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Individual and sex-related differences in pain and relief responsiveness are associated with differences in resting-state functional networks in healthy volunteers

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ABSTRACT

Pain processing is associated with neural activity in a number of wide-spread brain regions. Here, we investigated whether functional connectivity at rest between these brain regions is associated with individual and sex-related differences in thermal pain and relief responsiveness. Twenty healthy volunteers (ten females) were scanned with functional magnetic resonance imaging in resting condition. Half an hour after scanning, we administered thermal pain on the back of their right hand, and collected pain and relief ratings in two separate runs of twelve stimulations each. Across the whole group, mean pain ratings were associated with decreased connectivity at rest between brain regions belonging to the default mode and the visual resting-state network. In men, pain measures correlated with increased connectivity within the visual resting-state network. In women instead, decreased connectivity between this network and parietal and prefrontal brain regions implicated in affective cognitive control **were associated with** both pain and relief ratings. Our findings indicate that the well documented individual variability and sex-differences in pain sensitivity may be explained, at least in part, by network dynamics at rest in these brain regions.

Introduction

Human pain is a complex experience involving the interaction of a wide range of sensory, cognitive, affective, environmental and genetic factors (Rainville, 2002). This multifactorial nature is well represented in the variety of brain regions that support the processing of pain, which include the primary and secondary somatosensory cortex, thalamus, insula, anterior and midcingulate cortex, and medial prefrontal cortex (PFC) (Apkarian *et al*, 2005). The

multifaceted nature of the network of regions involved in pain processing has led to the idea that the subjective experience of pain may be better represented in patterns of connectivity rather than regional activity, and that the investigation of network dynamics may help understand the emergence of pain (Boly *et al*., 2007).

Functional connectivity, which measures the temporal correlation of neural activity in spatially distinct brain regions, is thus an ideal candidate to study the dynamics of the pain network. Recently, the study of brain networks has emphasized that the functional architecture of the brain is reflected by intrinsically connected networks, whose regional activity is correlated during the resting state, and modulated by external inputs (Raichle *et al*., 2001). This line of research has demonstrated that the brain is organized into multiple resting-state networks (RSN), such as the default mode network (DMN) (Shulman *et al*., 1997) and other networks involving frontal, parietal and primary sensory regions (Damoiseaux *et al*., 2006; van den Heuvel *et al*., 2009). The relevance of these RSN for pain processing is demonstrated by two lines of evidence. First, investigations on healthy individuals have demonstrated that the brain regions that normally respond to a noxious stimulation are functionally connected at rest (Mayhew *et al*., 2014). Second, abnormal patterns of functional connectivity within and between RSN were reported in clinical pain syndromes (Bolwerk *et al*., 2013; Pujol *et al*., 2014).

RSN have recently become a primary target of work on individual differences. Specific patterns of functional connectivity at rest have been associated with cognitive abilities and dispositional traits (Santarnecchi *et al*., 2014, **2015**), suggesting that resting-state functional connectivity can explain individual differences in a number of cognitive and psychological domains. So far however, little attention has been paid to the relationship between resting-state functional connectivity and individual variations in experimentally-induced responses to pain. In the current study, we aimed to investigate this relationship

using experimentally-induced thermal pain. Given the well documented evidence that men and women differ in the way they experience and respond to pain (Fillingim *et al.*, 2009), we further aimed to explore sex differences in the association between resting-state connectivity and pain ratings. Finally, building on recent observations of sex differences in the neural processing of relief (Galli *et al*., 2013), we investigated whether men and women differed in relief ratings and associated resting-state functional connectivity. To this aim, we delivered brief noxious stimulations to the hand of male and female participants. In separate runs participants gave continuous ratings of pain and relief, and these ratings were correlated with functional connectivity at rest **recorded in a separate fMRI session performed half an hour before the noxious stimulation**.

