations). Sex was included in the model as evidence suggests sex differences in pain and in brain networks [30,46,76].

Regression Analysis

We performed a linear regression analysis to determine whether the baseline connectivity results explained overlapping or non-overlapping variance in peak pain using IBM SPSS Statistics for Macintosh v.24 (IBM Corp., Armonk, N.Y., USA). We included a composite measure for the ascending nociceptive system findings (thalamic seed) and the descending modulatory system findings (PAG seed) in the regression analysis. Specifically, composite measures were calculated as the mean of the connectivity values from each seed to each resultant target that were significantly correlated to peak pain. The regression model included peak pain as a dependent variable, with sex, thalamic connectivity composite, and PAG connectivity composite as independent variables. The variance inflation factor (VIF) was calculated to evaluate whether the model was susceptible to collinearity. To ensure the reliability and stability of the model, we performed a leave-one-out cross-validation, fitting the

regression model to the training dataset and calculating the predicted peak pain for the left out, testing participant. This was done iteratively for every subject. The predicted peak pain score for each subject was then correlated with their actual peak pain score using a Pearson's correlation.

Sample size calculation

We have computed a priori power analysis using G*Power [29]. For an effect size of Cohen's *d*=0.2, power of 80% and alpha of 0.05, we needed 17 participants to test differences in tooth pain for 5 days using a repeated measures ANOVA. This sample size is consistent with a previous study evaluating pain trajectories with separators over 5 days [20].

RESULTS

Pain Ratings

Participants rated a median peak pain intensity following the placement of the separator at 17.2 [5.08-31.83] [IQR] mm. Pain ratings were the highest within two days, then declining after the second day (Fig. 1, see Fig. S1 for individual pain ratings). Pain ratings were significantly different across days (χ^2 = 12.51, P = 0.014). Post-hoc paired t-tests revealed that pain ratings on Day 2 (median [IQR] = 8.5 [1.0-25.50] mm) were significantly greater than pain ratings on Day 5 (median [IQR] = 3.3 [1.0-12.42] mm; Dunn-corrected P = 0.044).

Psychological ratings

Participants rated moderate trait anxiety (38.3 ± 11.37 (mean ± standard deviation (SD)), and mid-level somatosensory amplification score (16.3 ± 5.76) (mean ± SD). Peak pain ratings were not correlated with trait anxiety (r = 0.12, P = 0.55) nor somatosensory amplification scores (r = 0.21, P = 0.31).

Neuroimaging

No structural correlates of peak pain intensity

We found no structural correlates of peak pain intensity in our cohort. Specifically, no brain grey matter region's volume correlated with peak pain intensity. Furthermore, DTI metrics of the right cisternal segment of the trigeminal nerve (mean ± SD: FA = 0.38 ± 0.095; MD = 0.0019 ± 0.00035; RD = 0.0015 ± 0.00037; AD = 0.0027 ± 0.00031) did not correlate with peak pain intensity (FA: r = 0.004, P = 0.98; MD: r = 0.004, P = 0.98; RD: r = 0.022, P = 0.92; and AD: r = -0.031, P = 0.88).

Stronger thalamo-insular rsFC correlates with peak pain intensity

We found that rsFC between the left thalamus and bilateral insula significantly correlated with peak pain: L Thalamus-Insula (peak MNI coordinates (X,Y,Z) = -38, -8, 0, cluster size: 743 mm^3) at $r^2 = 0.62$ and L Thalamus-R Insula (peak MNI coordinates (X,Y,Z) = 38, 0, -14, cluster size: 513 mm^3) at $r^2 = 0.54$, cluster-mass $P_{FDR} < 0.05$ (Fig. 2). In other words, participants who had stronger connectivity between these regions rated their peak pain rating as higher.

Weaker PAG-default mode network rsFC correlates with peak pain intensity

We found that PAG rsFC to nodes of default mode network (DMN) significantly correlated negatively with peak pain intensity: the right medial prefrontal cortex (mPFC; peak MNI coordinates (X,Y,Z): 12 44 -12, cluster size : 456 mm^3) at $r^2 = 0.66$ and the left posterior cingulate cortex (PCC; peak MNI coordinates (X,Y,Z): -12 -46 28, cluster size: 254 mm^3) at $r^2 = 0.60$, cluster-mass $P_{FDR} < 0.05$ (Fig. 3). In other words, participants who had weaker connectivity between these regions rated their peak pain as higher.

