Manuscript #378

Cerebral Mechanisms Underlying the Effects of Auditory Stimuli During Submaximal Exercise

PCI Registered Reports

Recommender's Comments:

I have now received two very helpful and constructive reviews of your Stage 1 submission. As you will see, the evaluations are broadly positive while also noting several areas for potential improvement, including additional key methodological details, steps to address error/bias in the fNIRS measurements, clarity and scope of exclusion criteria, and suitability of the proposed analyses and controls. In revising, please also include the fNIRS template noted in David Mehler's review (e.g. in an Appendix, referred to in the main text).

I look forward to receiving your revised manuscript and response in due course.

Response: Thank you for extending an invitation to submit a revised draft of our Stage 1 manuscript. We appreciate the valuable input from both you and the two expert reviewers. Your insightful feedback has been instrumental in shaping our revisions. Below, we have meticulously addressed each reviewer's comments and concerns point-by-point. For ease of reference, we have provided page numbers that correspond to the revised manuscript file.

Reviewers' Comments:

Reviewer 1 (Anonymous)

This study aims to determine how the point of onset of cerebral oxygenation decline during an incremental exercise protocol is modulated by an auditory stimulus (i.e., music). Three conditions were compared: asynchronous music, audiobook control, and no-audio control. During the cycling task (a constant work rate exercise protocol), brain oxygenation will be recorded using a continuous-wave fNIRS system. Medial and dorsolateral prefrontal cortex (PFC), primary motor cortex and lateral parietal cortex will be measured over the two cortices.

Response: We thank you for the time devoted to reading our contribution.

Title: Cerebral oxygenation or correlates should be used in the title. But not cerebral mechanisms that were not assessed per se.

Response: In response to your suggestion, we have made the necessary amendments to the title (see p. 1, l.1–2).

Overall, objective and hypotheses are clearly exposed and based on a strong scientific background. However, there are some potential concerns to tackle.

Response: We have diligently addressed each of your insightful comments and provide point-by-point responses below.

Introduction as a whole is quite long; the two first pages (up line 80) could be synthesized in order to move quickly on the main topic delivered by this original investigation.

Response: Thank you for your recommendation regarding the structure of the Introduction. We have revised it accordingly, making it more concise and focused. We have reduced the length of the introduction by one page (see pp. 3–6).

From line 108, lines 124-126, regarding fNIRS studies during incremental (upright) cycling exercise, some first relevant studies that showed the typically curve of brain oxygenation over the PFC are lacking (doi: 10.1016/j.resp.2006.08.009 and doi: 10.1007/s00421-007-0568-7). Note that brain deoxygenation occurs after the second ventilatory threshold. There are plenty of research works during cycling incremental exercise with fNIRS mainly over the PFC. Regarding a submaximal exercise at a constant power output, the brain deoxygenation is not so referenced and likely does not occur each time. Please clarify / comment on the real possibility to observe a deoxygenation pattern during submaximal constant exercise.

Response: We thoroughly appreciate your valuable suggestions for additional references, which we have now incorporated into the manuscript (see p. 5, l. 109–112; p. 5, l. 115–117). We contend that we have piloted the proposed protocol (see Figure 6, p. 32) and that our protocol, which does indeed have a constant workload (once 5% above VT1 is reached) and proceeds to the point of volitional exhaustion (see Figure 2), does induce brain deoxygenation. Note that one of the papers to which you kindly directed us shows that prefrontal deoxygenation commences from the respiratory compensation point (Bhambhani, 2007), which will be surpassed in our proposed protocol (i.e., en route to volitional exhaustion). Following a warm-up, the protocol starts the exercise phase at a relatively high intensity, thereby bypassing the early stages of an incremental test. Moreover, we are employing a within-subjects design and the approach up to and including the constant load will be strictly standardised across participants, with reference to the physiological event of VT1, identified by means of HRV (see Supplementary File 2). Thus, we have full confidence in our protocol in regard to salient physiological data. An additional (practical) reason for which we chose not to use an incremental exercise task is that there was resistance to this in the ethical approval process (induces anxiety in participants). We secured ethical approval prior to registering this protocol (#D2021-001).

L 98. “Recently”. First studies that have measured brain oxygenation (and not metabolism) were proposed about 15-20 years ago.

Response: We appreciate you bringing this to our attention. This was an oversight on our part that has now been remedied (see p. 4, l. 86–89).

L 101. Replace “tool” by “technique” or “method”

Response: We have amended the text in accord with your recommendation (technique; see p. 4, l. 89–90).

L 105. What is referring to “Exercise metabolism”? through oxygen consumption measurement?
Up to 10 Hz: fNIRS device for a while can sample up to 50 Hz.

Response: We have used the words employed by Herold et al. (2018; i.e., tissue oxygenation; see p. 4, l. 91–p. 5, l. 93). We have changed "10 Hz" to "50 Hz", albeit in the *f*NIRS literature we could find very few studies that go up to 50 Hz (e.g., Lin & Lin, 2016).

