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# Decreased Default Mode Network Connectivity Following 24 Hours of Capsaicin-induced Pain Persists During Immediate Pain Relief and Facilitation

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**Abstract:** Prolonged experimental pain models can help assess cortical mechanisms underlying the transition from acute to chronic pain such as resting-state functional connectivity (rsFC), especially in early stages. This crossover study determined the effects of 24-hour-capsaicin-induced pain on the default mode network rsFC, a major network in the dynamic pain connectome. Electroencephalographic rsFC measured by Granger causality was acquired from 24 healthy volunteers (12 women) at baseline, 1hour, and 24hours following the application of a control or capsaicin patch on the right forearm. The control patch was received maximum 1 week before the capsaicin patch. Following 24hours, the patch was cooled and later heated to assess rsFC changes in response to pain relief and facilitation, respectively. Compared to baseline, decreased rsFC at alpha oscillations (8-10Hz) was found following 1hour and 24hours of capsaicin application for connections projecting from medial prefrontal cortex (mPFC) and right angular gyrus (rAG) but not left angular gyrus (lAG) or posterior cingulate cortex (PCC): mPFC-PCC (1hour: $P < .001$ , 24hours: $P = .002$ ), mPFC-rAG (1hour: $P < .001$ , 24hours: $P = .001$ ), rAG-mPFC (1hour: $P < .001$ , 24hours: $P = .001$ ), rAG-PCC (1hour: $P < .001$ , 24hours: $P = .004$ ). Comparable decreased rsFC following 1hour and 24hours ( $P \leq 0.008$ ) was found at beta oscillations, however, decreased projections from PCC were also found: PCC-rAG ( $P \leq 0.005$ ) and PCC-lAG ( $P \leq 0.006$ ). Pain NRS scores following 24hours ( $3.7 \pm 0.4$ ) was reduced by cooling ( $0.3 \pm 0.1$ ,  $P = .004$ ) and increased by heating ( $4.8 \pm 0.6$ ,  $P = .016$ ). However, neither cooling nor heating altered rsFC. This study shows that 24hours of experimental pain induces a robust decrease in DMN connectivity that persists during pain relief or facilitation suggesting a possible shift to attentional and emotional processing in persistent pain.

**Perspective:** This article shows decreased DMN connectivity that might reflect possible attentional and emotional changes during acute and prolonged pain. Understanding these changes could potentially help clinicians in developing therapeutic methods that can better target these attentional and emotional processes before developing into more persistent states.

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**Key words:** EEG, granger causality, resting state functional connectivity, DMN, prolonged pain.

Resting-state functional connectivity (rsFC) assessing the temporal correlations between brain regions is a metric that provides insight into brain alterations during chronic pain.<sup>5,6,17,34,42</sup> One brain

network that exhibits consistent alterations in rsFC during chronic and acute pain<sup>1,2,9</sup> is the default mode network (DMN), a major part of the dynamic pain connectome. The dynamic pain connectome consists of

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distinct brain networks that communicate with each other to give rise to the pain experience including the DMN, the salience network, and the ascending nociceptive and descending modulation pathways.<sup>47,48</sup> The DMN serves as a "sentinel" to consciousness by regulating internal and external attention to ensure responsiveness to the external world.<sup>29,81,87</sup> The DMN consists of a subset of cortical regions including the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), and bilateral angular gyrus (AG).<sup>79</sup> At rest, these regions exhibit high connectivity that decreases (deactivated) during goal-directed tasks and cognitively demanding stimuli.<sup>25,26,79</sup> DMN deactivation may signify a shift from internal processes (involving daydreaming or internal attention to self-referential thoughts) to external processes (involving external attention to the surrounding and vigilance to ongoing changes in the environment) necessary for responding to various contextual demands.<sup>3</sup> The absence of this deactivation is linked to poor cognitive functioning in humans<sup>4,46,94,102</sup> and animals.<sup>23</sup>

Recent evidence shows abnormalities in the cross-network communication involving the DMN and other networks in individuals with chronic pain.<sup>14,34,37,51,105</sup> Given that DMN regions are heterogeneous and differentially sensitive to different stimuli,<sup>52,90</sup> it is equally important to examine how connectivity within the DMN is affected during chronic pain. Unfortunately, there are mixed findings regarding the effect of chronic pain on DMN connectivity. Some studies reported increased DMN connectivity during chronic pain<sup>50,55,106</sup> that can be attributed to maladaptive enhanced focus on internal pain-related thoughts and emotions. Other studies reported decreased DMN connectivity during chronic pain<sup>2,9,13,34</sup> suggesting a maladaptive and persistent state of heightened attention/vigilance.

The mechanisms underlying the transition from acute to chronic pain are still unknown. One possibility may be that an acute response to pain initiates the process or that the development of chronic pain stems from unknown distinctive mechanisms. Alshel et al. examined the effects of both acute and chronic pain on DMN connectivity. They reported decreased connectivity for acute and chronic pain and suggested a shared mechanism,<sup>2</sup> which disagrees with other reports of increased DMN connectivity during chronic pain.<sup>50,55,106</sup> The increases in DMN connectivity may indicate a distinct underlying mechanism for chronic pain.

Experimental models of prolonged pain for hours/days (eg, topical capsaicin<sup>31</sup> or intramuscular injection of nerve growth factor<sup>19,91</sup>) may bridge the gap in our knowledge on the cortical mechanisms underlying DMN reorganization during the transition from acute to chronic pain, especially during the early stages. Therefore, this study aimed to examine changes in DMN connectivity as assessed by electroencephalography (EEG) following 1-hour- and 24-hour-capsaicin application. Moreover, given that cortical reorganization during chronic pain may be independent of changes in pain intensity,<sup>53,98,103</sup> this study aimed to determine the

stability of the 24-hour-pain-related connectivity changes in response to immediate cooling or heating. It was hypothesized that 1) compared to the pain-free baseline and control condition, DMN connectivity would decrease following 1 hour and 24 hours of capsaicin application, and 2) the connectivity decrease present 24 hours-post capsaicin application would not be influenced by changing pain intensity perception by cooling or heating the primary application site.

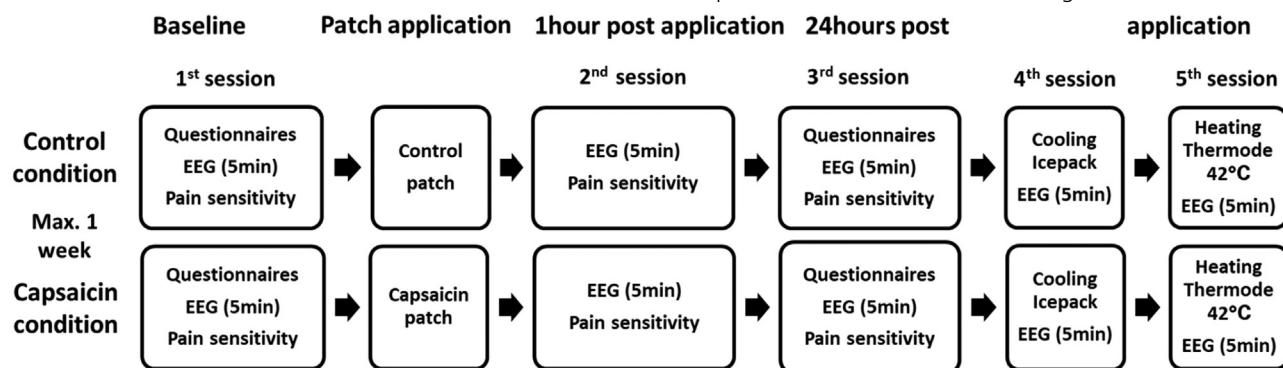
## Methods

### Participants

Twenty-four healthy right-handed volunteers (12 women, age  $27.5 \pm 5.1$  years, mean  $\pm$  SD) were recruited online and through flyers posted at Aalborg University to participate in the study. Edinburgh Handedness Inventory<sup>73</sup> was used to assess handedness. Participants reported no neurological or psychiatric disorders, no pregnancy, no use of pain medications, no current or chronic pain, and no significant medical disorders. Moreover, participants were instructed to refrain from coffee or alcohol consumption at least 6 hours before the experiment onset. Sample size calculations were based on our previous work on connectivity and pain,<sup>1</sup> the effect size ranged between ( $\eta^2=0.27-0.43$ ). The aim of this study was to assess DMN connectivity in 2 conditions (capsaicin/control) across 3 time points: baseline, 1 hour, and 24 hours. Using the smallest effect size ( $\eta^2=0.27$ ,  $d=0.6$ ), sample size was calculated using G\*power 3.1.9.6 (Kiel Universität, Germany) with 80% power and an alpha level of 0.05% for ANOVA repeated measures-within factors design: 1 group and 6 within group measurements (3 time points and 2 conditions). Due to the large effect size, a minimum of 5 participants was required to show significant within factors interaction or main effect. To be more conservative, a moderate effect size (0.3) was used, and a minimum of 20 participants was required. To account for possible dropouts, 24 participants were recruited. Study procedures were conducted in accordance with the Helsinki Declaration, approved by the local ethics committee (N-20190057) and registered at ClinicalTrials.gov (NCT05158309). Signed informed consent was given by all participants before the experiment. Data collection took place at Aalborg University in Denmark between May 31, 2021 to November 10, 2021.

