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Individual differences in conditioned pain modulation are associated with functional connectivity within the descending antinociceptive pathway

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Abstract

The perception of pain and ability to cope with it varies widely amongst people, which in part could be due to the presence of inhibitory (antinociceptive) or facilitatory (pronociceptive) effects in conditioned pain modulation (CPM). This study examined whether individual differences in CPM reflect functional connectivity (FC) strengths within nodes of the descending antinociceptive pathway (DAP). A heat-based CPM paradigm and resting-state functional magnetic resonance imaging (rs-fMRI) were used to test the hypothesis that an individual's capacity to exhibit inhibitory CPM (changes in test stimuli [TS] pain due to a conditioning stimulus [CS]) reflects FC of the subgenual anterior cingulate cortex (sgACC), periaqueductal gray (PAG), and rostral ventromedial medulla (RVM). A total of 151 healthy participants (72 men, 79 women) underwent CPM testing and rs-fMRI. Three types of CPM were identified based on the effect of the CS on TS pain: (1) Antinociception: CS reduced TS pain in 45% of participants, (2) No-CPM: CS did not change TS pain in 15% of participants, and (3) Pronociception: CS increased TS pain in 40% of participants. Only the Antinociceptive subgroup exhibited FC between the left sgACC and PAG, right sgACC and PAG, and RVM and PAG. Furthermore, only the Antinociceptive subgroup exhibited a correlation of both left and right sgACC-RVM FC (medium effect sizes) with CPM effect magnitude. Women, compared with men were more likely to be categorized as pronociceptive. These data support the proposition that FC of the DAP reflects or contributes to inhibitory CPM.

Keywords: Conditioned pain modulation, Functional connectivity, sgACC, PAG, RVM

1. Introduction

The pain experience varies widely amongst people in part due to different capacities to modulate pain.[28,29,76] In humans, the assessment of conditioned pain modulation (CPM) can be used to study the functionality of an individual's endogenous descending inhibitory pathway, said to reflect pain modulation capability associated with the "pain inhibits pain" phenomenon.[2,48,85] The CPM effect as well as measures of pain perception show variability across individuals[29,36,49,86] and may reflect, at least in part, the balance between the activity of the ascending nociceptive sensing pathway (ANP) and descending antinociceptive pathways (DAP) within the dynamic pain connectome (DPC).[13,17,41]

Traditionally, the ANP and the DAP can be viewed as 2 systems of the dynamic pain connectome[53] that contribute to how pain is sensed and modulated. The core of the "top-down" DAP, which modulates the activity of nociceptive neurons in the spinal dorsal horn in what is classically viewed as inhibitory, includes the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) brain regions.[24] In humans, CPM has been used as a proxy to assess descending pain modulation pathway function.[36] The CPM effect in humans has been thought to reflect, at least in part, the phenomenon first discovered and studied in animals known as diffuse noxious inhibitory controls (DNIC).[57] The DNIC effect refers to the attenuation of noxious stimulus-evoked activity from stimulation applied to the receptive field of spinal cord nociceptive neurons by a noxious conditioning stimulus (CS) applied to a different (remote) body part.[56–58,60,81] Similarly, the CPM paradigm has been used to evaluate pain modulation efficacy by assessing how pain evoked by an acute test stimulus (TS) applied to one area of the body can be modulated by a CS that is applied to another area of the body.[36]

There is accumulating evidence that the CPM effect, contrary to the original belief, has wide intersubject variability ranging from

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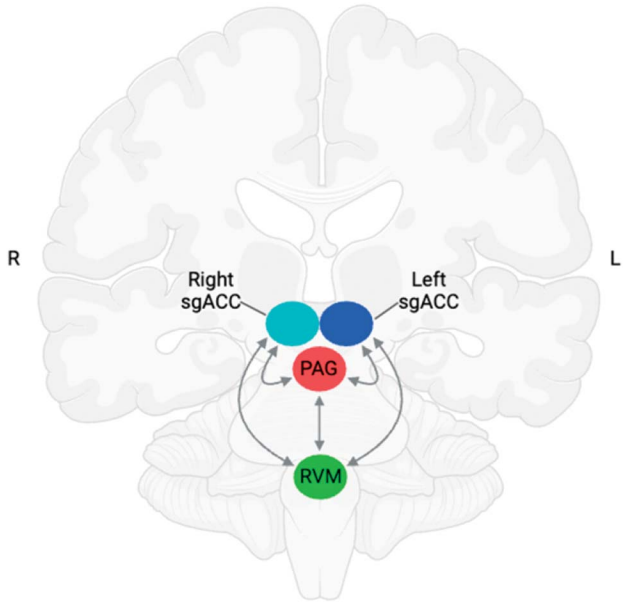

Figure 1. Regions of interest within the descending antinociceptive pathway. Coronal view of the brain depicting the approximate location of regions of interest and FC pairings examined in this study. Figure created using BioRender (https://app.biorender.com/). PAG, periaqueductal gray; RVM, rostroventral medulla; sgACC, subgenual anterior cingulate cortex.

pain reduction (pain inhibition, ie, antinociception) to no effect on pain increases (pain facilitation, ie, pronociception).[22,29,34,75] However, the mechanisms underlying the variability of the CPM effect are not established. One possibility is that the functionality of the DAP underlies whether an individual exhibits the classic inhibitory CPM effect, no CPM effect, or a facilitatory CPM effect.

In this study, we examined CPM in a large group and correlated individual CPM effects with resting-state fMRI FC, a method commonly used for functional connectivity analysis,[18,51,83] between key regions of the DAP (PAG, RVM, sgACC in healthy individuals) (**Fig. 1**) to test the hypotheses that CPM is positively associated with FC within the DAP. In addition, we explored whether there are sex differences in CPM capability and its association with FC of the DAP.

2. Methods

2.1. Study participants

This study consisted of data from 151 healthy controls (79 women; mean ± SD age = 26.2 ± 4.6 years, 72 men; mean ± SD age = 27.0 ± 5.3 years). Written consent was obtained from all study participants for this study approved by the University Health Network ethics board. Potential study participants were excluded if they had the following: (1) any contraindication for having an MRI; (2) self-reported chronic pain (ie, pain lasting longer than 3 months); (3) current pain; (4) a history of psychiatric or neurological disorders; (5) a major surgery within the past 2 years; (6) aged 40 years of age or older to avoid confounds due to age-dependent reduction of CPM[37]; (7) left-handedness to control for brain lateralization; (8) an initial test stimulus pain rating of less than 25 of 100.

Before testing, participants were asked to refrain from the following: (1) wearing eye and face makeup (as it may contain traces of metal contraindicated for MRI); (2) drinking coffee within 1 hour before the scan; (3) drinking alcohol or taking recreational drugs 8 hours before this study; (4) using hair products such as gel or hairspray or washing their hair right before the scan (as these may contain traces of metal).

This study analysed imaging and psychophysical data in healthy individuals collected in part for previous studies of chronic pain in our laboratory.[9,13,14,22,29,43,76] We note that data from a total of 203 participants were deemed potentially eligible for this study. However, 52 participants were excluded from the analysis, mostly due to incomplete data collection, but also because of left-handedness, a TS1 pain rating score of lower than 25, or for 6 participants, an fMRI signal intensity dropout of higher than 50% of the mean intensity within all nonzero voxels in either the left or right sgACC seeds.

2.2. Neuroimaging session

Neuroimaging data were acquired using a 3T MRI (GE Medical Systems, Chicago). The neuroimaging acquisition included a high-resolution T1-weighted anatomical scan (180 axial slices; repetition time (TR) = 7.8 milliseconds; echo time (TE) = 3 milliseconds; inversion time (TI) = 450 milliseconds; field-of-view (FOV) = 256 mm × 256 mm; flip angle = 15°; 256 × 256 matrix; 1 × 1 × 1 mm^3 voxels, 1 mm slice thickness) that lasted for 9 minutes 56 seconds, and a T2*-weighted resting-state fMRI scan that lasted for a total of 9 minutes 14 seconds (echo-planner imaging sequence, 36 axial slices, TR = 2000 milliseconds; TE = 30 milliseconds; 64 × 64 matrix; 3.125 mm × 3.125 mm × 3.125 mm voxels, 3.125 mm slice thickness). The functional imaging slab was set to ensure coverage of the regions of interest (ROIs) (ie, from the primary somatosensory cortex to the RVM). For resting-state scans, participants were instructed to close their eyes and not to think about anything in particular and avoid thinking about structured thoughts, such as singing a song or counting.

