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Nam Nguyen, Ph.D.
Review Editor
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Contact
Philipp Taesler

September, 20th, 2016

Revision of JoVE55228

Dear Dr. Nguyen,

Thank you for your response dated September, 6th, 2016. We are grateful for the opportunity to revise our manuscript and your as well as the reviewers' constructive feedback. We took care to address all the concerns expressed and have carefully proofread the revised manuscript as per your instruction. Attached are detailed responses to the comments we received. We hope that our changes have substantially improved the manuscript. We have tracked all the changes in the revised document.

Kind regards

Philipp Taesler

Zertifikat Nr. QS-6568HH
und EM-8126HH



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Editorial comments:*1. Please abbreviate all journal titles.*

We have updated the citation style to use abbreviated journal names throughout the bibliography.

2. Please remove references from the Abstracts.

We have removed the references from the short and long abstracts now.

3. [...] We ask that you please reduce the number of instances of “QUEST” within your text. The term may be introduced but please use it infrequently and when directly relevant. Otherwise, please refer to the term using generic language.

We have taken care to use the term QUEST as sparsely as possible. We have used “estimation procedure” or “algorithm” to refer to the procedure, where possible.

4. Formatting: References – Please include DOI where available.

Our citation style has been updated to include DOIs. We have also added DOIs to our citation database where available.

5. Visualization: It is unclear whether Section 5 is performed in a GUI. Please include a single representative screen shot of software use to confirm. This should be submitted as a supplemental file. If this section consists of modifying code or scripting, it should not be highlighted for filming.

In the revised manuscript, we have removed the highlighting for filming from section 5. Instead, we have uploaded an animation detailing the estimation procedure, also in part to respond to the comments from reviewer #2, and explain the process in more technical detail. This animation could be incorporated into the video or supplied as supplemental material as per your preference.

*6. Additional detail is required:**-3.6 – How does one enable this?*

We have added a description of the button location on the stimulator (lines 164 & 183).

-4.1 – How is rating performed? Is the participant using a touch screen?

We have described the rating in more detail, it is performed using a standard computer mouse (line 188).

*-5.1 – How does one create QUEST sessions in the software?**-5.2 – How is this done in the software?**-5.3 – Where in the software is the intensity selected?**-5.4 – How is this done?**-5.5 – Where is this function found in the software?**-5.7 – How is this done in the software?*

We have added a schematic of the scripting/software process in supplemental materials (S1). We hope this will enable your readers to recreate the rating using the standard tools cited in the appropriate sections.

7. Discussion: Please discuss the future applications of the protocol.

To highlight future applications, we have added a concluding paragraph at the end of the discussion section (lines 399-403).

Reviewers' comments:**Reviewer #1:**

We thank Reviewer #1 for the time spent reviewing our manuscript and the constructive and positive feedback. We hope that our changes to the manuscript will address all of the expressed concerns.

Major Concerns:

-I do not agree with the authors about that there is a necessity to match the subjectively perceived pain intensity. For the investigation of a subject's pain sensitivity (between subjects analysis; citations 2 and 3) an identical stimulus is required. Some participants a more pain sensitive a show higher brain activity to the very same physical stimulus intensity. The necessity in these cases is to keep the stimulus intensity stable across subjects.

We agree with reviewer #1 on this, the phrasing in the original manuscript was not reflecting this. As we regularly use threshold paradigms, we are used to presenting subjectively identical stimuli tailored to individual thresholds and seem to have generalized to the processing stage absent mindedly. We rephrased the paragraph to reflect that there might be experimental settings, where the subjective perception is the quality which is sought to be kept constant by design (lines 54-57), for example when trying to disentangle changes in sensory input from purely perceptual decision processes.

-A major issue when applying pain stimuli to healthy participants is that they are not used to this type of stimulation. Before using any pain ratings for determining the experimental pain intensity, the subjects should be allowed to establish a more or less reliable pain scale for these stimuli. In my experience the participants struggle to recognise the difference between 4 and 5 on the numerical rating scale. This should be included in the procedure.

