

The association between pain-induced autonomic reactivity and descending pain control is mediated by the periaqueductal grey

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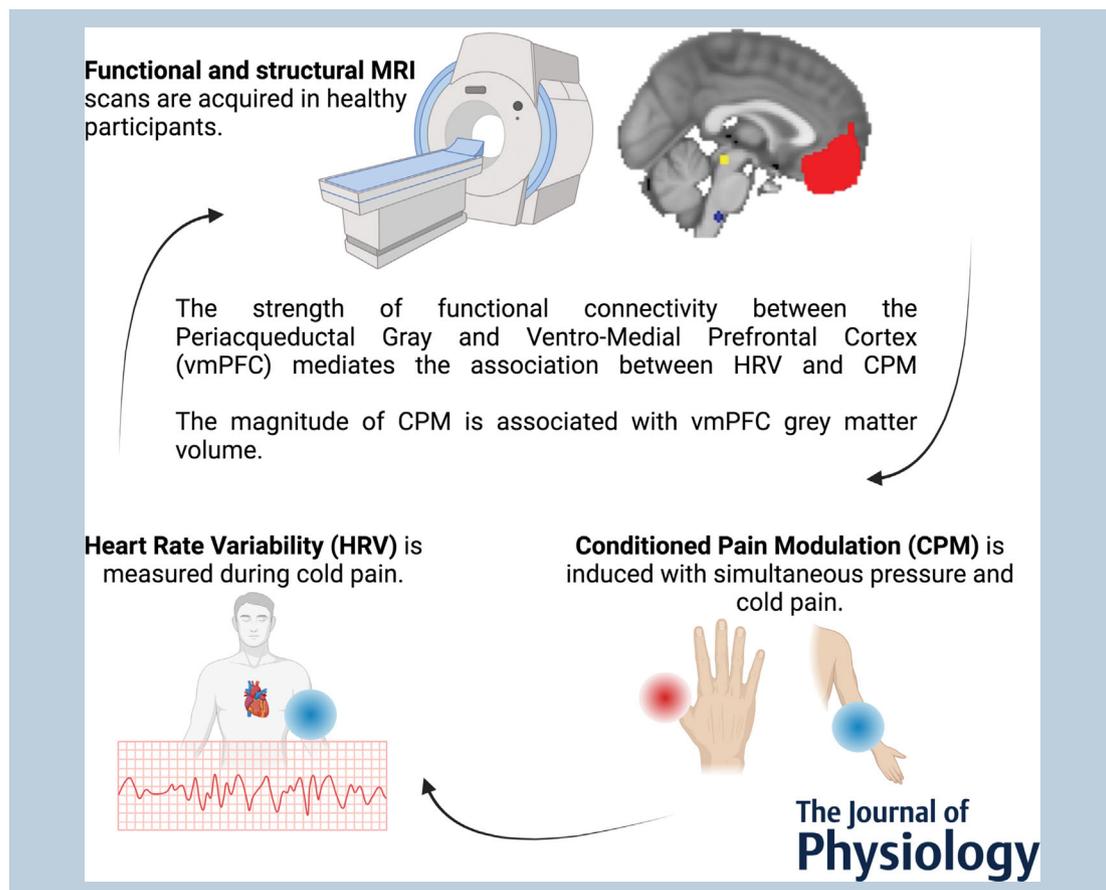
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Abstract There is a strict interaction between the autonomic nervous system (ANS) and pain, which might involve descending pain modulatory mechanisms. The periaqueductal grey (PAG) is involved both in descending pain modulation and ANS, but its role in mediating this relationship has not yet been explored. Here, we sought to determine brain regions mediating ANS and descending pain control associations. Thirty participants underwent conditioned pain modulation (CPM) assessments, in which they rated painful pressure stimuli applied to their thumbnail, either alone or with a painful cold contralateral stimulation. Differences in pain ratings between 'pressure-only' and 'pressure + cold' stimuli provided a measure of descending pain control. In 18 of the 30 participants, structural scans and two functional MRI assessments, one pain-free and one during cold-pain were acquired. Heart rate variability (HRV) was simultaneously recorded. Normalised low-frequency HRV (LF-HRVnu) and the CPM score were negatively correlated; individuals with higher LF-HRVnu during pain reported reductions in pain during CPM. PAG-ventro-medial prefrontal cortex (vmPFC) and PAG-rostral ventromedial medulla (RVM) functional connectivity correlated negatively with the CPM. Importantly, PAG-vmPFC functional connectivity mediated the strength of the LF-HRVnu-CPM association. CPM response magnitude was also negatively correlated with vmPFC GM volume. Our multi-modal approach, using behavioural, physiological and MRI measures, provides important new evidence of interactions between ANS and descending pain mechanisms. ANS dysregulation and dysfunctional descending pain modulation are characteristics of chronic pain. We suggest that further investigation of body-brain interactions in chronic pain patients may catalyse the development of new treatments.

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Abstract figure legend The aim of this study is to investigate which brain regions mediate the association between the autonomic nervous system (ANS) and descending pain modulating mechanisms. To achieve this, healthy controls underwent a conditioned pain modulation protocol, with the aim of exploring the functioning of their descending pain modulating mechanisms. In a subgroup of these participants, we also acquired structural and functional MRI data, alongside physiological data (i.e. Heart Rate Variability -HRV- during cold pain). Overall, we observed that the CPM response was negatively associated with ventro-medial prefrontal cortex grey matter (vmPFC) and positively associated with the strength of functional connectivity between the periaqueductal grey area (PAG) and the vmPFC. Importantly, PAG-vmPFC functional connectivity mediated the strength of the association between HRV and CPM.

Key points

- Heart rate variability (HRV) is associated with descending pain modulation as measured by the conditioned pain modulation protocol (CPM).
- There is an association between CPM scores and the functional connectivity between the periaqueductal grey (PAG) and ventro-medial prefrontal cortex (vmPFC).
- CPM scores are also associated with vmPFC grey matter volume.
- The strength of functional connectivity between the PAG and vmPFC mediates the association between HRV and CPM.
- Our data provide new evidence of interactions between the autonomic nervous system and descending pain mechanisms.

Introduction

Nociception is modulated by descending pain control systems in the brainstem and spinal cord, under the direct influence of higher order brain areas. In humans, the efficiency of these systems can be measured using

protocols such as conditioned pain modulation (CPM) (Yarnitsky, 2010). During a typical CPM trial, participants subjectively rate responses to a painful test stimulus, both on its own, and in the presence of an additional painful conditioning stimulus (Yarnitsky *et al.* 2015). Here, a reduction in the pain induced by the test stimulus

when coincident with the conditioning stimulus indicates efficient descending pain control. CPM responses are, however, variable, and a reduction in pain during conditioning stimulus application is only observed in some individuals (Kemp *et al.* 2019). Individual differences in CPM responses may relate to factors such as age or gender (Edwards *et al.* 2003; Granot *et al.* 2008), emotions (Roy *et al.* 2009), personality (Nahman-Averbuch *et al.* 2016) or the type of protocol adopted (Kemp *et al.* 2019). Despite substantial inter-individual variability, reports indicate CPM responses predict post-operative pain (Yarnitsky *et al.* 2008) and pharmacological treatment response (Yarnitsky *et al.* 2012), thus showing potential as a biomarker.

