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DISSERTATION

Effect of Head-Down Tilt Bed Rest on Brain Structure and Function

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Preface

This thesis is based on the following three peer-reviewed publications:

K. Brauns, A. Friedl-Werner, M. A. Maggioni, H. C. Gunga, and A. C. Stahn, "Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity," *Front. Physiol.*, vol. 12, Feb. 2021, doi: 10.3389/fphys.2021.638669.

K. Brauns, A. Werner, H.-C. Gunga, M. A. Maggioni, D. F. Dinges, and A. Stahn, "Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization," *Sci. Rep.*, vol. 9, no. 1, p. 16610, Dec. 2019, doi: 10.1038/s41598-019-52555-1.

K. Brauns, A. Friedl-Werner, H.-C. Gunga, and A. C. Stahn, "Effects of two months of bed rest and antioxidant supplementation on attentional processing," *Cortex*, vol. 141, pp. 81–93, Apr. 2021, doi: 10.1016/j.cortex.2021.03.026.

Accordingly, parts of this work are adopted from the original manuscripts.

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Abstract

English version

Introduction: Future long-duration exploratory missions to the Moon and Mars will expose several stressors to the human body that are likely to result in adverse cognitive and behavioral conditions. To assure mission success, it is critically to ascertain the neurobehavioral risks associated with such endeavors. Head-down tilt bed rest provides a unique analog to study some of the effects of spaceflight on Earth.

Methods: As part of a randomized controlled study conducted by the French Institute for Space Medicine and Physiology in Toulouse, France in 2017, twenty young healthy men underwent 60 days of bed rest. Participants were randomly assigned to either a treatment group (n = 10) receiving a daily antioxidant supplement or a control group (n = 10) not receiving any treatment. To assess brain changes in response to prolonged immobilization, electroencephalography was employed both at rest, and while performing two standardized cognitive tasks measuring selective attention and emotion processing at different time points before, during, and after the bed rest phase.

Results: Irrespective of the assigned groups, distinct decreases in resting state spectral power were observed within the delta, theta, alpha, and beta frequency bands across all electrode sites with the onset of bed rest. An extended duration of the bed rest exposure did not further change this response. Spectral power returned to baseline level eight days after the cessation of bed rest. Additionally, data revealed a significant decrease of efficiency in the selective attention task after 60 days of bed rest that was corroborated by changes in event-related potential (ERP) amplitudes across several brain regions regardless of the assigned groups that did not recover eight days after bed rest. In addition, significantly lowered ERP amplitudes at frontal and parietal regions in response to positive and negative pictures of the emotion task were found in participants tested after one month of bed rest compared to a control group tested before bed rest. Source localization confirmed a bilateral lower activity in the posterior cingulate gyrus, insula and precuneus in the bed rest group for emotional, but not neutral stimuli.

Conclusion: The present work provides evidence for the adverse effects of long-duration bed rest on neurophysiology and behavior that could not be mitigated by the antioxidant supplement. Future research is needed to elucidate the mechanisms underlying these effects to promote the development of efficient target-specific countermeasures to assure human survival in space.

Deutsche Version

Einleitung: Zukünftige Langzeit-Weltraummissionen zum Mond und Mars werden den menschlichen Körper verschiedenen Stressoren aussetzen, die höchstwahrscheinlich negative kognitive und verhaltensspezifische Auswirkungen haben können. Um den Erfolg dieser Missionen zu gewährleisten, ist es von entscheidender Bedeutung, die neurophysiologischen und verhaltensbezogenen Risiken, die mit solchen Unternehmungen einhergehen, zu ermitteln. Bettruhe in Kopftieflage bietet ein einzigartiges Analogmodell, um auch auf der Erde einige der Auswirkungen der Raumfahrt auf den Menschen zu untersuchen.

Methodik: Im Rahmen einer randomisiert-kontrollierten Studie, die 2017 vom französischen Institut für Weltraummedizin und Physiologie in Toulouse, Frankreich, durchgeführt wurde, unterzogen sich zwanzig junge, gesunde Männer 60-tägiger Bettruhe. Die Teilnehmer wurden nach dem Zufallsprinzip entweder einer Behandlungsgruppe (n = 10), die täglich ein antioxidatives Nahrungsergänzungsmittel einnahm, oder einer Kontrollgruppe (n = 10), die keine Behandlung erhielt, zugewiesen. Um die Veränderungen des Gehirns als Reaktion auf die andauernde Inaktivität zu beurteilen, wurde ein Elektroenzephalogramm in Ruhe und während der Durchführung zweier standardisierter kognitiver Aufgaben zur Messung der selektiven Aufmerksamkeit und der Emotionsverarbeitung zu verschiedenen Zeitpunkten vor, während und nach der Bettruhephase aufgezeichnet.

Ergebnisse: Unabhängig von der Gruppenzuordnung konnten deutliche Abnahmen der spektralen Leistungsdichte im Ruhezustand in den Delta-, Theta-, Alpha- und Beta-Frequenzbändern über alle Elektroden mit dem Beginn der Bettruhe beobachtet werden. Eine längere Dauer der Bettruhe änderte diese Reaktion nicht weiter. Die spektrale Leistungsdichte kehrte acht Tage nach Beendigung der Bettruhephase auf das Ausgangsniveau zurück. Zusätzlich wurde eine signifikant verringerte Effizienz während der Bearbeitung der selektiven Aufmerksamkeitsaufgabe nach 60 Tagen Bettruhe festgestellt, die mit veränderten Amplituden der ereigniskorrelierten Potenziale (EKP) in zahlreichen Hirnregionen einherging. Dieses Verhalten war unabhängig von den Gruppen und erholte sich auch acht Tage nach Bettruhe noch nicht. Darüber hinaus zeigten die Daten signifikant verringerte EKP-Amplituden in frontalen und parietalen Hirnregionen als Reaktion auf die positiven und negativen Bilder der Emotionsaufgabe bei Teilnehmern, die nach einem Monat Bettruhe getestet wurden, im Vergleich zu einer Kontrollgruppe, die vor der Bettruhe getestet wurde. Die Quellenlokalisation bestätigte eine geringere Aktivität im bilateralen posterioren cingulären Cortex, in der Insula und im Precuneus in der Bettruhegruppe für emotionale, aber nicht für neutrale Stimuli.

Schlussfolgerung: Die vorliegende Arbeit liefert weitere Nachweise für die negative Auswirkung von Langzeit-Bettruhe auf die Neurophysiologie und das Verhalten, die durch die Einnahme eines antioxidativen Nahrungsergänzungsmittels nicht gemildert werden konnte. Zukünftige Untersuchungen sind notwendig, um die Mechanismen, die diesen Effekten zugrunde liegen, aufzuklären und die Entwicklung effizienter zielgerichteter Gegenmaßnahmen zu fördern, um das Überleben der Menschen im Weltraum zu sichern.