2.3. Preprocessing of functional magnetic resonance imaging data

All imaging data were preprocessed using version 5.0.1 of the FMRIB Software Library (FSL) and the fMRI Expert Analysis Tool (FEAT) within it. The first 4 volumes of all participants' resting-state scans and data from nonbrain voxels were removed using FEAT's Brain Extraction Tool (BET).[47] Motion correction was performed using Motion Correction FMRIB's Linear Image Registration Tool (MCFLIRT).[47] MCFLIRT was also used to register each participant's functional images to their skull-stripped T1-weighted anatomical image and for nonlinear registration to Montreal Neurological Institute (MNI) 152-2 mm space with FMRIB's Nonlinear Image Registration Tool. OptiBet was used to skull-strip T1-weighted anatomical images, and each subject's resting-state image then underwent linear registration to their T1-weighted anatomical image.[64] Additional respiratory and cardiac cycle noise was removed using aCompCor, a component base noise reduction method[11] as previously described.[52] Six motion parameters in addition to scanner-related noise as well as the top 5 white matter and cerebrospinal fluid (CSF) components were regressed out. A 4-mm full-width half maximum (FWHM) Gaussian spatial smoothing kernel was applied to the data before being filtered through a temporal filter with a high-pass cut-off of 0.01 Hz and a low-pass cut-off of 0.1 Hz. Cut-offs were selected to remove scanner-related drift and physiological noise, respectively.

2.4. Functional connectivity analysis

The PAG and the RVM were the focus of this study given that they are key, core regions of the descending antinociceptive pathway. A 3-mm radius spherical seed in the PAG (MNI: 0, −32, −10) and a 2-mm radius spherical seed in the RVM (MNI: 0, −34, −50) (**Fig. 2**). Both of these seed locations have been previously published based on fMRI activity due to noxious stimuli in healthy controls.[6,72] A third and fourth spherical seed, both 6 mm radius, were placed in the left and right sgACC (MNI: −5, 34, −4 and MNI: 5, 34, −4, respectively) (**Fig. 2**). Given the anatomical location of the sgACC, we considered susceptibility effects and signal dropout.[72,83] As such, we used an exclusion criteria of a BOLD signal intensity dropout higher than 50% of the mean intensity within all nonzero voxels in either the left or right sgACC seeds as done previously.

Static FC was quantified between each pair combination of brain regions of interest (seed-to-seed). To do such, the mean time series of each region was first extracted and then a Pearson correlation was run between the 2 mean time series. Finally, a Fisher *r-to-z* transformation was completed for each brain region pair.

2.5. Psychophysics protocol and evaluation of conditioned pain modulation

Participants were comfortably seated in an office chair at a desk inside of a separate quiet testing room. Thermal stimuli were delivered using 30 × 30 mm contact thermodes placed on each volar forearm (approximately 15 cm above the wrist) (QSense device, Medoc Ltd, Ramat Yishai, Israel) (**Fig. 3A**). Before the CPM test, a familiarization paradigm was used to determine each participant's pain[50]; the thermode temperature at which they verbally defined the pain intensity as a rating of 50 of 100 with 0 being "no pain" and 100 being "the worst pain imaginable." During the familiarization paradigm, predetermined stimuli were applied using the left volar forearm thermode in the following order: 44°C, 45°C, 43°C, 46°C, 42°C, and 47°C if the participant looked comfortable had not yet up to that point reported a pain rating that was greater than 75 of 100. A baseline and interstimulus temperature of 35°C was held between each familiarization test stimuli and the ramp-up rate was 2°C/second to reach the target temperatures. Once reached, the target temperatures were held for a total of 6 seconds and participants were asked at the 3 seconds mark to rate their evoked pain intensity. After 6 seconds had elapsed, the decreasing thermode temperature speed was 1°C/second back to baseline. For each individual, their Pain50 stimulus temperature was estimated from this familiarization paradigm and reconfirmed during a subsequent habituation paradigm that used stimulus timings and ramp rates identical to the CPM paradigm. Both TS and CS thermode temperatures were manually set, assuring a percept-matched stimulus of Pain50.

The timeline of the CPM paradigm is depicted in **Figure 3B**. The right volar forearm thermode was used to deliver the TS, and the CS was delivered to the left volar forearm. A baseline temperature of 35°C was maintained in between raises to the respective target temperature of Pain50 for each participant, which was predetermined in the aforementioned familiarization paradigm.

The first TS (TS1) was delivered 5 seconds after the start of the CPM paradigm. The right thermode increased at a speed of 2°C/second from baseline (35°C) to the target Pain50 temperature for each respective participant. Once attained, the temperature was held for 10 seconds before returning back down to baseline at a speed of 1°C/second. At the 7-second mark while the TS temperature was being held at the target temperature, participants were asked to verbally rate their pain to TS1 from 0 to 100. Back at baseline, the thermode remained at 35°C for 69 seconds before ramping up again to deliver the second TS (TS2). The ramp speed and timing of TS2 is identical to TS1. After ramping down back to the baseline after TS2, the thermode remained at 35°C for 85 seconds before the third TS (TS3) was delivered in the same manner at TS1 and TS2, with participants verbally rating their pain intensity from 0 to 100 at 7 seconds after reaching the target temperature.

On the left volar forearm, the CS was delivered 40 seconds from the start of the CPM paradigm. The 35°C baseline and target temperatures for each participant were kept consistent with the TS stimuli. A ramp-up rate of 1°C/second was applied to the CS as the target temperature was attained from baseline and then held for 100 seconds before returning to baseline at a rate of 1°C/second. During the 100 seconds, participants were asked to verbally rate their pain out of 100 3 times, at 10 seconds, 30 seconds, and 60 seconds after the CS had reached its target temperature. Data from TS3 are not reported in this study but have been shown in our previous study.[29]

In line with previous studies indicating the CPM effect or efficacy of CPM can range from pain inhibition to pain facilitation,[2,19,29] this study used a scale ranging from −100 to 100 as possible CPM percent effect scores. Specifically, this effect score represents the magnitude of each individual's response during the CPM paradigm that was calculated from

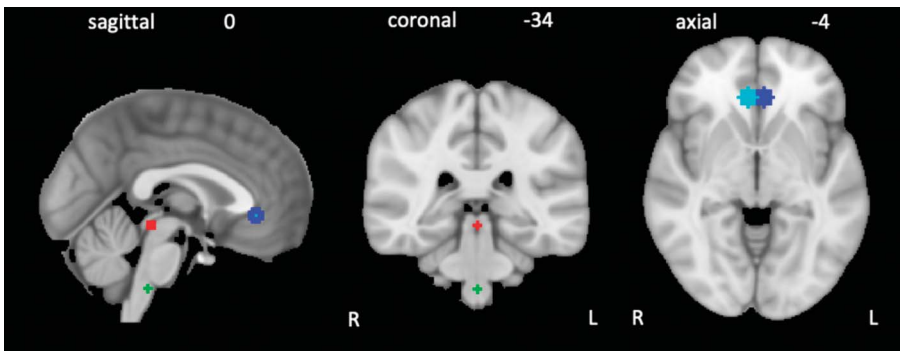

Figure 2. Descending antinociceptive pathway regions of interest. The sagittal brain slice at X = 0 shows the PAG ROI in red (3 mm rad; MNI 0, −32, −10), RVM ROI in green (2 mm rad; MNI 0, −34, −50), and left sgACC ROI in dark blue (3 mm rad; MNI −5, 34, −4). The coronal brain slice at Y = −34 shows PAG and RVM ROIs. The axial brain slice at Z = −4 shows left sgACC and right sgACC ROI in light blue (3 mm rad; MNI 5, 34, −4). PAG, periaqueductal gray; RVM, rostroventral medulla; sgACC, subgenual anterior cingulate cortex; Rad, radius; MNI, Montreal Neurological Institute.