The bi-directional interaction between the cardiovascular system and pain is another important component of descending pain control (Bruehl & Chung, 2004). In healthy individuals, acute pain increases sympathetic arousal and blood pressure (Kyle & McNeil, 2014). By contrast, a reduction in pain perception is reported in healthy participants during spontaneous or induced high blood pressure (Saccò *et al.* 2013). Similarly, unmedicated patients with hypertension report lower pain sensitivity and higher pain thresholds, a condition termed as blood pressure-related hypoalgesia (Olsen *et al.* 2013; Ottaviani *et al.* 2018). Several studies have suggested that high blood pressure may protect against clinical pain (Maixner *et al.* 1997; France & Katz, 1999; Bruehl *et al.* 2002; Hagen *et al.* 2005), entertaining the possibility that the baroreflex – a homeostatic mechanism that controls blood pressure – may also be involved in pain regulation (Suarez-Roca *et al.* 2019). An association has been also described between blood pressure and the CPM response (Chalaye *et al.* 2014), supporting the role of baroreceptors in signalling the cardiovascular state of the body to the brain in pain control (Suarez-Roca *et al.* 2019). Carotid baroreceptor stimulation results in reduced pain sensitivity in both hypertensive and normotensive individuals (Edwards *et al.* 2003), suggesting that interactions between the ANS and descending pain control systems may involve baroreflex activation (Dayan *et al.* 2018). Heart rate variability (HRV), an indirect marker of autonomic control of the heart, has also been shown to be related to the perception of pain. Increased low-frequency HRV, a measure specifically linked to baroreflex efficiency

(Moak *et al.* 2007; Rahman *et al.* 2011) has been associated with reduced subjective ratings of unpleasantness to cold pain and higher thresholds for low-to moderate levels of thermal pain (Appelhans & Luecken, 2008). Pain appraisal might play an important role in modulating autonomic responses to pain (Mischkowski *et al.* 2018), which might in turn explain the association between descending pain modulation and psychological variables such as, for example, pain catastrophising (Traxler *et al.* 2019). Autonomic measures such as skin conductance and heart rate can predict within-subject perceptual changes in pain and pain ratings (Loggia *et al.* 2011). This further supports the notion that ANS responses are more than just a nociceptive reflex and that they play a crucial role in the modulation of pain perception.

Perhaps associations between the ANS and pain control are not surprising, given the overlap between brain structures involved in ANS regulation (via the baroreflex) and those involved in pain. For example, the ventro-medial prefrontal cortex (vmPFC), anterior cingulate cortex, thalamus and insula are involved both in the regulation of body autonomic arousal (Benarroch, 2012; Beissner *et al.* 2013; Sklerov *et al.* 2018) and in pain perception and elaboration (Peyron *et al.* 2000; Benarroch, 2006). Animal studies indicate that the periaqueductal grey (PAG) and the rostral ventromedial medulla (RVM) have well-defined roles in descending pain control (Bouhassira *et al.* 1992; Harris, 1996; Villanueva *et al.* 1996). In humans, pain control mechanisms have been related to functional networks involving the PAG and the mPFC (Bogdanov *et al.* 2015) and to resting-state functional connectivity (FC) between the PAG and RVM (Yelle *et al.* 2009; Harper *et al.* 2018); but also between the subnucleus reticularis dorsalis and cingulate and dorsolateral prefrontal areas (Youssef *et al.* 2016). These same brain structures, namely, the cingulate cortex, insula, amygdala and PAG, are also associated with reduced baroreflex sensitivity, particularly the ability of the baroreflex to control transient BP fluctuations in response to physiological stress (Gianaros *et al.* 2012).

The neurovisceral integration model (Thayer & Lane, 2000) provides a framework for investigating inter-relationships between descending pain control mechanisms and autonomic functioning in the brain. This model theorises that increased HRV provides an

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individual with flexibility to adapt in response to changing physiological and environmental demands, governed by a 'central autonomic network' (CAN) (Benarroch, 1993), incorporating the prefrontal cortex (anterior cingulate, insula, orbitofrontal and ventromedial cortex), amygdala, hypothalamus and brain stem (including the PAG and RVM). The dynamic and bi-directional interaction between the CAN and the cardiovascular system affects cognition, executive functioning and emotions (Thayer *et al.* 2009).

Here, we sought to determine brain mechanisms underlying the putative association between descending pain control and autonomic regulation systems in a group of healthy participants. We employed resting-state fMRI (rs-fMRI) and voxel-based morphometry (VBM) as measures of brain function and structure, respectively, focusing our attention on the PAG as a major hub for both autonomic and pain processing. We also examined HRV as an index of autonomic reactivity to pain and performed a behavioural experiment examining descending pain control. We hypothesised that connectivity between the PAG and other pain-related structures would mediate the association between the ANS and descending pain control mechanisms. We also hypothesised an involvement of the RVM, given its role in descending pain controls, as documented by animal studies (Tobaldini *et al.* 2019), and vmPFC in light of recent studies in healthy volunteers and chronic pain patients (Moont *et al.* 2011; Harper *et al.* 2018).

Methods

Ethical approval

The study was approved by the King's College London Research Ethics Committee (HR-16/17-4769) and was conducted in accordance with the *Declaration of Helsinki*, except for registration in a database. All participants were informed about the experimental procedures by reading an information sheet. All participants provided written informed consent prior to commencing experimental sessions and were informed that they could withdraw from the experiment at any time.

Participants

Thirty healthy participants took part in the CPM experiment (12 women, 18 men, mean age across the group = 26.4; SD = 4.7 years). Out of these 30 participants, 18 (6 women, 12 men, mean age across the group = 27.1; SD = 5.1 years) took part in the CPM, MRI and physiology experimental sessions (detailed below). The 18 participants who took part in the MRI study are a subset of a larger group, in whom the relationship between rs-fMRI and HRV

has been described elsewhere (Hohenschurz-Schmidt *et al.* 2020). Therefore, 30 participants in total were considered for the behavioural (CPM) analyses whilst 18 participants only were considered for the regression analysis with MRI and HRV data. All participants were right-handed as assessed by the Edinburgh handedness inventory (Oldfield, 1971). Exclusion criteria included MRI contraindications, history of brain injuries, hypertension, neurological or psychiatric disease, and alcohol or drug abuse. To minimise the potential effects of menstrual cycle-related hormone fluctuations on pain responses (Vincent & Tracey, 2010) and descending pain modulating effects (Tousignant-Laflamme & Marchand, 2009), female participants were all tested within the follicular phase of their menstrual cycle. Furthermore, to minimise the influence of diurnal variations on pain responses (Strian *et al.* 1989) and on rs-fMRI networks activity (Jiang *et al.* 2016), participants were always tested at the same time during the day across the three experimental sessions. At the beginning of each visit, participants were tested for drug use (urine drug test) and alcohol consumption (alcohol breathalyser).

Experimental procedure

Participants took part in one familiarisation session, one scanning session and one post-scanning CPM session. At the beginning of each experimental session, participants completed the 'state' subscale of the State-Trait Anxiety Inventory (STAI) (Spielberger *et al.* 1983), in order to evaluate for possible differences in anxiety levels between the three testing sessions.

Familiarisation and thresholding session

During the familiarisation session, participants became accustomed to the neuroimaging environment (in a simulated 'mock' scanner), and with the cold-pain and pressure-pain stimulation protocol. The cold pain stimulation was delivered via an aluminium probe (4 × 20 cm), attached to the volar surface of their right forearm, through which cold water (2°C) was constantly circulated by means of two chillers.