1 Introduction

Man's yearning to explore the space is as old as mankind itself. However, it was not until 1961 that the first manned space flight took place. Currently, national space agencies and private companies are striving for further deep space exploration and human settlement. For example, the National Aeronautics and Space Administration (NASA) is planning their next manned flight to the moon for 2024. The aim of this program is to build a modular space station in the lunar orbit that provides vital support for a sustainable, long-term human return to the lunar surface. It could later also serve as a staging point for flights to Mars. These future missions will require astronauts to live and work in an isolated, confined and hostile environment for prolonged time periods exposing them to numerous physiological, environmental, and psychosocial stressors including social isolation and confinement, radiation, increased CO_2 levels, hypokinesia, and weightlessness [1]. Within the past decades, a large body of research has already been performed to understand the impact of spaceflight on the human body. To date, the main focus of these investigations has been on the cardiovascular system, the endocrine system, and the musculoskeletal system [2]. However, due to the extended duration of stays in space, psychosocial circumstances and neurobehavioral adaptations are becoming increasingly important. This is supported by NASA's Human Research Roadmap [3] identifying cognitive and behavioral conditions as well as mental disorders as one of the prime risk factors of prolonged spaceflight. Thus, studies elucidating the neurobehavioral changes associated with such endeavors are critically needed before future missions can take place. These studies should ideally be performed in space. However, implementing experiments onboard the International Space Station involves high costs, is restricted to specific experimental setups, and limited to small sample sizes. Therefore, different Earth-based analog models have been developed to fully unveil the effects of spaceflight on the human body. These analogs include parabolic flights, isolation studies, dry and wet immersion, and bed rest in horizontal and head-down tilt position. Certainly, all these models can only partially replicate the stressors associated with spaceflight. To study the physiological effects of microgravity on ground, head-down bed rest (HDBR) has predominantly been used in the past because it can be conducted over extended time periods.

1.1 Head-down Bed Rest

After several decades of research, it has been determined that a minus 6 degrees head-down tilt position provides the best way for simulating the physiological adaptations to microgravity.

This came firstly from anecdotal reports of Russian cosmonauts [4]. When returning to Earth after long-term spaceflights, they complained about the feeling of falling out of the bed. Consequently, the lower part of the bed was raised to eliminate this sensation. Because of this, investigators experimented with head-down positioning to accentuate the fluid redistribution and thus better approximate what is observed during spaceflight. Tilting angles used ranged from minus 4 to minus 15 degrees but minus 6 degrees emerged as the most common one [5].

Nowadays, space agencies use bed rest studies from several days up to three months to mimic the physical deconditioning, and cephalic fluid shifts, and to a certain degree, also sensory deprivation and confinement observed during standard space missions. To date, the model has been employed in several studies to examine the neurophysiological, behavioral, and cognitive adaptations to inactivity and semi-isolation. Recent studies using magnetic resonance imaging (MRI) have shown that prolonged bed rest is associated with structural and functional brain changes. For instance, Roberts et al. (2015) reported an upward and posterior brain shift, increased density of brain tissue at the vertex, and contraction of adjacent cerebrospinal fluid (CSF) spaces [6]. Koppelmans and colleagues (2017) observed widespread increases in gray matter (GM) volume in posterior brain regions and decreases in frontal areas [7]. In addition, alterations in prefrontal cortex functioning [8], changes in the activation of the frontal, parietal, cingulate and temporal cortices [9], as well as decreases in GM volume within the bilateral frontal lobes and increases along the left primary sensorimotor cortex were found [10]. Though MRI can be considered the gold-standard to assess structural and functional brain changes it suffers from a poor temporal resolution. EEG is capable of assessing cortical responses in the order of milliseconds. In particular, event-related potentials (ERPs), i.e., electrical potentials induced by specific internal or external events can provide information about a broad range of cognitive and affective processes. Still, data on electrocortical adaptations during long-term bed rest are scarce. Previous studies using immediate postural changes or short-term head-down tilt of up to two hours reported decreases in EEG power at rest [11–13], but no study so far investigated the effects of long-term bed. As part of this thesis, data from one experiment elucidating how a prolonged duration of HDBR affects resting state activity is therefore presented [14]. Moreover, studies using ERPs to assess changes in neurobehavioral processing during bed rest are lacking. Given that physical activity enhances cognitive processes, including attention, executive function, and memory, promotes mental well-being, and supports psychological health [15–17], it can be expected that physical inactivity provokes significant neurobehavioral impairments. Accordingly, bed rest has previously been shown to induce decrements in executive function [18,19] and deteriorations in emotion recognition processing during a Flanker task

[20]. However, other studies reported no changes or improvements in tasks assessing several aspects of cognitive functioning in response to bed rest [21–25]. This work therefore shows data from two experiments [26,27] using established paradigms measuring selective attention and emotion processing to further improve the knowledge about the effects of bed rest on brain and behavior as well as the underlying electrocortical processes.

In addition to assessing the consequences of spaceflight on human physiology, bed rest studies also provide the opportunity to develop and test potential countermeasures to mitigate these effects. Previously tested countermeasures included resistive exercise to minimize loss of bone density and muscle mass, aerobic exercise to maintain cardiorespiratory fitness, or artificial gravity to reduce the effects of body unloading and fluid shifts [28]. Despite the success and unique accomplishment of these approaches, they require considerable crew time and hardware. Countermeasures that are easy to apply, are timesaving, and target different organ systems, are therefore critically needed. A nutritional supplement taken during mealtime could have the potential to meet these requirements. Prolonged physical inactivity is thought to be associated with increased oxidative stress that might trigger a persistent systemic low grade inflammation [29]. For instance, 45 days of bed rest have been shown to elevate the release of immune cells and inflammatory cytokines (interleukins) that are important regulators of immune responses and inflammatory reactions. This was confirmed by a 60-day bed rest study revealing significant alterations in immune cell populations and cytokine concentrations [30]. These inflammatory conditions that are usually associated with a wide range of diseases such as hypertension, hyperglycemia, and insulin insensitivity, can also be expected to systematically affect brain function and cognition [31,32]. Dietary substances that inhibit or alleviate the inflammatory responses could therefore be potent in attenuating the potential maladaptive neurophysiological effects of long-duration immobilization. One such group of substances shown to have strong antioxidant properties are polyphenols, which are a family of naturally occurring organic compounds. In the past, the health benefits of polyphenols have been linked to their capacity to directly scavenge free radicals and other nitrogen species. However, recent findings suggest that polyphenols may activate adaptive cellular stress response pathways triggering the production of several types of cytoprotective proteins including neurotrophic factors, protein chaperones, antioxidant and phase II enzymes, and antiapoptotic proteins [33]. Polyphenols are found extensively in fruits, vegetables, and beverages and are thus typically ingested as part of the normal diet. For instance, fruits such as grapes, apples, and berries contain up to 300 mg of polyphenols per 100 g fresh weight. Cereals, dry legumes, and chocolate as well as wine, tea,