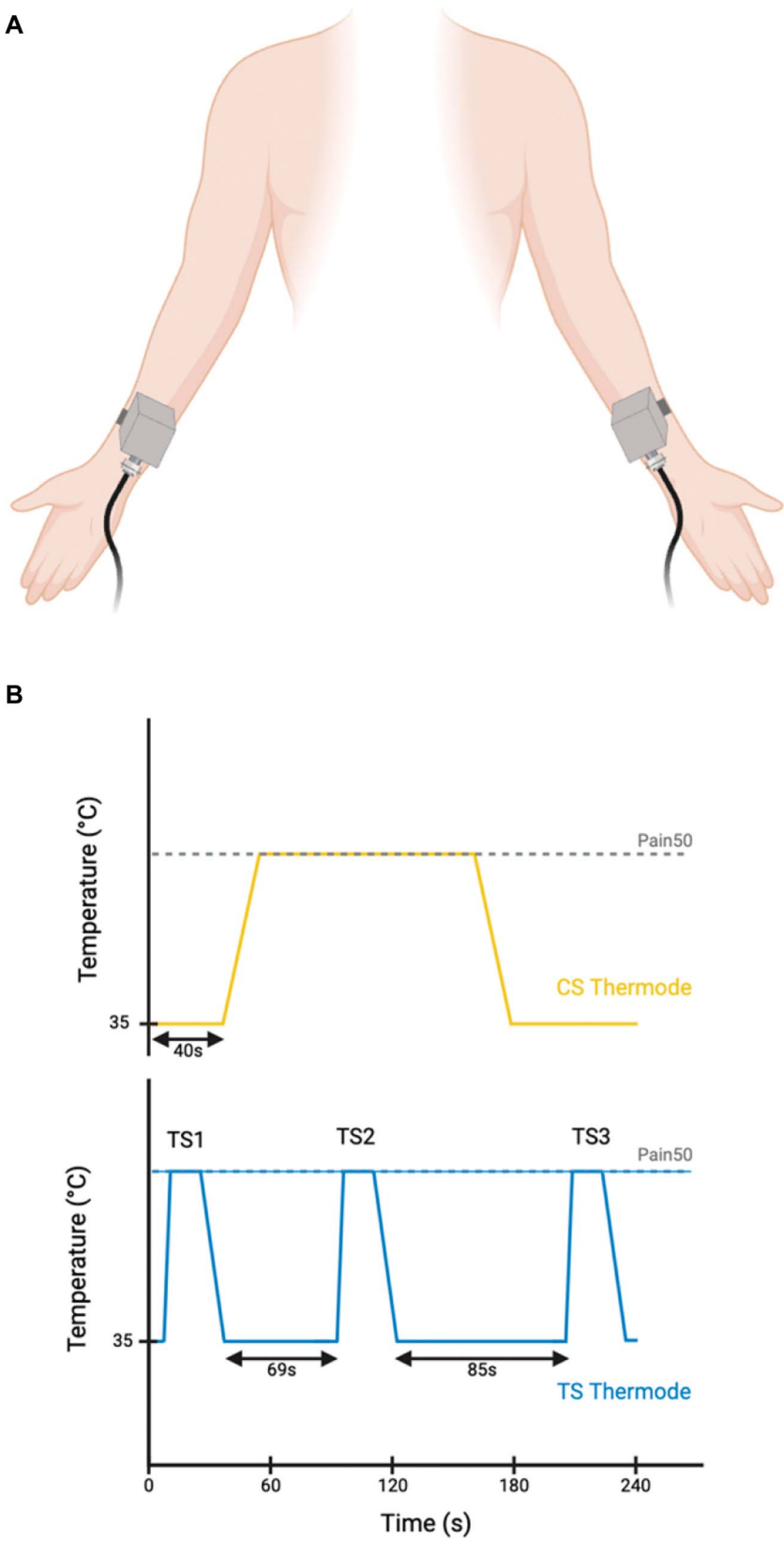

Figure 3. CPM paradigm and setup schematic. (A) Timeline of 3 test stimuli (TS) were delivered (blue line), and participants verbally rated their pain each time on a numerical rating scale from 0 to 100. One sustained conditioned stimulus (CS) was delivered (yellow line), and participants rated their pain at 10 seconds, 30 seconds, and 60 seconds after onset. The right panel shows the physical setup of thermode stimuli and volar forearm placement. (B) Artistic rendering depicting the physical setup of thermode stimuli and volar forearm placement. Figures created using BioRender (https://app.biorender.com/).

the percent difference in TS2 pain intensity ratings from TS1 pain intensity ratings, dividing the value by TS1 as follows:

$$\text{CPM Effect \% Change} = ([\text{TS2 rating} - \text{TS1 rating}]/\text{TS1 rating}) \times 100\%$$

Thus, responses can be either negative or positive representing pain inhibition or pain facilitation, respectively.[36] A score of 0% indicates no CPM effect.

Similar to previous studies of CPM,[45,54,65,75,87,89] participants were divided into 1 of 3 separate subgroups based on the directionality of their CPM effect score: (1). Individuals with negative values, indicating classical inhibitory CPM, were denoted as the CPM/Antinociception subgroup. (2) Those with values of 0, indicating no presence of CPM effect, were denoted as the No-CPM subgroup. (3) Individuals with positive values, indicating faciliatory CPM, were denoted as the Pronociception subgroup. The separation of these subgroups and their CPM effect changes can be seen in Figure 1, supplemental digital content, http://links.lww.com/PAIN/C179.

2.6. Statistical analyses

All statistical analyses were performed using GraphPad Prism (version 7.05), Microsoft Excel 2010, and SPSS (28.0.1.1) and corrected for multiple comparisons. As the first step of characterizing the CPM effect within all participants, raw CPM pain ratings changes from TS1 to TS2 were compared. Shapiro-Wilk normality tests were used to determine whether parametric or nonparametric 2-tailed *t* tests ensued. A Mann-Whitney test was completed for the whole group and either Mann-Whitney or Welch tests within each subgroup separately. To evaluate and compare overall CPM effects within each subgroup, a one-way analysis of variance (ANOVA) was conducted followed by post hoc Tukey tests. Between-subgroup comparisons of FC for each brain ROI pair were conducted using 2-sided 1 sample *t* tests in comparison to 0. Next, to characterize FC between brain ROI dyads across all participants and subsequently within each subgroup, another set of one-way ANOVAs (and associated post hoc Tukey tests) was completed. A final set of one-way ANOVAs and post hoc Tukey tests were used to compare the FC correlation of each of the 5 ROI pairings between the 3 subgroups.

To address our original hypothesis and explore the potential brain-behaviour relationship between DAP FC and CPM effect, Fisher r-to-z transformations were conducted on brain region dyad FCs before being correlated to the CPM effect. In accordance with previously conducted normality tests, Pearson correlations were then used for normally distributed datasets and Spearman correlations for non-normally distributed datasets. *P*-values were corrected for multiple comparisons using Benjamini-Hochberg tests.

Finally, to explore sex differences in CPM, a two-step analysis was performed. First, Mann-Whitney or Welch *t* tests were conducted to compare CPM effect magnitude between the sexes at a whole-group level and then within each separate subgroup. Second, a chi-squared analysis using a 2×3 contingency table (and respective post hoc residual analyses) was completed to assess for probability of subgroup categorization in relation to sex.

3. Results

3.1. Study participants

A total of 151 participants were included in this study (72 M, 79 F) (**Table 1**). The mean ± SD age of all participants was 26.6 ± 5.1 years. There was no statistical age difference by sex (women: 26.2 ± 4.6 years; men: 27.0 ± 5.3 years; $P = 0.753$) or between the 3 subgroups of CPM response (CPM/Antinociception: 26.1 ± 4.4 years; No-CPM: 27.0 ± 5.0 years; Pronociception: 27.0 ± 5.0 years, $P > 0.05$ for all).

3.2. Conditioned pain modulation effect and subgroup characterization

The demographic data and descriptive CPM statistics for all 151 participants (72 M, 79 F) are shown in **Table 1**. For the whole group, the mean ± SD raw CPM effect (ie, TS2-TS1) was −3.1 ± 18.2, and the CPM effect expressed as a percent change in pain intensity ratings from the TS1 ratings was −3.4 ± 34.1%. The skewness of the data was −0.0566 and the kurtosis was 3.69, therefore distribution did not have significant skew, and there was slight positive kurtosis. The CPM raw change scores are shown in Figure 1 (see supplemental digital content, http://links.lww.com/PAIN/C179) for completeness but were not used further in this study. Instead, the CPM effect expressed as a % change was used for all analyses due to its normal distribution which normalizes against the variability in TS1 ratings.

Amongst the group of participants, there was a wide range of CPM effects from −100% to +112.5% (**Fig. 4**). That is, in some participants, the test stimulus pain either increased, while in others it decreased, or did not change at all in the presence of the CS. Given these 3 types of effects of the CS on TS pain, response subgroups were delineated as follows.

(1) The classic inhibitory CPM effect whereby the TS pain was decreased during the CS and so designated as the "CPM/Antinociception subgroup" (45% of participants, 36 M: 32 F)
(2) No change in TS pain during a CS, and so designated as the ("No-CPM subgroup," 15% of participants (15 M: 8 F)
(3) A facilitatory CPM effect whereby the TS pain increased during the CS, and so designated as the "Pronociception subgroup," 40% of participants (21 M: 39 F)

Delineation of these subgroups was informed by previous categorizations of people showing signs of antinociception and pronociception representing more inhibitory or faciliatory CPM, respectively, along a nociception spectrum.[36,54,87] For further description, see the Methods section.

