Effects of Auditory Stimuli During Submaximal Exercise on Cerebral Oxygenation

_____[1], _____[2], _____[1], _____[3] and _____[1]

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Author Note

The study data and materials will be shared openly as part of the publication of the article. The proposed protocol has been reviewed and accepted by the ethics committee of the University of _____ (ref. D2021-001). The study will receive financial support from _____. The authors have no competing financial interests to declare.

Abstract

Asynchronous music has been commonly used to reduce perceived exertion and render the exercise experience more pleasant. Research has indicated that in-task asynchronous music can reallocate an individual's attentional focus to task-unrelated signals and increase the use of dissociative thoughts. Nonetheless, the brain mechanisms that underlie the purported benefits of music during exercise remain largely unknown due to the severe motion-related restrictions of popular neuroimaging techniques. *f*NIRS represents a non-invasive imaging method that is particularly suited to exercise-related protocols given its high tolerance to motion artifacts. With use of *f*NIRS, the purpose of the proposed study will be to determine the point of onset of cerebral oxygenation decline during exercise and how this is influenced by the presence of asynchroneous (ambient) motivational music. A continuous-wave *f*NIRS system will be used to record the prefrontal, motor, and parietal hemodynamic responses of 24 participants who will perform a cycle-ergometry exercise protocol. The objective will be to test the hypothesis that brain oxygenation changes will be observed earlier when participants exercise with an audiobook or in silence, when compared with exposure to asynchronous music. The results will shed light on the neurophysiological mechanisms that underlie the well-documented ergogenic and psychological effects of music.

Keywords: cerebral oximetry; cycling; physical activity; prefrontal activity; ventilatory threshold

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Casual observers cannot help but notice the almost symbiotic relationship that exists between music and physical activity. This relationship has been fuelled by rapid development in the digital technology that underlies music delivery and a growing recognition that well-selected music can enhance the experience of physical activity (Terry et al., 2020). In the exercise domain, music is used to partially block negative bodily signals from entering focal awareness, elevate affective states, and provide a rhythmic cue that can prolong physical effort (Bigliassi et al., 2017; Karageorghis et al., 2018).

In the exercise context, an ergogenic aid can be broadly defined as a technique or substance used for the purpose of enhancing or prolonging performance (Thein et al., 1995). Music is an oft-used ergogenic aid in this context (see Karageorghis, 2020, for a review). During an exercise task, there are two main ways in which music can be applied: synchronously and asynchronously. The phenomenon observed when an exerciser synchronises their movements with the rhythmical qualities of music is commonly referred to as auditory-motor synchronisation (Karageorghis & Terry, 1997). In recent years, two main forms of auditory-motor synchronisation have been proposed: (a) *active synchronisation*, in which individuals consciously synchronise their movement rate with the music tempo; and (b) *passive synchronisation*, in which the music tempo is automatically adjusted to match the movement rate of the exerciser (Karageorghis, 2020). The application of asynchronous or ambient music, by way of contrast, does not involve synchronisation between an exerciser's movements and the rhythmical qualities of a piece of music. Asynchronous music represents the most widely used form of music application during individual exercise routines (Karageorghis, 2020).

Asynchronous music has been commonly used to reduce perceived exertion and render the exercise experience more pleasant (Karageorghis et al., 2017; Kawabata & Chua, 2021). Collectively, studies have indicated that in-task asynchronous music can reallocate an individual's attentional focus to task-unrelated signals, increase the

frequency of dissociative thoughts, and consequently ameliorate the effects of fatigue-related symptoms (e.g., limb discomfort, increased respiration rate; Bigliassi et al., 2018; Karageorghis & Priest, 2012). Jones et al. (2014) reported that even high-intensity exercise performed at 5% above the first ventilatory threshold (i.e., the point during exercise at which breathing becomes laboured) is rendered more pleasant by the presence of asynchronous music. In the proposed study, music will be applied in the asynchronous mode during an exercise protocol that concludes with volitional exhaustion.

A clutch of studies has indicated that music-induced cerebral phenomena may contribute to exercise performance (for a review, see Karageorghis, 2020). Through adjustments of neural dynamics, music-related interventions were found to guide attention away from the unpleasant sensations caused by exercise-related tasks (Bigliassi et al., 2019; Bigliassi et al., 2016). Reallocating attention outwardly during exercise was associated with reduced frontal–central connectivity (Bigliassi et al., 2017) and increased activation of the left inferior frontal gyrus (Bigliassi et al., 2018). Furthermore, the parietal cortex was found to be implicated in the conscious awareness of bodily sensations through neural inputs from thalamocortical neurones (Crossman & Neary, 2014). Most of the aforementioned electroencephalogram (EEG) and functional magnetic resonance imaging (*f*MRI) studies used relatively simple motor tasks (e.g., isometric handgrip, ankle-dorsiflexion task) that are somewhat disconnected from ecological physical activities (e.g., cycling, running). This is due mainly to the severe motion-related methodological restrictions of current brain-imaging technologies (Karageorghis et al., 2018).

A neuroimaging technique used to assess brain metabolism is functional near-infrared spectroscopy (*f*NIRS), which entails a non-invasive imaging method that quantifies chromophore concentration resolved from the measurement of near-infrared light attenuation, temporal or phasic changes. This technique is particularly salient to exercise-related protocols given its high tolerance for motion artefacts (Leff et al., 2011). In addition, the neurophysiological mechanisms that underlie the influence of

attentional manipulation on tissue oxygenation during exercise can be investigated with an acceptable degree of temporal resolution (up to 50 Hz; Vitorio et al., 2017).

*f*NIRS is a technique that has proven to be effective in the examination of cortical oxygenation during exercise (Herold et al., 2017). Notably, performance of a cycling task increased prefrontal (i.e., medial prefrontal cortex [mPFC] and dorsolateral prefrontal cortex [dlPFC]) oxygenation that became stable over time (Tempest et al., 2017). Similar results were reported by Jones and Ekkekakis (2019) across the dlPFC during recumbent cycling. Specifically, these authors showed that higher levels of right dlPFC oxygenation were associated with lower ratings of affective valence for participants who reported a preference for low-intensity exercise. They suggested that the observed dlPFC activity was associated with the cognitive regulation of unpleasant affective responses to exercise. This was experienced to a greater degree by participants with low preference-for-exercise levels when compared to their high-preference-for-exercise counterparts.

