Journal of Visualized Experiments

# Psychophysically anchored, robust thresholding in studying pain related lateralization of oscillatory pre-stimulus activity

--Manuscript Draft--

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| **Manuscript Number:** | JoVE55228R1 |
| **Full Title:** | Psychophysically anchored, robust thresholding in studying pain related lateralization of oscillatory pre-stimulus activity |
| **Article Type:** | Invited Methods Article - JoVE Produced Video |
| **Keywords:** | Pain threshold; Psychophysics; Thresholding; QUEST; behavior; Experimental pain; Pre-stimulus activity; Electroencephalography |
| **Manuscript Classifications:** | 5.1.370.376.300: Electroencephalography (EEG); 5.1.370.685: Psychophysics; 6.2.463.593.710.560: Pain Threshold; 6.2.830.816.444: Pain |
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| **Abstract:** | In perceptual studies, it is often important to objectively assess the equality of delivered stimulation across participants or to quantify the intra-individual sensation magnitude that is evoked by stimulation over multiple trials. This requires a robust mapping of stimulus magnitude to perceived intensity and is commonly achieved by psychophysical estimation methods such as the staircase procedure. Newer, more efficient procedures like the QUEST algorithm fit a psychophysical function to the data in real time while at the same time maximizing the efficiency of data collection. A robust estimate of the threshold intensity between painful and non-painful perception can then be used to reduce the influence of variations in sensory input in subsequent analyses of oscillatory brain activity. By stimulating at a constant threshold intensity determined by an adaptive estimation procedure, the variance in the ratings can be directly attributed to perceptual processes. Oscillatory activity can then be contrasted between "pain" and "no-pain" trials directly, yielding activity that closely relates to perceptual classification processes in nociception. |
| **Author Comments:** | |
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| **Question** | **Response** |
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**TITLE:**
*Psychophysically anchored, robust thresholding in studying pain related lateralization of oscillatory pre-stimulus activity*

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**KEYWORDS:**
Pain threshold, Psychophysics, Thresholding, QUEST, Behavior, Experimental pain, Pre-stimulus activity, Electroencephalography

**SHORT ABSTRACT:**
Psychophysical methods such as the QUEST estimation procedure can efficiently yield robust estimates of the stimulation intensity at which non-painful sensations transition into painful sensations. By stimulating repeatedly at the threshold intensity, the variability in rating responses can directly be attributed to perceptual classifications in subsequent analyses.

**LONG ABSTRACT:**
In perceptual studies, it is often important to objectively assess the equality of delivered stimulation across participants or to quantify the intra-individual sensation magnitude that is evoked by stimulation over multiple trials. This requires a robust mapping of stimulus magnitude to perceived intensity and is commonly achieved by psychophysical estimation methods such as the staircase procedure. Newer, more efficient procedures like the QUEST algorithm fit a psychophysical function to the data in real time while at the same time maximizing the efficiency of data collection. A robust estimate of the threshold intensity between painful and non-painful perception can then be used to reduce the influence of variations in sensory input in subsequent analyses of oscillatory brain activity. By stimulating at a constant threshold intensity determined by an adaptive estimation procedure, the variance in

the ratings can be directly attributed to perceptual processes. Oscillatory activity can then be contrasted between “pain” and “no-pain” trials directly, yielding activity that closely relates to perceptual classification processes in nociception.

**INTRODUCTION:**

When conducting behavioral experiments involving human participants, it is important to be able to closely control the intensities of presented stimuli. Using stimuli of equal intensity for all participants, however, will in some settings introduce the bias of subjective perception. For some perceptual qualities such as pain, there are high inter- and intra-individual variations in perceived intensity at a constant stimulus level[1,2]. For experiments that assume equal subjective percepts it is thus a necessity to match the subjectively perceived intensity across participants. This is also important when examining perception at threshold level, e.g. between pain and non-painful stimulation. Psychophysics research has addressed these kind of problems for decades, and today there are sophisticated but easy to use methods available to achieve robust psychophysical anchoring.

