Markus Ploner: The proposed study aims to investigate the relationship between ongoing oscillations in the brain and the perception of pain. To this end, the authors propose a paradigm in which pain is modulated by changing noxious stimulus intensity and expectations of upcoming pain in 30 healthy human participants. Expectations will be modulated by presenting visual cues indicating upcoming pain intensity.

The study is well-planned, and the manuscript is mostly clear and convincing. However, it might benefit from clarifications and added details:

We appreciate your insightful comments and suggestions, which have greatly contributed to improving the quality of our manuscript. We have carefully considered each of your points and have made the necessary revisions to address them. We would like to respond to your specific concerns as follows:

1. **Framework.** The proposed study aims to investigate the relationship between ongoing oscillations and pain perception. To this end, they propose a cue-based expectation paradigm to modulate pain. However, there are numerous possibilities to modulate pain. They might explain why they will particularly use expectation to modulate pain. Moreover, they might consider recent EEG studies on expectation effects on pain (Bott et al., 2023; Strube et al., 2023).

We agree with you that there are multiple ways to change pain perception. Taking this into account, we created a bundle of investigations, which all use different “modes” to change pain perception (i.e., modulating attention, expectation, and stimulus saliency). In this bundle, we aim to investigate both cognitive top-down factors as well as bottom-up modulations of pain perception. These investigations will complement each other in the search for a relationship between changes in pain perception and the modulation of ongoing oscillations. Therefore, we are not interested in the mechanisms behind the expectation effect specifically, but we rather use it as a tool to change pain perception in a controlled manner.

We thank you for mentioning the two recent investigations and have taken them into consideration for the introduction (p. 4).

2. **Hypotheses.** The authors should specify whether the hypotheses on the relationships between ongoing oscillations and pain perception are directed or undirected, i.e. do they expect positive or negative relationships for the different frequency bands?

We expect to observe a positive relationship between perception and modulation of oscillations at the frequency of interest. This means that if the pain perception increases, we expect to see a larger amplitude at the frequency of interest (i.e., change in modulations of oscillations congruent to the change in pain perception, as described on p. 5). Further, we expect to see similar modulations in all frequency bands. We are aware that differences in modulation of the different frequency bands have been shown in the time domain (event-related de-/synchronization) of the alpha and beta, and theta and gamma band, respectively. Yet, these findings do not directly translate into the analysis using FT-OO, in which we analyze the signal in the frequency domain in a non-phase-locked manner. Nevertheless, because we believe that investigating the direction of the modulation of the oscillations of interest can help us better characterize these activities, we also plan to perform additional analyses.
in the time domain on the signal averaged across stimulation cycles as in Mulders et al. (2020). As these analyses are exploratory and will be performed post hoc, we will currently not include them in the Stage 1 of our Registered Report.

3. **Participants. The authors should describe their sampling strategy and the inclusion and exclusion criteria in more detail. How will they recruit participants? Will they perform convenience sampling? How are gender and ethnicity accounted for?**

Participants will be recruited via social media, as well as posters on campus and word-of-mouth. We do not plan to include a convenience sampling, as long as participants adhere to the exclusion criteria. We aim to recruit a balanced sample in terms of gender. Regarding ethnicity, we do not expect differences between participants of different ethnicities. Moreover, since most participants will be students of the UCLouvain campus in Brussels we can expect to recruit a rather diverse group of ethnicities, reflecting the population of the city. Overall, this should lead to generalizable results. This is now detailed on p. 5.

4. **Experimental procedure. The paradigm should be specified in sufficient detail to replicate the findings. However, essential information is lacking. What will be the stimulation site? What will the latencies be between visual cue and expectation rating, expectation ratings and thermal stimulation, and between thermal stimulation, auditory cue, and pain intensity ratings? A figure detailing the paradigm might be helpful.**

The thermode delivering the thermonociceptive stimulation will be placed on the volar forearm of the dominant arm of the participant, as now specified on p. 8. The latencies between the different parts of the experiment are self-paced by the experimenter. Prompts to rate the expected and experienced intensity ratings will be given after participants had time to look at the cue and after the end of the stimulation, respectively. A figure was added to the manuscript illustrating the paradigm (p. 10).