The sensation of discomfort and pain is often an indication to the organism that exercise should be surceased. These signals become more intense at the respiratory compensation point (RCP); the moment during exercise at which minute ventilation starts to become excessive in relation to exhaled carbon dioxide. Studies that have used *f*NIRS to evaluate mPFC and dlPFC haemodynamics have reported a decrease in cerebral oxygenation at intensities above the RCP (e.g., Ochi et al., 2018; Rupp & Perrey, 2008). The reduced availability of oxygen in the brain might influence central nervous system motor output, and constitutes a signal that eventually leads to a sharp degradation in exercise performance.

The cerebral haemodynamic phenomena that are observed during high-intensity exercise have blood-related concomitants, insofar as blood pH is reduced with the onset of anaerobic metabolism (Bhambhani et al., 2007). Nonetheless, the directionality of the relationship between cerebral haemodynamics and blood lactate is presently only a matter for speculation (i.e., whether it is a cerebral mechanism that instigates the onset of lactic acid generation; c.f. Quistorff et al., 2008). The patterns of cerebral

(de)oxygenation observed in the PFC at the RCP are associated with the neural control of the musculature as well as processing of emotions, thoughts, and afferent feedback from the working muscles and internal organs. In order to counteract the effects of fatigue, the dlPFC relies on motivational signals travelling through the mesocortical and mesolimbic systems. Insufficient motivation (either conscious or unconscious) to maintain movement execution is likely to cause deoxygenation of the central executive network and task disruption (Bigliassi et al., 2022). At acute levels of brain deoxygenation, the organism is driven towards the discontinuation of exercise (Ekkekakis, 2009; Perrey, 2008).

Music can be used to prolong physical effort, possibly through the neurophysiological effects that it has at, or close to, the RCP (Bigliassi et al., 2017; Karageorghis et al., 2018). Two hypotheses have been offered to account for the neurophysiological mechanisms that underlie the effects of music during exercise and physical activity: (a) music delays the decrease in prefrontal oxygenation and shifts "the entire oxygenation curve towards higher levels of exercise intensity" (Karageorghis, 2020, p. 942); (b) music delays the increase in prefrontal oxygenation due to a reallocation of attention towards exteroceptive cues (Karageorghis et al., 2017; see Figure 1). Notably, Jones and Ekkekakis (2019) reported an increase in dlPFC oxygenation over time during recumbent cycling, but no such difference was observed between a music condition and a no-music control. In this study, however, participants did not continue cycling until volitional exhaustion but stopped after 15 min. Accordingly, it is plausible that, rather than attenuate prefrontal oxygenation, the application of music delayed the decline that accompanies volitional exhaustion.

Objectives and Hypotheses

The purpose of the proposed study will be to determine the point of onset of cerebral oxygenation decline during an incremental exercise protocol and how this is modulated by the presence of asynchronous music. More specifically, we will assess the effects of pleasurable auditory stimuli (i.e., music) on the cerebral oxygenation curve during a cycle ergometry exercise task. The task will be executed under three

conditions: asynchronous music, an audiobook control and a no-audio control. The audiobook condition will be included to control for the effects of auditory attentional distraction that is devoid of musical components (e.g., melody and harmony). Brain oxygenation will be recorded using a continuous-wave *f*NIRS system over the bilateral mPFC, dlPFC, primary motor cortex and lateral parietal cortex.

We hypothesise that the decrease in prefrontal (i.e., mPFC and dlPFC) oxygenation will be observed earlier under conditions in which participants exercise in silence or with an audiobook when compared with exposure to asynchronous motivational music (H_1). Exercise in silence or with an audiobook will lead to less prefrontal (H_2) and parietal (H_3) activation when compared to exercising with music. In addition, as a sanity check for the effect of music exposure on prefrontal and parietal brain activity, we hypothesise that the occipital cortex activation will not differ among the experimental conditions (i.e., negative control; H_4). We ran a series of pilot tests to confirm that the proposed experimental protocol is logistically feasible and that planned analyses will allow us to test the research hypotheses (see Methods section).

Methods

Participants

Volunteer adults will be eligible if in the age range 18–35 years, recreationally active, and apparently healthy. Recreationally active is defined as those who engage in 45–90 min of moderate-intensity exercise (3–6 metabolic equivalents [METs]) 2–4 times a week over the previous 6 months (see Kelleher et al., 2010). To be included in the study, participants will need to have brought a recent (under 12 months) medical certificate from their personal physician stating that they are fit to engage in high-intensity physical exercise. Participants will be excluded from the study if they self-report: (a) exercising > 5 times per week at moderate intensity, (b) incidents of motor dysfunction, (c) hearing deficiency, (d) epilepsy, or (e) head trauma (i.e., loss of consciousness for more than 5 min). They will be compensated for their time (i.e., €40 for the completion of all four trials).

The sample size for the critical statistical test of each research hypothesis was calculated using R with the "pwr" and "TOSTER" packages (the code is available here: https://doi.org/10.5281/zenodo.6261358). The required sample size has been computed for paired-samples t tests, which are the critical statistical tests (see Table 1). The *f*NIRS results of Ozawa et al. (2019) were used as a parameter for H_1–H_2 across the mPFC. For H_1–H_2 across the dlPFC and H_3, the *f*NIRS results of Oh et al. (2018) were used. For H_4, the *f*NIRS results of Guérin et al. (2021) were used. For H_1–H_2, the power analysis indicated that 30 participants would be required for the mPFC ($d = 0.64$; $\alpha = .02$; 1-$\beta = .90$) and nine participants for the dlPFC ($d = 1.38$; $\alpha = .02$; 1-$\beta = .90$. In addition, nine participants would be required for H_3 ($d = 1.37$; $\alpha = .02$; 1-$\beta = .90$) and 36 participants for H_4 ($d = 0.62$; $\alpha = .02$; 1-$\beta = .90$; see Table 1). Accordingly, a sample of 36 participants will be recruited for the proposed study.

The small telescopes approach was used to determine the smallest effect size of interest (SESOI; i.e., the difference that is considered too small to be meaningful; Simonsohn, 2015). Accordingly, the SESOI was set to the effect size that an earlier study would have had 33% power to detect (Lakens et al., 2018)[1]. The *f*NIRS results of Oh et al. (2018) were used as parameters for H_1–H_4, with a one-tailed test for H_1–H_3, and a two-tailed test for H_4. The SESOI computations were performed using R (the code is available as supplementary material here: https://doi.org/10.5281/zenodo.6261358) and the outputs are displayed in Table 1.

Experimental Procedures

The study will consist of four sessions. There will be a minimum recovery period of 48 hours between session. Participants will be advised to refrain from engaging in physical activity the day of the experiment. They also will be advised to avoid intense physical activity the day before the experiment.

