The relationship between brain cortical activity and brain oxygenation in the prefrontal cortex during hypergravity exposure

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The prefrontal cortex (PFC), situated in the anterior part of the frontal lobe of the brain, serves function in cognitive control and emotional processing (7, 19), with an important role in mental performance and psychosocial well being, with the PFC involved in many aspects of stress, including controlling stress response (4). Cognitive performance, emotional control, and stress response are all important factors for the demanding workload and the confined, stressful conditions of long-duration spaceflight missions, and therefore it is important to understand how artificial gravity may affect these factors.

Imaging techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) are the most advanced imaging techniques that can be used in measuring brain activity. However, due to technical and logistical limitations, it is not practical, nor possible, to use such techniques in environments such as hypergravity during centrifugation. Electroencephalograph (EEG), integrated with electrotomography, provides a solution. EEG records the summation of simultaneous activations of multiple neurons within the cortex. An in-depth description of the underlying neural mechanisms, and formation and recording of EEG signals can be obtained from Kirschstein and Kohling (14). EEG combined with electrotomography is used to specify the source of cortical activity within the brain, known also as source localization. A limitation of previous EEG attempts is a poor spatial resolution, thus the source of activity cannot be accurately localized. Electrotomography is inexpensive compared with other imaging techniques, easy to operate, non-invasive (26), and a validated tool for localizing cortical activity (12).

Near-infrared spectroscopy (NIRS) is a non-invasive method used to measure oxygenation and de-oxygenation of tissues within the human body and has been proposed to be used as a measure of brain activity. Brain activation is thought to be accompanied by increases in regional cerebral blood flow and oxygen metabolic rate, with neuronal activation accompanied by an increase in oxyhemoglobin (O$_2$Hb) and a decrease in deoxyhemoglobin (HHb) (2, 18, 20).

Possible effects of artificial gravity on PFC activity may be linked to cardiovascular changes as the legward fluid shift experienced during $+G_z$ may reduce cerebral perfusion, thus lowering oxygenation of the PFC (13). Therefore, the effects of hypergravity during $+G_z$ exposure on cerebral perfusion and oxygenation could be factors behind a reduction in an individual’s mental and cognitive performance observed during hypergravity (9, 17). Although changes in cerebral perfusion have been studied in hypergravity, the relationship between PFC oxygenation and cortical activity has had little study.

ARTIFICIAL GRAVITY, PRODUCED through centrifugation, has been proposed as a method to counteract the microgravity-induced physiological deconditioning of the human body during long-duration spaceflight, by producing a 1G or greater environment intermittently (5). Besides a positive impact of artificial gravity on the musculoskeletal and cardiovascular system (5), its currently remains unclear what effect artificial gravity has on the neurocognitive system.

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Within this study, it is hypothesized that hypergravity induced by a short-arm human centrifuge will reduce oxygenation within the PFC, which will be mirrored by changes in electrocortical PFC activity.

**METHODS**

Participants. Twelve healthy participants without athletic background, aged 27.2 ± 4.5 yr [men (n = 6): 28.8 ± 4.4 yr; women (n = 6): 25.5 ± 4.4 yr], participated in the study. Participants had a varied amount of experience in hypergravity exposure, and none reported taking any medication during the study. In a preexperiment briefing, the participants were informed about the experimental protocol and possible consequences of the acceleration (nausea, dizziness, etc.). Each participant provided written, informed consent before participating and was required to pass a pretesting medical examination, carried out by trained personnel from the German Aerospace Center (DLR). Ethics approval was obtained through the “Ethik-Kommission der Arztekammer Nordrhein,” and was in compliance with national legislation and the Code of Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association (Declaration of Helsinki).

