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Sustained perturbation in functional connectivity induced by cold pain

Elena Makovac[1,2] | Ottavia Dipasquale[1] | Jade B. Jackson[1,2] | Sonia Medina[1,2] | Owen O'Daly[1] | Jonathan O'Muircheartaigh[1,3,4,5] | Alfonso de Lara Rubio[1] | Steven C. R. Williams[1] | Stephen B. McMahon[2] | Matthew A. Howard[1]

[1]Department of Neuroimaging, King's College London, London, UK

[2]Wolfson Centre for Age Related Diseases, King's College London, London, UK

[3]Sackler Institute for Translational Neurodevelopment, King's College London, London, UK

[4]Centre for the Developing Brain, King's College London, London, UK

[5]MRC Centre for Neurodevelopmental Disorders, King's College London, London, UK

Correspondence
Elena Makovac, Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, Box 89, De Crespigny Park, London SE5 8AF, UK.
Email: elena.makovac@kcl.ac.uk

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Abstract

Background: Functional connectivity (FC) perturbations have been reported in multiple chronic pain phenotypes, but the nature of reported changes varies between cohorts and may relate to the consequences of living with chronic-pain related co-morbidities, such as anxiety and depression. Healthy volunteer studies provide opportunities to study the effects of tonic noxious stimulation independently of these sequelae. Connectivity changes in task negative and positive networks, for example, the default mode and salience networks (DMN/SN), respectively, have been described, but how these and other connectivity networks, for example, those governing descending pain control are affected by the presence of tonic, noxious stimulation in healthy, pain-free individuals, remains unknown.

Method: In 20 healthy volunteers, we assessed FC prior to, during, and following tonic cold painful stimulation in the ventromedial prefrontal cortex (vmPFC), rostral anterior insula (rAI), subgenual anterior cingulate cortex (ACC) and periaqueductal grey (PAG). We also recorded subjectively reported pain using a computerised visual analogue scale.

Results: We saw DMN FC changes during painful stimulation and that inter-network connectivity between the rAI with the vmPFC increased during pain, whereas PAG-precuneus FC decreased. Pain-induced FC alterations persisted following noxious stimulation. FC changes related to the magnitude of individuals' subjectively reported pain.

Conclusions: We demonstrate FC changes during and following tonic cold-pain in healthy participants. Similarities between our findings and reports of patients with chronic pain suggest that some FC changes observed in these patients may relate to the presence of an ongoing afferent nociceptive drive.

Significance: How pain-related resting state networks are affected by tonic cold-pain remains unknown. We investigated functional connectivity alterations during and following tonic cold pain in healthy volunteers. Cold pain perturbed the functional connectivity of the ventro-medial prefrontal cortex, anterior insula, and

the periacquaductal grey area. These connectivity changes were associated with the magnitude of individuals' reported pain. We suggest that some connectivity changes described in chronic pain patients may be due to an ongoing afferent peripheral drive.

1 | INTRODUCTION

Resting state functional magnetic resonance imaging (rs-fMRI) is a useful tool for investigating brain networks underlying pain processing in healthy participants and perturbations in these networks in chronic pain patients. Pain is an emergent property; a multi-system response to perceived threats to the body, comprising peripheral and central, autonomic, endocrine and immune components (Thacker & Moseley, 2012). Changes in functional connectivity (FC) from "task negative" to "task positive" networks (e.g. the "default mode" and "salience" networks – DMN/SN respectively) have been reported in various chronic painful conditions (Baliki, Mansour, Baria, & Apkarian, 2014; Schwedt et al., 2013; Seminowicz & Davis, 2007a). Functional alterations in the periaqueductal grey (PAG), a crucial hub in descending pain control, have been also reported in chronic pain (Brooks & Tracey, 2005; Hemington & Coulombe, 2015), but reports of network perturbations vary between patient cohorts and are often inconsistent, for example, increased PAG-ventromedial prefrontal cortex (vmPFC)/rostral ACC connectivity in patients with low back pain (Yu et al., 2014a), but reductions in the same regions in migraineurs (Jiang et al., 2016). Connectivity differences may reflect alternate clinical phenotypes, compensatory mechanisms, comorbidities such as depression or anxiety (Bair, Wu, Damush, Sutherland, & Kroenke, 2008), attentional fluctuations or the tendency to engage in mind wandering (Kucyi & Davis, 2015; Kucyi, Salomons, & Davis, 2013). Healthy volunteer models provide an opportunity to study the effects of evoked stimulation independently of these sequelae.

Reports of FC changes in response to acute, peripherally mediated noxious stimulation in healthy volunteers (Alshelh et al., 2018; Zhang et al., 2014) have informed the central representation and elaboration of nociceptive mechanisms, but those examining responses to tonic painful stimulation remain scarce. Experimentally induced tonic muscle pain (Alshelh et al., 2018) decreased oscillatory power in the main DMN hubs (posterior cingulate cortex- PCC, inferior parietal cortex, and vmPFC) and a pilot study of pain-inducing intramuscular hypertonic saline injection described insula-DMN connectivity changes (Zhang et al., 2014). How experimentally induced noxious tonic stimulation in healthy volunteers effects other pain-related functional networks, for example, descending pain control, or how these networks interact with one another, remains unknown.

