Ultra-high field imaging reveals increased whole brain connectivity underpins cognitive strategies that attenuate pain

Enrico Schulz[1,2], Anne Stankewitz[2], Anderson M Winkler[1], Stephanie Irving[2], Viktor Witkovsky[3], Irene Tracey[1]

[1] Wellcome Centre for Integrative Neuroimaging, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, UK

[2]Department of Neurology, Ludwig-Maximilians-Universität München, 81377 Munich, Germany

[3] Department of Theoretical Methods, Institute of Measurement Science, Slovak Academy of Sciences, 841 04 Bratislava, Slovak Republic

Corresponding author:
Enrico Schulz
Neurologische Klinik und Poliklinik
Marchioninistr. 15
81377 München
Email: eschulz@med.lmu.de

ABSTRACT

The perception of pain activates a number of brain regions and processes that are involved in its sensory, emotional, cognitive, and affective aspects; all of which require a flexible functional connectivity between local and distant brain regions. Here, we investigate how the attenuation of pain with cognitive interventions affects the strength of these connections by pursuing a whole brain approach in order to assess every cortical connection that contributes to successful pain relief.

While receiving 40s trials of tonic cold pain, 22 healthy participants were asked to utilise three different pain attenuation strategies: (a) non-imaginal distraction by counting backwards in steps of seven, (b) imaginal distraction by imagining a safe place, and (c) cognitive reappraisal. During a 7T fMRI recording, participants were asked to rate their pain after each single trial. We related the trial-by-trial variability of the attenuation performance to the trial-by-trial functional connectivity of the cortical data. Across all three conditions, we found that a higher performance of pain attenuation was predominantly associated with higher functional connectivity between all regions.

Of note, we observed an association between low pain and high connectivity for regions that belong to the core areas of pain processing, i.e. the insular and cingulate cortices. For one of the cognitive strategies (safe place), the performance success of pain attenuation was explained by diffusion tensor imaging metrics of increased white matter integrity.

Therefore, successful cognitive interventions to ameliorate pain and improve clinical outcomes would require the strengthening of cortical connections.

SHORT TITLE

Cognitive Strategies Increase Brain Connectivity to Attenuate Pain.

INTRODUCTION

An increased perception of pain is generally associated with increased cortical activity; this has been demonstrated for a number of brain regions and processes involved in sensory, emotional, cognitive, and affective aspects of pain (1,2). Given the threatening nature of pain, the information processed from these different aspects have to be integrated and assessed to compute an appropriate decision and subsequent action (3). To do so, pain-processing brain regions are required to exchange information, which entails increased functional connectivity between relevant cortical and subcortical regions (4,5). Conversely, less is known about connectivity changes during decreased pain, although many studies highlight decreased neuronal activity with some studies highlighting selective changes in coupling between brain regions (6).

Such studies have largely investigated the network activity of the pain system by quantifying the covariation of the fluctuating blood-oxygen-level dependent (BOLD) activity. Changes of this covariation of cortical signals have then been related to conditions that represent different levels of pain experience. Villemure & Bushnell (2009) and Ploner et al. (2011), for example, investigated the influence of different levels of emotion and attention on pain-related cortical connectivity (5,6). Both studies observed an increase of connectivity for the conditions that increased the intensity of pain; i.e. increased attention towards painful stimuli was associated with more negative emotions.

A further study found that a change in pre-stimulus cortical connectivity patterns from the anterior insula to the periaqueductal grey (PAG), which is part of the descending pain modulatory system (7), determined whether a subsequent nociceptive stimulus was perceived as painful or not (8). Supporting that observation, other investigations have similarly reported increased functional connectivity between the PAG and the perigenual anterior cingulate cortex (pACC) for conditions associated with decreased pain intensity perception (placebo, shift of attention) (9-11). A recent study even showed that the structural integrity, as measured using diffusion tensor imaging (DTI) of white matter tracts between brain regions coupled with this descending pain modulatory system, was significantly correlated to the effectiveness of transcranial direct current stimulation brain stimulation in alleviating pain (12).

Therefore, all studies to date point to the relevance of connectivity patterns in pain modulation; yet, excluding an increased connectivity to the descending pain modulatory system's PAG, the precise nature of cortical connectivity during decreased pain is unclear

and limited. Using ultra-high field functional magnetic resonance imaging (fMRI) to provide enhanced signal-to-noise ratio (SNR) to facilitate single-trial analysis, we explored the functional connections that contribute to the attenuation of pain by means of three different cognitive interventions: (a) a non-imaginal distraction by counting backwards in steps of seven; (b) an imaginal distraction by imagining a safe place; and (c) reinterpretation of the pain valence (cognitive reappraisal). These cognitive strategies are hypothesised to be represented in the brain by a complex cerebral network that connects a number of brain regions, where:

(1) The effective use of a cognitive strategy that is successful for pain attenuation results in an increase of functional connectivity between task-related brain regions.
(2) Decreased connectivity is expected between cortical areas that are involved in the processing and encoding of pain intensity, e.g. sub-regions of the insular cortex, the cingulate cortex, somatosensory cortices, and PAG.
(3) Increased connectivity is hypothesised for the descending pain control system, particularly for the connection between the pACC and the PAG.
(4) Divisions of the insular cortex and their connections to frontal and somatosensory regions play a key role through their high relevance in integrating sensory information.

Unlike previous research paradigms, the present experimental procedure aims to approximate clinical treatment procedures by using a novel pain stimulation approach that produces longer lasting pain experiences. Healthy participants were asked to utilise cognitive strategies in order to attenuate the experience of pain during 40s of cold stimulation. We pursued a whole-brain parcellation approach (13) in order to assess every cortical connection that contributes to successful pain relief.

RESULTS

Overall, we found an increase of connectivity during pain attenuation: trials rated as low pain as a consequence of utilising a cognitive strategy had stronger connectivity compared with trials of the unmodulated pain condition that were rated as high pain. Therefore trials with high pain are coupled with low connectivity, and trials with low pain are coupled with high connectivity.

We pursued a whole-brain approach by subdividing the cortex into 180 regions per hemisphere plus 21 subcortical regions (13) and related cortical connectivity to pain ratings at single trial level. This approach was facilitated by an increased SNR as a result of ultra-high field recording, as well as by a more reliable assessment of single trial data from longer lasting painful stimulation and an extended task application. For each of the three conditions, we merged the 11 trials of the cognitive interventions with the 11 unmodulated pain trials, which has two major advantages:

(i) First, it takes the within-subjects variable performance of the pain attenuation attempts into account; e.g. a more successful attempt to attenuate pain is considered to cause a different cortical connectivity than a less successful attempt.

(ii) Second, we also take into account the more natural fluctuation of the unmodulated pain trials.

The findings are represented in confusion matrices, depicting the pain intensity-related connectivity between all brain regions. Positive relationships (*red*) show connectivities that were increased in particularly effective trials (performance encoding). For all tasks, we confirmed our first hypothesis by showing that an increased connectivity of *task-processing* brain regions is related to particularly successful attempts to attenuate pain. However, contrary to our second hypothesis, we found increased brain activity for successful single trials also in *pain-processing* regions. The increased connectivities are therefore suggested to initiate mechanisms of cortical activity suppression, such as shown in our previous publication (14). Negative relationships (*blue*) represent cortical connections that are disrupted in successful trials to attenuate pain. Disruptions were hypothesised to occur for the core regions of pain processing, such as for the various subregions of the insular, cingulate, and somatosensory cortices. However, we found that these regions predominantly showed increased connectivity during successful trials of pain attenuation (see above).

(1) Counting. We aimed to detect patterns of connectivity changes that are related to successful trials during counting. The attenuation of pain during counting is predominantly related to an increase of cortical connectivity in several brain regions, with the exception of decreased connections involving the right temporo-parieto-occipital junction (Figure 1A). The detailed matrix of statistical results can be found in the supplementary material (Supplementary Spreadsheet 1).