Procedure. The study was completed at the German Aerospace Center (DLR) in Cologne, Germany, providing access to the European Space Agency Short-Arm Human Centrifuge (SAHC), used for the present experiment in hypergravity. The participant was supine throughout centrifugation, harnessed within a nacelle, with the head toward the center of rotation, so that the centrifugal and gravitational acceleration is toward the feet (+Gz acceleration). Gravitational acceleration was measured and is reported at heart level due to interindividual differences in +Gz levels at the feet because of differences in participants’ heights. Baseline measurements were taken during 10 min of 0.5 rpm rotation. Post baseline, gravitational acceleration was increased to 0.6 +Gz for women and 0.8 +Gz for men for a 10-min period. Following this, gravitational acceleration was increased by 0.1 +Gz for 3 min and repeatedly increasing by 0.1 +Gz for 3-min intervals in a stepward manner until the participant reached a level at which presyncope occurred and the centrifugation was terminated. Presyncope was determined through subjective symptoms (head vacuum feeling, daze feeling, omnidirectional vertigo, sudden sensation of heat, nausea, sweating, grayout) and objective symptoms (systolic blood pressure dropoff of >15 mmHg, decline of the heart rate of >15 beats/min suddenly, or the subject showing a significant high tachycardia). To monitor heart rate, a 12-lead electrocardiogram (ECG) was fitted to the participant, and blood pressure was monitored using a blood pressure cuff, both combined into a Philips intellivue XDS solution for viewing on a dedicated monitor as well as a Finometer (Portapres, TVO, Amsterdam, The Netherlands), giving beat-by-beat measurements. Termination of centrifugation was determined by a medical monitor. In addition, video and audio loops, along with a panic button, were available for the participants’ safety during the centrifugation procedure. Participants were asked to keep their head fixed on a head cushion to prevent vestibular input, which might lead to nausea. Throughout the procedure, a hood was placed over the head, so that the participant is in darkness, limiting visual stimuli, helping to prevent nausea.

Due to the sex differences at which +Gz level centrifugation was started and the interindividual differences at which +Gz level the participants became presyncope and centrifugation terminated, the amount of participants completing each different +Gz level was different. To overcome this problem, data were taken from baseline and the last three +Gz levels completed before presyncope for all participants. This created four levels of +Gz: baseline, level 1, level 2, and level 3.

**EEG recording.** An EEG actiCAP (Brain Products, Munich, Germany) with 32 Ag-AgCl electrodes arranged in the international 10–20 system on positions Fp1, Fp2, F3, F4, F7, F8, Fz, FC1, FC2, FC5, FC6, C3, C4, Cz, CP1, CP2, CP5, CP6, P3, P4, P7, P8, Pz, PO9, PO10, T7, T8, TP9, TP10, O1, O2, and Oz was mounted on the participants’ heads. Each electrode was referenced to a reference electrode that was mounted in the triangle of Fp1, FP2, and Fz. There was also a ground electrode. Electrodes were fitted with Electro-Gel (Electro-Cap International) for signal transduction. During recording, impedance of all electrodes was checked and did not exceed 10 kΩ. The analog signal of the EEG was amplified, converted to digital signals, and stored using a Brain Vision amplifier and RecView software (Brain Products). During EEG recordings, the participants had their eyes closed and were asked to neither move nor speak, to produce a state of rest. Recordings lasted for 2 min at baseline and each +Gz level.

**Electromagnetic tomography.** Electromagnetic tomography uses EEG recordings integrated with mathematical algorithms, such as “standardized low-resolution brain electromagnetic tomography” (LORETA) (24). LORETA uses the extracranial signals recorded by EEG, produced by spatial summation of highly synchronized postspike potentials occurring in large clusters of neurons, and models the cortex as a collection of volume elements (voxels) in the digitized Talairach atlas (22). LORETA corresponds to the three-dimensional distribution of electric neuronal activity that has maximum synchronization in terms of strength between neighboring neuronal areas, represented by adjacent voxels (22). The localization capabilities of LORETA compared with other methods shows LORETA to be the most accurate, whereas other methods are especially incapable of localizing deep sources (23). From LORETA recordings, cortical current density can be determined, which is the measure of the density of flow of electrical charge, calculated in microampere (μA/mm²), localized to a specific brain region, such as the frontal cortex. Cortical current density defines the electrical activity of neurons in a specific area, allowing for the comparison of brain regions using a non-invasive, versatile method, such as EEG.

**EEG data analysis.** EEG data were processed using the Brain Vision Analyzer 2.0 (Brain Products). The data were first filtered to remove interfering frequencies. The data were then segmented into 4-s sections, with the first and last 10 s of the 2-min recording rejected to eliminate any timing issues relating to the subject not being in a state of rest, producing a data recording period of 100 s for baseline and each +Gz level. A systematic protocol for excluding artifacts followed, including careful visual inspection of all EEG data, with removal of electrodes, which had poor or no signal, then interpolated using surrounding electrodes. Spectral analysis was completed to identify any artifacts occurring at high frequencies, undetected by previous artifact rejection processes. Unexplained interfering frequencies above 70 Hz were found to be present in all channels and thus were removed. The whole artifact rejection procedure was cross-checked by a second experienced and uninvolved person. The data were averaged across the 100-s recordings. LORETA analysis of the prefrontal cortex was completed using Brain Vision Analyzer 2.0. Average cortical current density was calculated for each 100-s period at baseline and +Gz levels. Logarithm transformation was applied to cortical current density values before statistical analysis, producing ln[Power(μA²/mm⁴)].

