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Assessing the specificity of the relationship between brain alpha oscillations and tonic pain

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ABSTRACT

Recent research proposed that the slowing of individual alpha frequency (IAF) could be an objective marker of pain. However, it is unclear whether this research can fully address the requirements of specificity and sensitivity of IAF to the pain experience. Here, we sought to develop a robust methodology for assessing the specificity of the relationship between alpha oscillations and acute tonic pain in healthy individuals. We recorded electroencephalography (EEG) of 36 volunteers during consecutive 5-minute sessions of painful hot water immersion, innocuous warm water immersion and aversive, non-painful auditory stimulus, matched by unpleasantness to the painful condition. Participants rated stimulus unpleasantness throughout each condition. We isolated two regions of the scalp displaying peak alpha activity across participants: *centro-parietal* (CP) and *parieto-occipital* (PO) ROI. In line with previous research our findings revealed decreased IAF during hot compared with warm stimulation, however the effect was not specific for pain as we found no difference between hot and sound in the CP ROI (compared to baseline). In contrast, the PO ROI reported the same pattern of differences, but their direction was opposite to the CP in that this ROI revealed faster frequency during hot condition than controls. Finally, we show that IAF in both ROIs did not mediate the relationship between the experimental manipulation and the affective experience. Altogether, these findings emphasize the importance of a robust methodological and analytical design to disclose the functional role of alpha oscillations during affective processing. Likewise, they suggest the absence of a causal role of IAF in the generation of acute pain experience in healthy individuals.

1. Introduction

The study of the relationship between the experience of pain and its neural substrates is a remarkable example of reverse inference in the scientific endeavour, and it has seen consensus moving from the hypothesis of no specialized pain centre in the brain (Melzack, 1990; Melzack and Loeser, 1977) to the belief of a "pain matrix" specifically coding the experience of pain (Apkarian et al., 2005; Ingvar, 1999; Tracey and Mantyh, 2007; Treede et al., 1999) until the acceptance, once again, of the lack of pain-specific brain responses as measured with current neuroimaging technology (Davis et al., 2015; Iannetti and Mouraux, 2010; Salomons et al., 2016). However, the endemic lack of neural specificity in the study of pain did not (after all) discourage the search for markers of modulatory mechanisms involved in pain and similar aversive/unpleasant affective bodily states.

In this respect, the examination of the temporal characteristics of pain gains a central role. Most experimental studies induced phasic/transient acute pain (milliseconds to seconds). Other studies investigated the experience associated with a longer stimulation (minutes and repeated sessions). According to our qualitative assessment, a greater deal of research has investigated the effects of brief acute pain rather than prolonged tonic pain on neural responses (Verne et al., 2004). Over the years, only a few neuroimaging studies focused on the neural correlates of tonic pain by means of both hemodynamic and electrophysiological techniques (e.g., Casey et al., 1996; Huber et al., 2006; Schreckenberger et al., 2005). Relevant to our research, past studies interpreted decreased electroencephalography (EEG) alpha power during prolonged pain (compared to pre-stimulus activity) as reflecting changes

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in pain perception (Chen and Rappelsberger, 1994; Giehl et al., 2014; Li et al., 2016; Nir et al., 2012; Peng et al., 2015). However, others concluded that there was no specific EEG change associated with pain (e.g., Chang et al., 2001; Huber et al., 2006; Ploner et al., 2017 for a review). More recently, the occurrence of decreased alpha as well as increased gamma power were reported (Peng et al., 2015; Schulz et al., 2015). Importantly, while some of these studies indicated correlation between the modulation of the magnitude in both alpha and gamma frequency and pain perception, other studies described dissociation between them. For example, Schulz et al. (2015) reported no significant relationship between pain intensity and the magnitude of brain oscillations in the theta, alpha and beta range, but hinted to a pain-specific role of prefrontal gamma oscillations during tonic ongoing pain. The same research group later replicated this pattern (Nickel et al., 2017). Nevertheless, they also revealed an increased connectivity between the sensorimotor and prefrontal region through alpha frequency during tonic pain (Nickel at al., 2019).

To date, the functional role of brain oscillations in prolonged experimental pain is not fully established. The systematic assessment of alpha oscillations, and particularly its frequency, has fuelled pain neuroscientists' hope to discover a mechanistic role, similarly to other research domains (Cecere et al., 2015; Cooke et al., 2019; Di Gregorio et al., 2022; Foxe and Snyder, 2011; Klimesch, 1999; Mierau et al., 2017; Migliorati et al., 2019; Samaha and Postle, 2015).

The study of measures of alpha frequency has been recently adopted in the context of neurogenic inflammatory pain models (i.e., intradermal capsaicin) in healthy humans (Furman et al., 2018). This approach allows induction of hyperalgesia, which is one of the main symptoms in chronic pain conditions (Reichling and Levine, 2009). Importantly, Furman et al. (2018) found that individual alpha frequency (IAF) slowed with the increase of pain sensitivity. A finding interpreted as potentially relying on similar mechanisms that may underpin slowed alpha oscillations observed in clinical pain patients compared to healthy controls (de Vries et al., 2013; Lim et al., 2016; Sarnthein et al., 2006; Walton et al., 2010). These studies lend support to the idea that the slowing of IAF, particularly in its lower range (8–9.5 Hz), may contribute to the generation of clinical and chronic pain.

However, there is also evidence of increased alpha frequency during prolonged (5 min) tonic thermal pain in healthy individuals, and a positive correlation between the increase of alpha frequency and the increase of pain perception (Nir et al., 2010). In their most recent study Furman et al. (2019) suggest that the slowing of IAF correlates with prolonged pain rather than repeated consecutive phasic painful stimulation. Crucially, this correlational finding has been interpreted as evidence of IAF being a reliable biomarker of pain.

We surmised that the lack of control conditions able to discriminate the oscillatory activity associated with baseline resting-state alpha from unpleasant and neutral sensory stimulation could confound the interpretation of the IAF functional significance. More importantly, there is no study attempting to disentangle whether alpha brain oscillations preferentially reflect painful stimulation rather than threatening non-painful (but equally unpleasant) sensory stimulation.

Here, we devised a methodology apt to grant a fine-grained assessment of oscillatory changes specifically locked to each different brain and perceptual state. Considering the previously discussed implication of alpha oscillations during tonic pain, we addressed whether acceleration or deceleration of alpha would distinguish tonic pain compared to several control conditions. Furthermore, we tested the strength of the relationship between IAF and pain perception. To attain our goal, we recorded EEG on healthy volunteers during exposure to consecutive 5 min sessions of painful hot water immersion, innocuous warm water immersion and an aversive prolonged non-painful auditory stimulation. This aimed to dissect the specificity of alpha frequency for the prolonged hot water immersion. To establish a perceptual/experiential compatibility between the two conditions, we focused our psychophysical assessment on the unpleasantness of their experience. The rationale being that unpleasantness is the most distinctive feature of pain (Merskey et al., 1979; Price, 2000) while being strongly correlated with the intensity, salience, and the homeostatic threatening value of the somatosensory stimulation (Borsook et al., 2013; Price, 2000; Price et al., 2002). Participants rated stimulus unpleasantness throughout each condition. We also asked participants to sit still with eyes closed and eyes open right before and after the three experimental conditions. The latter were expressed as a function of the baseline closed and open eyes conditions in separate analyses.

