

Cue-based modulation of pain stimulus expectation: do ongoing oscillations reflect changes in pain perception? A Registered Report

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Abstract

A promising stream of investigations is targeting ongoing neural oscillations and whether their modulation could be related to the perception of pain. Using an electroencephalography (EEG) frequency tagging approach, sustained periodic thermonociceptive stimuli perceived as painful have been shown to modulate ongoing oscillations in the theta, alpha and beta bands at the frequency of stimulation. Nonetheless, it remains uncertain whether these modulations are indeed linked to pain perception. To test this relationship, we modulated pain perception using a cue-based expectation modulation paradigm and investigated whether ongoing oscillations in different frequency bands mirror the changes in stimulus perception. 40 healthy participants were instructed that a visual cue can precede either a high or low intensity stimulation. These cues were paired with 3 different levels of sustained periodic thermonociceptive stimuli (low, medium, high). Despite a strong effect of expectation on the perceived stimulus intensity, this effect was not reflected in the modulation of the ongoing oscillations, thus suggesting a potential dissociation of pain perception and these oscillatory activities. Rather, it seems that the intensity of stimulation is the primary generator of the responses collected using an EEG frequency-tagging approach. Importantly, these results need to be confirmed by further investigations that could allow detecting smaller effects than originally estimated.

1. Introduction

The synchronization of information across different brain regions through the flexible activity of ongoing neural oscillations has in recent years been associated with the processing of pain in the human brain (Ploner et al., 2017). Current investigations showcased the benefits of using an EEG frequency-tagging approach paired with the application of slow sustained periodic nociceptive stimuli for the exploration of the characteristic of pain-related ongoing oscillations (Colon et al., 2017; Colon et al., 2012; Leu et al., 2023; Mulders et al., 2020). In particular, this approach allows to differentiate between cortical activity related to the applied stimulus and unrelated activity by “tagging” responses at the frequency of stimulation and its harmonics. As such, a periodic modulation was found at the frequency of stimulation in the aforementioned investigations in the alpha, beta and theta frequency bands. Expanding this approach to investigations using intracerebral EEG in patients undergoing a presurgical evaluation of focal epilepsy, Liberati et al. (2019) found a preferential modulation of ongoing oscillations at the frequency of stimulation in the alpha and theta frequency band following thermocceptive stimulation in comparison to non-nociceptive vibrotactile stimuli. These results suggest that the modulation of ongoing oscillations could be related to nociception and/or the perception of pain. Yet, the functional relationship between ongoing oscillations and the perception of pain remains unclear. If there is in fact a link between these two factors, we expect that a modulation of pain perception should lead to a congruent change in the modulation of ongoing oscillations.

Expectation is a powerful cognitive modulation factor that can strongly influence the subjective experience of pain. While there are numerous ways to influence an individual’s expectation towards a painful stimulus, the modulation can be categorized into placebo analgesia, nocebo hyperalgesia and stimulus expectancies (reviewed by Atlas and Wager (2012)). Importantly, while the former two categories rely on the application of an inert substance or intake of a fake drug, stimulus expectations achieve a modulation of pain perception solely by the association of pain-predictive cues (Atlas et al., 2010; Hauck et al., 2007; Jepma et al., 2018; Keltner et al., 2006; Lobanov et al., 2014).

To further understand the modulatory effects of expectation on pain perception, recent investigations studied its effects on ongoing neural oscillations. The application of a placebo analgesic as well as nocebo hyperalgesic intervention both led to an increase in post-treatment resting-state alpha activity (Albu & Meagher, 2016; Huneke et al., 2013). Even before the application of an expected painful stimulus, suppression of alpha frequency band activity has been observed in EEG as well as MEG investigations (Babiloni et al., 2006; Franciotti et al., 2009). Similarly, a visual cue-based expectation modulation paradigm found a cluster of activity between 1-30 Hz when a painful stimulus was expected (Strube et al., 2021). While similar results were found by Nickel et al. (2022) regarding pre-stimulus activity in a predictive coding paradigm, changes in pain perception induced by expectation did not seem to have an effect on the modulation of ongoing oscillations. Another recent investigation found that while expectations and prediction error did not lead to any changes in local brain activity at the regions of interest (ROI), they did modulate the interregional connectivity within the chosen ROIs in the alpha and gamma frequency band (Bott et al., 2023). As discussed by Nickel and collaborators, it could be possible that commonly used approaches to analyze oscillatory activity are not sufficient to unravel the complexity of pain perception, as higher-order cortical processes such as the contextual modulation of pain might not be rigorously time-locked to the application of a painful stimulus. We aimed to overcome this limitation by using an EEG frequency tagging approach, which allowed us to more clearly differentiate between activity related to the applied stimulus and other ongoing activity. Moreover, by using long-lasting periodic sustained stimuli, we aimed to capture high-level processes related to stimulus expectation to a larger extent than it is possible in the analysis of relatively brief and sudden stimuli.

We employed a cue-based stimulus expectation modulation paradigm to investigate whether changes in stimulus perception induced by expectation will lead to congruent changes in the modulation of ongoing oscillations at the frequency of stimulation and its harmonics. Based on Atlas et al. (2010) and Keltner et al. (2006), we expect (1) that the information (cue) presented

to participants before a nociceptive stimulus can influence the expectations towards that stimulus and consequently (2) alter the perception of this stimulus. Specifically, we hypothesized that if the same medium intensity stimulus is presented with a cue indicating the following stimulus would have a low intensity, the rating of perception would be lower than if the same stimulus is presented with a cue indicating that the stimulus would be highly intense. As demonstrated in previous investigations from our lab (Colon et al., 2017; Leu et al., 2023; Liberati et al., 2019; Mulders et al., 2020), we expected (3) the ultra-slow sustained thermonociceptive stimulation to elicit a periodic response in the different frequency bands at the frequency of stimulation and its harmonics. If the modulation of ongoing oscillations is indeed functionally related to pain perception, we hypothesized (4) that the aggregated amplitudes at the frequency of interest will exhibit a change in modulation congruent to the changes in stimulus perception induced by the cue-based expectation modulation. This would provide evidence that there is an association between the modulation of ongoing oscillations and pain perception.

2. Methods

The Stage 1 manuscript of this Registered Report (RR) has been formally registered on the Open Science Framework (OSF) by PCI RR after receiving in-principal-acceptance (<https://osf.io/adnf4>). ~~The OSF project repository associated with this RR can be found under the following link: <https://osf.io/9ud7x>. All anonymized raw data sets, analysis codes and pre-processing pipelines, and digital study materials~~ are available in the public archive of Harvard Dataverse (<https://doi.org/10.7910/DVN/40ZRQR>).

2.1. Participants

We recruited 40 healthy participants. A detailed sample size rationale as well as a discussion of the expected effect sizes can be found in the Supplementary Materials. Participants who have neurological diseases, psychiatric disorders, or recent upper limb trauma upon direct questioning were excluded from the study. In addition, those who have taken paracetamol, nonsteroidal anti-inflammatory drugs (NAIDs), or acetylsalicylic acid within 12 hours before the

assessment were also ~~be~~ excluded. Before the assessment began, written informed consent was obtained from all participants, who were also informed that they have the option to withdraw from the study at any time. We recruited participants between the ages of 18 and 35 (mean \pm std. dev.: 23.65 \pm 3.45), with the aim of achieving a gender-balanced sample size (17 males / 23 females). Participants were recruited via an established Facebook group, as well as posters on campus and word-of-mouth.