2. Materials and methods

2.1. Subjects

Twenty right-handed volunteers (10 males, age range 24-46 years, mean age 29 years; 10 females, age range 22-47 years, mean age 28 years) with no previous experience of thermal pain stimulation experiments participated in the study. Exclusion criteria were history of neurological, psychiatric or cardiovascular disease, any current or regular pain in the last six months, any regular use of analgesic or other drugs that alter central nervous system function in the last six months. Female participants reported regular menstrual cycles and were in the following cycle phases: menses (two participants), follicular (5-13 days from first day of menses, three participants), ovulation (14-16 days from first day of menses, three participants) and luteal (17-28 days from first day of menses, two participants). The study was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki.

2.2. Thermal stimulation

Thermal stimuli were delivered using the Medoc Pathway (Medoc Advanced Medical Systems, Ramat Yishai, Israel) and a 30x30 mm contact thermode. All thermal stimulations were applied by the same female experimenter (GG).

Thermal pain threshold and tolerance. To determine the temperature to be used in the pain/relief task, we first measured individual pain threshold (PTh) and pain tolerance (PTo) on the right volar forearm with an ascending method of limits (Yarnitsky & Ochoa, 1990). Starting from a baseline temperature of 32 C°, temperature increased 0.7 C° every second until it reached PTh, PTo, or until a maximum of 52 C° if no threshold was reported. For PTh trials, subjects were instructed to say 'painful' when the thermal percept first became painful. For PTo trials, subjects were instructed to say 'stop' when they felt they could no longer tolerate the pain (Fillingim *et al*., 1998). This procedure was repeated for eight consecutive trials, the first four trials were used for PTh measurement and the last four for PTo measurement. The average temperature of the last three trials of PTh and PTo was calculated, and the resulting PTo was then used as stimulation temperature for the pain/relief task. This was on average 48.0 C° (48.0 C° for females and 48.1 C° for males). Mean PTh was instead 44.3 C° (44.3 C° for females and 44.2 C° for males). There was no difference between females and males in either PTh (independent samples t test, $p = 0.967$) or PTo ($p = 0.876$), and no correlation between age of participant and PTh ($p = 0.422$) or PTo ($p = 0.659$).

Pain/relief task.The thermal stimulation procedures for the pain/relief task were similar to those employed in a previous study (Leknes *et al.*, 2008). In total, twenty-four noxious stimulations were delivered to the back of the right hand. These were divided in two runs of twelve stimulations each, one run for pain rating and the other for relief rating. The two runs were separated by a gap of approximately five minutes, and their presentation order was

counterbalanced across subjects. In both runs, each stimulation started with a baseline temperature of 32 C°, which rapidly increased (37.5 C° rise in one second) until the destination temperature, corresponding to the subject's PTo (Figure 1). This temperature was then maintained for three seconds, and returned to baseline with a return rate of 30 C°/sec. An interval of 60 seconds preceded the beginning of the next stimulation. During the noxious stimulation, subjects were in front of a computer screen where a VAS for pain or relief ratings was presented. Subjects were instructed to rate only pain in the pain run, and only relief in the relief run. To explain the relief sensation, we asked them to imagine the relief that they would feel when, after holding on a painfully hot cup for some seconds, they were finally able to put the cup down (Leknes *et al*., 2008). Participants rated their level of pain or relief continuously by moving a pointer along the VAS with the mouse, from 0 (corresponding to absence of pain or relief) to 10 (maximum perceived pain or relief). As shown in Figure 1, a graded scale of colour or grayscale intensity from left to right coded the intensity of pain or relief sensations. A few practice trials were delivered before the start of the experiment to familiarize participants with the rating procedure and to stabilize their perception of maximum pain and relief as anchor points for the VAS. The position of the pointer along the scale was continuously recorded from the moment the pointer moved from 0 to the moment the pointer returned to this position after the rating. This allowed us to compute pain and relief ratings using three different parameters. The peak indicated the maximum rating along the VAS scale, the mean the mean rating, and the area-under-curve (AUC) the total pain or relief. To assess whether differences in anxiety levels could affect sex differences in pain sensitivity, at the end of the pain/relief task subjects completed the Spielberg State-Trait Anxiety Inventory (Spielberger, 1976). There was no difference between men and women in either state (independent samples t test, $P = 0.308$) or trait ($P = 0.532$) anxiety.