Based on previous research, we tested the confirmatory hypotheses that (1) individual alpha power (IAP) is reduced during prolonged hot water compared with warm immersion, (2) IAF is slowed in prolonged hot water compared with warm immersion, (3) slowing of IAF during hot water immersion would predict increase in unpleasantness ratings within this condition. We also tested the explorative hypothesis that (4) IAF is slowed during prolonged hot water immersion compared with an equally unpleasant auditory experience.

2. Materials and methods

2.1. Participants

A total of 43 participants volunteered to take part in the study which was approved by the ethics committee of the University of Essex. Seven participants were excluded. One participant disclosed to have had taken a painkiller prior to the experiment. Another participant failed the perceptual matching procedure (see below for detail). Data from the 5 remaining participants were excluded due to technical issues with EEG recording.

This resulted in a final sample size of 36 participants (22 females, mean age: 25.36, age range: 20–56). Twenty-four participants self-identified as White/Caucasian, 6 identified as Asian/Pacific Islander, and 6 as Other. All participants had normal or corrected-to-normal vision and normal hearing. Prior to attending, the recruited volunteers were asked to complete a questionnaire to ensure that they had no history of neurological, psychiatric or pain disorders that could interfere with the study or jeopardise their safety.

2.2. Sensory and pain stimulation

We used immersion in hot water to induce a tonic sensation of thermal discomfort. We asked the participants to immerse their left hand up to the wrist in a 30L tank (RW-3025P, Medline Scientific) with constantly circulating hot water, initially set to 45°C. This specific temperature has previously been shown to induce a moderate level of pain in healthy subjects (e.g., Granot et al., 2008). Importantly, we instructed our participants to focus on the unpleasantness of their experience as this would have been the dimension by which they would have assessed the other experimental conditions too.

To create a neutral (non-painful) control condition, participants immersed their left hand up to the wrist in the same water tank, with the water temperature set to be 6°C lower than the temperature used for the hot condition. This 6°C reduction was selected based on pilot sessions to define the optimal temperature reduction to obtain a minimally unpleasant/no unpleasant water temperature. We concluded that a 6°C reduction was the most effective quantity in reducing unpleasantness without producing a floor effect on unpleasantness in most of the pilot trials.

Finally, to create an unpleasant auditory stimulus with a level of discomfort comparable to that of the hot water a constant high-frequency tone (5000 Hz, Saw Tooth waveform) was created in Audacity (v. 2.0.5) software and played through a pair of headphones (NC-40, Lindy; noise cancelling was set to off). This condition was devised to control for the negative affect contribution to the alpha oscillations. While being not painful, this condition allowed us to exclude that the modulation of alpha activity is not brought about by non-pain-related negative affective

states. The volume of the auditory stimulus was determined through the perceptual matching process detailed in the following section.

2.3. Perceptual matching procedure

Anecdotally, some participants reported initial difficulty associated with the qualitative difference between the experience of somatosensory pain and the discomfort associated with the non-painful and non-noxious but distressing sound. Nevertheless, all the participants eventually reported a satisfying matching, and confirmed the hot temperature as a painful experience. We informed the participants that the sound would not have had a "painful" quality but that they were required to strive to detect a loudness level that would have generated a similar level of unpleasantness. They were specifically required to focus on the unpleasantness of the sensory stimulation. The goal of the matching procedure was to ensure that the unpleasantness of the auditory stimulus and hot water condition did not significantly differ from one another during the experimental session.

During the EEG experiment, participants were seated ~65cm from a screen, with the water bath placed to their left, and a mouse and volume adjustment knob within easy reach of their right hand. Participants wore headphones throughout the full procedure.

Participants were required to place their left hand up to the wrist into a hot bath (45°C) and were instructed to find a comfortable position and to keep their hand as still as possible. Towels were used for padding to keep any pressure on the participant's wrist at a minimum, this was done to avoid loss of sensation and to maximise their level of comfort. Participants were then asked to rate the unpleasantness of their sensation on an onscreen visual analogue scale (VAS), with verbal anchors at 0 ("No unpleasant") and 100 ("Intolerable unpleasantness") and numerical markers at 25, 50 and 75. The scale appeared every 10 seconds for 2.5 minutes. If unpleasantness was consistently rated between 50 and 75, the participant then progressed to the next stage of the matching process. If the participant could not tolerate 45°C, the temperature was reduced by 0.5°C and the matching procedure was started again from the beginning. Similarly, if the participant consistently rated the unpleasantness below 50 on the VAS, the temperature was increased by 0.5°C and the matching procedure started again. This correction process was completed as many times as deemed necessary to ensure that ratings were within the required range. Yet participant's safety and comfort were maximised.

After approximately 2.5 minutes (15 VAS ratings) at the final selected temperature, participants were offered a short break, before the auditory stimulus began playing through the headphones. Participants were instructed to remain in the water bath whilst the auditory block took place and were asked to increase the volume of the sound such that the unpleasantness of the auditory stimulus matched the unpleasantness of the sensation elicited by the hot immersion. For the following ~2.5 minutes, participants rated the affective component associated with the auditory stimulus on the onscreen VAS (using the identical scale from the previous block). Again, participants were offered a short break before commencing the final matching block.

In the final block participants were asked to remove their hand from the hot water. Here they only listened to the auditory stimulus in isolation for a further ~2.5 minutes, whilst continuing to rate the unpleasantness on the onscreen scale (once again using the same scale as before). Before the start of this further rating phase, they were reminded to adjust the volume to maintain a level of unpleasantness equal to the magnitude of unpleasantness they recalled during the hot immersion. This further rating phase allowed the participant to compensate potential overestimation associated with the multisensory enhancement occurring in the previous rating task. Prior to commencing the matching block participants were asked to refrain from touching the volume control knob any more during the study once the matching task was completed. This level of volume (and the corresponding water temperature) was then used for that participant for the remainder of the experimental procedure.

2.4. EEG recording

Prior to any measurements, sixty-two Ag/AgCI electrodes (Easycap, BrainProducts GmbH, Gilching, Germany) were mounted to obtain EEG recordings (Synamps RT, Neuroscan, Compumedics). The electrodes were carefully placed according to the 10-20 International System. The impedance of all electrodes was kept below 10 kΩ, and the EEG signal was amplified and digitised at 1000 Hz. The online reference was placed upon the left earlobe and the ground was located at electrode position AFz. Data were re-referenced to a secondary offline reference placed upon the participant's right earlobe.

2.5. Experimental design and procedure

Fig. 1 depicts the experimental design and procedure. Each participant was submitted to five experimental conditions: 'eyes closed', 'eyes open', 'hot', 'warm', 'sound'. Closed and open eyes conditions were baseline EEG measurements requiring no sensory rating, whereas the three tonic sensory conditions required ratings of unpleasantness. Prior to the perceptual matching task, the EEG cap was mounted upon the scalp, and the signal was quality-checked. No recording took place during the matching task. During the experimental task the EEG was recorded in seven separate blocks corresponding to each separate baseline (eyes-open and eyes closed) and sensory condition (hot, warm, sound). Eyes closed and eyes open (2.5 minutes each) were recorded both at the beginning and at the end of the experimental session (their trial order was counterbalanced across participants).