### Experimental Design

This crossover study included 2 conditions (control/capsaicin) separated by maximum 1 week, with each condition consisting of 5 sessions (Fig 1). Given that 24-hour-capsaicin patch application may result in residual effects lasting 21 days after patch removal,<sup>57</sup> the Control - condition always preceded the capsaicin - condition. Participants were informed that they will experience both capsaicin and control conditions in separate days without specifying which condition will be first. They



**Figure 1.** Experimental design. EEG, electroencephalography, Pain sensitivity, mechanical pain sensitivity assessment.

were also informed that there will be one patch for each condition with the capsaicin patch containing chilli as the main ingredient and the control patch having no effect. However, at the time of application, participants were not aware which patch was applied. It is also noteworthy that participants have not previously tried the capsaicin patch. The first session consisted of a series of baseline measures for each condition, including EEG recordings and mechanical pain sensitivity assessment, followed by patch application (control or capsaicin). In the second and third sessions following 1hour and 24hours, EEG recordings and mechanical pain sensitivity measures were repeated.

In the fourth and fifth sessions following 24hours of patch application, the patch (control or capsaicin) was cooled then heated to relieve or enhance ongoing pain, respectively. An ice pack was placed on the patch for 5 minutes to cool the area. A thermal stimulator (30 × 30 mm probe; Pathways Model ATS, Medoc Ltd, Israel) was applied over the patch with a baseline probe temperature of 32°C, which changed to 42°C at a rate of 3°C/second<sup>36</sup> to heat the area. The temperature remained constant at 42°C for 5 minutes, followed by a return to baseline at a rate of 5°C/second. EEG signals were recorded throughout the 5-minute cooling/heating procedures. The 2 procedures were performed consecutively, and participants reported pain intensity scores immediately after each procedure.

### Experimental Pain Model

An 8% topical capsaicin patch (Transdermal patch, 'Qutenza', Astellas, 5 × 10 cm) was used to induce cutaneous pain over the volar part of the dominant right forearm (about 5 cm from the wrist). For the control condition, an adhesive transparent patch of the same size was applied to the same location. The capsaicin patch was applied according to the manufacturer's instructions using nitrile gloves. The patches were covered with 2 layers of medical tape (Fixomull stretch, BSN medical, Victoria, Australia) to maintain blinding.

A numerical rating scale (NRS) anchoring from 0 (no pain) to 10 (worst imaginable pain) was used to assess the pain intensity. Throughout the first 1 hour of the patch application, participants were instructed to report their NRS pain ratings every 5 minutes. For the

remaining 23 hours, participants kept a pain diary reporting pain intensity every hour except for sleeping hours. Three pain parameters were then calculated for the 1-hour and 24-hour measurements: average pain reflecting intensity across all the 5-minute NRS reports (1hour) or across all the 1-hour NRS reports (24hours), current pain intensity as the NRS pain level reported at the end of 1-hour or 24-hour measurements, and the peak pain intensity as the highest NRS pain score reported during 1hour or 24hours.

### Mechanical Pain Sensitivity Assessment

Mechanical pain sensitivity was assessed by mechanical pain thresholds (MPT) using a set of 7 weighted pin-prick stimulators consisting of steel tubes ending with a tip contact diameter of 0.25 mm and exerting forces of 8, 16, 32, 64, 128, 256, and 512 mN (MRC Systems GmbH, Germany). The stimulators were applied in the area (2 × 5cm) surrounding the patch (control/capsaicin) at a rate of 2 seconds on, 2 seconds off in an ascending order,<sup>82</sup> starting with 8 mN until the subject perceived the stimulator as sharp, pricking, or stinging (first supra-threshold value). Once the first painful stimulus was perceived, the test direction changed in descending order until the subject perceived the stimulator as blunt (first subthreshold value). This ascending/descending testing was repeated 5 times and the final threshold was calculated as the geometric mean of these 5 ascending/descending stimuli series. MPT was measured at baseline, 1hour, and 24hours following patch application. Prior to the assessment, participants were familiarized with the test at the same location of the left forearm.

### Electroencephalographic Acquisition

For each participant, EEG signals during the capsaicin and control conditions were recorded at the same time of the day. During the recordings, participants were seated on a comfortable chair in a light-dimed and sound-attenuated room and were instructed to stay still and relax without falling asleep. For each condition (control or capsaicin), EEG signals were recorded for 5 minutes with eyes closed at 5 time-points: Baseline, 1hour, 24hours (following patch application), during

cooling and heating the patch following 24 hours of application (Fig 1).

EEG data were collected using an EEG cap consisting of 64 electrodes (g.GAMMA cap2, g.tec, Austria). The cap was positioned according to 10-5 system with Cz placed on the vertex of the head.<sup>19</sup> To monitor eye movement, an additional EEG electrode (Fp1) was placed above the left eye. EEG signals were amplified (50000x) with a sampling rate of 1200 Hz (g.Hlamp bio-signal amplifier, g.tec, Austria). The electrode impedances were maintained below 5 k $\Omega$ .

### Electroencephalographic Processing

Brain Electrical Source Analysis (BESA Research 7.1, GmbH, Gräefelfing, Germany) was used to process and analyze EEG data. Data were high-pass filtered at 0.53 Hz, low-pass filtered at 175 Hz, and notch-filtered at 50 Hz. Each 5-minute EEG recordings were segmented into 2-second epochs, and screened visually for evident artifacts. Using independent component analysis (ICA) in BESA, the epochs were then screened again for eye movement- and cardiac-related artifacts and distorted components were excluded from further analyses. The epochs were then scanned further in BESA to exclude epochs having artifacts not related to cardiac or eye movement. An epoch was marked bad and excluded from further processing, if its amplitude exceeded 120  $\mu$ V or fell below 0.07  $\mu$ V or it contained amplitude jumps of > 75  $\mu$ V between 2 sampling points. Epochs were also considered bad during which the signal was smaller than the threshold criterion of 0.01  $\mu$ V. Connectivity analysis was performed on each session's screened, accepted, and artifact-cleaned epochs.

### Default Mode Network Connectivity

EEG data were transformed in BESA to source space using a built-in pre-defined resting-state source montages for the DMN.<sup>1,85</sup> This source montage was created based on a source localization method called multiple discrete sources. Specifically, the time series of neuronal activity within the DMN was reconstructed using a multiple discrete sources approach, a source localization method that offers a stable linear transformation of EEG data into source space with limited "cross-talk" between source areas.<sup>88</sup> In this method, the recorded data were interpolated onto the standard 81 electrodes, after which the source waveforms were calculated employing spatial filters that rely on a 4-shell ellipsoidal head model.<sup>88</sup> Source analysis takes data away from the scalp into the brain, reducing the effect of volume conduction and field spread caused by scalp electrodes.<sup>62,88</sup> To ensure the specificity of the DMN and increase the sensitivity of the DMN sources, the resting state DMN montage in BESA includes 6 pre-defined noise sources: left and right occipital, left and right frontal, medial frontal, and medial parietal cortices. Regional sources of the DMN areas and the noise sources were positioned at the locations assigned by Montreal Neurological Institution (MNI) based on the DMN system identified by

Power et al.<sup>79</sup> Four major DMN regions were selected: mPFC, PCC, left AG (lAG) and right AG (rAG).<sup>79</sup>

BESA connectivity 1.0 (MEGIS Software GmbH, Gräefelfing, Germany) was used to examine connectivity between the pre-defined DMN sources (mPFC, PCC, bilateral AG). The data were transformed using the complex demodulation method to the time-frequency domain. Complex demodulation is a technique relying on time-frequency analysis and describes the amplitude and phase of a specific frequency component of a time series, offering a uniform frequency resolution across the analysis bandwidth.<sup>32</sup>

Analysis of brain oscillations contributing to the DMN activity revealed a positive correlation with alpha and beta activity,<sup>11,35,39,59,68,87,97</sup> 2 neuronal oscillations playing an essential role in chronic pain.<sup>42</sup> Our previous study showed no differences in pain-related connectivity at upper alpha (11 – 13 Hz),<sup>1</sup> which is also supported by studies showing that lower alpha (8 – 10 Hz), but not upper alpha (11 – 13 Hz) oscillations, are involved and may explain individual differences in pain processing and perception.<sup>44,69</sup> Therefore, this DMN study focused on the lower alpha range (8 – 10 Hz) and beta (14 – 30 Hz).