All procedures were performed in accordance with the relevant guidelines and regulations. The local Research Ethics Committee approved all experimental procedures (Commission d’Ethique Hospitalo-Facultaire Saint-Luc UCLouvain, B403201316436).

2.2. Sample size calculation

Previous investigations in this lab have shown that 15-20 participants are sufficient to observe the modulation of neural oscillations induced by a sustained periodic nociceptive stimulation (Colon et al., 2017; Mulders et al., 2020). This is largely due to the high signal-to-noise ratio in the periodic responses to the ultra-slow 0.2 Hz sustained periodic stimulation, which can even be differentiated from noise at an individual level (Colon et al., 2017). Other investigations using cue-based expectation modulation while acquiring EEG data recruited between 10 and 20 participants per experiment (Albu & Meagher, 2016; Atlas et al., 2010; Hauck et al., 2007; Keltner et al., 2006; Koyama et al., 2005) and more recent investigations recruited between 40 and 48 participants (Bott et al., 2023; Nickel et al., 2022).

We thus used a simulation-based approach to calculate appropriate power and sample size estimation to reach sufficient statistical power and detect a specific effect in a linear mixed model (LMM). The calculations were conducted using R Statistical Software (Version 4.1.0, R Core Team 2021) and the R package “simr” (Green & MacLeod, 2016). The regression model used for the simulated LMM was built as follows: $amplitude \sim temperature + cue + temperature:cue + (1|subject)$, as detailed in our hypotheses plan and statistical analysis section. The simulated model is based on Mulders et al. (2020). This publication was chosen since the same stimulation and analysis techniques (i.e., frequency tagging of ongoing

oscillations) as proposed in this investigation were used to analyze differences in modulation of ongoing oscillations induced by different stimulation surface areas. The LMM interaction between temperature and surface in their investigation had an intermediate effect size of $\eta^2_p=0.060$ for the phase-locked response. We simulated the LMM based on the mean and standard deviations obtained for the phase-locked response using a small-variable surface of the contact-heat thermode probe for the stimulation (equaling our HH condition, mean =0.59 μV , sd = 0.33 μV) and a small-fixed surface of stimulation (equaling our LL condition, mean =0.41 μV , sd = 0.31 μV). The values for the medium intensity conditions (HM (mean = 0.545 μV , sd =0.349 μV) and LM (mean= 0.455 μV , sd = 0.291 μV)) were estimated based on the percentual difference in rating between these conditions that we observed in our behavioral pilot study (18%) (see Supplementary Materials). This percentage is similar to the difference observed in the ratings between HM and LM condition in Atlas et al. (2010). We therefore calculated the mean between our chosen HH and LL values, lowered it by 9% for the condition LM and increased it by 9% for the condition HM. These values reflect our assumption that a stimulus that is expected to be more painful will lead to larger amplitude at the frequency of stimulation and vice versa. The output of this LMM (based on intercept (0.809), slopes for temperature, cue and interaction (-0.228, -0.483, 0.444), residual variance (0.107) and random intercept (0)) was then fed to the LMM-specific sample size simulation.

In the power estimation, we specifically tested for the interaction effect between *temperature* and *cue*, since this is our main comparison of interest. Additionally, interactions usually have a lower effect size compared to main effects and are therefore more critical for the calculation of the adequate sample size. According to the sample size simulation, a sample size of 25 participants would enable us to reach a statistical power of 0.9 while using an alpha level of 0.02. To avoid missing out on any effect and to account for the potential exclusion of participants from the final data analysis (e.g., due to artifacts in the EEG signal), we decided to increase the sample size to 40 participants.

We considered recruiting a sample that would inform us not only about the effect size of interest, but that would also be able to detect the smallest effect that one could possibly be interested in (Dienes, 2021). The necessary sample size was calculated by obtaining the 80% confidence interval of the LMM and replacing the model estimates with the lower bound estimates of the confidence interval. This updated model was used for the simulation of the power to sample size relationship, resulting in a recommendation to test 150 participants. Unfortunately, limited resources do not allow us to test such a large cohort, and we decided to test only for the more conventional effect size of interest. In consequence, a non-significant result in the LMMs will not necessarily prove the absence of an effect but could also be due to the sample size which might not be large enough to detect effects that are smaller than expected (as noted in the Hypotheses Table).

2-2-2.3. *Thermonociceptive stimulation*

Thermonociceptive stimuli were delivered using a thermal cutaneous stimulator (TCS II, QST.Lab, Strasbourg, France) with the square T11 probe, which is set with 5 micro-Peltier elements (each ~181 mm²) whose temperature can vary at rates of up to 75°C/s and which can be activated individually. The full surface was used in this experiment, covering a rectangular area of 9 cm². A sustained periodic stimulation with a frequency of 0.2 Hz was applied and the baseline temperature of stimulation was set to 35°C. The peaks of the stimulation varied from 44°C for the low intensity condition, over 47°C for the medium intensity condition to 50°C for the high intensity condition (illustrated in Figure 1). Each sustained periodic stimulation comprised 10 stimulation cycles, lasting a total of 10x5 seconds per stimulus, similar to Liberati et al. (2019), Mulders et al. (2020) and Leu et al. (2023). Shorter cycle durations were chosen compared to previous investigations to avoid subjecting the participants to a large number of thermonociceptive stimuli. Inter-stimulus-intervals ~~will be~~ were variable and self-paced by the experimenter to allow participants to provide intensity ratings. The thermode was placed on the volar forearm of the dominant arm of the participants and was displaced after each trial to avoid habituation or sensitization.

2.3.2.4. *Experimental procedure*

2.3.1.2.4.1. *Expectation cue*

The visual cues, adapted from Keltner et al. (2006), were displayed on a monitor in front of the participants. The cues consisted of a colored square (blue for low intensity and red for high intensity stimulation), covering the full screen. In the middle of the square, the word “low” or “high”, respectively, was displayed (illustrated in Figure 1). The participants received verbal instructions identifying each cue and which stimulus intensity it is associated with. The cue was presented to the participants prior to the onset of each stimulus and remained visible during the stimulation (illustrated in Figure 2). A pilot study was conducted prior to data collection to ensure that the chosen paradigm would be able to modulate subjects’ pain perception (see Supplementary Materials).

2.3.2.2.4.2. *Cue-based expectation modulation*

Five blocks of stimuli were implemented, each block consisting of 8 trials (each trial consisted of 50 s of sustained periodic stimulation), adapted from Atlas et al. (2010). The first block was used to establish the link between the expectation cue and the stimulation temperature and consisted of only 4 trials, which were not considered for the analysis. Therefore, in this block, the cue for low intensity was always paired with a low intensity stimulus (LL) and the cue for high intensity was always paired with a high intensity stimulus (HH). The second block also started with two trials of matching conditions (LL / HH), followed by a randomized sequence of trials including unmatched cue / temperature combinations. In the unmatched conditions, medium intensity stimuli were paired with either a cue for high intensity (HM) or a cue for low intensity (LM). Blocks 3 to 5 were random in the sequence of conditions. In each block, each condition needed to be presented two times, resulting in a total of 8 trials per condition for the analysis. The experimenters (as well as the participants) were blinded regarding the condition that was to be applied; thus neither knew whether the current stimulus was a matched or unmatched condition.

