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

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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 aim to modulate pain perception using a cue-based expectation modulation paradigm and investigate whether ongoing oscillations in different frequency bands mirror the changes in pain perception. Participants will be instructed that a visual cue can precede either a high or low intensity stimulation. These cues will be paired with 3 different levels of sustained periodic thermonociceptive stimuli (low, medium, high). In an initial associative learning phase, participants will learn to associate the low cue with the low stimulus intensity (LL) and the high cue with the high stimulus intensity (HH). In the experimental trials, the same cues will also be followed by medium intensity stimuli (LM and HM respectively). We expect that the stimuli delivered in the HM condition will be perceived as more intense compared to the LM condition, despite being delivered at the same temperature. Furthermore, based on previous findings, we expect that the sustained periodic stimuli will exert a modulation of ongoing oscillations at the frequency of stimulation. We hypothesize that the magnitude of this modulation will reflect the changes in pain perception induced by the cue-based expectation paradigm, therefore suggesting a link between ongoing oscillations and pain perception.

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 ; 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 thermonociceptive 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 (review 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 aim to overcome this limitation by using an EEG frequency tagging approach, which will allow 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 hope to be able 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 will employ a cue-based stimulus expectation modulation paradigm to investigate whether changes in pain 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 hypothesize 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 will 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 ; Liberati et al., 2019 ; Mulders et al., 2020), we expect (3) that 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 hypothesize (4) that the aggregated amplitudes at the frequency of interest will exhibit a change in modulation congruent to the changes in pain 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

All analysis codes and pre-processing pipelines will be uploaded to the OSF repository associated with this publication (https://osf.io/9ud7x/). All anonymized raw data sets will be made available in the public archive of Harvard Dataverse.

2.1. Participants

We will recruit 40 healthy participants. A detailed sample size rationale can be found in the Supplementary Materials. Participants who have neurological diseases, psychiatric disorders, or recent upper limb trauma upon direct questioning will be 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 will also be excluded. Before the assessment begins, written informed consent will be obtained from all participants, who will also be informed that they have the option to withdraw from the study at any time. We will recruit participants between the ages of 18 and 35, with the aim of achieving a gender-

balanced sample size. Participants will be recruited via an established Facebook group, as well as posters on campus and word-of-mouth.

All procedures will be 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. Thermonociceptive stimulation

Thermonociceptive stimuli will be delivered using a thermal cutaneous stimulator (TCS II, QST.Lab, Strasbourg, France) together 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 will be used in this experiment, covering a rectangular area of 9 cm^2. A sustained periodic stimulation with a frequency of 0.2 Hz will be applied and the baseline temperature of stimulation will be set to 35°C. The peaks of the stimulation will vary from 44°C for the low intensity condition, over 46.5°C for the medium intensity condition to 49°C for the high intensity condition (illustrated in Figure 1). Each sustained periodic stimulation will comprise 10 stimulation cycles, lasting a total of 10x5 seconds per stimulus, similar to Liberati et al. (2019), Mulders et al. (2020). Shorter cycle durations will be chosen compared to previous investigations to avoid subjecting the participants to a large number of thermonociceptive stimuli. Inter-stimulus-intervals will be variable and self-paced by the experimenter to allow participants to provide intensity ratings. The thermode will be placed on the volar forearm of the dominant arm of the participants and will be displaced after each trial to avoid habituation or sensitization.

2.3. Experimental procedure

2.3.1. Expectation cue

The visual cues, adapted from Keltner et al. (2006), will be displayed on a monitor. The cues will consist of a colored square (blue for low intensity and red for high intensity stimulation), covering the full screen. In the middle of the box, the word “low” or “high”, respectively, will be displayed (illustrated in Figure 1). The participants will receive verbal instructions identifying each cue and which stimulus intensity it is associated with. The cue will be presented to the participants prior to the onset of each stimulus and will remain visible during the stimulation (illustrated in Figure 2).