Ascending nociceptive and descending modulatory systems account for non-overlapping variance of peak pain

To determine whether thalamo-insular connectivity and PAG-DMN connectivity explained overlapping or non-overlapping variance in peak pain, we performed a regression analysis with both rsFC measures input into the same model. We included biological sex in the model. The combined model was

significantly related to peak pain, adjusted $r^2 = 0.78$, $F_{3,22} = 30.95$, $P < 0.001$. The VIF of all independent variables was < 2 indicating that the model did not suffer from collinearity [33,48]. Importantly, connectivity findings were independently associated with peak pain in this joint model (thalamo-insular connectivity, b = 0.42, $t_{22} = 3.42$, $P = 0.002$; PAG-DMN connectivity, b = -0.54, $t_{22} = -4.38$, $P < 0.001$) with thalamo-insular connectivity accounting for 10% of the unique variance in peak pain and PAG connectivity accounting for 17% of the unique variance in peak pain. Biological sex was not a significant predictor in this joint model (Sex [Male=1; Female=0], b = -0.15, $t_{22} = -1.64$, $P = 0.12$). Further, the leave-one-out cross validation revealed strong stability of the model as the correlation between the predicted peak pain ratings and actual peak pain ratings was r = 0.86.

DISCUSSION

Individual variability in pain is thought to be correlated to the structure and functional connectivity of nociceptive-responsive and pain modulatory regions. Here, we used an ecologically valid model of tonic orofacial pain to evaluate whether baseline trigeminal and grey matter structure, as well as rsFC of brain regions involved in nociceptive processing and pain modulation correlate with peak pain intensity, occurring days later. Pain intensity was highest within 48 hours (Fig. 1). Although we did not find any significant structural correlates of peak pain intensity; we did, however, find significant correlations between peak pain and rsFC of key nodes of both the ascending nociceptive and the descending pain modulatory systems. Peak pain intensity correlated with stronger thalamo-insular connectivity—a key set of brain regions involved in nociceptive processing (Fig. 2) and weaker rsFC between the PAG with the mPFC and PCC—core nodes of the DMN (Fig. 3). We further showed that together thalamo-insular and PAG-DMN connectivity explained 78% of the variance in pain ratings (in a model that also contained sex), and each accounted for significant variance, indicating that both ascending and descending systems determine peak pain ratings. Together, these data show that pre-existing rsFC networks, but not neural

structure, may serve as predictors of forthcoming pain intensity to an ecologically valid model of clinical low-level tonic orofacial pain.

Elastomeric separator placement is a common orthodontic procedure. These separators typically induce low-to-mid-level pain [16]. Our participants had a median of low-level pain compared to previous work in adolescents and adults, who reported moderate levels of pain [1,13,16,20]. However, these studies placed multiple separators between the first molar and second premolar, which could explain higher pain intensity ratings, compared to our study. Since the amount of pain is thought to be proportional to the magnitude of the orthodontic force applied to the teeth [39], increasing the number of separators can be used in future studies to elucidate the effects of different intensities of tonic nociceptive input. Nevertheless, our findings are consistent with these and other studies showing pain intensity is highest within two days of separator placement and resolves by within approximately five days [16,41,54]. Although peak pain intensity in our cohort was variable amongst participants (Fig. S1), the temporal pattern was similar to what has been reported, such that Day 2 had a significantly higher median rating than Day 5.

Separator placement is an ecologically valid noxious stimulus as it mimics naturally occurring painful experiences. The model captures environmental variability, such that the individual leaves the laboratory setting, while continuously receiving the stimulus for the duration of the separator placement and as the participant goes through their day. This ecological variability is fundamental to capturing individual differences in pain, particularly in its evolution and resolution. Therefore, this model circumvents the limitations of laboratory-based pain testing, where stimuli are usually brief (i.e., do not go beyond the experimental session), and only capture a brief snapshot of the experience in a controlled setting [62].