**NIRS recording.** Changes in PFC O2Hb and HHb were recorded using a two-channel portable NIRS-system (Oxymon, Artinis, The Netherlands) on the left and right prefrontal cortex of participants throughout centrifugation at a sampling frequency of 50 Hz. The NIRS transmitter and receiver diodes were fitted to the EEG-actiCAP in positions on the left and right prefrontal cortex. NIRS recordings were taken at the same time as EEG recordings.

**NIRS data analysis.** NIRS data were processed using OxySoft DAQ 2.1.6 (Oxymon). Variables analyzed were PFC O2Hb and HHb levels. According to the EEG analysis, the 100-s epochs during baseline and each +Gz level, up to the participants’ presyncope level, were averaged. Values produced are not absolute values but show relative changes in PFC O2Hb and HHb levels. As with the EEG data,
data were taken from baseline and the last three +Gz levels completed before presyncope.

Statistical analysis. A one-way repeated ANOVA was used to test for intra-individual changes in PFC log-transformed cortical current density, O2Hb, and HHb during the different levels of +Gz: baseline, level 1, level 2, and level 3. A Fisher’s least significant difference (LSD) post hoc test was used after the ANOVA to test where significance differences, if any, were found between the changing +Gz levels. A Pearson’s test of correlation was completed for PFC cortical activity vs. O2Hb and for PFC cortical activity vs. HHb to test for any relationship between PFC cortical activity and PFC oxygenation. Significance for all statistical testing was set at \( p < 0.05 \). Statistical testing was performed using the Statistica program 7.1 (StatSoft, Tulsa, OK).

RESULTS

Hypergravity exposure. All 12 subjects (6 men, 6 women) completed the procedure. Maximum +Gz level reached before presyncope occurred when the centrifugation stopped ranged between 0.8 +Gz to 1.4 +Gz (men: 1.1–1.3 +Gz; women: 0.8–1.4 +Gz).

Prefrontal cortex cortical activity. There was a statistically significant difference between +Gz levels as determined by one-way ANOVA \([F(3, 33) = 3.277; P < 0.05]\). A Fisher’s least significant difference post hoc test revealed that PFC cortical activity was statistically significantly higher at +Gz level 2 compared with baseline \((P < 0.05)\) and +Gz level 3 compared with baseline \((P < 0.05)\). No significant difference in PFC cortical activity was found between baseline and +Gz level 1 \((P = 0.397)\), +Gz level 1 and level 2 \((P = 0.164)\), +Gz level 1 and level 3 \((P = 0.063)\), and +Gz level 2 and level 3 \((P = 0.618)\). The results are summarized in Fig. 1.

PFC O2Hb level. There was a statistically significant difference in PFC O2Hb level between +Gz levels as determined by one-way ANOVA \([F(3, 33) = 19.674; P < 0.05]\). A Fisher’s least significant difference post hoc test revealed that PFC O2Hb level was statistically significantly lower at +Gz level 1 compared with baseline \((P < 0.05)\), +Gz level 2 compared with baseline \((P < 0.05)\), and +Gz level 3 compared with baseline \((P < 0.05)\). No significant difference in PFC O2Hb level was found between, +Gz level 1 and level 2 \((P = 0.927)\), +Gz level 1 and level 3 \((P = 0.121)\), and +Gz level 2 and level 3 \((P = 0.143)\). The results are summarized in Fig. 2.

PFC HHb level. There was a statistically significant difference in PFC HHb level between +Gz levels as determined by one-way ANOVA \([F(3, 33) = 15.861; P < 0.05]\). A Fisher’s least significant difference post hoc test revealed that PFC HHb level was statistically significantly higher at +Gz level 1 compared with baseline \((P < 0.05)\), +Gz level 2 compared with baseline \((P < 0.05)\), +Gz level 3 compared with baseline \((P < 0.05)\), and +Gz level 3 compared with +Gz level 1 \((P < 0.05)\). No significant difference in PFC HHb level was found between +Gz level 1 and level 2 \((P = 0.222)\), and +Gz level 2 and level 3 \((P = 0.094)\). The results are shown in Fig. 3.