Cold pain is an apposite choice. Cold allodynia is a frequent symptom in neuropathic pain (Bowsher & Haggett, 2005) and abnormal cold perception is a major symptom in cold complex regional pain syndrome (Eberle et al., 2009), and other neuropathic conditions including diabetic neuropathy, peripheral nerve injury and chemotherapy-induced neuropathy (Yin, Zimmermann, Vetter, & Lewis, 2015). Healthy volunteer evoked-response fMRI studies have reported activations in ACC, thalamus, insula, PAG and PFC during repeated, short-duration cold-water hand immersion (La Cesa et al., 2014; Wilcox et al., 2015). Increases in regional cerebral blood flow during tonic cold pain in healthy participants have been shown in the anterior cingulate, sensorimotor, premotor, and prefrontal cortices, anterior insula and thalamus (Casey, Minoshima, & Morrow, 1996). How tonic cold pain modulates FC networks in healthy participants remains unexplored. Here, we investigated FC in pain-related networks in response to and following administration of tonic noxious cold stimulation. We hypothesized that cold pain would alter connectivity in vmPFC, rAI, PAG and sgACC functional networks and that the magnitude of FC alterations would relate to individuals' subjectively reported pain.

2 | METHODS AND MATERIALS

2.1 | Participants

Twenty healthy participants took part in the experiment (9 women, 11 men, mean age across the group = 26.05; *SD* = 5.32 years). Pain ratings were obtained from 18 participants only (two participants did not attend the final session). All participants were right-handed as assessed by the Edinburgh handedness inventory (Oldfield, 1971). In addition to MRI contraindications, further exclusion criteria were history of brain injuries, hypertension, neurological or psychiatric disease, and alcohol or drug abuse. To minimize the potential effects of menstrual cycle-related hormone fluctuations on pain responses (Vincent & Tracey, 2010), female participants were all tested within the follicular phase of their menstrual cycle. Furthermore, to minimize the influence of diurnal variation on pain responses (Hodkinson et al., 2014) and on rs-fMRI network activity (Jiang et al., 2016), participants were always tested at approximately the same time for each visit. At the beginning of each visit, participants were tested for drugs of abuse (urine drug test) and alcohol consumption (alcohol breathalyser). All participants provided written informed

consent. The study was approved by the King's College London Research Ethics Committee.

2.2 | Experimental procedure

Participants took part in an initial familiarisation session, followed by two identical scanning sessions and one post-scanning session. During the familiarisation session, participants became accustomed with the neuroimaging environment (in a mock scanner) and with the tonic cold-pain stimulation. The cold pain stimulation was delivered via an aluminium probe (4 × 20 cm), attached to the right inner forearm, through which cold water (2°C) was constantly circulated by means of two chillers. The constant circulation of the water ensured the stability of the temperature, which was also constantly monitored via a feedback loop. Participants were given a button-box to respond if the pain became intolerable.

During the scanning sessions (Session 2 and 3), participants underwent three 6-min resting state investigations: baseline (Pre-cold); cold-pain (Cold-pain) and post-cold recovery (Post-cold). During each resting state period, participants were instructed to rest with their eyes open, and keep their focus on the fixation cross-presented at the centre of the screen, without thinking of anything and not falling asleep. To further explore the perception of cold pain, participants were tested during a post-scan session, where they were presented with the same 6-min cold stimulation and a 6-min post-cold interval. In this session, participants were asked to provide subjective ratings of the 6-min cold and post-cold sessions on a visual analogue scale (VAS) ranging from 0 (indicating no pain) to 100 (indicating maximal imaginable pain in an experimental context).

2.3 | MRI acquisition and preprocessing

MR images were acquired on a GE MR750 scanner equipped with a 32-channel receive-only head coil (NovaMedical). Structural volumes were obtained using a high-resolution three-dimensional magnetization-prepared rapid gradient-echo sequence (TR = 7,312 ms, TE = 3.02 ms, flip angle = 11°, slice thickness = 1.2 mm, 196 sagittal slices, FOV = 270 × 270 mm). Functional MRI data were collected with a T2*-weighted descending multi-echo imaging (EPI) sequence sensitive to blood oxygenation level dependent (BOLD) signal (TR = 2 s, TE1 = 12 ms, TE2 = 28 ms; TE3 = 44 ms; flip-angle 80°, 32 slices, FOV 240 × 240 in plane, voxel size 3.75 × 3.75 mm, 3 mm slice thickness, 0.4 mm inter-slice gap).