A simple, classical method of mapping the intensity of a stimulus to an individual sensation magnitude is the staircase method[3]. Hereby, the intensity of successive stimuli is increased or decreased, until there is a change in the participant’s response relating to the desired threshold or position on the subjective sensation scale. Repeating this process a number of times yields a plausible estimate of the reversal point. Classical methods, however, fail to make use of all the information contained in each rating trial. This leads to an unnecessarily high number of trials required to reach convergence. Methods such as (linear) regression or function fitting might fail, if the assumptions for the relationship between stimulus intensity and sensation magnitude are wrong or do not hold for the tested stimulus range. The adaptive procedures not only yield a robust point estimate for a certain subjective intensity, but do so more efficiently. Especially for longer experiments, which heavily rely on accurate estimation of a threshold or sensation magnitude, it is necessary for the psychophysical method to be both robust and at the same time efficient with respect to the number of required trials. This is especially important in fields such as pain research, where the total exposure to painful stimulation should be kept as low as possible for the participants’ benefit.

Although the classical staircase methods are still widely used, e.g. in quantitative sensory testing, the use of more advanced estimation methods that make better use of the acquired information across trials is steadily increasing. In the case of the maximum likelihood estimation method QUEST[4,5] used here, this is probably due to the readily available implementation in the popular Matlab PsychToolbox[6] suite. The modern, revised version of this procedure is superior to classical estimation methods both in robustness and the low number of trials required to arrive at a sufficient estimate, if used with the right settings[7].

The rationale behind the QUEST procedure is to fit a Weibull function to the incoming data to model the psychophysical transformation between stimulus intensity and sensation magnitude. The parameters for the psychophysical Weibull function are in part given by the experimenter, e.g. the steepness of the function or the offset due to the false positive rate and responder

inconsistency. The positioning of the parameter of interest along the intensity dimension is approximated by the procedure using Bayesian maximum likelihood estimation. Hereby, a probability distribution is assumed over the location of the target parameter, i.e. the threshold intensity. Given a sensible prior assumption for such a distribution, the algorithm will determine the most informative intensity that the participant should respond to. For the current implementation of the procedure, this is the mean of the prior probability distribution[8]. For each successive trial, the prior probability distribution is in essence multiplied with the likelihood of the participant's given response at the tested stimulation level, as characterized by the Weibull function. Every response will be used to continuously update the probability distribution estimate for the threshold parameter. This procedure is repeated until a satisfying estimate is produced. The procedure is more efficient than a simple regression because it makes immediate use of the collected responses to determine which stimulation intensity to test next. Also, the procedure will specifically probe around the point of interest, e.g. a threshold or certain sensation intensity. Using only testing data from such a limited range in regression would lead to an unstable estimate, making adaptive procedures more robust in settings where only relatively low numbers of trials are feasible.

Such robust psychophysical anchoring can be used to measure changes in pain sensitivity over time, modulatory effects in hyperalgesia/allodynia research or analgesic effects in pharmacological interventions, amongst other settings. Another interesting prospect of being able to anchor stimuli to the intensity just at the threshold between two sensory continua is to examine subjective perception across the transition from non-painful to painful sensation[9–11]. This scenario is very interesting because if the pain threshold has been robustly estimated, pain and no-pain conditions can be contrasted in electroencephalographic (EEG) activity, for example, without changing the physical stimulus intensity[12]. This allows for the observation of pain-specific perceptual processes under constant stimulus conditions by examining the difference in brain activity between trials rated as painful and non-painful.

We will demonstrate how to use the readily available implementation of adaptive estimation in PsychToolbox to robustly determine the individual pain threshold in an EEG experiment where the contrast between pain and no-pain activity is examined for lateralization effects, depending on the stimulation site. Since the stimulation intensity can be kept constant after the thresholding procedure, it is not necessary to account for EEG activity co-varying with stimulus intensity in the subsequent analysis.

**PROTOCOL:**

The experiment has been approved by the ethics commission of the Hamburg medical association (PV4509).

**1. Participant selection**

1.1. Beyond standard selection criteria, such as fitness for pain stimulation, head implants or pre-existing neurological conditions, make sure the participants are not suffering from acute or chronic pain, are not taking any pain medication, and have no known history of substance

abuse. Participants should also not have taken part in any pharmacological studies during the four weeks prior to the experiment.

1.2. Include participants of any gender, yet take care to only include female participants that are using hormonal contraceptives[13,14] to minimize the effect of cyclic changes in pain perception.