Participants were then presented with the 3 types of sensory conditions (i.e., tonic hot, tonic warm, tonic sound), which were counterbalanced across participants to control for order effects, and asked to rate the level of unpleasantness on the VAS every 10 seconds. During these trials (and the open eyes baseline condition) a central fixation cross was presented to participants to hold their attention. Participants were first submitted to the tonic hot condition, for example, for ~5 minutes (30 VAS ratings), then to the tonic warm condition for another ~5 minutes and finally to the tonic sound condition for a final ~5 minutes. Finally, a post procedure baseline measurement was once again recorded. As before, participants rested again with their eyes open (or closed) for 2.5 minutes and closed (or open) for a further 2.5 minutes. Between each block participants were offered a 5-minute resting period.

3. Data analysis

3.1. Identification of alpha oscillatory activity and analysis of its power and frequency

The continuous EEG data were pre-processed with EEGLAB – an open-source tool-box run within the MATLAB environment (Delorme and Makeig, 2004). Data from individual participants were first re-sampled at 500 Hz and then band-pass filtered from 0.9 to 100 Hz (filter order 8). To isolate the power and frequency of the alpha rhythm we first applied a band-pass filter from 7 to 13 Hz. Next, we off-line re-referenced the data to the right earlobe and then to the full EEG set-up, thus obtaining average reference. We then ran the extended version of Infomax independent component analysis (ICA).

Next, we clustered the ICA components using the mixing matrices based on the ISCTEST approach (Hyvärinen, 2011; Hyvärinen and Ramkumar, 2013) using a false positive and discovery rate of 0.05. We proceeded to inspect each cluster with more than 30 components. Out of the frontal, fronto-temporal, central, centro-parietal, temporal, temporo-parietal, occipital, parieto-occipital, and multiple scalp distribution clusters, we identified peak alpha only in central, centro-parietal, occipital and parieto-occipital clusters. To further facilitate the extraction of functionally significant activity we designated two regions of interest (ROI). We labelled the central and centro-parietal clusters (n=4; #3,14,26,32) as the bilateral *centro-parietal* (CP) ROI and the occipital

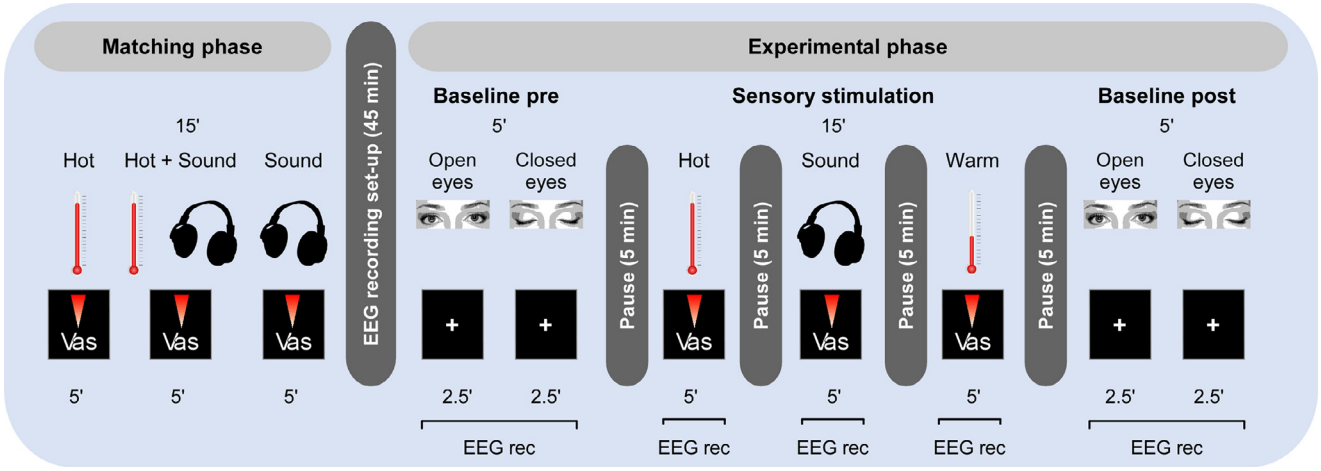

Fig. 1. Procedure and design. Participants were comfortably seated with a water bath placed to their left, and a mouse and volume adjustment knob within easy reach of their right hand. Participants wore a pair of headphones throughout (see main text for a full description). They first underwent a matching procedure to determine the water temperature and volume level that induced a target unpleasantness rating (range: 50-75) on a visual analogue scale (VAS) ranging between 0 ("not unpleasant") and 100 ("intolerable unpleasantness"). The resulting parameters were then used in the experimental phase, during which the EEG was recorded in different blocks. The figure shows one of the possible combinations of events. The order of the different blocks was counterbalanced across participants. Baseline recordings (pre and post) consisted of two 2.5 minutes blocks (eyes open or closed). A fixation cross was shown on screen while participants were simply asked to sit in a comfortable position and relax. After a 5 minutes break, participants completed the tonic Hot, Sound and Warm blocks. Throughout these three blocks, participants rated the unpleasantness of the sensation every 10 seconds on the same VAS as in the matching phase.

and parieto-occipital clusters (n=3; #12,21,27) as the bilateral *parieto-occipital* (PO) ROI.

Next, we segmented each experimental condition into regular non-overlapping epochs of 5 seconds length and filtered these using the unmixing matrices from the clusters we found. We calculated the power-spectral density (PSD) by transforming each of these epochs (which were in component space at this time) in the frequency domain using a multitaper frequency transformation (Hanning windows, 2-40 Hz, 0.2 Hz bin width) via the use of Fieldtrip (Oostenveld et al., 2011). Finally we calculated the centre of gravity (CoG; Klimesch et al., 1993) of the alpha peak in a 7-13 Hz window of interest using the following equation:

$$CoG = \frac{\sum_{i=1}^{n} f_i a_i}{\sum_{i=1}^{n} a_i}$$

With n being the number of frequency bins in the window of interest, f_i being the frequency and a_i the amplitude represented by the i^{th} bin. Amplitude and frequency were then stored individually for each cluster, participant, condition, and trial. It is worth noting that a series of different approaches have been developed to assess alpha frequency and extract a summary index of it (e.g., Grandy et al., 2013; Lodder and van Putten, 2011). However, by extracting a weighted mean of the selected frequency range, CoG is meant to reduce the impact of non-canonical individual alpha distributions such as split or multiple peaks (Chiang et al., 2011).

Finally, the amplitudes and frequencies of the two clusters (CP and PO) were exported for statistical analysis (see next Section).

3.2. Statistical analyses

Ratings, alpha power, and frequency

All analyses were performed with Jamovi (*The Jamovi Project*, 2020). Normality was assessed for all the data using Q-Q plots. The perceptual matching ratings obtained during the calibration phase were analysed using T-test. We adopted mixed model analyses for unpleasantness ratings and alpha oscillations in the main experiment. Single trial unpleasantness ratings, CoG alpha power and frequency for each participant across tonic hot, warm and sound conditions were submitted to an a priori established linear mixed model (LMM) with restricted maximum likelihood (REML) estimation (Satterthwaite method for degrees of freedom) as implemented in Jamovi (v. 1.5; Gallucci, 2019; R Core Team, 2021; *The Jamovi Project*, 2020). We added random intercepts for participants and trials, as well as a random slope for the effect of the sensory condition.

Unpleasantness ratings~1 + Condition + (1 + Condition | Participants) + (1 | Trial)

Due to the within-subject nature of the experimental design we specified the random coefficients as correlated. The warm condition was used as default control level for the estimation of fixed effects. Therefore, for a direct comparison between hot and sound we recalculated the model using sound as a default comparison term. T values are reported as measures of effect size.