Brain structure is thought to reflect, and even predict individual differences in behaviour and cognition [40]. A limited of number of studies have reported baseline gray matter correlates of acute laboratory-based thermal pain stimuli [27,28,32] in various brain regions. In individuals with trigeminal

neuralgia, diffusion metrics of the trigeminal nerve have been used to predict treatment responsiveness [38]. However, our study did not identify any significant structural correlates of peak pain, even with a directed search of regions identified in the rsFC analysis. Notably, previous gray matter studies had substantially larger sample sizes and/or small effect sizes. Therefore, our sample size may have been too small to detect subtle effects. For the trigeminal nerve, the FA values in our cohort were within a normative range, whereas those for the affected nerve in trigeminal neuralgia cohorts were abnormally low [38]. Therefore, it is likely that structural white matter abnormalities may predict improvement after treatment; whereas FA variability (and other diffusion metrics) may not be good predictors of nociceptive transmission in health. Therefore, neural structure may not be a suitable predictor for this ecologically valid model of orofacial pain in otherwise healthy individuals.

Brain networks are continuously active at rest, including those involved in nociceptive processing and pain modulation [69]. Previous studies in healthy adults show increased rsFC between brain regions involved in pain processing before receiving nociceptive stimuli, i.e. pain anticipation [15,56] and following nociceptive stimulation [64]. Analogous to the current findings, a recent study demonstrated that data-driven, multivariate analyses of rsFC in a pain-free state predicted individual differences in pain sensitivity during later testing [73]. Their measure of pain sensitivity was a 'composite' based on a weighted average of heat, cold and mechanical pain thresholds delivered 1-3 days from the time of resting-state functional imaging. Notably, pain thresholds measure a brief static point at which the stimulus becomes painful for the individual [65], and do not reflect most real-life painful experiences. In contrast, our study shows that both baseline ascending and descending systems determine individual peak pain induced by an ecologically valid tonic noxious stimulus.

We investigated the rsFC of the thalamus, a major relay region of the ascending nociceptive pathways [24]. The thalamus and insula are structurally connected. Evidence from primates and humans show efferent and afferent projections from the dorsal thalamus to the insular cortex [9]. The dorsal thalamus projects to the insula from the ventral anterior, centromedian, ventral posterior medial nuclei

[8]. Specifically, there is evidence that the ventral medial posterior thalamus projects to posterior insula [23-25]. There is a functional dichotomy in the involvement of the insula in pain perception: the anterior insula is closely associated with the cognitive and/or modulatory aspect of pain, whereas the posterior and mid-insula are associated with lower-level, sensory-discriminative features of pain, such as location and intensity [17,50,78]. Some have even proposed that the posterior insula/opercular region could serve as primary pain cortex [31]. Our insular clusters are located in the posterior and mid-insula, and therefore receive direct input from the thalamus, and may reflect the first cortical relays of nociceptive information processing. Therefore, our study advances this finding by showing that baseline thalamo-insular connectivity can determine peak pain intensity, and thus supports the sensory-discriminative role of the insula in pain.

Pain perception is a fine balance between nociceptive and antinociceptive mechanisms [14]. Early evidence in rodents [63] and humans [37] implicate the PAG in pain inhibitory mechanisms. More recent evidence suggests that the PAG is involved in both facilitatory and inhibitory pain mechanisms [47]. Our results show that peak pain intensity was negatively correlated with PAG-DMN rsFC. The PAG receives input from ascending spinal/trigeminal systems, descending input from cortical subcortical regions. Further, it projects to brainstem pain modulatory circuits, and is a source of opioidergic pain modulation [34,47,49]. Specifically, the dorsolateral and lateral subregions of the PAG receive input from the mPFC and PCC [35,34,45,47]. The mPFC, PCC and lateral parietal cortices are regions whose activity is highly correlated and form the DMN [61]. DMN activity increases when the individual is not attending to external stimuli and is involved in monitoring the internal milieu which would include monitoring nociceptive signals. We found that individuals who felt less pain had a stronger baseline PAG-DMN rsFC. Previous evidence shows that stronger PAG-DMN rsFC is associated with the tendency to disengage from pain, in other words mind wander away from pain [43]. Thus, high baseline connectivity between the PAG and DMN may enable greater engagement of descending systems in order to disengage from pain. Conversely, weaker PAG-mPFC rsFC in patients with chronic low back pain

was related to higher pain intensity conditions, suggesting dysfunction in the descending pain modulation [80]. As such, our study further postulates that the strength of PAG-DMN rsFC at baseline in the healthy individuals determines peak pain intensity days later. Whether this rsFC could predict either the development or maintenance of chronic pain requires further investigation.