Granger causality was selected as a connectivity measure and calculated in the frequency domain<sup>28</sup> employing a non-parametric spectral factorization approach<sup>20</sup> at alpha (8 – 10 Hz) and beta (14 – 30 Hz) bands. This connectivity measure assesses the directionality of information flow between EEG signals,<sup>99</sup> ie, when a past value of a signal Y can predict a future value of a signal X, then Y is said to Granger-causes X.<sup>30</sup> rsFC at lower alpha and beta oscillations was computed at ten DMN connections (mPFC-PCC, mPFC-rAG, mPFC-lAG, PCC-mPFC, PCC-rAG, PCC-lAG, rAG-PCC, rAG-mPFC, lAG-PCC, lAG-mPFC) as the average of rsFC scores for all the artifact-free epochs in each sub-condition. To assess DMN specificity, possible communication between the noise sources and the DMN regions was examined prior to investigating the DMN regions at lower alpha (Table S1) and beta (Table S2) oscillations. No significant effects for condition or time factors were found. This suggests that any observed pain-related change in connectivity within the DMN is less likely to be attributed to any external source.

### Statistics

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, N.Y., USA). All data are presented as the mean and standard error of the mean (SEM). Statistical significance was shown as  $P < .05$  (unless stated otherwise), and the associated effect size was presented as  $\eta^2$  or Cohen's d. Normality was assessed using kurtosis and skewness and their corresponding z scores, and variables were considered normal when z scores did not exceed  $\pm 1.96$ .<sup>24</sup> A paired-samples t-test was used to assess the difference between 1 hour and 24 hours for NRS pain scores. Ten separate repeated measures analysis of variance (ANOVA)



were performed to assess rsFC at ten pairs: 1) mPFC-PCC, 2) mPFC-rAG, 3) mPFC-IAg, 4) PCC-mPFC, 5) PCC-rAG, 6) PCC-IAg, 7) rAG-PCC, 8) rAG-mPFC, 9) IAg-PCC, 10) IAg-mPFC. For each ANOVA, the within-subject factors were *time* (baseline, 1hour, 24hours), *condition* (control, capsaicin). Because of multiple ANOVAs (10 pairs), *P*-value for accepting significant main effects or interactions was Bonferroni-corrected to  $P < .005$  (ie, 0.05/10). Where appropriate, post-hoc analyses (Bonferroni-corrected) were performed. To assess whether changes in rsFC following the 24-hour application were affected by pain relief or exacerbation, repeated measures ANOVAs with within-subject factors as *time* (24hours, 24-hours-cooling, 24-hours-heating) and *condition* (control, capsaicin) were performed. A paired-samples *t*-test was used to assess the difference between current pain following 24hours and current pain following heating. When normality was violated, Wilcoxon signed-rank test was used to assess the difference between current pain following 24hours and current pain following immediate cooling. Spearman's correlation was used to assess the relationship between NRS pain intensity scores and the change in rsFC following 1hour and 24hours among connections that exhibit a significant change in response to capsaicin application. Due to multiple correlation analyses, the significance level for the correlations was Bonferroni-corrected based on the number of correlations performed. To investigate whether secondary pin-prick hyperalgesia was associated with the experimental pain model, repeated-measures ANOVA was performed on the mechanical pain threshold (MPT) with factors *time* (baseline, 1hour and 24hours) and *condition* (control and capsaicin).

## Results

All participants were included in the analyses.

**Table 1. ANOVA Findings Comparing Lower Alpha (8-10Hz) Resting State Functional Connectivity (rsFC) Between Baseline and After 1hour and 24hours (Time) of Capsaicin-induced Pain and Control (Condition) (N = 24) at Ten DMN Connections (mPFC-PCC; mPFC-IAg; mPFC-rAG; PCC-mPFC; PCC-IAg; PCC-rAG; rAG-PCC; rAG-mPFC; IAg-PCC; IAg-mPFC). F-values and P-values are From the Repeated Measures ANOVA (Significance Accepted at  $P < .005$  is Indicated in Bold, Bonferroni Corrected Due to Multiple ANOVAs)**

DMN CONNECTION	MAIN EFFECT		INTERACTION	
	CONDITION	TIME	CONDITION X TIME	
mPFC-PCC	$F(1,23)=17.58, P < .001, \eta^2=0.43$	$F(2,46)=6.02, P = .005, \eta^2=0.21$	$F(2,46)=6.16, P = .004, \eta^2=0.21$	
mPFC-IAg	$F(1,23)=2.13, P = .157, \eta^2=0.09$	$F(2,46)=11.22, P < .001, \eta^2=0.33$	$F(2,46)=6.21, P = .004, \eta^2=0.21$	
mPFC-rAG	$F(1,23)=10.64, P = .01, \eta^2=0.26$	$F(2,46)=6.02, P < .001, \eta^2=0.32$	$F(2,46)=7.72, P = .001, \eta^2=0.25$	
rAG-mPFC	$F(1,23)=1.95, P = .176, \eta^2=0.08$	$F(2,46)=14.31, P < .001, \eta^2=0.38$	$F(2,46)=6.56, P = .003, \eta^2=0.22$	
rAG-PCC	$F(1,23)=0.004, P = .952, \eta^2=0$	$F(2,46)=11.59, P < .001, \eta^2=0.36$	$F(2,46)=6.86, P = .002, \eta^2=0.23$	
IAg-mPFC	$F(1,23)=2.82, P = .107, \eta^2=0.11$	$F(2,46)=4.88, P = .012, \eta^2=0.18$	$F(2,46)=2.01, P = .145, \eta^2=0.08$	
IAg-PCC	$F(1,23)=0.054, P = .818, \eta^2=0$	$F(2,46)=7.27, P = .002, \eta^2=0.24$	$F(2,46)=2.81, P = .071, \eta^2=0.11$	
PCC-mPFC	$F(1,23)=2.79, P = .109, \eta^2=0.11$	$F(2,46)=2.02, P = .145, \eta^2=0.08$	$F(2,46)=0.246, P = .783, \eta^2=0.01$	
PCC-IAg	$F(1,23)=1.52, P = .229, \eta^2=0.06$	$F(2,46)=5.46, P = .007, \eta^2=0.19$	$F(2,46)=1.56, P = .222, \eta^2=0.06$	
PCC-rAG	$F(1,23)=1.69, P = .207, \eta^2=0.15$	$F(2,46)=7.27, P = .001, \eta^2=0.06$	$F(2,46)=1.18, P = .315, \eta^2=0.04$	