2.3. fMRI scanning and analysis

Neuroradiological aquisition. Approximately half an hour before the thermal stimulation session, subjects underwent fMRI scanning. MRI examinations were performed at a 1.5 Tesla Philips Intera Scanner (Philips Medical Systems, Best, The Netherlands). Anatomical data were obtained through a T1-weighted Fast Field Echo 1-mm thick image of the entire brain (TE = 4.6 ms, TR = 30.00 ms, flip angle = 30.00, FOV = 250 mm, matrix 256x256, slice number = 150), acquired in the axial plane parallel to the anterior and posterior commissures, as well as a Fluid Attenuation Inversion Recovery (FLAIR) image for lesion detection. Functional data were acquired using an fMRI BOLD T2-weighted sequence during rest condition (TE = 40ms, TR = 2500ms, 200 scans, 23 interleaved slices). Subjects were instructed not to focus their thoughts on any particular topic while keeping their eyes closed. Only subjects with a negative response to lesion detection on T1/T2-weighted images entered statistical analysis.

fMRI preprocessing. Functional image preprocessing and statistical analyses were carried out using SPM8 (Statistical Parametric Mapping; http://www.fil.ion.ucl.ac.uk/spm/). The first five volumes of functional images were discarded for each subject to allow for steady-state magnetization. Echo-Planar Images were (i) corrected for inhomogeneity using fieldmaps regression, then (ii) stripped of skull and other non-cerebral tissues, (iii) slice-timed using interleaved descending acquisition, (iv) realigned and (v) resliced to the mean volume for head motion correction. Two recent studies suggested that head motion during MRI scanning may produce significant changes on functional connectivity estimation and consequently over local and global topological indexes used for brain robustness quantification (Power *et al*., 2012; Van Dijk *et al*., 2012). To tackle this issue we used the time series interpolation procedure based on displacement indexes proposed by Power and colleagues (Power *et al*., 2012), i.e. Frame-wise displacement (FD) and RMS variance of the temporal derivative

(DVARS). Therefore, functional time points showing FD > 0.5 mm and DVARS > 0.5 have been interpolated using a cubic spline function. Structural images were co-registered to the mean volume of functional images and subsequently segmented using routine in SPM8. Hidden Markov Random Field model was applied in order to remove isolated voxels. To obtain a more accurate spatial normalization we applied the SPM8 DARTEL (Diffeomorphic Anatomical Registration Through Exponentiated Lie) module (Ashburner, 2007), creating a customized gray matter template from all subjects' segmented images. A nonlinear normalization procedure with subsequent affine-only normalization to the Montreal Neurological Institute (MNI) template brain, and voxel resampling to an isotropic 3x3x3 mm voxel size, were then applied to functional images. Linear trends were removed to reduce the influence of the rising temperature of the MRI scanner and all functional volumes were band-pass filtered at .01 Hz<f<.08 Hz to reduce low-frequency drifts.

Functional connectivity analysis and statistical models. BOLD images were parcellated using the Anatomical Labeling Atlas (Tzourio-Mazoyer *et al*., 2002), leading to 90 time series corresponding to cortical and subcortical brain regions. The connectivity between each region with the others was computed as a Pearson correlation coefficient, with the coefficients distribution being re-centered for each subject using a Fisher r-to-z transform. The first set of analyses was based on the whole sample investigating the pattern of correlations between pairwise functional connectivity and peak pain, peak relief, mean pain, mean relief, AUC Pain and AUC relief, using a linear multivariate regression at a statistical threshold of $P < 0.05$ **unless otherwise stated**. Given the potential interaction between gender, brain connectivity and individual response to painful stimulation, six Analysis of Variance (ANOVA) models were built including biological sex and each pain and relief score as factors. Between-group analyses were based on the following parameters: an uncorrected $P = 0.001$ (two-sided) threshold for pairwise connectivity, and a P = 0.05 Family Wise Error

(FWE) correction for multiple comparisons (corrected for connection intensity) for the network-level statistics. To reduce the risk of alpha-type errors and/or the identification of spurious connectivity due to the high-dimensionality of the fMRI dataset, Network-Based-Statistic method for multiple comparisons correction was applied in both regression and ANOVA analyses (Zalesky *et al.*, 2012).