Relationship between PFC cortical activity and oxygenation. The result of the Pearson’s test of correlation between PFC cortical activity and PFC O2Hb (Fig. 4) was insignificant \((R = -0.250; p < 0.05)\).
Previous research under hypergravity conditions, through centrifugation, have shown and described the effect of +Gz exposure on cerebral perfusion and oxygenation using NIRS (1, 16, 25) but did not relate the deoxygenation to cortical activity. Results from the present study agree with previous findings, since PFC O₂Hb was shown to decrease during hypergravity exposure compared with normal gravitational conditions, mirrored by an increase in PFC HHb level with increasing +Gz level. Furthermore, it could be shown that this decrease in oxygenation is not mirroring a decreased cortical activity, since EEG combined with electrotomography showed an increase in PFC cortical activity on exposure to hypergravity in this study. No significant relationship was discovered between PFC cortical activity and the reduced oxygenation of the PFC during hypergravity exposure.

Relationship between PFC activity and oxygenation. This study has shown no significant correlation between PFC activity and oxygenation during hypergravity exposure. There is, however, a clear relationship between hypergravity exposure and PFC activity, and PFC oxygenation, separately.

A slowing of dominant EEG frequencies (2–8 Hz, mainly in the frontal regions), indicating increased cortical arousal, has been found at high gravitational levels (greater than +5Gz) before gravity-induced loss of consciousness (G-LOC) (10, 30, 33). This was attributed to a decrease in oxygen levels in the brain leading to degraded neuronal functioning, probably as a protective mechanism (32). Before G-LOC, Wilson et al. (33) found a widespread, high-frequency activity at ~33 Hz, starting at 40% of +Gz, at which individuals lost consciousness. At this point, cerebral regional oxygen saturation remained unchanged to normal gravity conditions (11), although broad declines in mean arterial pressure and mean blood flow velocities in the middle cerebral artery have been found between +2Gz and +3Gz (21).

Although it is difficult to compare different G loads achieved on a long-arm and short-arm centrifuge (due to the G gradient present on a short-arm centrifuge), it is suggested that a reduction in oxygenation of the PFC was not the cause of the increased PFC activity observed during hypergravity exposure in the present study.

The PFC has been shown to be linked to stress (4), with an increase in activity related to increased stress and decreased activity when relaxed. Hypergravity, produced through centrifugation, produces a novel environment rarely experienced in everyday life. The psycho-physiological challenges imposed by hypergravity will result in strain placed on the individual; thus it is reasonable to predict that hypergravity exposure will result in increased stress and, therefore, an increase in PFC activity. An increase in stress and arousal during hypergravity has been shown with increases in mean cortical activity, with an increase in beta activity and reduction in alpha activity measured by EEG, increased stress hormones, and decreased rating of perceived physical health and motivation (28). The increase in PFC activity observed is most likely the product of the stressful and novel environment of hypergravity rather than a physiological response, supported by the insignificant correlation between PFC activity and PFC oxygenation.

NIRS as a measure of cortical activity. The use of NIRS as a measure of cortical activity has been proposed as a method that would allow for measurement with easy use under changing gravitational conditions or exercise (31). This is based on the hypothesis that an increase in brain activity is accompanied by an increase in regional cerebral blood flow and oxygen metabolic rate, thus an increase in O₂Hb in a specific region would signal an increase in cortical activity. The presented study found no significant correlation between PFC O₂Hb level or between HHb level and PFC cortical activity, leading to the conclusion that changes in cortical activity level cannot be predicted through regional oxygenation levels measured by NIRS, at least not if a shift of blood volume occurs simultaneously. The slight negative correlation found between PFC cortical activity and O₂Hb levels further argues against the use of NIRS as a measure of cortical activity, since this suggests a
decrease in PFC $O_2$Hb levels with increasing PFC cortical activity, opposite to what has been suggested. During exposure to hypergravity and the resulting shift of blood volume out of the brain, the reduction in $O_2$Hb and the increase in Hb in the PFC may be so great that changes in oxygenation levels due to neuronal activity may be hidden, although NIRS has been proven to have a good temporal resolution, with the ability to investigate millisecond-range neural signals during normal conditions (6). If changes in brain oxygenation resulting from changes in neuronal activity are too small to be detected and distinguished from hypergravity-induced changes in cerebral blood flow, NIRS could not be used as a measure of cortical activity during environments of large changes of cerebral blood flow such as hypergravity and microgravity.