We found that some regions show a particularly strong connectivity: the right insula, the left and right temporal cortices, the left parietal cortex, as well as higher order visual regions in occipito-temporal areas. The best connected area is the right middle insula (Figure 1C, $p<0.05$, PALM corrected). Indeed, some prominent connectivity patterns are noticeable: pain attenuation-related connections from the right insular sub-regions are always connected to insular sub-regions from the contralateral hemisphere, but not to other ipsilateral insular regions. In addition, areas in the left medial wall of the parietal cortex (Brodmann area 7) are functionally connected to a right posterior cortical region that stretches from higher order visual areas (lateral occipital cortex) to the posterior medial temporal cortex. The homologue left occipito-temporal region is functionally connected to the right inferior parietal lobe (subregions PFt and PFop). Regions in the left superior and middle temporal cortex are strongly connected with several sections of the insular cortex. Extended regions in the left superior parietal cortex (Brodmann area 5) and the posterior cingulate cortex are functionally connected with the right middle insular cortex (Figure 1C, Supplementary Spreadsheet 1).

Measures of structural connectivity (DTI fibre tracking) did not explain interindividual differences in modulating task-related functional connections in the counting condition.

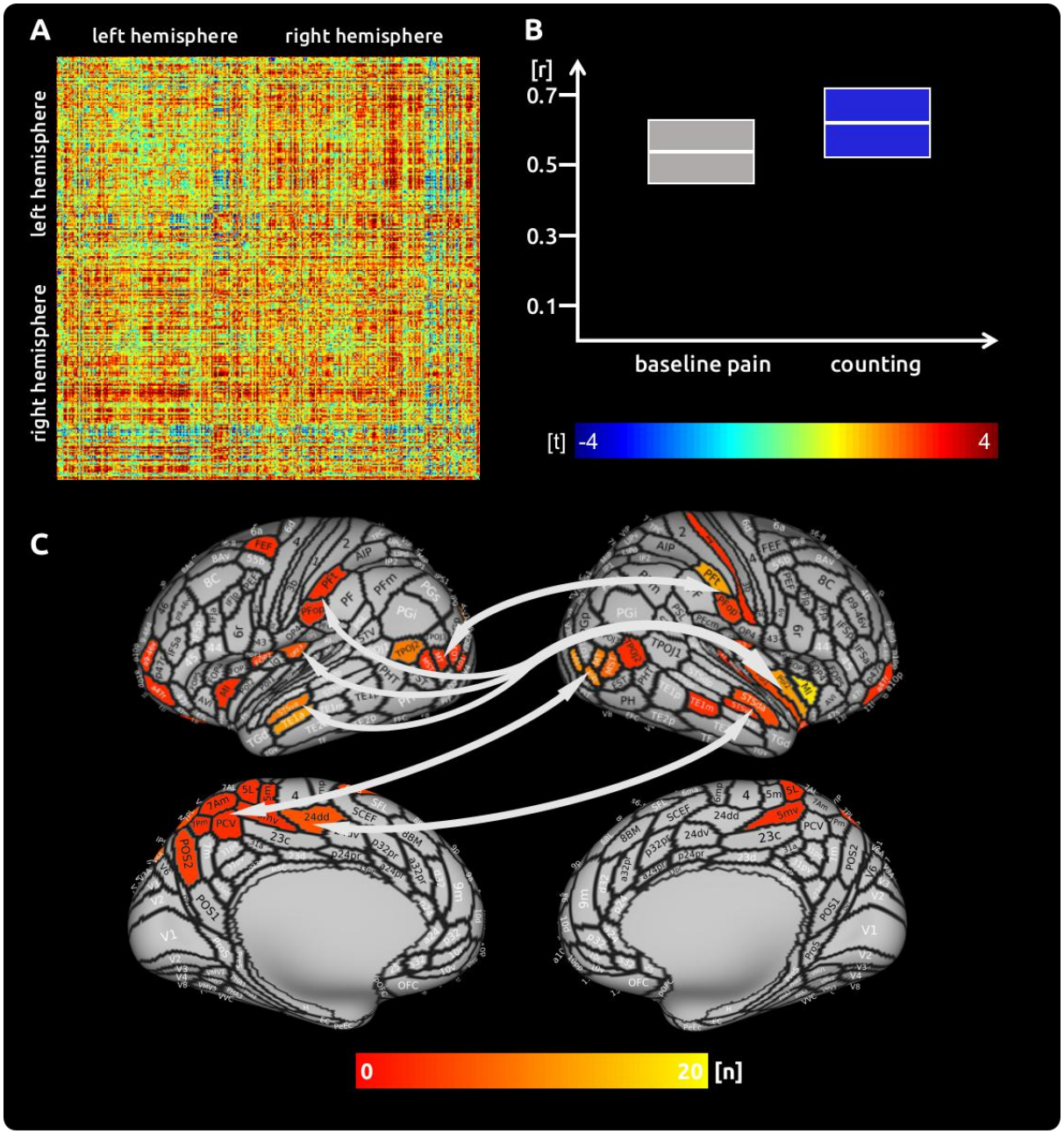

Figure 1. Counting: (A) the confusion matrix shows the statistical results. The values are mirrored along the principal diagonal of the matrix. A single red dot represents the varying connectivity between two specific brain regions and indicates that a stronger cortical connectivity in a single trial is related to a decrease in pain perception (performance encoding). These findings are the result of the higher connectivity in the trials of the counting task compared to unmodulated pain trials. (B) data from the confusion matrix averaged across all subjects, connections and trials (for illustration purposes only). (C) Depiction of the cortical regions as defined by the Glasser parcellation. The right middle insular cortex has the most connections where connectivity changes are shown to significantly modulate pain intensity.

(2) *Safe place.* During the imagining condition, we found an increase of connectivity across all cortical regions when compared to the unmodulated pain condition (Figure 2A). The detailed matrix of statistical results can be found in the supplementary material (Supplementary Spreadsheet 2). There is no negative relationship between single trial

connectivity and pain intensity. Besides the well-connected right insular cortex, we observed attenuation-related connectivity changes in right parietal (BA 5) and left superior parietal cortices (BA 7). Further well-connected areas include a frontal language area (BA 55b) as well as motor and premotor areas. The right posterior insular cortex is connected to the left parietal cortex (BA 7). The right precentral areas are functionally interconnected with prefrontal and orbitofrontal areas, the right parietal cortex (BA 5), and the left superior parietal cortex (BA 7). The right “belt” regions are functionally connected to prefrontal and orbitofrontal areas (Figure 3C).

For the safe place condition only, we found that the strength of fibre connections mediates the strength of the functional connectivity. Some subjects made use of their better structural connectivity, as measured by the number of streamlines obtained from fibre tracking. Strong structural connectivities are related to a better ability to modulate the functional connectivity in order to attenuate pain. This applies especially to connections between frontal regions (IFSp and Brodmann area 8C) and the secondary somatosensory cortex (SII). Further functional connections that are supported by the strength of fibre connections projected to memory-related areas (presubiculum of the hippocampus and entorhinal cortex).