Rs-fMRI datasets were pre-processed by combining different elements of established software toolboxes: AFNI (Cox, 1996), the Advanced Normalization Tools (ANTs) (Avants et al., 2011) and FSL (Smith et al., 2004). By acquiring multiple echo images per slice, multi-echo fMRI permits identification of non-BOLD related sources of signal, preserving signals of interest (Dipasquale et al., 2017). This is of importance for pain studies, where pain-induced body movements or gross physiological changes may provide sources of artefactual non-BOLD-related signal alterations. Pre-processing steps were implemented in AFNI, and included volume re-alignment for motion correction, time-series de-spiking and slice time correction. After pre-processing, functional data were optimally combined (OC) by taking a weighted summation of the three echoes using an exponential T2* weighting approach (Posse et al., 1999). The OC data were then de-noised with the Multi-Echo Independent Component Analysis (ICA) approach implemented by the tool meica.py (Version v2.5 beta; AFNI; Kundu et al., 2013; Kundu, Santin, Bandettini, Bullmore, & Petiet, 2014), given its proven effectiveness in removing physiological and motion-related noise and increasing temporal signal-to-noise ratio (Dipasquale et al., 2017; Kundu et al., 2013). Briefly, multi-echo principal component analysis was first used to reduce the data dimensionality in the OC dataset. Spatial ICA was then applied on one echo, and the independent component time-series were fitted to the pre-processed time-series from each of the three echoes to generate ICA weights for each echo. These weights were then fitted to the linear TE-dependence and TE-independence models to generate F-statistics and component-level κ and ρ values, which respectively indicate BOLD and non-BOLD weightings. The ρ metrics were then used to identify non-BOLD-like components to be regressed out of the OC dataset as noise. For further technical details on ME-ICA see (Kundu et al., 2015).

Using FSL, we regressed out the white matter (WM) and cerebrospinal fluid (CSF) signals, high-pass temporal filtered the data with a cut-off frequency of 0.005 Hz and spatially smoothed them with a 5 mm FWHM Gaussian kernel. Each participant's dataset was co-registered to its corresponding structural scan with an affine registration and normalised to standard MNI152 space (with a non-linear approach) resampled to $2 \times 2 \times 2$ mm^3 using ANTs. A detailed description of registration parameters is provided in the Supplementary Material.

2.3.1 | Seed-based fMRI analysis

Anatomical ROIs were constructed using the Marsbar toolbox (http://marsbar.sourceforge.net/) implemented in SPM 12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12). We focused our interest in vmPFC as the central hub of the DMN, rAI as the central hub of the SN and often involved in chronic pain syndromes (Cottam, Iwabuchi, Drabek, Reckziegel, & Auer, 2018), and the sub-genual ACC

(sgACC) and PAG (both central hubs in the descending pain modulation pathway; Yu et al., 2014a). We created a 12 mm-radius sphere for the vmPFC (MNI 0 50 –8), 5 mm-radius sphere for the rAI (MNI = 36 20 1), 10 mm-radius sphere for the sgACC (MNI = −3 15 –10) and 3 mm-radius sphere for the PAG (MNI = 0 –30 –12). See Supplementary Material for a graphical representation of our ROIs across different brain views (Figure S1). The volumes of the spheres differed according to the anatomical constraints imposed by each region. Coordinates were selected based upon those reported in the literature (Schweinhardt et al., 2006; Tzourio-Mazoyer et al., 2002; Yu et al., 2014a). The average resting state fMRI time-series in each ROI was extracted for each participant and scan and used as a regressor in a first level SPM analysis, to perform a seed-to-whole brain regression analysis and explore networks associated with each of our seed regions.

Next, the first-level contrast images were entered into a flexible factorial design, with Condition (Pre-cold, Cold-Pain, Post-cold) and Session (Time 1, Time 2) as main factors. Planned comparisons were performed for the following contrasts: Pre-cold versus Cold-pain, Pre-cold versus Post-cold and Cold-pain versus Post-cold. Contrast images were masked with an explicit binary grey matter mask (derived from SPM-12 grey matter tissue probability maps, thresholded at a minimum 40% probability of being grey matter).

2.3.2 | Assessment of relationships between FC and self-reported pain

To investigate FC alterations directly associated with subjective ratings of Cold-pain (as indexed by VAS), we performed second level multiple linear regression general linear model (GLM) analyses. Inputs to the GLM were contrast images obtained at first level by subtracting Cold-pain and Pre-cold (ΔFC Cold-pain - Pre-cold) and between Post-cold and Pre-cold condition (ΔFC Post-cold - Pre-cold), with VAS pain ratings as covariates of interest. For illustrative purposes, parameter estimates for regions that showed significant correlations with VAS scores were extracted and plotted.

2.3.3 | Statistical inference

All mass-univariate voxelwise analyses were thresholded using an initial cluster-forming threshold of $p = .001$ and a Gaussian random field theory family wise error (FWE)-corrected (Worsley, Evans, Marrett, & Neelin, 1992) cluster-significance threshold of $p = .05$. Parameter estimates averaged across voxels in the significant cluster were extracted and plotted for illustrative purposes.

3 | RESULTS

3.1 | Pain ratings

Following six-minute cold stimulation, participants gave an average pain rating of 44.94 ($SD = 21.82$) on a 0–100 VAS scale. At the end of the six-minute post-cold pain recovery phase, the participant pain intensity (rated on the VAS) was 1.50 ($SD = 3.37$).

3.2 | Functional connectivity baseline networks

We first validated the existence of brain networks associated with each of our chosen seed regions at Baseline, prior to examining the effects elicited by tonic noxious cold stimulation.

3.2.1 | Baseline FC (vmPFC seed)

FC analysis of the vmPFC seed showed the core DMN network, consisting of the PCC/precuneus, bilateral parietal regions and PFC (Figure 1a).