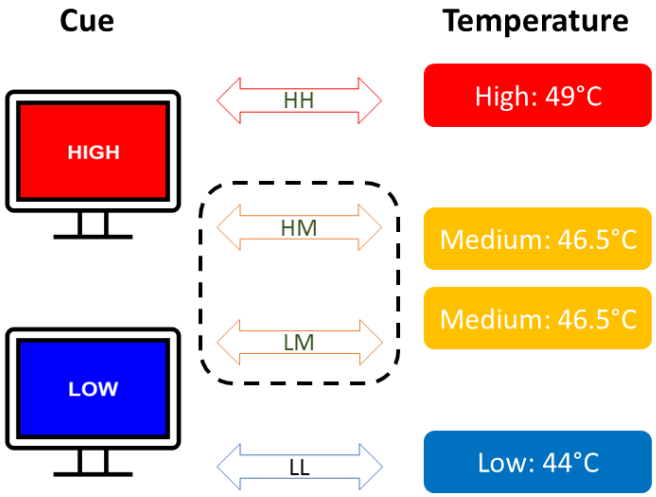

Figure 1: Cue-based expectation modulation paradigm, adapted from Atlas et al. (2010). Cues indicating a high respectively low intensity stimulus were adapted from Keltner et al. (2006).

2.3.2. Cue-based expectation modulation

Five blocks of stimuli will be implemented, each block consisting of 8 trials (each trial consists of 50 s of sustained periodic stimulation), adapted from Atlas et al. (2010). The first block will be used to establish the link between the expectation cue and the stimulation temperature and will consist of only 4 trials, which will not be considered for the analysis. Therefore, in this block, the cue for low intensity will always be paired with a low intensity stimulus (LL) and the cue for high intensity will always be paired with a high intensity stimulus (HH). The second block will also start 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 will be paired with either a cue for high intensity (HM) or a cue for low intensity (LM). Blocks 3 to 5 are random in the sequence of conditions. In each block, each condition needs to be presented two times, resulting in a total of 8 trials per condition for the analysis. The experimenter will be blinded regarding the condition that will be applied; thus they will not know whether the current stimulus is a matched or unmatched condition.

2.4. Behavioral measures

Participants will have to rate 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 will be labeled “no perception” and the higher extremity will be labelled “the most intense perception you can imagine”. The time-interval between the rating and the start of the stimulation will be variable. During the thermonociceptive stimulation, participants will be instructed to sit as still as possible to generate an artifact-free EEG signal. After the stimulation, participants will hear a beep sound which will indicate the end of the trial. Participants will then have 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).

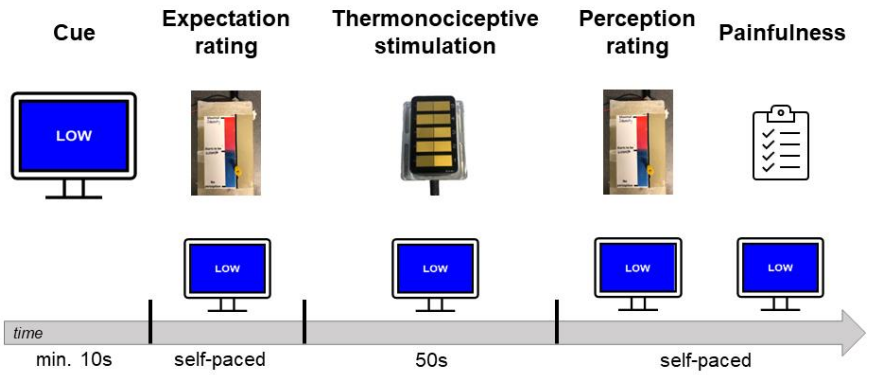

Figure 2: Trial design for one example stimulus, using a cue for a low intensity. VAS ratings will be given on a scale from min: no perception to a max: “most intense perception imaginable”. Participants will be asked to evaluate the painfulness of the stimulus with a simple yes/no answer.