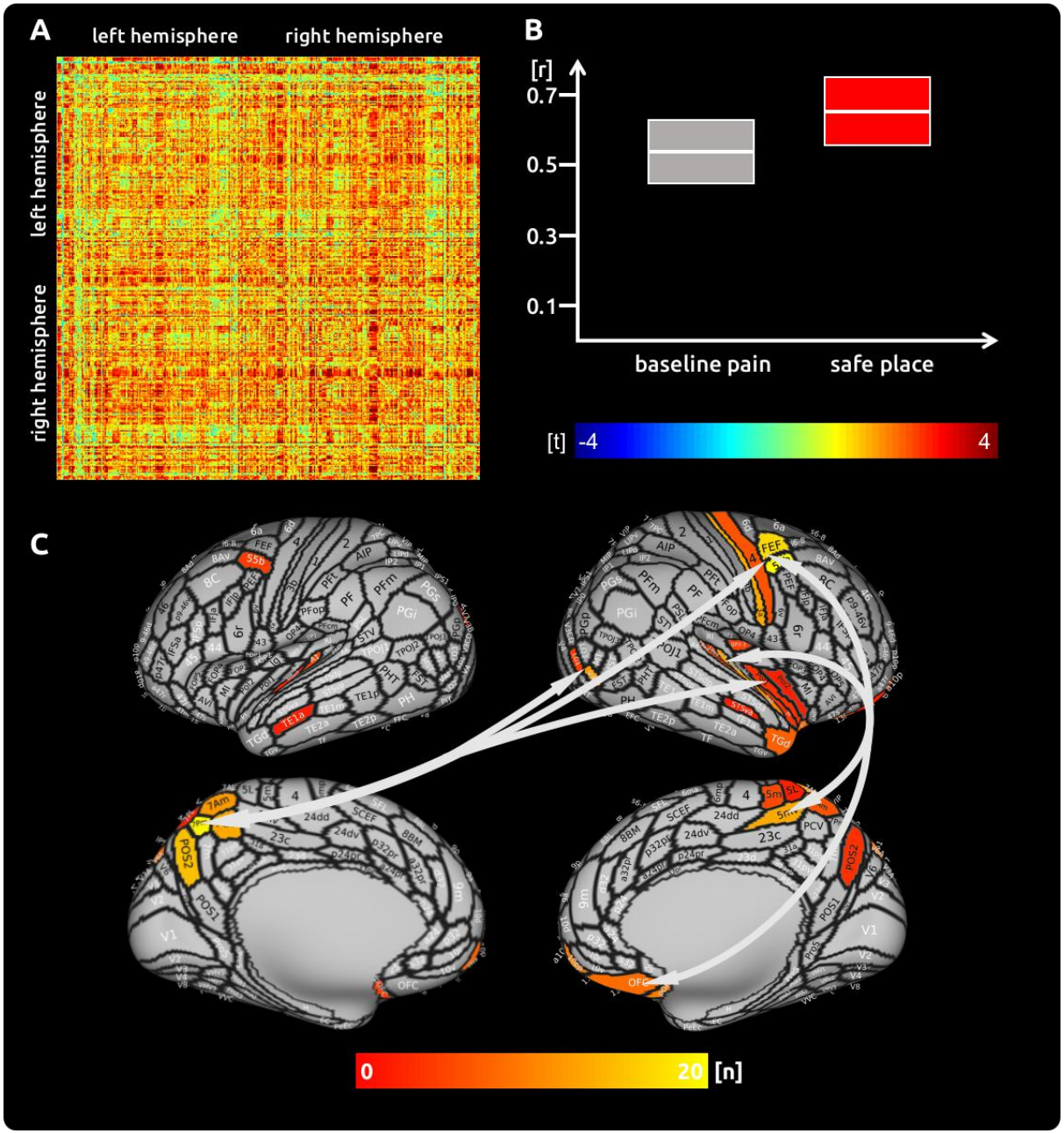

Figure 2. Safe place: (A) the confusion matrix shows the statistical results. The values are mirrored along the principal diagonal of the matrix. A single red dot represents the varying connectivity between two specific brain regions and indicates that a stronger cortical connectivity in a single trial is related to a decrease in pain perception (performance encoding). These findings are the result of the higher connectivity in the trials of the imagination task compared to unmodulated pain trials. (B) data from the confusion matrix averaged across all subjects, connections and trials (for illustration purposes only). (C) Depiction of the cortical regions as defined by the Glasser parcellation. The left parietal cortex and right premotor areas have the most connections where connectivity changes are shown to significantly modulate pain intensity.

(3) *Reappraisal.* While executing cognitive reappraisal we found a pain attenuation-related increase of functional connectivity compared to the unmodulated pain condition across the entire cortex (Figure 3A). The detailed matrix of statistical results can be found in the supplementary material (Supplementary Spreadsheet 3). Decreased functional connectivity

has not been observed. Connections that included frontal premotor and insular sub-regions contributed to a decrease of pain (Figure 3C). However, the main hub of connectivity was located in the medial parieto-occipital cortex. Besides other regions, the area V6A is interconnected with several insular and frontal premotor areas, some of which control eye movements. The structural characteristics between cortical regions did not contribute to an enhanced functional connectivity for reappraisal.

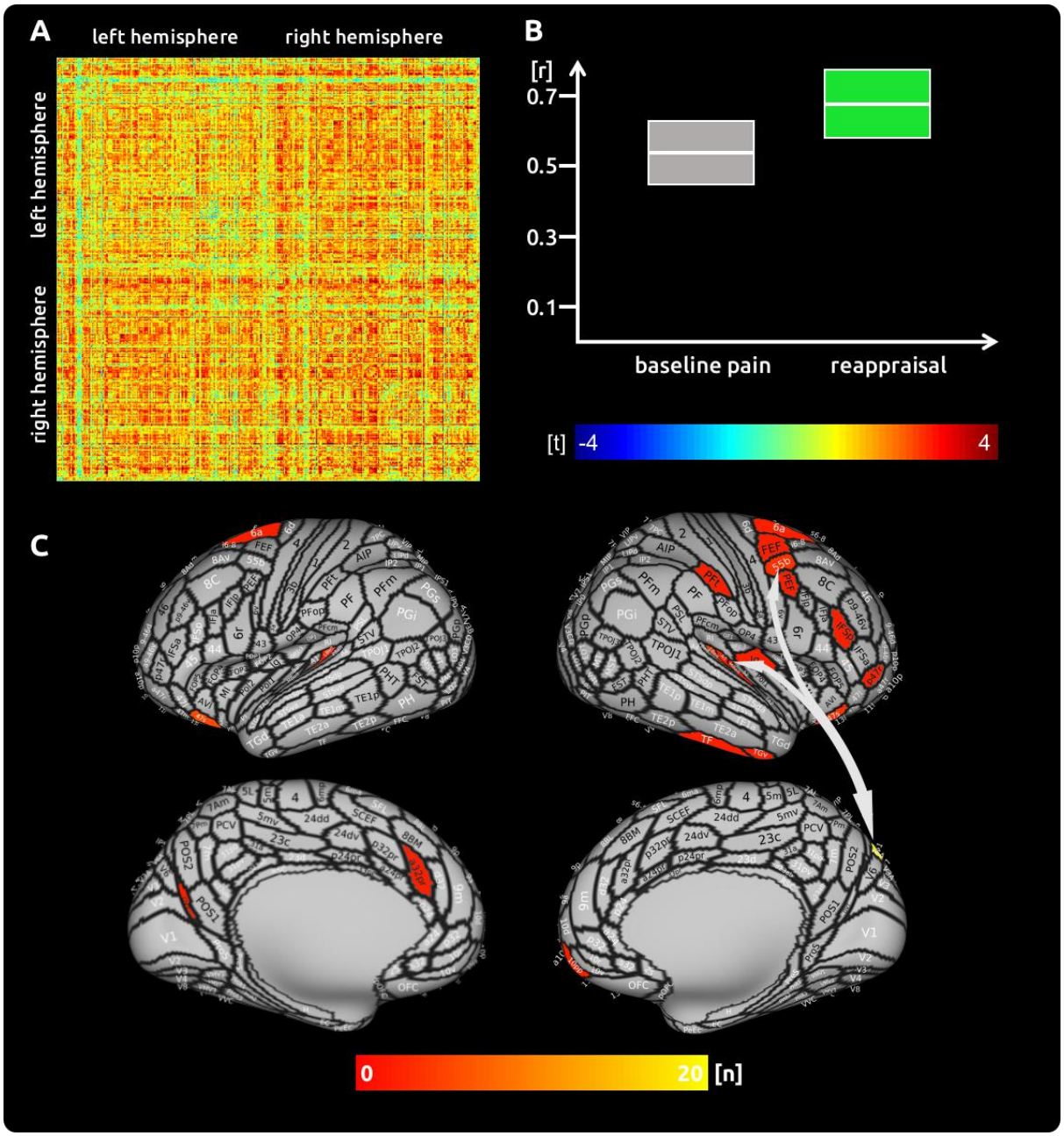

Figure 3. Reappraisal: (A) the confusion matrix shows the statistical results. The values are mirrored along the principal diagonal of the matrix. A single red dot represents the varying connectivity between two specific brain regions and indicates that a stronger cortical connectivity in a single trial is related to a decrease in pain perception (performance encoding). These findings are the result of the higher connectivity in the trials of the reappraisal task compared to unmodulated pain trials. (B) data from the confusion matrix averaged across all subjects, connections and trials (for illustration