3.2.2 | Baseline FC (rAI seed)

FC analysis of the rAI seed resulted in the identification of the SN network, comprising the following regions: sgACC and middle ACC, bilateral insula, bilateral supramarginal gyrus and cuneal cortex (Figure 1b).

3.2.3 | Baseline FC (sgACC seed)

FC analysis of the sgACC seed showed a network comprising the frontal medial cortex, thalamus, bilateral amygdala and bilateral parahippocampal gyrus (Figure 1c).

3.2.4 | Baseline FC (PAG seed)

FC analysis of the PAG seed showed a network comprised of the bilateral parahippocampal/amygdalar areas, middle cingulate cortex, anterior insular cortex, and prefrontal medial cortex (Figure 1d), in line with other published studies (Yu et al., 2014b).

3.3 | Changes in FC networks from baseline, during and following cold pain

Next, we investigated modulation of baseline FC networks in each seed, during tonic noxious cold stimulation (Cold-pain)

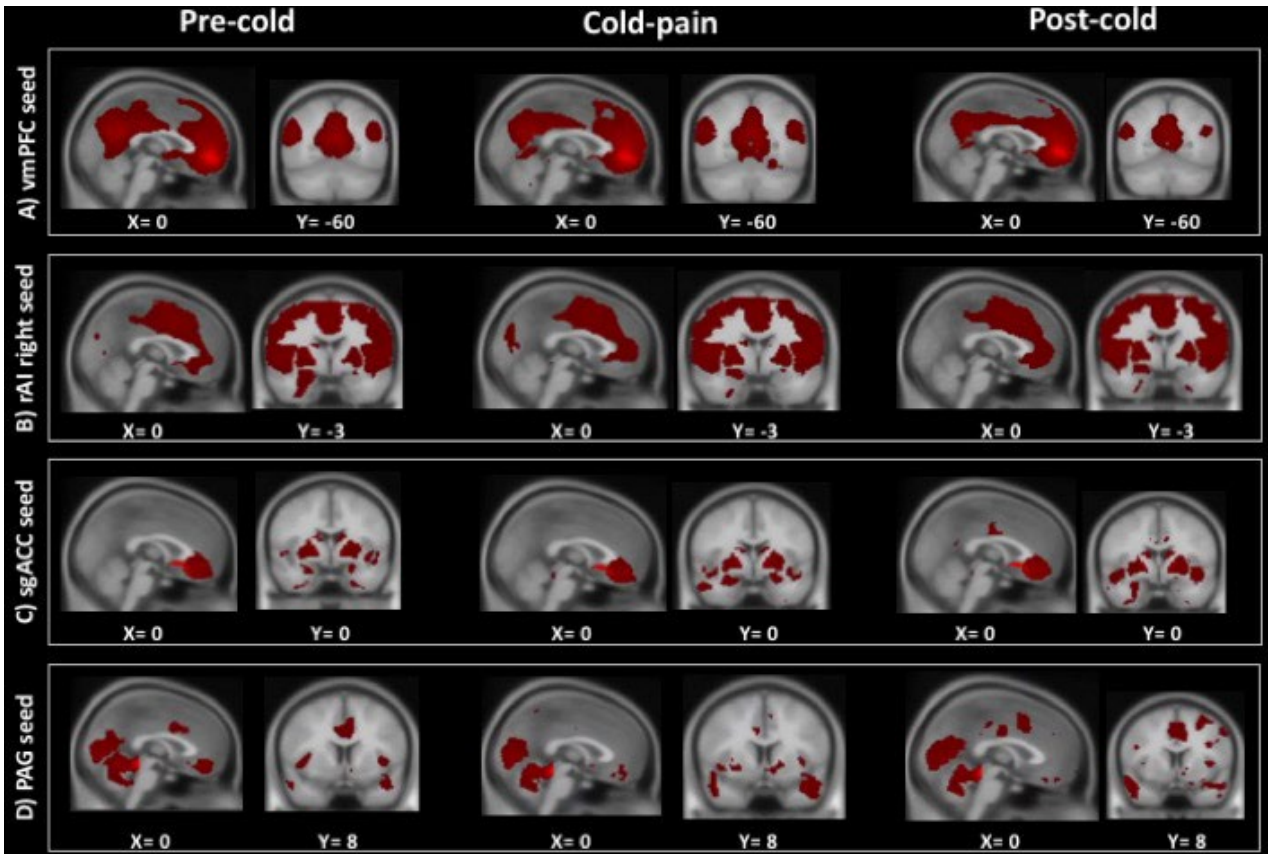

FIGURE 1 Resting-state FC networks for the four seed regions (vmPFC, rAI, sgACC, PAG). Demonstration of FC networks during Pre-cold, Cold-pain and Post-cold pain period. Results (in MNI space) are reported at a FWE cluster-level corrected threshold of $p = .05$

and in a second assessment period immediately following cold pain (Post-cold).

3.3.1 | FC changes (vmPFC seed)

When comparing Pre-cold to Post-cold only, we observed a decrease in FC between the vmPFC and precuneus/PCC, and an increase in FC between the vmPFC and lateral middle frontal gyrus/frontal pole (Figure 2a2 and a3; Table 1; further detail provided in Figure S2).