The same LMM formula was applied to the alpha power and frequency that were expressed as a function of both the open and closed eyes baseline (pre and post recordings were averaged per baseline type; cf. Fig. 1). Specifically, we expressed the sensory conditions and event-related power in decibels, according to the following formula:

$$dB = 10 * \log10\left(\frac{sensory\ x_i}{baseline\ \bar{x}}\right)$$

However, as the result obtained with the closed eyes baseline overlapped with the open eyes, we will report only the results obtained with the open eyes baseline according to the rationale that the open eyes baseline provides a better alpha activity benchmark for the comparison with the sensory conditions.

The p values obtained with the t-tests and LMM (except from the likelihood ratio test – LRT for the test of random effects) were corrected using the Benjamini–Yekutieli two-stage procedure to account for false discovery rate (Benjamini et al., 2006), with the threshold for significance set at $p<0.05$.

3.3. Relationship between alpha oscillations and unpleasantness rating

Finally, we addressed the relationship between alpha oscillations as expressed above and unpleasantness ratings collected during the sensory conditions. We implemented mediation analysis whereby alpha EEG responses were treated as intermediate mediating variables (M) between the sensory conditions (X) and unpleasantness ratings (Y). A simple transmittal approach (Rungtusanatham et al., 2014) allowed us to test

Table 1
Linear mixed model of unpleasantness ratings. Fixed effects estimates.

Fixed Effects	**Estimate**	**SE**	95% CI Lower	**95% CI Upper**	**df**	**t**	P
Intercept	45.56	1.16	43.59	47.90	35.58	39.13	< .001
H - W	63.25	1.74	60.17	67.78	35.00	36.31	< .001
S - W	59.69	2.10	55.93	63.89	35.00	28.38	< .001
S - H	-3.57	1.83	-6.99	0.01	35.00	-1.95	0.06

Notes. Number of Observations: 3240, Participants: 36. Trials: 30. Standard error (SE); Confidence interval (CI). Significance corrected using FDR (i.e., q values).

the hypotheses that power would not mediate the relationship between X and Y whereas frequency would mediate the relationship. Mediation analyses were run separately for frequency and power indices whereas both ROIs were included in the model as distinct mediators, according to the following formula:

Unpleasantness ratings ~ CP ROI+ PO ROI + Condition.

Akin to the LMM, we used dummy contrasts for the factor condition whereby tonic warm was used as default control level. Results were obtained using bias-corrected bootstrapping of confidence intervals (1000 repetitions) and report indirect (mediated), direct (unmediated) and total effects according to the JAMM package in Jamovi (Gallucci, 2019). The package estimates the coefficients using the maximum likelihood method implemented in *laavan* R package. Betas were obtained as standardized parameters of the path model, thus producing an index of mediation and effect size (Preacher and Kelley, 2011).

4. Results

4.1. Perceptual matching

Mean (±SD) temperature (°C) and sound pressure level (dB) resulting from the matching procedure and chosen for the experiment were 44.50 (±0.49)°C and 90.84 (±11.41) dB, respectively. One participant's unpleasantness ratings were lost due to a technical software fault. Both distributions were in the desired range as the average ratings were beyond VAS 50 of unpleasantness (hot: 66.87±6.99; sound: 69.59±9.77). Paired t-tests confirmed that the participants successfully achieved a matched experience of unpleasantness for the hot and sound condition (t_{34}=-1.62; p=0.09; [-6.12, 0.69]; d=-0.27) before starting the experiment.

4.2. Unpleasantness ratings

The LMM successfully converged and explained 94% of the variance (R_c^2=0.94). The fixed effects explained the 85% alone (R_m^2=0.85). Fixed effects are summarised in Table 1. The factor 'condition' was significant ($F_{2,35}$=705.61, p<0.001). The effect was accounted for by a large difference between the tonic hot and warm (t_{35}=36.31; p<0.001) as well as tonic sound and warm (t_{35}=28.38; p<0.001), whereas no difference between sound and hot was observed (t_{35}=-1.95; p=0.06). Indeed, participants rated both tonic hot (67.83±9.18) and sound (64.27±11.93) as similarly unpleasant, thus indicating the matching procedure was successful. Fig. 2 displays the distribution of average VAS unpleasantness ratings for each condition and the difference between conditions as revealed by the fixed effects estimates. The likelihood ratio test (LRT) indicated the random intercept for participants improved the model (LRT_5=1525.22; p<0.001). Table 1 reports the fixed effects estimates and random effect variance.

4.3. Alpha oscillations

Figures 3 and 4 show the grand-median spectral profiles and related boxplots illustrating both CoG frequency and power profiles across the three conditions (panel A), as well as scalp topographies of the alpha

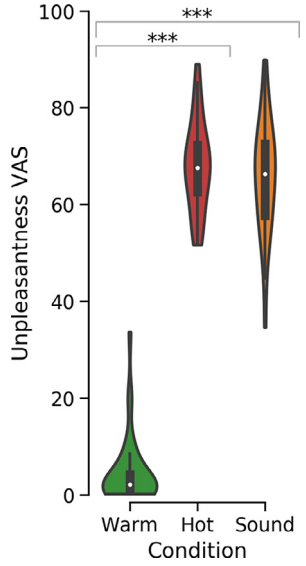

Fig. 2. Unpleasantness ratings. Violin-plots representing the median of individual average unpleasantness ratings in the three sensory conditions (x axis). The boxes represent the inner quartiles while whiskers represent data within 1.5 times the inner quartile range. The outer shape is a Kernel density estimation of showing the distribution density of the ratings. Asterisks represent statistical two-tailed significance (***p<0.001). Note that the tonic hot and sound conditions do not differ in unpleasantness while both differ from the somatosensory control condition (i.e., tonic warm).

ICs clusters displaying the mean power at the frequency of the CoG computed from the signal using the back-projection of the selected alpha ICs (panel B).

4.4. IAF analysis

Tables 2 and 3 report the fixed effects estimates and random effect variance for the two topographical ROIs.

4.5. Bilateral central-parietal (CP) ROI as function of open eyes alpha

The LMM successfully converged and explained 31% of the IAF variance (R_c^2=0.31). The fixed effects explained the 0.20% alone (R_m^2=0.002). Fixed effects are summarised in Table 2. The factor 'condition' approached significance ($F_{2,34.8}$=3.01, p=0.06). The estimates indicated a significant difference between IAF observed during the tonic hot and warm ($t_{33.5}$=-2.44; p=0.02). Fig. 3 (panel B) shows how this difference is accounted for by a slower frequency during tonic hot. A similar pattern, though insignificant, can be observed for the comparison of tonic sound against warm ($t_{35.3}$=-1.52; p=0.10). No difference between tonic sound and hot could be observed ($t_{33.0}$=0.36; p=0.44). The likelihood ratio test (LRT) indicated that the factor 'condition' as random effect across participants improved the model (LRT_5=150.75; p<0.001).