and coffee also significantly contribute to the usual polyphenolic intake. In addition, polyphenols can be supplemented using such dietary extracts as green tea, spearmint, grape seed, or ginger. These plant-based extractives are considered to have higher levels of phenolic content and therefore provide stronger antioxidant properties. Given these antioxidant and anti-inflammatory characteristics it is not surprising that polyphenols have been shown to promote cognitive processes including but not limited to executive functions, working memory performance and attention while reducing the risk for neurodegenerative disorders [34,35]. Both, acute and regular supplementation with polyphenols have been demonstrated to modulate resting state electrocortical activity [36–38] and enhance performance in a variety of cognitive tasks assessing different subdomains of attention, i.e., sustained attention, selective attention, and complex attention [39–41]. It can thus be assumed that a nutritional supplement containing polyphenols could be effective in counteracting the potential adverse effects of bed rest on resting state electrocortical activity and neuroelectric processing of attention.

In summary, data on the effects of long-term bed rest on electrocortical activity are lacking so far. There is evidence from short-term studies suggesting that resting state power decreases with the onset of bed rest. Data from behavioral studies are inconclusive, showing deteriorations, no changes or even improvements. An antioxidant supplement may be able to enhance cognitive processes and preserve from the potential maladaptive effects of prolonged bed rest.

1.2 Hypotheses

The aim of the current thesis was to investigate the effects of long-term head-down tilt bed rest on brain function using EEG at rest and while performing cognitive tasks. To address this aim, the following hypotheses have been equally evaluated as part of the integrated publications:

1) The postural change to head-down tilt leads to a decrease in EEG resting state spectral power with the onset of bed rest irrespective of the treatment. With increasing duration of bed rest spectral power will further be affected [14].
2) One month of bed rest leads to a cortical inhibition of affective picture processing as indicated by reduced event-related potentials and changes in self-reported evaluations of the stimuli [26].
3) Two months of bed rest negatively affects attentional resource allocation as indexed by reductions in task performance and concomitant neuroelectric measures. These changes can be mitigated by an antioxidant supplement due to its neuroprotective properties [27].

2 Materials and Methods

The experiments described in this synopsis were part of the randomized controlled study "Effects of a nutritional cocktail consisting of antioxidant and anti-inflammatory supplements to prevent the deconditioning induced by 60 days of antiorthostatic bed rest" that was sponsored by the European Space Agency and carried out by the French Institute for Space Medicine and Physiology in Toulouse, France in 2017. The study was approved by the local ethics committee and registered at ClinicalTrials.gov under NCT03594799. In the following paragraphs, the study design, experimental procedures, data analyses and statistics are summarized. For more detailed information see the original publications [14,26,27].

2.1 Participants

Due to the time-consuming as well as physically and psychologically demanding nature of the study, potential candidates had to undergo a multistage selection process comprising a telephone interview, an Air Force Class III equivalent physical examination and a psychological screening. Exclusion criteria included but were not limited to cardiovascular diseases, musculoskeletal or neurological dysfunctions, gastroesophageal reflux, osteopenia, or osteoporosis. In addition, volunteers with a history of mental illness, the presence of psychological disorders, a lack of tolerance for close spaces, the unwillingness to share a hospital room with another participant, or the inability to tolerate 60 d of confinement to bed rest were excluded after the psychological screening. From more than 500 applicants, twenty men (mean age: 34 ± 8 years; mean height: 176 ± 5 cm; mean body mass: 74.0 ± 7 kg; n = 17 right-handed) passed the screening process and were enrolled in the study. All participants provided written informed consent before participating in the study and received a monetary compensation for their participation.

Table 1. Subjects' group characteristics at baseline for CTRL and TREAT.

	TREAT (n = 10)	**CTRL** (n = 10)
Age (y)	34.8±7.5 (24-44)	33.5±7.5 (20-45)
Height (cm)	176.1±4.7 (168-184)	176.1±5.2 (69-184)
Body Mass (kg)	73.1±5.7 (66.6-83.5)	74.9±8.8 (61.6-86.0)
BMI (kg/m^2)	23.6±1.6 (21.9-25.5)	24.1±2.1 (21.1-27.7)

Data are means and standard deviations (min-max); TREAT, bed rest group receiving antioxidant supplementation; CTRL, bed rest control group not receiving supplementation; BMI, Body Mass Index.

On the first day of the bed rest phase the participants were randomly allocated to one of two groups by the study coordinator. The intervention group (TREAT) received a daily antioxidant

supplement in terms of six pills (two pills per meal) during their bed rest phase. This dose was equivalent to 741 mg polyphenols, 2.1 g omega-3 fatty acids, 168 mg vitamin E and 80 µg selenium. The participants of the control group (CTRL) did not receive any treatment. A comparison of demographic group characteristics is displayed in Table 1. There were no significant differences in any subject characteristics at the beginning of the study (all $p > 0.57$).

2.2 Study Design

The bed rest study comprised 15 days of baseline data collection (BDC), 60 days of -6 degrees head-down tilt bed rest (HDBR) and 15 days of recovery (R). The study protocol was highly standardized. Participants were accommodated in double bedrooms. Regular sleep-wake cycles were maintained with lights off at 11 pm and scheduled wake-up between 6:30 and 7:00 am. The diet was provided by a specialized team of nutritionists regulating the daily caloric intake according to the personal energy requirements and served on a fixed schedule three times per day. The intake of liquids was not limited but recorded through intake and output diaries. During their leisure time, participants had access to various media, such as books, games, video, radio, and internet. Contact with family and friends via phone or email was allowed, though visitors were not permitted. During the bed rest period, the participants had to remain in a lying position with at least one shoulder touching the bed at all times, including while eating and maintaining personal hygiene. Strict adherence to this position was monitored by video cameras.

A total of 16 research teams were involved in the study. Accordingly, the volunteers participated in more than 100 different experiments. The three experiments presented within the scope of this thesis include EEG data collections (1) at rest [14], (2) while administering an oddball task [27], and (3) during an emotion task [26] that were done at different time points during the study. The experimental schedule is displayed in Fig. 1a. In the following, these three experiments will briefly be summarized.

