JoVE62082R1 | Rebuttal Document
"Objectively Assessing Sports Concussion Utilizing Visual Evoked Potentials"

Dear Vineeta Bajaj,

The following rebuttal document is in direct response to the comments raised by the editorial and reviewers of the manuscript; JoVE62082R1. The authors responses are found directly under each **raised concern or comment** in *non-bolded italics text*.

All additional revisions to the manuscript have been made with track changes in the latest manuscript sent by Vineeta Baja on Saturday the 30th of January, titled "62082_R1_RE".

On behalf of all the authors, I would like to thank you and the reviewers for their time and consideration of this manuscript. Additionally, the extension for revision addressing was greatly appreciated, and it is my hope that the newly submitted manuscript addresses all raised concerns.

Kind Regards,

Dylan Mahony.
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Editorial Comments:

1. The editor has formatted the manuscript to match the journal's style. Please retain and use the attached version for revision.

*The attached version of the manuscript (*62082_R1_RE) was retained in this revision, with all edits marked via track changes.

2. Please address all the specific comments marked in the manuscript.

All comments marked in the manuscript were directly addressed via responses, with appropriate description to any changes and the utilization of track changes.

3. Please address all the reviewer's comments.

All comments marked in the manuscript were directly addressed via responses, with appropriate description to any changes and the utilization of track changes. Any reviewer comments that were not directly marked in the manuscript were addressed by the authors in this response document.

4. Once done please ensure that the protocol section length is no more than 10 pages and the highlight (only for the protocol section) is no more than 3 pages including headings and spacings. Please ensure that the highlighted section makes a cohesive story.

The submitted manuscript adheres to these journal guidelines, with the protocol section being amended to no more than 10 pages and highlighted for a maximum of 3 pages in an order that generates a cohesive story. For convivence of the editor, a separate document (JoVE62082R1 Filming Script) has been submitted.

Reviewers' Comments:

Reviewer #1:

Manuscript Summary: The manuscript looks at differences in the signal to noise-ratio for a 15 Hz amplitude of the EEG waveform in the occipital lobe in response to a flickering visual stimulus at 15HZ for 30 seconds as a function of time of assessment and concussion status among rugby players. The authors also utilize 2 EEG-based assessment systems and compare their findings.

Major Concerns: The authors seem to have addressed most of the reviewer's major concerns although the reviewer did not find an accompanying cover letter itemizing point-by-point revisions and had to compare the revised version with the original manuscript.

The authors apologize for the lack of accompanying cover letter, however a rebuttal document and revised manuscript with track changes applied were supplied (submitted to the JoVE editorial online manager; "JoVE62082 Rebuttal Document" as outlined by the editor.

Reviewer #2:

Manuscript Summary:
This is a revision of a manuscript that begins to provide a methodology/protocol for assessing steady-state visual-evoked potentials in human. However, like the prior version it is missing critical steps and critical controls to ensure a repeatable procedure across laboratories. This is a serious issue and using the described methodology cannot readily recreate this type of study with reproducibility.

Major Concerns:

Section 2.2.1: SSVEP luminosity: The authors state to use an smart phone presentation of a .mp4 of a 15 hz flicker with a random number. Given that the brightness of the stimulus is a critical part of the SSVEP (e.g. Mouli S. and Palaniappan R. ISCAE At: Newcastle 2016. 10.1109/ICSAE.2016.7810188) and can vary from model to model of smartphone devices,

the authors should report what the luminosity values are and how these values are determined.

It was stated in section 2.1.2 of the manuscript that the LCD smartphone utilized had its displays brightness turned to maximum. The exact make and model of the smartphone used is also recorded in the table of materials of which is referenced in section 2, ultimately allowing for a reader to establish the luminosity value range that was used. However, as pointed out by Reviewer 2, the authors agree these values are import to SSVEP, and hence have been added to the protocol to guide readers to an appropriate range of luminosity used.

Section 2.2.1: The authors indicate in this revision that a number is presented and altered at a 5-second interval "to encourage sustained attention". Are the subjects required to do anything with the presented numbers other than " focus on the focal number"? If the authors require sustained attention for this task than the instructions read to participants and task need to be designed as such. One possibility is to require the subject to report whether a particular number was shown (i.e. 5) or whether a number was presented in color.

No, the subjects are not required to do anything other than focus on the focal number. As stated, this is to encourage sustained attention, but not to enforce it. This is because sustained attention was not required to generate a SSVEP response.

Section 3 Is this section referring to the use of the emotive headset and software? If so, state that in this section.

The authors originally directly stated the use of the Emotiv Headset and software in an early revision of this manuscript, however as per JoVE's guidelines in which no commercial language is to be used, the reference was replaced with a more general term "14 channel EEG headset" of which is referenced in the table of materials.

Section 3.3 What is the impedance range required for the hardware. The reviewer assumes that the author is referring to the emotive headset and software. Is this range the same as that reported 12.4? If so, state that in this section.

The reviewer is correct in their assumption, this section is referring to the emotive headset and software. However, as mentioned above, this commercial language was removed due to JoVE guidelines. The impedance range of good contact quality of the system is to be taken as below 20k Ohms impedance. The authors have included this range in this section.