5. **Behavioral measures. The rating scales and their anchoring should be detailed and explained. The rating scale assesses the intensity of general perception rather than the intensity of pain. As the study aims at investigating brain-pain relationships a rating scale assessing pain intensity might be more appropriate.**

We agree that differentiating between pain and intensity is indeed a rather complex issue. Here we chose to use a scale of intensity, since the low intensity stimulation trials are likely to be perceived as not painful and would lead to ratings of zero on a scale of pain. Nevertheless, we assume a linear relationship between perceived intensity and pain for the trials that are perceived as painful. A similar scale of intensity (and not pain) was used in previous experiments that involved both painful and non-painful stimuli (Horing et al., 2019; Hu et al., 2015; Liberati et al., 2020; Mulders et al., 2020).

6. **Specificity. Pain is associated with many different perceptual, cognitive, emotional, and physiological processes which are not specific to pain. Thus, relationships between pain and...**
brain activity can equally well reflect other pain-associated but not pain-specific processes. Studies investigating brain-pain relationships therefore often contain control conditions. The authors might explain why the proposed study does not include a control condition such as non-painful thermal stimulation, as their previous studies did.

We agree with the you that it is indeed difficult to dissociate pain from the mentioned processes. Importantly, in this investigation, we do not claim pain-specificity of our intervention. To clarify this, we readapted the wording in the introduction (p.5). In fact, we agree with the perspective that demonstrating that a neural response is pain-specific requires testing a very large spectrum of possible stimuli (Mouraux & Iannetti, 2018), i.e. to disentangle the effects of painfulness, intensity, saliency, processing of thermal information, activation of the spinothalamic system, etc. Because the current investigation protocol is already quite lengthy, we decided to not include other modalities of stimulation as a comparison. Nevertheless, it is important to point out that our aim is not so much to identify a response that is specific to pain, but to shed light on the association between the modulation of pain intensity and ongoing neural oscillations.

7. Blinding. The authors should specify whether the experimenters will be blinded during recordings and analyses.

The experiment will be blinded during the recording, since the person applying the thermonociceptive stimuli does not know which condition is applied (matched/unmatched). This is now specified on p. 9. Furthermore, a pre-defined analysis pipeline has been implemented to prevent any bias. This analysis pipeline is already accessible in the OSF repository associated with this Registered Report (https://osf.io/9ud7x/), which is now mentioned in the manuscript on p. 5.

8. Analysis. The procedure resulting in “aggregated amplitudes” should be specified in more detail.

To aggregate the amplitude at the frequency of stimulation and its harmonics, the EEG signal in the frequency domain will be cut into slices of 0.2 Hz (= frequency of stimulation), beginning at 0.1 Hz after the onset of the stimulation. This creates 2558 slices with a length of 0.2Hz, all with the expected peak in the middle of the slice. These slices will then be averaged, and the resulting amplitude multiplied by the number of slices to sum up all the harmonics. This procedure is now explained in more detail on p. 14.

9. Negative findings. In the design table, questions 4 and 6 are most important. It is specified that negative findings would mean that ongoing oscillations might not be related to pain perception. This is a rather vague interpretation. The authors might think about clearer interpretations of negative findings. Using Bayesian rather than frequentist statistics might help with the interpretation of negative findings.

We agree with your comment. To get more insight into potential negative findings, we will apply a Bayesian interference approach in which we will compare H₀ (the model used in the main analysis, including the interaction term) to a model that only includes the main and random effects, but no interactions (H₁). The ratio between these two models expressed in a Bayes Factor (BF₁₀) will be used
to assess the validity of the previous rejection of $H_0$ using the frequentist approach. The interpretation of the $B_{10}$ will be based on the interpretation table proposed by Lee and Wagenmakers (2013).

These adaptations are mentioned in the manuscript in the analysis table (pp. 19-20) as well as in the main text on p.17.

10. **Code/data sharing.** The authors should specify whether they will share the data and the codes for stimulations and analyses. If they share, it should be specified where code and data will be available. If they do not share, this should be justified.

All original data sets will be publicly available on the Harvard Dataverse domain. All pre-processing and analysis codes will be / are shared in the OSF repository associated with this Registered Report (https://osf.io/9ud7x/). These details were added to the Stage 1 manuscript in the beginning of the Methods section (p. 5).

11. **References.** For some details, the authors refer to a study under review (Leu et al., 2023). As this information is not available so far, the authors should provide the details in the current manuscript rather than referring to unpublished manuscripts.

We agree that it is not ideal to refer to an unpublished study. We have therefore removed this reference in the manuscript and will add it in the Stage 2 manuscript if the paper has been published by then (currently under stage 2 review at Cortex, the stage 1 manuscript is available in the OSF repository under the following link: https://osf.io/738uq). Additionally, we added a description of the aggregation method the reference was referring to on p. 14 (see also response to point 8 of this review).