Thank you for point this out. We have updated the paragraph detailing the pre-thresholding “familiarization” stage to reflect this. We also tried to stress, that a wide variety of intensities should be presented (repeatedly) to allow the participants to evaluate the rating range (lines 194-207). The two-staged process we describe, where the familiarization stage also serves (informally) for the experimenter to get a rather coarse starting range for the two QUEST runs, is in our experience well suited to accomplish both. The estimation is rather robust, as long as the starting points are reasonably well within the high/low are of the participant’s sensory continuum. We also added encouragement to query the participant verbally of his/her use of the rating scale (line 199).

Minor Concerns:

-I have my concerns about the scale which is somatosensory on the left side and nociceptive on the right. I am aware that there's is no other solution for that but the authors should discuss this as potential issue for the algorithm.

This is an important hint, thank you. We have mentioned this in the appropriate section (lines 353-361) and have given recommendations to rephrase the testing as a simple “yes/no” two-alternative forced choice task, wherever necessary, when more detailed ratings are not necessary for further analysis.

-An alternative method to determine the best stimulus intensity would be a simple regression approach. To determine a subjective "5", a number of different stimuli could be applied that also include some extreme ratings (2, 3 or 8 or even 9). The authors may show the superiority of the QUEST method compared to this rather simple regression.

This is a very good comment, thank you. We had initially tried to use regression in the first stage of the task to gauge, where good starting points for QUEST would be. Sadly, we did not save the data for these estimations, and we did not compare the reliability of the regression estimates with the QUEST estimate over the course of the experiment (since we would have had to stimulate at different intensities twice, then). We have, however, tried to give intuitive reasons, why a point estimate by QUEST might be more robust for a single intensity level than a regression or function fitting approach (lines 67-71 and 99-104).

-What is meant with "suppressing sensory components" and "sensory EEG activity can be factored out"? Does that refer to cortical processes or does that also include any variability in the periphery.

This question points out a flaw in the phrasing of the original manuscript, thanks! We have taken care to make this section more concise. The intended meaning was in fact the latter one, i.e. that the external input is kept constant (i.e. a constant stimulus of known subjective intensity), so that variability can be attributed to both, peripheral and central variability. Our aim here was to make a distinction between external and internal processes, not between internal peripheral and central processes. We have updated the phrasing to better reflect this (lines 113-155 and 212-222).

-There are further studies by Gross/Ploner et al. (Plos Biol, PNAS) who also used stimuli at the pain threshold. They should be cited as well.

We have now included citations for the Plos. Biol. And PNAS papers (citations 10 & 11).

-Did the authors see some habituation or sensitisation in their data. The repeated stimulation may shift the pain threshold. However, this may only apply to higher stimulus intensities such as the subjective "5". For this and the other reason mentioned above, it would be extremely valuable for other researchers to include a thresholding at "5".

To answer this, we have been very pleased with the stability of the threshold estimate in the present experiment. Figure 2 shows that the ratings are stable across testing blocks and show no general tendency of habituation or sensitization over time. We agree with reviewer #1 that this is probably because we stimulated at the pain threshold. Unfortunately, we do not have data for higher level stimulations e.g. as the pain scale midpoint of 5. However, we have added a paragraph to point out potential problems in thresholding higher intensities (lines 342-346).

Additional Comments to Authors:

-The QUEST procedure is not just suitable for determining the threshold. Stimuli at the pain threshold haven't been used that often. Most researchers use the subjective "5" (out of 10; or 50 out of 100) for painful stimulation. It might be interesting for these readers to see a further example with that example (optional).

We agree with reviewer #1, as stated above, however, we don't have data to show this empirically. In the revised manuscript, we have, however, stressed that the QUEST estimation is not limited to the threshold, but works for other intensities as well (lines 64, 102, 342-344).

-Representative results: It would be interesting to see whether the authors could replicate the findings by Gross et al. (Plos Biol, 2007), who found differences in the gamma range but no differences for the low-frequency evoked activity. In my view, these post-stimulus activities are more interesting and could be included in the manuscript (particularly if the results differ). The authors should also include the separate topographies for the conditions (pain/no-pain).