Next, participants were individually thresholded for pressure pain. A laboratory-built pressure-inducing thumb device was used to apply pressure on the right-side thumbnail. Participants received an ascending series of pressure stimuli and were asked to state when they felt the first sensation of pain (pain threshold) and when they reached a 70 on a visual analogue scale (VAS) from 0 (no pain) to 100 (maximum pain tolerance). Three intermediate pressure values between these thresholds were created to provide five equally spaced pressure values, starting with the threshold and ending with the highest values corresponding to each individuals' VAS 70/100. For

example, if the minimum pain threshold was represented by a pressure of 200 kPa in the ascending series and the maximum pain threshold of >70 was reached with a pressure of 600 kPa, the randomised series would consist of pressures of 200, 300, 400, 500 and 600 kPa. The five pressure values were then presented to the participant in a random order, each repeated three-times (total number of new stimulations $n = 15$). After each stimulation, the participant was required to give a rating on a VAS scale. Based on the 15 pressure values and 15 subjective pain ratings, a polynomial regression function was used to determine each individual's representation of VAS 50, 60 and 70 (Kosek *et al.* 2017). These values were then used in our CPM protocol (third session).

MRI scanning session and physiological recording

During the scanning sessions, participants underwent three fMRI scans, each of 6 min in duration: a baseline; a cold-pain and a post-cold recovery block. The investigation of the post-cold recovery phase aimed at identifying pain-related carry over effects and the results from this analysis have been reported elsewhere (Makovac *et al.* 2019). A multi-echo resting-state fMRI (rs-fMRI) approach was adopted to mitigate problems related to pain-induced movements, which are an important source of non-BOLD artefacts in pain studies (Siegel *et al.* 2016; Dipasquale *et al.* 2017; Moayedi *et al.* 2018). During each period, participants were instructed to keep still with their eyes open, focusing on a fixation cross presented at the centre of the screen, without thinking of anything or falling asleep.

MRI 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 = 7312 ms, TE = 3.02 ms, flip angle = 11° , slice thickness = 12 mm, 196 sagittal slices, FOV = 270 mm). Multi-echo rs-fMRI datasets were acquired using a T2*-weighted blood oxygenation level dependent (BOLD) sensitive echo planar imaging (EPI) sequence (TR = 2 s, TE1 = 12 ms, TE2 = 28 ms; TE3 = 44 ms; flip-angle 80° , 32 slices, 3 mm slice thickness, 240 mm FOV, voxel size $3.75 \times 3.75 \times 3$ mm). During rs-fMRI, heart rate was monitored using an inbuilt MRI-compatible finger pulse oximeter (General Electric) recorded digitally from an analogue output waveform at a sample rate of 40 Hz, fitted to the participants' dominant right index finger.

CPM session

Participants were tested during a third session (on a different day from the scanning session), where they

underwent a CPM protocol, in order to test descending pain modulatory mechanisms.

CPM experimental protocol

During CPM, short-duration noxious pressure test stimuli were delivered to the right-hand thumbnail prior to, simultaneously with, and immediately following a noxious cold conditioning stimulus applied to the left-hand forearm. The intensity of the pressure test stimuli was determined individually for each participant, based on the previously described thresholding procedure, to synthesise pressures to induce pain ratings of 50, 60 and 70 on a 0–100 VAS. Given the lack of unanimous consensus regarding the optimal psychophysical characteristics of the test stimulus and conditioning stimulus in eliciting the CPM effect, we randomly delivered pressure pain stimuli corresponding to the three subjective intensities (50, 60 and 70 VAS), to explore the effect of test stimulus intensity on the CPM response. To account for potential carry-over effects of the conditioning stimulus, test stimuli were presented simultaneously with the conditioning stimulus, and for 6 min following the conditioning stimulus.

Participants underwent three separate experimental runs (refer to Fig. 1): (i) test stimuli only (Baseline condition), and two identical CPM runs (CPM RUN1 and CPM RUN2; Fig. 1), separated by a 15 min break. CPM investigations were split into two runs to provide an adequate number of events per experimental condition but to minimise response habituation and/or peripheral sensitisation. The CPM runs comprised two 6 min mini-blocks, the first during which cold stimulation was present (CPM Cold-ON condition), the second without cold stimulation (CPM Cold-OFF condition). During the Baseline condition, participants rated the intensity of pain evoked by pressure pain stimuli, on a computerized VAS ranging from 0 (no pain) to 100 (maximal imaginable pain). Twenty-four pressure test stimuli were delivered in a random order to their right thumbnail, eight per class of stimulus intensity (50, 60 and 70 VAS). Each test stimulus had a duration of 2 s, followed by an 6 s VAS rating period then 5 s inter-trial interval. The total duration of the Baseline run was 9.2 min. During the CPM run (CPM RUN1 and CPM RUN2), participants were instructed to rate the pressure pain test stimuli (as per the Baseline condition), and not the painful cold conditioning stimulus applied to their left forearm. Finally, participants subjectively rated the intensity of the painful cold conditioning stimulus using a computerized VAS ranging from 0 (no pain) to 100 (maximal imaginable pain).

Statistical analysis

All data are expressed as mean and SD. Normality of the variables was examined using Shapiro-Wilks tests.

Non-normally distributed variables were logarithmically transformed before proceeding with further analyses. The Benjamini-Hochberg procedure was used for multiple-comparisons correction (Thissen *et al.* 2002).

State anxiety

A one-way within-subject ANOVA was used to explore for differences in state anxiety among the three experimental sessions (thresholding, scanning session, CPM session). Possible associations between state anxiety, HRV and CPM were investigated by means of Pearson's correlation coefficient. Statistical threshold was set at $P < 0.05$.

CPM analysis

CPM Cold-ON and CPM Cold-OFF conditions were obtained by averaging pressure ratings from CPM RUN1 and CPM RUN2 (Fig. 1). Raw data from the separate runs are shown in Table 1. Δ CPM Cold-ON and Δ CPM Cold-OFF scores were calculated by subtracting Baseline VAS rating values from the CPM Cold-ON and CPM Cold-OFF conditions ratings, respectively. Negative Δ CPM values indicated a decrease in perceived pressure pain intensity during the conditioning stimulus (Δ CPM Cold-ON) or following the conditioning stimulus (Δ CPM Cold-OFF), whereas positive values indicated an increase in perceived pain. A two-way within-subject ANOVA was used to explore the main effect of pressure (50, 60 and 70 VAS), condition (Baseline, CPM Cold-ON, CPM Cold-OFF), and the pressure \times condition interaction.

Table 1. Mean and standard deviation for pain rating in the five experimental conditions and three pressure pain conditions (corresponding to the individual VAS of 50/100, 60/100, and 70/100)

	Pressure levels		
	50	60	70
Pressure only	31.5 (17.2)	38.3 (16.9)	45.3 (18.8)
CPM Cold-ON RUN1	29.7 (20.3)	36.3 (19.7)	41.0 (17.8)
CPM Cold-ON RUN2	34.3 (20.6)	41.8 (21.2)	45.6 (20.9)
CPM Cold-OFF RUN1	36.2 (17.2)	41.4 (20.2)	46.7 (20.0)
CPM Cold-OFF RUN2	40.5 (19.7)	42.1 (22.3)	49.3 (20.0)

Data obtained from $n = 30$ participants.