2.5. Pilot Study

A behavioral pilot study was conducted to assess whether the changes in the stimulation paradigm compared to the original publication (Atlas et al., 2010) would still lead to the desired modulation of participants' pain perception. Specifically, we needed to ensure that the effect of the expectation modulation paradigm on the perceived intensity would last for the entire stimulation, which was extended from 10s in Atlas et al. to 50s in the present paradigm to accommodate for the frequency tagging approach. To achieve this, participants rated their perception of the thermonociceptive stimuli continuously *during* the application of the stimulus on the VAS, allowing to track the time course of their perception over the entire stimulation. 10 healthy participants (24.3 ± 2.9, 2 males) were recruited. The procedure of the pilot was identical to the experimental setup described previously, with the addition of the continuous rating.

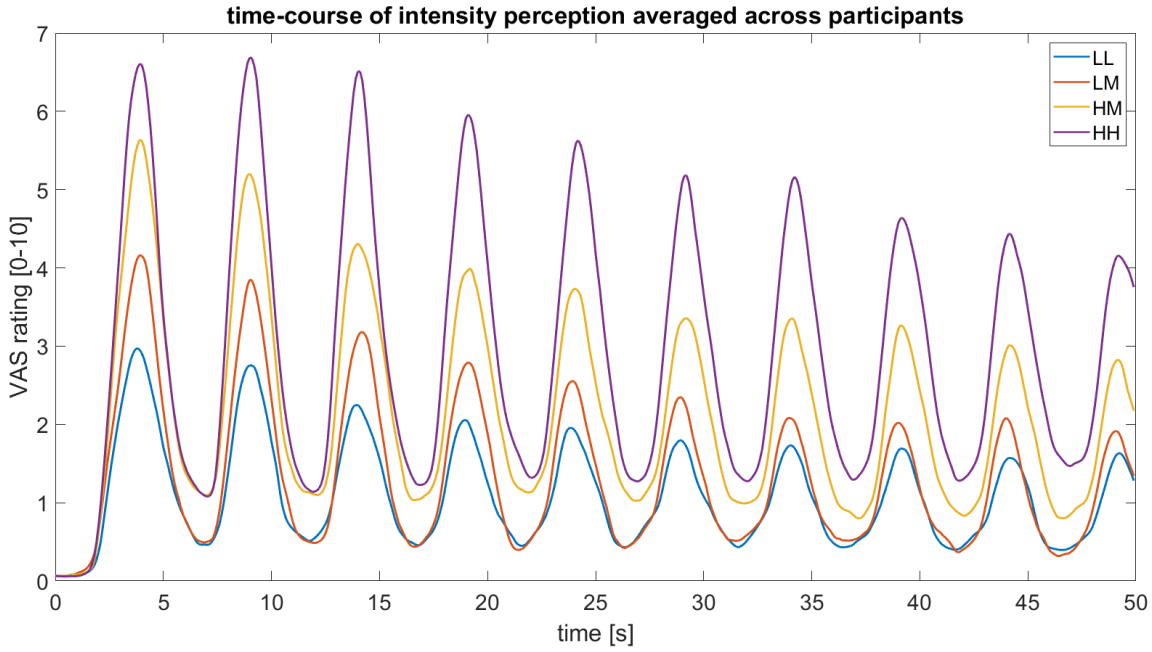

Figure 3. Average of the continuous rating across participants for each condition (LL: cue for low intensity stimulation + low stimulation temperature, LM: cue for low intensity stimulation + medium stimulation temperature, HM: cue for high intensity stimulation + medium intensity stimulation, HH: cue for high intensity stimulation + high intensity stimulation).

The results of the continuous rating were analyzed qualitatively, while the rating of perceived stimulation intensity was assessed using the LMM proposed in the main experiment (rating ~ cue*temperature+(1|subject)). The independent variables had two levels (cue: low, high and

temperature: matched, unmatched). A clear difference could be observed between all 4 conditions in the continuous ratings (Figure 3). Importantly, condition LM led to ratings that were noticeably lower compared to condition HM, despite the stimuli being delivered using the same temperature. This difference seems to persist over the course of the trial, encouraging us that this paradigm is indeed effective for the slow sustained periodic stimulation paradigm we chose instead of a short tonic heat application.