2.2.1 Impact of 60-Day Bed Rest on Resting State Electrocortical Activity

The first experiment was used to assess the time course of changes in resting state electrocortical activity [14]. All participants were repeatedly subjected to EEG data collections at rest. Three minutes of eyes-closed EEG recordings were performed eight days before HDBR (BDC-8), on days 7, 31, and 60 of bed rest (HDBR7/31/60), and once eight days after HDBR (R+7,

R+0 was the first day of the recovery phase). Unlike the other experiments of this thesis, baseline and recovery data (BDC-8 and R+7) were recorded in a seated position and not in head-down tilt posture. During HDBR participants stayed in -6 degrees head-down tilt for the course of the data collection.

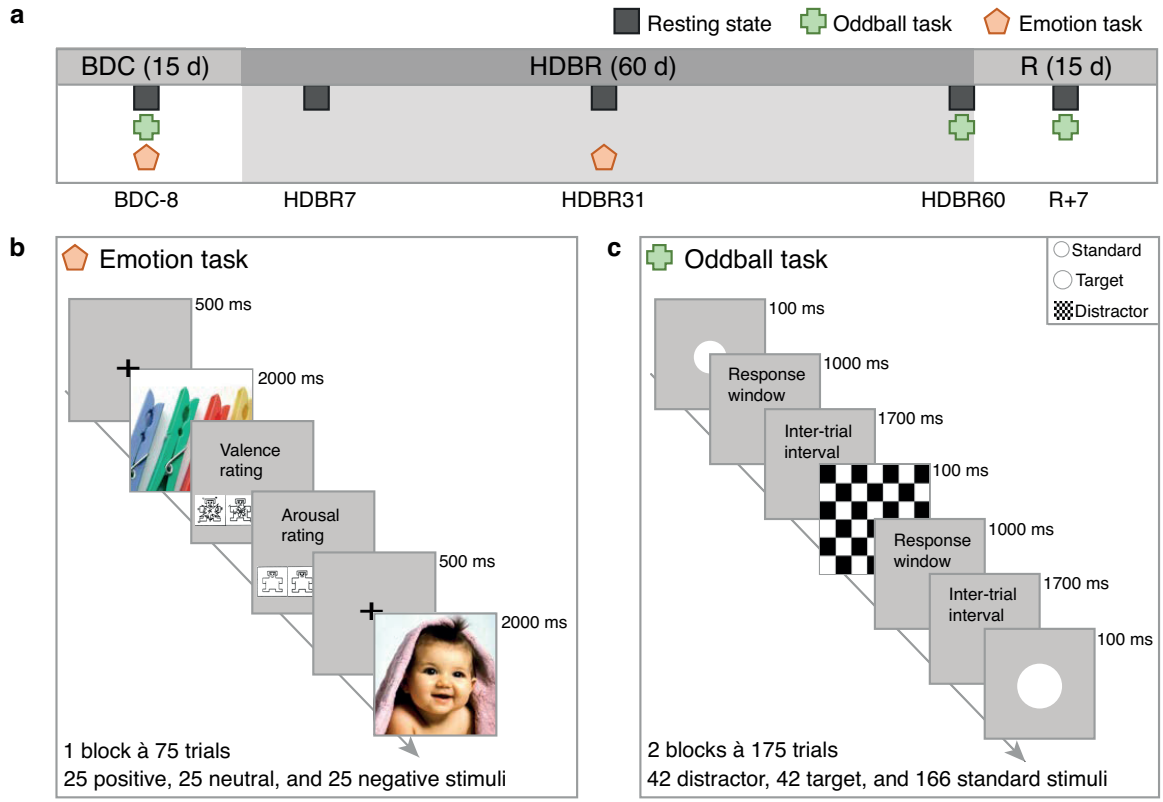

Figure 1. General study design. In **panel a**, the experimental schedule is displayed. Data was collected in a -6 degrees head-down tilt position on all testing days for all experiments except for the resting state that was performed in a seated position during baseline (BDC) and recovery (R). The emotion task was administered either before bed rest (BDC-8) or after 31 days of bed rest (HDBR31) in a counterbalanced fashion. **Panel b** provides a schematic overview of the emotion task used to assess affective processing. A schematic representation of the three-stimulus visual oddball paradigm is shown in **panel c**.

2.2.2 Impact of 30-Day Immobilization on Affective Picture Processing

The second experiment was employed to assess affective picture processing [26]. For this, the participants of the bed rest study were randomly divided into two groups. Half of the participants were tested eight days before undergoing bed rest (BDC-8), the other half after one month of bed rest (HDBR31). The group that was tested on BDC-8 served as a control for the group that was tested on HDBR31. Importantly, both experimental cohorts did not differ in age and anthropometric factors (all $p > 0.74$). To reduce potential confounding effects of body posture, both groups were tested in -6 degrees head-down tilt after at least 30 minutes of adaptation to this position.

Fig. 1b provides a schematic overview of the emotion task used as part of this experiment. In brief, 75 standardized stimuli selected from the IAPS dataset [42], including unpleasant (n = 25, e.g., scenes of violence, threat and injuries), pleasant (n = 25, e.g., sporting events, erotic scenes) and neutral pictures (n = 25, e.g., household objects, landscapes) were presented in a random order. Before each trial, a central fixation cross appeared for 500 ms. Pictures were displayed on the screen for 2000 ms. After each picture presentation participants were asked to rate the arousal and valence of their emotional perception using two independent 9-point Likert scales that ranged from very unpleasant/not arousing at all to very pleasant/very arousing. The rating was performed without any time constraints.

2.2.3 Impact of 60-Day Bed Rest on Attention and its Neuroelectric Basis

The third experiment was employed to assess the impact of two months of bed rest on selective attention [27]. EEG data were collected from all participants while performing a three-stimulus oddball task eight days before bed rest (BDC-8), after 60 days of HDBR (HDBR60), and eight days after bed rest (R+7). To account for potential postural effects, participants were tested in a -6 degrees head-down tilt position on all measurement days after at least 15 minutes in this position.

A schematic overview of the three-stimulus oddball paradigm is given in Fig. 1c. In sum, participants were instructed to respond to an infrequent target stimulus as quickly and accurately as possible by pressing a button while ignoring all other stimuli. Target stimuli were defined as 55-mm diameter white circles occurring with a probability of 0.12. A 47-mm diameter white circle that was presented with a probability of 0.76 was used as the standard stimulus. A full-screen checkerboard that was presented with a probability of 0.12 served as a distractor stimulus. All stimuli were shown for 100 ms, with a 1000-ms response window and a 1700-ms inter-trial interval. The experiment consisted of two blocks of 175 trials each, resulting in a total of 42 target and 42 distractor stimuli. Performance was assessed by reaction time (RT) in milliseconds and response accuracy to the target stimuli in percent. An efficiency score was calculated by averaging the z-transformed accuracy and speed summary scores.