## Capsaicin Application Following 1hour and 24hours

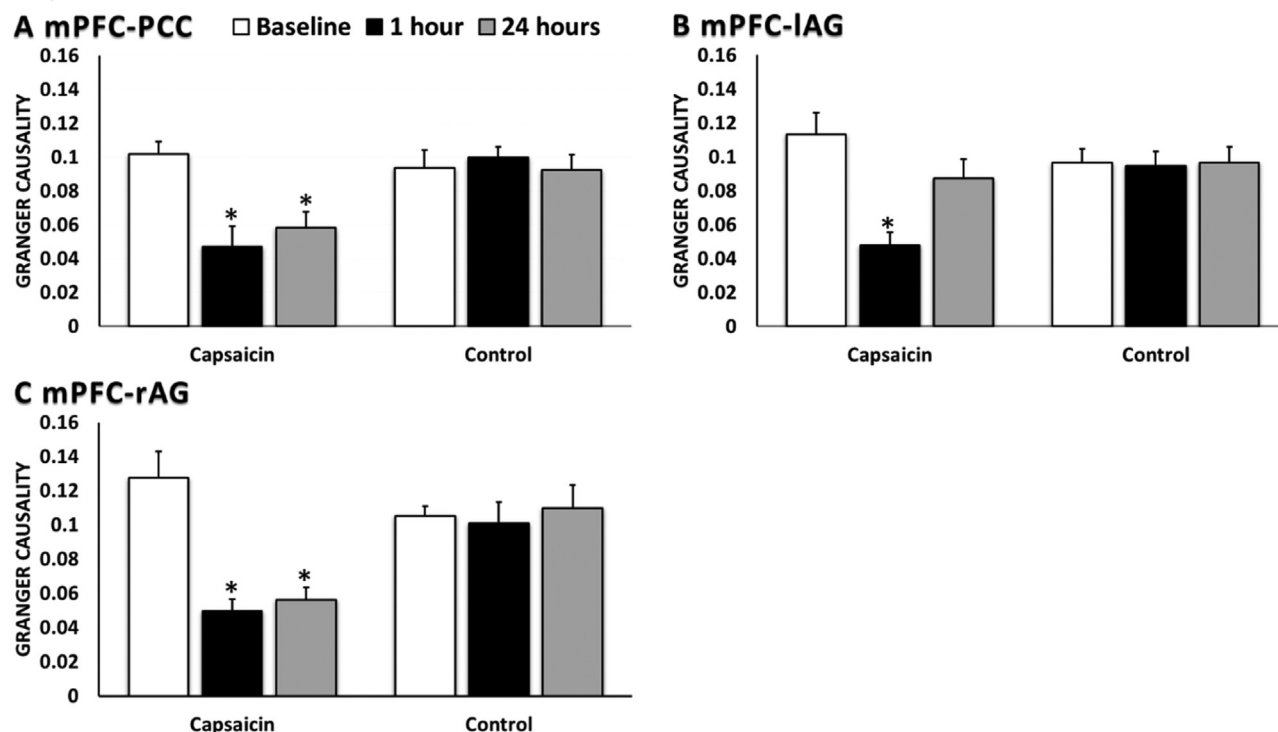
Current pain NRS scores for capsaicin following 1hour and 24hours were  $5.9 \pm 0.4$  and  $3.8 \pm 0.4$ , respectively, which were higher than control  $0.4 \pm 0.2$  and  $0.1 \pm 0.0$ . Similarly, average capsaicin pain NRS scores were  $4.3 \pm 0.4$  and  $5.4 \pm 0.4$ , which were higher than control  $0.3 \pm 0.1$  and  $0.2 \pm 0.1$ . Finally, capsaicin peak pain NRS scores ( $6.5 \pm 0.4$  and  $7.4 \pm 0.4$ ) were higher than control ( $0.7 \pm 0.2$  and  $0.7 \pm 0.2$ ).

There was a difference in NRS pain scores between 1hour and 24hours post capsaicin application across all 3 pain parameters. Current pain NRS scores decreased between 1hour and 24hours (paired *t*-test:  $t(23)=5.65, P < .001, d=1.9$ ). In contrast, average pain and peak pain NRS scores increased (paired *t*-test: average pain NRS:  $t(23)=3.06, P = .006, d=1.3$ ; peak pain NRS:  $t(23)=3.10, P = .005, d=1.8$ ).

## Default Mode Network Connectivity at Lower Alpha (8-10Hz) Oscillations Following Capsaicin Application

Only changes at connections showing significant time X condition interaction, where the capsaicin condition but not the control condition showed significant change, were considered as pain-related changes.

Connections projecting from the mPFC (mPFC-PCC, mPFC-IAg, mPFC-rAG): ANOVAs revealed a condition x time interaction for the 3 connections (Table 1). Post hoc analysis showed a decrease in lower alpha rsFC following capsaicin-induced pain compared to baseline for the 3 connections following 1hour (mPFC-PCC:  $P < .001, d=1.1$ , Fig 2A; mPFC-IAg:  $P < .001, d=1.1$ , Fig 2B; mPFC-rAG:  $P < .001, d=1.2$ , Fig 2C) and for 2 connections following 24hours (mPFC-PCC:  $P = .002, d=0.80$ , Fig 2A; mPFC-rAG:  $P = .001, d=0.85$ , Fig 2C) but not significantly reduced for mPFC-IAg following 24hours ( $P = .195$ ,



**Figure 2.** Mean (+ SEM, N = 24) Granger causality reflecting lower alpha (8-10Hz) resting state functional connectivity (rsFC) between baseline (white bars), 1hour (black bars) and 24hours (grey bars) measurements for capsaicin and control conditions at DMN connections projecting from the mPFC (A: mPFC-PCC; B: mPFC-IAG; C: mPFC-rAG). Significantly lower compared to baseline in the capsaicin but not control condition based on the post hoc analysis of the time x condition interaction (\*,  $P < .05$ ).

$d=0.41$ , Fig 2B). Interestingly, rsFC at 3 connections did not differ significantly between 1hour-and 24-hour measurements except for mPFC-IAG (mPFC-PCC:  $P = .847$ ,  $d=0.22$ , Fig 2A; mPFC-IAG:  $P = .014$ ,  $d=0.63$ , Fig 2B; mPFC-rAG:  $P > .05$ ,  $d=0.14$ , Fig 2C). For the control condition, there was no significant change in lower alpha rsFC compared to baseline for the 3 connections following 1hour and 24hours (Fig 2A – C).

Comparing capsaicin and control conditions, the lower alpha rsFC for the capsaicin condition was lower than control following 1hour for the 3 connections (mPFC-PCC:  $P < .001$ ,  $d=0.90$ , Fig 2A; mPFC-IAG:  $P < .001$ ,  $d=1.2$ , Fig 2B; mPFC-rAG:  $P = .004$ ,  $d=0.65$ , Fig 2C) and for 2 connections following 24hours (mPFC-PCC:  $P = .005$ ,  $d=0.63$ , Fig 2A; mPFC-rAG:  $P = .002$ ,  $d=0.69$ , Fig 2C) but not significantly lower for mPFC-IAG following 24hours ( $P = .576$ ,  $d=0.11$ , Fig 2B).

Connections projecting from the rAG (rAG-PCC, rAG-mPFC): ANOVAs revealed a condition x time interaction for both connections (Table 1). Post hoc analysis showed a decrease in lower alpha rsFC following capsaicin-induced pain compared to baseline for the 2 connections at 1hour (rAG-mPFC:  $P < .001$ ,  $d=1.07$ , Fig 3A; rAG-PCC:  $P < .001$ ,  $d=0.95$ , Fig 3B) and 24hours (rAG-mPFC:  $P = .001$ ,  $d=0.88$ , Fig 3A; rAG-PCC:  $P = .004$ ,  $d=0.72$ , Fig 3B). There was no significant difference in connectivity between 1-hour- and 24-hour measurements (rAG-mPFC:  $P > .05$ ,  $d=0.12$ , Fig 3A; rAG-PCC:  $P > .05$ ,  $d=0.19$ , Fig 3B). For the control condition, there was no significant change in lower alpha rsFC compared to baseline

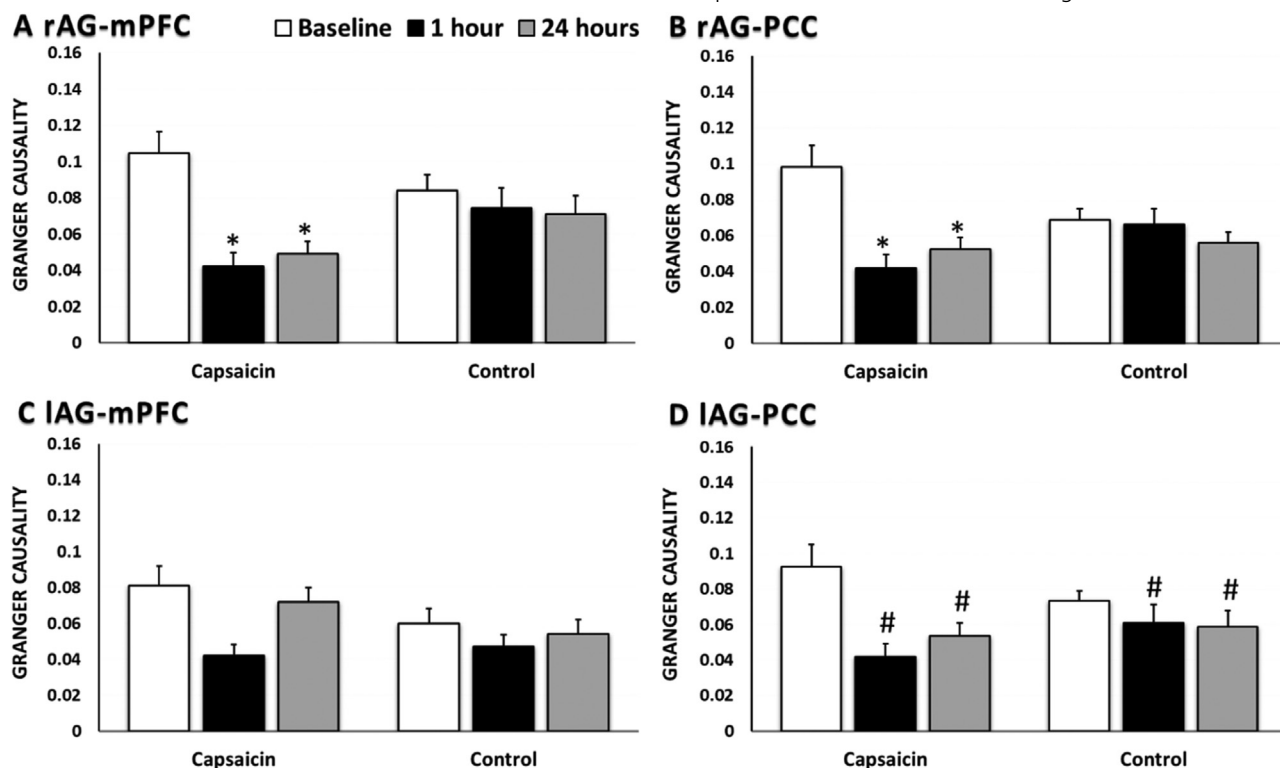
at both connections following 1hour and 24hours (Fig 3A–B).