We completely agree with reviewer #1, although we also think that since the pre-stimulus time range has traditionally been mostly used as a baseline period, it might have been under-researched. Hence our specific interest in this time-period. We think it is important to screen the ongoing activity before stimulus onset for systematic influence on subsequent processing stages, especially, since using baselines makes the assumption, that activity in these pre-stimulus time ranges is purely random. That said, we are also very eager to find out, whether our data shows the same patterns as described in Gross et al. (2007). Since the policy on the scope of results is rather restrictive in JoVE due to the method focus, we have only included our primary results – which the whole study design and data preprocessing was aligned with. The data we collected has been skewed towards a long, clean, artifact free pre-stimulus time range, and we only have about 0.5s of mid-quality data after stimulus onset, since participants knew, that after the stimulus, blinking, getting ready for the rating etc. was not as heavily discouraged as during the pre-stimulus time range. Thus our data is not best suited for a full featured analysis of the stimulus processing stage. However, we have included a somewhat similar (albeit superficial – i.e. no spatial filtering/source-space) analysis in the supplemental material (S3), and hope, that this placement will not displease reviewer #1, it is in no way meant to. On the contrary, we will take great care in future studies to obtain clean post-stimulus data, in the hope of integrating it with the results of Gross et al. and other post-stimulus literature, possibly in a way more suitable for an extended synthesis of conclusions.

-The authors mention the importance of optimally adjusting the parameters for the fitting procedure. The author should also give some advice for the adjustment and should also include a "bad" or implausible example other readers can learn from.

Thank you for pointing out this important omission. We have put more information on this in the notes in section 5 (lines 225-229). Also, we have reviewed the examples for the parameters given in the beginning of this section for accuracy, and added a reminder in the discussion (lines 358-361).

Reviewer #2:

We thank Reviewer #2 for taking the time to review our manuscript and the detailed and helpful remarks. We hope that we were able to adequately address all concerns.

Manuscript Summary:

- I felt that a detailed explanation of the QUEST algorithm is missing from the presentation. The technique is based around the advantages that QUEST have over other threshold estimation protocols, so I think it deserves some discussion.

Thank you for pointing this out. We have tried to remedy this by adding a detailed paragraph about the rationale and methodology behind the estimation process at lines 89 through 104.

- On the other hand, I am not sure that the visual presentation of the experimental setting will be of much use to the neuroscience/neurology community. The video will present standard techniques to place EEG and stimulation electrodes. Then the video will show a subject performing the rating of electrical stimuli of different amplitudes, which is also a standard procedure that does not need to be presented visually.

This is also a very good point, which we were concerned about as well. Hence, we have uploaded an animated illustration of the estimation procedure including the Bayesian estimation process. We are yet unsure, whether this will be added as part of the video, which we would hope for, or whether it will be added as supplemental material. In any case we hope that the illustration together with the information in the paragraph we have added in response to reviewer #2's previous comment will help the reader-/viewership to get a better insight into the psychophysical estimation process in QUEST.

Additional comments:

-Please provide a detailed explanation of the stimulation parameters such as duration of single pulses (biphasic?) and the frequency of presentation of such pulses.

Thank you for pointing out this omission. We have added information on the stimulation parameters in the beginning of section 3 (lines 157-161).

-Please provide a detailed explanation on how the activity on figure 3C was calculated.

We have added this information in the figure legend for Figure 3 (line 330), since there is no designated data analysis section.

-Sentence on line 85 needs revision.

Thanks, we have corrected the phrasing.

-Line 112: Please specify "standard selection criteria".

We have added our most important selection criteria as examples (line 130-131).

-Briefly describe the VAS scale.

Thank you for pointing this out. We have added a short explanation on lines 188-190.

-In Materials, the electric stimulator is not listed.

Corrected. We have added the details for the DigiTimer in the Materials now.

-Sentence on line 289 needs revision.

Thank you, we have removed the extra “from”.