1.3. Before administering any kind of stimulation, make sure participants have given informed consent in writing.

**2. EEG setup**

2.1. Select an appropriate cap size and prepare the EEG electrode setup as per the system's instruction manual.

2.2. Set the sample rate and high/low cutoff as well as the impedance limits of the recording equipment (recommended: 500 Hz, 0.5 Hz high-pass filter, impedances < 20 kΩ).

2.3. Make sure the stimulation device and the EEG device are not electrically coupled by running the EEG system on battery.

2.4. Ensure that any link between the EEG system and the computer controlling the electrical stimulation device is potential-free.

**3. Electrical stimulation setup**

3.1. To best make use of the time resolution of the EEG recording, keep the electrical stimulation as short as possible. Set the stimulator to a single, monophasic stimulation pulse with 1 ms duration and 400 V maximum voltage. If a more intense pain level is needed, or the exact timing of the post-stimulus EEG recording does not take precedence, other stimulation protocols can be used.

3.2. Make sure the electrical stimulator is switched on but the output to the electrode is switched off. For the DS7A stimulator the switch labeled "OUTPUT" to the right of the device should be in the down position.

3.3. Locate the landmark(s) that identify the chosen stimulation site. For a stimulation at the hand, use the muscle between thumb and index finger (abductor/flexor pollicis brevis). Ask the participant to lay their hand on a flat surface with all digits stretched out and apposed. Identify the stimulation site by bisecting the distance between the first knuckles of the thumb and index finger.

3.4. Clean the skin by applying electrode preparation gel. Make sure not to use alcohol or disinfectant, which might leave residue on the skin that can lead to irritation or unreliable stimulation.

3.5. Attach the stimulation electrode and fasten it in place with textile tape.

3.6. Ask the participant to find a comfortable position for the hand and to try not to move the hand during the experiment, if possible. For the participant's convenience, place a soft tissue under the hand to absorb any humidity, depending on the surface permeability.

3.7. Enable the stimulator's output by switching the "OUTPUT" switch to the upward position.

**4. Determine starting points**

4.1. Instruct the participant on how to operate the rating scale on the screen using the mouse. The left half represents non-painful sensations; the right half corresponds to a standard pain VAS scale, providing a visual equivalent for a range of continuous sensation intensities in the form of a horizontal line. Point out to the participant that the absolute center point of the scale cannot be selected. Provide the participant with the standardized instructions about the anchor points[15] (Table 1).

4.2. Give the participant the opportunity to get comfortable with the rating process by applying stimuli of varying intensity and recording the responses. Use the information gathered during this phase to get an estimate for two intensities that consistently evoke strong but non-painful sensations (low-point) and moderately painful sensations (high-point), respectively. Continue the stimulation for about 25-30 trials or until satisfied with having reached good estimates. During this time, it can be beneficial to query the participant for verbal feedback on the intensities and the subjective similarity of repeatedly presented stimulus intensities.

4.3. Try to pick the intensities randomly to evoke responses around the scale center. For best results, do not simply increase or decrease the intensities linearly, and also explore the more extreme ends of the painful side. This phase should also give the participant the opportunity to get accustomed to the potentially unfamiliar stimulation and establish some reference for a consistent rating range. Because of this, it is advisable to apply intensities from the whole range of possible stimulus intensities, while also repeating some intensities.

4.4. Once satisfied with having obtained estimates for both a high-point and low-point starting intensity, inform the participant that the first part of the experiment is about to start and (s)he should keep on rating as practiced while random stimulus intensities are presented.

**5. Determine Threshold**

Note: The QUEST algorithm requires some parameters to be specified before starting estimation. Those parameters include the steepness of the psychophysical function (beta, typically 3.5), the fraction of trials where a random answer is expected (delta, typically 0.01), and the fraction of trials where a positive response is expected even though no stimulation is given (gamma, no recommendation). For Bayesian estimation, the range (SD) of the expected ratings and the spacing of possible responses (grain) must be specified. For a VAS, grain should be set to the resolution of the scale (typically 1), and the SD should be set large enough to

include both the scale zero point and the maximum possible intensity plus some safety margin. The recommendations and "typical" values given here are explained in detail in the QuestCreate source code included with PsychToolbox[6,16]. For pain at the threshold, a gamma value of at most 0.01 should be plausible. The estimation method is relatively robust in terms of misspecification of the parameters, however for settings with only few trials, failure to specify sensible parameters might increase the uncertainty of the final estimate. If the standard deviation is set too low, the procedure will have problems converging on estimates that lie outside the area spanned by the standard deviation around the prior guess for the parameter. Thus it is important to rather err on the side of a too large standard deviation.