[1] The effect sizes used for the sample size computation and SESOI are two distinct entities. More precisely, the sample size computation serves to ensure that the study is appropriately powered (i.e., good probability that the statistical test will detect an effect that actually exists), while the SESOI serves to ensure that a true effect exists (for further details, see Sullivan & Feinn, 2012).

Session 1 will entail screening, administration of questionnaires, and protocol habituation. Sessions 2–4 will be administered in a fully counterbalanced order and comprise cycling (a) with asynchronous music (120–123 beats per minute [bpm]), (b) with an audiobook (audio control), (c) without any extraneous auditory stimuli (i.e., ambient noise control). The procedure used for the selection of motivational music tracks is presented in Supplementary File 1.

During Session 1, the participant will read an information sheet, be afforded an opportunity to ask questions and sign an informed consent form. Participants will perform an incremental $\dot{V}O_{2max}$ test on a cycle ergometer (Ergomedic 874E, Monark, Vansbro, Sweden) to determine a work rate representative of 5% above the first ventilatory threshold (VT1; for details on its determination, see Supplementary File 2). Five percent above VT1 will be computed for each participant using the heart rate variability index of root mean square of successive differences (see Karapetian et al., 2008). Participants will also be administered several questionnaires relating to (a) socio-demographic and anthropometric details, (b) self-reported physical activity level (International Physical Activity Questionnaire, IPAQ; Craig et al., 2003), (c) motivation to engage in physical activity (Behavioural Regulations in Exercise Questionnaire, BREQ-3; Markland & Tobin, 2004) and (d) tolerance of exercise intensity (Preference for and Tolerance of the Intensity of Exercise Questionnaire, PRETIE-Q; Carlier et al., 2017).

During Sessions 2–4, participants will undergo an exercise test on the cycle ergometer. The ambient temperature will be controlled with the use of a climate-control system to maintain 20°C. Participants will cycle at a constant rate of 63 rpm (revolutions per minute) to avoid synchronisation of the pedal revolutions with the tempo of the music tracks (i.e., 120–123 bpm). After a 5-min warm up at 5% below VT1 and a 1-min transition phase performed at VT1, the resistance of the cycle ergometer will be increased so that the participant exercises at 5% above VT1. For the experimental conditions, the auditory stimulus (i.e., asynchronous music or audiobook) will be played to the participant from 1 min before the end of the warm-up session up

to the point at which they reach volitional exhaustion. The session will be terminated when the participant is no longer able to maintain the prescribed pedal rate of 63 rpm for a period > 10 s[2] (see Figure 2). Thereafter, there will be a 3-min active warm down at 63 rpm at an intensity of 5% below VT1.

Data Acquisition and Processing

Questionnaires

Core affect (Feeling Scale and Felt Arousal Scale; Hardy & Rejeski, 1989; Svebak & Murgatroyd, 1985), perceived exertion (Borg Category Ratio-10 scale, CR10; Borg, 1982) and attentional focus (Attention Scale; Tammen, 1996) will be assessed during the cycle ergometer exercise (i.e., at the beginning and end of warm up, every 2.5 min into the 5% above VT1 stage, at the beginning and end of the active recovery stage, and at the end of passive recovery; see Figure 2). Physical activity enjoyment (Physical Activity Enjoyment Scale, PACES; Delignières & Perez, 1998) and remembered pleasure (visual analogue scale developed by Zenko et al., 2016) will be assessed at the end of each experimental session.

Cardiorespiratory Monitoring

Respiratory-rate monitoring will be facilitated by use of TSD201 respiratory effort transducer, connected to a MP150 Biopac device (Biopac Systems, Goleta, USA). This respiratory belt will be placed around the chest wall, at the level of the sternum. The fs will be set to 250 Hz. Data acquisition was facilitated by the AcqKnowledge software that is included in the MP system. Heart rate will be assessed by means of a Polar system (H10 Polar strap) and the HRV Logger app (correction = workout). The *f*NIRS technique measures cerebral oximetry, which is strongly associated with respiratory and cardiac functioning (Pinti et al., 2019). Using spectral analysis (Welch's

[2] The duration of the exercise will thus vary in accord with the individual's physiological capacity. Nonetheless, because the recruited participants will all have similar physical fitness levels (i.e., recreationally active), the 5%-above-VT1 phase should be rather brief and its duration fairly consistent among participants. If outliers are detected, they will be removed prior to the subsequent analyses (see Statistical Analyses subsection).

estimation method), both heart and respiratory rates can be identified in the *f*NIRS signal. The ability to identify these two frequency components will serve to ensure the validity of *f*NIRS measures.

Head Blood-Volume Pulse Assessment

To control for extra-cerebral noise, non-cortical haemodynamic responses will be monitored by means of a photoplethysmograph sensor (Shimmer3 GSR+ unit; Shimmer, Dublin, Ireland) that will be attached to the participant's earlobe. In accord with the SPA-*f*NIRS guidelines (Scholkmann et al., 2022), the recorded blood-pulse volume (frequency of sampling [fs] = 128 Hz) will be regressed from the collected *f*NIRS signals to account for non-cortical haemodynamic responses that represent potential confounds.

fNIRS Headset Shift Monitoring

Performing a motor task (e.g., cycling) can cause a shift in the position of the *f*NIRS headset. If a headset shift occurs during an experimental session, the exact source of recorded haemodynamic signals is rather difficult to determine. Thus, a motion capture technique (Qualisys MoCap, Götebord, Sweden) will be used to detect shifts in the *f*NIRS headset within each experimental session. Specifically, one passive marker will be taped to the participant's right temple and two markers to the *f*NIRS headset.

To verify the occurrence of an *f*NIRS headset shift, the surface of the planar triangle connecting the 3D markers will be computed over a 30-s timing window (a) at the beginning of the warm-up phase and (b) 30 s before volitional exhaustion (see Equation 1; Guérin et al., 2021).

$$\overrightarrow{M_0M_1}(t) \cdot \overrightarrow{M_0M_2}(t) = \begin{pmatrix} x_1(t) - x_0(t) \\ y_1(t) - y_0(t) \\ z_1(t) - z_0(t) \end{pmatrix} \cdot \begin{pmatrix} x_2(t) - x_0(t) \\ y_2(t) - y_0(t) \\ z_2(t) - z_0(t) \end{pmatrix} \quad (1)$$

where 0 is the temple marker, 1 is the first headset marker, 2 is the second headset marker and t is the time point. The percentage of variation between the two values will be calculated. An *f*NIRS headset shift will be detected if this value exceeded 15% (i.e., 10 mm). A participant's entire data set will be removed prior to further analyses if a

*f*NIRS headset shift is detected in at least one session (see Figure 5). Any excluded participants will be replaced to ensure that $N = 36$.

fNIRS Data

The *f*NIRS technique will be used to monitor the brain activity of participants. This technique consists of placing light source and detector optodes on the surface of the scalp. Adjacent sources and detectors of infrared light are ~3 cm apart. The depth of analysis into the cortex is 0.5–2.0 cm with the system that will be used in the proposed study (FOIRE-3000/16; Shimadzu, Kyoto). The system's light beam emanates from three lasers (class 1M) at three wavelengths of 780, 805 and 830 nm. The equipment contains 16 light sources (multicomponent glass bundle fibres) and 16 detectors (multi-alkali photomultipliers detectors).