Comparing capsaicin and control conditions showed that the lower alpha rsFC for the capsaicin condition was lower than control following 1hour for the 2 connections (rAG-mPFC:  $P = .032$ ,  $d=0.47$ , Fig 3A; rAG-PCC:  $P = .048$ ,  $d=0.44$ , Fig 3B) and following 24hours for the rAG-mPFC ( $P = .034$ ,  $d=0.46$ , Fig 3A) but not significantly lower for rAG-PCC following 24hours ( $P = .663$ ,  $d=0.10$ , Fig 3B).

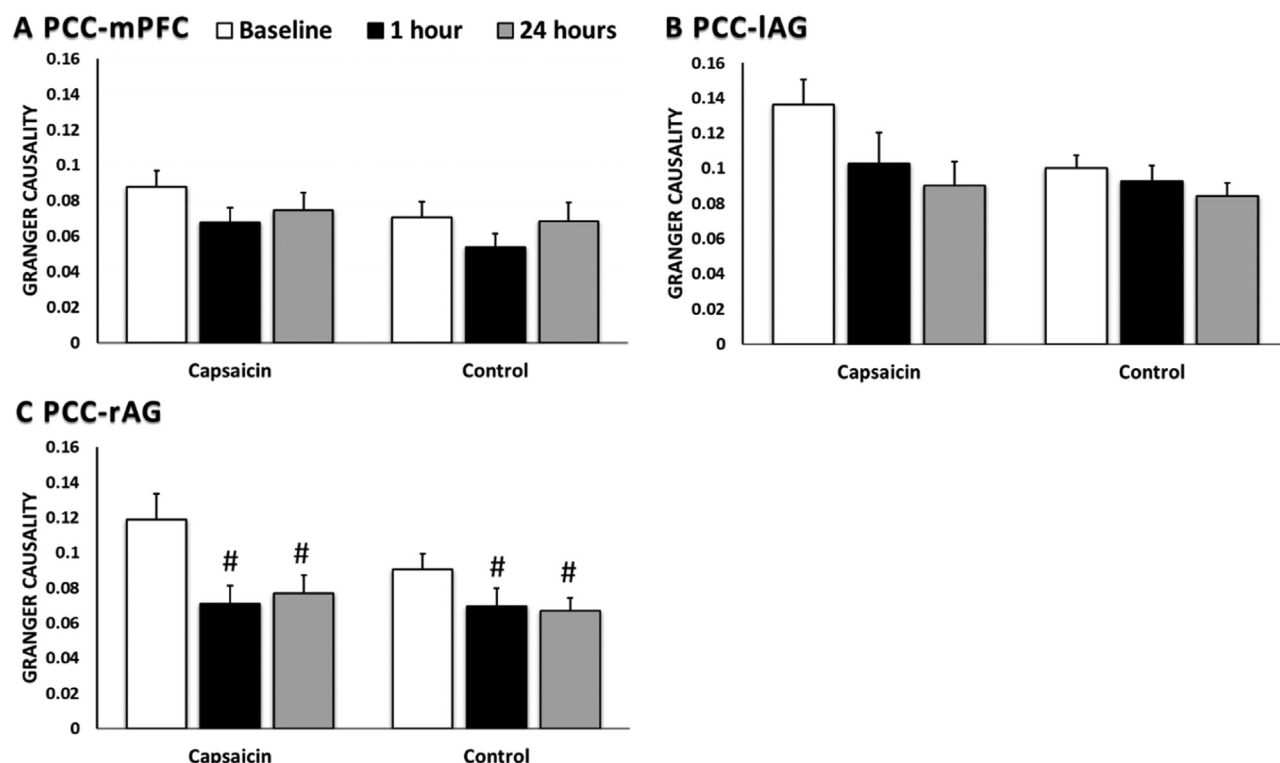
Connections projecting from the IAG (IAG-PCC, IAG-mPFC): ANOVAs revealed no significant time x condition interaction for both connections (Table 1). No significant main effect was reported for IAG-mPFC (Table 1, Fig 3C). There was, however, a significant main effect for time at IAG-PCC (Table 1). Post hoc analysis showed that regardless of the condition, lower alpha rsFC for IAG-PCC was significantly lower following 1hour ( $P = .005$ ,  $d=0.70$ , Fig 3D) and 24hours ( $P = .012$ ,  $d=0.69$ , Fig 3D).

Connections projecting from the PCC (PCC-mPFC, PCC-IAG, PCC-rAG): ANOVAs revealed no significant time x condition interaction for any of the 3 connections (Table 1). No significant main effect was reported for PCC-mPFC (Table 1, Fig 4A) or PCC-IAG (Table 1, Fig 4B). There was, however, a significant main effect for time at PCC-rAG (Table 1). Post hoc analysis showed that regardless of the condition, alpha rsFC was significantly lower for PCC-rAG following 1hour ( $P = .005$ ,  $d=0.71$ , Fig 4C) and 24hours ( $P = .026$ ,  $d=0.61$ , Fig 4C).