204 purposes only). (C) Depiction of the cortical regions as defined by the Glasser parcellation. The region
205 V6A in the parieto-occipital cortex has the most connections where connectivity changes are shown to
206 significantly modulate pain intensity.

207 (4) *Conjunction analysis*. We did not find any pain-related connectivity changes present in all
208 three conditions.

DISCUSSION

Here, we aimed to explore how functional and structural connections in the brain contribute to executing cognitive tasks that attenuate pain (14,15) by utilising a single-trial analysis approach afforded by ultra-high field imaging. Across three experimental conditions, 20 healthy participants were asked to (a) count backwards, (b) imagine a safe and happy place, and (c) apply a cognitive reappraisal strategy. All strategies resulted in significant pain relief when compared to the unmodulated pain condition. We applied a whole-brain approach on the basis of brain parcellation definitions (13) and explored connectivity patterns during single attempts to attenuate pain. We further explored whether functional connections are facilitated by axonal fibre connections, measured with DTI.

Across all cognitive interventions, our results revealed an *increase* of connectivity pattern throughout the cerebral cortex for all three interventions; a higher functional connectivity was related to particularly successful single attempts to attenuate pain. Therefore, the unmodulated pain trials - which were experienced as considerably more painful - exhibited a lower functional connectivity compared to pain trials during cognitive tasks. This finding has two implications:

First, increased connectivity in *task-related* regions is necessary to successfully execute the respective cognitive tasks.

S*econd*, contrary to our hypothesis and previous findings, increased connectivity with *pain-related* brain regions (e.g. insular cortex, ACC, or somatosensory cortices) is related to successful attenuation trials with decreased intensities of pain. These increased connectivities are required to actively suppress the *activity* in regions known to contribute to pain processing (16) and are further modulated in the respective task (14). The neuronal activity of these pain-related brain regions are most likely to be actively inhibited, such as by GABAergic neurons in the insular cortex (17,18), and thus contribute to a lower pain experience by impeding the processing of pain in this region.

Counting. For the cognitively-demanding counting task, we found a number of well-connected regions that contribute directly or indirectly to the reduction of pain intensity. These regions are located in the parietal and occipito-temporal cortices, overlapping with the modulation of BOLD activity during counting tasks (14,19). Increased connectivity during counting occurred for connections with pain processing areas, such as divisions of the insular cortex, the posterior cingulate cortex, and the primary and the secondary somatosensory cortices. The highest number of connections to other brain regions during the counting task was found for the right middle insular cortex. Although our analyses do not

allow for any assumptions on directionality, the many pain-related functional connectivities between left parietal areas (high BOLD activity) and right insular sub-regions (low BOLD activity) suggest a suppression effect on the insular areas (see (14)).

Disrupted connectivities during the counting task were observed for the right temporo-parieto-occipital junction (TPJ) to the right posterior insula, as well as to temporo-occipital areas. Given the involvement of the TPJ in attentional processing (20,21), elevated focus on the task may have decreased the transmission during task execution but increased the transmission for the unmodulated pain trials (14). The counting trials are further suggested to require a visual support by imagining the numbers in space (22). Visual areas in the *left* occipito-temporal cortex connect to and suppress right parietal opercular areas. We also found visual support located in the *right* occipito-temporal cortex that is functionally connected to parietal areas, which in turn suppress insular activity.

Safe place. Similar to the counting condition, we found regions in the left and right parieto-occipital cortex to be highly connected to other brain regions. Notably, the parietal cortex is functionally connected without a rise of regional BOLD activity (see(14)). This effect shows that brain regions can play an important role in pain processing via an exchange of information, where low-scale modulations of cortical activity are not causing large metabolic effects. Moreover, the strong connectivity pattern between left parietal and right insular regions suggests an active suppression of insular regions initiated by the parietal cortex (as reflected by *increased* functional connectivity between these regions).

We found well-connected regions in the precentral gyrus: area 55b has been shown to be active during listening to stories in the language task of the Human Connectome Project dataset (13). Therefore, the increased connectivity in area 55b may be related to the narrative aspects of the imaginary task in which the participants may recall being actively involved in an event of pleasure and happiness. The premotor and motor areas in the precentral gyrus in particular may reflect the motor aspect of the imagination task (23,24). They are connected to orbitofrontal areas which are thought to initiate top-down pain suppression of ascending pathways (9,25).

For the safe place condition only, we found that the ability to functionally utilise certain pathways is mediated by the strength of axonal fibre connections. These anatomical characteristics are suggested to help the participants with stronger fibre connections to better attenuate pain. This applies especially to connections between middle frontal regions (IFSp and 8C) to the secondary somatosensory cortex (SII). Further functional connections

that are supported by the strength of fibre connections project from the frontal cortex to memory-related limbic areas (presubiculum of the hippocampus, entorhinal cortex), which could facilitate memory retrieval for the imagination of pleasant scenes (26-30).

Reappraisal. The best connected region during cognitive reappraisal is located in the higher order visual cortex, area V6A, which is mainly interconnected with insular and frontal premotor areas. Area V6A is known to contribute to spatial object localisation; a study on monkeys shows that V6A cells are active when executing reaching movements independent of visual or oculomotor processing (31). These cells have also been found to encode body-centred spatial localisation (32). The use of V6A and its connection to other brain areas could help the participants - as required by the task - to focus on the stimulated body site. However, this focussing should be considered as a prerequisite and does not necessarily imply any pain attenuation. Yet the focus on pain has been shown to increase pain perception and pain-related cortical activity (33-35). Therefore, as found in the present investigation, the connections from the inferior frontal cortex, the anterior cingulate cortex, the frontal pole, and orbitofrontal cortex are additionally required to utilise cognitive reappraisal (36,37) in order to ultimately attenuate the experience of pain (14).

Analysing pain-related functional connections in the human brain. Unlike previous studies, we almost exclusively found a lower functional connectivity for trials and conditions of higher pain intensity, which could be caused by differences in experimental design and analysis strategies.

In neuroimaging, functional connectivity is considered a joint phase-locked oscillation of spatially distant cortical regions. Task-based connectivity analyses predominantly utilise a seed-based approach to determine the functional connectivity between a predefined seed region and one or more distant brain regions; such analyses can only take into account the short period during which a task is being executed. However, exact connectivity measures between brain regions would require a sufficient number of samples to quantify the joint in-phase increases and decreases of the BOLD response. In order to estimate a reliable measure of connectivity, we applied a relatively long time window (~30s, 15 data points) for inflicting pain, for executing the cognitive task, and for reliably determining the connectivity of a single trial. The strong focus on extended stimulation makes the present investigation difficult to compare with previous work. For instance, a study by Villemure & Bushnell (5) sampled every 4s but analysed a relatively short time window of 5s painful stimulation to investigate connectivity. Another study analysed a single data point (3s analysis window, sampling of 3s) before nociceptive laser stimulation to predict pain intensity (8). A further study used 3 data points for connectivity analyses of an experiment in which the pain

311 stimulations lasted 10s (4). A repeated stimulation at the frequency of the recording
312 (application of 5 brief laser pain stimuli every 3s sampled with a TR of 3s) makes it difficult to
313 separate the connectivity aspects from the general increase of the BOLD response (6).