We further demonstrate that our rsFC findings have partially overlapping, but unique contributions to peak pain intensity induced by separator placement. Together thalamo-insular and PAG-DMN rsFC in the pain-free state, predict peak pain intensity at 78%, days later. Specifically, our model found medium to large unique contributions of the ascending nociceptive system (10%) and descending pain modulation (17%). However, sex did not show meaningful individual contributions to the variance observed in our data. Thus, the majority of the explained variance is overlapping between the ascending and descending systems—individuals with higher PAG-DMN connectivity also tended to have lower thalamic-insular connectivity and this connectivity phenotype is associated with lower peak pain. However, as mentioned above, each system still showed a significant unique contribution beyond this shared component. We found this model to be reliable and stable based on the cross-validation correlation.

In sum, we suggest that the pre-existing state of the ascending and descending pathways may lead to pain susceptibility and that the synergy of both systems is required to determine a painful response to an ecologically valid nociceptive stimulus.

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AUTHORS CONTRIBUTIONS

LA: data analysis and drafting of manuscript; MPM: revising the manuscript; AB: data analysis; KCJH: data collection; IC and MM: conception and study design, revising the manuscript. All authors have approved the final version and agree to be accountable for all aspects of the work.

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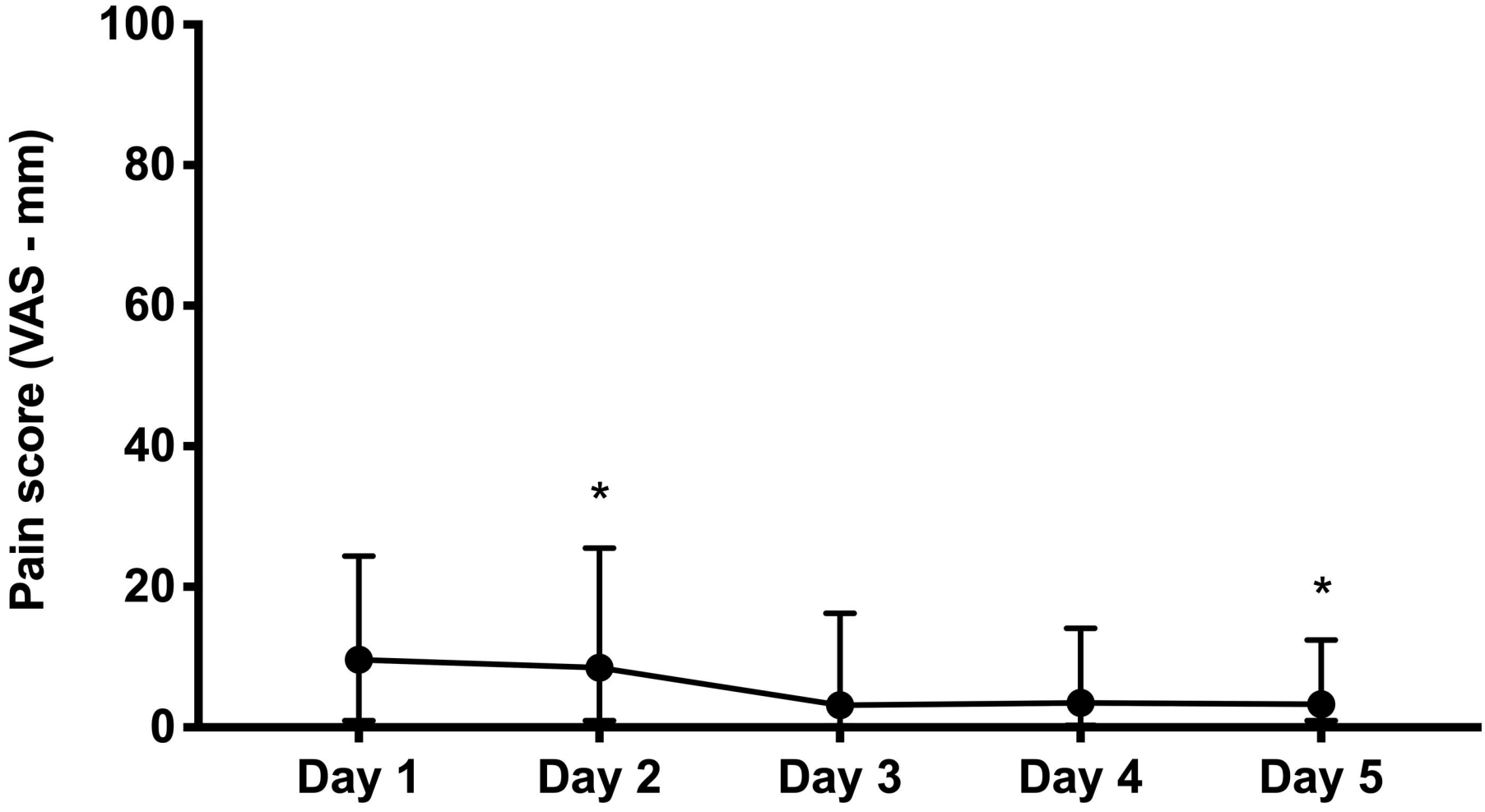
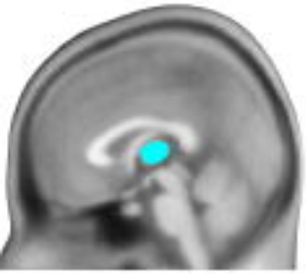
FIGURES