The ratings of expected and perceived intensity are shown in Figure 4. The cue appeared to be effective in influencing participants' expectation towards the following stimuli, as the cue for high intensity stimulation led to quite high expected levels of stimulus intensity, while the cue lor low intensity stimulation had the opposite effect. Additionally, the expected and perceived intensity levels are almost the same for the matched conditions (LL and HH). While the expectations did not change for the unmatched conditions (LM and HM), the perceived intensities graduated more towards the middle of the scale, reflecting that the stimulation temperature was at a medium intensity for both conditions. Yet, the most important contrast lies in the difference between the perceived stimulation intensity for LM and HM. Although the temperature of stimulation was the same for the two conditions, perceptions of intensity varied significantly (main effect of interaction: $F(227,1)=73.561$, $p<0.0001$; *pairwise comparison*, unmatched temperature high cue vs low cue $p<0.0001$) between the conditions. This is consistent with the continuous ratings and indicates that even when asked to provide a single rating describing the overall perception of the stimulation, the effect of the cue-based expectation modulation paradigm persists. These results confirm the effectiveness of the chosen paradigm to change the subjective intensity perception of the applied stimuli towards the presented cue.

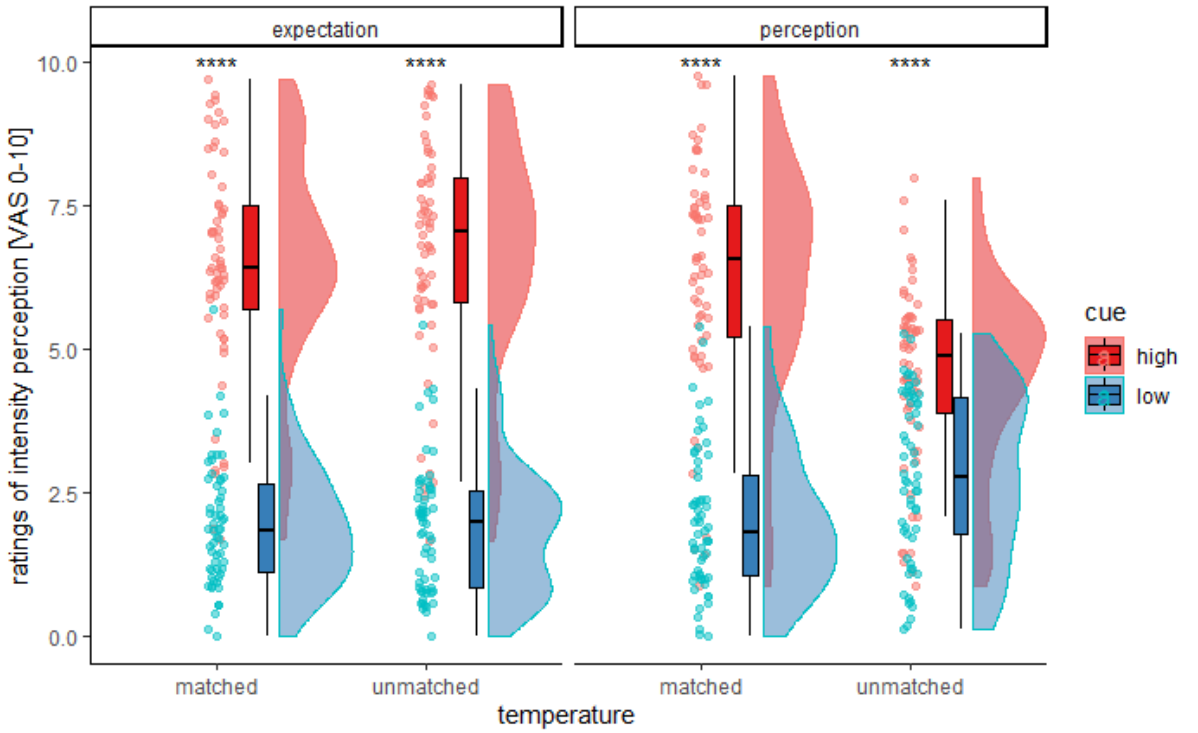

Figure 4. Averaged ratings of expected and perceived intensity of stimulation, given before and after each stimulus. The ratings were converted to a scale from 0-10 for readability. Matched conditions: low cue + low intensity stimulation, high cue + high intensity stimulation: unmatched conditions: low cue + medium intensity stimulation, high cue + medium intensity stimulation. Pairwise comparison; p<0.0001:****.