314 Therefore, the different methodological approaches might have caused our findings to
315 contradict previous studies, in which high levels of pain were shown to increase cortical
316 connectivity between pain processing brain regions (4-6). Villemure & Bushnell (5) and
317 Ploner et al. (6) found a stronger connectivity in pain processing brain regions for conditions
318 that increased the intensity of pain (i.e. increased attention, more negative emotion). The
319 connectivity of the inferior frontal cortex for an emotional condition, and the connectivity of
320 the superior parietal cortex, and the entorhinal cortex for the attentional condition were found
321 to modulate cortical processes (5).

322 Other studies investigated the connectivity in the descending pain control system and
323 observed an increase of connectivity between the perigenual ACC and the PAG during a
324 pain-relieving placebo intervention (9). Given the lower signal-to-noise ratio in mid-brain
325 areas, this finding could not be replicated in any of the present conditions with the current
326 whole-brain approach and a strict correction for multiple comparisons (38). By lowering the
327 statistical threshold, we found a modulation of pain intensity-dependent functional
328 connectivity from the PAG to regions that contribute to pain processing, such as the anterior
329 ventral insula ($t>2$), the midcingulate cortex ($t>2.5$), and the nucleus accumbens ($t>3$),
330 indicating a stronger connectivity for the pain condition with cognitive modulation.

331 Further studies directly investigated the functional connectivity in the brain in response to
332 different intensities of pain stimuli. Sprenger et al. (2015) found an increase of connectivity in
333 subcortical nuclei for the higher of two pain conditions. Similarly, an increased connectivity
334 has been found in response to cold pain stimulation. The authors reported a significant
335 correlation across the entire time course of the experiment between predefined regions that
336 are known to be involved in the processing of pain (39). As discussed above, our data
337 showed that the decrease of pain is predominantly related to an increase of cortical
338 connectivity in both *pain-related* regions (e.g. subregions of the insular cortex) and *task-*
339 *related* brain regions (subregions in the frontal and parietal cortex).

340 *Summary.* The present investigation resembles a clinical intervention in which a pain patient
341 would be taught to utilise cognitive strategies to attenuate pain. Here, we investigated which
342 cortical connection contributes to particularly successful trials to attenuate pain. In contrast
343 to previous research, we revealed an increased connectivity for the single attempts that

resulted in lower percepts of pain. This applies to the classical pain processing regions (e.g. insula, cingulate cortex, and somatosensory cortices). Although we found different connectivity patterns for all interventions, the general mechanism was universally valid. The present findings are suggested to open a new window in the understanding of cortical processes that are associated with high levels of long-lasting tonic or chronic pain. As a consequence, clinical treatments that would aim to decrease cortico-cortical connections are suggested to have a rather detrimental effect on pain relief in patients suffering from chronic pain. Future studies would be needed to investigate the effect of cognitive interventions on the intracortical connectivity in pain patients.

METHODS

Twenty two healthy human subjects (18 female/4 male) with a mean age of 27±5 years (21 - 37 years) participated in the experiment. Two of the female subjects were excluded as a result of insufficient data quality. All subjects gave written informed consent. The study was approved by the Medical Sciences Interdivisional Research Ethics Committee of the University of Oxford and conducted in conformity with the Declaration of Helsinki.

The experiment has been described in detail in our previous publication (14) and consisted of four conditions (see table 1) across 4 separate blocks, where each block comprised of 12 trials from the same condition. In all conditions and trials the subjects received cold pain stimuli on the dorsum of their left hand delivered by a thermode (Pathway II; Medoc Ltd, Ramat Yishai, Israel). The subjects were prompted to rate pain intensity and pain unpleasantness. A numerical and a visual analogue scale (VAS), ranged between 0 - 100 in steps of 5 points, was used to assess the pain ratings. The endpoints of the scale were determined as no pain (0) and the maximum pain the subjects were willing to tolerate (100). Single trial ratings were recorded after each trial.

Table 1: Conditions and Instructions.

(0) pain, non-modulated	Concentrate only on the pain.
(A) attentional shift	Count backwards from 1000 (+x) by sevens.
(B) imaginal strategy	Imagine that you are in a safe and happy place that you know very well. That place has the colours you like and you hear the music you like. There are only people around that you want to have around you. You feel well and comfortable.
(C) cognitive reappraisal	Concentrate on the cool and tingling sensations in your arm and reinterpret these sensations as not painful.

The thermode temperature for painful stimulation for each subject was determined in an extensive practise session one week prior to scanning and was individually adapted to a VAS score of 50. The 40s of painful stimulation were then preceded by a rest period of 10s at 38°C thermode temperature. The first 10s were not included in the analysis. The mean temperature of cold pain application across subjects was 7°C with a standard deviation of 3.6°C. In order to avoid habituation effects, the thermode temperature during painful stimulation was oscillating with ⅛ Hz at ± 3°C (40,41).

376 *Data Acquisition.* Imaging data were acquired on a 7T Siemens MRI scanner. Each volume
377 comprised 34 axial slices of 2 mm thickness and 2 × 2 mm in-plane resolution with 1mm gap
378 between slices. The repetition time (TR) was 1.96s, the echo time (TE) was 25ms (flip angle
379 90°), the field of view (FOV) was 220 × 220 mm, and the matrix size was 110 × 110 pixels. A
380 T1-weighted structural image (isotropic 1mm^3 voxel) was acquired for the registration of the
381 functional images to the MNI (Montreal Neurological Institute) template. Two sequences of
382 diffusion tensor images (DTI) were recorded with L>>R and R>>L phase encoding direction.
383 64 directions were recorded with a TR of 9.3s, a TE of 63ms, and an acceleration factor of 2.
384 The length of the edge of the isotropic voxels was 1.2 mm.

385 *Image processing - preprocessing of functional connectivity data.* The data were
386 preprocessed with FSL (42). The preprocessing of the *functional data* consisted of brain
387 extraction, high-pass filtering with a frequency cutoff of 1/90 Hz, a spatial normalisation to
388 the MNI template, a correction for head motion during scanning registered to the MNI
389 template, and a spatial smoothing (6mm FWHM). The data were further semi-automatically
390 cleaned of artefacts with independent component analysis (ICA) (43,44). The number of
391 components had been set *a priori* to 200. Artefact-related components were removed from
392 the data. The design matrix for painful stimulation, including the temporal derivative, were
393 then regressed out from the data in Matlab (The Mathworks, USA).

394 *Image processing - preprocessing of structural connectivity data.* Preprocessing of DTI data
395 was performed using FSL. FSL preprocessing included (i) correcting susceptibility induced
396 distortions (“topup”), (ii) skull stripping (“bet”), (iii) corrections for eddy currents and head
397 motion (“eddy”), and (iiii) determining the strength of structural connectivity between cortical
398 regions (“bedpostx” and “probtrackx”) defined by the Glasser atlas.

399 *Image processing - extraction of regions of interest data.* The time series of functional
400 volumes were converted to MNI space and subsequently projected to surface space by
401 using the “Connectome Workbench” package. We used a template that allowed to project
402 from 3D standard MNI space to 2D surface space. Regions of interest (ROIs) were defined
403 by subdividing the cortical surface into 180 regions per hemisphere (13). Six further regions
404 that are important for the processing of pain, such as the PAG, the thalamus and the
405 amygdala, were also included. Latter ROIs were based on the Oxford Atlas, implemented in
406 FSL.