Heart rate variability analysis

Inter-beat interval values were visually inspected, and potential artefacts were manually removed. Inter-beat-intervals were then entered into Kubios HRV Standard ver. 3.0.2 (Tarvainen *et al.* 2014) and further artefact correction was applied using the lowest correction threshold appropriate for individual artefact severity. To capture the most salient autonomic response to pain (without the interfering effect of habituation), low-frequency HRV (LF-HRV; 0.04–0.15 Hz) was derived from recordings of the initial 3 min during cold-pain application. LF-HRV is a measure of interest in acute pain studies as it is an index of baroreflex activity (Goldstein *et al.* 2011; Rahman *et al.* 2011). Recent meta-analytic works have highlighted the influence of acute experimental pain on LF-HRV (Koenig *et al.* 2014). Next, LF-HRV data were normalised. Normalised units (n.u.) of HRV spectral data express the relative value

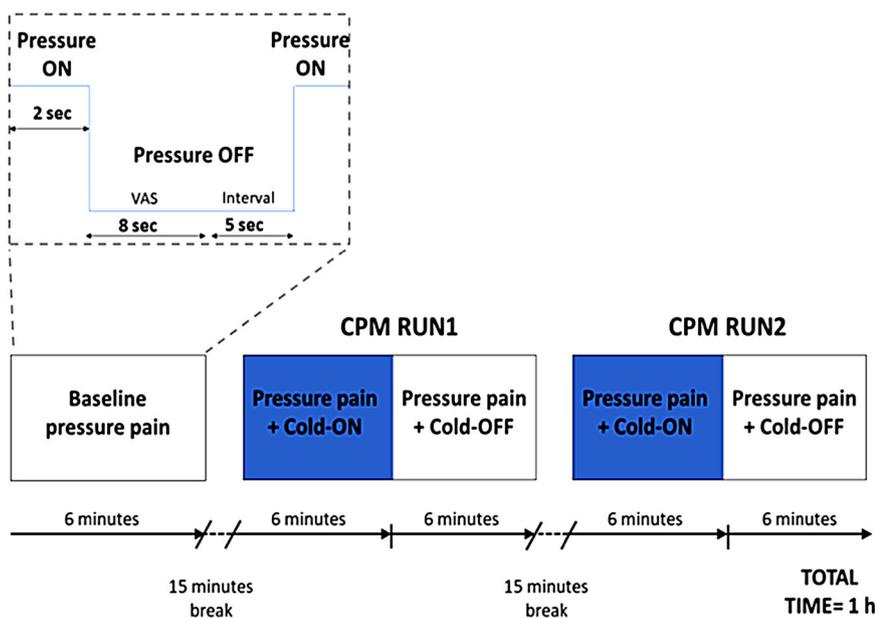


Figure 1. A graphical representation of the experimental protocol

Participants were required to rate 2 s painful pressure stimuli for 6 min (Baseline condition), followed by a 15 min break. During CPM, participants rated pressure pain with a simultaneous contralateral cold stimulation for 6 min (CPM Cold-ON condition), followed by ratings of pressure pain without the contralateral cold (CPM Cold-OFF condition). [Colour figure can be viewed at wileyonlinelibrary.com]

of each frequency component in relation to the total power spectrum minus the very low frequency band (VLF): $LF-HRV_{nu} = LF (ms^2) / (\text{total power} (ms^2) - VLF (ms^2))$ (Tarvainen *et al.* 2014). Raw (non-normalised), LF-HRV reflects an influence of both the sympathetic and parasympathetic nervous systems (Reyes del Paso *et al.* 2013). Nevertheless, in the case of normalized units (necessary in studies with a small sample size) LF (and high frequency-HF) normalized measures become linearly associated, and therefore provide a more generalized index of autonomic reactivity (Burr, 2007). A time-based HRV measure – the root mean square successive difference, rMSSD – was also calculated. RMSSD is an HRV measure which is sensitive to changes in parasympathetic tone (Laborde *et al.* 2017), providing complementary information to LF-HRV analyses.

Rs-fMRI pre-processing

Analysis of Functional NeuroImages (AFNI, Cox, 1996), the Advanced Normalization Tools (ANTs) (Avants *et al.* 2011) and the FMRIB Software Library (FSL, Smith *et al.* 2004) toolboxes were used to process multi-echo rs-fMRI data. The volumes were first re-aligned and slice timing-corrected. Next, multi-echo sequences were optimally combined (OC) by taking a weighted summation of the three echoes using an exponential T2* weighting approach (Posse *et al.* 1999). A multi-Echo ICA approach (implemented by the tool meica.py, Version v2.5 beta9) (Kundu *et al.* 2013) was adopted to de-noise the OC-data. Multi-Echo ICA is an effective method for the removal of physiological and motion-related noise, resulting in a significant increase in the temporal SNR (Kundu *et al.* 2013; Dipasquale *et al.* 2017). 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 κ and 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). Lastly, the white matter (WM) and cerebrospinal fluid (CSF) signals were regressed-using FSL. A high-pass temporal filter (with a cut-off frequency of 0.005 Hz) and spatial smoothing with a 5 mm full-width at half-maximum (FWHM) Gaussian kernel were then applied. 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) with a $2 \times 2 \times 2 \text{ mm}^3$ resampling using ANTs.

VBM pre-processing

T1-weighted volumes from all participants were visually reviewed to exclude the presence of macroscopic artefacts, prior to pre-processing in SPM12 (Statistical Parametrical Mapping, <http://www.fil.ion.ucl.ac.uk/spm/>) using the DARTEL algorithm. In brief, the MR images were bias-field corrected to correct non-uniformities then segmented into grey matter (GM), white matter and cerebro-spinal fluid sections using a priori tissue probability maps in International Consortium of Brain Mapping (ICBM) template space. The individual GM images were subsequently normalized to the Montreal Neurological Institute (MNI) template with a $1.5 \times 1.5 \times 1.5 \text{ mm}^3$ voxel size and modulated to maintain original individual GM volumes. Finally, all GM images were smoothed with 5 mm FWHM isotropic Gaussian kernel. Total intra-cranial volume (ICV), age and gender were used as covariate of no interest in subsequent GLM analyses (see VBM Analyses).

Seed-based fMRI: first level analysis

An ROI for the PAG (a 3 mm-radius sphere; MNI = 0, -32, -10; Coulombe *et al.* 2017) was constructed using the Marsbar toolbox implemented in SPM 12 (<http://marsbar.sourceforge.net/>). The average resting state fMRI time-series for the ROI was extracted from smoothed images for each participant and scan and used as a regressor in a 1st level SPM analysis, to explore the network of areas associated with the seed region.

Assessment of relationships between brain FC, brain structure and self-reported pain and HRV measures

For both seed-based fMRI and VBM analyses, we sought to determine brain regions positively or negatively associated with $\Delta\text{CPM Cold-ON}$, with LF-HRVnu in response to cold, or with the interaction between the two variables (i.e. the interaction term obtained by multiplying $\Delta\text{CPM Cold-ON}$ with LF-HRVnu). We further explored the association between brain structure/FC and both pressure pain threshold and cold pain ratings to determine whether our results were dependent on descending pain modulating pathway or rather on more general characteristics of the stimuli (i.e. pressure pain threshold or subjective perception of cold pain) adopted in our protocol.