2.4.2.5. *Behavioral measures*

Participants rated the expected intensity of stimulation on a visual analog scale (VAS) using a 10 cm ungraduated sliding ruler right after seeing the cue before the beginning of the stimulation. The lower extremity of the VAS was labeled “no perception” and the higher extremity was labelled “the most intense perception you can imagine”. The time-interval between the rating and the start of the stimulation was variable. During the thermonociceptive stimulation, participants were instructed to sit as still as possible to generate an artifact-free EEG signal. After the stimulation, participants heard a beep sound which indicated the end of the trial. Participants then had to indicate on the VAS how intense they perceived the thermonociceptive stimulation overall, as well as whether they perceived the stimulation as painful or not (as illustrated in Figure 2).

2.5.2.6. *EEG recordings*

EEG was recorded using an elastic electrode-cap with 64 active, pre-amplified Ag-AgCl electrodes (BioSemi, Netherlands), which were arranged according to the international 10-10 system. To ensure a clean signal, the direct-current offset was kept below 30 mV. All electrodes were re-referenced offline to the average electrode activity. The recorded signal was stored in the BioSemi ActiView software for offline analyses.

2.6.2.7. *EEG analysis*

The EEG recordings were analyzed using the Letswave7 (www.letswave.org) toolbox in MATLAB (2022a The MathWorks).

2.6.1.2.7.1. *Analysis of the phase-locked response*

To isolate activity related to the applied stimulus, we made use of the frequency tagging analysis approach (Regan, 1989). According to the rationale of this approach, a periodic stimulation elicits a periodic activation of higher order neurons, which in turn leads to a periodic EEG response at the frequency of stimulation and its harmonics (Colon et al., 2012; Mouraux et al., 2011). This approach has been used extensively in our lab over the past years, (Colon et al., 2014; Colon et al., 2017; Colon et al., 2012; Leu et al., 2023; Liberati et al., 2019; Mulders

et al., 2020), leading to a standardized analysis approach: First, events were created based on the triggers related to the onset of the stimulation. Each trigger received a label according to the condition it preceded (HH, LL, HM, LM). Then, slow drift and high frequency power line noise were removed using a Butterworth band-pass filter between 0.05 Hz and 40 Hz. Epochs were segmented into segments of 0-50s, relative to the onset of the stimulation, creating one file per event code containing all 8 trials of this condition. Electrodes P9, P10 and Iz were removed, since due to their placement on the EEG cap, they frequently only record muscular noise rather than brain activity. All signals were re-referenced to the average of the remaining electrode set. Then, an Independent Component Analysis (Fast ICA algorithm) (Hyvarinen & Oja, 2000) was employed to detect artifacts due to eye movement or other muscular artifacts and remove them. The ICA was computed for each subject separately across all conditions at the same time, using the “runica” algorithm (Bell & Sejnowski, 1995), decomposing the full rank data matrix into 61 independent components. Therefore, the same components were removed in each condition for each subject. Additionally, trials with an amplitude larger than $\pm 500 \mu\text{V}$ (Colon et al., 2014) on any of the electrodes were excluded. Any participant with less than 5 trials at this stage would have been removed from the data set, but this did not occur. Finally, the average signal was calculated for each participant and each condition, and then transformed into the frequency domain using a discrete Fourier transform (FFT) (Frigo & Johnson, 1998). Residual noise was partially removed by subtracting the average amplitude of the signal measures at 2-5 neighboring frequencies, at each electrode and at each frequency bin.

Since the periodic response elicited by the ultra-slow sustained periodic stimulation is not a perfect sinewave, the peaks in the amplitude of the frequency spectrum do not only appear at the frequency of stimulation itself, but also at its harmonics. To account for this, the amplitude at the frequency of stimulation and its harmonics was aggregated and the resulting amplitude at the frequency of interest (FOI) was used for the statistical analysis. To aggregate the harmonics, the signal was cut into chunks of 0.2 Hz length, starting at 0.1 Hz. Therefore, in

each chunk, the signal in the middle corresponds to the harmonic of the frequency of stimulation. Then, the chunks were averaged, and the resulting amplitude was multiplied by the number of ~~averaged~~ chunks for which the average has been calculated. The whole electrode set was ~~be~~ taken into account for this procedure.

2.6.2.2.7.2. *Analysis of the modulation of ongoing oscillations*

The analysis of the modulation of ongoing oscillations was almost identical to the previously outlined analysis. To investigate the effect of our stimulation on the periodic modulation of the amplitude of ongoing neural oscillations within different frequency bands (theta: 4-8 Hz, alpha: 8-12 Hz, and beta: 12-40 Hz), the EEG signal was additionally filtered using a 4th order Butterworth filter for each frequency band after calculating the ICA and re-referencing of the electrodes in the remaining signal. Another additional step was the estimation of the envelopes of the signal, which was computed using a Hilbert transform. The following steps were equal to the procedure described for the phase-locked response, including the aggregation of the signal amplitudes at the frequency of stimulation and its harmonics. The amplitude at the FOI was used for the statistical analysis in each frequency band and the whole electrode set was considered.

2.7.2.8. *Statistical analysis*

Statistical analysis was done using R Statistical Software (Version 4.1.0, R Core Team 2021) and MATLAB (2020b The MathWorks). The significance level of $p < 0.05$ was set for the behavioral analysis and LMMs. The LMM was fitted using REML and a Kenward-Roger approximation to produce appropriate type I error rates for smaller sample sizes was used to test the significance of the results. All explicit formulas / equations for the statistical analysis can be found in the hypotheses table.

2.7.1.2.8.1. *Behavioral data*

To assess whether the cue affected the rating of expected stimulus intensity, an LMM with the independent variable (IV) *cue* and dependent variable (DV) *expectation rating* was used. The factor *subject* accounted for the variation of the regression model intercept across participants

and was therefore a random effect in the model. This model was the positive control for the factor *cue*; if the cue would not be effective in influencing the expected stimulus intensity, we had to assume that the cue-based expectation modulation paradigm in this experiment failed.

Further, we employed another LMM to analyze the effect of *cue* (2 levels: low, high) and stimulation *temperature* (2 levels: matched, unmatched) (IV's) on the rating of perceived stimulus intensity perception (DV). We further wanted to assess the interaction between these two factors on the intensity rating. As in the aforementioned LMM, *subject* was used as a random effect. We hypothesized that the medium intensity stimulation paired with the high intensity cue (HM) would lead to a higher rating of perceived stimulus intensity compared to the medium stimulus paired with the low intensity cue (LM).

2.7.2.2.8.2. *Periodic response*

To assess whether the amplitude at the FOI was significantly different from zero, a right tailed multi-sensor cluster-based permutation test using Wilcoxon signed-rank test as test statistic was used. To do this, for each condition, the corresponding data was merged into one file, containing all participants. The test compared each signal to 0, using a Bonferroni corrected alpha level of 0.0125 (the standard alpha level 0.05 divided by the number of conditions). The threshold for the cluster-based permutation was also set to 0.0125, and 2000 permutations were computed. The multi-sensor analysis was set to a threshold of 0.161, which sets the threshold for the sensor connection so each channel has 4 neighbors on average. This approach allowed to control for a non-normal distribution of the data, while taking potential type I error inflation due to multiple testing into account. The electrode with the highest test statistic will be chosen for further analysis.

Based on previous results from this lab (Colon et al., 2017; Leu et al., 2023; Mulders et al., 2020) we expected a periodic response in the EEG signal elicited by the sustained periodic stimulation significantly larger than zero.

To investigate whether the high intensity cue paired with the medium intensity stimulation (HM) would lead to a higher amplitude at the FOI for the phase-locked EEG signal compared to the low intensity cue paired with the medium intensity stimulation (LM), we used a LMM with the following factors: stimulation *temperature* and *cue* as independent (fixed) variables (IV) with an interaction, while *subject* was a random factor. The *amplitude* at the FOI was used as dependent variable (DV).