The *f*NIRS headset holding the optodes will be placed on the participant's head in accord with the International 10–20 system guidelines for standard electrode positions (Jasper, 1958). In the proposed study, the brain regions of interest will be the bilateral dlPFC (Brodmann areas [BAs] 9 and 46), medial prefrontal cortex (BAs 10 and 11), lateral parietal cortex (BA 39 and 40) and primary visual cortex (BA 17). Thus, a 26-channel model (11 sources and 15 detectors) will be designed in order to cover the brain ROIs over both the left and right hemispheres (see Figure 4). The fOLD toolbox (*f*NIRS Optodes' Location Decider; Morais et al., 2018) will be used to guide the selection of optimal optode positioning with respect to the brain ROIs[3] (see Supplementary File 3).

A system calibration will be conducted at the beginning of each experimental session by means of automatic adjustment using LabNIRS to verify that all optodes are emitting correctly. In case that the amount of light detected will be insufficient, the participant's hair will be pushed back beneath each problematic source–detector couple until data can be reliably collected. The sampling frequency will be set at 10 Hz (i.e., temporal resolution of 100 ms).

[3] The obtained optode array will be the same for all participants because the *f*NIRS headset is rigid and does not facilitate customisation of optode positioning.

To control for the quality of acquired *f*NIRS data, the power-spectral density will be computed using Welch's estimation method for each participant, session and channel. The frequency corresponding to maximal peak in the 100–250 bpm range will be detected in the power-spectral density of the raw *f*NIRS data (for a similar procedure, see Pinti et al., 2019). To guarantee that the identified frequency is the genuine heart-rate frequency, it will be compared to the heart-rate measurements provided by the Polar system, with a tolerance threshold of 10 bpm (Guérin et al., 2021, 2022). A channel will be excluded if heart rate frequency is not found in the *f*NIRS signals (see Figure 3). The number of excluded channels will be reported in the final manuscript in the interests of transparency. A participant's entire data set will be removed prior to further analyses if all channels pertaining to at least one region of interest (ROI) are excluded on this basis. Any excluded participants will be replaced to ensure that $N = 36$.

The presence of the heart pulse is a necessary but not sufficient condition to ensure the quality of *f*NIRS data (Pollonini et al., 2016). Thus, the QT-NIRS toolbox (Quality Testing of Near-Infrared Scans; Hernandez & Pollonini, 2020) will be used to identify channels with poor optical coupling though the computation of the scalp-coupling index (cardiac filter = 2.5–4 Hz; time window = 5 s; λ = 805 and 830 nm). For a given participant and channel, *f*NIRS signals characterised by a scalp-coupling index < 0.7 for at least 10% of the time segment of interest (i.e., 5%-above-VT1 phase) will be removed prior to further analyses. As for the power-spectral density check, a participant's entire data set will be removed prior to further analyses, if all channels pertaining to at least one ROI are excluded on this basis. Any excluded participants will be replaced to ensure maintenance of $N = 36$.

Correction for motion artefacts will be performed using wavelet filtering (interquartile range = 0.5) in Homer 3 (v1.58.0; Massachusetts General Hospital, Boston, MA). The motion-corrected data will be visually inspected to ensure that the selected interquartile range value is well suited to the *f*NIRS data. For a given participant, channels in which motion artefacts will still be visible (i.e., high-frequency

spikes and/or baseline shifts) will be removed prior to further analyses. To reject both cardiac and breathing rates along with parts of Mayer oscillations, a lowpass filter set at 0.1 Hz will be applied (see Figure 5).

For each participant and condition, the *f*NIRS data between the beginning and end of the 5%-above-VT1 phase will be extracted and referred to as a trial. The mean haemodynamic response function (HRF) will be computed for each ROI (i.e., mPF, dlPFC, motor cortex, parietal cortex). For each trial i, a polynomial regression will be fitted to the HRF. Thereafter, the decrease in cerebral oxygenation D_i will be defined as the time point at which the polynomial regression reaches its maximal value (see Figure 6). To account for possible differences in exercise duration among participants, D_i will not be expressed in absolute time but rather as a percentage of the 5%-above-VT1 phase (e.g., if a participant exercises at 5% above VT1 for 10 min and the maximal value of the polynomial regression is reached at 9 min, D_i will correspond with 90%). To estimate the amplitude of changes in oxygenation during a trial, a linear regression will also be fitted to each HRF from the beginning of the 5%-above-VT1 phase to D_i (see Mandrick et al., 2013, for a similar procedure). The amount of cerebral oxygenation will be identified by the slope coefficient of the linear regression, referred to as β_i (see Figure 6).

Statistical Analyses

The statistical analyses will be performed using RStudio (v.1.2.5019). The raw data files and the associated data processing algorithms (pre-processing, statistics and visualisations) will be available as supplementary materials.

Data Eligible for Analysis

Participants characterised by a duration of the 5%-above-VT1 phase unusually short or long will be removed prior to further statistical analyses. Data will be screened for univariate outliers using standardised scores (i.e., z scores). Participants with z scores $> \pm 3.29$ will be excluded and replaced to ensure that $N = 36$.

Classic Null-Hypothesis Significance Tests

Data from the questionnaires will be analysed by means of one-way repeated-measures (multivariate) analysis of variance (RM [M]ANOVA; audio condition [music, audiobook, control]). The cardiorespiratory data will also be analysed and reported in a supplementary file. Because HbO_2 benefits from a better signal-to-noise ratio (see Gervain et al., 2011), only D_{HbO2} and β_{HbO2} will be used to support or refute the hypotheses. Nonetheless, HHb indices will also be analysed and the findings reported in the interests of transparency. D_{HbO2} and β_{HbO2} will be analysed for each ROI (see Suzuki et al., 2004) by means of RM ANOVAs for H_1–H_3. The critical statistical tests used to confirm or disconfirm hypotheses will be the associated pairwise t tests from the post hoc analyses (see Table 1).