Section 3.7 Possibly a typo and the authors did not delete this section?

This section is not a typo. The authors believe the reviewer has misread the section, due to the in-text reference to section 3.4 of which aligns with the section numbering of the rest of the protocol.

Section 3.9 Are .edf files HIPAA compliant and de-identified? If so, state that in this

section.

No as this study was conducted within Australia and not held to HIPAA compliance. De-identification of patient data is covered by the description of the labels used, with no personal information besides the subject's initials being stored in the file or its label.

Section 3.11.2 How are the authors cleaning the cardboard VR frame with isopropyl alcohol and not having it degrade? How often is the cardboard replaced?

The authors carefully cleaned the cardboard VR frame, and ensure any excess moisture was wiped off the cardboard with a dry lint free tissue. The VR frame did not show signs of degradation and hence was not needed to be replaced due to the small sample size of the studies population. However, the authors agree degradation may occur in larger sample size studies.

Section 4: What time point after injury is the SSVEP performed? There is a well-defined time course of electrophysiological changes that occur post-injury. Prescribing a time range that the assessment is given is likely critical to minimize variability in these data.

The time point of post-injury assessment is stated in section 4.2, as "within 72 hours of the incident"

Section 8: The use of FFT for these data is an outdated methodology for estimation of a biosignal in the frequency domain. Use the S-transform, Thompson's multi-taper method, or other applicable approach that limits spectral bleeding and can provide confidence intervals to compare spectra.

While the authors agree FFT is a basic form of transformation, it is still appropriate for analysis of an EEG signal in the frequency domain. Additionally, the stimulus was held at a fixed frequency (15 Hz), resulting in a signal that is effectively captured in the frequency domain.

Is there a finite amount or minimum amount of time/data used for analysis?

No, the entire data capture of the EDF files was used for analysis as started in section 8.

There is no discussion of artifact detection and/or removal.

There is discussion of artifact detection and removal is section 3.2, and 12.4.
A figure was provided of common artifacts (figure 4), and a clear statement was made that SSVEP data that contained any of these artifacts was/ should be discarded.

Section 10: This section refers to a SSVEP system and figure 7A. Figure 7A is of a power

spectrum. Are the authors referring to the HeadSafeIP Nurocheck system? if so, state that.

The authors are confused by this comment, as Figure 7A is not of a power spectrum density (PSD) but rather the SSVEP system used. The reviewer is correct in his assumption that the authors are referring to the NUROCHEK system, however as previously stated to adhere to JoVEs guidelines the use of commercial terminology was not used.

Section 11: Compumedics reference system:
- the part number is wrong for the Grael 4k Amplifier this should be 928-0002-02. The Profusion EEG 5 software is only available in Australia.

The part number has been updated to the reviewers believed number. The study was conducted in Australia, and hence Profusion EEG 5 software was used.

Section 12: It is unclear what SSVEP and EEG hardware the authors are referring to. Is this all HeadsafeIP hardware or a mixture of Compumedics and HeadsafeIP?

This section involves the use of both hardware, as outlined in section 12.2 and figure 6. The SSVEP device is used as the visual stimulus throughout the section, but the recording electrodes are alternated between the HeadsafeIP hardware and the Compumedics Hardware.

Section 12.15: Flash stimuli can provoke epileptic seizures especially in the 15-25 Hz range (R. S. Fisher. et al. Epilepsia, vol. 46, no. 9, pp. 1426-1441, 2005.). This is considerable concern with patients may be at risk for seizure including those whom suffer mild traumatic brain injuries. Other than the exclusion criteria and generally asking whether patients experienced abnormal reactions including headache or dizziness, was a seizure assessment performed?

No, the authors were strict on the exclusion criteria that any individual with a history of seizures, epilepsy, fits (or other forms of convulsions) was not to participate, and hence no seizure assessment was performed.

Section 15: Given homegrown Matlab scripts can be programmatically different, provide a link to these analysis scripts for data reproducibility.

The authors are willing to provide the general analysis scripts that were used for MATLAB based analysis, including the raised functions within the manuscript.

Are the statistical tests corrected for multiple comparisons or performed as a within subject design?

As stated in section 9.1. a Bonferroni correction was applied to correct for multiple comparisons.

Reviewer #3:

In my opinion, the paper is very interesting, the methodology is well designed, and the technical side is flawless.

Minor Concerns:
- lack of reference to the VEP ISCEV standard (although it is a clinical standard and the test arrangement was non-standard in this paper):Odom JV, Bach M, Brigell M, Holder GE, McCulloch DLL, Mizota A, TormeneAP (2016) ISCEV standard for clinical visual evoked potentials - (2016update). Doc Ophthalmol 133(1):1-9VEP Standard (from Springer)
- According to the VEP methodology (as per the ISCEV standard, but also due to the distance from the VEP signal source), the reference electrode should be placed at Fz and not at P1; for the grounding electrode the recommended location (according to the ISCEV standard and theinternational 10-20 system for EEG) is different than the one used in this paper (P2);
- the graph in Fig.10 lacks units on the axes.

The authors agree with Reviewer 3's minor concerns and have addressed the lack of units on the axes of Figure 10.