2.7.3.2.8.3. *Modulation of ongoing oscillations*

As for the phase-locked signal, we examined whether the amplitude at the FOI was significantly larger than zero with a right tailed multi-sensor cluster-based permutation test using Wilcoxon signed-rank test as test statistic. The electrode with the highest test statistic was used for the continuation of the analysis.

We expected the amplitude at the FOI to be significantly larger than zero in all frequency bands (Colon et al., 2017; Leu et al., 2023; Liberati et al., 2019). To test our main hypotheses, we used a LMM with the same structure as described above. The IVs were *temperature* and *cue*, while *subject* was added as a random factor, accounting for the variation in the regression model between participants. Finally, *amplitude* was the DV in this model. A separate LMM was calculated for the amplitude at the FOI in each frequency band.

We hypothesized that the amplitude at the FOI would be larger if the medium intensity stimulation was preceded by a high intensity cue (HM) compared to a low intensity cue (LM). If this was the case and the cue-based expectation modulation would change intensity perception in the same direction, these results would suggest that the modulation of ongoing oscillation could be functionally related to pain perception.

2.7.4.2.8.4. *Outliers*

Any participant unable to complete data acquisition would have been excluded from the analysis. This was not the case in our data set. Further, any data points that violated the LMM assumptions after fitting the LMM were identified using a Shapiro-Wilk test to assess the

normal distribution of the data as well as a Levene's test, testing the data set for homoscedasticity. In case the data did not conform to normality, a log-transform was applied, which conforms data to the assumption of normality by correcting right-skewed data into a more normal form (Bland & Altman, 1996). Any data point that still violated any of the assumptions after the transformation or disproportionately affected the dataset after fitting the LMM was removed from the data set and was not replaced. To ensure that we will still reach the targeted sample size and statistical power, a slightly larger group of participants will be recruited than required by the sample size calculation. Additionally, data points that over-proportionally influence the data set were identified using Cook's Distance [D]. This method calculates how much the fitted values of a given data set change if just one data point is removed. The influence of a data point is expressed in the "distance" D; the larger it is, the more influential the data point (Cook, 1977). Therefore, any data point exceeding a D of 1 was removed from the data set. Cook's distance was calculated for each datapoint within a condition. Thus, for each condition and frequency band, a separate calculation will be done. Overall, data points exceeding the threshold for Cook's distance were identified only in the phase-locked response. Results without removed outliers are reported in the Supplementary Materials.

3. Results

3.1. Behavioral results

The cue significantly influenced the expectation of the participants regarding the upcoming thermonociceptive stimulation ($F(1,1239)= 6108, p<0.001, \eta^2_p= 0.83-$). The perception of the stimulation was also significantly modulated by both cue and stimulation temperature, as well as their interaction (Table 1, Figure 3). Post-hoc pairwise comparisons revealed that the medium intensity stimulation was perceived as significantly more intense when preceded by a high intensity cue compared to a low intensity cue ($t(1237)=25.950, p<0.001$). Figure 4 illustrates the perception ratings for each participant in all conditions. Overall, stimuli were perceived as painful in condition HH (78.75% of trials), while this was rarely the case for

condition LL (1.25% of trials). The medium intensity condition was perceived as mostly non-painful when preceded by a low intensity cue (LM, 9.7% rated as painful). Yet, the same stimulation preceded by a high intensity cue (HM) was rated as painful in more than twice as many trials (22.8% of trials).

3.2. EEG

3.2.1. Phase-locked response

The Wilcoxon signed-rank test showed that a periodic modulation on the phase-locked response was only found in the HH condition. No significant periodic modulation was found in any of the other conditions. The electrode with the largest modulation was Fz, which was subsequently chosen as the electrode of interest for the remaining analysis of the phase-locked response. The data points of one subject were partially removed because they were identified as outliers, even after using a log-transform to increase the normality of the data distribution. Hence statistical analysis was done on the log-transformed data. The original amplitudes are shown in the figures to increase the intuitive understanding of the graphs. The LMM showed a significant effect of cue as well as of the temperature x cue interaction on the amplitude at the FOI (Table 1). Post-hoc pairwise comparison revealed a significant difference between the matched conditions (HH vs LL, $p < 0.001$), but no significant difference between the unmatched conditions (HM vs LM, $p = 0.11$). Further analysis revealed a significant difference between conditions HH and HM ($p < 0.0001$) but not between LM and LL ($p = 0.073$).

3.2.2. Modulation of ongoing oscillations

Similar to the phase-locked response, only condition HH lead to a significant modulation at the FOI in the different frequency bands. The electrode with the largest modulation in the theta, alpha and beta frequency band were C2, PO3 and FCz. Scalp topographies illustrating the obtained Wilcoxon test-statistics can be found in Figure 5.

As for the phase-locked response, all data sets were log-transformed to conform to the assumption of normality. While the statistical analysis was done on the log-transformed data, we chose to show the original amplitudes in the plots for enhanced readability. The LMM's in

the different frequency bands all yielded similar results (Table 1). While significant effects of cue, stimulus temperature and their interaction were observed in almost all frequency bands, all post-hoc pairwise comparisons revealed that the difference in amplitude at the FOI was not significant between conditions that were presented using the same stimulation temperature (HM and LM). Yet, in all comparisons, condition HH led to significantly larger amplitudes at the FOI than condition LL (illustrated in Figure 6). Additional pairwise comparisons revealed that in all frequency bands, condition HH elicited significantly larger amplitude at the FOI than condition HM ($p=0.011$, $p<0.001$, $p<0.001$, respectively, for theta, alpha and beta frequency band), while no significant differences were observed between conditions LM and LL. Illustrations of the spectra up to 1 Hz for each frequency band can be found in the Supplementary Materials.

4. Discussion

The cue-based expectation modulation paradigm, which was adapted from Atlas et al. (2010) and Keltner et al. (2006), influenced the participants' stimulus intensity expectation and perception as intended; both positive controls regarding the behavioral aspect of the experiment have been met. The visual cue changed the expectation of the participants towards the upcoming stimulus and participants anticipated the correct level of stimulus intensity based on the cue they were given. Moreover, the cue also significantly changed the stimulus intensity perception of the participants in the medium intensity stimulation trials: participants experienced the stimulation as more painful and intense when shown a cue indicating a high stimulus intensity compared to being shown a cue for a low intensity stimulus. Consequently, given our hypothesis, we expected the modulation of ongoing oscillations to reflect these cue-based changes in stimulus perception during the stimuli delivered at a medium intensity (HM and LM).

A significant modulation of ongoing oscillations at the frequency of interest was only found in the high intensity stimulation condition. Additionally, while significant differences were found for the main effects and interactions between cue and stimulation temperature, post-hoc pairwise comparisons uncovered that these differences were mainly driven by the conditions HH and LL; the conditions of interest HM and LM did not differ significantly in their modulation at the frequency of interest for the specified electrodes in any of the analyzed frequency bands. Moreover, significant differences in amplitude found between HH and HM indicate the intensity at which the stimulus is delivered is mainly contributing to the amplitude observed at the frequency of interest, and not the expected level of stimulus intensity. This suggests a potential dissociation of stimulus intensity perception and the modulation of ongoing oscillations measured using scalp EEG.