5.1. Create two QUEST sessions with the parameters given above. Start one from the high-point intensity and one from the low-point. Information about the implementation logic of the estimation process can be found in the supplemental material (S1).

5.2. Randomly select a probe intensity of one of the two runs given by the respective QuestMean function.

5.3. Set the electrical stimulator to the probe intensity. If a different intensity than the one suggested by the algorithm needs to be applied or the suggested intensity is out of range, feed the presented intensity back into the QuestUpdate function in step 5.5.

5.4. Trigger the stimulus.

5.5. After the participant has rated the stimulus, run QuestUpdate for the respective estimation session and supply it with the actual stimulus intensity presented as well as the participant's rating.

5.6. Continue running rating trials until the estimates are stable or a predefined stopping criterion (> 40 trials) has been reached.

5.7. Record the mean threshold estimate between both estimation runs, starting from the high and low starting point as given by QuestMean.

5.8. Allow the participant to take a break at this stage, if desired.

**6. Stimulate at threshold level**

Note: It is possible to adjust the rating and block count to your needs as long as it is tolerable to the participant.

6.1. Inform the participant that for the remaining part of the experiment, more blocks with random stimulation will be following and they should keep rating as they did before. If needed, refresh the instruction on the scale anchor points.

6.2. Start the EEG recording.

6.3. Set the electrical stimulator to the mean threshold estimate obtained in step 5.7 and keep the setting constant throughout the rest of the experiment.

6.4. Start a rating block (30 trials) and observe the data quality of the EEG recording. Depending on the EEG data quality, run 4-5 rating blocks and allow the participant to take short breaks in between the blocks.

Note: Try to keep social interaction with the participant to a minimum during these breaks or standardize the interaction as much as possible.

6.5. When finished, stop the EEG recording, switch the stimulator output to off, and remove the electrode. Debrief the participant after removing the EEG cap.

**REPRESENTATIVE RESULTS:**

Using a rating scale split into one half for non-painful and one half for painful sensations (Figure 1a), constant stimulation can be applied over many trials while still yielding ratings across the scale midpoint (Figure 1b). This way, changes in sensory input can be avoided, and the rating outcome can be directly related to intrinsic perceptual classification processes related to pain.

[Please insert Figure 1 here.]

The two estimation runs starting from the “non-pain” low-point and “pain” high-point converge on robust threshold estimates. Taking the mean of both estimates yields the final threshold estimate, while the bias induced by the starting intensity is reduced (Figure 2a). The subjective stimulation intensity evoked by repeated stimulation at the estimated threshold is stable across multiple blocks of 30 trials each within one experimental session (Figure 2b).

[Please insert Figure 2 here.]

By splitting the concurrently recorded EEG data into trials that were rated as “painful” and “non-painful”, respectively, the oscillatory activity can be contrasted post-hoc. This yields activity differences which coincide with perceptual decisions about the same stimulus being categorized as strong sensation or as pain. Figures 3a and 3b show these differences for a time-window before the painful stimulus is presented (-0.8 s to 0 s before stimulus onset) and the theta-band frequency range (4-7 Hz), which have previously been shown to be connected to subsequent perceptual classification in pain[17]. The thresholding paradigm enables the examination of such pre-stimulus differences in oscillatory activity linked to subsequent perceptual classification of pain, independent of stimulus magnitude.

[Please insert Figure 3 here.]

By changing the side of the stimulation between groups, these pre-stimulus effects can further be disentangled from any lateralization effects in stimulus expectation. Figure 3c shows the

sum of activity across both groups (left hand/right hand), highlighting pre-stimulus theta activity, that is common to the perceptual classification of non-pain versus pain irrespective of the site of stimulation.