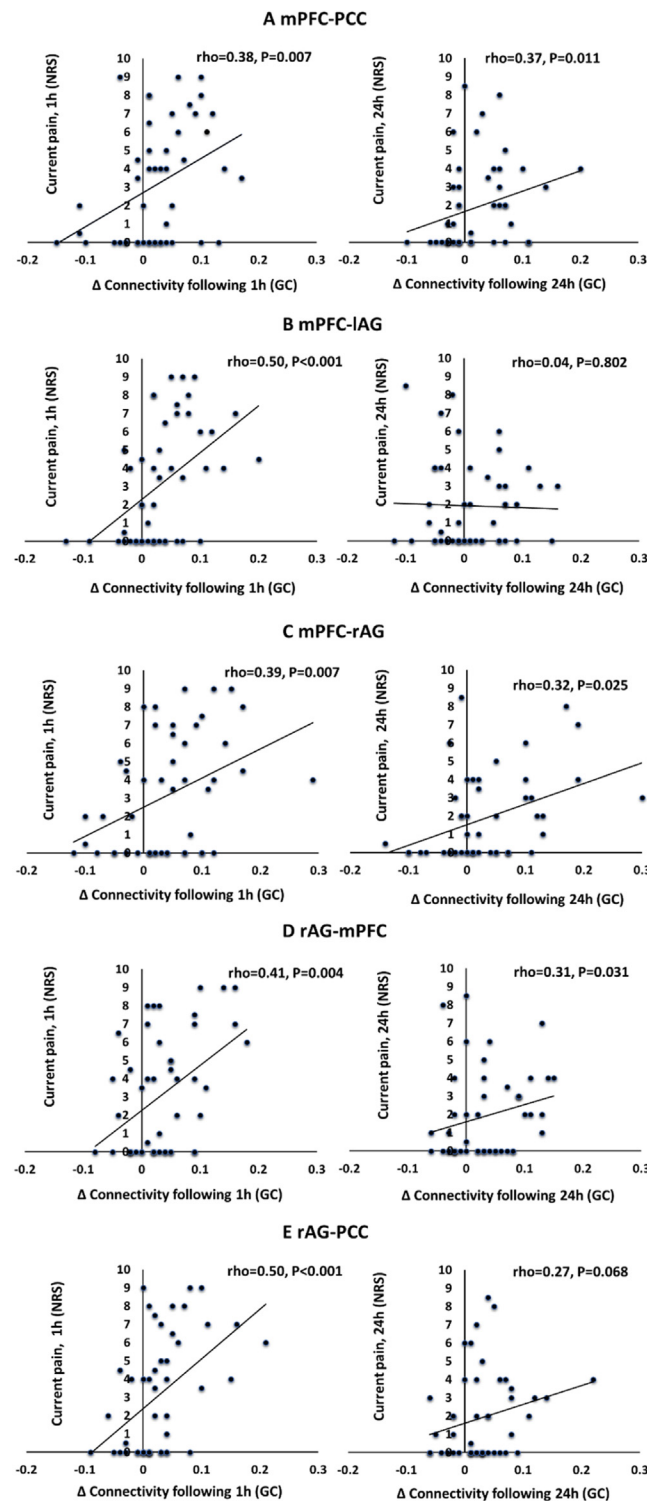




**Figure 3.** Mean (+ SEM, N = 24) Granger causality reflecting lower alpha (8-10Hz) resting state functional connectivity (rsFC) between baseline (white bars), 1hour (black bars) and 24hours (grey bars) measurements for capsaicin and control conditions at connections projecting from the bilateral AG (A: rAG mPFC; B: rAG-PCC; C: IAG-mPFC; IAG-PCC). Significantly lower compared to baseline in the capsaicin but not control condition based on the post hoc analysis of the time x condition interaction (\*,  $P < .05$ ). Significantly lower compared to baseline in both the capsaicin and control conditions based on the post hoc analysis of the time effect (#,  $P < .05$ ).



**Figure 4.** Mean (+ SEM, N = 24) Granger causality reflecting lower alpha(8-10Hz) resting state functional connectivity (rsFC) between baseline (white bars), 1hour (black bars) and 24hours (grey bars) measurements for capsaicin and control conditions at DMN connections projecting from the PCC (A: PCC-mPFC; B: PCC-IAG; C: PCC-rAG). Significantly lower compared to baseline in both the capsaicin and control conditions based on the post hoc analysis of the time effect (#,  $P < .05$ ).



**Figure 5.** Spearman's correlation between current pain intensity following 1hour and 24hours (NRS: numerical rating scale) and the change in lower alpha (8-10Hz) rsFC between baseline and 1hour and 24hours measurements for capsaicin-induced pain and control at DMN connections that exhibit significant change following capsaicin application (A: mPFC-PCC, B: mPFC-IAG, C: mPFC-rAG; D: rAG-mPFC; E: rAG-PCC). Significant correlations accepted at  $P < .005$ , due to multiple correlations.

### **Correlation between current pain and DMN connectivity at alpha oscillations**

For the connections exhibiting a significant decrease following capsaicin-induced pain, there was a positive significant correlation between current pain NRS scores

following 1hour and the decrease in alpha rsFC between baseline and 1hour (Fig 5B, 5D, 5E) except for mPFC-PCC (Fig 5A) and mPFC-rAG (Fig 5C). There was, however, no significant correlation between current pain NRS scores following 24hours and the decrease in rsFC between baseline and 24hours (Fig 5A–5E). These results indicate

a greater decrease in rsFC associated with a greater increase in pain intensity but only following 1-hour application.

### **Secondary Mechanical Hyperalgesia and Decrease in DMN Connectivity**

The ANOVA of the MPT revealed a time  $\times$  condition interaction ( $F(2,46)=65$ ,  $P < .001$ ,  $\eta^2 = 0.71$ ). Post hoc analysis showed that compared to baseline, MPT decreased following 1 hour ( $P < .001$ ,  $d=2.6$ ) and 24 hours ( $P < .001$ ,  $d=3.6$ ). There was also a decrease in MPT between 1 hour and 24 hours ( $P = .004$ ,  $d=0.75$ ). For control, no significant change in MPT was found between baseline and 1 hour ( $P = .312$ ,  $d=0.34$ ) and 24 hours ( $P = .057$ ,  $d=0.52$ ) or between 1 hour and 24 hours ( $P = .520$ ,  $d=0.29$ ). The comparison between capsaicin and control revealed that the MPT for the capsaicin condition was lower than control following both 1 hour ( $P < .001$ ,  $d=2.1$ ) and 24 hours ( $P < .001$ ,  $d=3.7$ ).

Interestingly, in the 4 connections showing a significant decrease in alpha rsFC following 24 hours, there was a trend toward a positive correlation between the decrease in alpha rsFC (between baseline and 24 hours) and the decrease in MPT (between baseline and 24 hours) for 2 of the 4 connections (mPFC-rAG:  $\rho=0.34$ ,  $P = .018$ ; rAG-mPFC:  $\rho=0.34$ ,  $P = .018$ ) but not mPFC-PCC ( $\rho=0.27$ ,  $P = .065$ ) and rAG-PCC ( $\rho=0.15$ ,  $P = .312$ ), indicating that the decreased DMN connectivity following 24 hours may be related to secondary hyperalgesia.

### **Immediate Cooling and Heating Following 24hours of Capsaicin Application**

Two participants were excluded from further analysis because of technical problems with the heating protocol. Cooling the capsaicin patch following 24 hours decreased NRS pain score from  $3.7 \pm 0.4$  to  $0.3 \pm 0.1$  [Wilcoxon signed-rank test:  $Z=-4.04$ ,  $P < .001$ ], and heating the patch increased NRS scores from  $3.7 \pm 0.4$  to  $4.8 \pm 0.6$  [Paired t-test:  $t(21)=2.62$ ,  $P = .016$ ,  $d=2.2$ ]. Following 24 hours of control application NRS pain scores were not significantly changed by cooling (from  $0.1 \pm 0.0$  to  $0.1 \pm 0.1$ ) or heating (from  $0.1 \pm 0.0$  to  $0.0 \pm 0.0$ ) the patch.

### **The Effect of Immediate Cooling and Heating on Default Mode Network Connectivity Following 24hours of Capsaicin Application**

As connections projecting from mPFC and rAG are the only connections showing significant changes following capsaicin-induced pain (mPFC-PCC, mPFC-IAG, mPFC-rAG, rAG-mPFC, rAG-PCC), these connections were further analysed to assess the stability of these changes following 24 hours. ANOVAs revealed no significant time main effect or interaction for any of the 5 connections (Table 2, Fig 6A–6E), indicating

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that cooling the patch down or heating it up did not affect alpha rsFC following 24 hours of capsaicin at these connections. There was, however, a significant main effect for condition at mPFC-PCC and rAG-mPFC (Table 2), indicating that, following 24 hours, capsaicin condition was associated with lower alpha rsFC compared to control (Fig 6A,6D).

### **Default Mode Network Connectivity and Capsaicin-induced Pain at Beta Oscillations (supplementary materials)**

Comparable rsFC decrease was found at beta and alpha oscillations following 1 hour and 24 hours of capsaicin-induced pain. However, ANOVAs revealed significant interaction for the connections projecting from mPFC, rAG, and PCC (Table S3). Following 1 hour, the decrease in beta rsFC includes connections projecting from mPFC, rAG as well as PCC (mPFC-PCC, Fig S1A; mPFC-IAG, Fig S1B; mPFC-rAG, Fig S1C; rAG-mPFC, Fig S2A; rAG-PCC, Fig S2B). No significant change was shown in connections projecting from the IAG (IAG-mPFC, Fig S2C; IAG-PCC, Fig S2D). For PCC, no significant change in PCC-mPFC (Fig S3A) was found, but significant change was reported for PCC-rAG (Fig S3B) and PCC-IAG (Fig S3C). The decrease in beta rsFC persisted 24 hours later except for mPFC-IAG, which went back to baseline. Additionally, ANOVAs revealed no significant interaction for any of the 7 connections affected by pain in response to cooling or heating following 24 hours (Table S4, Fig S4A–S4G).