While the frequency-tagging approach has been used numerous times in our labs, the present stimulation parameters diverged from previous experiments in their duration as well as in the stimulus temperatures. Notable, stimuli were shorter (10 instead of 15 cycles) and intensities lower (44°C and 47°C instead of only 50°C) compared to the previous publications using this technique (Colon et al., 2017; Leu et al., 2023; Mulders et al., 2020). While the numbers of cycles were carefully chosen following the re-analysis of the data sets of these previous investigations, it was less clear how much the stimulation temperature would affect the modulation of ongoing oscillations. As shown by Colon et al. (2017), these slow sustained periodic stimuli recruit predominantly C-fibers, for which the activation threshold lies around 37°C to 49°C (Schmidt et al., 1997; Treede et al., 1990). Since we were not able to detect a significant modulation at the frequency of interest for the low and medium stimulation intensities, one possible explanation would be that the temperatures were ~~simply~~ too low to recruit enough afferent fibers to elicits a periodic activation of higher order neurons. Especially given the temperature of the low intensity stimulus, which was set just around the threshold for pain perception for most people, it seems reasonable that no large effects would be seen. Importantly, while we measured the neural responses to all 4 conditions, we were mainly

interested in the responses to stimuli using the medium stimulation intensity (47°C). For conditions HM and LM it was indeed rather surprising to not find a periodic response at the frequency of interest. This might be a result of the lower stimulation temperature in combination with the very slow stimulation paradigm, as temporal summation (nociceptor discharge frequency > 0.5 Hz) is thought to be one of the means through which innocuous warm stimuli can activate nociceptive C-fibers (Tillman et al., 1995). While the low frequency of stimulation was chosen due to its superior signal-to-noise ratio (Colon et al., 2017), a higher temperature for the medium intensity trials might have led to a larger response at the frequency of interest. Yet, as we tried to create a paradigm that had 3 clearly discernable stimulation temperatures that were also tolerable for the high intensity stimulation, the range of temperatures to choose from for the paradigm was limited.

Nonetheless, while the response at the frequency of interest might have not been significant for certain conditions, we could theoretically still observe difference in the modulation of the ongoing oscillations at that frequency. Importantly, those differences will have to be interpreted with caution, as the observed responses were clearly rather small and not statistically different from zero. As expected, a clear-significant difference between high and low intensity stimulation was found in all frequency bands, with the intensity of stimulation as the main differentiator. For both conditions, the expectations matched the applied stimulus. The comparison between the conditions preceded by the high expectation cue showed a similar pattern; the more intense stimulation led to a larger response at the frequency of stimulation in all frequency bands. Thus, Neither the expectation of a similar-high intensity stimulus nor the mismatch-in-between-expected-and-perception-perceived-stimulus-intensity for condition HM seemed to have an influence on the recorded amplitude.

On the contrary, the medium intensity conditions (HM and LM) did not show any differences in their modulation, despite the strong effects the visual cues had on the intensity perception associated with the stimulation. While various investigations have found effects of pain stimulus expectation on EEG correlates (Hauck et al., 2007; Huneke et al., 2013; Strube et al.,

2023; Thomaidou et al., 2021; Tiemann et al., 2015; Wager et al., 2006), other recent publications found a similar dissociation between stimulus perception and oscillatory activity. Albu and Meagher (2016) recorded an increase in oscillatory activity between 8 and 10 Hz related to thermociceptive stimuli following a placebo treatment. Yet, these changes were correlated to the catastrophizing of the individual and not the perceived pain intensity or unpleasantness. In a paradigm comparable to the one used in the present study, Nickel et al. (2022) found a similar modulation of thermociceptive stimulus perception based on visual cues, but failed to detect related changes in either event-related potentials (ERPs) or oscillatory activity in their time-frequency analysis.

With our frequency-tagging approach, we aimed to overcome some of the pitfalls of other - more standard - EEG analyses, such as the focus on phase-locked responses and brief stimuli. However, it appears that scalp EEG might not have the specificity or spatial resolution to relay the complex network of activity that shapes human pain perception when the activity of a specific electrode is considered. Nevertheless, this does not imply that EEG cannot be used to assess relevant information on how neural activity guides pain perception.

On one hand, expanding traditional scalp EEG assessments to connectivity analyses and source modeling has been shown to be a promising tool to further understand the complex processes that integrate sensory and contextual information. Bott et al. (2023) have provided interesting results using the same cue-based expectation paradigm as in Nickel et al. (2022), expanding the analysis from local oscillatory activity to interregional connectivity in a network of 6 pre-selected regions of interest associated with pain perception (such as contralateral primary somatosensory cortex, anterior cingulate and prefrontal cortices). While sensory information was mostly encoded in local activity, expectations were shaped exclusively by interregional connectivity, predominantly in the alpha frequency band from the prefrontal to the somatosensory cortex. These results highlight that the perception of pain, which depends on both sensory as well as contextual factors, can hardly be measured by a single electrode measured using scalp EEG. Thus, future EEG investigations should focus rather on the

connectivity between different brain regions to further unravel how pain perception emerged from neural activity.

On the other hand, ~~in~~ intracerebral EEG could overcome some limitations of scalp EEG. Previous investigations from our lab have shown that thermnociceptive stimuli (delivered in the same slow sustained periodic manner) are preferentially modulated in the human insula compared to non-nociceptive vibrotactile stimuli (Liberati et al., 2019). By using electrodes which are implanted in patients undergoing pre-surgical evaluation for focal refractory epilepsy, it is possible to assess the activity of brain regions which are difficult to assess using scalp EEG due to the depth of their locations with a very high spatial as well as temporal resolution (Bradley et al., 2017).

Finally, the statistical power given our sample size should be considered for the interpretation of our results. As outlined in our hypotheses table, while we had enough power (given the limitations of an alpha level of 0.02 and target power of 0.9) to find expected effect sizes based on previous investigations and our initial estimation, our sample size did not allow us to reach a statistical power ~~(given the limitations of an alpha level of 0.02 and target power of 0.9)~~ that would allow us to find the “*smallest possible effect that we would still be interested in*” (Dienes, 2021). Frequently, the targeted effect size is the observed effect in previous literature; yet, this approach might lead to the lack of support for rejection of a hypotheses only because the effect might have been smaller than in previous investigations and not because there was truly no effect (Dienes, 2021). Additionally, since the amplitudes related to the medium intensity stimulation were smaller than expected, this could have led us to overestimate the initial effect size. The large range of the confidence intervals of the post-hoc estimated effect sizes suggest that our effect size is not very precise, and a different investigation might yield different results. Thus, even though we did not find any modulations in the ongoing oscillations related to the differences in pain perception induced by the cue-based expectation modulation, we cannot fully reject this hypothesis based on our ~~investigation study~~ alone and further investigations are needed to confirm our results.

Conclusion

Despite a strong effect of the visual cues on stimulus perception, no significant differences were observed in the modulation of ongoing oscillations at the frequency of interest between the conditions of interest (medium intensity stimulation preceded by either a cue for a high or a low stimulation intensity). These results could suggest a dissociation between stimulus perception and the modulation of ongoing oscillations measured using scalp EEG. While our results parallel other recent investigations using a cue-based expectation modulation, we cannot exclude that our sample size was not large enough to detect a potentially small effect and definitive conclusions should not be drawn.

It appears that more advanced analysis procedures (such as source localization and connectivity analysis) or more spatially precise recordings of neural activity (such as iEEG) could be more beneficial to understand how our perception of pain emerges from neural activity.

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Conflict of interest

The authors declare no conflict of interest.