**Figure 1: Experiment description.**
(a) The rating scale with the left side spanning non-painful sensation and the right side spanning painful sensation. (b) Procedure used for data collection. 40 thresholding trials followed by 4-6 blocks of constant stimulation (30 trials each). The blocks had a jittered 3-5 s inter-trial interval (ITI). The rating scale appeared 0.25 s after stimulation.

**Figure 2: Stability of the threshold estimates.**
(a) Data for a single participant showing the algorithm converging on two estimates, one for a high intensity starting point, one for a low intensity starting point. To minimize the influence of the starting point, both threshold estimates were averaged (dashed line). (b) Stability of the rating medians over the course of the experiment under constant stimulation at the estimated threshold across all participants (n=25).

**Figure 3: Power differences between non-pain and pain.**
Data has been transformed to time-frequency domain using a multi-taper method. Depicted are Theta frequencies between 4-7 Hz and before stimulus onset (-0.8 s to 0 s). (a) Power difference specific to the subsequent classification of the stimulus to the left hand as painful. Data adopted from Taesler & Rose[17] (n=15). (b) Power specific to classification of a stimulus to the right hand as painful (n=10). (c) Common Theta activity between (a) and (b), independent of the stimulated side (n=25). The topo-plot shows the sum of the lateralized differences between painful and non-painful stimulation. For individual pain/no-pain topographies (S2) as well as a comparison to pre-existing post-stimulus data[10] (S3) please refer to the supplemental materials.

**Table 1: Definition of rating scale anchor points.**
Since the middle of the scale cannot be chosen, the ratings can also be dichotomized into a two-alternate-forced-choice (2AFC) dataset between non-pain and pain.

**DISCUSSION:**
Here we used the well theoretically founded QUEST method to efficiently estimate a robust psychophysical threshold between non-pain and pain perception. Using constant stimulation at this threshold enables an analysis of perceptual decisions independent of changes in stimulus magnitude. While we examined threshold intensity at the transition point between innocuous and noxious sensation domains, other points along the pain scale (e.g. 50 on a 100-point pain scale) can also be anchored with the here presented estimation method. In these cases, care has to be taken to account for habituation or sensitization effects across the course of the experiment. Such effects are more likely to occur for higher stimulation intensities.

One critical step in this procedure is to optimally adjust the necessary parameters for the psychophysical function to be fitted by the adaptive procedure. Another important issue is the instruction given to the participant regarding the anchoring of the response scale. The

participant should have a clear understanding of where to range in the subjective intensities on the scale. It is thus very important to standardize and repeat these instructions, whenever necessary, to avoid introducing any bias into the ratings. Specifying a scale that is split into a non-painful and painful side might prove difficult to handle for some participants, since both sensory continua might differ in their respective sensitivity. In this case, when the information from the split-scale is not needed for further analysis, the estimation procedure can also be carried out as a two-alternative forced choice paradigm. Here, the participant just has to decide, whether a stimulus was perceived as painful or not. In case of problems with the rating scale, the estimation will be robust, as long as the participant's response about a stimulus being painful or non-painful is veridical and false responses are within the limits specified by the delta and gamma parameters.

In cases where the initial thresholding does not converge upon a plausible estimate or rating irregularities become evident, the experiment should be interrupted and restarted. In such cases, it might help to ask the participant about their interpretation of the scale and their subjective perception of the stimulation. If technical errors such as a loose electrode or a faulty connection to the stimulator can be ruled out, it might be helpful to ask the participant about their strategies for dealing with pain. Participants who regularly deal with pain in martial arts or high-performance sports, for example, might exhibit irregular responses despite passing the initial screening. Additionally, social interaction with the participant after the beginning of the experiment and during the breaks should be standardized, as not to induce any effects of experimenter demeanor or induced compliance.

The method outlined here has been demonstrated to be very robust within one experimental session. However, there might be substantial differences in thresholds in the same participants when measured over multiple sessions and days. This might in part be due to circadian changes in pain susceptibility or to changes in arousal or motivation. For heat pain, this has also been shown to be an effect of differing interpretation of the scale range over multiple sessions[18]. These problems could be reduced by re-training participants on the scale anchoring in each session and averaging multiple stimulations into one aggregated rating per trial[19]. An additional concern is that reattaching the electrode in a different session might not yield the exact same stimulation intensity and thus may change the estimated threshold.