12. **Errors.** In the design table, the DV for the first question should likely be the expectation rating rather than the perceived intensity rating. On p.15, third paragraph, amplitude is likely the DV rather than the IV.

We thank you for your attention to detail and have corrected the errors.
Zoltan Dienes: The paper proposes to investigate the neural oscillatory correlates of pain perception by creating conditions in which the physical stimulation is the same but cyclic changes in pain perception are different: The same medium stimulation creating the perception of relatively high vs low pain, based on expectation differences.

We would like to thank the Reviewer for his detailed feedback on the statistical analysis proposed in our manuscript. We hope that by improving on the raised points we made our statistical methods more rigorous and reproducible.

1. **I did wonder if any result that did emerge would be a reflection of specifically pain perception rather than say a difference between intense vs weak sensation more generally, or more strongly attended vs somewhat less strongly attended stimuli. This is an issue the discussion could address in the final Stage 2 - unless there is a quick answer that could be given in the introduction.**

As pointed out by the Reviewer, disentangling pain perception from attention and other cognitive processes is complex. In this investigation, we do not claim to investigate a primarily pain-specific phenomenon. More so, we are aiming to change pain perception to observe a possible relationship with the modulations of ongoing oscillations. How exactly we change the perception of pain is at this point of secondary concern, whether it is solely the effect of expectation, or additional effects of attention are mixed in should not change the validity of our results. Indeed, this investigation is part of a larger project in which we are using different ways to modulate pain perception (i.e., by modulating attentional states or the saliency of the applied stimulus) and assess the effect these changes have on the modulations of ongoing oscillations. We added a short disclaimer in the introduction which clarifies that we are not aiming to observe pain-specific effect, but rather pain-associated ones (p. 5).

2. **Not being an EEG expert I will comment on the statistics, and specifically the power calculation. Power is one means by which a justification could be given for why a non-significant result should be taken seriously. Or to put it another way, if one is to use frequentist hypothesis testing, power needs to be calculated in such a way that a non-significant result could be taken seriously. The aim of power is to control the long term risk of missing an effect of interest. That is, one should ensure power is calculated with respect to any effect that could be of interest. That is, it should be calculated with respect a roughly minimal interesting effect size. Thus, PCI RR guidelines say "power analysis should be based on the lowest available or meaningful estimate of the effect size." Some thoughts here may be useful: [https://doi.org/10.1525/collabra.28202].**

As far as I can make out, the authors used a value for relevant parameters based on a past paper. BTW the authors do not provide enough information to reproduce their calculations - please provide exact numbers with justification why they were chosen in particular. The value obtained in a past paper does not define the value that one is prepared to miss out on. Presumably an effect half the size found previously would still be of theoretic interest - and one wouldn't want to miss out on it.
Power must be calculated for each test in the Design Template separately, with due sensitivity to the nature of that DV.

Take the predicted effect of perceived pain on the modulation of neural oscillations. The extent of the modulation must depend on the extent of the perceived pain difference. There are data that indicate what the modulation is estimated to be for a particular known pain perception difference, based on past work; in the simplest case of one such study, one could draw a line from that point of oscillation modulation vs pain difference to (0,0). The authors have from their pilot an estimate of the pain difference they are likely to obtain. So the degree of modulation, in raw units, can be estimated for the pain difference they are likely to obtain. But what we want is the roughly smallest possible difference. So put an 80% CI on the estimated modulation in the first step from a past paper, and repeat the procedure, drawing a line from there to (0,0).

Numbers of trials will affect the population by-participants Cohen’s d. Make sure when using past studies one takes into account any difference in quantity of data used in the previous and current study (durations over which data are collected, number of trials).

I know this is FAR more work than is usually done in non-RRs. But RRs are an opportunity to tighten up on our scientific inference, so we have a chain of inference that actually holds together, at least roughly.

We thank the Reviewer for this in-depth discussion of the problems associated with the estimation of sample sizes. We indeed based our model on values of previous studies. Here it should be mentioned that we created a random dataset based on means and standard deviations of those previous studies, and then used those to calculate the LMM.

After reading the mentioned literature, we adapted our model as follows: The data of Mulders et al. (2020) was used to compute a linear mixed model that resembled ours. The publication of Mulders and colleagues 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. We simulated the linear mixed model based on the mean and standard deviations obtained in the alpha frequency band for the stimulation using a small variable surface of stimulation (equaling our HH condition) and a small fixed surface of stimulation (equaling our LL condition). The values for the medium intensity conditions (HM, LM) were estimated based on the difference in rating between these conditions that we observed in our behavioral pilot study (18%). 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 amplitudes at the frequency of stimulation and vice versa.

