Dear Dr. Karakashevska,

Thank you for your resubmission of your Stage 1, both the reviewers and I were impressed by your detailed reply and edits to your manuscript.

Felix Klotzsche considers your Stage 1 very close to ready for acceptance and has provided some valuable additional suggestions for quantifying the EEG data quality with the VR-headset. They also provide useful considerations for publishing the experimental code and handling participants who wear glasses.

We thank the reviewer for the useful suggestions. We have now addressed the comments and made a minor change to the manuscripts. Please see below.

From my perspective as your recommender, your edits regarding your exclusion criterion and sampling plan are thorough.

I invite you to address each of Felix Klotzsche's comments and resubmit your Stage 1.
Yours sincerely,
Grace Edwards

Review by Felix Klotzsche, 26 Sep 2024 09:39

I thank the authors for their extensive response to the points I raised in the first round of the review process and the reworking of the manuscript. Especially the reordering and reformulation of the hypotheses and their relation to each other as well as the new Study Design Table are helpful for the reader to understand the aim and scope of the planned study. Also, the additional explanations regarding the power analysis and how to tackle the challenge of optional stopping are valuable, just as the fact that the authors now plan to make use of the eye tracking data. The new figures (4 and 6), demonstrating the (virtual) setup of the experiment clarified (almost) all my questions regarding this aspect. Overall, I am impressed by the quality and rigor of the RR and I wish the authors good luck and fun with the data collection. I am looking forward to reading the paper with the actual data.

Please find a few minor comments/suggestions below which I wanted to share with the authors. They should not further keep them from conducting the study as planned or require revising the manuscript again but might be useful for the upcoming steps:

1) I appreciate the work and thought that the authors put into the question regarding a potential drop in signal quality due to the VR setup. Overall, I share the opinion and experience of the authors that in their setup the negative impact on EEG data quality should be rather low, especially for "slow"/low-frequency components like the SPN. However, I think that the number of rejected epochs based on a fixed amplitude threshold is not a sufficient

measure for the quality of EEG data. Adding a VR headset (in static conditions) will primarily add noise with power in rather "high" frequencies (for EEG; i.e., beta band and upwards) but not necessarily high amplitudes (see for example Weber et al., 2021, and I can confirm this from my own experience). Such noise sources (e.g., muscles, line noise and harmonics) will not lead to trial rejection due to high amplitudes but might decrease the signal-to-noise ratio overall (e.g., smaller ERP amplitudes), depending on the definition of the "signal". How to quantify this effect is controversial and I am not aware of a silver bullet. In case the authors want a (in my opinion) more reliable numeric criterion (than the number of rejected epochs) to compare/assess data quality, they could, for example, make use of a metric which was recently suggest by Luck et al (2021). Alternatively, to dispel doubts of future readers/reviewers, the authors may argue that the SPN reigns in (low) frequency ranges which are less affected by noise due to a VR headset (especially as they will use a lowpass filter). This claim could further be strengthened by comparing the power spectra of the VR and the non-VR data, which the authors now plan to collect.

These are relevant points. We agree that high frequency noise may be increased by the VR headset (although our piloting suggests this is not always obvious during EEG recording). High frequency low amplitude artifacts are less problematic than other artifacts because they affect all conditions equally and cannot produce SPN differences. Our pipeline applies a 25Hz low pass filter and the SPN is a long, low frequency difference wave.

We agree that trial exclusion is not the best metric to capture high frequency artifacts in the EEG signal check. We will thus also run a time frequency analysis using the fieldtrip toolbox. This is now explained in the paper:

"Frequency and amplitude are inversely related in EEG. Trial exclusion can indirectly measure prevalence of high amplitude artifacts (which tend to be low frequency) but not of high frequency artifacts (which tend to be low amplitude). High amplitude artifacts can be increased by VR apparatus (Weber et al., 2021). We are less worried about high frequency artifacts because 1) they are removed by the 25Hz low pass filter in our analysis pipeline, and 2) because the SPN is a low frequency signal. High frequencies are unlikely to be confound that can explain observed SPN modulations.

However, we will also investigate high frequency artifacts in the signal quality check EEG data. We will run an alternative pipeline using Fieldtrip toolboxes in eeglab (This is available on https://osf.io/sw8hm, and was used for supplementary gamma band analysis in Makin et al., 2021).

This an exploratory analysis that will begin with visualization of time frequency plots and sequential topographies. It could be that the VR headset increases power in higher frequency bands at some spatiotemporal clusters. This

would be an interesting methodological contribution for VR-EEG researchers, but not a problem for interpreting the SPN results."

2) I still could not find the Unity scenes on OSF (only some StreamingAssets and a C# project file). But the new figures 4 and 6 are very helpful in understanding (most of) the specifics of the scene setup. Nevertheless, for hands-on researchers nothing is more useful than running/trying an experimental setup/code themselves. It would be great if the authors could share the final Unity project (or at least a compiled demo scene) at latest when publishing the manuscript.

We have now updated the OSF repository to contain a demo scene here:

We have now uploaded a demo scene to OSF in the *Stimuli and Experiment* folder. We will upload all the materials necessary to run the experiment at Stage 2.

3) The authors plan to test participants with "corrected to normal vision". In case this includes participants wearing glasses, please keep in mind the following challenges:
- Eye tracking quality might be lower in these participants (in my experience, it's still acceptable for many cases, but for studies where eye tracking places a crucial role, we normally exclude people with glasses)
- To fit glasses into the Vive Pro HMD, it might be necessary to adjust the lens distance (https://www.vive.com/hk/support/vive-pro-hmd/category_howto/adjusting-the-lens-distance.html), which changes the FOV (not to confuse with the individual adjustment of the IPD). The authors might want to keep this hardware setting stable across all participants.

It is true that glasses interfere with eye-tracking, and we will not have participants in the experiment wearing glasses throughout the experiment. The corrected-to-normal vision participants will be asked to wear contact lenses at the time of the experiment if they wisht to participate. We have now added the clarification in the manuscript:

"We will use a sequential approach to recruitment, with a minimum participant sample of 48 and a maximum of 120. All participants will have normal or corrected-to-normal vision and no history of neurological conditions, based on self-reported information. To prevent interference with eye tracking, participants who use corrective lenses will be asked to wear contact lenses during the experiment. Upon arrival at the lab, participants' interpupillary distance (IPD) will be measured using a ruler."

I hope my comments are of use to the authors & I am happy to be part of the process.

Best regards,
Felix Klotzsche

References:
Luck et al. (2021): https://pubmed.ncbi.nlm.nih.gov/33782996/
Weber at al. (2021): https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8645583/