Using an adaptive estimation procedure such as QUEST, in each iteration, the full set of information from all prior thresholding trials is used to determine the optimal intensity for the next test intensity. This decreases the number of necessary trials while increasing the robustness against inconsistent ratings during thresholding compared to classical methods such as the staircase. The thresholding process could be further optimized by independently gathering data in a pilot experiment to better estimate the slope of the psychophysical function for the desired modality or stimulus type[7].

Even though the theoretical foundation of the algorithm presented here is sound and we have demonstrated that a robust estimate for exhaustive experiments can be obtained, there are already improved techniques available, that further reduce the number of trials needed to

reach robust threshold estimates. These optimized Bayesian methods not only promise less biased results for low trial numbers but also try to fit the position as well as the slope of the psychophysical function in one iteration[20].

By using such advanced estimation methods, future research in areas relying on the anchoring of subjective perception can benefit. For one, these algorithms reduce the strain on participants and thus help make the experimental setting more ecologically valid. Additionally, they improve accuracy, not only in threshold experiments, but potentially in all self-report measures suited for psychophysical procedures - a property especially useful for research in the clinical setting.

**ACKNOWLEDGMENTS:**
This work has been funded by the Transregional Collaborative Research Centre TRR169 "Crossmodal Learning: Adaptivity, Prediction and Interaction" / German Research Foundation (DFG). The authors thank Stephanie Shields for helpful comments on the manuscript.

**DISCLOSURES:**
The authors declare that they have no competing financial interests.

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Figure 1

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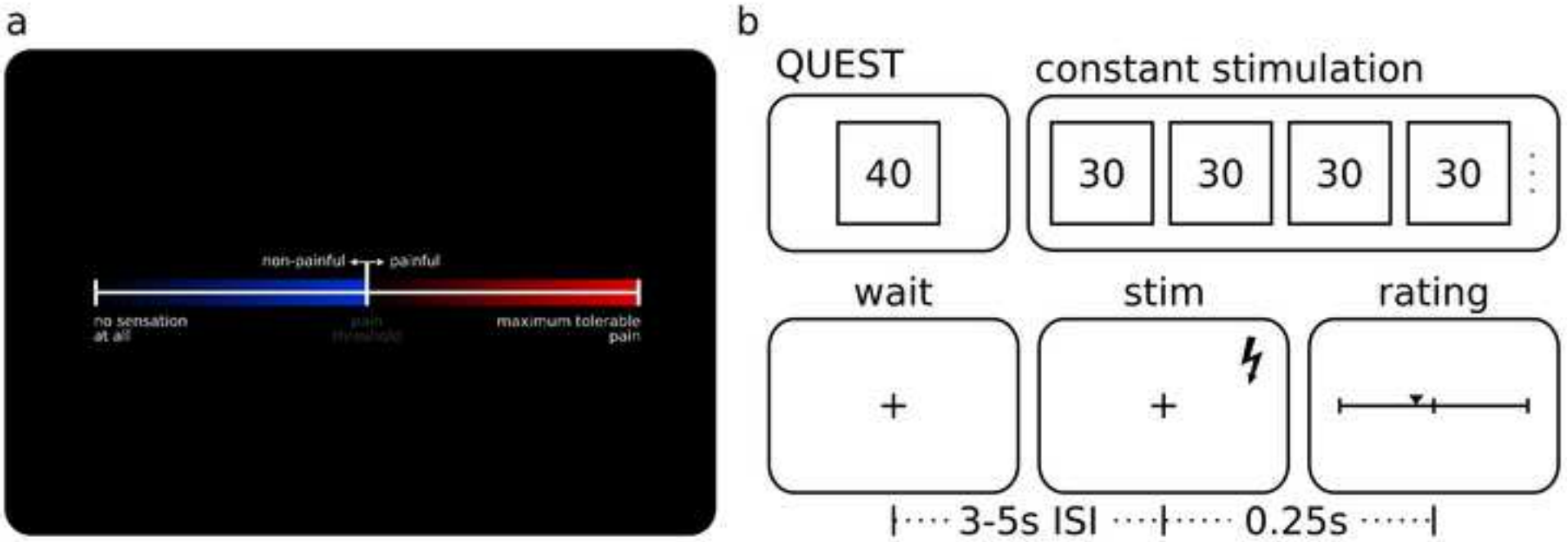