## **Discussion**

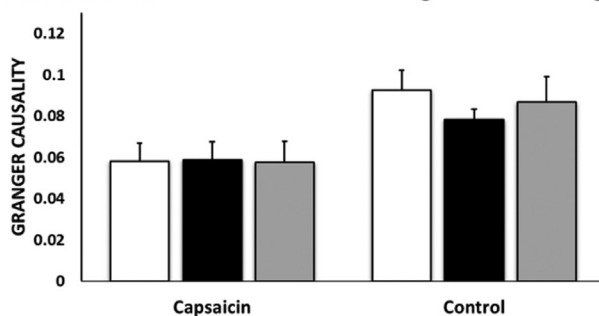
This study examined the effects of 1 hour and 24 hours of capsaicin application on the DMN connectivity at lower alpha and beta oscillations. One hour of capsaicin exposure was associated with decreased rsFC at the connections projecting from the mPFC (to PCC, rAG, and IAG) and the rAG (to mPFC and PCC) at lower alpha and beta oscillations, and from the PCC (to rAG and IAG) at beta oscillations. Although current pain intensity at 24 hours was lower compared to 1 hour, the decrease in DMN connectivity observed at 1 hour was maintained 24 hours later. The only exception was for the mPFC-IAG, where connectivity went back to baseline. Furthermore, cooling or heating the capsaicin patch 24 hours later did not alter the decrease in DMN connectivity.

Consistent with previous research, the present study supports decreased DMN connectivity during acute pain.<sup>1,2</sup> For prolonged pain (24 hours), interestingly, the decreased DMN connectivity was present in 2 distinct communication loops: 1) between the mPFC and rAG occurring at both lower alpha and beta oscillations and 2) between the PCC and the rAG occurring at beta oscillations.

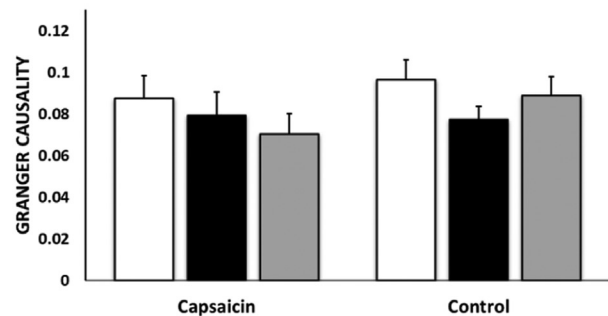
**Table 2. ANOVA Findings Comparing Lower Alpha (8-10Hz) Resting State Functional Connectivity (rsFC) Between 24-hour Application, 24-hour-cooling and 24-hour-heating (Time) for Capsaicin and Control (Condition) (N = 24) at DMN Connections That Exhibited Significant Change Following Capsaicin Application (mPFC-PCC; mPFC-IAg; mPFC-rAG; rAG-mPFC; rAG-PCC). F-values and P-values are From the Repeated Measures ANOVA (Significance Accepted at  $P < 0.01$  is Indicated in Bold, Bonferroni Corrected Due to Multiple ANOVAs)**

DMN CONNECTION	MAIN EFFECT		INTERACTION	
	CONDITION	TIME	CONDITION X TIME	
mPFC-PCC	$F(1,21)=13.15, P = .002, \eta^2 = 0.39$	$F(2,42)=0.294, P = .747, \eta^2 = 0.01$	$F(2,42)=0.428, P = .655, \eta^2 = 0.02$	
mPFC-IAg	$F(1,21)=0.494, P = .490, \eta^2 = 0.02$	$F(2,42)=1.04, P = .364, \eta^2 = 0.05$	$F(2,42)=0.788, P = .461, \eta^2 = 0.04$	
mPFC-rAG	$F(1,21)=5.16, P = .034, \eta^2 = 0.20$	$F(2,42)=0.423, P = .658, \eta^2 = 0.02$	$F(2,42)=2.93, P = .065, \eta^2 = 0.12$	
rAG-mPFC	$F(1,21)=9.65, P = .005, \eta^2 = 0.32$	$F(2,42)=0.671, P = .517, \eta^2 = 0.03$	$F(2,42)=0.894, P = .417, \eta^2 = 0.04$	
rAG-PCC	$F(1,21)=0.008, P = .913, \eta^2 = 0$	$F(2,42)=0.648, P = .528, \eta^2 = 0.03$	$F(2,42)=0.304, P = .739, \eta^2 = 0.01$	

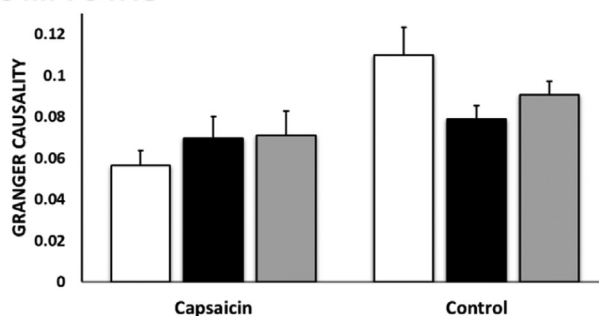
**A mPFC-PCC** □ 24hours ■ 24h-Cooling ▒ 24h-Heating



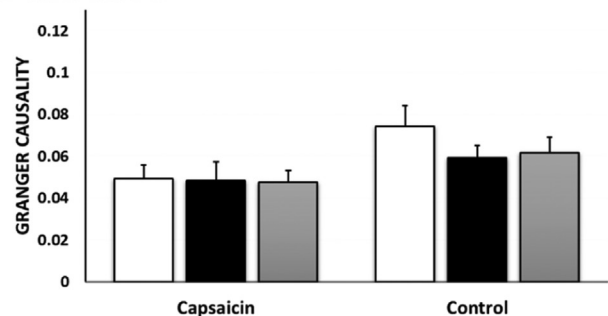
**B mPFC-IAg**



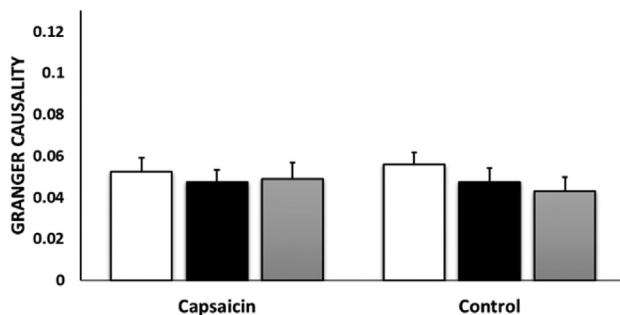
**C mPFC-rAG**



**D rAG-mPFC**



**E rAG-PCC**



**Figure 6.** Mean (+ SEM, N = 24) Granger causality reflecting lower alpha (8-10Hz) resting state functional connectivity (rsFC) at 24hours measurement (white bars), patch cooling at 24hours (black bars) and patch heating at 24hours (grey bars) for capsaicin and control conditions at DMN connections that exhibited significant changes following capsaicin application (A: mPFC-PCC, B: mPFC-IAg, C: mPFC-rAG; D: rAG-mPFC; E: rAG-PCC).

### ***Interaction Loop Between mPFC and rAG: Attention and Emotions at Lower Alpha and Beta Oscillations***

Granger causality assesses the directionality of the information flow<sup>30,99</sup> between 2 brain regions involved in a given connection. In this study, pain-related decrease in DMN connectivity occurred in 2 pairs of connections: 1 projecting from the mPFC to the rAG and another projecting from the rAG to the mPFC. This information flow of decreased connectivity or pain-related communication loop took place following 1 hour of pain and was sustained 24 hours later. There is mounting evidence linking the activity of the right posterior parietal cortex, including the rAG, to attention reorientation and regulation.<sup>10,15,18,56,86,89,92</sup> Additionally, the rAG exhibits strong connections with the ventral attention/salience network.<sup>49</sup> Interestingly, temporary interruption of the AG activity using transcranial magnetic stimulation (TMS) hinders performance in tasks requiring attention reorientation.<sup>84</sup> During pain, decreased rAG connectivity may reflect enhanced external attention.<sup>1</sup> The mPFC, on the other hand, plays a critical role in emotion regulation<sup>22,96,104</sup> through its connection with limbic structures, especially the amygdala, a key structure in the emotional processing circuit.<sup>70</sup> For pain, reduced glutamate in the mPFC among chronic pain patients is linked to emotional dysregulation.<sup>67</sup> Based on these findings, the communication loop between mPFC and rAG during prolonged pain may indicate an interaction between emotion and attention. The involvement of the mPFC in the top-down modulation of emotionally biased attention has been shown by previous studies.<sup>45,96</sup> The presence of this continuous communication loop between the mPFC and rAG during prolonged pain may explain the continuous interaction between emotions and attention characterizing chronic pain.<sup>7,12,43,80,83</sup> The presence of this loop may also explain the difficulty in deciphering the unique effects of attention and emotions on pain processing.<sup>77</sup> However, documenting the affective and attentional states of the participants would provide better insight into the interaction between emotion and attention during prolonged pain.