Normality will be checked in each cell of the analysis using the Shapiro–Wilk test. Where normality is violated, for nonself-reported data, a transformation will be used in accord with the nature of the distribution curve (e.g., log10, square root; see Figure 5). Where Mauchly's test indicates violations of the sphericity assumption, Greenhouse–Geisser corrections will be applied to the F test. Bonferroni adjustments pairwise/multiple comparisons will be used where necessary to identify where differences lie. In accord with the stipulations of the periodical *Cortex*, the significance level will be set at $p < .020$ for all analyses. Partial eta squared and Cohen's d effect sizes will be reported alongside each inferential analysis.

Outcome-Neutral Validation Tests

A negative control condition will be included by placing two additional channels over the occipital brain region (Broadmann's area 17). This region is involved primarily in visual perception and so its activation should not differ in response to the experimental conditions. To confirm that similar haemodynamic responses of the primary visual cortex will be observed regardless of the audio condition (H_4), two one-sided tests (TOSTs) will be used (Lakens et al., 2018). In this procedure, the results of both t tests needed to reach significance in order for equivalence to be claimed. Statistically nonsignificant differences will provide a means by which to confirm that

observed mPFC, dlPFC and parietal differences are related to the audio manipulations. If differences are detected over the occipital brain region, the mean occipital HRF will be removed from all other HRFs (for a similar rationale, see Guérin et al., 2021). TOSTs will be computed using the TOSTER R package for paired-samples t tests (Lakens, 2017).

Anticipated Timeline for Completion of the Proposed Study

If the present contribution were to be accepted for publication, data collection would be conducted within a 6-month timeframe. We estimate the time for data pre-processing and analysis to take a further 2 months. Accordingly, we are likely to submit our Stage 2 manuscript within 9 months of acceptance of the present Stage 1 manuscript.

Open Practices

Pilot data and codes are available on a public Zenodo repository (https://doi.org/10.5281/zenodo.6261358). All anonymised raw and processed data supporting the reported analyses will be archived in this repository at the point of Stage 2 submission. Methodological details pertaining to the proposed study were preregistered using the *f*NIRS preregistration template developed by Schroeder et al. (2023; see Supplementary File 4).

CRediT Author Statement

First author: Conceptualisation; Methodology; Formal analysis; Data curation; Software; Visualisation; Writing – original draft; Writing – review & editing. Second author: Conceptualisation; Methodology; Formal analysis; Supervision; Writing – review & editing. Third author: Writing – review & editing. Fourth author: Conceptualisation; Methodology; Writing – review & editing. Last author: Conceptualisation; Funding acquisition; Resources; Supervision; Writing – review & editing.

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Table 1

Estimated Required Sample and Effect Sizes

Question	Hypothesis	Sampling plan	Analysis plan	Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis	Interpretation given different outcomes	Theory that could be shown wrong by the outcomes
The decrease in prefrontal oxygenation will be observed earlier under conditions in which participants exercise in silence or with an audiobook, when compared with exposure to asynchronous motivational music.	$D_{\text{HbO2,mPFC}}$ will be larger during the music condition vs. the audiobook and silence conditions.	$N = 30$ ($d = 0.64$; $\alpha = .02$; 1-$\beta = .90$)	Pairwise t tests	Small telescopes approach ($d_{\text{SESOI}} = 0.28$).	The hypothesis will be accepted if the statistical test is significant ($p < .020$) and the associated Cohen's $d > d_{\text{SESOI}}$.	Karageorghis et al.'s (2017) Hypothesis A (see Figure 1) logically extended to mPFC activity.
	$D_{\text{HbO2,dlPFC}}$ will be larger during the music condition vs. the audiobook and silence conditions.	$N = 9$ ($d = 1.38$; $\alpha = .02$; 1-$\beta = .90$)	Pairwise t tests	Small telescopes approach ($d_{\text{SESOI}} = 0.38$).		Karageorghis et al.'s (2017) Hypothesis A (see Figure 1).

Continued

Question	Hypothesis	Sampling plan	Analysis plan	Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis	Interpretation given different outcomes	Theory that could be shown wrong by the outcomes
Less prefrontal activation will be observed when participants exercise in silence or with an audiobook, when compared to when they exercise with music.	$\beta_{\text{HbO2,mPFC}}$ will be larger during the music condition vs. the audiobook and silence conditions.	$N = 30$ ($d = 0.64$; $\alpha = .02$; 1-$\beta = .90$)	Pairwise t tests	Small telescopes approach ($d_{\text{SESOI}} = 0.28$).	The hypothesis will be accepted if the statistical test is significant ($p < .020$) and the associated Cohen's $d > d_{\text{SESOI}}$.	Role of the mPFC in appraisal and expression of negative emotion as proposed by Etkin et al. (2011).
	$\beta_{\text{HbO2,dlPFC}}$ will be larger during the music condition vs. the audiobook and silence conditions.	$N = 9$ ($d = 1.38$; $\alpha = .02$; 1-$\beta = .90$)	Pairwise t tests	Small telescopes approach ($d_{\text{SESOI}} = 0.38$).		Karageorghis et al.'s (2017) Hypothesis B (see Figure 1).
Less parietal activation will be observed under conditions in which participants exercise in silence or with an audiobook, when compared to when they exercise with music.	$\beta_{\text{HbO2,lPC}}$ will be larger during the music condition vs. the audiobook and silence conditions.	$N = 9$ ($d = 1.37$; $\alpha = .02$; 1-$\beta = .90$)	Pairwise t tests	Small telescopes approach ($d_{\text{SESOI}} = 0.38$).	The hypothesis will be accepted if the statistical test is significant ($p < .020$) and the associated Cohen's $d > d_{\text{SESOI}}$.	Role of the parietal cortex to facilitate the selection of relevant signals proposed by Bigliassi (2021).