L 130. Previous references (see above) have showed this idea, as indicated in a first review on the topic of fNIRS during incremental exercise where brain deoxygenation phenomenon was presented (doi: 10.1016/j.ymeth.2008.04.005.).

Response: We have duly added your suggested reference (see p. 6, l. 127–129).

L 120-130. This section is quite important in the rationale of the present study. They are several hypotheses explaining the possible deoxygenation occurrence at such exercise intensities that were proposed in the three doi references above and in Rooks et al. (2011) but for incremental exercise test only.

Response: Following your recommendation, we have revised this paragraph to offer further insights regarding the cause of brain deoxygenation, considering competing hypotheses (see p. 5, l. 106–p. 6, l. 129). We have also explained above the reasons for which we chose to use a non-incremental test (see response pertaining to lines 107–114). Note that Rooks et al. (2011) did not use *f*NIRS technology – they took respiratory and non-cortical haemodynamic measures.

L168-175. These tests are precious. It is a shame that non-cortical haemodynamics variables will be not considered as proposed by the current guidelines; even if the exercise (physiological) load (power output in watts) is judged similar, the conditions could modulate affective response (emotional states) and so autonomic nervous system accordingly. This in turn may change the brain oxygenation patterns. It is suggested to implement the autonomic nervous activity assessment during the three conditions as more control.

Response: We fully concur with your remark concerning non-cortical haemodynamic variables. Because we do not have *f*NIRS apparatus with short-channel facilities in our laboratory, we added an assessment of the head blood-volume pulse through a photoplethysmograph sensor (see p. 11, l. 256–262). In accord with the SPA-*f*NIRS guidelines (Scholkmann et al., 2022), the recorded autonomic nervous activity will be regressed from the collected *f*NIRS signals to remove the physiological confounding effects (see p. 11, l. 262–265, Figure 5, p. 31). In addition, details regarding heart- and respiratory-rate monitoring were already included in the manuscript (now located at p. 10, l. 248–252). Statistical analyses of the cardiorespiratory data will be performed and reported in a supplementary file, as is now mentioned in the manuscript (see p. 15, l. 367–368).

Regarding the methods, they are well presented and appear suitable for testing the hypotheses.

Response: We thank you for your positive comments.

L219. What is the exact incremental protocol test for determining VO2max? What are the criteria for selecting VO2max values?

Response: Kindly note that these details were included in Supplementary File 2.

L220. Based on a typical incremental VO2max test, ventilatory thresholds can be determined accurately, mainly VT2, with the help of gas respiratory exchanges.
Concerning the hypothesis on the decrease of brain oxygenation during incremental test, literature showed extensively that this phenomenon occurred around VT2. It is unclear why VT1 is here targeted.

Response: It is not that we are 'targeting' VT1, rather it is that VT1 is the start point of our exercise protocol, and given how challenging it will be for participants to maintain this intensity, they will soon drift to RCP and beyond, towards volitional exhaustion. The protocol that we have selected is 'bearable' from a psychological standpoint (i.e., we can get it through an ethics panel [imagine starting at VT2!]) and holds high ecological validity, as it represents how a cardiovascular workout might be conducted by individuals who are recreationally active, albeit such a workout would seldom end in voluntary exhaustion. Accordingly, for practical reasons and with an eye on the generalisability of our findings, we decided to use VT1 as a starting point.

L 222. It is not clear also and surprising to use the heart rate to setup 5% above VT1 in terms of exercise intensity. Please comment on this choice as compared to power output

Response: The use of deflection points in heart-rate *variability* (and not heart rate) has been well-documented in the literature to compute VT1 (see e.g., Barreto-Silva et al., 2018; Bigliassi et al., 2017; Karapetian et al., 2008; Queiroz et al., 2018). Our rationale is also clearly detailed in Supplementary File 2.

L241. 63 rpm is selected as a criterion. It is surprising and not argued.
Overall, the exercise protocol testing is unusual in the field of exercise physiology and does not seem the best one for showing brain deoxygenation. It becomes confusing sometimes when exercise term is cited. Brain deoxygenation occurence and its determinants are different across the type of exercise protocol (incremental vs. constant).

Response: The selection of 63 rpm was made to prevent synchronisation between pedal revolutions and the tempo of the music tracks (120–123 bpm). This justification was already included in the manuscript (now located at p. 9, l. 225–227).

L255. Cardiorespiratory. This is inexact. Only cardiac activity was used here. Again, respiratory measurements are lacking. They are required for determining the exercise intensity domains (see doi: 10.14814/phy2.14098).

Response: We confirm that *both* cardiac and respiratory activity will be monitored, as previously stated in our manuscript (now located at p. 10, l. 247–p. 11, l. 258). We have bolstered the paragraph relating to cardiorespiratory monitoring to make this point clear (see p. 10, l. 248–252).

L322. Low pass filter of 0.1 Hz is often required (doi: 10.1117/1.NPh.4.4.041403)

Response: Thank you for your remark and the proposed study. We have modified this sentence accordingly (see p. 14, l. 337–339).