2.3 Data Acquisition and Pre-Processing

All data collections were performed in a dimly lit and sound-attenuated room in the morning between 8.30 am and 1.30 pm. The electrocortical activity was continuously recorded and synchronized with the stimuli using a 32-channel amplifier (actiCHamp, Brain Products GmbH,

Germany). Electrodes were attached to an EEG cap (actiCap, Brain Products GmbH, Germany) according to the International 10/20 System using F_z as the reference electrode. Eye movements and eye blinks were monitored via tin electrooculogram electrodes (B18 Multitrodes, EASYCAP GmbH, Germany). Collected data were processed offline using EEGLAB and ERPlab, two toolboxes embedded in MATLAB (version R2015b, The MathWorks, Inc., Natick, Massachusetts, United States). As described in detail in the original publications, data processing included filtering, visual inspection, interpolation of channels with a poor signal quality, baseline correction, and artifact removal [14,26,27]. An automated exclusion procedure was used, that rejected epochs which exceed a pre-defined slope and minimum/maximum threshold. Artifact-free data were used for the respective analyses of the three different experiments as described below.

2.4 Data Analysis and Statistics

All data analyses were performed in MATLAB and all statistical analyses were run using the software package R [43] (version 3.6.3) unless stated otherwise. The level of significance was set at $\alpha = 0.05$ (two-sided) for all tests ($p < 0.05$).

2.4.1 Impact of 60-Day Bed Rest on Resting State Electrocortical Activity

The resting state EEG data were analyzed by fast Fourier transform to calculate absolute (μV^2/Hz) power density within the theta (0.5 to 4 Hz), delta (4 to 7.5 Hz), alpha (7.5 to 12.5 Hz), and beta (12.5 to 35.0 Hz) domain. After clustering the electrodes into four regions of interest (anterior-left, anterior-right, posterior-left, posterior-right) the averaged activity values were exported for statistical analysis. For each frequency band, linear mixed models were run with *Participants* as random effects (random intercepts), and *Time* (BDC-8, HDBR7, HDBR31, HDBR60, R+7), *Group* (TREAT, CTRL), *Laterality* (left, right), *Region* (anterior, posterior), and their interactions as fixed factors. To assess changes from baseline simple comparisons were performed between the baseline recording (before HDBR) and all subsequent time points using pre-planned contrasts corrected for multiple comparisons. For further details on data analyses and statistics see the original publication [14].

2.4.2 Impact of 30-Day Immobilization on Affective Picture Processing

Artifact-free EEG data from the emotion task were used to create epochs ranging from 200 ms before stimulus presentation to 800 ms after stimulus onset which were separately averaged for positive, negative, and neutral stimuli. For each stimulus type, the late positive potential (LPP)

was assessed as the average voltage between 400 and 700 ms following picture onset while the P300 was quantified as the average voltage between 280 and 350 ms after stimulus presentation. The mean P300 and LPP amplitudes were averaged for anterior and posterior electrodes, respectively and exported for statistical analysis. Separate mixed model ANOVAs for each region (anterior, posterior) and ERP component (P300, LPP) were employed using *Stimulus Type* (negative, neutral, positive) and *Group* (BDC-8, HDBR31) as fixed factors and *Participants* as a random factor. Self-reported evaluations of emotional valence and arousal were analyzed by linear mixed models with the same parameters. Simple comparisons between each condition and both groups were performed using pre-planned contrasts with corrections for multiple comparisons. Additionally, the eLORETA software [44] (version 20181116) was used to identify the neural sources of the electrocortical responses. Independent sampled *F*-tests were used to test for differences in estimated cortical current density between groups in all emotional conditions and both time frames. Statistical significance was assessed using a non-parametric permutation test with 5000 randomizations that determined the critical probability threshold (F_{crit}) with corrections for multiple testing. Additional details on data analyses and statistics are provided in the original publication [26].

2.4.3 Impact of 60-Day Bed Rest on Attention and its Neuroelectric Basis

The cleaned oddball EEG data were segmented into epochs including 100 ms of pre-stimulus baseline and 1000 ms of stimulus-dependent data and averaged for correctly identified target and distractor trials. Two timeframes were evaluated to assess the responses to distractor and target stimuli, i.e., 250 to 550 ms following the onset of the distractors and 300 to 700 ms after target presentation. Statistical analyses were performed using cluster-based permutation tests carried out in MATLAB [45,46] in order to detect electrode sites that collectively show changes in the ERP waveforms between groups and testing days. Separate linear mixed model ANOVAs were run on the performance measures, i.e., RT, accuracy, and efficiency treating *Participants* as a random factor, and *Time* (BDC-8, HDBR60, R+7), *Group* (TREAT, CTRL) and their interaction as fixed factors. Simple comparisons between the initial recording and both subsequent points in time were performed via pre-planned contrasts with corrections for multiple comparisons using a false discovery rate procedure. More detailed information on data analyses and statistics is given in the original publication [27].

3 Results

In the following chapter, a brief summary of the main results of the three experiments, i.e., resting state EEG, affective processing of emotional stimuli, and electrocortical processing of selective attention, is provided. For a comprehensive overview of all results and full statistical reports see the respective publications [14,26,27].