3.3.2 | FC changes (rAI seed)

During Cold-pain, compared to Pre-cold, FC increases were observed between rAI and vmPFC regions (both DMN hubs) (Figure 2b1; Table 2). The same pattern was observed when comparing Pre-cold with Post-cold, with an additional increase in rAI-PCC FC and a decrease in FC with the orbital frontal cortex and superior frontal gyrus during Post-cold condition (Figure 2b2; Table 2, Figure S3 for a detailed representation). No differences in FC using this seed were observed when comparing Cold-pain with the Post-cold period. These data indicate that tonic noxious cold increases FC between the rAI and areas of the DMN, and that these FC perturbations persist following painful stimulation.

3.3.3 | FC changes (sgACC seed)

No differences in FC networks associated with the sgACC seed were evident when comparing Pre-cold with the Cold-pain condition or Post-cold recovery periods, nor between Cold-pain and Post-cold periods.

3.3.4 | FC changes (PAG seed)

During Cold-pain, a decrease in FC was observed between the PAG and the PCC, when compared to Pre-cold (Figure 2c1). No differences in FC networks associated with the PAG seed were evident when comparing Pre-cold with Post-cold recovery periods, nor between Cold-pain and Post-cold periods (Table 3).

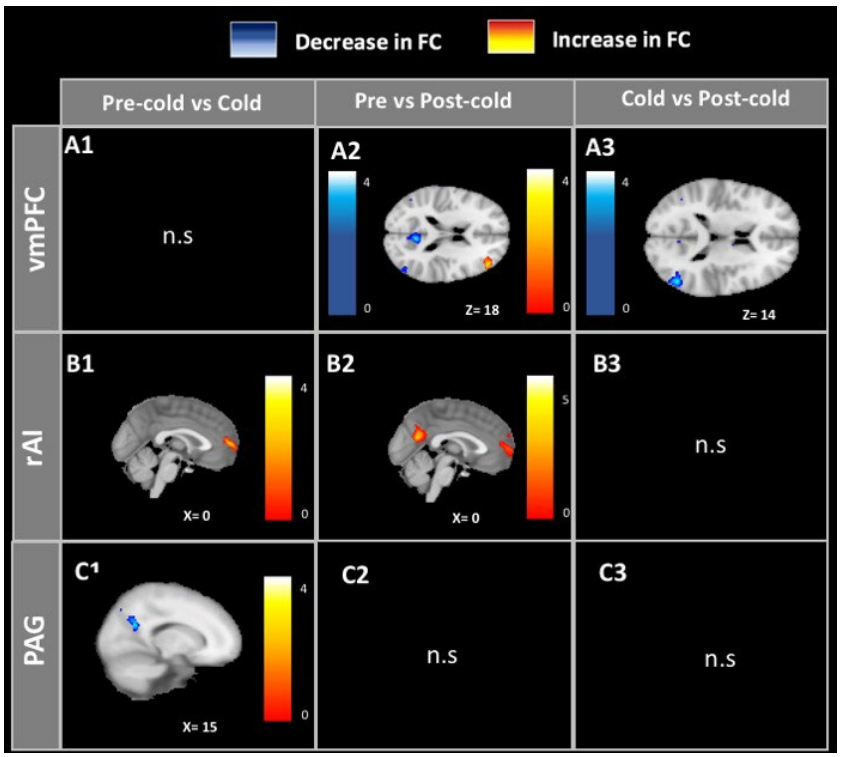

FIGURE 2 Functional connectivity alterations during Cold-pain and in the Post-cold recovery phase. FC Changes from Pre-cold to Cold-pain and Post-cold period. Red/yellow regions indicate FC increases between the seed regions (vmPFC, rAI, PAG) and the rest of the brain; blue areas indicate FC decreases. For the rAI and PAG seeds, FC was altered when comparing Cold-pain to the Pre-cold period (top to bottom; b1-c1). For the vmPFC and for the rAI seed, FC was altered also when comparing the Post-cold period to Pre-cold (top to bottom; a2-b2). Less pronounced alterations were observed when comparing the Post-cold period to the Cold-pain period for the vmPFC seed (a3). (n.s = non-significant results). See also Supplementary Material (Figures S2–S4) for further detail. Results (in MNI space) are reported at a FWE cluster-level corrected threshold of $p = .05$

TABLE 1 Brain areas showing significant functional connectivity alterations during Cold-pain and Post-cold pain recovery period, compared to Pre-cold baseline, for the vmPFC seed

Contrast	Brain area	*k*	Cluster-level p (FWE)	Voxel-level p (FWE)	*t*	MNI *xyz*	Effect size (*d*)
Cold-pain > Post-cold	Lateral occipital/parietal lobule	**330**	**0.031**	*n*.s.	4.49	−52 −68 18	1.46
Pre-cold > Post-cold	Precuneus	**442**	**0.010**	***n*.s**	4.64	−6 −58 18	1.51
Pre-cold < Post-cold	Lateral middle frontal gyrus	**4.13**	**0.013**	*n*.s.	4.34	−44 40 18	1.41

Abbreviations: ACC: anterior cingulate cortex; *n*.s.: non-significant.