2.6. EEG recordings

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

2.7. EEG analysis

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

2.7.1. Analysis of the phase-locked response

To isolate activity related to the applied stimulus, we will make 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 ; Liberati et al., 2019 ; Mulders et al., 2020), leading to a standardized analysis approach: First, events will be created based on the triggers of the stimulation. Each trigger will receive a label according to the condition it preceded (HH, LL, HM, LM). Then, slow drift and high frequency power line noise will be removed using a Butterworth band-pass filter between 0.05 Hz and 40 Hz. Epochs will be 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 will be removed, since due to their placement on the EEG cap, they frequently only record muscular noise rather than brain activity. All signals will be re-referenced to the average of the electrode set. Then, an Independent Component Analysis (Fast ICA algorithm) (Hyvarinen & Oja, 2000) will be employed to detect artifacts due to eye movement or other muscular artifacts and remove them. The ICA will be 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 30 independent components. Therefore, the same components will be removed in each condition for each subject. Additionally, trials with an amplitude larger than ± 500 µV (Colon et al., 2014) on any of the electrodes will be excluded. Any participant with less than 5 trials at this stage will be removed from the data set. Finally, the average signal will be 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 will be 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 will 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 are summed up and the resulting amplitude

at the frequency of interest (FOI) is used for the statistical analysis. To sum up the harmonics, the signal is 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. To sum up the harmonics, the chunks will be averaged, and the resulting amplitude will be multiplied by the number of chunks that were averaged. The whole electrode set will be taken into account for this procedure.

2.7.2. Analysis of the modulation of ongoing oscillations

The analysis of the modulation of ongoing oscillations will be 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 will be additionally filtered using a 4^{th} order Butterworth filter for each frequency band after calculating the ICA and re-referencing of the electrodes in the remaining signal. Another additional step is the estimation of the envelopes of the signal, which will be computed using a Hilbert transform. The following steps are 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 is used for the statistical analysis in each frequency band and the whole electrode set is considered.

2.8. Statistical analysis

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

2.8.1. Behavioral data

To assess whether the cue will affect the rating of expected stimulus intensity, an LMM with the independent variable (IV) *cue* and dependent variable (DV) *expectation rating* will be used. The factor *subject* will account for the variation of the regression model intercept across participants and is therefore a random effect in the model. This model will be the positive control for the factor *cue*; if the cue is not effective in influencing the expected stimulus intensity, we will have to assume that the cue-based expectation modulation paradigm in this experiment failed.

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

2.8.2. Periodic response

To assess whether the amplitude at the FOI will be significantly different from zero, a right tailed multi-sensor cluster-based permutation test using Wilcoxon signed-rank test as test statistic will be used. To do this, for each condition, the corresponding data will be merged in to one file, containing all participants. The test will compare 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 will also be set to 0.0125, and 2000 permutations will be computed. The multi-sensor analysis will be 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 will allow 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 ; Mulders et al., 2020) we expect a periodic response in the EEG signal elicited by the sustained periodic stimulation significantly larger than zero. If the periodic response at the FOI is not significantly greater than zero for any of the electrodes, we will have to assume that we were unable to induce a periodic modulation of the EEG signal and the data will therefore be unusable since the basic premise of the investigation will not be reached.

To investigate whether the high intensity cue paired with the medium intensity stimulation (HM) will 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 will use a LMM with the following factors: stimulation *temperature* and *cue* as independent (fixed) variables (IV) with an interaction, while *subject* is a random factor. The *amplitude* at the FOI is used as dependent variable (DV).

2.8.3. Modulation of ongoing oscillations

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

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

We hypothesize that the amplitude at the FOI will be larger if the medium intensity stimulation is preceded by a high intensity cue (HM) compared to a low intensity cue (LM). If this is the case and the cue-based expectation modulation will change intensity perception in the same

direction, these results would suggest that the modulation of ongoing oscillation could be functionally related to pain perception.