Seed-based second level analyses. For the second-level seed-based regression analyses, we calculated the ΔFC of the PAG network in response to cold pain, by

subtracting baseline FC maps from those during cold pain. Δ FC maps were then used for regression analyses within the framework of the general linear model with Δ CPM, LF-HRVnu, and the Δ CPM \times LF-HRVnu interaction term as covariates of interest; these analyses were performed to explore brain areas in which FC during pain was associated with descending pain modulation and with the interaction between descending pain modulation and autonomic reactivity to pain. Age and gender were used as covariates of no interest.

VBM second level analyses. Statistical analyses were performed on smoothed GM maps within the framework of the general linear model. As in the seed-based fMRI analysis, Δ CPM, LF-HRVnu (during cold) and the Δ CPM \times LF-HRVnu interaction term were used as variables of interest in regression analyses, whereas intra-cranial-volume volume was used as a nuisance covariate to consider potential confounding effects of individual differences in head size.

Small volume correction analyses for a priori-hypothesized regions of interest. To test *a priori* hypotheses, small volume correction (SVC) analyses were performed in the vmPFC, PAG (for the structural analyses only) and rostral ventromedial medulla (RVM). A 5 mm radius spherical mask was created for the PAG (MNI = 0, -32, -10). For the RVM, three separate 3 mm radius spheres were created, centred at MNI (x,y) = [0, -34], at three contiguous rostro-caudal levels from

MNI (z) = [-53 to -49], as described in Marciszewski *et al.* (2018). The three contiguous spheres were then merged resulting in a final single ROI. Finally, an anatomical mask was defined for the vmPFC, derived from the SPM-12 anatomy toolbox (Eickhoff *et al.* 2005). A graphical representation of the three ROIs is provided Fig. 2.

For each participant, we extracted GM volume values and FC parameter estimates from the vmPFC, PAG and RVM masks. These values were used for further mediation analyses. GM volume values and FC parameter estimates were also extracted from significant voxels and plotted for illustrative purposes (and to explore for possible outliers). Parameter estimates from significant voxels were also used for additional correlation analysis, to investigate whether Δ CPM related brain areas were associated with LF-HRVnu and vice versa.

Statistical inference. For both types of analyses, statistical thresholds were set to $P < 0.05$ - FWE-corrected at cluster level (Worsley & Friston, 1995) (cluster size defined using uncorrected voxel-level threshold $P < 0.001$). Exploratory analyses were also performed using a less stringent cluster forming threshold ($P < 0.005$).

Correlation and mediation analyses

Mediation analysis was performed to ascertain whether pain and ANS related brain regions mediated the relationship between autonomic reactivity to pain and

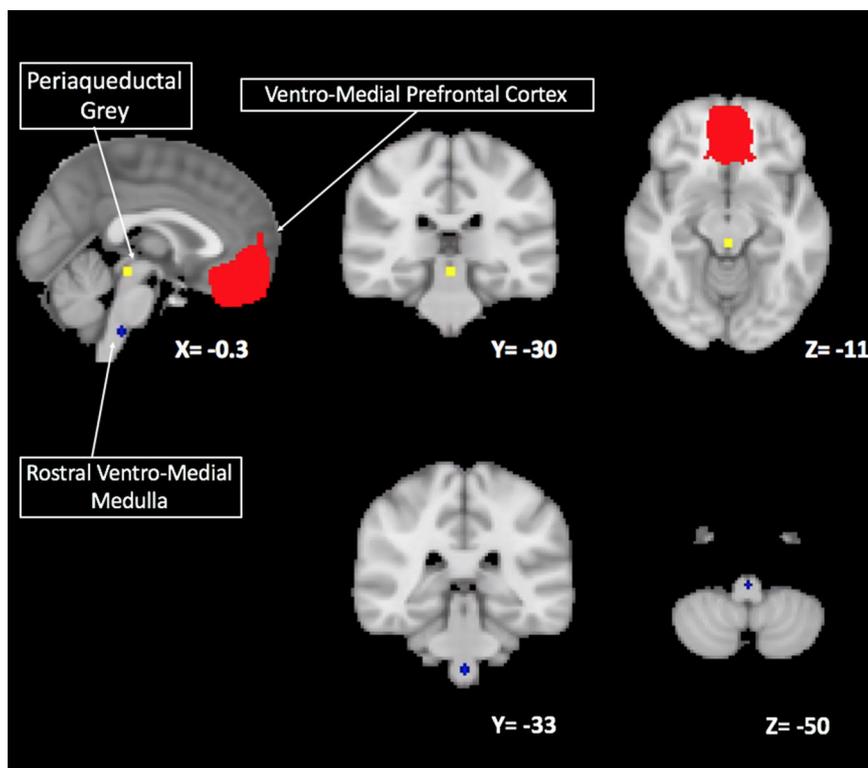


Figure 2. Graphical representation of the regions of interest (ROIs) adopted for the small volume correction [Colour figure can be viewed at wileyonlinelibrary.com]

CPM response. Data analysis was performed with SPSS 22.0 for Windows (SPSS Inc, USA). First, Pearson's correlation coefficient was used to explore the association between LF-HRVnu during cold and Δ CPM ratings (Δ CPM = (pressure + cold) – (pressure only baseline)). Next, the mean parameter estimates were extracted from SVC masks (PAG, RVM and vmPFC) were entered in a mediation analysis using PROCESS (Hayes, 2012) in IBM SPSS version 23.0 (IBM Corp., Armonk, NY). LF-HRVnu was introduced as an independent variable, Δ CPM was the outcome variable, and brain functional and structural parameter estimates from SVC masks were possible mediators. The indirect effect was tested with 20,000 percentile-corrected bootstrap confidence intervals (95%).

Results

Subjective pain ratings following cold pain

Following 6 min 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 6 min post-cold pain recovery phase, the participant pain intensity (rated on the VAS) was 1.50 (SD = 3.37).

Effect of anxiety

We did not observe a significant difference in state anxiety between the three experimental sessions ($F(2,48) = 1.03$, $P = 0.37$).

VAS ratings during CPM

Mean and standard deviation for pain rating in the five experimental conditions (Baseline condition, CPM RUN1 Cold-ON and Cold-OFF, CPM RUN2 Cold-ON, and Cold-OFF) and three pressure pain conditions (VAS 50/100, 60/100 and 70/100) are reported in Table 1.

Ratings from conditions CPM RUN1 and CPM RUN2 were averaged, separately for Cold-ON and Cold-OFF. We computed a within-subject ANOVA analysis with two factors (pressure (50, 60, 70 VAS)) and (condition (Baseline, Cold-ON, Cold-OFF)). Logarithmic transformation was applied to achieve normality of the data. A main effect of pressure was found ($F(2,58) = 31.02$, $P = 0.001$), driven by higher VAS ratings in response to 60-pressure stimuli comparing to 50-pressure stimuli (mean (SD) = 31.5 (5.8) vs. 38.3 (16.9) for 50 and 60 pressure respectively, $t(20) = 6.92$, $P = 0.001$); higher ratings to 70-pressure stimuli (mean (SD) = 45.8 (18.2)) when compared to both 60-pressure (mean (SD) = 40.0 (18.2); $t(29) = 5.87$, $P = 0.001$) and 50-pressure stimulations (mean (SD) = 34.3 (17.5); $t(29) = 8.27$,

$P = 0.001$). A main effect of Condition was also observed ($F(2,58) = 4.84$, $P = 0.016$), driven by an increase in overall perceived pain intensity in the CPM Cold-OFF condition (mean (SD) = 42.8 (19.8)) when compared to Baseline (mean (SD) = 38.4 (17.3), $t(29) = 2.43$ $P = 0.02$) and CPM Cold-ON trials (mean (SD) = 38.9 (17.9), $t(29) = 3.0$ $P = 0.005$). No significant difference was observed when comparing CPM to Baseline ($t(29) = 0.33$, $P = 0.74$). No significant effect of the condition \times pressure interaction was observed ($F(2,58) = 2.01$, $P = 0.12$). Given the lack of significant interaction with pressure levels, data were averaged across different pressure conditions for further analyses.