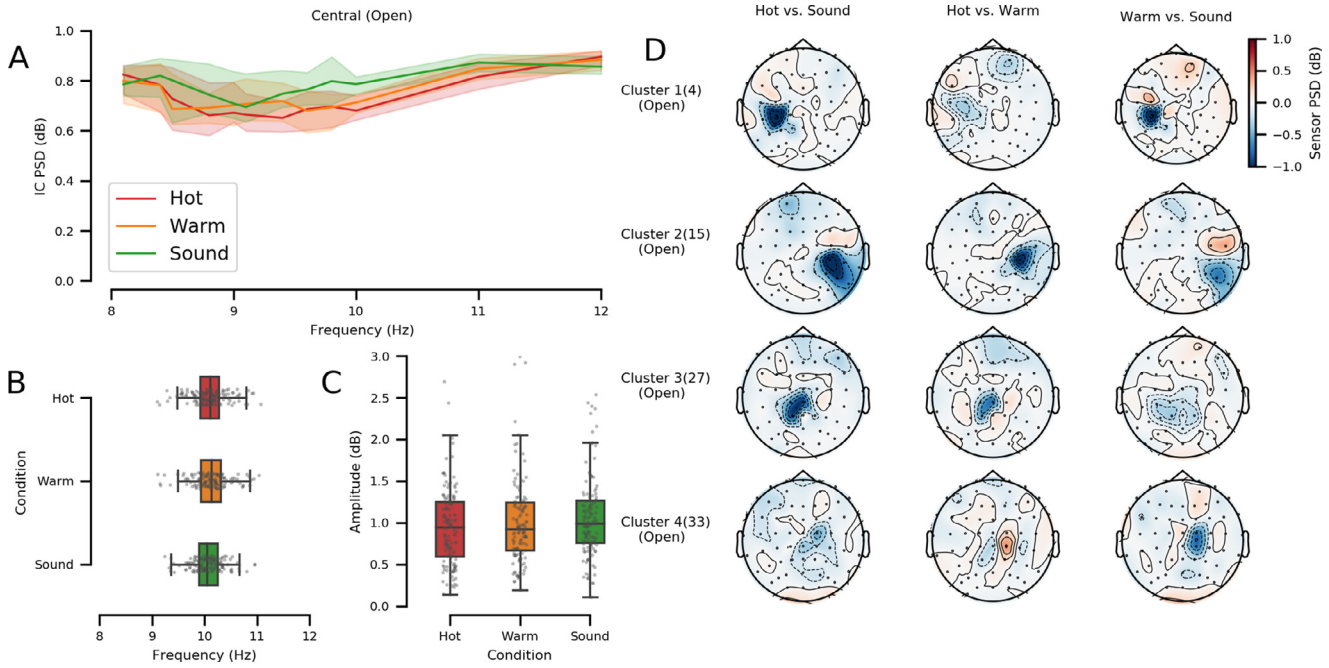

Fig. 3. Bilateral central-parietal (CP) ROI as function of alpha baselines. Panel A. grand-median (shaded area: 95% confidence interval) PSD plots obtained following the IC alpha identification phase. Note how the alpha oscillations in the sensory stimulation conditions show similar magnitude for both baseline conditions. Boxplots representing the median of individual average CoG IAF (B) and IAP (C) highlight important variability and narrow difference across conditions. The boxes represent the inner quartiles while whiskers represent data within 1.5 times the inner quartile range. Single dots indicate individual values. Note the reduced frequency and power in the hot compared with warm condition. Panel D. scalp topographies of mean power differences in the alpha CoG range between conditions per each alpha baseline and across IC clusters into sensors space.

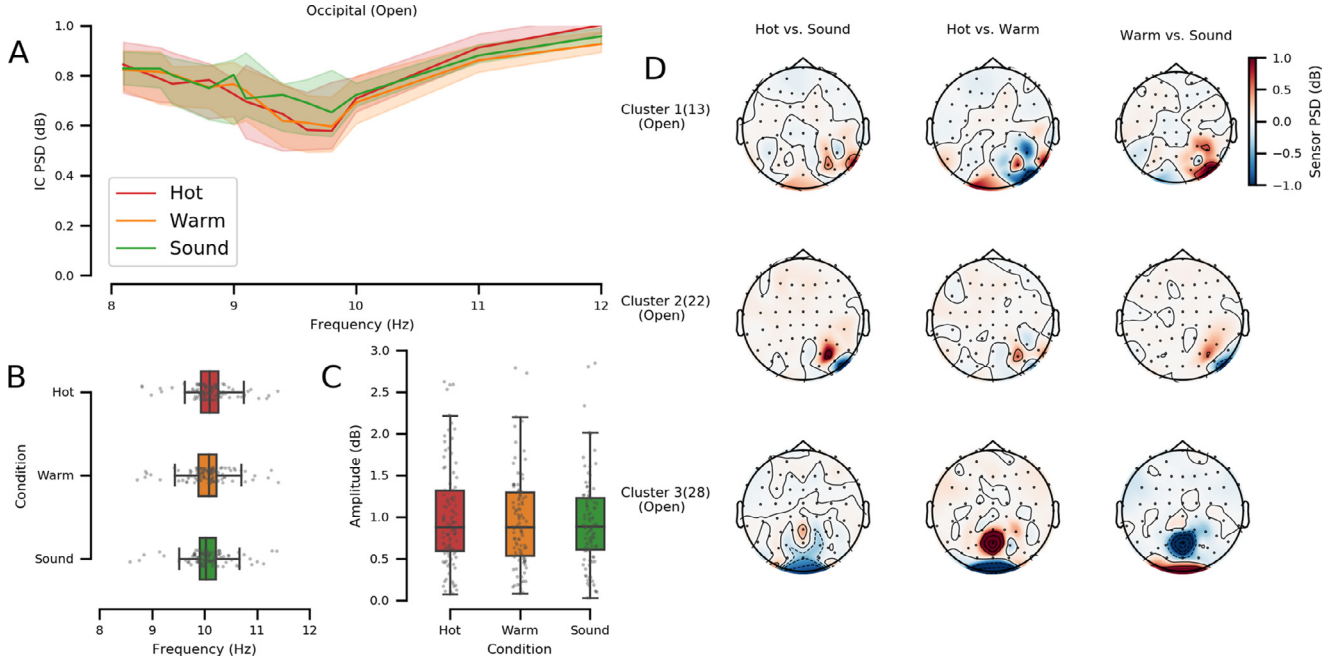

Fig. 4. Bilateral parietal-occipital (PO) ROI as function of alpha baselines. Panel A. grand-median (shaded area: 95% confidence interval) PSD plots obtained following the IC alpha identification phase. Note how the alpha oscillations in the sensory stimulation conditions show similar magnitude with both baselines. Boxplots representing the median of individual average CoG IAF (B) and IAP (C) highlight important variability and narrow difference across conditions. The boxes represent the inner quartiles while whiskers represent data within 1.5 times the inner quartile range. Single dots indicate individual values. Note the increase in frequency compared with CP ROI in Fig.3, particularly during the hot condition. Panel D. scalp topographies of mean power differences in the alpha CoG range between conditions per each alpha baseline and across IC clusters into sensors space.

Table 2
Linear mixed model of IAF quantified over the CP ROI as function of open eyes alpha activity. Fixed effects estimates.

Fixed Effects	Estimate	SE	95% CI Lower	95% CI Upper	Df	T	P
Intercept	-0.001	0.015	-0.03	0.03	35.12	-0.08	0.94
H - W	-0.02	0.01	-0.03	-0.004	33.51	-2.44	0.02
S - W	-0.01	0.01	-0.03	0.003	35.24	-1.52	0.10
S - H	0.003	0.01	-0.01	0.02	33.04	0.36	0.44

Notes. Number of Observations: 8481, Participants: 36. Trials: 140. Standard error (SE); Confidence interval (CI). Significance corrected using FDR (i.e., q values).