Figure 2

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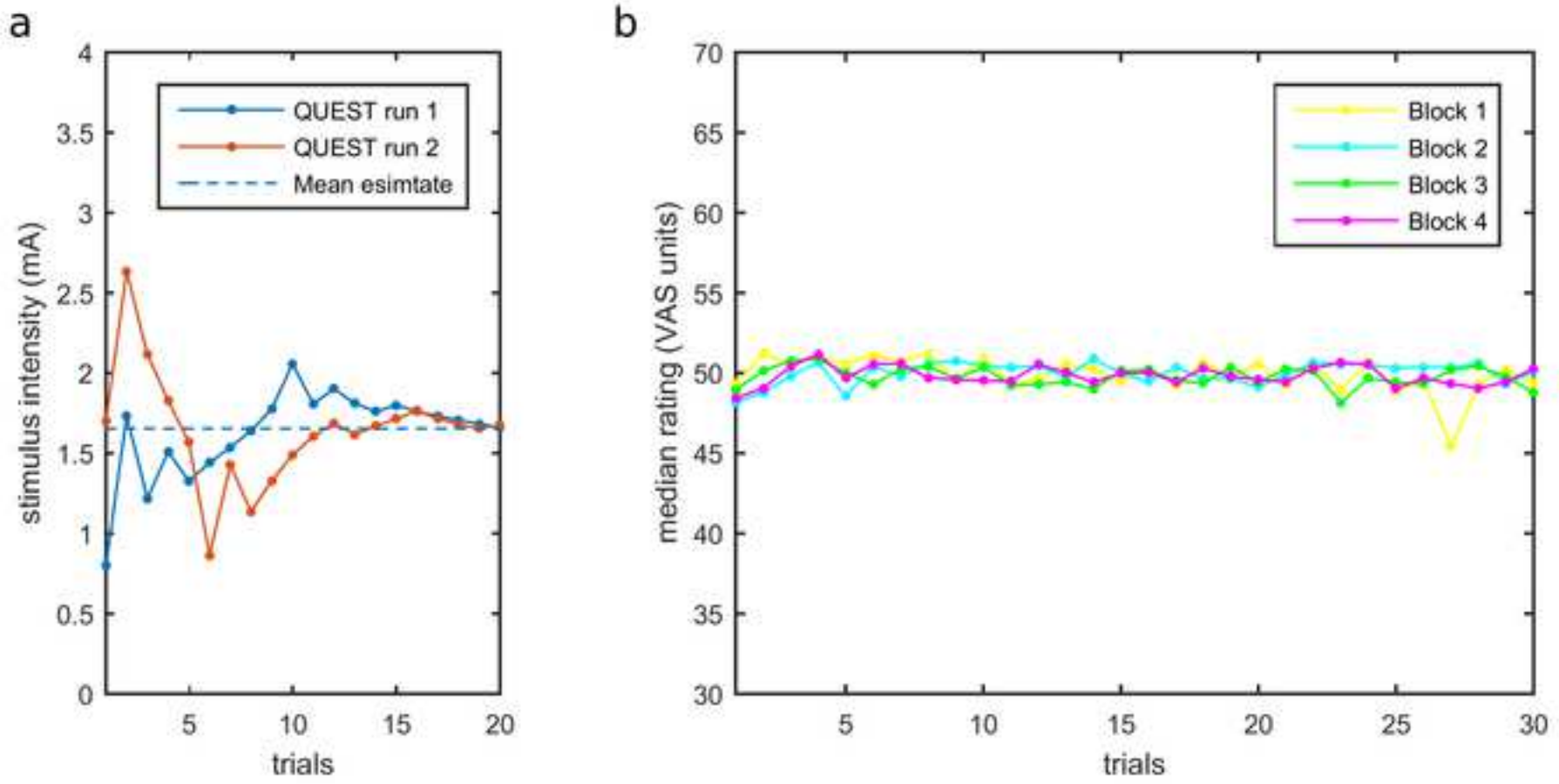