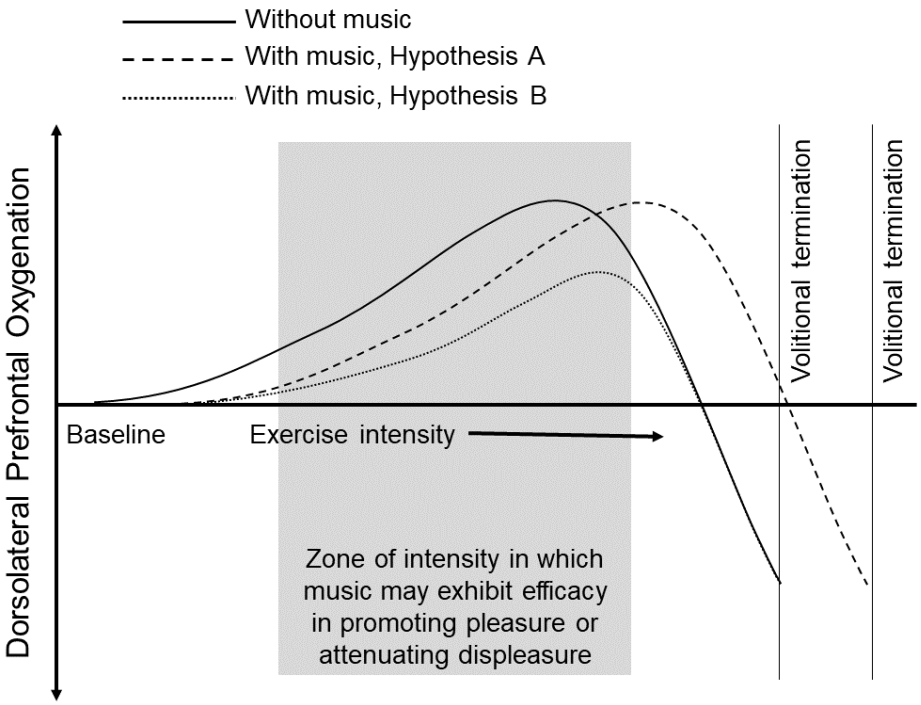
Continued

Question	Hypothesis	Sampling plan	Analysis plan	Rationale for deciding the sensitivity of the test for confirming or disconfirming the hypothesis	Interpretation given different outcomes	Theory that could be shown wrong by the outcomes
Similar haemodynamic responses of the occipital cortex will be observed across conditions.	$\beta_{\text{HbO2,motor}}$ will be similar during the music condition, audiobook and silence conditions.	$N = 36$ ($d = 0.62$; $\alpha = .02$; 1-$\beta = .90$)	TOSTs	Small telescopes approach ($d_{\text{SESOI}} = 0.62$).	The hypothesis will be confirmed if both t tests are significant.	Not applicable (control condition).

Note. Statistical power, planned analyses and critical statistical tests for each research hypothesis. mPFC = medial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; lPC = lateral parietal cortex; RM ANOVA = repeated-measures analysis of variance; TOSTs = two one-sided t tests; SESOI = smallest effect size of interest.

Figure 1

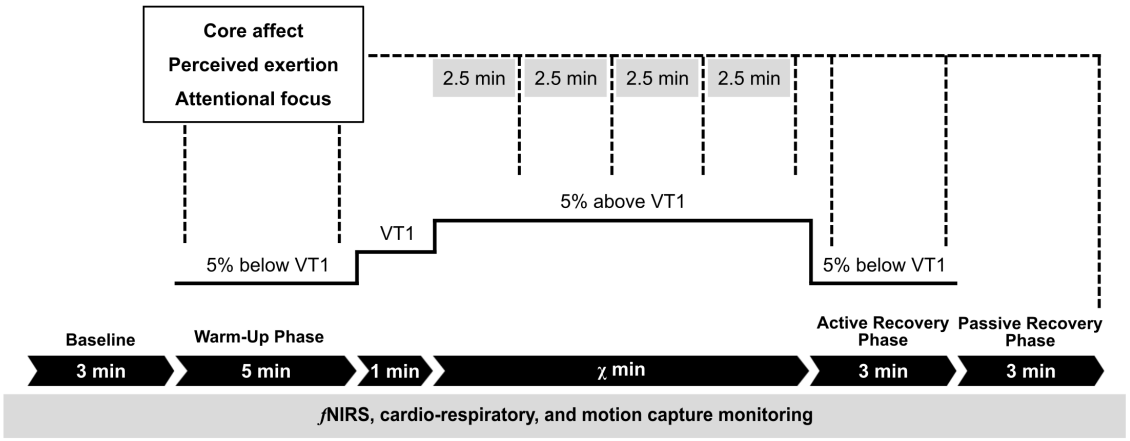
Schematic Representation of the Hypothetical Neurophysiological Mechanisms Underlying the Effect of Music During Exercise



Note. Reproduced from Karageorghis, C. I., Ekkekakis, P., Bird, J. M., & Bigliassi, M. (2017). Music in the exercise and sport domain: Conceptual approaches and underlying mechanisms. In M. Lesaffre, P.-J. Maes & M. Leman (Eds.), *The Routledge companion to embodied music interaction*, p. 288. Copyright 2017 by Routledge. Reprinted with permission through PLSclear.

Figure 2

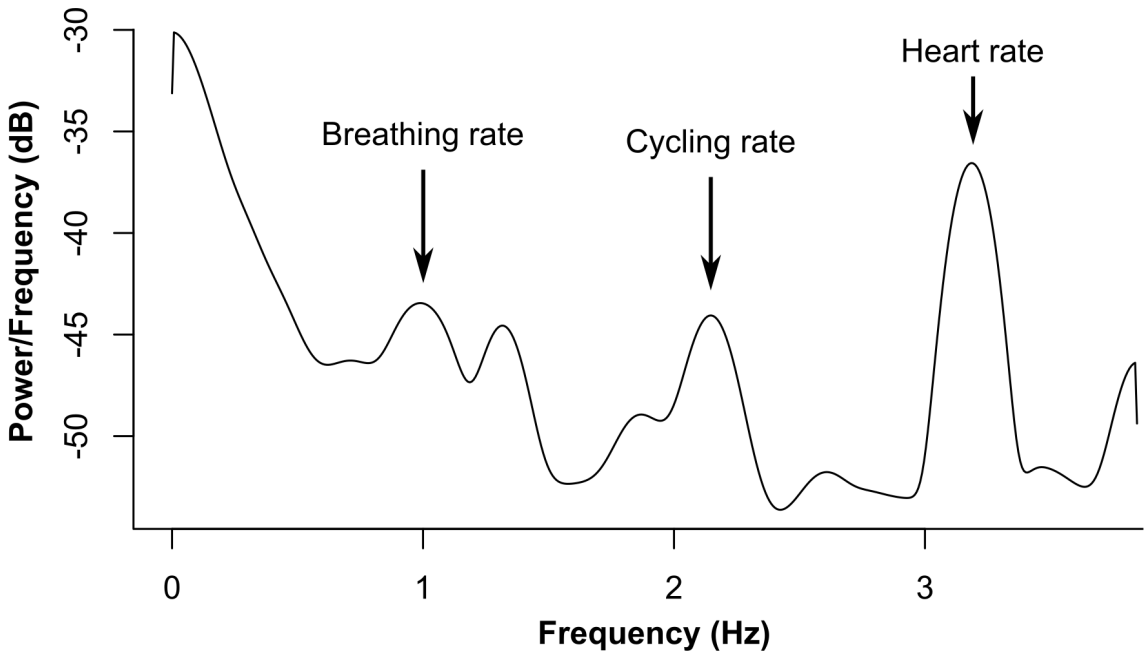
Experimental Protocol for the Proposed Study



Note. VT1 = first ventilatory threshold.