Table 3
Linear mixed model of IAF quantified over the PO ROI as function of open eyes alpha activity. Fixed effects estimates.

Fixed Effects	Estimate	SE	95% CI Lower	95% CI Upper	Df	T	p
Intercept	0.02	0.02	0.01	0.05	35.30	1.02	0.32
H - W	0.02	0.01	0.005	0.03	29.65	2.72	0.01
S - W	<0.001	0.01	-0.01	0.01	34.96	<0.001	0.55
S - H	-0.02	0.01	-0.03	-0.005	30.29	-2.80	0.01

Notes. Number of Observations: 8481, Participants: 36. Trials: 140. Standard error (SE); Confidence interval (CI). Significance corrected using FDR (i.e., q values).

Table 4
Linear mixed model of IAP quantified over the CP ROI as function of open eyes alpha activity. Fixed effects estimates.

Fixed Effects	Estimate	SE	95% CI Lower	95% CI Upper	Df	t	p
Intercept	-8.21	0.91	-9.90	-6.38	35.53	-9.02	<0.001
H - W	-1.21	0.34	-1.83	-0.51	32.49	-3.52	0.003
S - W	0.18	0.34	-1.83	-0.51	34.95	0.59	0.36
S - H	1.39	0.32	0.77	1.99	33.79	4.38	<0.001

Notes. Number of Observations: 8481, Participants: 36. Trials: 140. Standard error (SE); Confidence interval (CI). Significance corrected using FDR (i.e., q values).

Table 5
Linear mixed model of IAP quantified over the PO ROI as function of open eyes alpha activity. Fixed effects estimates.

Fixed Effects	Estimate	SE	95% CI Lower	95% CI Upper	Df	T	p
Intercept	-17.71	0.92	-19.63	-16.01	35.34	-19.28	<0.001
H - W	0.56	0.32	-0.07	1.19	34.04	1.77	0.08
S - W	0.54	0.34	-0.16	1.20	34.53	1.57	0.10
S - H	-0.02	0.24	-0.50	0.45	33.57	-0.08	0.54

Notes. Number of Observations: 8481, Participants: 36. Trials: 140. Standard error (SE); Confidence interval (CI). Significance corrected using FDR (i.e., q values).

4.6. Bilateral parietal-occipital (PO) ROI as function of open eyes alpha

The LMM successfully converged and explained 36% of the variance (R_c^2=0.36). The fixed effects explained the 0.20% alone (R_m^2=0.002). The factor 'condition' significantly explained the changes in IAF ($F_{2,33.9}$=5.48, p=0.01). The effect was explained by a difference between the tonic hot and warm ($t_{29.6}$=2.71; p=0.01), whereas no difference between sound and warm was observed ($t_{35.0}$=0.0002; p=0.55). However, tonic sound and hot were significantly divergent ($t_{30.3}$=-2.81; p=0.01). Fig. 4 (panel A, left bottom) highlights the increase of speed in alpha oscillations across the sensory conditions and particularly highlights the faster frequency during hot condition compared with the other two sensory conditions. The LRT indicated that the factor 'condition' as random effect across participants improved the model (LRT_5=82.61; p<0.001).

4.7. IAP analysis

Tables 4 and 5 report the fixed effects estimates and random effect variance for the two topographical ROIs.

4.8. Bilateral central-parietal ROI as function of open eyes alpha

The LMM successfully converged and explained 53% of the IAP variance (R_c^2=0.53). The fixed effects explained the 1% alone (R_m^2=0.01). Table 4 summarises the fixed effects. The factor 'condition' was significant ($F_{2,34.3}$=10.37, p=0.001). Fig. 3 (panel C) shows how this difference is accounted for by a substantial power reduction during tonic hot than warm ($t_{32.5}$=-1.21; p=0.003) and by a decrease of power during tonic hot compared with sound (t33.8=4.83; p<0.001) whereas there was no difference between sound and warm (t_{35}=0.59; p=0.36). The LRT indicated that the factor 'condition' as random effect across participants improved the model (LRT_5=181.99; p<0.001).

4.9. Bilateral parietal-occipital ROI as function of open eyes alpha

The LMM successfully converged and explained 50% of the variance (R_c^2=0.50). The fixed effects explained the 0.1% alone (R_m^2=0.001). The factor 'condition' was not significant ($F_{2,34.1}$=1.64, p=0.14). Fig. 4 highlights the overall lower power reduction of the sensory conditions when expressed as a function of open eyes alpha but otherwise no different across the sensory conditions (see Table 5 too). The LRT

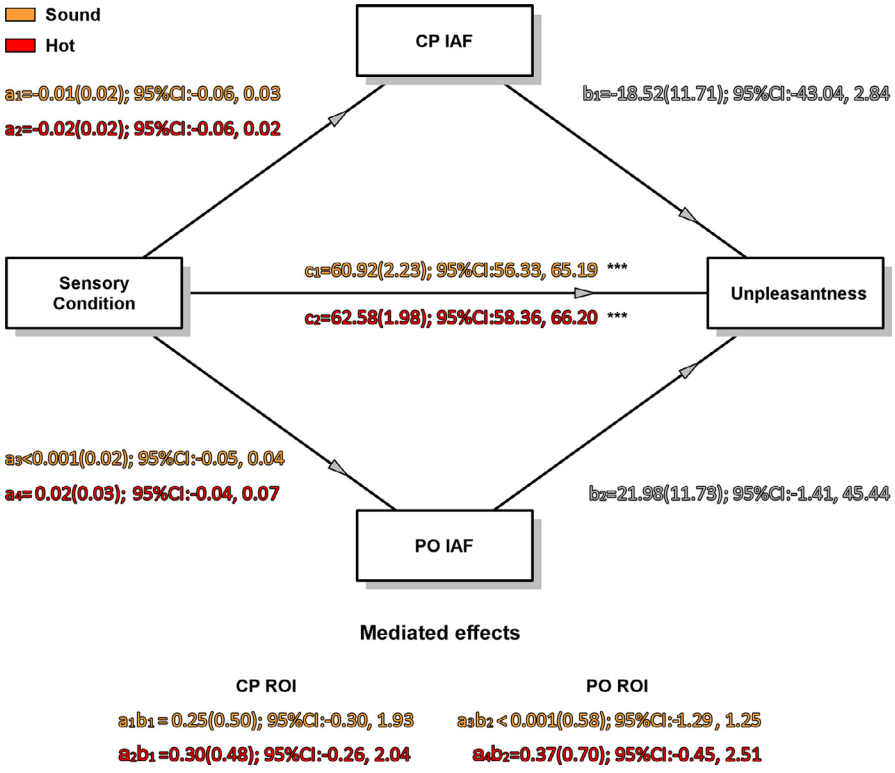

Fig. 5. Diagram of the general mediation model analysis of IAF. Parameter estimates of IAF with their SE and CI for the direct (c), indirect (mediated effects; bottom inset), and the components of the indirect effects (a•b). Notes. Contrasts: Sound-Warm, orange; Hot-Warm, red. CIs are computed with Bias corrected bootstrap method. ***p<0.001.

indicated that the factor 'condition' as random effect across participants improved the model (LRT_5=130.47; p<0.001).