Figure 3

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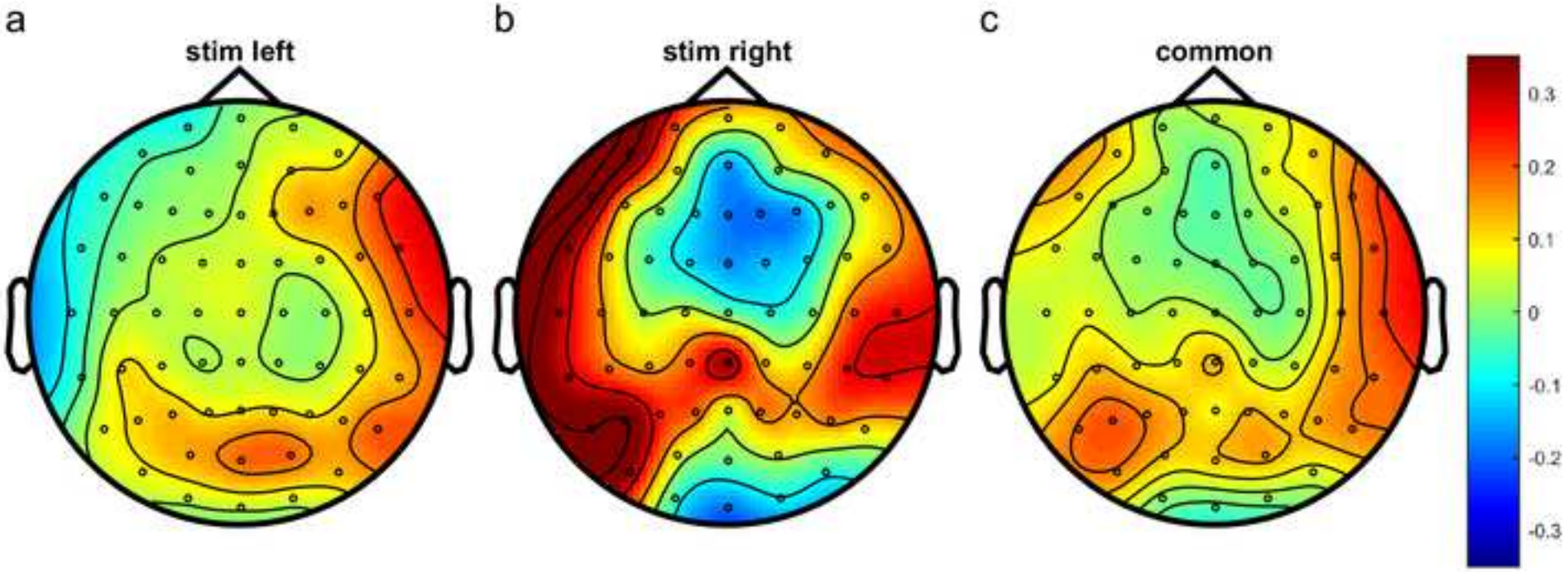

Table 1



| Scale position | Instruction | Score |
|---|---|---|
| Leftmost | “No sensation at all” | 0 |
| Left to center | “The strongest sensation, that is not yet painful” | 49 |
| Center | “Pain threshold - this point cannot be selected” | 50 |
| Right to center | “Painful sensation” | 51 |
| Rightmost | “Maximum tolerable pain” | 100 |

| Name of Material/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| EasyCap electrode cap | EasyCap, Woerthsee-Etterschlag, Germany | CUCHW-58 | |
| actiCap active Ag/Cl EEG electrode set | BrainProducts GmbH, Gliching, Germany | - | |
| SuperVisc EEG eletrode gel | EasyCap, Woerthsee-Etterschlag, Germany | V16 | |
| BrainAmp EEG amplifier | BrainProducts GmbH, Gliching, Germany | BrainAmp Standard | |
| PsychToolbox-3 | Mario Kleiner / Open Source | - | Available at http://psychto |
| Matlab | MathWorks, Natick, MA | Matlab R2015b | |
| DigiTimer DS7A constant current electrical stimulator | DigiTimer Ltd., Hertfordshire, United Kingdom | DS7A | |

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Author(s): Taesler, P + Rose, M.

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Nam Nguyen, Ph.D.
Review Editor
JoVE

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

**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 us 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 212-122).

*-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”.

Animated Figure (video and/or .ai figure files)