3.1 Impact of 60-Day Bed Rest on Resting State Electrocortical Activity

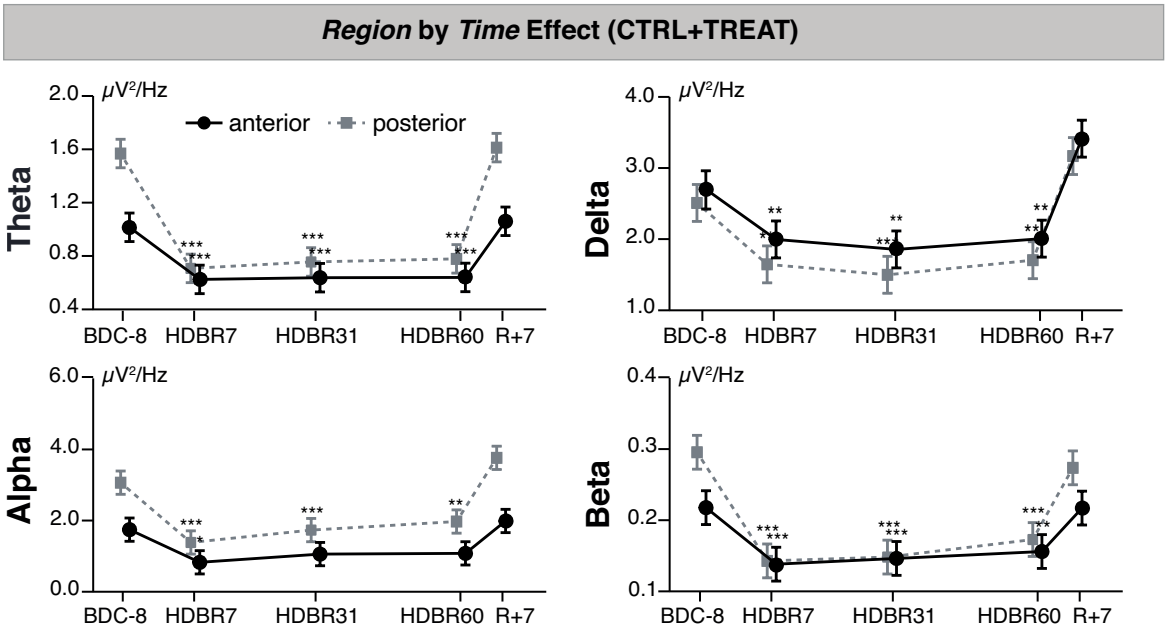

Figure 2. Time courses of resting state electrocortical activity for theta, delta, alpha, and beta power at anterior and posterior sites (pooled groups). Data show estimated marginal means and standard errors of the *Time* by *Region* (dashed grey line: anterior, solid black line: posterior) interaction. BDC-8, 8th day before head-down bed rest; HDBR7/31/60, 7th/31st/60th day of head-down bed rest; R+7, 8th day after head-down bed rest (the first day of recovery was R+0); *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to baseline.
This figure is based on Brauns et al. (2021a) [14].

As described in detail by Brauns et al. (2021a), the mean changes in EEG spectral power were very similar between groups (TREAT, CTRL) for all frequency domains which was confirmed by mixed model analyses [14]. There were neither significant main effects of *Group* (all $p > 0.441$) or *Laterality* (all $p > 0.436$) nor significant interactions between *Group* and *Laterality*, *Time*, or *Region* (all $p > 0.075$) for any frequency band. However, significant main effects of *Time* and *Region*, and their interaction (all $p > 0.001$) were observed. These effects were found for all frequency bands except delta (*Time* by *Region*: $p = 0.983$). Contrasts (*Time* by *Region*,

Fig. 2) revealed that all power indices significantly decreased during the bed rest period at posterior sites, reaching a plateau already at the seventh day of bed rest. A similar though less pronounced pattern was observed for the anterior region showing significant decreases in all frequency bands but alpha. In both regions the reductions in EEG spectral power returned to baseline after the cessation of bed rest. The decrease in absolute power during HDBR was related to a reduced spectral power across all electrode sites. There were no differences between the data collected during the bed rest period. This was revealed by a mixed model ANOVA showing no significant interactions of *Time* and *Region* for any of the investigated frequency bands (all $p > 0.615$). For a full summary of all results and statistics see the original publication [14].

3.2 Impact of 30-Day Immobilization on Affective Picture Processing

Table 2. Subjective ratings for positive, neutral and negative pictures for the groups tested before bed rest (BDC-8) and after one month of bed rest (HDBR31).

	BDC-8		**HDBR31**	
	Valence	Arousal	Valence	Arousal
Positive Pictures	7.6±0.6	5.7±1.2	7.6±0.9	5.7±1.6
Neutral Pictures	4.6±0.9	2.4±1.2	5.2±0.8	2.2±1.2
Negative Pictures	2.6±0.5	5.7±1.0	2.6±0.7	5.7±2.2

Subjective ratings are based on 9-point Likert scales ranging from very unpleasant/not arousing at all to very pleasant/very arousing. Data are means and standard deviations.

As shown by Brauns et al. (2019), the self-reported ratings of both groups, i.e., the group tested before bed rest (BDC-8) and the group tested after one month of bed rest (HDBR31) were consistent with IAPS normative data for all three picture categories ([42] as cited in [26]). Statistical analyses yielded a significant *Stimulus Type* effect for valence ($p < 0.001$) and arousal ($p < 0.001$) irrespective of the subgroups. Positive slides were scored as more arousing ($p < 0.001$) and more pleasant ($p < 0.001$) than neutral ones. Negative slides were evaluated as less pleasant ($p < 0.001$) and more arousing ($p < 0.001$) than neutral pictures. The subjective valence and arousal ratings are summarized in Table 2. The analysis did not reveal any significant effect of *Group* or a *Group* by *Stimulus Type* interaction for neither valence (*Group*: $p = 0.948$; *Group* x *Stimulus Type*: $p = 0.879$) nor arousal (*Group*: $p = 0.909$; *Group* x *Stimulus Type*: $p = 0.928$).

Mixed model analyses of P300 amplitudes revealed significant main effects of *Stimulus Type* (anterior: $p < 0.001$; posterior: $p = 0.034$) and its interaction with *Group* (anterior: $p = 0.002$; posterior: $p = 0.002$) as well as a significant *Group* effect ($p = 0.004$) at posterior sites. No

interaction effects were observed for the LPP amplitudes (anterior: $p = 0.061$; posterior: $p = 0.125$). However, a significant *Group* effect at posterior ($p = 0.026$) and anterior electrodes ($p = 0.048$) and a significant *Stimulus Type* effect at posterior sides ($p < 0.001$) was found. As depicted in Fig. 3a, diminished P300 amplitudes for neutral stimuli compared to emotional ones were observed in the group tested before bed rest (BDC-8). In both brain regions, these differences did not appear for the group tested after one month of bed rest (HDBR31). Likewise, there were significant differences between LPP components induced by positive and neutral stimuli at frontal and parietal electrodes and a significantly smaller LPP amplitude in the frontal area induced by negative pictures compared to neutral pictures in the BDC-8 group only.