Image processing - computation of single trial functional connectivity scores. The time courses for all voxels of cortical activity for a specific region of the Glasser Atlas, e.g. the middle insula, were extracted. We computed principal component analyses (PCA) separately for each ROI and subject and selected the first component (Matlab, The MathWorks, Inc., USA). The plateau phase of the last ~30s of painful stimulation (15 data points) has been extracted from each region and trial for each subject and condition. Outliers were removed from the data. These 15 data points determined the connectivity for a brain region for a given trial. Correlation coefficients were computed for each trial and for each ROI with the remaining 370 ROIs. The single trial correlation coefficients were Fisher Z-transformed and fed into group-level statistical analysis.

Image processing - structural connectivity data. DTI data were also analysed in FSL. The processing steps included a median filter, a correction for susceptibility distortions, and fibre tracking from the same aforementioned brain regions (Glasser parcellation - see above).

Statistical modelling: The statistical analysis for the connectivity between cortical regions has been performed in Matlab. To explore the relationship of fluctuating cortical connectivity and the variable pain experience, we computed linear mixed effects models (LMEs) that related the single trial correlation coefficients between two brain regions to the pain intensity scores (14,45). Each condition in the model included the data for the respective intervention plus the trials of the unmodulated pain condition (for more details regarding the statistical analyses see (14). The model is expressed in Wilkinson notation https://www.mathworks.com/help/stats/wilkinson-notation.html). Statistical thresholds were determined by PALM software (38).

painrating ~ func_conn + (1| subject)

We further analysed whether individual differences in functional connectivity could be explained by individual structural characteristics of the brain. In other words, we analysed whether the functional connectivity that leads to a single subject's successful pain attenuation is facilitated by that subject's high number of fibre tracts. In a similar vein, a poor functional connectivity that is not able to contribute to pain attenuation might be caused by a low number of fibre tracts.

painrating ~ func_conn:struc_conn + (1| subject)

437 We considered only functional connections with a t-value >2 as potentially modulated by
438 structural connections.

REFERENCES

(1) Tracey I, Mantyh PW. The Cerebral Signature for Pain Perception and Its Modulation. Neuron 2007 August;55(3):377-391.
(2) Wiech K, Ploner M, Tracey I. Neurocognitive aspects of pain perception. Trends Cogn Sci (Regul Ed) 2008 August;12(8):306-313.
(3) Wiech K, Tracey I. Pain, decisions, and actions: a motivational perspective. Frontiers in neuroscience 2013 January;7:46.
(4) Sprenger C, Finsterbusch J, Büchel C. Spinal cord-midbrain functional connectivity is related to perceived pain intensity: a combined spino-cortical FMRI study. J Neurosci 2015 March 11,;35(10):4248-4257.
(5) Villemure C, Bushnell MC. Mood influences supraspinal pain processing separately from attention. J Neurosci 2009 Jan 21,;29(3):705-715.
(6) Ploner M, Lee MC, Wiech K, Bingel U, Tracey I. Flexible cerebral connectivity patterns subserve contextual modulations of pain. Cereb Cortex 2011 March;21(3):719-726.
(7) Sprenger C, Eichler I, Eichler L, Zöllner C, Büchel C. Altered Signaling in the Descending Pain-modulatory System after Short-Term Infusion of the μ-Opioid Agonist Remifentanil. J Neurosci 2018 03 07,;38(10):2454-2470.
(8) Ploner M, Lee MC, Wiech K, Bingel U, Tracey I. Prestimulus functional connectivity determines pain perception in humans. Proc Natl Acad Sci U S A 2010 January 5,;107(1):355-360.
(9) Eippert F, Bingel U, Schoell ED, Yacubian J, Klinger R, Lorenz J, et al. Activation of the opioidergic descending pain control system underlies placebo analgesia. Neuron 2009 August;63(4):533-43.
(10) Sprenger C, Eippert F, Finsterbusch J, Bingel U, Rose M, Büchel C. Attention modulates spinal cord responses to pain. Curr Biol 2012 Jun 5,;22(11):1019-1022.
(11) Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, et al. Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci 2002 April;22(7):2748-2752.
(12) Lin RL, Douaud G, Filippini N, Okell TW, Stagg CJ, Tracey I. Structural Connectivity Variances Underlie Functional and Behavioral Changes During Pain Relief Induced by Neuromodulation. Sci Rep 2017 Feb 02,;7:41603.
(13) Glasser MF, Coalson TS, Robinson EC, Hacker CD, Harwell J, Yacoub E, et al. A multi-modal parcellation of human cerebral cortex. Nature 2016 Aug 11,;536(7615):171-178.
(14) Schulz E, Stankewitz A, Witkovský V, Winkler AM, Tracey I. Strategy-dependent modulation of cortical pain circuits for the attenuation of pain. Cortex 2019 Jan 04,;113:255-266.
(15) Devine DP, Spanos NP. Effectiveness of maximally different cognitive strategies and expectancy in attenuation of reported pain. J Pers Soc Psychol 1990 April;58(4):672-678.
(16) Tracey I. Imaging pain. Br J Anaesth 2008;101(1):32-39.
(17) Thiaucourt M, Shabes P, Schloss N, Sack M, Baumgärtner U, Schmahl C, et al. Posterior Insular GABA Levels Inversely Correlate with the Intensity of Experimental Mechanical Pain in Healthy Subjects. Neuroscience 2018 09 01,;387:116-122.
(18) Watson CJ. Insular balance of glutamatergic and GABAergic signaling modulates pain processing. Pain 2016 10;157(10):2194-2207.
(19) Johansen-Berg H, Matthews PM. Attention to movement modulates activity in sensori-motor areas, including primary motor cortex. Exp Brain Res 2002 Jan;142(1):13-24.
(20) Mars RB, Sallet J, Schüffelgen U, Jbabdi S, Toni I, Rushworth MFS. Connectivity-based subdivisions of the human right "temporoparietal junction area": evidence for different areas participating in different cortical networks. Cereb Cortex 2012 Aug;22(8):1894-1903.
(21) Kucyi A, Hodaie M, Davis KD. Lateralization in intrinsic functional connectivity of the temporoparietal junction with salience- and attention-related brain networks. J Neurophysiol 2012 Dec;108(12):3382-3392.
(22) Amalric M, Dehaene S. Origins of the brain networks for advanced mathematics in