3. Results

3.1 Pain/relief task

Values of peak, mean and AUC in men and women were contrasted with independent-samples t-tests, separately for pain and relief runs. The Bonferroni correction was applied to reduce the risk of false positives in the presence of multiple comparisons. As evident in Table 1, in general women gave higher ratings of pain and relief compared to men. However, after correcting for multiple comparisons this difference was only significant for mean pain (t_{18} = 2.857, P =0.011). Similar to Leknes et al. (Leknes *et al.*, 2008), pain and relief ratings were significantly correlated (peak values: r = 0.872, P < 0.001; mean values: r = 0.800, P < 0.001), indicating that the more intense the perceived pain, the higher the rating of relief. For AUC, this correlation only approached statistical significance (P = 0.059). To test whether pain and relief ratings were correlated differently in men and women, we transformed the correlation coefficients for peak and mean values with the Fisher's r-z transformation and compared these values between men and women using an independent samples t-test. There was no sex difference in the correlation between pain and relief ratings (Ps > 0.062).

3.2 fMRI

As evident in Figure 2 and 3, the resting-state connectivity profile of a number of brain regions encompassing the prefrontal and temporal lobes were able to explain the variance of individual ratings of mean pain across the whole sample (r^2=18%, $P < 0.02$). One connectivity pattern consisted of decreased right-lateralized connectivity within DMN brain regions such as the gyrus rectus and the medial, superior and inferior portions of the orbital gyrus. Another pattern included a decrease in the connectivity values between these regions and the right hippocampus, right parahippocampus, right middle frontal gyrus, left frontal inferior operculum, right inferior parietal cortex and left visual cortex. In addition, a more local network included a number of lateral and medial temporal lobe structures and the right amygdala**. Interestingly, the observed decrease in connectivity affected positive connections (Figure 3), suggesting a weaker intrinsic connectivity between the aforementioned regions, instead of an enhanced negative correlation.** The only pairwise connectivity showing a positive correlation with mean pain ratings was the one connecting the left visual cortex and the right supramarginal gyrus (Figure 2).

To further characterize the patterns of decreased connectivity involving DMN structures, which seem to largely contribute to individual differences in pain responsiveness across the whole sample, we conducted an additional seed-based analysis. We selected the right hippocampus as seed-region and its BOLD time course was correlated with mean pain ratings ($P < 0.05$ FDR corrected). The results showed a significant negative correlation with other DMN nodes like right angular gyrus, bilateral posterior cingulate cortex and medial prefrontal cortex (Figure 4).

Peak and AUC of pain ratings, as well as mean, peak and AUC of relief ratings did not correlate significantly with any pairwise connectivity pattern when considering the whole sample.

Men and women did not differ in their global connectivity profile, regardless of whether we set a liberal (two tailed, FDR corrected $P < 0.05$ for ROI-ROI statistics, $P < 0.01$ uncorrected for Network-Based statistics) or a more conservative threshold (two tailed, FDR corrected $P < 0.05$ for ROI-ROI statistics, FDR corrected $P < 0.05$ for Network-Based statistics). When we analysed sex-differences in connectivity as a function of pain and relief ratings, we found six almost identical patterns of connectivity profiles for mean ($F_{2,18} = 2.43$, $P = 0.017$, Cohen's $D = 0.26$), peak ($F_{2,18} = 2.56$, $P = 0.023$, Cohen's $D = 0.29$) and AUC ($F_{2,18} = 2.34$, $P = 0.011$, Cohen's $D = 0.24$) of pain ratings and mean ($F_{2,18} = 2.38$, $P = 0.015$, Cohen's $D = 0.24$), peak ($F_{2,18} = 2.45$, $P = 0.017$, Cohen's $D = 0.29$) and AUC ($F_{2,18} = 2.19$, $P = 0.014$, Cohen's $D = 0.19$) of relief ratings. Figure **5** shows that a higher correlation between individual ratings for all these measures and increased functional connectivity between bilateral nucleus, bilateral calcarine cortex, left inferior parietal lobe and left middle frontal gyrus was found in women compared to men. Instead, a higher correlation between pain ratings and the connectivity between the right cuneus and the left calcarine cortex was found in men compared to women. No other brain regions' connectivity profile showed a significant interaction with pain or relief ratings.