2.8.4. Post-hoc Bayesian interference

Negative results for hypotheses 4 will be further analyzed on their validity using a Bayesian interference approach. This is used to assess whether the null hypothesis was rightfully rejected. All analysis will be conducted using the "BayesFactor" package (Morey et al., 2015) in R. Default parameter values will be used. We will test the association between H_0 (LMM including the interaction between cue and temperature) and H_1 (same model, without interaction). The ratio between these two models will be expressed in a Bayes Factor (BF_{10}). This factor will then be interpreted based on the interpretation table proposed by Lee and Wagenmakers (2013).

2.8.5. Outliers

Any participant unable to complete data acquisition will be excluded from the analysis. Further, any data points that violate the LMM assumptions after fitting the LMM will be removed from the data set. They will be identified using a Shapiro-Wilk test to test the normal distribution of the data as well as a Levene's test, assessing the data set for homoscedasticity. In case the data does not conform to normality, a log-transform will be 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 will still violates any of the assumptions after the transformation or disproportionately affects the dataset after fitting the LMM will be removed from the data set and will not be replaced. This will lead to the exclusion of this participant from the analysis. 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 will be 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 will be removed from the data set. Cook's distance will be calculated for each datapoint within a condition. Thus, for each condition and frequency band, a separate calculation will be done.

2.9. Hypotheses table

Question	Hypothesis	Sampling plan (power analysis)	Analysis Plan	Interpretation given different outcomes
Behavioural response				
(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 η^2=0.058.	rating_expected = cue + (1|subject) - DV: expected *intensity rating* - IV: *cue* - random coefficient: *subject*	*Positive control:* If the rating matches our expectations, we confirm that the cue is influencing pain expectations as intended. If not, the experiment cannot be used.
(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 η^2=0.058.	rating_perceived = temperature * cue + (1|subject) - DV: perceived *intensity rating* - IV: *temperature, cue* - random coefficient: *subject*	*Positive control:* 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.
Time locked, phase-locked response				
(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 η^2=0.083.	Multi-sensor cluster-based permutation Wilcoxon signed-rank test of aggregated and averaged amplitudes at FOIs.	*Positive control:* A periodic response shows that the stimulation paradigm induces the expected neural activity. If this is not the case, the basic assumption for the frequency-tagging approach used in this investigation is not met. *
(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 0.98 with an alpha level of 0.0502, 30 40 participants will be enrolled. Calculations are based on power simulations using the simr	amplitude_FOI = temperature * cue + (1|subject) - DV: *amplitude* at the FOI - IV: *temperature, cue* - random coefficient: *subject*	A change in modulation congruent to the change in intensity perception would reveal a possible connection between perceived pain and ongoing oscillations. A non-corresponding change would indicate that ongoing oscillations might not be related to pain perception. This

		package in R (Green et al., 2016). This sample size will allow us to detect an effect around η^2_p =0.09 ~~See above~~		interpretation will be tested using a post-hoc Bayesian interference analysis.*
Time locked, non-phase locked response				
(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 η^2 =0.083.	Multi-sensor ~~C~~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).*
(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 η^2_p=0.09	amplitude_FOI_OO† = temperature * cue + (1|subject) - DV: *amplitude* at the FOI for each frequency band - IV: *temperature, cue* - random coefficient: *subject* † 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. If the amplitudes are not modulated by the change in cue, it would indicate that the modulation of ongoing oscillations and pain perception might not functionally connected. This interpretation will be tested using a post-hoc Bayesian interference analysis.*
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). *: As we were not able to recruit for the sample size that would 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. Due to the limited sample size, it is possible that we will miss a potentially small effect. Post-hoc analyses will be used to help uncover whether this is the case.				

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Supplementary Materials

I. 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), but effect sizes or power calculations were rarely mentioned.

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 ~ 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 η^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 section 2.5). 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. This sample size will give us the power to detect an estimated effect size of η^2_p =0.09 for the interaction between cue and temperature in the phase-locked response.

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).