492 expert mathematicians. Proc Natl Acad Sci U S A 2016 May 03,;113(18):4909-4917.
493 (23) Xie F, Xu L, Long Z, Yao L, Wu X. Functional connectivity alteration after real-time fMRI
494 motor imagery training through self-regulation of activities of the right premotor cortex. BMC
495 Neurosci 2015 May 01,;16:29.
496 (24) Szameitat AJ, Shen S, Sterr A. Motor imagery of complex everyday movements. An
497 fMRI study. Neuroimage 2007 Jan 15,;34(2):702-713.
498 (25) Bingel U, Schoell E, Herken W, Buchel C, May A. Habituation to painful stimulation
499 involves the antinociceptive system. Pain 2007;131(1-2):21-30.
500 (26) Schultz H, Sommer T, Peters J. The Role of the Human Entorhinal Cortex in a
501 Representational Account of Memory. Front Hum Neurosci 2015;9:628.
502 (27) Braskie MN, Small GW, Bookheimer SY. Entorhinal cortex structure and functional MRI
503 response during an associative verbal memory task. Hum Brain Mapp 2009
504 Dec;30(12):3981-3992.
505 (28) Dalton MA, Maguire EA. The pre/parasubiculum: a hippocampal hub for scene-based
506 cognition? Curr Opin Behav Sci 2017 Oct;17:34-40.
507 (29) Hodgetts CJ, Voets NL, Thomas AG, Clare S, Lawrence AD, Graham KS. Ultra-High-
508 Field fMRI Reveals a Role for the Subiculum in Scene Perceptual Discrimination. J Neurosci
509 2017 03 22,;37(12):3150-3159.
510 (30) Montchal ME, Reagh ZM, Yassa MA. Precise temporal memories are supported by the
511 lateral entorhinal cortex in humans. Nat Neurosci 2019 Feb;22(2):284-288.
512 (31) Fattori P, Kutz DF, Breveglieri R, Marzocchi N, Galletti C. Spatial tuning of reaching
513 activity in the medial parieto-occipital cortex (area V6A) of macaque monkey. Eur J
514 Neurosci 2005 Aug;22(4):956-972.
515 (32) Hadjidimitrakis K, Bertozzi F, Breveglieri R, Fattori P, Galletti C. Body-centered, mixed,
516 but not hand-centered coding of visual targets in the medial posterior parietal cortex during
517 reaches in 3D space. Cereb Cortex 2014 Dec;24(12):3209-3220.
518 (33) Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention
519 modulates pain in humans using functional MRI. Brain 2002 February;125:310-319.
520 (34) Hauck M, Lorenz J, Engel AK. Attention to painful stimulation enhances gamma-band
521 activity and synchronization in human sensorimotor cortex. J Neurosci 2007
522 August;27(35):9270-9277.
523 (35) Peyron R, Garcia-Larrea L, Gregoire MC, Costes N, Convers P, Lavenne F, et al.
524 Haemodynamic brain responses to acute pain in humans: sensory and attentional networks.
525 Brain 1999 September;122 (Pt 9:1765-1780.
526 (36) Buhle JT, Silvers JA, Wager TD, Lopez R, Onyemekwu C, Kober H, et al. Cognitive
527 Reappraisal of Emotion: A Meta-Analysis of Human Neuroimaging Studies. Cerebral cortex
528 (New York, N.Y.: 1991) 2013 June;24(11):2981-2990.
529 (37) Tracey I. Getting the pain you expect: mechanisms of placebo, nocebo and reappraisal
530 effects in humans. Nat Med 2010 November;16(11):1277-83.
531 (38) Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference
532 for the general linear model. Neuroimage 2014 May 15,;92:381-397.
533 (39) Wilcox CE, Mayer AR, Teshiba TM, Ling J, Smith BW, Wilcox GL, et al. The Subjective
534 Experience of Pain: An FMRI Study of Percept-Related Models and Functional Connectivity.
535 Pain Med 2015 Nov;16(11):2121-2133.
536 (40) Lautenbacher S, Roscher S, Strian F. Tonic pain evoked by pulsating heat: temporal
537 summation mechanisms and perceptual qualities. Somatosens Mot Res 1995;12(1):59-70.
538 (41) Stankewitz a, Schulz E, May A. Neuronal correlates of impaired habituation in response
539 to repeated trigemino-nociceptive but not to olfactory input in migraineurs: an fMRI study.
540 Cephalalgia 2013;33(4):256-265.
541 (42) Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM. FSL. Neuroimage
542 2012 Aug 15,;62(2):782-790.
543 (43) Salimi-Khorshidi G, Douaud G, Beckmann CF, Glasser MF, Griffanti L, Smith SM.
544 Automatic denoising of functional MRI data: combining independent component analysis and
545 hierarchical fusion of classifiers. Neuroimage 2014 Apr 15,;90:449-468.
546 (44) Griffanti L, Salimi-Khorshidi G, Beckmann CF, Auerbach EJ, Douaud G, Sexton CE, et

al. ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging. Neuroimage 2014 Jul 15,;95:232-247.

(45) Schulz E, May ES, Postorino M, Tiemann L, Nickel MM, Witkovsky V, et al. Prefrontal Gamma Oscillations Encode Tonic Pain in Humans. Cereb Cortex 2015 Nov;25(11):4407-4414.