4. Discussion

The current study aimed to investigate whether functional connectivity at rest predicted individual differences in pain and relief sensitivity. We delivered noxious heat stimulations to the back of the right hand, and participants gave continuous ratings of pain and relief using a VAS. We measured total, maximum and mean pain and relief and correlated these ratings with brain functional connectivity at rest obtained in a separate fMRI scan.

Across the whole sample, individual variability in mean pain responsiveness was **associated with** decreased functional connectivity at rest in a wide-spread network of brain regions, including right-lateralized ventromedial PFC regions, right hippocampus, right parahippocampus, right inferior parietal cortex, right midfrontal PFC and left frontal operculum. In addition, mean pain ratings were associated with decreased local connectivity within the ventromedial PFC and within bilateral temporal lobe structures. The only increase in functional connectivity associated with mean pain ratings was between the occipital cortex and the supramarginal gyrus.

The ventromedial PFC and inferior parietal lobe are core hubs of the DMN (Buckner *et al*., 2008; Raichle *et al*., 2001), a set of brain regions that decrease their activity during the execution of cognitive tasks (Raichle *et al*., 2001; Shulman *et al*., 1997) and are functionally connected in a resting state (Fox *et al*., 2005). The hippocampus and the parahippocampal gyrus are also generally considered part of the DMN (Buckner *et al*., 2008; Vincent *et al*., 2006; Ward *et al*., 2014). Our findings thus indicate that individuals with weaker intrinsic brain connectivity within the DMN are more prone to be responsive to pain. The specific functions of the DMN are still a matter of debate. One possible account is that this network supports the broad monitoring of the external environment while individuals are at rest (Buckner *et al*., 2008). Alternatively, it may be involved in spontaneous, self-referential and internally directed cognitive activities, such as mental simulation, mentalizing and imagery (Buckner *et al*., 2008). One idea to interpret our finding of decreased DMN connectivity in relation to pain sensitivity is based on the notion that self-referential activity and introspective thought patterns under some circumstances function as competitors to the instantiation of pain and protect against excessive pain sensitivity (Buckner *et al*., 2008; Emerson *et al*., 2014; Starr *et al*., 2010). Within this view, individuals with weaker connectivities in the DMN may be less engaged in, or more easily disengaged from, self-

referential thought processes. As a consequence, these processes would not compete efficiently with those generating the nociceptive experience, and pain responsiveness would thus be affected. Our study is certainly not unique in showing an association between pain and the DMN. Functional connectivity within core hub regions of the DMN is disrupted in acute and chronic pain (Baliki *et al*., 2008; Kornelsen *et al*., 2013; Napadow *et al*., 2012; Pujol *et al*., 2014), and cortical thickness of these brain regions is inversely correlated with pain sensitivity (Emerson *et al*., 2014). In addition, event-related fMRI and positron emission tomography (PET) studies consistently revealed pain-induced deactivations in a number of regions belonging to the DMN (Coghill *et al*., 1998; Kong *et al*., 2010; Lui *et al*., 2008; Moulton *et al*., 2006; Seminowicz & Davis, 2007; Yelle *et al*., 2009). Interestingly, Kong *et al*. (2010) demonstrated that the DMN regions showing fMRI signal decreases during administration of pain were also functionally connected at rest in the same subjects. This finding suggests that the intrinsic dynamics of the DMN contribute to shape brain activations during noxious stimulation.