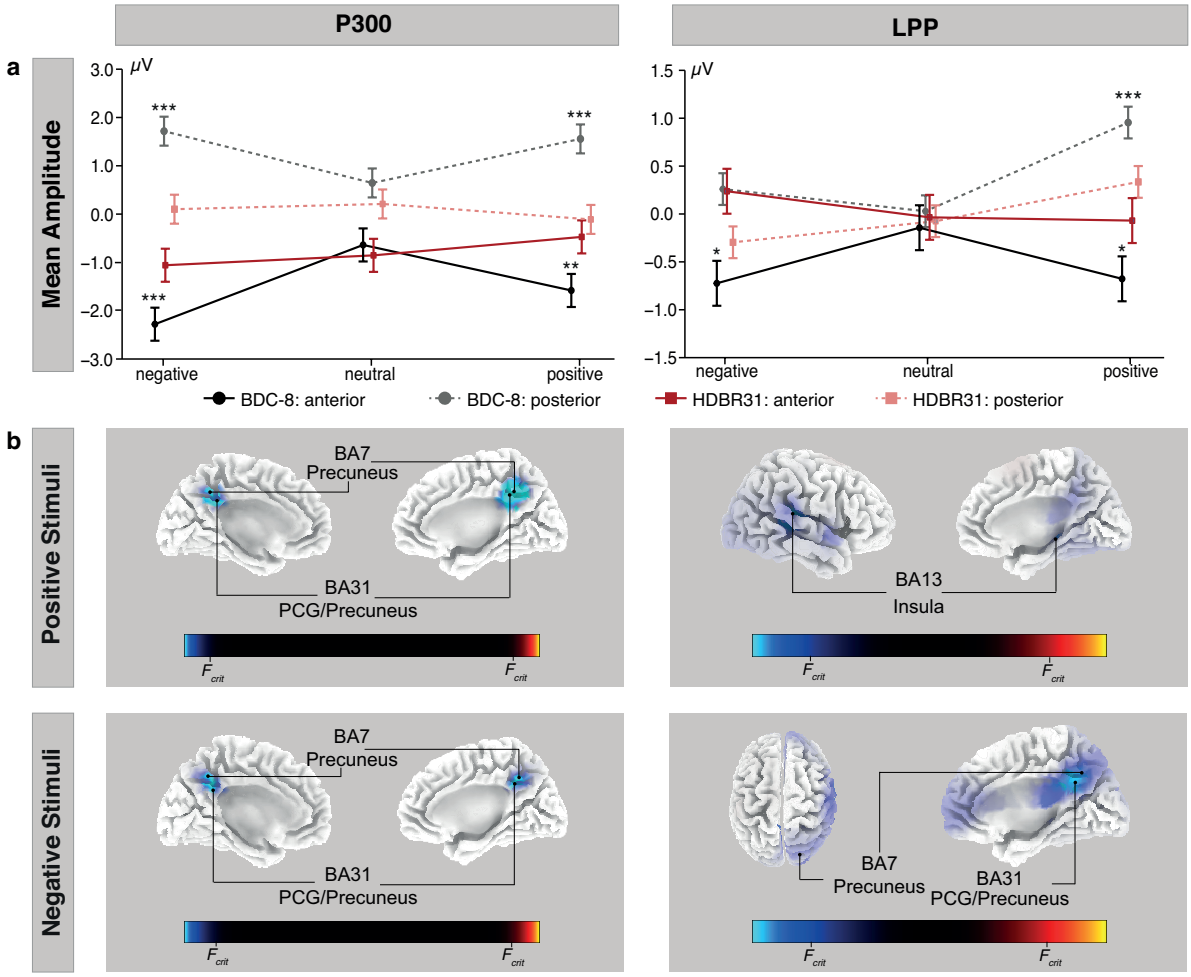

Figure 3. Electrocortical responses to emotional and neutral stimuli. **Panel a** depicts averaged ERP amplitudes to negative, neutral, and positive stimuli for the P300 and LPP components in participants tested eight days before bed rest (BDC-8, reddish squares) and participants tested after one month of bed rest (HDBR31, greyish circles) at anterior (solid lines) and posterior sites (dashed lines). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to neutral pictures. **Panel b** shows statistical parametric maps indicating differences in current source density between BDC-8 and HDBR31 for negative and positive stimuli and both ERP components. The color scale depicts *F*-values for group differences of brain activity. Blue colors highlight decreased activity in HDBR31 compared to BDC-8. F_{crit} critical probability threshold, PCG posterior cingulate gyrus, BA Brodmann area.
This figure is based on Brauns et al. (2019) [26].

Source localization revealed a significantly lower cortical activation in participants of the bed rest group compared to the control group in the right insula (BA 13, $p < 0.05$, Fig. 3b, upper right panel) for the averaged LPP evoked by positive pictures. Similarly, comparing the P300 components of both groups yielded lower cortical activations in the bilateral precuneus and the bilateral cingulate gyrus (BA 31/7, $p < 0.05$, Fig. 3b, upper left panel) in HDBR31 participants. The comparison of both groups unveiled a diminished cortical activity at the same locations (BA 31/7; all $p < 0.05$, Fig. 3b, lower panels) in bed rest participants when processing negative pictures for both ERP components. No significant differences were found comparing the data of the control and bed rest group for mean P300 and LPP amplitudes evoked by neutral stimuli. A detailed description of all results and statistics is given in the original publication [26].

3.3 Impact of 60-Day Bed Rest on Attention and its Neuroelectric Basis

As stated in Brauns et al. (2021b), the analysis of the behavioral data revealed a significant change over *Time* irrespective of the groups ($p = 0.010$) for the efficiency score [27]. There was neither a main effect of *Group* ($p = 0.22$) nor interactions with *Time* ($p = 0.132$). Contrasts assessing the effect of *Time* confirmed a reduction in efficiency from 0.24 at baseline to -0.08 at HDBR60 ($p = 0.019$). Efficiency further decreased to -0.16 during the recovery period ($p = 0.009$). The analysis of response accuracy and RT did not yield any significant main or interaction effects (all $p > 0.097$). Descriptive statistics for both groups are provided in Table 3.

Table 3. Behavioral characteristics of the oddball paradigm per *Group* and *Time*.

	TREAT			**CTRL**		
	BDC-8	HDBR60	R+7	BDC-8	HDBR60	R+7
RT (ms)	559.5±27.1	571.9±26.8	541.4±16.8	613.6±25.2	570.9±19.1	568.5±19.1
Accuracy (%)	87.9±2.4	84.3±4.1	82.4±4.3	89.5±2.8	83.3±4.4	86.9±2.5
Efficiency	0.02±0.21	-0.06±0.13	-0.36±0.23	0.47±0.25	-0.11±0.13	0.04±0.12

Data are means and standard deviations. TREAT, bed rest group receiving antioxidant supplementation; CTRL, bed rest control group not receiving the supplement; RT, reaction time; BDC-8, 8th day before head-down bed rest; HDBR60, 60th day of head-down bed rest; R+7, 8th day after head-down bed rest (the first day of recovery was R+0).