The distribution of Δ CPM (CPM Cold-ON and CPM Cold-OFF) values is represented in Fig. 3. During the CPM Cold-ON condition, 16 participants reported an increase in pain rating (i.e. positive Δ CPM), and 14 participants reported a decrease in pain ratings (i.e. a negative Δ CPM). During the CPM Cold-OFF condition, 22 participants reported a positive Δ CPM, whereas 8 participants reported a negative Δ CPM.

Exploratory correlation analyses

Exploratory analyses were performed to investigate whether the magnitude of the CPM response was related to either the characteristics of the test stimulus, the conditioning stimulus, or to participants' anxiety levels. Results indicated that the magnitude of the CPM Cold-ON response (Δ CPM Cold-ON) was not associated with cold pain ratings ($P = 0.92$), with individual pressure pain thresholds ($P = 0.81$), or state anxiety levels during the CPM session ($P = 0.83$).

Correlation between HRV and CPM response

We observed a negative correlation between LF-HRVnu during cold pain and Δ CPM Cold-ON ($r = -0.49$, $P = 0.041$), where individuals with the highest increase in LF-HRVnu during cold pain exhibited the strongest decrease in pressure pain rating during CPM (Fig. 4). We did not observe a significant association between baseline LF-HRVnu and Δ CPM Cold-OFF ($P = 0.17$). Similarly, we did not observe a significant correlation between RMSSD and both Δ CPM Cold-ON ($P = 0.65$) and Δ CPM Cold-OFF ($P = 0.14$). Given the lack of correlation between RMSSD and CPM, further associations between this HRV measure and brain structure and functional networks were not pursued.

Seed-based analysis

Seed-based FC analysis of the PAG showed a network comprised of the bilateral parahippocampal/amygdalar

did not observe any association between PAG FC and the $\Delta\text{CPM Cold-ON} \times \text{LF-HRVnu}$ interaction term.

Exploratory analyses of the association between PAG FC and pressure pain threshold and cold-pain ratings. We did not observe any positive or negative association between PAG ΔFC and baseline pressure pain ratings.

Associations between brain structure, CPM score and autonomic reactivity (LF-HRVnu) during cold pain

We observed a negative correlation between $\Delta\text{CPM Cold-ON}$ score and vmPFC volume (Fig. 5B). These results indicate that individuals with negative ΔCPM values exhibited larger vmPFC GM volumes. We did not observe any association between GM volume and LF-HRVnu during cold, nor between GM volume and the $\Delta\text{CPM} \times \text{LF-HRVnu}$ interaction term.

Exploratory analyses of the association between GM volume and pressure pain threshold and cold-pain ratings. We did not observe any positive or negative association between GM volume and baseline pressure pain ratings.

Correlation between CPM associated PAG functional connectivity estimates, grey matter volume and LF-HRVnu

We performed *post hoc* correlation analyses to explore associations between CPM related brain areas and

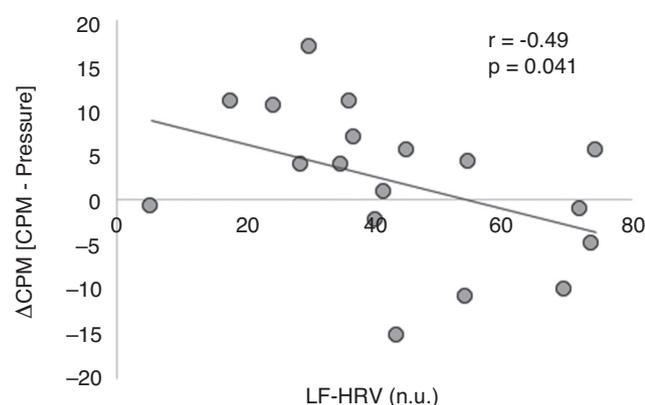


Figure 4. The association between $\Delta\text{CPM Cold-ON}$ and LF-HRVnu, investigated in 18 participants using Pearson's correlation coefficient

A negative correlation between $\Delta\text{CPM Cold-ON}$ (CPM Cold – ON-Baseline pressure pain) and LF-HRVnu was observed, indicating that individuals with the highest LF-HRVnu during cold pain exhibited a decrease in pressure pain rating during simultaneous contralateral cold pain. n.u. = normalized units.

LF-HRVnu. We observed a positive correlation between the strength of PAG-vmPFC FC with LF-HRVnu, indicating that individuals with the highest increase in LF-HRVnu during pain had also the strongest FC among these the PAG and the vmPFC when engaged in descending pain modulation (Fig. 6).

Mediation analysis

We observed a mediating role of PAG-vmPFC FC in the relationship between LF-HRVnu and the CPM effect. The total effect of LF-HRVnu on ΔCPM was significant (path C; $t = -2.23$, $P = 0.041$). Adding PAG-vmPFC FC as the mediator, the direct effect of LF-HRV on ΔCPM was no longer significant (path C'; $t < 1$, $P = 0.31$), whereas the indirect path via PAG-vmPFC FC was significant (95% CI $[-0.27, -0.08]$). Therefore, the effect of LF-HRVnu on ΔCPM was largely mediated by PAG-vmPFC FC (Fig. 7).

Discussion

ANS activity can influence pain perception (Saccò *et al.* 2013). This interaction might involve descending pain modulatory pathways. In this healthy volunteer study, we used rs-fMRI and VBM to investigate brain areas underlying one's ability to engage descending pain modulatory mechanisms (as measured by CPM) and autonomic reactivity to cold pain (as measured by the LF-HRVnu index). We observed an association between LF-HRVnu during cold pain and the magnitude of an individual's CPM response. The CPM response was associated with PAG-PCC, PAG-vmPFC and PAG-RVM FC, and with PAG and vmPFC GM volume. The same brain areas were also associated with LF-HRVnu during cold pain. The strength of the association between CPM and LF-HRVnu was mediated by PAG-vmPFC FC in response to tonic cold pain. Our results provide evidence for the mechanisms through which central, peripheral and pain systems interact, indicating that an adaptive autonomic reaction to pain is involved in descending pain inhibition.