Importantly, by basing our power on the interaction effect in the modulation of ongoing oscillations, we are already calculating the sample size estimation based on the comparison with the smallest effect, as modulations of ongoing oscillations generally don’t lead to large amplitudes even without cognitive interventions, and these amplitudes are always much smaller than the ones of phase-locked responses (Colon et al., 2017; Mulders et al., 2020). Additionally, since we are using a much larger probe (full surface: 9 cm\(^2\), ~181 mm\(^2\) for each of the 5 stimulation zones) than it was used in the
investigation by Mulders and colleagues (~24 mm² per stimulation zone, two zones used to elicit the responses used in the simulation), and in its full surface, we can expect to elicit larger amplitudes due to spatial summation. We therefore consider the values used in the simulation of the LMM to reflect the smallest effects we could obtain. The simulated LMM was used to simulate a sample size using rather conservative parameters for power threshold (0.9) and alpha level (0.02). Thus, the sampling size simulation resulted in a recommendation to recruit 40 subjects to reach the specified statistical power. To account for potential dropouts (due to e.g., artifacts in the signal or non-completion of the experiment), we will recruit 43 participants in total. We have added all necessary parameters and values to the main manuscript to ensure the reproducibility of our sample size simulation (pp. 6-7).

To put these sample size into a context of previous investigations. We would like to mention that 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). Our sample size therefore seems to be adequate for the planned investigation.

3. p 14 "a right tailed multi-sensor cluster-based permutation test using Wilcoxon signed-rank test as test statistic will be used". Can a reference be given for why this controls for multiple testing? Also describe, or give a reference for the exact procedure.

The Wilcoxon signed-rank test allows for non-normality of the data. Further we will account for potential type I error inflations due to the 64 comparisons against 0 by applying a Bonferroni correction. Therefore, the standard alpha of 0.05 is divided by the number of conditions (4) which we are comparing (Bland & Altman, 1995). Thus, we will apply an alpha level of 0.0125, now specified on p. 16.

4. “taking potential type II error inflation due to multiple testing into account.”. Did you mean Type I?

We apologize for the typographical error in the previous version of our manuscript, and we have adapted it accordingly.

5. “A separate LMM is calculated for the amplitude at the FOI in each frequency band.” How will familywise error be controlled? Note: Power must be determined given the family wise error correction used.

Since, for each frequency band, a different amplitude at the frequency of interest will be extracted, we assume it will not be necessary to control for a family-wise error.
6. "normality and linearity will be assessed visually". As this will be done once the data are collected, it allows analytic flexibility - choices could be made based on the p-values obtained. Could a blind analysis procedure be used? (That is, the condition labelling is removed or scrambled and the data with IV information removed given to an analyst just to make this decision).

We agree with the Reviewer that the proposed visual assessment of the LMM assumptions is not an optimal approach. To avoid any bias, we propose to use objective measures instead, specifically a Shapiro-Wilk test to test the normal distribution of the data set as well as a Levene’s test to test for heteroscedasticity (specified on pp. 17-18).

7. How often do the MLMs fail to converge with this sort of data? Make sure there is no analytic flexibility left over here: Describe how convergence will be ensured without analytic flexibility.

So far, this problem has never occurred using similar LMMs and data sets, partially because the models are rather simple (Leu et al., 2023 (under review); Liberati et al., 2019; Liberati et al., 2020; Mulders et al., 2020). Frequently, the problem of convergence arises in models with relatively small data sets (>50 sampling units) (Maas & Hox, 2004, 2005), which is not the case for the LMMs used in this investigation. To nevertheless ensure that there is no flexibility left in the analysis of the LMM, we will ensure convergence by manually increasing the number of fitting iterations.
**Chris Chambers:** This is a promising proposal to investigate the causal relationship between pain perception and neural oscillations. I particularly appreciated the interventional nature of the design, which stands in contrast to the predominance of studies that focus on correlations between behaviour and oscillations. The method is generally strong (with appropriate inclusion of pilot data to validate the primary methodology). I have a few comments/suggestions for the authors to consider in revision:

We appreciate the time and effort that you put in this response and are grateful for the insightful comments and constructive suggestions. We have carefully considered your feedback and hope that our point-by-point response will address your concerns.