4.10. Relationship between ratings of unpleasantness and IAF

Unstandardized parameter estimates (±SE) for the model are shown in Fig. 5. Regressing CP ROI IAF on sensory conditions showed that neither tonic sound nor tonic hot significantly changed CP ROI IAF (β=-0.07, z=-0.62, p=0.53; β=-0.09, z=-0.78, p=0.43). Regressing PO ROI IAF on sensory conditions showed a similar pattern (β<0.001, z=-0.002, p=0.99; β=0.08, z=0.66, p=0.51]. Regressing unpleasantness ratings on CP ROI IAF, PO ROI IAF, and sensory conditions showed that the two ROIs had opposite influence on the perceptual outcome (unit increase of CP ROI IAF predicted reduced unpleasantness whereas unit increase of PO ROI IAF predicted its increase), however neither of them achieved statistical significance (β=-0.05, z=-1.58, p=0.11; β=0.07, z=1.87, p=0.06). Finally, significant direct effect of the sensory conditions remained after the mediators were modelled (β=0.93, z=27.30, p<0.001; β=0.96, z=31.59, p<0.001).

Mediation analysis showed that both IAF ROIs did not significantly mediate the effects of the sensory conditions on unpleasantness ratings, neither of tonic sound (β=0.004, z=0.51, p=0.61; β<-0.001, z=0.001, p=0.99) nor of tonic hot (β=-0.007, z=-0.63, p=0.53; β=0.01, z=0.54, p=0. 59).

4.11. Relationship between ratings of unpleasantness and IAP

Unstandardized parameter estimates (±SE) for the model are shown in Fig. 6. Regressing CP ROI IAP on sensory conditions showed that neither tonic sound nor tonic hot significantly changed CP ROI IAP (β=0.02, z=0.14, p=0.88; β=-0.11, z=-0.96, p=0.33). Regressing PO ROI IAP on sensory conditions showed a similar pattern (β=0.05, z=0.43, p=0.67; β=0.04, z=0.37, p=0.71). Similar to the IAF pattern, regressing unpleasantness ratings on CP ROI IAP, PO ROI IAP, and sensory conditions showed that the two ROIs had opposite influence on the perceptual outcome. However, IAP pattern was reversed compared to IAF: unit increase of CP ROI IAP predicted increased unpleasantness whereas unit increase of PO ROI IAF predicted its decrease. And yet, neither of them achieved statistical significance (β=0.05, z=1.75, p=0.08; β=-0.004, z=-0.13, p=0.90). Finally, the significant direct effect of the sensory conditions remained after the mediators were modelled (β=0.94, z=27.84, p<0.001; β=0.97, z=34.13, p<0.001).

Mediation analysis showed that both IAP ROIs did not significantly mediate the effects of the sensory conditions on unpleasantness ratings, neither of tonic sound (β<0.001, z=0.13, p=0.90; β<-0.001, z=-0.05, p=0.96) nor of tonic hot (β=-0.005, z=-0.76, p=0.44; β<-0.001, z=-0.05, p=0.96).

5. Discussion

The objective of our study was to test the pain-specificity of EEG alpha oscillations against neutral and perceptually matched unpleasant non-painful stimulation (Fig 1, right). Our preliminary matching task allowed participants to equalize their perceptual experience by anchoring their sensory evaluation on the unpleasantness of both the hot painful immersion and the distressing prolonged high pitch sound (Fig. 1, left). All the participants were eventually successful in calibrating the two negative affective experiences (Fig. 2, Table 1). This important methodological feature, together with the introduction of two different types of resting baseline alpha conditions, allowed us to quantify the specificity of alpha oscillatory activity (particularly IAF) as an index of prolonged pain stimulation and its relationship with subjects' perception.

Our findings revealed an unexpected dissociation between the two ROIs. First, the IAF slowed during prolonged hot water immersion compared to warm immersion but not to the tonic sound over the centro-parietal region of the scalp (Fig. 3-B, D, Table 2). Second, the IAF increased during prolonged hot water immersion compared to the two sensory control conditions at the parieto-occipital region (Fig. 4-B, D, Table 3). Third, we replicated the expected magnitude reduction of alpha oscillations during prolonged hot water immersion compared with the other two sensory control conditions over the centro-parietal region

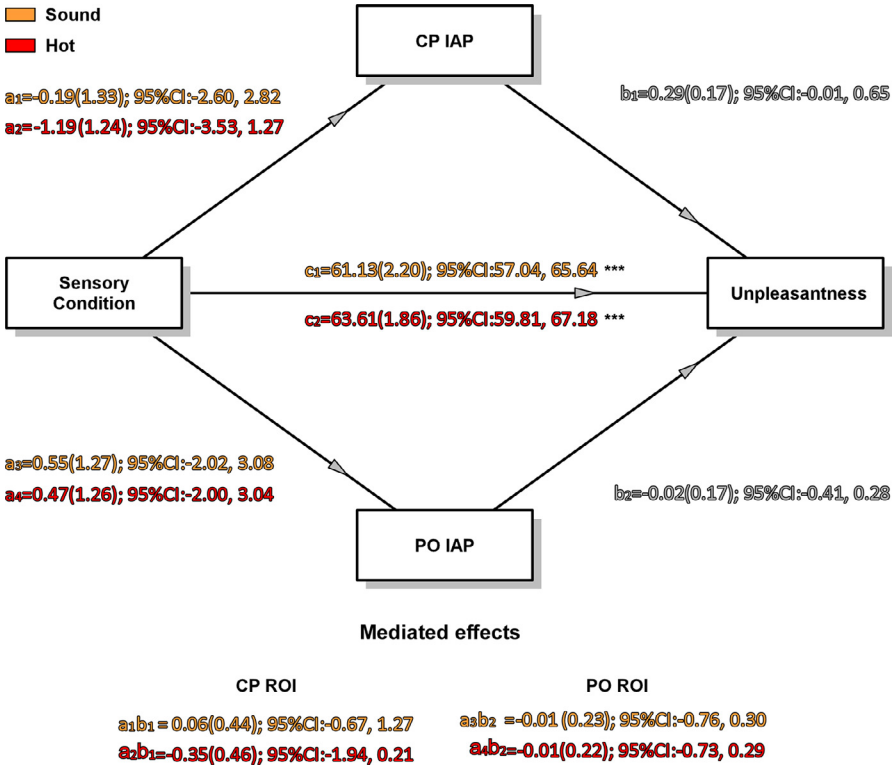

Fig. 6. Diagram of the general mediation model analysis of IAP. Parameter estimates of IAP with their SE and CI for the direct (c), indirect (mediated effects; bottom inset), and the components of the indirect effects (a•b). Notes. Contrasts: Sound-Warm, orange; Hot-Warm, red. CIs are computed with Bias corrected bootstrap method. ***p<0.001.

of the scalp (Fig. 3 C,D, Table 4) but no difference at the PO ROI (Fig. 4 C,D, Table 5). Fourth, the mediation analysis of IAF did not support a causal role of frequency in mediating the perceptual experience (Fig. 5). However, the analysis also suggested that the dissociation between the two ROIs might deserve further investigation in future research. Indeed, while the centro-parietal clusters showed that increase of frequency predicted reduced unpleasantness (and thus frequency decrease was associated with greater unpleasantness), the increase of frequency at the parieto-occipital clusters predicted increase of unpleasantness. Interestingly, the mediation analysis of IAP showed an opposite pattern whereby increase of power within the centro-parietal clusters predicted increased unpleasantness whereas increase of power in the parieto-occipital clusters predicted its decrease. However, this pattern as well as their mediational contribution were not significant either (Fig. 6), thus preventing us to draw any further conclusion on the relationship between alpha oscillations and the experience of unpleasantness.