**Implications for artificial gravity use during spaceflight.** The present study, as well as others, found an increase in PFC activity during hypergravity, most likely attributable to psychological stress. This could pose a problem for the use of a SAHC as a countermeasure for astronauts during spaceflight, since an individual would be exposed to hypergravity on a daily basis (5). Due to the role of the PFC in cognitive function and emotional processing, the changes in activity observed during hypergravity could possibly affect these.

It is important to consider the effects of microgravity in relation to PFC cortical activity and oxygenation during the use of hypergravity in space, since the present study only considers changes from normal gravitational conditions; however, during spaceflight, astronauts will transit from microgravity to hypergravity. Schneider et al. (26) discovered changes in frontal activity during weightlessness, concluding these changes were most probably emotionally related rather than due to a hemodynamic shift. Whether microgravity changes in PFC activity persist or return to normal during prolonged microgravity exposure and whether these changes affect the changes in PFC activity seen during hypergravity is yet to be observed.

The use of artificial gravity as a replacement for current countermeasures, e.g., exercise, may not be a full solution to the physiological problems experienced during spaceflight. Exercise is not only used as a countermeasure for the physiological de-conditioning of spaceflight but also the psychological effects due to confinement, workload, stress, etc. (27), and has been shown to be beneficial for mental well being (8) and cognitive performance (3). The present study, as well as previous studies, show hypergravity exposure also increases psychological stress, which could be additive to the general stress of spaceflight. It is therefore important that exercise can be combined with artificial gravity to have both physiological and psychological benefits and for artificial gravity not to be a replacement for the exercise countermeasures currently employed during spaceflight but to be complementary, possibly combined through human-powered centrifugation (15) or exercises that can be completed while on a SAHC.

The success of artificial gravity use as a spaceflight countermeasure relies on determining the optimal duration, frequency, $+G_z$ level, and combination with other countermeasures to provide the greatest physiological benefits, while maintaining psychosocial well being and mental performance.

**Limitations and future challenges.** Although this study produced measurements of brain cortical activity and oxygenation, it is limited by the fact that no measure of psychological stress was collected. Since the increase in PFC cortical activity was not correlated with PFC oxygenation, this study suggests the increased activity is related to an increase in psychological stress, as witnessed in previous studies (29). Future studies should combine measurement of PFC cortical activity with both oxygenation and psychological stress measurements during hypergravity exposure to confirm an increase in PFC activity during hypergravity is not related to oxygenation but to increased stress.

Since PFC oxygenation was only determined by NIRS and other physiological variables such as blood pressure, cardiac output, or cerebral blood flow were not obtained, it can only be assumed that the reduced PFC $O_2$Hb and increased Hb were caused by a hemodynamic shift due to the hypergravity exposure. However, in such experimental conditions as hypergravity, NIRS is a feasible, non-invasive method for the assessment of PFC oxygenation.

In regard to the gravitational profile used in this study, not all participants experienced the same $+G_z$ levels; this limits comparison between specific gravitational loads. The 3-min periods of increasing $+G_z$ levels is not consistent with a gravitational profile expected to be used for artificial gravity use as a spaceflight countermeasure; also, it would be expected that astronauts will be exposed to artificial gravity on a frequent basis, unlike the subjects in this study, which must be considered when extrapolating results from this study for use in relation to artificial gravity use during long-duration spaceflight. The gravitational profile used, however, allowed for observation of changes across a range of $+G_z$ levels of exposure, allowing for comparisons to be made.

In conclusion, the present study has observed an increase in PFC cortical activity, as measured by electroencephalography and electrotomography, and a decrease in PFC oxygenation, as measured by NIRS, during hypergravity exposure provided by a SAHC. Since an increase in brain cortical activity would normally result in an increase in the oxygenation of the underlying tissue, it is concluded that NIRS might not be an appropriate tool to measure brain cortical activity under hypergravity when a possible change in oxygenation caused by neural activity is superimposed by a redistribution of blood volume. The increase in PFC activity was attributed to an increase in psychological stress during hypergravity exposure rather than a physiological response. Due to there being no relationship between PFC activity and oxygenation, it remains questionable whether NIRS can be used as an accurate measure of brain activity during hypergravity. Considerations, in relation to increased PFC activity during hypergravity exposure must be taken into account when artificial gravity as a possible countermeasure for long-duration spaceflight is considered. Further research must be completed to confirm that measurement of brain activity through NIRS during hypergravity cannot be used due to the hemodynamic shift experienced. The optimal duration, frequency, $+G_z$ level, and combination with other countermeasures to give both physiological and psychological benefits must be researched in the future before artificial gravity can be employed as a countermeasure for long-duration spaceflight.

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