Comparing between subgroups, the TS1 pain rating scores did not differ significantly (CPM/Anti vs No-CPM $P = 0.20$; CPM/Anti vs Pro $P = 0.09$; No-CP Mvs Pro $P = 0.98$, FWE-corrected for multiple comparisons) but the TS2 ratings were significantly higher in the No-CPM (mean ± SD = 50.0 ± 11.1) and Pronociception subgroups (mean ± SD = 63.6 ± 10.7) when compared with the CPM/Antinociception subgroup (mean ± SD = 35.4 ± 14.1) ($P < 0.01$ for both, FWE-corrected for multiple comparisons) (**Table 1**). At a whole-group level, heat stimuli temperature of the TS thermode (mean ± SD) was 45.4 ± 1.9° and 46.0 ± 2.0° for the CS thermode temperature. For subgroup analyses, the mean temperature of the TS thermode was 45.5 ± 2.1°C for the CPM/Antinociception subgroup, 46.9 ± 2.1°C for the No-CPM subgroup, and 46.3 ± 1.9°C for the Pronociception subgroup. Last, when compared with men (35%), there was a higher ratio of women (65%) within the Pronociception subgroup.

With respect to changes in TS pain intensity ratings, there was a significant decrease in TS2 ratings (49.0 ± 17.9), compared with TS1 ratings (52.1 ± 10.6), suggesting an overall inhibitory CPM effect ($P < 0.05$) at a whole-group level (see Fig. 1A, supplemental digital content, http://links.lww.com/PAIN/C179). In support of predetermined subdivision into subgroups based on

Table 1

Demographic information and descriptive statistics for CPM of all participants by subgroups.

Variable	Whole group	CPM/Antinociception subgroup	No-CPM subgroup	Pronociception subgroup	*P* A = CPM/Anti vs No-CPM B = CPM/Anti vs pro C = No-CPM vs pro
N (M [%], F [%])	151 (72 [48%], 79 [52%])	68 (36 [53%], 32 [47%])	23 (15 [65%], 8 [35%])	60 (21 [35%], 39 [65%])	
Age (y)	26.6 ± 5.1	26.1 ± 4.4	26.6 ± 7.0	27.0 ± 5.0	A n.s B n.s C n.s
CPM effect raw change	−3.1 ± 18.0	−19.0 ± 13.6	0 ± 0	13.1 ± 8.3	A* B* C*
CPM effect % change	−3.4 ± 34.1	−34.6 ± 22.2	0 ± 0	28.1 ± 20.8	A* B* C*
TS1 pain intensity (NRS 0-100)	52.1 ± 10.6	53.4 ± 11.0	50.0 ± 11.1	50.5 ± 9.5	A n.s B n.s C n.s
TS2 pain intensity (NRS 0-100)	49.0 ± 17.9	35.4 ± 14.1	50.0 ± 11.1	63.6 ± 10.7	A* B* C*
TS temperature (°C)	45.4 ± 1.9°C	45.5 ± 2.1°C	46.9 ± 2.1°C	46.3 ± 1.9°C	A† B‡ C n.s
CS temperature (°C)	46.0 ± 2.0°C	45.0 ± 3.9°C	45.9 ± 1.9°C	45.8 ± 1.8°C	A n.s B n.s C n.s

Values include mean ± SD. *P* values are corrected for multiple comparisons.
* $P < 0.0001$.
† $P < 0.01$.
‡ $P < 0.05$.
CPM, conditioned pain modulation; TS, test stimulus; CS, conditioned stimulus; NRS, numeric rating scale (verbal); n.s, not significant.

the CPM effect, TS2 pain ratings (35.4 ± 14.1) were significantly lower than TS1 ratings (53.4 ± 11.0) in the CPM/Antinociception ($P < 0.0001$) (Supp Fig. 1B). By contrast, the Pronociception subgroup had significantly higher TS2 pain ratings (63.6 ± 10.7) compared with TS1 ratings (50.5 ± 9.5) ($P < 0.0001$) (see Fig. 1C, supplemental digital content, http://links.lww.com/PAIN/C179).

3.3. Functional connectivity analysis

The individual and group-level FC characteristics of the ROI dyads examined are shown in **Figure 5**. The detailed FC analysis by CPM grouping is provided in supplemental digital content (see Table, http://links.lww.com/PAIN/C179), and several prominent findings are described here: The between-subgroup analysis of

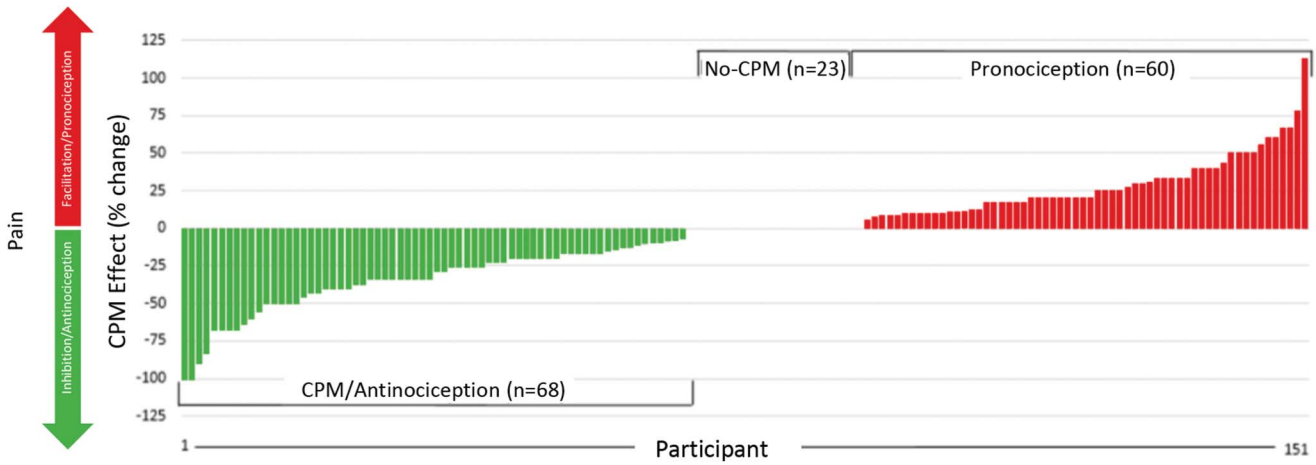

Figure 4. CPM effect (%) change distribution for all participants. The CPM effect is calculated using the following formula: ([TS2 − TS1]/TS1)×100. Negative values represent antinociception and pain inhibition, and positive values present pronociception and pain facilitation. Distribution of individual CPM effects for all 151 participants. Subgroups were identified and labeled on the graph. ****$P < 0.001$. CPM, conditioned pain modulation.

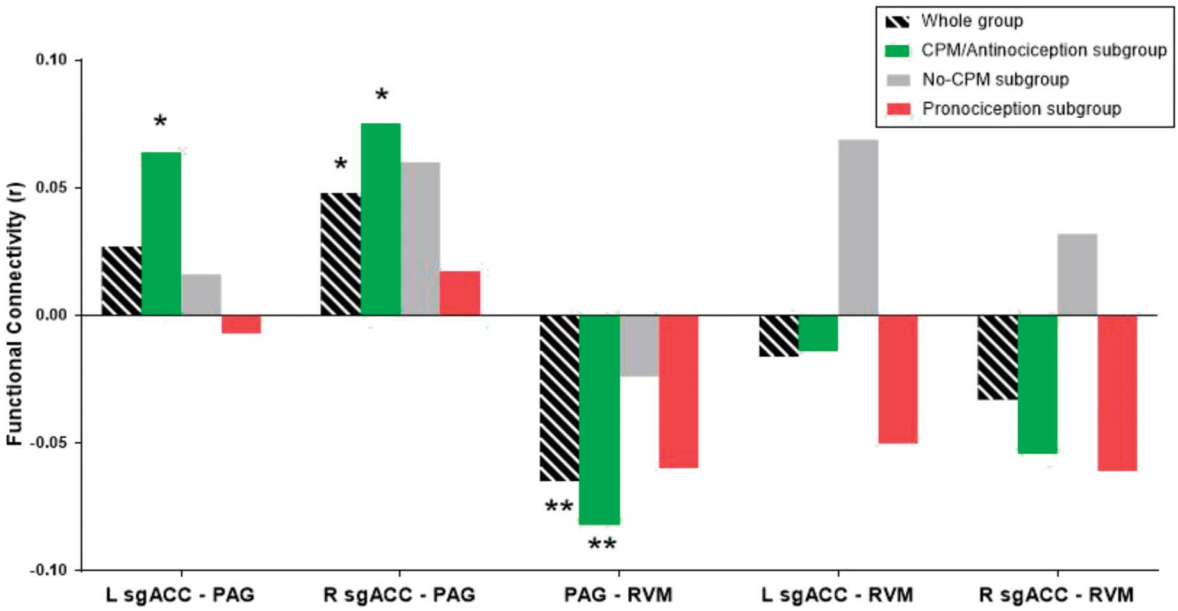

Figure 5. Between-group characterization of FC for each descending antinociceptive pathway brain region pair. Mean group functional connectivity (FC) ± SE expressed in Pearson correlation (r) for the whole group and each of the 3 subgroups. From left to right: left sgACC with PAG FCs, FC was positively significant in the CPM/Antinociceptive subgroup ($P < 0.05$); right sgACC with PAG FCs, FC was positively significant in the whole group ($P < 0.05$) and CPM/Antinociceptive subgroup ($P < 0.05$); PAG with RVM FCs, FC was negatively significant in the whole group ($P < 0.01$) and CPM/Antinociceptive subgroup ($P < 0.01$); left sgACC with RVM FCs; right sgACC with RVM FCs. ROI, region of interest; L sgACC, left subgenual anterior cingulate cortex; R sgACC, right subgenual anterior cingulate cortex; PAG, periaqueductal gray; RVM, rostroventral medulla. *$P < 0.05$; **$P < 0.01$.