We have demonstrated an association between each person's autonomic reactivity to pain and the efficiency of their descending pain control systems, namely a negative association between an LF-HRVnu during tonic cold pain and a decrease in subjective ratings of pressure pain during simultaneous application of a cold pain. Our findings accord with one previous study in patients with fibromyalgia showing that systolic blood pressure responses during the cold pressor test are positively associated with the CPM inhibiting effect, suggesting that chronic pain might be linked to a reduced blood pressure response to the conditioning stimulus during CPM (Chayale *et al.* 2014). Our study, in healthy participants, supports the existence of a functional pain

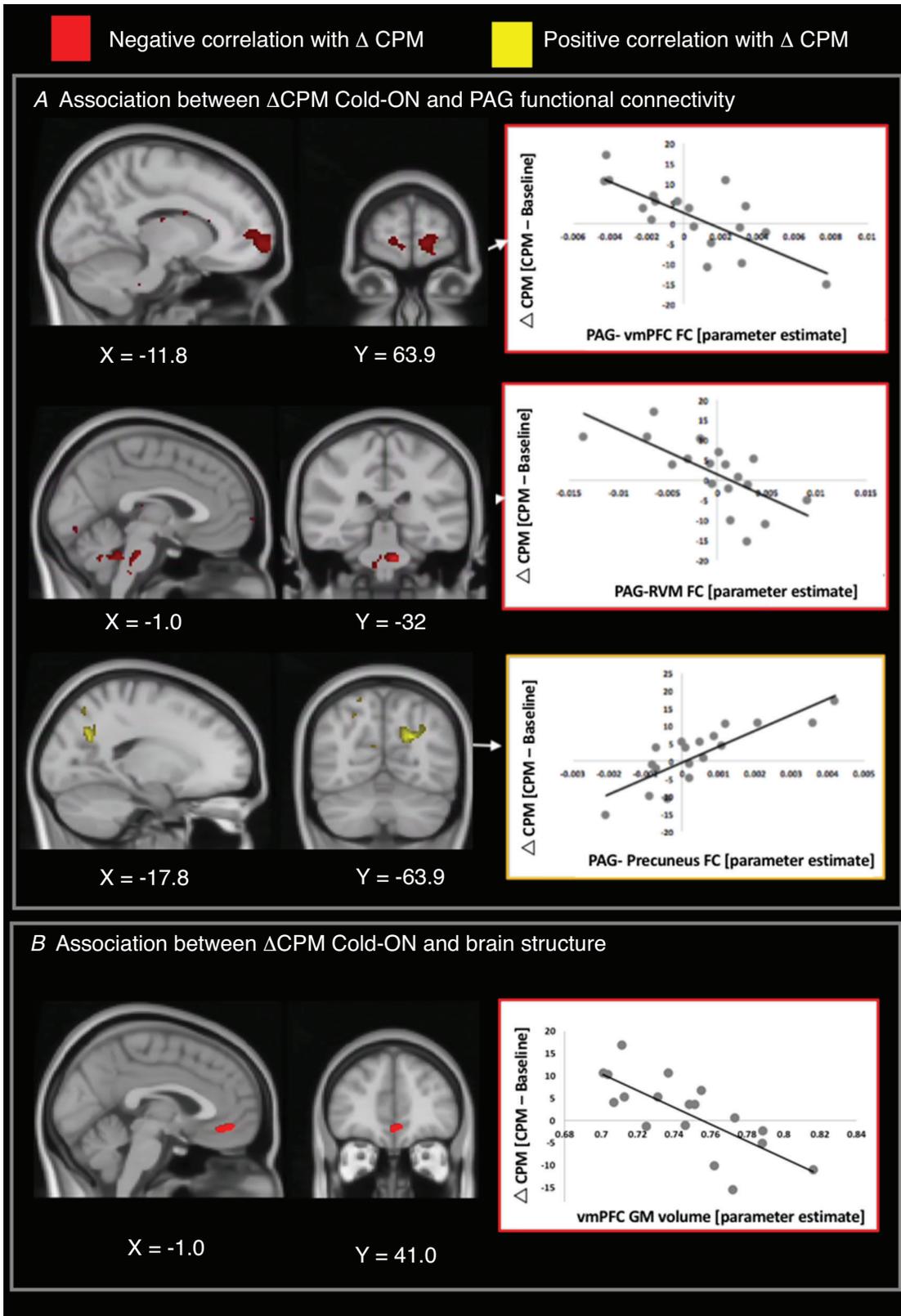


Figure 5. Association between CPM and brain structure and function, explored in 18 participants
 A, functional connectivity between the PAG and both the vmPFC and RVM was negatively associated with Δ CPM Cold-ON (CPM Cold-ON-Baseline pressure). B, Δ CPM Cold-ON was negatively associated with vmPFC GM volume. [Colour figure can be viewed at wileyonlinelibrary.com]

inhibitory feedback system described in animal models that is responsible for blood pressure-related hypoalgesia (Ghione, 1996). This model describes a reflexive increase in sympathetic arousal during pain, resulting in an increase in blood pressure (Reis *et al.* 1989). The increase in blood pressure stimulates baroreceptors, whose afferent information activates descending pain modulatory pathways, modulating perceived pain, and regaining homeostasis (Bruehl & Chung, 2004). In this work, we focused our interest on the relationship between ANS reactivity to a tonic cold painful stimulus and CPM response. However, both test and conditioning stimuli are likely to produce acute ANS reactions. Future studies should consider not only ANS reactions to conditioning stimuli, and test stimuli in isolation, but

also potential effects of combined ANS reactions elicited during simultaneous presentations of these stimuli and their relationship to the CPM response.

Our next aim was to investigate relationships between CPM response, brain FC and brain structure, and to investigate whether the HRV-CPM relationship is mediated by a common neural network. For the FC analyses, we considered the PAG as our seed region. The PAG has a known columnar organisation with functionally discrete subfields (Bandler & Shipley, 1994). While others have attempted to parcellate the PAG into its ventrolateral and dorsal components in their FC analyses (Segerdahl *et al.* 2018), this was not practical at our acquisition resolution. Here, we favoured increasing our signal to noise ratio at the cost of slightly lower spatial resolution. We suggest that individuals planning similar experiments might consider higher magnetic field strengths, (for example, 7 Tesla) and/or limited field of view acquisitions with bespoke receiver coils, to facilitate more focused investigations of PAG subfields, although note that additional challenges are likely in their implementation (Sclocco *et al.* 2018). The PAG is considered a core region involved in descending pain modulation that projects to the RVM, the output pathway for pain control to the dorsal horn of the spinal cord (Mtui *et al.* 2021). The PAG also receives cortical projections, originating principally from the mPFC (Neafsey *et al.* 1986; An *et al.* 1998); stimulation of these cortical areas can induce analgesia (Benarroch, 2012). We observed that participants who reported a reduction in pressure pain in the presence of a tonic painful conditioning stimulus, also had increased FC strength between the PAG and both the vmPFC and RVM. This result, although obtained with a more liberal cluster-forming threshold, accords with our hypotheses. Nevertheless, other fMRI studies in humans investigating brain activity underpinning CPM have provided contrasting results. In a study by Piché and colleagues (2009), a sustained increase in orbito-frontal cortex activity during the application of a cold contralateral conditioning stimulus was associated with a decrease in subjective response to evoked painful electrocutaneous test stimuli. Similarly, Bogdanov and colleagues described that individual differences in CPM (i.e. reduction in painful laser heat ratings) related to levels of PFC activation in response to the early part of the cold conditioning stimulus (Bogdanov *et al.* 2015). Youssef and colleagues, on the other hand, suggested that engagement of the prefrontal and cingulate areas interferes with the generation of CPM (Youssef *et al.* 2016). In contrast to the studies of Piche and Bogdanov, the conditioning stimulus adopted in this later study was hypertonic saline-induced muscle pain, suggesting that the discordance in results between studies is likely to relate to the specific pain modalities and/or stimulation methodology adopted in the CPM investigation

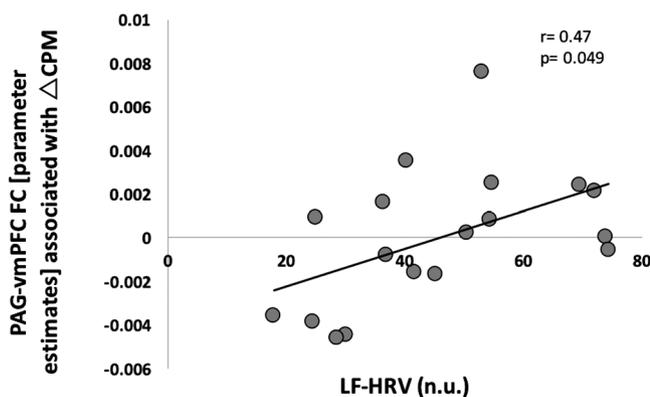


Figure 6. Correlation between LF-HRVnu and parameter estimates of CPM associated FC between PAG and vmPFC

The association was investigated in $n = 18$ participants, by means of Pearson's correlation coefficient.