3.4 | Relationships between FC and self-reported pain

We conducted a series of whole-brain voxelwise correlation analyses with VAS score as an explanatory variable, to investigate FC changes in each of our seed regions between Pre-cold, Cold-pain and Post-cold periods associated with the subjective perception of tonic cold pain.

3.4.1 | VAS- delta FC (PFC) relationships

We observed a negative correlation between Cold-pain VAS ratings and the change in FC between the vmPFC and precuneus/PCC, when comparing both Cold-pain and Post-cold periods with the Pre-cold condition (Figure 3a). A positive correlation was observed between the pain scores and the same contrasts (ΔFC Cold-pain -Pre-cold and ΔFC Post-cold - Pre-cold), for the FC between PFC

TABLE 2 Brain areas showing significant functional connectivity alterations during Cold-pain and Post-cold pain recovery period, when compared to Pre-cold baseline, for the rAI seed

Contrast	Brain area	*k*	Cluster-level p (FWE)	Voxel-level p (FWE)	*t*	MNI *xyz*	Effect size (*d*)
Pre-cold < Cold-pain	vmPFC	**510**	**0.003**	*n*.s.	4.00	4 62 4	1.3
Pre-cold < Post-cold	Precuneus/PCC	**1,497**	**<0.001**	**0.003**	5.40	−2 −62 20	1.75
	Frontal orbital cortex, right	**366**	**0.015**	*n*.s.	4.37	52 34 −10	1.42
	Frontal orbital cortex	**322**	**0.022**	***n*.s**	4.19	2 60 4	1.36
	Superior frontal gyrus, left	**150**	***n*.s.**	0.039	4.88	−24 22 38	1.58

n.s.: non-significant.

TABLE 3 Brain areas showing significant functional connectivity alterations during Cold-pain and Post-cold pain recovery period, when compared to Pre-cold baseline, for the PAG seed

Contrast	Brain area	*k*	Cluster-level p (FWE)	Voxel-level p (FWE)	*t*	MNI *xyz*	Effect size (*d*)
Pre-cold > Cold	Posterior cingulate cortex	285	**0.042**	*n*.s.	4.14	16 −60 26	1.36

n.s.: non-significant.

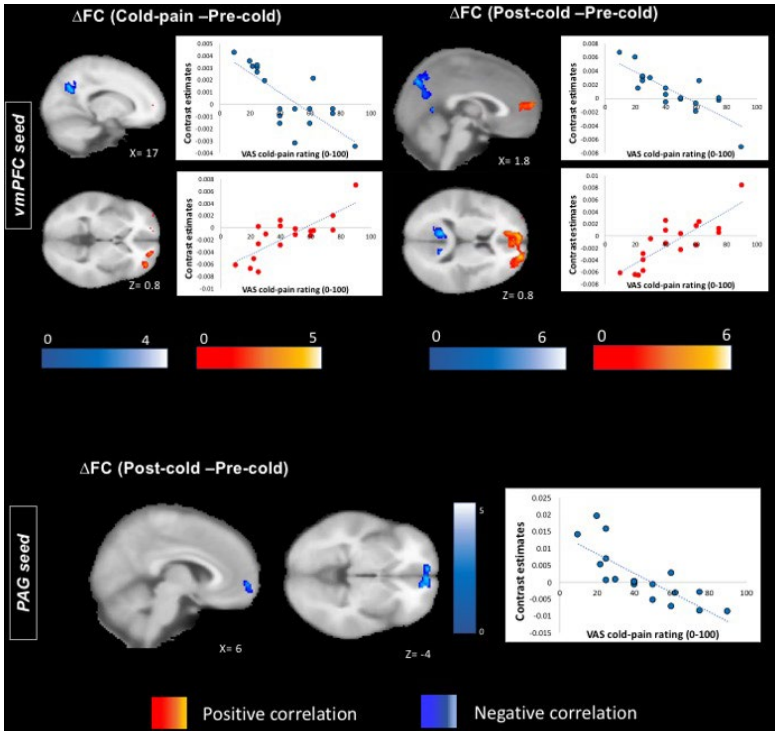

FIGURE 3 Correlation between changes in functional connectivity (delta-FC) and VAS pain ratings. Brain regions (in MNI space) showing a significant association between VAS ratings of cold pain and the change in FC between the Pre-cold and Cold-pain condition (ΔFC Cold-pain–Baseline) and Pre-cold and post-cold (ΔFC Post-cold–Baseline), for the vmPFC and PAG seeds. Supplementary Material (Figures S5 and S6) provide further details. Results (in MNI space) are reported at a FWE cluster-level corrected threshold of $p = .05$

and dorsolateral PFC (Figure 3, Table 4, and Figure S5 for a detailed representation). These results indicate that in individuals with higher self-reported cold pain, the magnitude of FC decrease between prefrontal and posterior areas of the DMN and the magnitude of FC increase between vmPFC and dorsolateral PFC was greatest.