FC for each ROI pair revealed that both left and right sgACC with PAG FC was positively significant ($P < 0.05$) in the CPM/Antinociception subgroup. The right sgACC-PAG pair was also significant at a whole-group level ($P < 0.05$). In addition, the PAG-RVM dyad showed significance at both a whole-group level ($P < 0.01$) and within the CPM/Antinociception subgroup ($P < 0.01$).

When considering all participants, there was a positive FC correlation between the left sgACC-PAG dyad and the right sgACC-PAG dyad (**Fig. 6**). The PAG-RVM, left sgACC-RVM, and right sgACC-RVM dyads showed negative FC correlation (**Fig. 6**). Furthermore, a significant difference was found between the left sgACC-PAG dyad ($P < 0.05$, FWE-corrected for multiple comparisons), PAG-RVM dyad ($P < 0.05$, FWE-corrected for multiple comparisons), and right sgACC-PAG dyad ($P < 0.01$, FWE-corrected for multiple comparisons) (**Fig. 6**). Within the CPM/Antinociception subgroup, a similar trend was seen, with a significant difference in the same 3 dyads: left sgACC-PAG dyad ($P < 0.01$, FWE-corrected for multiple comparisons), PAG-RVM dyad ($P < 0.05$, FWE-corrected for multiple comparisons), and right sgACC-PAG dyad ($P < 0.01$, corr) (**Fig. 6**). No significant differences were found between any of the dyads of interest within the No-CPM (**Fig. 6**) or Pronociception subgroup (**Fig. 6**).

Next, between-subgroup comparisons of each of the 5 brain ROI dyad FCs revealed that the Pronociception subgroup displayed significantly more negative FC, or stronger anticorrelation, in the R sgACC-PAG dyad when compared with the CPM/Antinociception subgroup ($P < 0.01$, FWE-corrected for multiple comparisons) (**Fig. 7**). No other significant differences were found among the subgroups.

3.4. Relationship between conditioned pain modulation and functional connectivity of the descending antinociceptive pathway

Analyses were done to test the hypothesis that CPM is positively associated with FC within regions of the DAP. The first analysis was done to examine this for the whole-group level and showed that there were no significant correlations (L sgACC-PAG $P = 0.157$ rho = 0.118; R sgACC-PAG $P = 0.532$ rho = 0.052; PAG-RVM $P = 0.967$ rho = 0.003; L sgACC-RVM $P = 0.210$ rho = 104; R sgACC-RVM $P = 0.255$ rho = 0.095) between the magnitude of a subject's CPM and their FC between any of the brain dyads of interest within the DAP (see Fig. 2, supplemental digital content, http://links.lww.com/PAIN/C179). To note, a slight negative trend can be seen within the PAG-RVM dyad to CPM effect comparison, opposing the general directionality of other correlations.

Given that no significant correlations were found at the whole-group level after correcting for multiple comparisons, subsequent analyses were conducted at the subgroup level for each of the 3 separate subgroups. Within the CPM/Antinociception subgroup, a medium effect size was seen when comparing the CPM effect with FC between the left sgACC and RVM ($P = 0.032$ rho = 0.268) as well as the right sgACC and RVM ($P = 0.029$ rho =0.029) (**Fig. 8**). A small but visible effect size was also present in the L sgACC-PAG ($P = 0.221$ rho = 0.155) dyad (see Fig. 3, supplemental digital content, http://links.lww.com/PAIN/C179).

Within the No-CPM subgroup, there was no significant correlation of CPM with FC of any of the brain region dyads as CPM effect scores were all zero.

In the Pronociception subgroup, no significant correlations were found between ROI dyad FCs and the CPM effect (see Fig. 4, supplemental digital content, http://links.lww.com/PAIN/C179). Although not significant, there were trends similar to those seen in the CPM/Antinociception subgroup as well as the whole group for the L sgACC-PAG, L sgACC-RVM, and R sgACC-RVM dyads to CPM effect comparisons.

3.5. The effect of sex on the conditioned pain modulation effect

To examine the interaction between sex and CPM, we compared the CPM effects for men and women at the whole-group level,

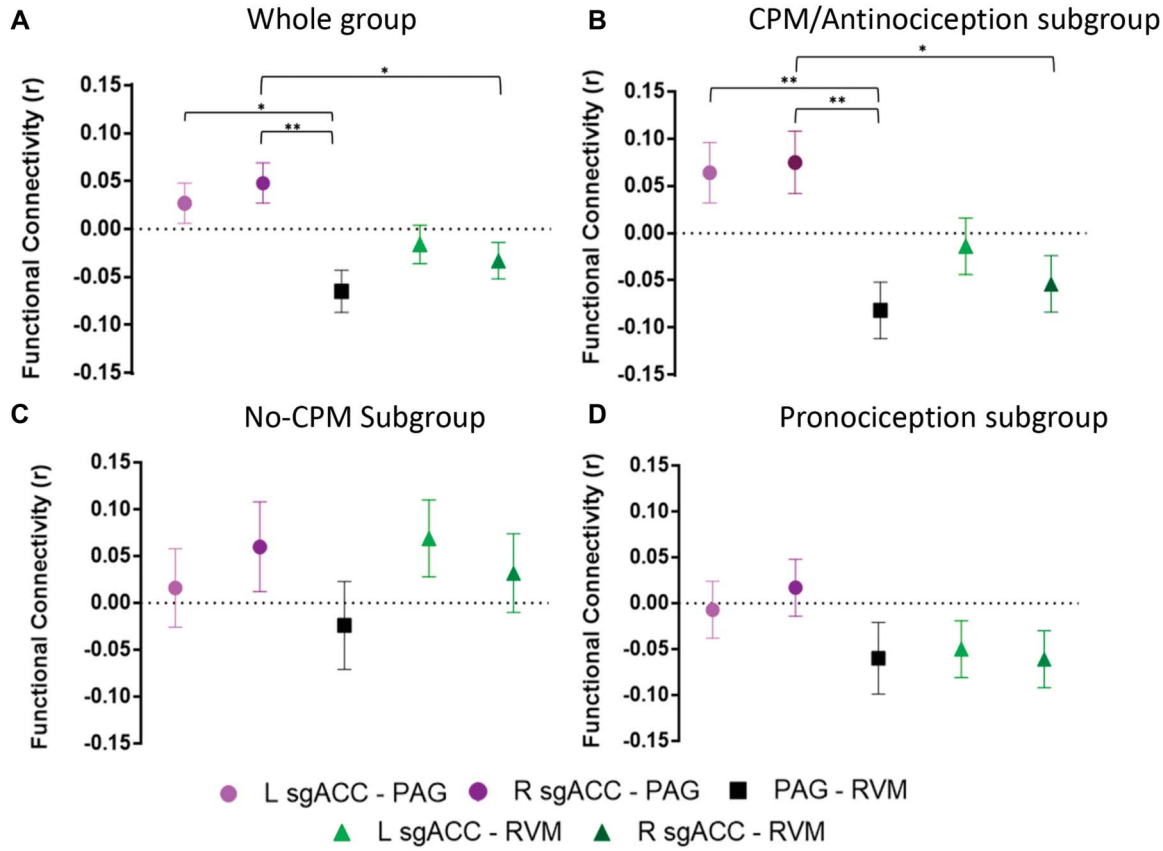

Figure 6. Within group characterization of FC for each descending antinociceptive pathway brain region pair by CPM subgrouping. Mean group functional connectivity (FC) ± SE expressed in Pearson correlation (r) for each of the 5 ROI dyads of interest. (A) Whole-group plot. (B) CPM/Antinociception subgroup plot. (C) No-CPM subgroup plot. (D) Pronociception subgroup plot. L sgACC, left subgenual anterior cingulate cortex; R sgACC, right subgenual anterior cingulate cortex; PAG, periaqueductal gray; RVM, rostroventral medulla. *$P < 0.05$; **$P < 0.01$. *P*-values corrected for multiple comparisons.

and within the subgroups, within the No-CPM subgroup, and within the Pronociception subgroup. There was no significant effect of sex on the magnitude of the CPM effect within each of the subgroups (whole group, $P = 0.085$; CPM/Antinociception subgroup $P = 0.315$; Pronociception subgroup $P = 0.928$) (**Fig. 9**).