One quite unexpected finding was that individual and sex-related differences in pain responsiveness correlated with increased and decreased functional connectivity at rest involving the occipital cortex. The involvement of visual brain regions in normal and clinical pain has been reported in previous studies (Khan *et al*., 2014; Kong *et al*., 2010, 2013; Peltz *et al*., 2011; Pujol *et al*., 2014), but has been mostly left uncommented. Brain regions within the medial inferior occipital lobe have consistently shown coherent fluctuations at rest in the BOLD signal, to the extent that they have been identified as a separate, 'visual resting state network' (Damoiseaux *et al*., 2006; Smith *et al*., 2014). The functional significance of this network is not yet entirely clear, but one idea is that functional connectivity in this region during rest may reflect visual imagery (Wang *et al*., 2008). Mental images of pain can be very clear and vivid, and generally include proprioceptive elements such as temperature or

pressure, and anatomical representations (Gosden *et al*., 2014). It is thought that mental imagery of pain may shape the experience of pain (Gosden *et al*., 2014) and interestingly, subjective reports in the vividness of mental imagery correlate with neural activity in the visual cortex (Cui *et al*., 2007). Although further investigations are clearly needed to reveal the interactions between visual imagery and pain, we speculate that the incorporation of visual images into the sensory experience contributes to pain responsiveness.

The analysis of sex differences suggests that this mechanism may be particularly relevant in male participants. In men, pain responsiveness was associated with increased functional connectivity within the visual resting state network, specifically, between the left cuneus and the right primary visual cortex. This finding is consistent with previous studies that showed increased connectivity in males within the visual resting state network (Filippi *et al*., 2013; Smith *et al*., 2014), and may indicate that men make greater use of mental images when evaluating sensory experiences, as demonstrated for evaluative processes in other domains (Christakou *et al*., 2009; Thomsen *et al*., 2000). In females instead, pain ratings were associated with functional connectivity in a wider network of brain regions, involving bilateral inferior visual areas, the left inferior parietal cortex and the left midfrontal gyrus. The left inferior parietal cortex plays a key role in multisensory processing, including multimodal evaluation of thermal pain (Moulton *et al*., 2012). The right midfrontal gyrus instead is associated with cognitive control, including suppression of emotional memories (Depue *et al*., 2007), emotional reactivity (Ochsner *et al*., 2012) and cognitive regulation of negative emotions (Ochsner *et al.,* 2004). Our findings thus indicate that a common network of visual areas is associated with pain sensitivity in men and women. However, the additional involvement of parietal and frontal brain regions in women may suggest that pain ratings in women are based to a larger extent on affective control processes and cognitive control of negative affect. Our findings confirm previous evidence of sex differences in functional

connectivity at rest (Biswal *et al*., 2010; Filippi *et al*., 2013; Kilpatrick *et al*., 2006; Smith *et al.*, 2014; Wang *et al*., 2008, 2014) and suggest that the higher responsiveness to pain in women evidenced in our pain/relief task and previous investigations (Fillingim *et al*., 2009; Racine *et al*., 2012; Riley *et al*., 1998) may be explained, at least in part, by functional connectivity at rest involving the medial inferior occipital cortex in men, and parietal and frontal brain regions in women, perhaps reflecting different contributions of imagery and affective control processes in pain ratings. Critically, sex differences in the current study cannot be attributed to factors known to contribute to sex-related differences in pain perception such as age, anxiety levels, sex of the experimenter, or history with chronic pain (Greenspan *et al*., 2007), as these variables were all controlled for. Sex differences could instead be due to contribution of sex steroid hormones, which have a pivotal role in organizing structural and functional connections in the human brain (Peper & Koolschijn, 2012).

We did not find sex differences in relief ratings, although there was a tendency for higher relief ratings in women. **Whilst this negative result may be due to low statistical power, our current dataset** contradicts the hypothesis that motivated our investigation of relief, which based on previous findings (Galli *et al*., 2013) predicted higher sensitivity to relief in men. It should be noted however that in the study of Galli *et al*. (2013) the aversive stimulation that generated relief was a breathing restriction, which involves different physiological and psychological mechanisms compared to the noxious stimulation employed in the present study. These mechanisms could differ between men and women in such a way that men, for instance, may be more sensitive to the psychological aspects associated with dyspnea, and consequently to the relief from it. While some caution is warranted in embracing this interpretation, further work can verify its validity by directly manipulating the modality of aversive stimulation and assess how relief varies between men and women. Sex

differences instead emerged in the correlation between functional connectivity at rest and relief ratings. Whereas in women relief ratings were associated with the same network of brain regions that explained pain ratings, there was no evidence of similar brain-behaviour correlations in men, or when the whole sample was taken into account. These results indicate that, on the one hand, relief and pain ratings in women are based on largely overlapping neural networks at rest, perhaps reflecting common interoceptive and evaluation processes. On the other, they show that resting-state functional connectivity is not related to relief ratings in men, nor in general in our sample. Considering that relief has consistently been associated with the activation of brain regions involved in interoceptive and reward processing (Galli *et al*., 2013; Leknes *et al*., 2011; Peiffer *et al*., 2008), one could conclude that task fMRI may be more sensitive to detect relief-related brain responses.