CRedit statement

Conceptualization: CL, EG, GL

Data curation and analysis: CL

Funding Acquisition: CL, GL

Writing – original draft: CL

Writing – review & editing: CL, EG, GL

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Tables

| Dependent variable | cue | | | condition | | | interaction | | |
|-----------------------|----------------------|------------------|---------------------------|-----------------|------------------|---------------------------|-----------------|-----------------------|---------------------------|
| | <i>F</i> -value | <i>p</i> | η^2_p [95% CI] | <i>F</i> -value | <i>p</i> | η^2_p [95% CI] | <i>F</i> -value | <i>p</i> | η^2_p [95% CI] |
| Perception ratings | (1,1237) 3357.926 | <0.001 | 0.73 [0.71, .1] | 20.634 | <0.001 | 0.02 [0.01, .1] | 451.532 | <0.001 * | 0.27 [0.23, .1] |
| Phase-locked response | (1,114.68) 7.912 | 0.006 | 0.06 [0.01, .1] | 6.436 | 0.013 | 0.05 [0.01, .1] | 26.026 | <0.001 | 0.18 [0.09, .1] |
| Theta | (1,117) 4.992 | 0.027 | 0.04 [0.00, .1] | 4.588 | 0.034 | 0.04 [0.00, .1] | 2.248 | 0.147 | 0.02 [0.00, .1] |
| Alpha | (1,117) 10.761 | 0.001 | 0.08 [0.02, .1] | 4.470 | 0.037 | 0.04 [0.00, .1] | 8.861 | 0.004 | 0.07 [0.01, .1] |
| Beta | (1,117) 10.374 | 0.002 | 0.08 [0.02, .1] | 1.921 | 0.168 | 0.02 [0.00, .1] | 13.784 | <0.001 | 0.11 [0.03, .1] |

Table 1: Results of the Linear Mixed model with the formula *dependent variable ~ cue + condition + cue : condition + (1|subject)*. Significant *p*-values are highlighted in bold font. η^2_p was calculated as a measure of the partial effect size of each fixed effect, including its 95% confidence interval. Asterisks mark interactions that showed a significant difference in the post-hoc pairwise comparison between the conditions HM and LM (which used the same stimulus intensity, the only difference being the concomitantly presented cue). Electrodes of interest: Phase-locked: Fz, Theta: C2, Alpha: PO3, Beta: FCz.

| Question | Hypothesis | Sampling plan (power analysis) | Analysis Plan | Interpretation given different outcomes | Outcome |
|--|---|--|--|--|---|
| <i>Behavioural response</i> | | | | | |
| (1) Does the intensity cue influence the rating of expected pain? | A high pain cue will lead to a higher expected pain rating than a low pain cue. | See below. Expected detectable effect size is around $\eta^2=0.058$ (see Supplementary Materials for in-depth justifications). | rating_expected = cue + (1 subject) - DV: expected <i>intensity rating</i> - IV: <i>cue</i> - random coefficient: <i>subject</i> | <i>Positive control:</i> If the rating matches our expectations, we confirm that the cue is influencing pain expectations as intended. If not, the experiment cannot be used. | Hypothesis confirmed |
| (2) Do different cues differentially influence the perception of the same painful stimulus? | A medium pain trial paired with a high intensity cue will lead to a higher perceived intensity rating than a medium pain trial paired with a low intensity cue. | See below. Expected detectable effect size is around $\eta^2=0.058$ (see Supplementary Materials for in-depth justifications). | rating_perceived = temperature * cue + (1 subject) - DV: perceived <i>intensity rating</i> - IV: <i>temperature, cue</i> - random coefficient: <i>subject</i> | <i>Positive control:</i> A correct hypothesis would confirm that the expectation shapes the perception of the cue-associated stimuli. A dissociation of expectation and perception indicates that the cue-based paradigm was not successful at changing subjective intensity perception. | Hypothesis confirmed |
| <i>Time locked, phase-locked response</i> | | | | | |
| (3) Does the sustained periodic stimulation lead to a periodic EEG modulation at the frequency of interest? | The slow sustained periodic stimulation will elicit a periodic response at the frequency of interest. | See below. This sample size will allow us to detect an estimated effect size of around $\eta^2=0.083$ (see Supplementary Materials for in-depth justifications). | Multi-sensor cluster-based permutation Wilcoxon signed-rank test (Bonferroni corrected) of aggregated and averaged amplitudes at FOIs. | A periodic response shows that the stimulation paradigm induces the expected neural activity. Since the sample size is not sufficient to detect the smallest possible effect one would still be interested in, no definitive conclusions will be drawn from a non-significant result. | Hypothesis confirmed only for condition HH; no definitive conclusion possible (see sample size limitation). |
| (4) Does a cue-based expectation task modulate the EEG signal at the FOI in the frequency domain? | The amplitude at the FOI induced by the medium intensity stimulation will exhibit a larger modulation following a cue indicating high intensity than a cue for low intensity. | To reach a statistical power of 0.9 with an alpha level of 0.02, 40 participants will be enrolled. Calculations are based on power simulations using the simr package in R (Green et al., 2016). This sample size will | amplitude_FOI = temperature * cue + (1 subject) - DV: <i>amplitude</i> at the FOI - IV: <i>temperature, cue</i> - random coefficient: <i>subject</i> | A change in modulation congruent to the change in intensity perception would reveal a possible connection between perceived pain and ongoing oscillations. Since the sample size is not sufficient to detect the smallest possible effect one would still be interested in, no definitive conclusions will be drawn from a non-significant result. | Hypothesis disconfirmed; no definitive conclusion possible (see sample size limitation). |

| | | | | | |
|--|--|--|--|---|---|
| | | allow us to detect an effect around $\eta^2_p = 0.09$ (see Supplementary Materials for in-depth justifications). | | | |
| <i>Time locked, non-phase locked response</i> | | | | | |
| (3) Does a slow sustained periodic stimulation lead to a periodic neural response at the frequency of interest for the different frequency bands? | A periodic modulation will be elicited in all frequency bands. | See above. This sample size will allow us to detect an effect size of $\eta^2 = 0.083$ (see Supplementary Materials for in-depth justifications). | Multi-sensor cluster-based permutation and Wilcoxon signed-rank test of aggregated and averaged amplitudes at FOIs in the different frequency bands [†] . [†] One test for each frequency band (theta, alpha, beta) | A modulation at the frequency of stimulation indicates that sustained periodic stimulation leads to a periodic response also in the different frequency bands (Colon et al., 2017). Since the sample size is not sufficient to detect the smallest possible effect one would still be interested in, no definitive conclusions will be drawn from a non-significant result. | Hypothesis confirmed only for condition HH; no definitive conclusion possible (see sample size limitation). |
| (4) Does a cue-based expectation task modulate the ongoing oscillations in the different frequency bands in the time-frequency domain? | The medium intensity stimulation will lead to a larger modulation of amplitude at the FOI following a high intensity cue compared to the same stimulation followed by a cue for low intensity. | See above. This sample size will allow us to detect an effect size around $\eta^2_p = 0.09$ (see Supplementary Materials for in-depth justifications). | amplitude_FOI_OO [†] = temperature * cue + (1 subject) - DV: <i>amplitude</i> at the FOI for each frequency band - IV: <i>temperature, cue</i> - random coefficient: <i>subject</i> [†] One model for each frequency band (theta, alpha, beta) | A modulation of ongoing oscillations mirroring the level of intensity suggested by the cue would suggest that pain rating and ongoing oscillations might be functionally connected. Since the sample size is not sufficient to detect the smallest possible effect one would still be interested in (n=150) (Dienes, 2021), a non-significant result of this statistical test does not necessarily indicate that there is a definitive absence of an effect and no definitive conclusions will be drawn from a non-significant result | Hypothesis disconfirmed; no definitive conclusion possible (see sample size limitation). |

FOI: frequency of interest; DV: dependent variable; IV: independent variable; LMM: linear mixed model; amplitude_FOI: amplitude of phase-locked neural response; amplitude_FOI_OO: amplitude of non-phase locked neural response for each frequency band (theta, alpha, beta).