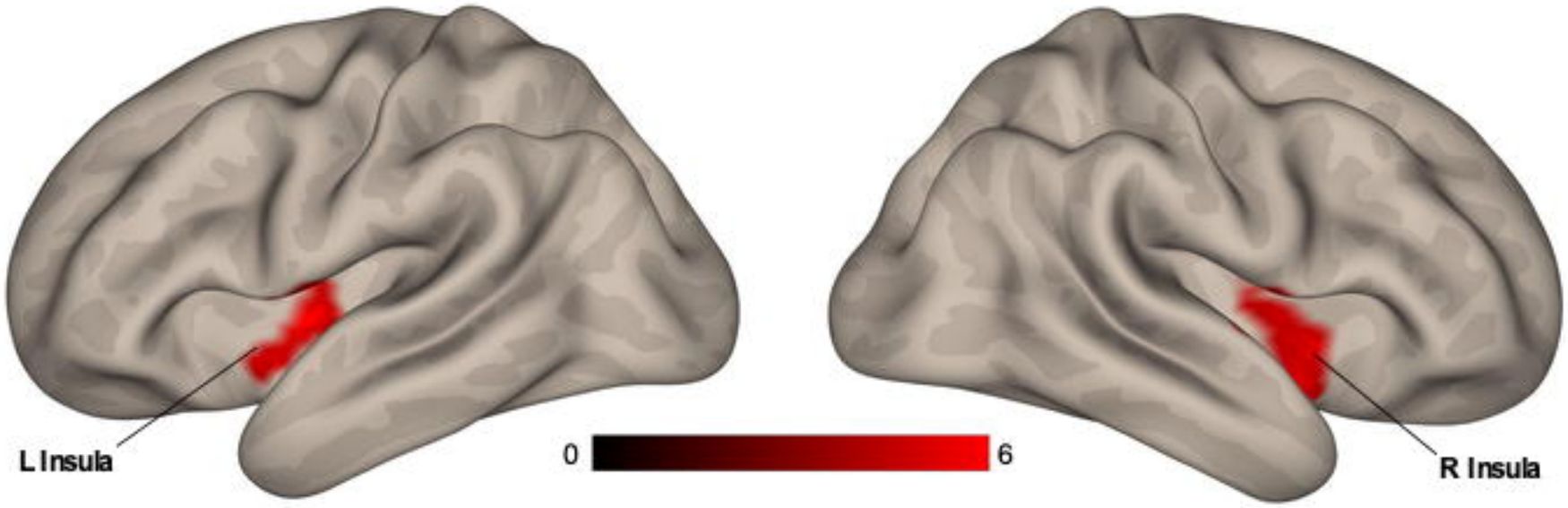
Fig. 1. Pain ratings in response to the orthodontic separator for five days. Self-report median pain ratings (IQR) are represented for all participants (n=26) per day. *Pain ratings on Day 2 were significantly increased compared to Day 5, significant at $P < 0.05$. *Abbreviations: IQR* interquartile range *VAS* visual analogue scale.