3.4.2 | VAS- delta FC (PAG) relationships

A negative correlation was observed between pain ratings and the change in FC (Cold-pain versus Pre-cold) between the PAG and the vmPFC (Figure 3c; Table 4; further detail in Figure S6), indicating that individuals with higher

TABLE 4 Brain areas showing significant functional connectivity associations with subjective pain ratings during the 6 min cold painful stimulation

Contrast	Brain area	Correlation	*k*	Cluster-level p (FWE)	Voxel-level p (FWE)	*t*	MNI *xyz*	Effect size (*d*)
vmPFC seed								
ΔFC (Pre-cold < Cold-pain)	dlPFC	**Positive**	**347**	**0.004**	*n*.s.	4.18	−28 40 24	1.43
	PCC/Precuneus	**Negative**	**349**	**0.004**	*n*.s.	4.92	14 −66 34	1.69
ΔFC (Post-cold < Pre-cold pain)	ACC/PFC	**Positive**	**1,331**	**<0.001**	*n*.s.	5.79	12 42 8	1.99
	Superior frontal gyrus	**Positive**			*n*.s.	5.48	0 60 12	1.88
	vmPFC	**Positive**			*n*.s.	4.76	10 46 2	1.63
	PCC/Precuneus	**Negative**	**1,743**	**<0.001**	*n*.s.	6.19	10 −70 30	2.12
PAG seed PAG seed								
ΔFC (Post-cold < Pre-cold pain)	vmPFC	**Negative**	**330**	**0.006**	*n*.s.	5.32	−12 60 −8	1.82

Abbreviations: ΔFC: change in functional connectivity; ACC: anterior cingulate cortex; AI: anterior insula; dlPFC: dorsolateral prefrontal cortex; PCC: posterior cingulate cortex; vmPFC: ventromedial prefrontal cortex.

self-reported pain had the strongest FC increase between PAG and the vmPFC when comparing Pre-cold to Post-cold recovery periods.

4 | DISCUSSION

In this healthy volunteer study, we used tonic noxious cold stimulation to induce FC perturbations in four major pain hubs (vmPFC, rAI, PAG and sgACC), eliciting alterations in the DMN, salience, central executive (CEN) and descending pain control networks. Several FC changes persisted following stimulation. Pain-induced network alterations were associated with participants' subjective pain ratings. We interpret these cold-induced network changes as responses by stimulus salience, interoceptive, autonomic and endogenous pain control systems to homeostatic threat. These FC changes in healthy individuals are qualitatively similar to those observed in chronic pain (Baliki et al., 2014; Kucyi et al., 2014), entertaining the possibility that elements of FC changes observed in response to processing of acute pain may also occur in patients, including, but not limited to, afferent nociceptive signalling. Our characterisation of the FC network changes in response to tonic experimental pain should facilitate appropriately powered, hypothesis-driven replication of this work increasingly acknowledged as necessary in studies with moderate sample sizes (Munafò et al., 2017).

Immediately following cold-pain, FC between major hubs of the CEN, vmPFC and dlPFC increased, whereas the FC between vmPFC and posterior DMN, precuneus/PCC and lateral parietal lobules decreased. In healthy individuals, the dlPFC has a causal regulatory influence on the vmPFC (Chen et al., 2013), where engagement of the CEN by an active task temporarily "shuts-down" the DMN. In another healthy volunteer study, increases in experimentally-induced secondary hyperalgesia were also associated with FC decreases between posterior and anterior areas of the DMN, and FC increases in frontal areas (Hansen et al., 2019). Reports of FC decreases between the PFC and precuneus also exist in several chronic pain conditions, including back pain, complex regional pain syndrome and osteoarthritis (Baliki et al., 2014). Similarly, increased FC between the dlPFC, bilateral rostral ACC and vmPFC has also been reported in fibromyalgia (Kong et al., 2019). However, we do not interpret our findings as sensitisation phenomena, as the time period following stimulation is too short to have fully developed; transient nociceptive hyperexcitability is more likely here (Gangadharan & Kuner, 2013). A follow-up scan after an appropriate amount of time to establish sensitisation and homotopic and heterotopic sensory testing (Koppert et al., 2001) could be employed to test sensitisation theories.

We observed increased FC between the rAI, a core node in the salience network, and the main hubs of the DMN during cold painful stimulation; these connectivity changes persisted in the post-pain period. These data and other healthy controls studies support theories of interactions between rAI, DMN and the CEN during the elaboration of salient stimuli including pain (Sridharan, Levitin, & Menon, 2008). Increased connectivity between the DMN and the insula has also been described in patients with diabetic neuropathic pain (Chen et al., 2013) and rheumatoid arthritis (Flodin et al., 2016). Increased insula-DMN coupling decreases after treatment in fibromyalgia and is associated with pain reduction (Napadow et al., 2010). Our data add to this knowledge by showing that increases in Insula-DMN FC can be induced experimentally and these perturbations persist beyond discontinuation of the noxious stimulus.