However, considering subgroups, there was a disproportionate CPM effect within sexes (**Fig. 9**). Specifically, among men, 50% (36 individuals) were categorized in the CPM/Antinociception subgroup, 21% (15 individuals) were categorized in the No-CPM subgroup, and 29% (21 individuals) were categorized in the Pronociception subgroup. Among women, 41% (32 individuals) were categorized in the CPM/Antinociception subgroup, 10% (8 individuals) in the No-CPM subgroup, and 49% (39 individuals) in the Pronociception subgroup. After dividing men and women for 2×3 contingency table analyses, the adjusted standardized residuals revealed that being of the female sex, as opposed to male sex, inferred a disproportionately higher likelihood of experiencing pain facilitation and being categorized as pronociceptive ($P = 0.011$, Bonferroni corr) (see Table 2, supplemental digital content, http://links.lww.com/PAIN/C179).

4. Discussion

This study sought to investigate whether the FC of the DAP could underlie the individual variability in the CPM. Thus, we examined brain-behavioural correlates of CPM with FC between key regions of the DAP (PAG, RVM, sgACC), and whether there are sex differences in CPM capability and these brain-behavioural relationships. Our key findings were as follows: (1) More than half of the participants exhibited a facilitatory or no CPM effect; (2) FC within the DAP was only present in the CPM/Antinociception subgroup; (3) the CPM effect correlated with FC of sgACC with the RVM only in CPM/Antinociception subgroup; (4) A faciliatory CPM effect (pronociception) has a higher likelihood in women than in men. These novel findings provide insight into potential contributors to the CPM and also point to the connectivity of the DAP as a potentially important consideration in developing therapeutic neuromodulation approaches to manage pain in individuals.

4.1. Intersubject variability in the conditioned pain modulation effect

This study adds to the growing literature that individuals can exhibit CPM effects ranging from classic inhibition (antinociception) to no CPM, to facilitation (pronociception).[36,54,87] It is not known why individuals differ in CPM[19,29,48,75,89] but here we demonstrated that FC of the DAP as 1 factor. Other individual characteristics such as resilience may relate to an individual's propensity to modulate pain.[2] Furthermore, past memory recollection and subsequent pain catastrophizing,[33] contextual factors (stimulus unpleasantness and suffering),[10,46] and the test environment (eg, stress environments, stimulus modality) can impact CPM.[8] Low inhibitory CPM in healthy individuals may be a risk factor for developing future pain.[7]

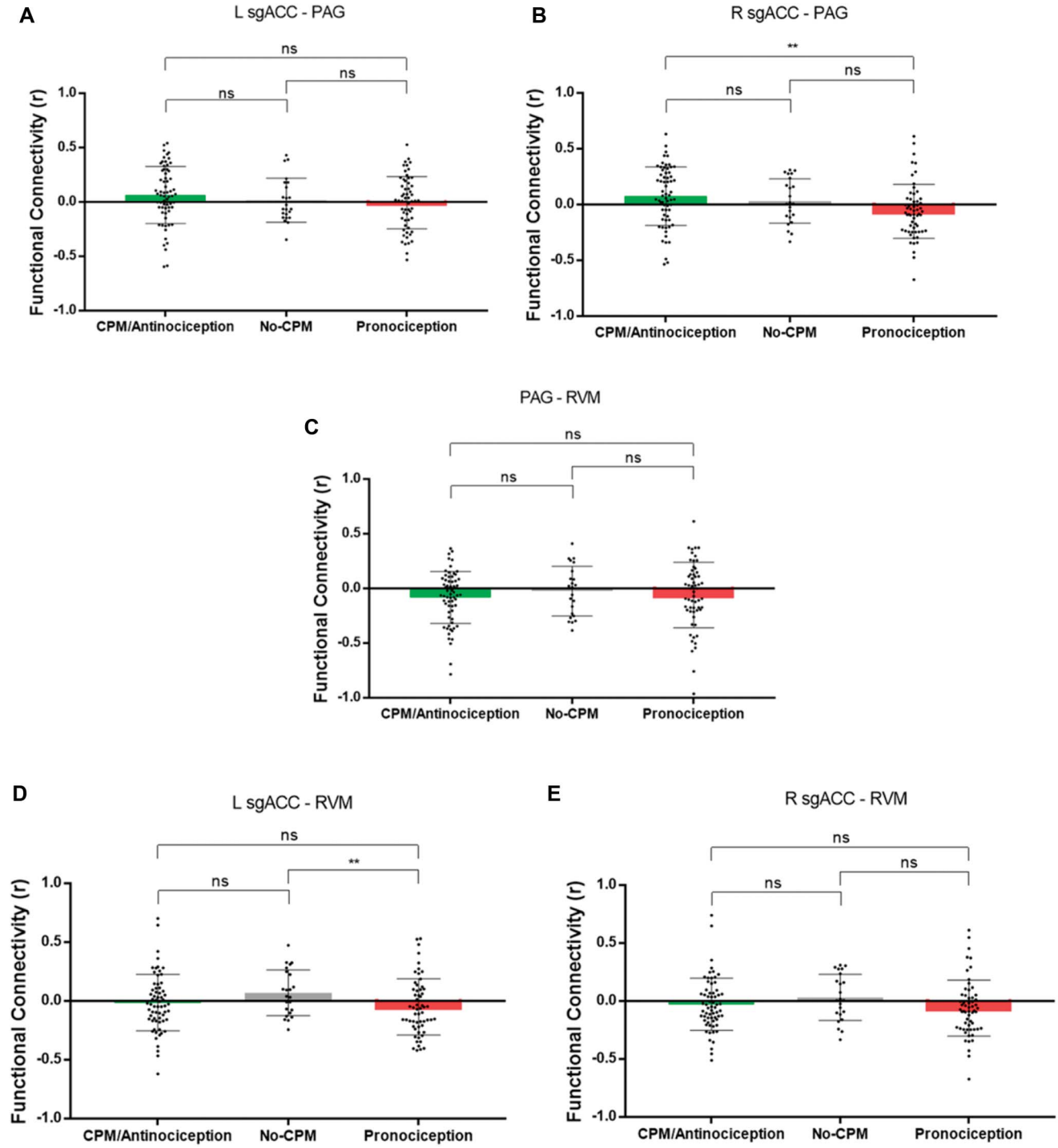

Figure 7. Brain region of interest dyad FC comparisons by subgroup. FC for each of the 5 dyads of interest expressed in Pearson correlation (r). (A) No significant between-subgroup differences in FC between the L sgACC and PAG. (B) Pronociception group presented significantly more negative FC of the R sgACC-PAG when compared with the CPM/Antinociception group. (C) No significant between-subgroup differences in FC between the PAG and RVM. (D) No significant between-subgroup differences in FC between the L sgACC and RVM. (E) No significant between-subgroup differences in FC between the R sgACC and RVM. $^{**}P < 0.01$. Significance ns indicates not significant. *P*-values corrected for multiple comparisons.