Fig. 2. Increased Thalamo-insular rsFC correlates with peak pain intensity. Resting-state functional connectivity (rsFC) between the left thalamus and bilateral insula before the insertion of the separator correlates with peak pain intensity measured on a 100-mm visual analogue scale (VAS), significant at cluster-mass $P_{FDR} < 0.05$.

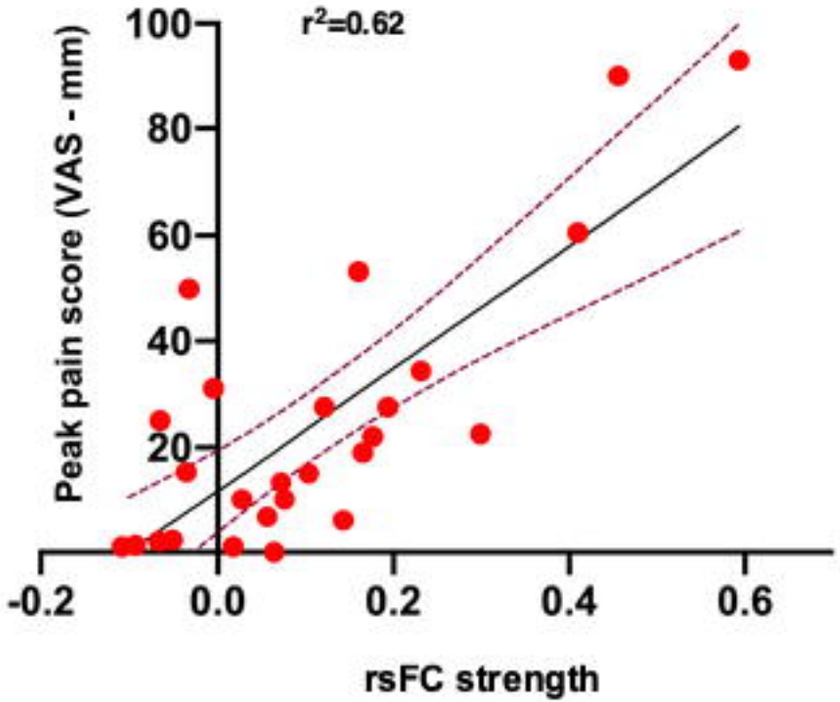
Fig. 3. Decreased PAG-DMN correlates with peak pain intensity. Resting-state functional connectivity (rsFC) of the periaqueductal gray (PAG) and core nodes of the default mode network (DMN)—the posterior cingulate cortex (PCC) and medial prefrontal cortex (mPFC)—before the insertion of the separator negatively correlates with peak pain intensity measured on a 100-mm visual analogue scale (VAS), significant at cluster-mass $P_{FDR} < 0.05$.