The PAG showed connectivity changes with the precuneus/PCC during noxious cold, compared to pain-free baseline assessment. The PCC/precuneus are engaged during assessment of the emotional valence (Vogt, 2005) and their

activity varies with arousal states (Vogt & Laureys, 2005). In healthy controls, increases in precuneus response during pain are associated with reduced pain sensitivity (Goffaux, Girard-Tremblay, Marchand, Daigle, & Whittingstall, 2014) and pain perceived during the application of non-noxious stimuli (i.e. allodynia) is associated with increased precuneus activity (Witting et al., 2001). A failure to down-regulate the PCC is related to cognitive dysfunctions, both in healthy subjects and patients (Weissman, Roberts, Visscher, & Woldorff, 2006). We suggest that our observations of decreased rAI-PCC anticorrelation and decreased PAG–Precuneus connectivity indicate that pain interferes with DMN control, and that these perturbations contribute to the complex relationships between pain and cognition (Seminowicz & Davis, 2007b).

During cold-pain, FC decreases between vmPFC and PCC, and increases between vmPFC and dlPFC, were associated with cold-pain intensity ratings. We suggest these noxious stimulation and post-stimulation FC effects relate to a multi-faceted subjective pain experience including, but not limited to, both sensory and affective components and more generalised processes of stimulus saliency (Legrain, Iannetti, Plaghki, & Mouraux, 2011). Increased PAG-vmPFC connectivity in the post-cold period also correlated negatively with pain ratings; individuals with the highest self-reported pain exhibited lower FC. A similar relationship has been previously described in patients with chronic low back pain (Yu et al., 2014a). Qualitative similarities in patterns of connectivity alterations in healthy volunteers and chronic pain patients need not necessarily stem from the same underlying mechanisms. For example, insula activity has been related to multiple pain mechanisms, from elaboration of a noxious peripheral drive in healthy participants (Peltz et al., 2011), to modulation and chronification of pain in chronic pain patients (Lu et al., 2016). We suggest that a multi-modal approach (perhaps including MRI data, behavioural and autonomic nervous system information) might offer improved characterisation of individual pain mechanisms, in turn facilitating meaningful comparisons between differing pain phenotypes. These considerations aside, our FC-VAS relationships still engender the working hypothesis that FC changes in chronic pain populations may include contributions from peripherally mediated afferent noxious signalling.

We observed that cold-induced rs-fMRI perturbations persisted in several functional networks during the post-cold condition. These findings accord with studies reporting BOLD responses that do not immediately return to baseline following completion of both cognitively demanding tasks (Barnes, Bullmore, & Suckling, 2009), and simpler paradigms such as fixed-duration button pressing (Tung et al., 2013). In the DMN and PAG networks, post-cold perturbations were associated with pain intensity ratings, suggesting that the persistence of these FC alterations is not a generalized rs-fMRI phenomenon, but relates to having experienced pain. In a study by Vaisvaser et al. (2013), prolonged DMN connectivity responses were described up to two hours following an acute psychosocial stressor and were inversely associated with cortisol release. Accordingly, persisting FC perturbations in our study may relate to increases in cortisol levels in response to cold pain. Relationships between cortisol and FC have been reported previously (Hodkinson et al., 2014) and salivary cortisol measurement is a tenable addition to further experiments. We suggest cautious interpretation of "back-to-back" fMRI pain assessments as results may be confounded.

Some of our observed FC changes are qualitatively similar to those reported in chronic pain patients, engaging the theoretical possibility that some FC changes in these patients might relate to the presence of ongoing nociceptive activity. While providing excellent experimental control, the sequelae of living with long term pain cannot be replicated in healthy volunteer models, placing limitations on how far we can extrapolate our findings to chronic pain. This caveat aside, there is evidence to support aberrant nociceptive signalling in chronic pain patients. For example, fibromyalgia nociceptive hyperexcitability is a contributor to pain maintenance, despite being historically considered to be a CNS-maintained disorder (Serra et al., 2014). Our consideration of the potential contribution of afferent nociceptive signalling in persistent pain adds to, rather than competes with, prevailing views of shifts from sensory-discriminative to affective aspects of chronic pain and associated FC changes (Baliki et al., 2012). In many patients multiple pathophysiological mechanisms contribute to their pain and these mechanisms are likely to differ across individuals and pain phenotypes (Wylde, Hewlett, Learmonth, & Dieppe, 2011). In an ideal future, FC network descriptions might disambiguate peripherally-mediated, from central nervous system-maintained chronic pain states. Identifying FC network "fingerprints" may facilitate precision medicine approaches that treat specific pathological pain mechanisms in individual patients (Borsook, Hargreaves, & Becerra, 2011; Rosa & Seymour, 2014).

In summary, in healthy volunteers we demonstrate FC changes in several major networks in response to tonic cold pain, several of which persist after noxious stimulation. Connectivity changes relate to subjective reports of pain intensity, recruiting multi-systemic pain control mechanisms. We envisage that our findings will stimulate future mechanistic investigations underpinning acute and chronic pain states.

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CONFLICTS OF INTEREST

None.

AUTHOR CONTRIBUTION

MAH, SCRW, SBM, EM, JBJ and SM have designed the experiment. EM has analysed the data, interpreted the results and wrote the manuscript. JBJ and SM have designed the experiment, collected data and revised the manuscript. ALR was involved in hardware realization and software optimisation. OOD, JOM, SCRW, SBM and MAH gave overall supervision to the project, and to the analysis of the data, and revision of the manuscript. All authors discussed the results and commented on the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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