Table 2: Hypotheses table defined prior to data collection, detailing hypotheses, analysis plans and interpretations given different outcomes. Further information on the sampling plan can be found in [section 2.2 and](#) the Supplementary Materials.

Figures

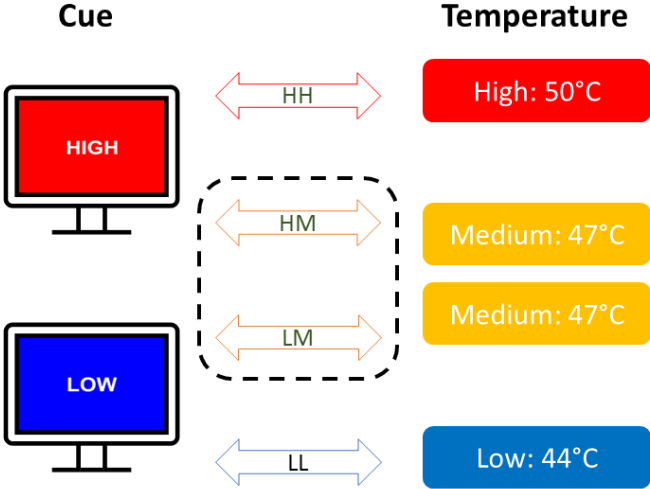


Figure 1: Cue-based expectation modulation paradigm, adapted from Atlas et al. (2010). Cues indicating a high respectively low intensity stimulus were adapted from Keltner et al. (2006).

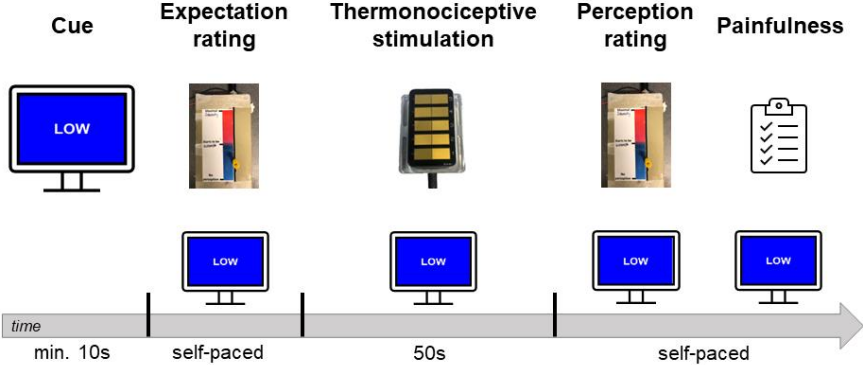


Figure 2: Trial design for one example stimulus, using a cue for a low intensity. VAS ratings were given on a scale from min: “no perception” to a max: “most intense perception imaginable”. Participants were asked to evaluate the painfulness of the stimulus with a simple yes/no answer.

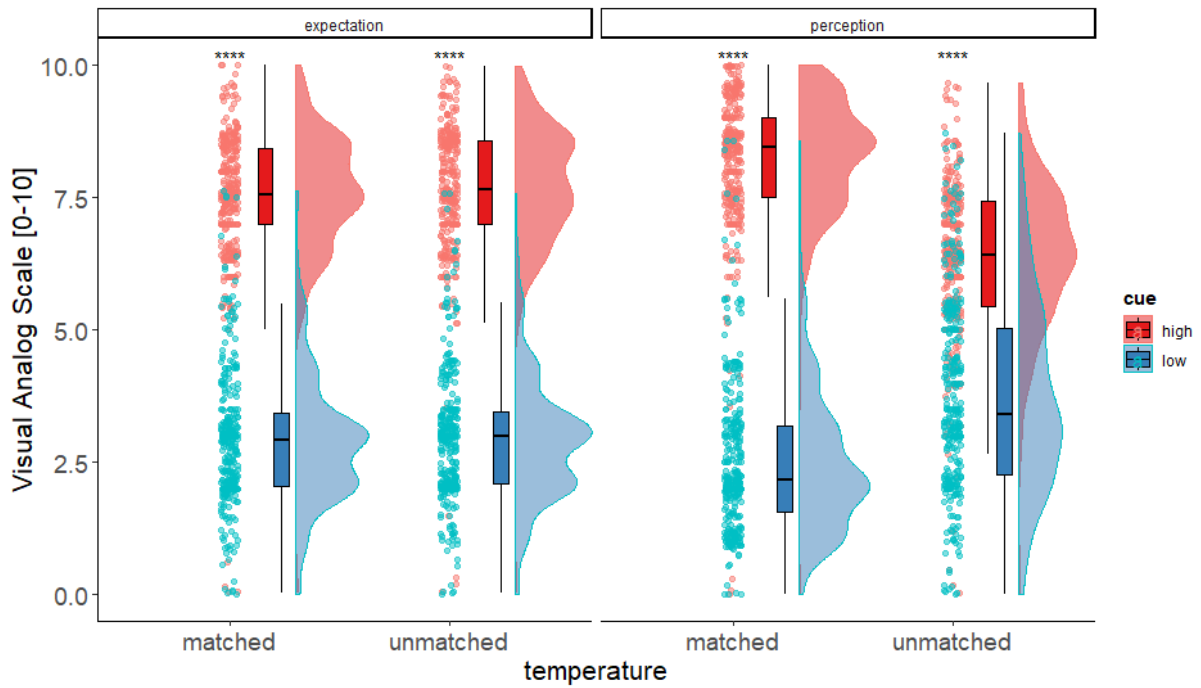


Figure 3: Ratings of expectation (left) and perception (right) collected on a Visual Analog Scale before and after each stimulation, illustrated in Raincloud plots (Allen et al., 2021). Dots indicate single subject ratings. Results of the post-hoc pairwise comparisons are indicated at the top; $p < 0.001 = ****$. “Matched” refers to conditions HH and LL, while “unmatched” refers to conditions HM and LM (cue / temperature).