Figure 3

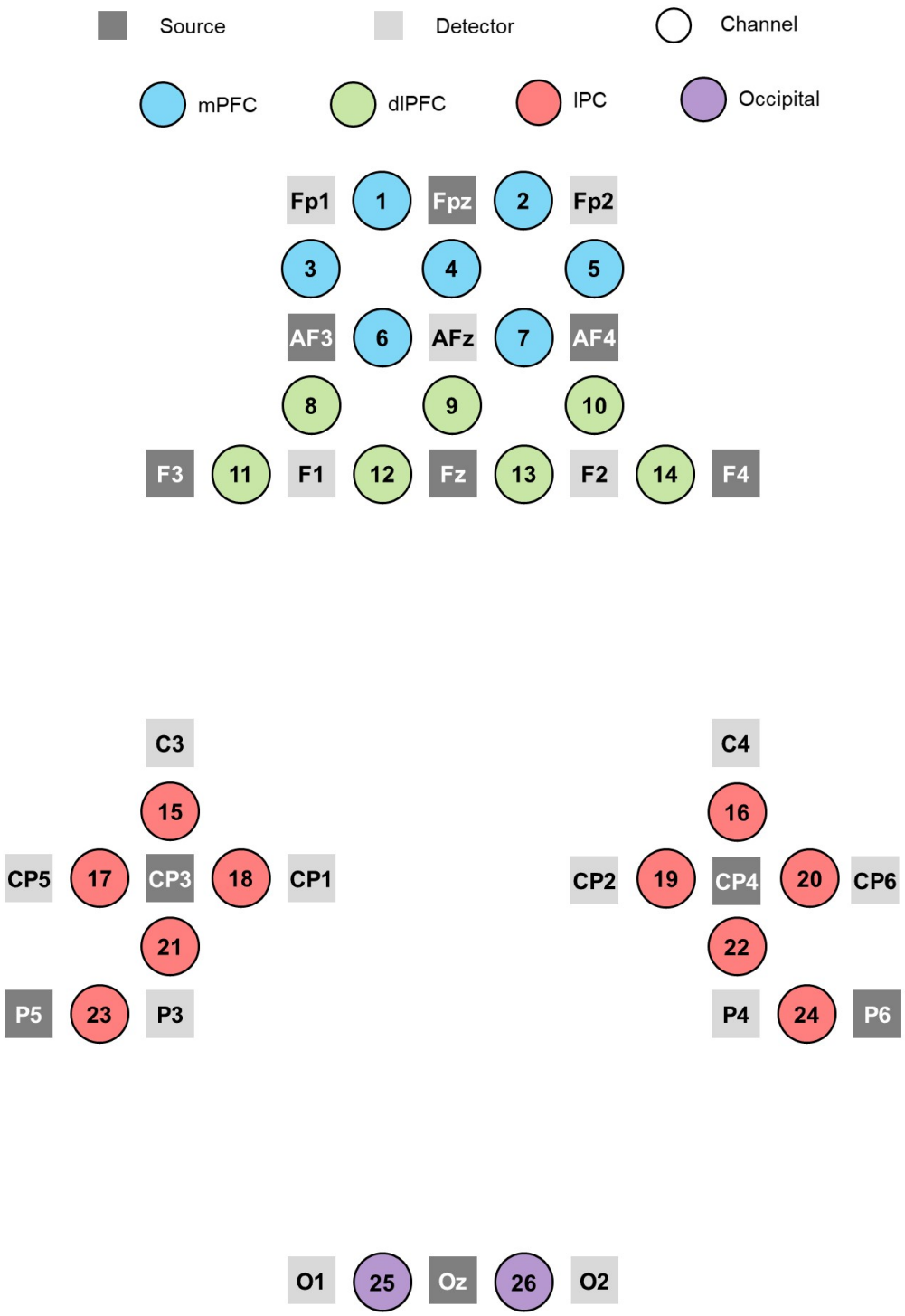
Welch Power-Spectral Density of the raw fNIRS Data



Note. The data were obtained from a pilot test.

Figure 4

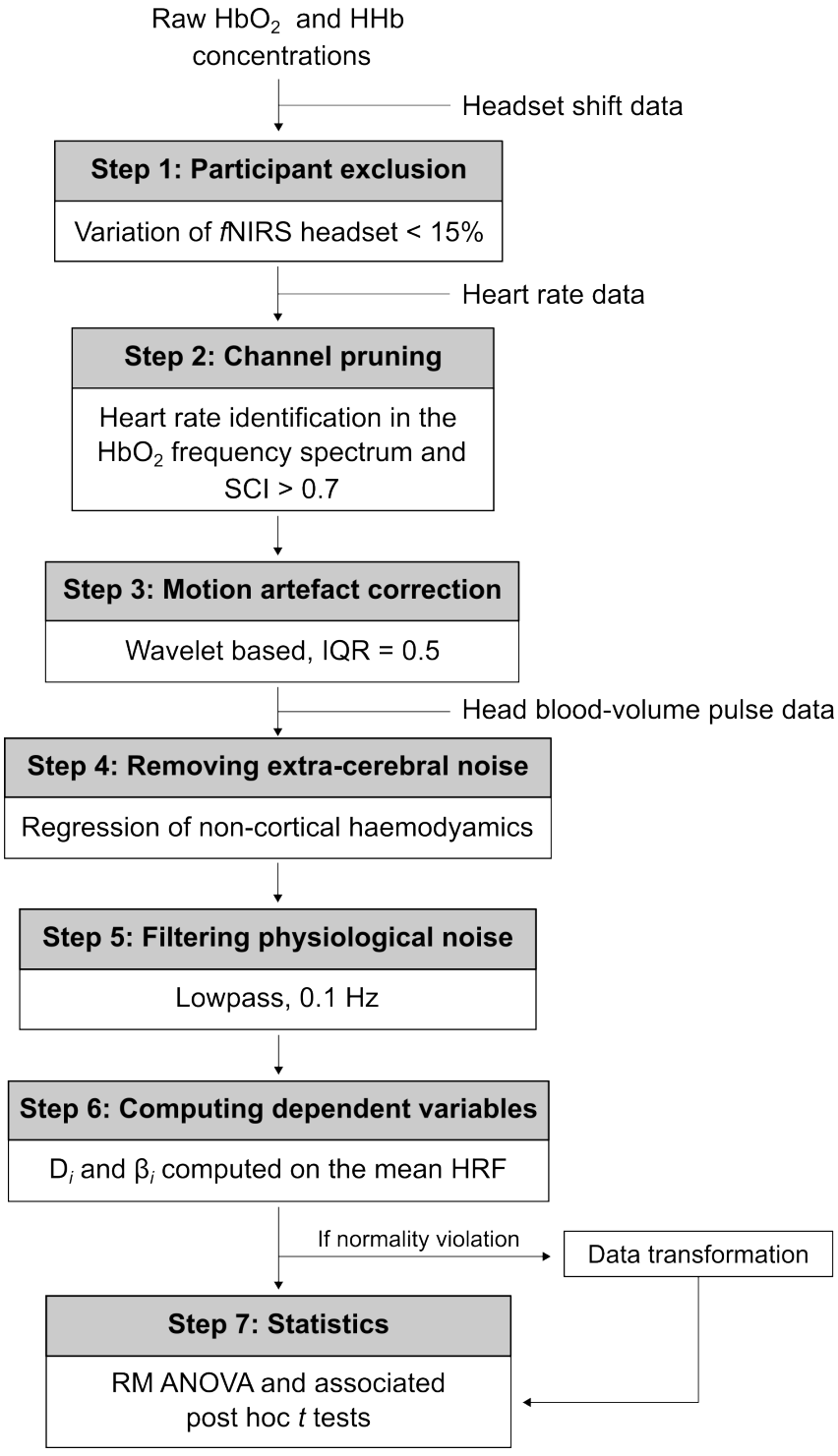
Diagrammatic Representation of the fNIRS Sources, Detectors and Channel Layout