Pain score (VAS - mm)
100
80
60
40
20
0
*
*
Day 1
Day 2
Day 3
Day 4
Day 5

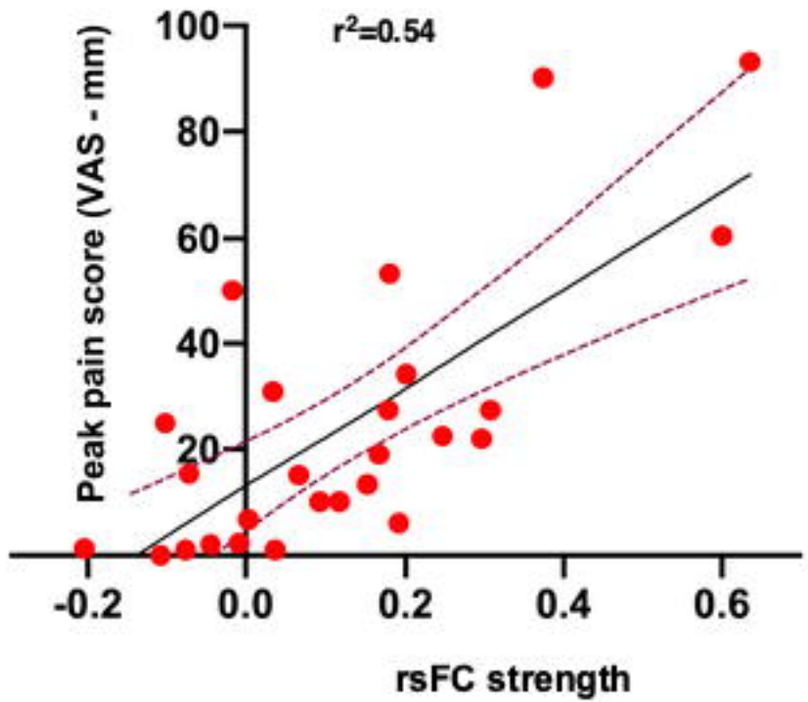




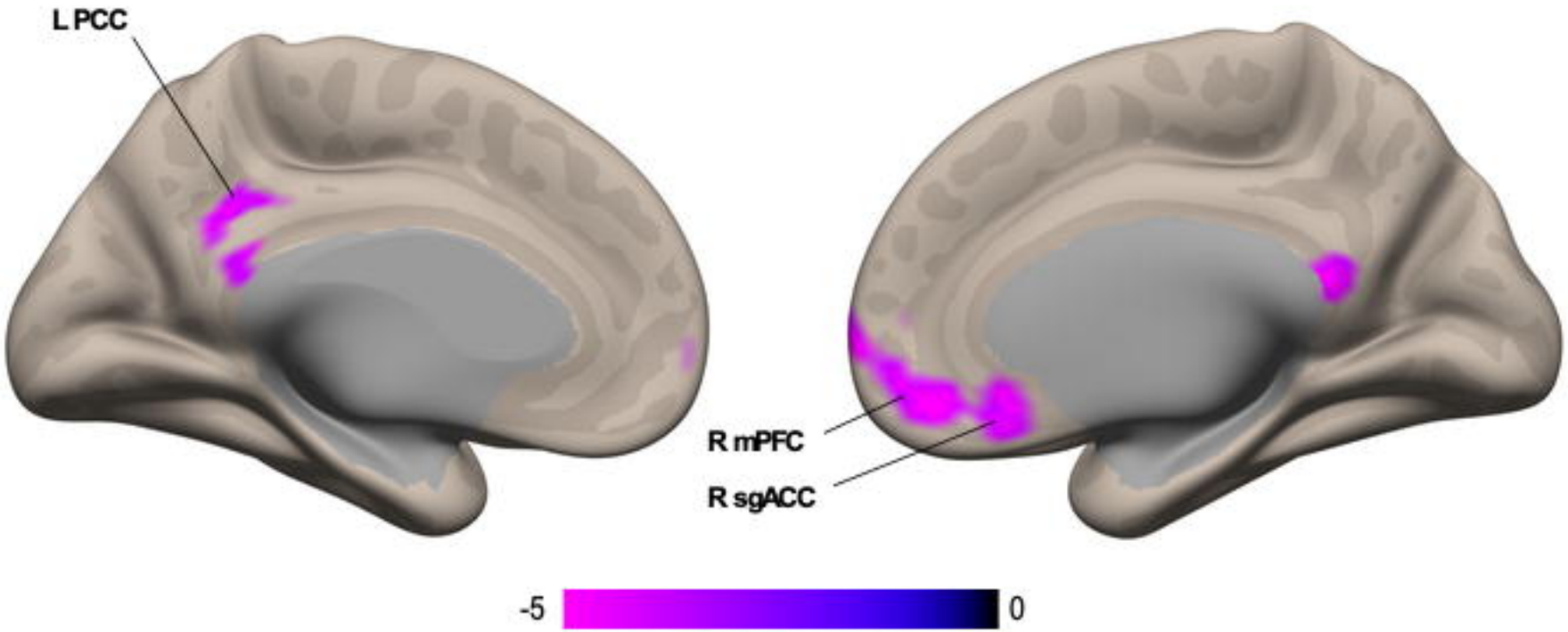
L Thalamus - L Insula

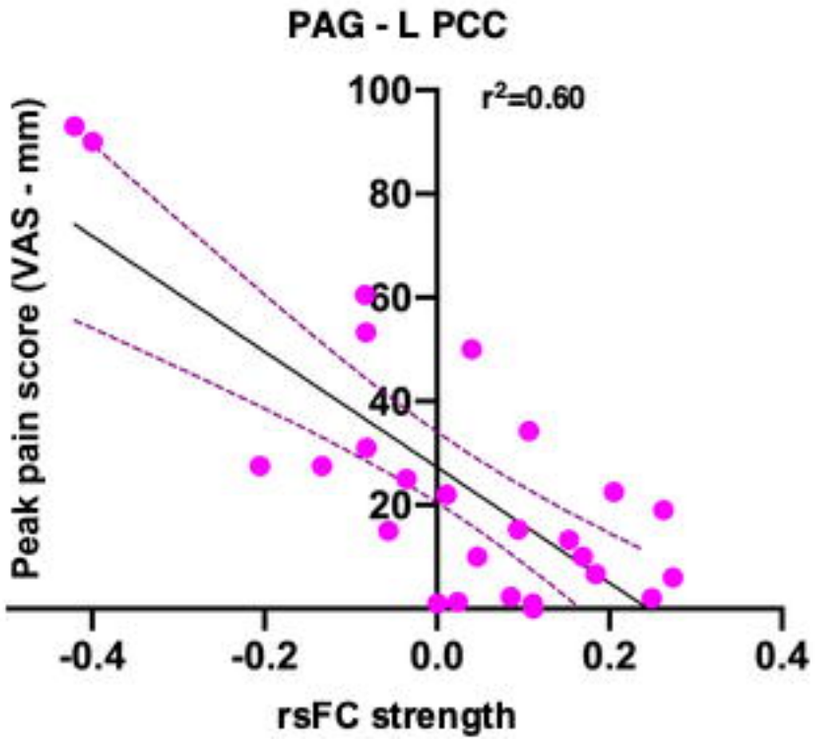


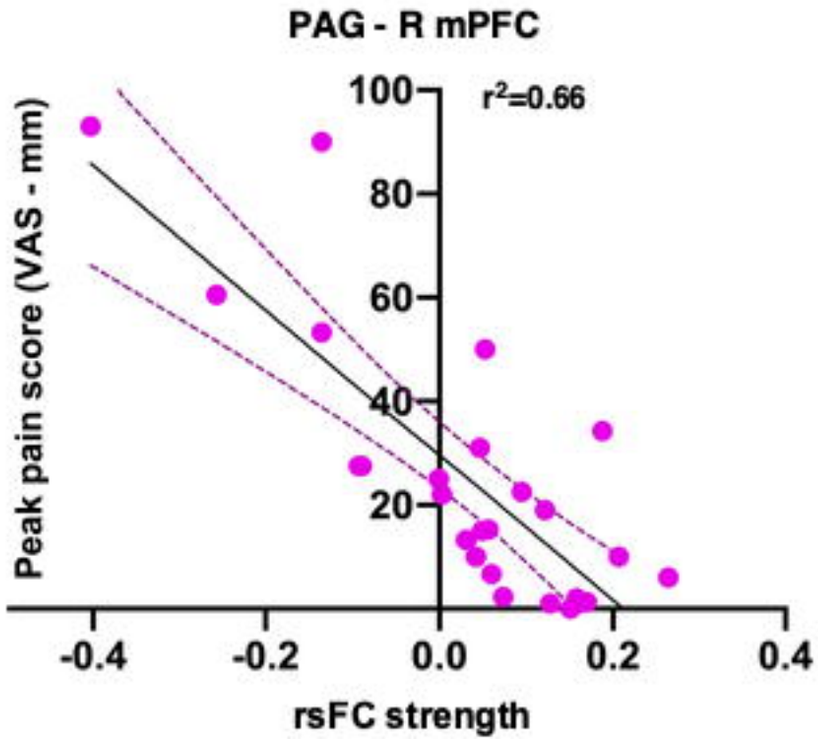
L Thalamus - R Insula



PAG seed


L PCC
R mPFC
R sgACC
-5
0


PAG - L PCC
r²=0.60
Peak pain score (VAS - mm)
100
80
60
40
20
-0.4
-0.2
0.0
0.2
0.4
rsFC strength


PAG - R mPFC
r²=0.66
Peak pain score (VAS - mm)
100
80
60
40
20
-0.4
-0.2
0.0
0.2
0.4
rsFC strength