1. **The design is tight but I wonder about the issue of functional specificity and, in particular, whether factors other than pain perception could explain any observed modulation of oscillatory activity.** For instance, could any change in oscillatory activity between LM vs HM reflect greater attention to the stimulus in the HM condition rather than greater perception of pain? One way to address this would be to insert some kind of additional stimulus into the pain-eliciting stimulus on 50% of trials (such as a short temporal gap or other transient) and include an attentional control task that, on some trials, requires the participant to decide whether the transient is present or absent (rather than making a pain judgement). By titrating the transient to a threshold level of detectability, you could determine whether the cue alters detection sensitivity, and thus whether attentional effects are likely to be mixed in with pain perception. If you then found evidence of no effect of the cue on detection sensitivity it would strengthen the causal link between oscillatory changes and pain perception, independently of attention. I suggest this merely as an option for the authors to consider at a conceptual level rather than a concrete design change (as the authors may have better ideas, or there may be valid reasons to discount this issue). Any changes to the design would require careful piloting.

We thank you for this thoughtful suggestion. We agree that we cannot completely exclude that a change in oscillatory activity might at least partially reflect greater attention to the stimulus rather than pain per se. Indeed, pain is intrinsically attention-grabbing and disentangling the two factors is not trivial. However, if the modulation of oscillations were related mostly to attention and not to perceived intensity, we would expect HH and HM to exert the same amount of modulation – as both are associated to a “high” cue – and we would expect LL and LM to exert the same amount of modulation – being both associated to a “low” cue. If the modulations exerted by HH and HM (and by LL and LM) are still differentiable, then we can exclude that these activities are predominantly attention-related.

Crucially, we would like to emphasize that we do not want to claim specificity of any observed effect to pain, but rather investigate the relationship between pain and neural oscillations. To this end, if attentional processes are also involved in the changes in perception, it should not hinder our conclusion.

2. **There are various points where additional methodological detail is needed to ensure that the methods are computationally reproducible and close of potential (inadvertent) researcher degrees of freedom.**
(a) *Independent Component Analysis (Fast ICA algorithm) -- please specify in advance all parameters for this analysis, and for all other EEG analysis steps that refer to general procedures. A Stage 1 RR must be computationally reproducible even when it refers to previous methods. Ideally an analysis script should be included as part of the submission.*

We thank you for pointing out that the parameters of analysis need to be specified in more detail to ensure reproducibility. The description of the ICA analysis has been updated accordingly (p. 13). The analysis script can be found in the OSF repository associated with this Registered Report ([https://osf.io/9ud7y/](https://osf.io/9ud7y/)) (specified in the manuscript on p. 5).

(b) *Outlier exclusion: visual exclusion is bias-prone unless done very rigorously using blinded analysts. Can it not be done using objective criteria?*

We thank you for pointing this out and have added objective criteria (a Shapiro-wilk test for normality and Levene’s test to test for homoscedasticity) to assess the data for potential outliers (pp. 17-18).

"Any data point that will still violate normality or linearity after the transformation or disproportionately affects the dataset after fitting the LMM will be removed from the data set and will not be replaced." Does this apply to data within participants? If so, how much of a dataset must be lost before the participant is excluded? Presumably excluded participants will be replaced to ensure that the minimum sample size is met?

If any single data point of a subject is identified as an outlier for a LMM, the subject will be completely removed from this specific model. To ensure that the sample size will still be met, we specified that we would recruit additional participants to the sample size specified in our calculations. Based on previous results from the lab we don’t expect to remove more than 10% of outliers within our sample. The Outlier section has been updated to clarify this (p. 18).

"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." Can the authors specify within which cells of the design these tests will be applied? Outlier exclusion can be applied in many different ways (collapsing across conditions or within the most specific cells -- please be specific)

This test will be applied across conditions, but separately for each LMM analysis.

3. **Minor points**

p11: "These results prove the effectiveness of the chosen paradigm to change the subjective intensity perception of the applied stimuli towards the presented cue." Suggest replacing "prove" with "confirm".

We agree with you that a more cautious phrasing would be better and have adapted the wording accordingly.
If the authors are able to increase the sample size to achieve power of 0.9 (rather than 0.8), it would open up the possibility of PLOS Biology being interested in this article as a PCI RR-interested journal (since they set a minimum 0.9 power requirement). In addition, if they increase power to 0.9 and decrease alpha to .02, it will release Cortex as a PCI RR-friendly outlet (see details here). I mention this for information only, as I appreciate the authors may face resource restrictions that prevent the necessary increase in sample size that would be required in each case, and the a priori evidence strength is (in my view) otherwise sufficient for PCI RR.

We thank you for providing us with this information. We adapted our sample size calculation to achieve a power of 0.9 using an alpha of 0.02 (see p. 8). These stricter guidelines should make it easier to find an appropriate journal for publication after Stage 2 reviews.
References