5.1. A bidirectional EEG pattern for alpha frequency during pain?

Our approach to activate the nociceptive system was based on a 5 minute immersion in hot water and is comparable to that used by several previous studies (Granot et al., 2008; Lautenbacher et al., 2008; Streff et al., 2010; Tousignant-Laflamme et al., 2005). The greater alpha power during closed and open eyes resting states is a well-established observation (Geller et al., 2014) as well as its suppression during sensory stimulation (e.g., Plöchl et al., 2016). In this respect, our finding is no surprise as both phasic and tonic nociceptive painful stimulation are commonly associated with alpha power suppression (Chang, Arendt-Nielsen, and Chen, 2002; Chen and Rappelsberger, 1994; Dowman, Rissacher, and Schuckers, 2008; Giehl et al., 2014; Peng et al., 2015).

Concerning alpha frequency, our findings seem to be consistent with either increased alpha frequency during tonic pain (Nir et al., 2010) and decreased frequency during an inflammatory model of pain (Furman et al., 2018). In keeping with Nir et al.'s findings (2010) we have detected an increase of IAF during the painful stimulation compared with the control sensory conditions over the parieto-occipital region. Conversely, the analysis of the centro-parietal region revealed lower frequency during the hot stimulation. This is in turn consistent with what found by Furman et al. (2018, 2020) with a clinically relevant approach whereby pain was induced by means of a intradermal capsaicin model, triggering prolonged (>15 min) inflammatory pain. Importantly, their more recent study (Furman et al., 2020) reported that "sensorimotor" (i.e., measured at the vertex of the scalp) baseline alpha (i.e., measured when the participants is at rest) is negatively correlated with pain sensitivity to both phasic thermal pain (similar to the one used in the present study) and capsaicin-induced inflammatory pain, even after weeks. In addition, they were able to classify pain sensitive individuals at their second measurement based on the resting alpha acquired during the first measurement, thus leading the authors to conclude that peak IAF is a reliable biomarker of prolonged pain sensitivity.

Due to the methodological differences we should be cautious in comparing findings. Yet, while we seem to support the slowing of alpha oscillations over the central and parietal region of the scalp (largely linked to cingulate, somatosensory and insular generators, Kim and Davis, 2020) we cannot claim this was specific to pain nor can we causally link this pattern to the participants' affective experience. It is noteworthy that Furman et al. (2020) grounded their conclusion on resting pain free alpha whereas we attempted to investigate the relationship between alpha oscillations during ongoing pain and the individual's ratings of the experience, net of their baseline resting alpha.

5.2. Methodological and mechanistic considerations

There are a series of reasons why we believe our work is reliable and robust. First, we relied on the largest sample size (n=36) in a within subject design compared to previous studies on healthy individuals (Chang et al., 2002; Dowman et al., 2008; Furman et al., 2018; Furman et al., 2020; Giehl et al., 2014; Li et al., 2016; Nir et al., 2010; Nir et al., 2012). Next, our experimental design allowed us to 1) assess alpha oscillations during both closed and open eyes resting state, 2) compare alpha oscillations recorded during hot painful stimulation against those recorded during warm and affectively neutral stimulation, and

even more, against those recorded during an equally negative affective stimulation. Moreover, we based the analytic approach to the extraction of alpha-related information on advanced signal processing while avoiding selective analysis and double dipping (Kriegeskorte et al., 2009). Specifically, we used an advanced data driven cluster analysis to identify those spatial regions of the EEG that would selectively represent the alpha oscillations at rest and rest only, based on the ISCTEST approach (Hyvärinen, 2011; Hyvärinen and Ramkumar, 2013). This technique was followed by the application of linear mixed modelling on the extracted centre of gravity summary index which is known to account for correlation among repeated observations within an individual compared with classical analysis of variance. In doing so, we learnt that modelling experimental conditions as random factors can improve the statistical estimate of IAF. We believe this is relevant information for future studies. By the same token, we bypassed correlation and implemented mediation analysis that, in combination with our experimental design, conveyed results we believe can be interpreted causally (VanderWeele, 2016).

Our approach revealed that there is not only "one" alpha activity to be deemed relevant during resting state activity, and that different brain regions may display opposite oscillatory patterns. In fact, there may be a rationale in co-occurring opposite frequency patterns across brain regions, possibly grounded on large scale anti-correlated functional networks (Klimesch, 2012; Li et al., 2021). In addition, there may be other factors determining which individuals respond to sensory stimulation with increase rather than decrease of the alpha rhythm. For example, future studies may address whether the temporal extent of the sensory stimulation is linked to a different alpha profile in the individual or if similarly unpleasant non-painful experiences are associated with lower alpha frequency during experimental models of clinical pain or in chronic pain individuals. Notwithstanding our large sample size, we should be cautious in extending the significance of our findings to brain activity in both acute and chronic pain as the type of experience induced in the present study (i.e., tonic/prolonged sensation) is relying on distinct physiological mechanisms than those involved in e.g. acute post-operative pain or chronic conditions associated with central sensitization (Gangadharan and Kuner, 2013; Pogatzki-Zahn et al., 2017).

Finally, our design allowed us to disentangle specific changes in EEG alpha frequency. The absence of a difference in alpha IAF for equally unpleasant auditory stimulation suggests that IAF may not be specifically reduced during prolonged pain compared with other negative affective bodily states. Nonetheless, the current findings invite more robust and comprehensive research on the role of EEG alpha rhythm during bodily threatening events. Such note of caution is particularly relevant in light of the recent suggestion to manipulate alpha oscillations through non-invasive brain stimulation (Arendsen et al., 2018; Hohn et al., 2019) or sensory entrainment (Ecsy et al., 2018).

In this vein, we recommend that future research would include proper baseline assessment (i.e., pre and post sensory stimulation) as well as non-painful but similarly unpleasant conditions as to quantify the scale and magnitude of the relationship between IAF and the experience of pain. Such methodological posture can also protect studies from spurious effects associated with alpha oscillations triggered by various uncontrolled emotional, attentional and memory processes that may influence perceptual processes (Klimesch, 1999; May et al., 2012).

5.3. Conclusive remarks

Altogether, our findings indicate that IAF can be a useful brain index of perceptual changes as it can discriminate between a hot painful and warm neutral sensation. However, we cannot offer support to the notion that IAF is also an index of specific negative valence affective changes as per the lack of difference the hot painful sensation and a high pitch unpleasant loud sound. In fact, our findings yield no support to the observation that slower alpha frequency may be functionally associated with the most distinctive feature of pain, i.e., its unpleasantness. Crucially, we identified a spatial dissociation between central and postcentral vs. parietal-occipital topographies reflecting a slowing vs. speeding of alpha frequency during the different sensory experiences. Therefore, we invite caution in interpreting IAF as a brain biomarker of pain sensitivity and underline the impact of methodological and statistical factors in this quest (Mouraux and Iannetti, 2018). All in all, our findings significantly add to the current state of the art by showing that the experimental design and data analysis approach have substantial impact on the direction and robustness of alpha modulations. Further research will need to address methodological aspects that impact on the specificity and sensitivity of IAF as brain marker of perceptual states.

Declaration of Competing Interest

The authors report no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119143.

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