In summary, the current investigation demonstrates that functional connectivity at rest in distinct brain networks including the default mode and the visual resting-state networks may reflect relatively stable processes that contribute substantially to individual and sex-related differences in pain responsiveness. Given the association between sensitivity to pain and risk of developing chronic pain (Staud *et al*., 2003), the investigation into the potential mechanisms that contribute to individual responsiveness to pain are not only relevant from a theoretical standpointbut also for its potential clinical implications. Furthermore, our investigation shows that network-based approaches are excellent candidates to study such individual differences and that the level of activation in specific regions may tell an incomplete story when highly-distributed processes such as pain processing are considered.

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Figure captions:

Figure 1: **Schematic illustration of a pain trial (a) and a relief trial (b).** In both trials, each stimulation started with a baseline temperature of 32 C°. This temperature then rapidly increased until the destination temperature, which corresponded to the subject's pain tolerance threshold and was maintained for three seconds. On different runs, subjects rated pain or relief continuously by moving a pointer from left to right with the mouse along the Visual Analogue Scale (VAS) depicted in the lower part of panel (a) and (b), in which 0 corresponds to absence of pain or relief, and 10 to maximum perceived pain or relief. As can be seen from the Figure, pain and relief intensities were coded with different colours to avoid overlapping sensation mappings. Gray squares indicate the time interval in which maximum pain and relief ratings were given.

Figure 2: Correlation between individual responses to experimentally-induced pain and functional connectivity across the whole sample (a) Significant correlation between M-Pain ratings and pairwise resting-state functional connectivity, showing a set of brain regions typically involved in nociceptive processing. Brain region labels also report (L) and (R) notation for left and right hemisphere. (b) Anatomical mapping of connectivities showing a significant correlation with pain responses. The colour code of each sphere represents the average intensity of the connectivity involving each node (light blue-dark blue for negative connectivity and yellow-red for positive connectivity).

Figure 3: Baseline functional connectivity patterns. The figure displays the spontaneous connectivity pattern between the twenty-one regions highlighted in the correlation analysis with pain responsiveness. Panel A shows the results when a more liberal threshold was set (uncorrected $P < 0.001$ for ROI-ROI statistics). In panel B a more stringent threshold was applied to highlight the strongest connections (FDR corrected *P*

< 0.05 for ROI-ROI statistics, FDR corrected *P* < 0.05 for Network-Based statistics). Colour bar indicates sign and magnitude of the correlation coefficients, showing a prevalent positive connectivity.

Figure 4: Default mode network connectivity. The figure depicts the pattern of negative correlation between spontaneous BOLD fluctuations in the seed region (right hippocampus, red sphere) and individual variability in response to painful stimuli. Colour bar represents Pearson product-moment correlation coefficient values (*P* < 0.05 FDR corrected).

Figure 5: Sex-related differences. Significant differences in the interaction between men and women's connectivity profile and mean pain and relief responses. The pattern is almost identical for pain and relief scores, and describes a major involvement of connections between the occipital, parietal and frontal lobes (*P*< 0.001 uncorrected at the pairwise level, *P* < 0.05 FWE corrected for network-based-statistic).

	Pain		Relief	
	Women	Men	Women	Men
Peak	7.63 (1.53)	6.30 (0.98)	7.36 (2.06)	6.80 (1.30)
Mean	4.21 (0.94)	3.06 (0.85)	4.34 (1.35)	3.62 (1.11)
AUC	97522 (2133)	76473 (2545)	112741 (4011)	104253 (4959)

Table 1: Mean values of all outcome measures for pain and relief ratings in men and women. Standard deviations are displayed in parentheses.