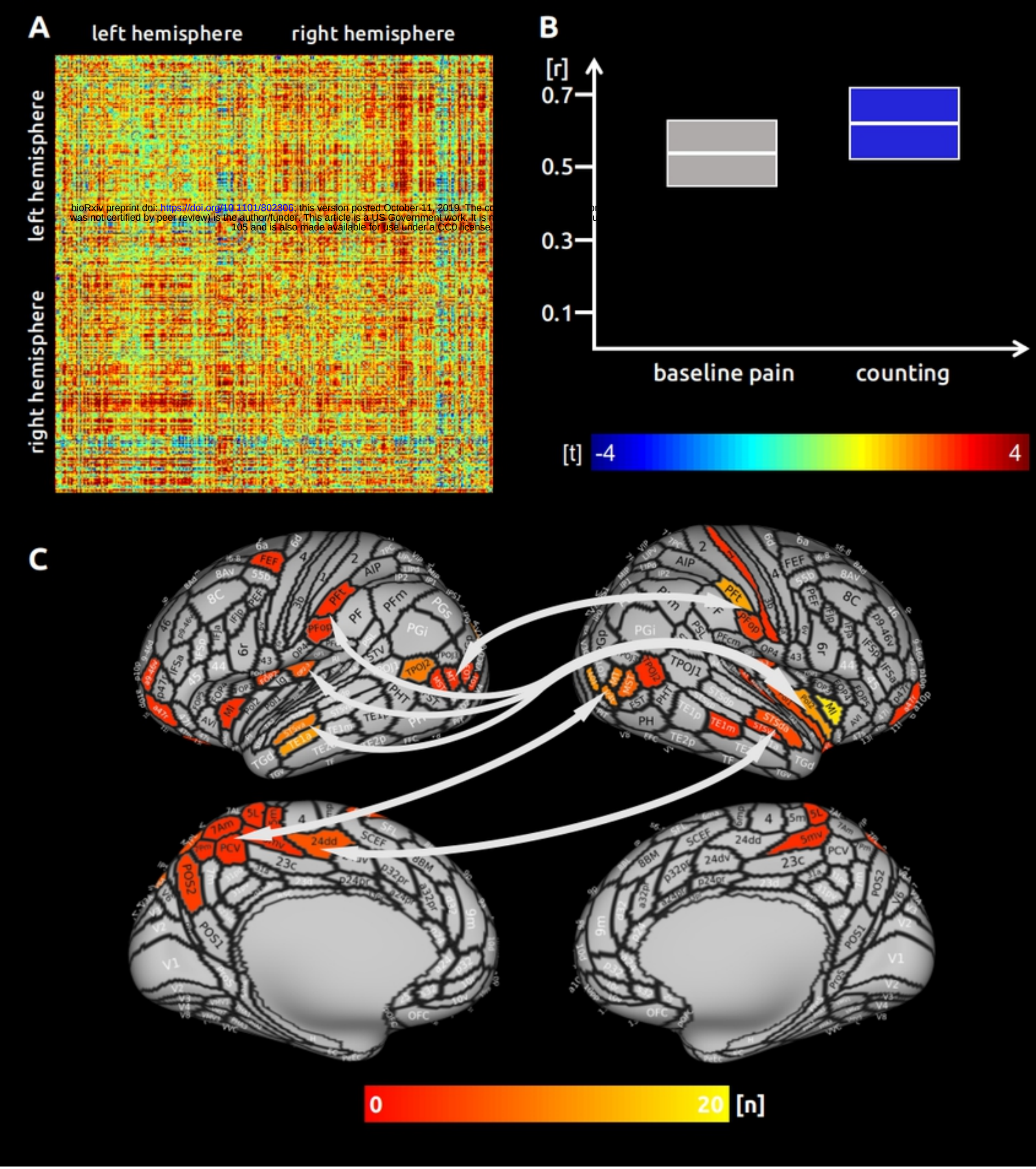

Figure 1

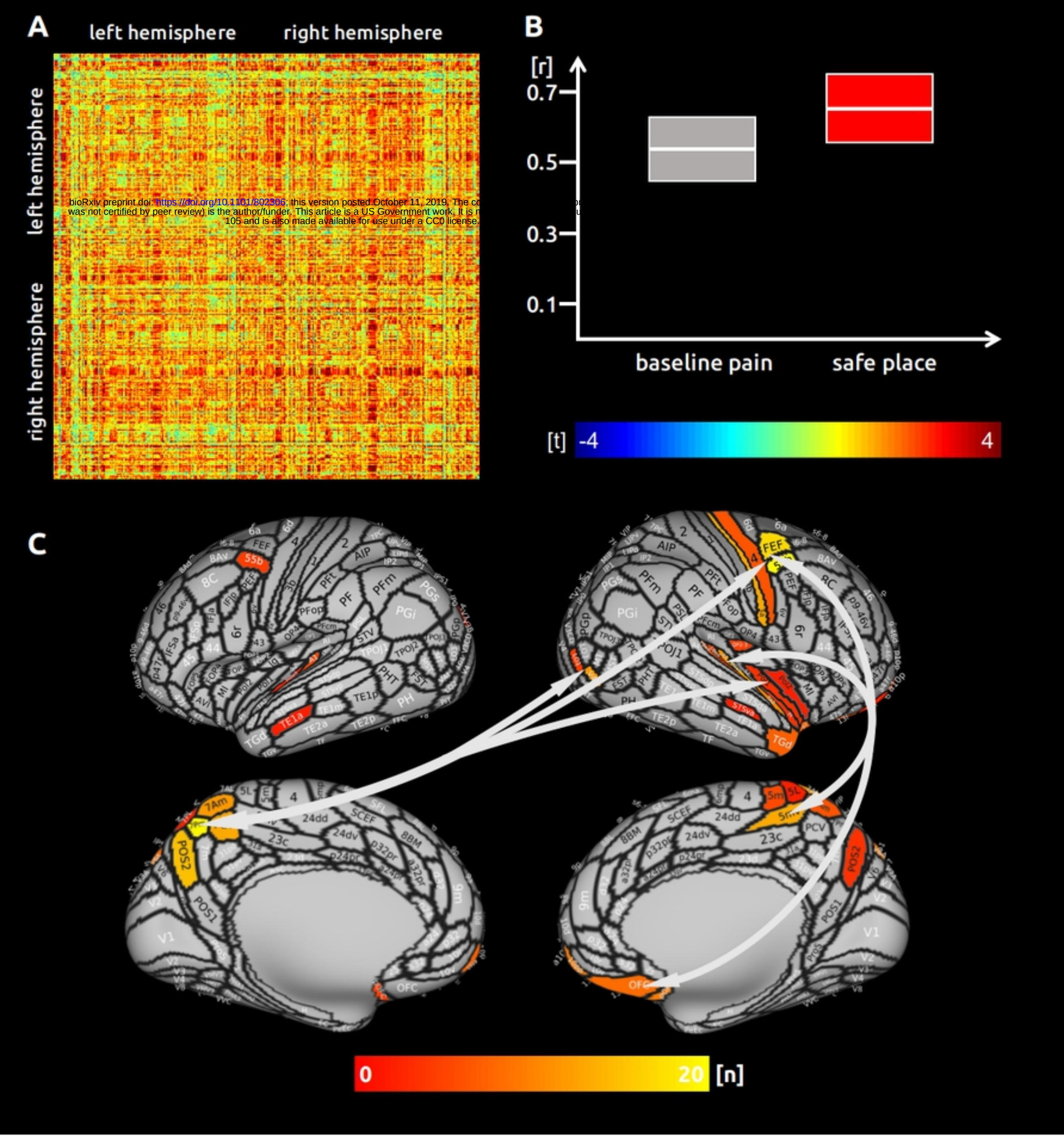

Figure 2

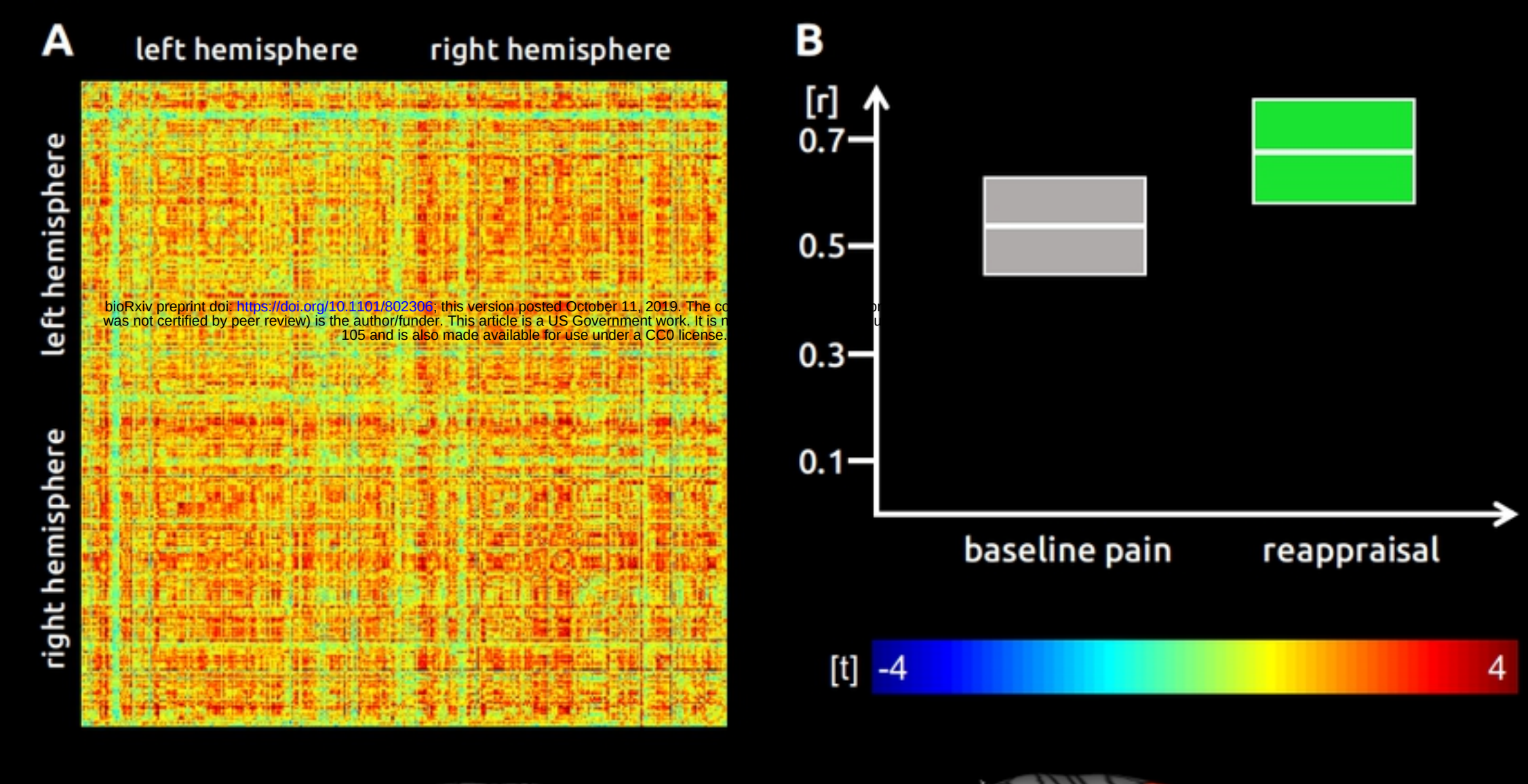

Figure 3