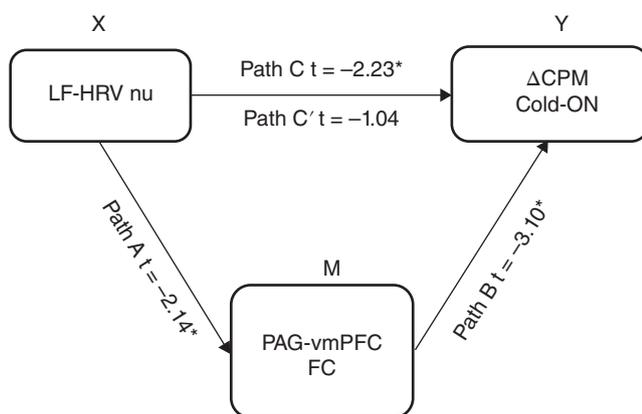


Figure 7. PAG-vmPFC functional connectivity mediates the association between LF-HRVnu and ΔCPM

Mediation analysis (performed in 18 participants) indicated that the association between autonomic reactivity during cold pain (LF-HRVnu) and the efficiency of the descending inhibitory pain mechanisms (Δ CPM) was strongly mediated by PAG-vmPFC functional connectivity (FC). The t value for each path is presented.

* $P < 0.05$.

(Kennedy *et al.* 2016; Kemp *et al.* 2019). In a resting-state fMRI study by Harper and colleagues, higher FC between the PAG, left insula and perigenual ACC was associated with more efficient inhibitory CPM responses in both healthy participants and patients with fibromyalgia, whereas PAG connectivity with the dorsal pons was associated with greater CPM inhibition only in the control group. Greater PAG connectivity to the caudal pons/rostral medulla was associated with descending pain inhibition in healthy participants, but with pain facilitation in fibromyalgia patients (Harper *et al.* 2018). Here, we provide new knowledge to the field by showing that the same brain structures are associated with both the cardiovascular responses to the conditioning stimulus and the efficiency of descending pain control. Our data indicate that the strength of FC between these structures is a mediator of the ANS-CPM association. Dysregulation of the three-way interaction between brain FC, efficiency of descending pain control mechanisms and autonomic reactivity might explain various symptoms of chronic pain conditions such as low HRV and impaired descending pain control compared to healthy controls (Lewis *et al.* 2012; Tracy *et al.* 2016). Future studies are required to investigate the existence and dysregulation of such a mechanism in chronic pain conditions.

Brain structure was also associated with the CPM effect. Specifically, individuals with larger vmPFC volumes exhibited an inhibitory CPM effect. Previous reports have described associations between GM volume reductions in the orbito-frontal cortex and the vmPFC (together with other areas such as the thalamus, insula, posterior cingulate cortex) and increased sensitivity to rectal pain stimuli in healthy volunteer participants (Elsenbruch *et al.* 2014). Reduction in vmPFC GM is found in patients with chronic complex regional pain syndrome (Geha *et al.* 2008), fibromyalgia (Kuchinad *et al.* 2007) and chronic back pain (Fritz *et al.* 2016). Similarly, patients with functional dyspepsia have decreased cortical thickness in prefrontal and dorso-lateral areas (Liu *et al.* 2018). A negative correlation was described between pain and dlPFC volumes in patients with chronic complex regional pain syndrome (Barad *et al.* 2014), whereas pain thresholds were correlated positively with dlPFC volumes in patients with chronic myofascial pain (Niddam *et al.* 2017). Here, our results show that individuals with larger vmPFC volumes report more efficient descending pain inhibition, whereas individuals with smaller GM volumes in this area showed pain facilitation during the application of the conditioning stimulus. Taken together these findings engender the working hypothesis that PFC volume reductions in multiple chronic pain conditions may be associated with impairments in descending pain control. Our data suggest that individuals with smaller vmPFC volumes exhibited pain facilitation (i.e.

an increase in pain with the contralateral presentation of the conditioning stimulus), whereas those with larger vmPFC volumes exhibited a reduction in pain (indicating efficient descending pain modulating mechanisms). We speculate that, in chronic pain patients, a reduction in vmPFC volume might be involved in the phenotypic switch between descending inhibition and pathological pain facilitation (Potvin & Marchand, 2016).

We acknowledge some methodological limitations in our study. For practical reasons, our heart rate data were acquired with a photoplethysmograph (PPG) rather than using electrocardiographic (ECG) recordings, the gold standard approach for deriving HRV measures. Some studies have shown that under certain conditions, PPG provides a good surrogate of ECG-derived HRV (Gil *et al.* 2010; Schäfer & Vagedes, 2013). We encourage future studies to replicate our findings with measures obtained from ECG. We also acknowledge that the types of painful stimulation adopted in this study, that is, pressure-pain delivered to the thumb nail bed, and cold-pain delivered via an aluminum probe, should be considered in further experiments. Both stimulation modalities are non-specific, and activate nociceptors both in the surface of the skin and in the deep tissue. Studies within our team are already in progress to further explore the involvement of different types of nociceptors in the interaction between ANS and CPM.

In conclusion, this study combined rs-fMRI, recording of the ANS responses to pain and assessments of descending pain control, providing mechanistic insights into the association between the ANS and pain in the brain. Our data adds to the large body of research focusing on the link between the individual differences in autonomic balance and physical and emotional well-being, underpinned by the PFC (Thayer *et al.* 2009). Associations identified between CPM response, resting-state connectivity networks involved in descending pain control and flexibility in HRV suggest that individuals with stronger engagement of the ANS in response to pain are also more efficient in triggering descending pain inhibition, which may lead to more expedient recovery of homeostasis. Some of our results should be considered exploratory, given their moderate sample size and (in a subset of our imaging findings) cluster forming thresholds. We encourage their replication in a novel, independent sample. Future studies should aim to shed light on potential dysregulation of these mechanisms in patients with chronic painful conditions, which in turn may signpost future therapeutic interventions for these patients. Therapies focused on stimulation of the ANS (such as HRV biofeedback, or baroreceptor stimulation) might help to regain appropriate levels of control over not only the ANS, but also mechanisms of endogenous pain control.

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Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interest

None.

Author contributions

E.M.: design of the experiment, data collection, data analysis and manuscript preparation. A.V.: data collection. D.H.S., O.D. and O.O.D.: data analysis and manuscript preparation. J.B.J.: design of the experiment, data collection, data analysis and manuscript preparation. S.M.: data collection and manuscript preparation. S.C.R.W., S.S.B. and M.A.H.: supervised the experiment planning, data collection, data analysis and manuscript preparation. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

descending pain modulation, heart rate variability, periaqueductal grey, resting state, voxel-based morphometry

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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