Reviewer 2 (David Mehler)

I congratulate the authors on this carefully designed fNIRS study to investigate cerebral mechanisms underlying the effects of auditory stimulation during exercise on psychological wellbeing.

Response: We would like to express our sincere gratitude for the time you dedicated to reading our contribution and for providing us with your valuable appraisal.

The submitted report provides in many aspects a detailed description of the methodology, the sampling plan and the analysis plan. To provide a more concise overview, I would kindly ask the authors to submit the Preregistration for fNIRS" template that was recently developed (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9993433/#r55; https://osf.io/hb4um/).

Response: Thank you for sharing this reference and the associated guide – we were unaware of its existence. We have duly filed the *f*NIRS preregistration template (see Supplementary File 4).

1) It appears from the manuscript that the fNIRS device does not use short-separation channels that are considered state-of-the-art to account for extracerebral noise: https://www.spiedigitallibrary.org/journals/neurophotonics/volume-10/issue-1/013503/Performance-comparison-of-systemic-activity-correction-in-functional-near-infrared/10.1117/1.NPh.10.1.013503.full
In fact, extracerebral noise is not mentioned as a confounding factor in the protocol. Please clarify how you intend to correct for it, also given that it can strongly bias results with false positive findings for oxygenated Hb in particular (see reference above). This point is also highly relevant in the context of exercise, which can further confound fNIRS result in the form of an increase in extracerabal oxygenated Hb (see for details here: https://www.sciencedirect.com/science/article/pii/S1053811923000290).

Response: Thank you for the suggested references, which we have consulted with due diligence. Although we fully concur with your remark regarding short-separation channels, we do not have the necessary equipment in our laboratory (i.e., the FOIRE-3000/16 Shimadzu equipment does not provide short-channel facilities). Nonetheless, following your comment and the analogous comment from Reviewer #1, we added an assessment of the head blood-volume pulse by means of a photoplethysmograph sensor as an index of extra-cortical noise (see p. 11, l. 259–262). In accord with the SPA-*f*NIRS guidelines (Scholkmann et al., 2022), the recorded autonomic nervous activity will be regressed from the collected *f*NIRS signals to remove the physiological confounding effects (see p. 11, l. 262–265, Figure 5, p. 31).

2) The protocol mentions that a substantial part of the data will be contiminated by motion artefacts. It is quite likely that significant proportions of data for some subjects may not be usable. The protocol should include clear data exclusion criteria with regards to motion and other aspects, and describe how it is ascertained that the target sample size will be achieved.

Response: The motion artefacts will be corrected by means of the wavelet filtering implemented in Homer 3, which should deal with most artefacts (see Brigadoi et al., 2014; Cooper et al., 2012; Molavi & Dumont, 2012). Nonetheless, as you suggested, we added an exclusion criterion concerning noisy data due to remaining excessive motion artefacts (see p. 13, l. 335–p.14 . l. 337). The target sample size ($N = 36$) will be achieved given that any

excluded participants will be duly replaced (see p. 12, l. 281–282; p. 13, l. 319–320; p. 13, l. 331).

3) To establish good quality for data, I would strongly suggest using the qt-nirs toolbox: https://github.com/lpollonini/qt-nirs

Response: Thank you for pointing this out. We added quality checks with the QT-NIRS toolbox as part of the *f*NIRS data preprocessing (see p. 13, l. 321–331; Figure 5, p. 31).

4) The sampling plan describes a smallest telescope approach to establish a SESOI, which I think is a val appoach given the risk for bias and the challenge in establishing a mechanistic SESOI. yet, sample size estimates seem based on effect size estimates in previous literature. Could the authors please clarify? Further, given their design, I am wondering whether (nested) Bayesian hypothesis testing may be more sensitive and robust, as it provides flexible stopping options?

Response: To justify our sample size, we decided to rely on statistical power, namely the probability of detecting an effect (i.e., not accepting the null hypothesis) provided that this effect exists. The sample size computation was not performed using the SESOI because the latter corresponds to the effect size that an earlier similar study would have had 33% power to detect. However, for a sample size justification, we want the power to be fixed at 80%, which corresponds to a β level of .20. We made this choice in a way that the Type I error is four times less likely to occur than the Type II error (see Cohen, 1988). The expected effect size was estimated from previous similar studies in terms of variables of interest and experimental design, as recommended by Lakens (2022).

Regarding the statistical analyses, none of the present authors are expert in Bayesian hypothesis testing. In addition, we believe that the null-hypothesis significance testing (NHST) is a suitable statistical approach to test our research hypotheses (see Lakens, 2021, for further considerations on the use of Bayesian statistics vs. NHST). Specifically, the limitations pertaining to the NHST have to do with (a) concluding a meaningful effect is absent after a non-significant result, or (b) misinterpreting a significant result as an important effect (Kline, 2013). Thus, in the proposed study, we will use the NHST only to test a priori research hypotheses regarding expected differences between conditions; in case an absence of difference is expected, a TOST procedure will be used (see p. 15, l. 377–390). In addition, the significant *p* values will be interpreted in a measured, considered manner by comparing the obtained effect sizes with the SESOI.

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