Note. Adjacent sources and detectors will be ~3 cm apart. mPFC = medial prefrontal cortex; dlPFC = dorsolateral prefrontal cortex; lPC = lateral parietal cortex.

Figure 5

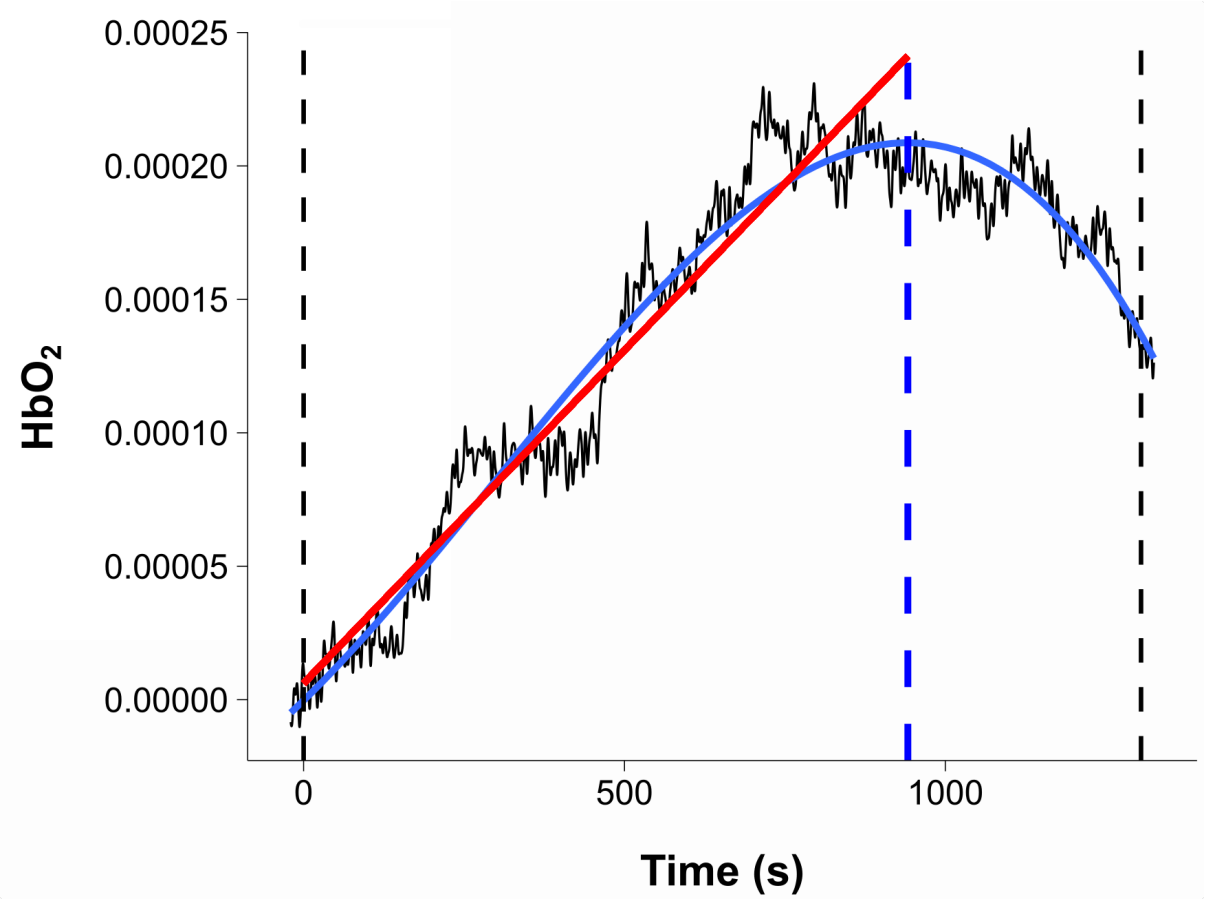
Processing Pipeline of the fNIRS Data



Note. *f*NIRS = functional near-infrared spectroscopy; SCI = scalp-coupling index; IQR = interquartile range; HRF = haemodynamic response function; RM ANOVA = repeated-measures analysis of variance.

Figure 6

Computation of the Dependent Variables on Orbitofrontal Cortex fNIRS Data



Note. The data were obtained from a pilot test. Dotted lines indicate the beginning and end of the 5%-above-VT1 phase. The polynomial regression is displayed in blue. The dotted blue line indicates the time point at which the maximal value of the polynomial regression is reached. The linear regression is displayed in red. Note that 0 on the x axis corresponds with the beginning of the 5%-above-VT1 phase. HbO_2 = oxygenated haemoglobin.