4.2. Sex differences in the conditioned pain modulation effect

This study found a greater proportion of women than men were categorized as pronociceptive (**Fig. 9**). Although some studies in healthy subjects show a higher proportion of women exhibiting inhibitory CPM when compared with men,[31,33,35,84] other studies did not find any sex differences in the CPM.[55,69,71,78,79] A recent review highlighted that despite there being a known sexual dichotomy, there has not been consistency in findings on sex differences in CPM.[73] Of note is that imaging studies from our laboratory have identified differences in the connectivity of the sgACC and PAG, potentially supporting this dichotomy.[15,23]

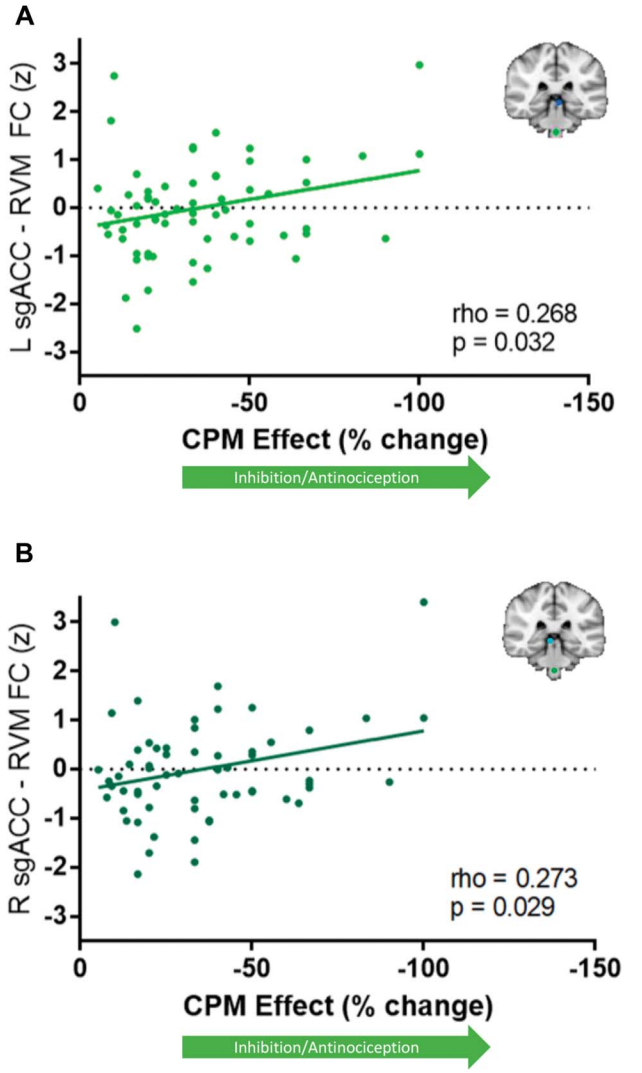

Figure 8. Correlation of CPM effect and FC between left and right sgACC with RVM in the CPM/Antinociception group. (A) Correlation plot of left sgACC-RVM FC vs CPM (rho = 0.268, $P = 0.032$). (B) Correlation plot of right sgACC-RVM FC vs CPM (rho = 0.273, $P = 0.029$). Dots represent individual ratings. Reported *P*-values are uncorrected for multiple comparisons. sgACC, subgenual anterior cingulate cortex; PAG, periaqueductal gray; RVM, rostroventral medulla.

4.3. Functional connectivity considerations: what is the data not telling us?

Functional connectivity is a measure of the degree of temporal synchrony of ongoing activity between brain regions. However, FC cannot distinguish anatomical connections, the directionality of influence,[38] or whether the lack of FC reflects a true absence of synchrony (ie, values cluster around zero) or a nullification caused by comparable inhibitory and excitatory inputs.

Correlated activity in 2 regions may also be mediated by a third structure that relays information between the 2, or be completely driven by an external region that induces concurrent activity in both areas even though those original regions may have no direct interaction.[20] This may explain the lack of FC found between sgACC and RVM in this study.

4.4. Functional connectivity within the descending antinociceptive pathway

The sgACC is involved in both nociceptive and antinociceptive mechanisms.[7,88] Furthermore, there are sex differences in FC between the sgACC and other relevant regions within the DPC.[50,72] The PAG to RVM projection is classically considered the core of the DAP.[63,67,74] This study only found anticorrelational relationships between the PAG-RVM and did not find FC between the sgACC and RVM. The mix of neuronal types within the RVM could have contributed to not finding negative correlations or the lack of a positive correlation. For example, in the RVM, there are neurons that exhibit different noxious-evoked activity and are thought to serve different roles in pain modulation, namely, ON and OFF cells which facilitate and modulate nociception, respectively.[3,27] These cells function complementarily[12] and work to modulate dorsal horn nociceptive transmission in parallel. However, ON cells do not directly inhibit OFF-cell activity, and their inactivity is not linearly related with OFF-cell function.[26,42]

The centrally located PAG has versatile and cross-functional roles in addition to being involved in nociception, such as regulation of autonomic function, and fear and reward processing.[80] A meta-analysis by Linnman et al. included 40 pain imaging studies of the PAG to examine its role in pain.[61] They reported a spread of activation likelihood within the PAG into a neighbouring cluster area representing activation in emotion-related studies involving the PAG, suggesting the difficulty in separating emotion from pain-related findings.[61]

Between-subgroup analyses revealed a significant difference in FC correlation between the CPM/Antinociception and Pronociception subgroups for the right sgACC-PAG dyad. This finding suggests a relationship between FC correlation and efficacy of descending pain modulation reflected in the CPM effect. However, this relationship was only apparent in categorization (ie, being in the CPM/Antinociception subgroup) but not the magnitude of the CPM effect. The former part of this finding supports many studies of CPM that show that individuals who are efficient modulators (experience antinociception) have greater FC between their PAG and various other pain processing regions.[39,40]

Within the CPM/Antinociception subgroup, the sgACC-PAG FC is in line with tracer studies demonstrating anatomical projections between the 2 regions in both primate[30] and human models.[70] FC between this pair may be relevant in understanding the prediction of treatment response for various conditions such as treatment-resistant depression.[32] The intrinsic functional synchrony found between the 2 during the resting state when no pain stimulus is applied provides further evidence in support of their involvement in pain modulation.[51] One study found a disruption of FC between the sgACC and PAG during tonic pain conditions.[66] Others have found functional synchrony between the PAG and sgACC to exhibit a positive correlation with the efficacy of DAP function.[1,13,59,77]

4.5. Is functional connectivity of the descending antinociceptive pathway related to the conditioned pain modulation effect?

We found a medium effect size in the relationship between CPM and sgACC-RVM FC. A potential contributor to this synchrony is through the PAG, given that anatomical PAG to RVM projections are known to mediate descending antinociceptive functions.[4] It is important to note that medium effect size was only found in the CPM/Antinociception subgroup. At a whole-group level, there was a similar trend and effect size, but statistical significance cut-offs were not met, suggesting that prior whole-group findings are likely driven by the CPM/Antinociception subgroup.