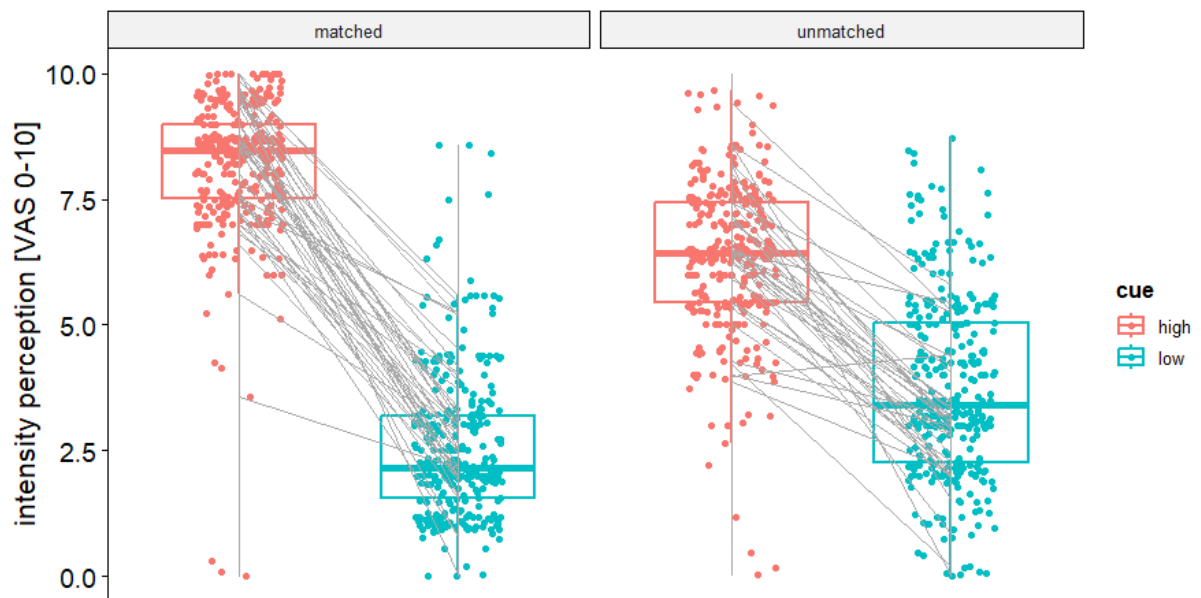


Figure 4: Ratings of intensity perception for each subject in each condition, given on a Visual Analog Scale (VAS). Grey lines connect the mean ratings of the same subject. Matched conditions equal the condition HH and LL, while unmatched conditions represent HM and LM (cue / temperature).

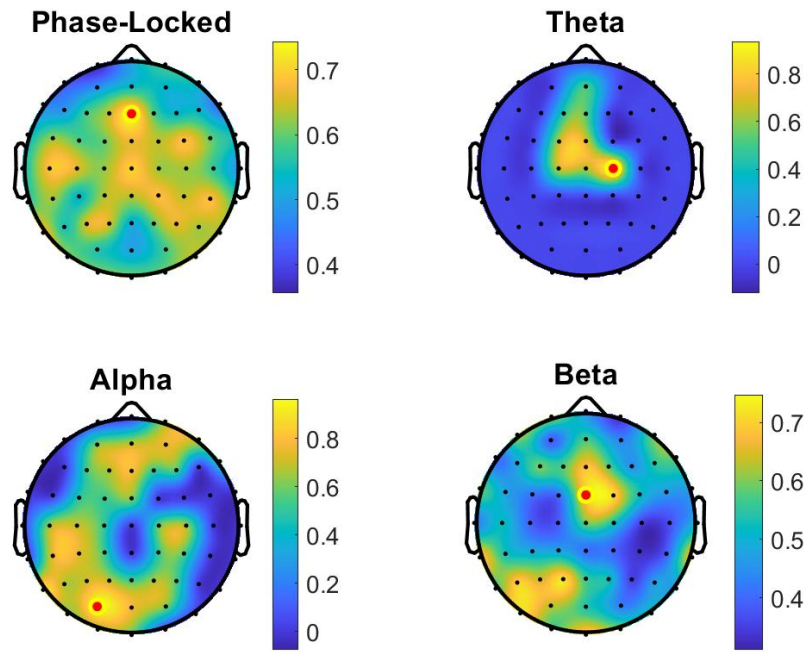


Figure 5: Topographies displaying the results of the multi-sensor cluster-based Wilcoxon signed-rank test for the condition HH (cue for high intensity + high intensity stimulation). The electrode with the largest test statistic – which was used for the subsequent statistical analysis – is marked with a red dot.

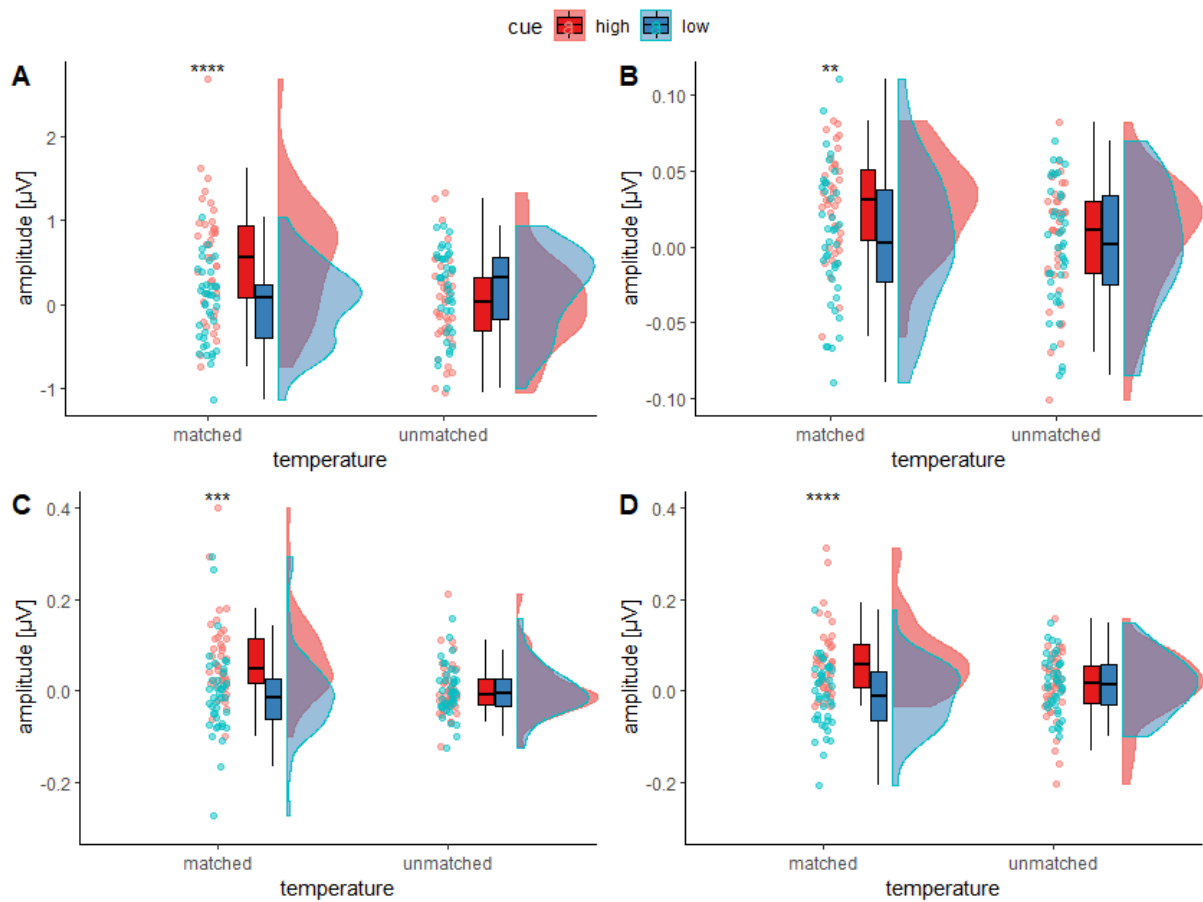


Figure 6: Raincloud plots (Allen et al., 2021) illustrating the amplitudes at the frequency of interest for **A:** phase-locked response, electrode Fz, **B:** theta frequency band (4-8 Hz), electrode C2, **C:** alpha frequency band (8-12 Hz), electrode PO3 and **D:** beta frequency band (12-40 Hz), electrode FCz. “Matched” refers to conditions HH and LL, while “unmatched” refers to conditions HM and LM (cue / temperature). Significant differences in the post-hoc paired t-test are noted as follows: $p < 0.01 = **$, $p < 0.001 = ***$, $p < 0.0001 = ****$.