### ***Interaction Loop Between PCC and the rAG: Attention and Motor Priming at Beta Connectivity***

A second communication loop showing decreased beta connectivity following 1 hour and 24 hours of pain was present between the rAG and PCC (rAG-PCC, PCC-rAG). This pain-related decrease in beta activity is consistent with previous pain studies,<sup>58,64,76</sup> and is thought to facilitate adaptive motor processing.<sup>27,63,66,93,101</sup> Decreased beta power may also signify a cognitive change caused by an external stimulus.<sup>38,72</sup> This cognitive change is encoded in the PCC, which serves as a "change detection region" due to its involvement in attention, learning and memory.<sup>54,74</sup> Therefore, the interaction between the rAG and PCC may reflect

### ***Capsaicin-induced Pain Persists During Immediate Pain Relief***

amplified attention (cognitive change) detected by the PCC, which through decreased beta activity permits motor processing required for potential adaptation such as withdrawal or avoidance.<sup>64</sup> Possible motor processing during pain is further supported by a TMS-EEG study demonstrating that pain (48 minutes) is associated with increased beta connectivity between the DMN and the primary motor cortex (M1).<sup>60</sup>

In summary, emotional and attentional processes at the acute stage of pain interact in continuous loops enhancing 1 another and persisting as pain progresses. The continuation of this interaction may ultimately lead to the persistence of pain. Therefore, addressing these loops at the acute stage by methods that target cognitive and behavioural distortions related to pain such as cognitive behavioural therapy might help to interrupt the transition to more persistent states.

### ***Possible Shift to Emotional Processing During Prolonged Pain***

DMN decreased connectivity following 1 hour and 24 hours of capsaicin-induced pain may be related to the sensitization of central pain mechanisms, which is supported by the presence of secondary mechanical hyperalgesia in the area surrounding the capsaicin patch following 1 hour and 24 hours. This sensitization may initially (1 hour) serve as an adaptive protective mechanism signalling pain and pain-avoidance mechanisms.<sup>53,100</sup> However, in the present study, despite the decrease in current pain intensity following 24 hours compared to 1 hour, the decreased connectivity and the sensitization persisted suggesting a maladaptive state of heightened attention depleting the individual cognitively and emotionally. The shift to this maladaptive state is further supported by the lack of correlation between DMN connectivity and current pain intensity following 24 hours. This study demonstrated that more decrease in DMN rsFC was associated with greater pain intensity following 1 hour (except for connections from mPFC to PCC and rAG), but not following 24 hours of pain. The lack of correlation at 24 hours may signal a shift in pain processing, where emotional and attentional processes play a more critical role than actual sensory inputs.<sup>53</sup> The lack of correlation between current pain intensity and DMN rsFC has also been reported during chronic pain.<sup>98</sup> DMN connectivity during chronic pain is, however, closely associated with pain annoyance and interference<sup>98</sup> indicating that chronic pain involves brain reorganization that affects the individual emotionally and cognitively beyond the mere feeling of pain.<sup>8</sup> Our results suggest that prolonged pain may involve an emotional/attentional shift that may contribute to that occurring during persistent pain. This shift can occur as early as 24 hours following the initial exposure to pain.

Unfortunately, there is a scarcity of EEG investigations targeting the effect of chronic pain on DMN connectivity. However, functional magnetic resonance imaging (fMRI)<sup>2,9,34,98</sup> and 1 EEG-fMRI study<sup>13</sup> revealed similar decrease in DMN connectivity in chronic pain



patients. This decreased connectivity during acute and prolonged pain suggests a possible shared mechanism underlying the different stages of pain. Other fMRI studies, however, showed increased DMN connectivity during chronic pain<sup>50,55,106</sup> suggesting distinctive mechanisms. These mixed results could be due to investigating different disorders, focusing on different nodes within the DMN, or using different analyses.

### ***Left Angular Gyrus Involvement in Prolonged Pain***

Interestingly, at both lower alpha and beta oscillations, projections from the IAG did not change significantly, which suggests less involvement of the IAG in prolonged pain processing compared to the rAG.<sup>1</sup> This finding is not surprising given that the decrease in DMN connectivity reported here is likely to be attention-related, and that rAG connections with the ventral attention/salience network are stronger than with IAG.<sup>49</sup> The heterogeneous response in connectivity within the DMN during prolonged pain agrees with previous DMN studies showing that, contrary to the common belief of lack of specialization within the DMN, DMN regions are differentially sensitive to different stimuli.<sup>1,3,52,90</sup>

### ***DMN Connectivity and Pain Relief and Facilitation Following 24hours of Capsaicin-induced Pain***

This study showed that the decrease in DMN connectivity following 24hours was maintained in response to immediate pain relief or further pain aggravation. This maintained connectivity could be related to sustained heightened attention to pain for a prolonged time, which could reflect a potential floor effect in attention-related connectivity decrease. Participants may be fully attentive to their pain beyond further attentional enhancement. Therefore, a short 5-minute cooling or heating was possibly insufficient to alter this sustained heightened attention and hence the maintained decrease in DMN connectivity. However, this full attention may augment the participants' pain sensitivity and hence the change in intensity scores. The maintained connectivity despite changes in pain intensity due to cooling and heating suggests that DMN decreased connectivity at 1hour or 24hours reported in this study may not be a result of pain salience only.

As there is a trend toward a positive correlation between decreased DMN connectivity and increased mechanical hyperalgesia following 24hours, this maintained connectivity could also be related to maintained secondary hyperalgesia. Capsaicin can activate the transient receptor potential vanilloid (TRPV1) receptors directly causing both primary and secondary mechanical hyperalgesia<sup>41,71</sup> or indirectly at an intracellular level<sup>53,61</sup> maintaining this hyperalgesia at room temperature or below.<sup>65,95</sup> Cooling the capsaicin patch could mask capsaicin-induced pain by eliciting cold sensations by way of TRPM8 receptors,<sup>40</sup> and hence the perceived

decrease in pain intensity occurring alongside the mechanical hyperalgesia. For heating, it was shown that a combination of heat and capsaicin has a synergistic or additive effect that enhances secondary hyperalgesia.<sup>75</sup> However, testing the capsaicin-heat model in 2 consecutive days, Dirk and colleagues reliably reported no difference in secondary hyperalgesia between capsaicin alone and a combination of capsaicin and heat.<sup>21</sup> Once mechanical hyperalgesia is maintained, it can last for up to 72 hours.<sup>33,78</sup> Therefore, a short 5-minute cooling or heating may be ineffective at altering such a strong cascade of events, that are most likely mediated spinally.

### ***Limitations***

This study has some limitations. When examining the relationship between experimentally induced pain intensity and the rsFC, we did not assess the variability in pain intensity during EEG recording. Recording continuous online pain ratings instead of momentary pain intensity (before or after recording), would allow for a better estimate of the pain-connectivity relationship.<sup>14,16</sup> Continuous rating during EEG, however, would introduce movement artifacts to the data. Neither the order of the control/capsaicin conditions nor the order of the cooling/heating protocols was randomized. Given the non-painful control, the non-randomization could introduce an expectation-related decrease in rsFC in the capsaicin condition as the control patch did not induce any pain, and only controlled for the time effect. However, there was no significant difference in rsFC between baseline recordings for the capsaicin and control sessions. The non-painful control condition may also introduce a salience or mood difference between the 2 conditions. However, if the change in connectivity is solely attributed to salience or mood, connectivity between 1hour and 24hours would change as the current pain salience/intensity, and likely negative mood was reduced significantly from 1hour to 24hours, but no significant change in connectivity was reported between 1hour and 24hours. Further, when increasing the pain intensity after 24hours by heating the patch (which likely increased salience and negative mood), connectivity did not change either. Taken together, the connectivity changes observed here cannot be due to salience or mood only. However, as salience and mood are inherent parts of pain, we cannot completely exclude the effect of salience or mood on the results. However, evaluating the emotional and attentional states of the participants would provide better insights into the effect of pain on DMN connectivity. Finally, adding an additional control condition with another modality such as low-intensity pain would provide a clearer image of the effect of capsaicin-related pain on connectivity.

### ***Conclusions***

This study revealed decreased DMN connectivity during prolonged pain occurring in continuous loops between regions linked to attentional and emotional

processes. These continuous loops can start as early as 1 hour following the initial painful exposure and persist 24 hours later. These loops were resistant to changes in pain intensity, which may reflect mechanisms contributing to the shift to more persistent pain conditions. The interaction between attentional and emotional processes has always been part of persistent pain characterization. However, this study is the first to show how this interaction manifests in continuous loops at the cortical level. Understanding these

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loops may help in developing therapeutic methods that can better target/break them before developing into more persistent states.

## Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jpain.2022.12.004>.

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