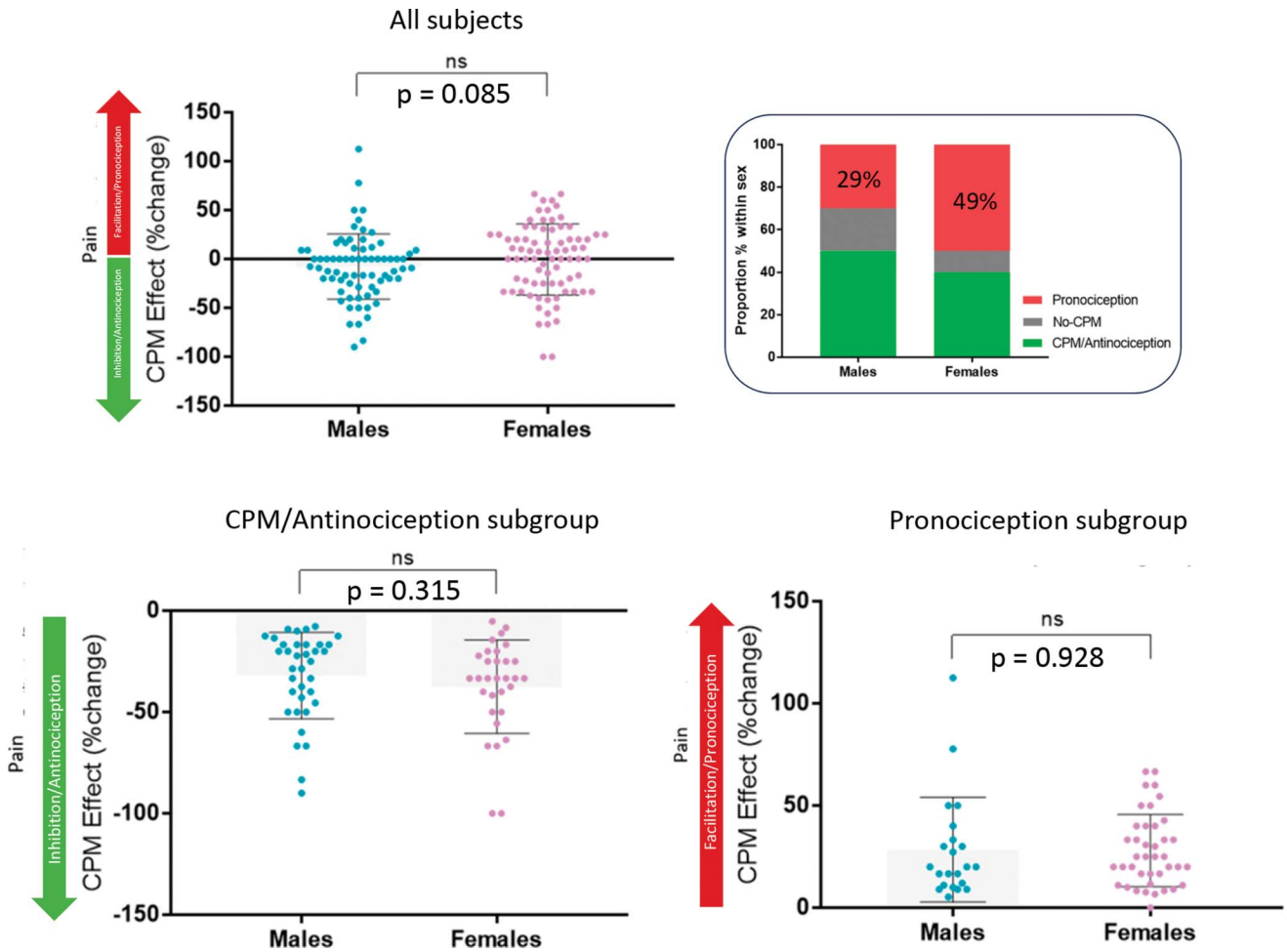

Figure 9. Sex differences in CPM effect and magnitude categorization. Top panel: left graph shows that in the whole group, there were no significant sex differences for CPM effect magnitude ($P = 0.085$); right bar graph shows the proportion of each subgroup type of CPM effect for men (left) and women (right). Bottom panel: left graph shows that within the CPM/Antinociception group, there were no significant sex differences for CPM effect magnitude ($P = 0.315$); right panel shows that within the Pronociception subgroup, there were no significant sex differences for CPM effect magnitude ($P = 0.928$). Dots represent individual ratings and Gy bars represent group means.

Our data demonstrate that the presence of significant FC between DAP regions is not a prerequisite for the presence of a brain-behaviour relationship between CPM efficacy and inter-DAP FC (**Fig. 10**). This is demonstrated in the CPM/Antinociception subgroup, as a positive correlation was found between sgACC-RVM FC when plotting against the CPM effect, but the same regions did not show significant FC between each other.

This result provides further support that the sgACC is involved in pain inhibition, in line with certain previous studies.[5,21,25]

4.6. Group-level analyses obscure subgroup-level correlations between conditioned pain modulation and descending antinociceptive pathway functional connectivity

Many studies of CPM consider the CPM effect as "pain inhibits pain," meaning that if an individual "displays a CPM response" they have reduced pain evoked by a TS in the presence of a CS giving an inhibitory effect.[44,60,62] However, studies that show the CPM effect as a range from pain inhibition to facilitation[16,29,68,89] are beginning to change this mindset within the field. Correlational analyses done on individual subgroups of the CPM effect may present results that risk being obscured when looking at a whole-group level (**Fig. 10**). Our results support the implementation of disaggregated analyses between CPM effect subgroups, as opposed to group analyses, as it may be crucial in characterizing underlying brain mechanisms of pain modulation.

4.7. Limitations

Despite our efforts to determine Pain50 before CPM testing, TS1 pain varied from 25/100 to 85/100. This variability in baseline TS pain may have impacted CPM effect scores. Furthermore, despite clear instructions to participants in terms of attending to and rating the pain evoked by the thermode delivering the TS, it is possible that a participant's attention could have shifted to the thermode delivering the CS or to something else in or outside the test room (eg, outside noise). However, we note that our previous study demonstrated that the CPM effect in our CPM protocol was not a result of habituation.[29] We also note the CPM effect reported in this study is based on a specific paradigm that conceivably could be different in some subjects than the CPM effect using a different paradigm. Finally, it was not possible to

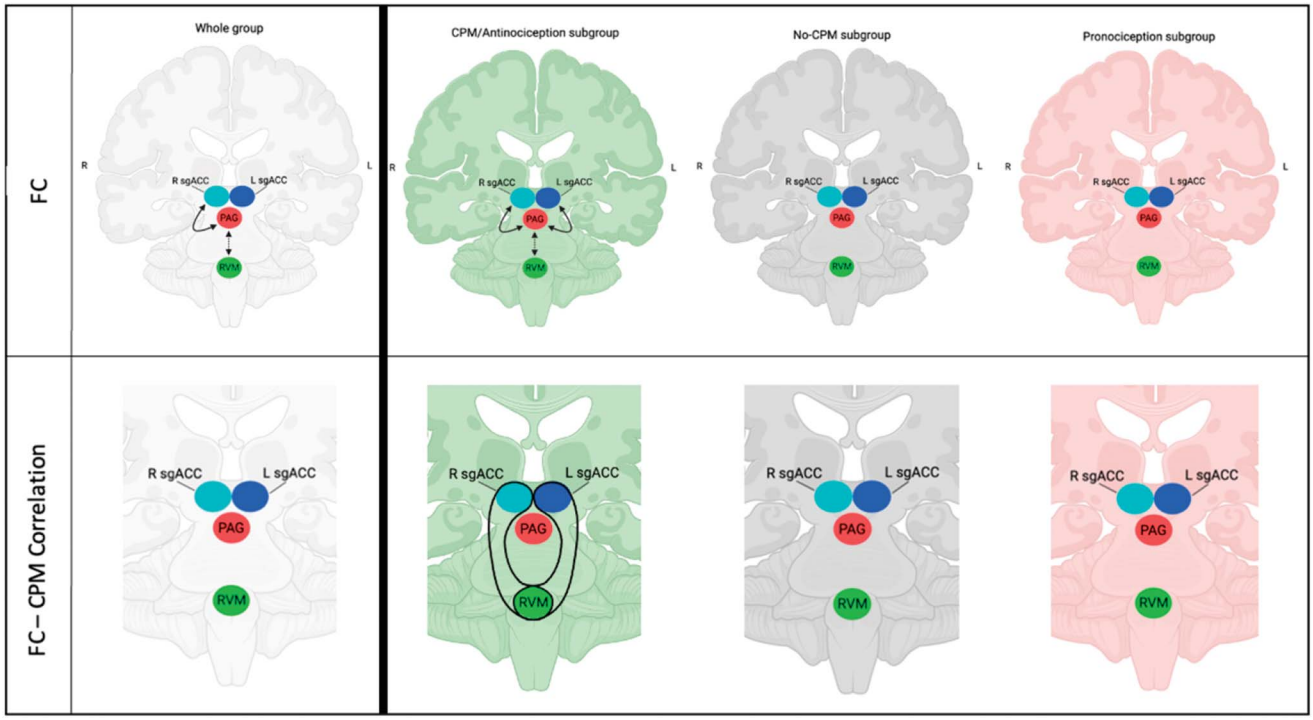

Figure 10. Summary of main findings of correlations between the dyads of regions of interest within the descending antinociceptive pathway and with CPM effect by subgroup. The top coronal brain slice (light gray) represents the whole group, which showed a significant positive correlation between right sgACC-PAG ($P < 0.05$) and a significant negative correlation between left sgACC-RVM ($P < 0.01$). The second top coronal brain slice (green) represents the CPM/Antinociception subgroup, which showed a significant positive correlation between left sgACC-PAG ($P < 0.05$), right sgACC-PAG ($P < 0.05$) and a significant negative correlation between left sgACC-RVM ($P < 0.01$). The third top coronal brain slice (dark gray) represents the No-CPM subgroup. The right top coronal brain slice (red) represents the Pronociception subgroup. The bottom left coronal brain slice (light gray) represents the whole group. The second bottom of brain slices (green) represents the CPM/Antinociception subgroup, which showed a medium effect size of correlation between left and right sgACC-RVM to CPM effect. The third bottom brain slice (dark gray) represents the No-CPM subgroup. The right bottom brain slice (red) represents the Pronociception subgroup. sgACC, subgenual anterior cingulate cortex; PAG, periaqueductal gray; RVM, rostroventral medulla. Arrows indicate a significant effect size relationship ($P < 0.05$). Solid arrows indicate a positive correlation. Dotted arrows indicate a negative correlation. Figure created with BioRender (https://app.biorender).

evaluate the potential effects of the menstrual cycle stage or oral contraceptives.[82]

5. Conclusions

Our findings show that an individual's propensity to modulate pain may reflect characteristics of their DAP efficiency. Individuals who do not exhibit antinociception may have diminished functioning of their DAP and therefore may benefit from neuromodulation therapeutics aiming to boost DAP FC. A better understanding of the complex variable interplay on pain modulation allows for a more complete characterization of an individual's nociceptive profile and has implications for pain treatment and management.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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