The factorial mass univariate analysis revealed a main effect of *Time* for the ERPs elicited by distractors (all cluster-wise $p < 0.001$) and targets (all cluster-wise $p < 0.05$). No significant effects were observed for *Group* (all cluster-wise $p > 0.36$) or its interaction with *Time* (all cluster-wise $p > 0.49$). Accordingly, simple comparisons were performed for pooled groups. For this, the difference waves between HDBR60 and BDC-8, and between R+7 and BDC-8 for the ERPs to distractors and targets was calculated. These difference waves were submitted to

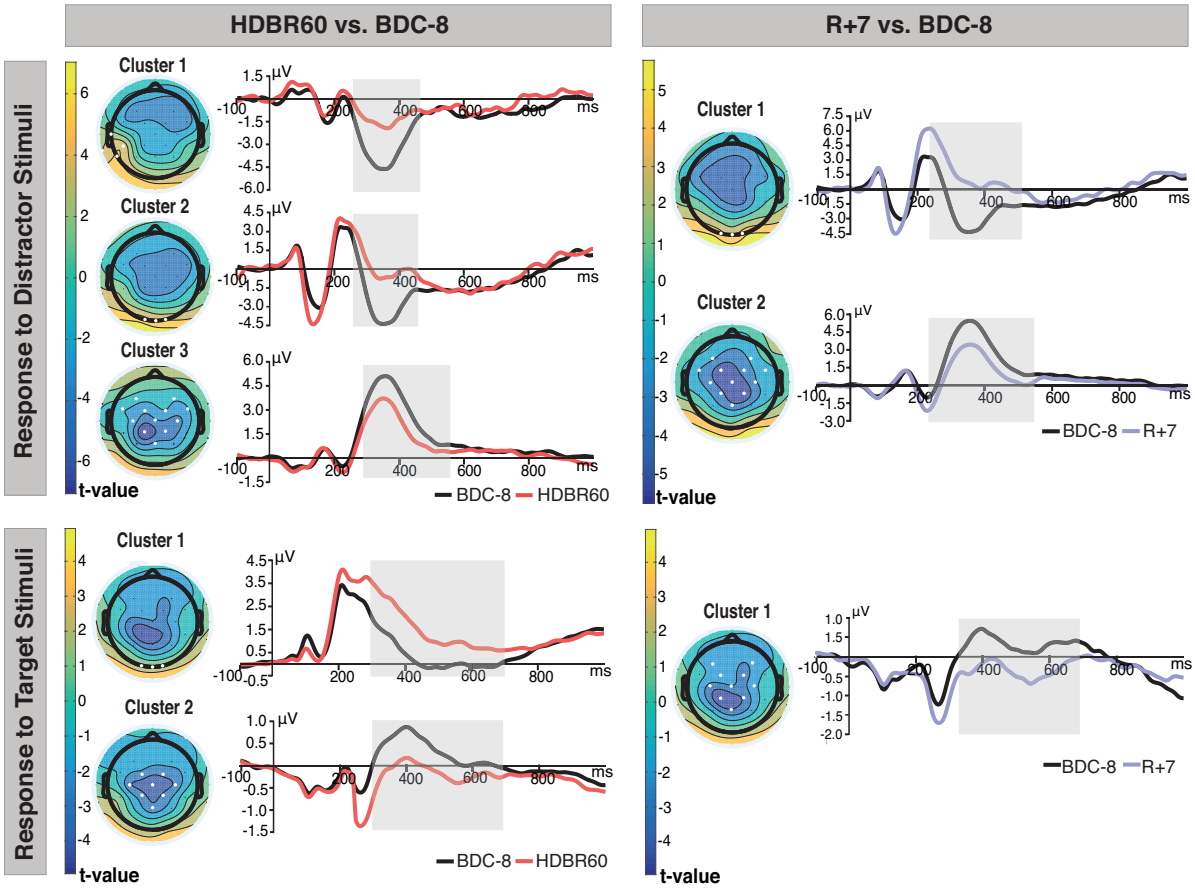

Figure 4. Electrocortical responses to target and distractor stimuli. ERP trace plots and topographical maps show results of the cluster-based permutation tests for distractors and targets comparing HDBR60 (60th day of head-down bed rest) with BDC-8 (8th day before head-down bed rest) and R+7 (8th day after head-down bed rest, R+0 was the first day of recovery) with BDC-8. The topographical maps show t-values for all significant clusters found by the cluster analyses. T-values are averaged over a 30-ms time window centered on the time point at which the Time effect was maximal. The electrodes comprising each cluster are indicated as white circles in the topographical maps. The trace plots depict grand averaged ERPs for each cluster pooled over the cluster electrodes with black lines indicating BDC-8, red lines depicting HDBR60, and blue lines representing R+7. The shaded areas indicate the time windows showing significant differences between measurement days.
This figure is based on Brauns et al. (2021b) [27].

separate cluster-based permutation tests. The analysis of the ERP waveform evoked by distractor stimuli (P3a component) revealed three significant clusters when contrasting HDBR60 and BDC-8 (Fig. 4, upper left panel) and two significant clusters when comparing R+7 and BDC-8 (Fig. 4, upper right panel). Compared to BDC-8, distractor stimuli showed diminished responses on HDBR60 in a large area comprising fronto-central and parietal electrodes (cluster-wise $p < 0.001$, temporal extent: 284 – 550 ms) as well as at left temporoparietal (cluster-wise $p = 0.031$, temporal extent: 263 – 470 ms) and occipital sites (cluster-wise $p = 0.019$, temporal extent: 267 – 467 ms). Accordingly, attenuated ERPs were found in a large cluster (cluster-wise $p < 0.001$, temporal extent: 250 – 550 ms) spanning fronto-central and parietal sites, and a

second cluster (cluster-wise $p = 0.012$, temporal extent: 250 – 530 ms) having an occipital topography when comparing R+7 with BDC-8. The analysis of the ERP responses to targets (P3b component) yielded slightly different results (Fig. 4, lower panel). A larger potential on HDBR60 than on BDC-8 was observed at occipital sites (cluster-wise $p < 0.001$, temporal extent: 300 – 700 ms) and a smaller one at fronto-central and parietal electrodes (cluster-wise $p = 0.021$, temporal extent: 300 – 696 ms). In a similar area encompassing fronto-central and parietal sites (cluster-wise $p = 0.002$, temporal extent: 332 – 696 ms), a decreased P3b amplitude was found when comparing R+7 to BDC-8. Further details on all results and statistics are provided in the original publication [27].

4 Discussion

The present experiments aimed to investigate the effects of long-term immobilization and antioxidant supplementation on electrocortical activity in young, healthy men. Resting state electrocortical power significantly decreased with the onset of bed rest [14]. Increasing time in bed did not affect this response. Task-related data revealed significant impairments of attentional processing after two months of bed rest [27] as well as blunted responses to emotional stimuli after one month of immobilization [26]. In contrast to the hypothesis, antioxidant supplementation did not mitigate the adverse neurobehavioral effects of prolonged bed rest.

The findings from the resting experiment point to a considerable effect of posture due to the bed rest induced fluid shift [14]. Although there is currently no evidence for an overall fluid increase within the intracranial compartment, MRI data from two long-term bed rest studies demonstrated changes in cerebrospinal fluid layer thickness and redistributions of CSF [6,47]. Due to its high conductivity CSF weakens the electric field and current density in the scalp. Any changes in CSF distribution could thus be expected to systematically affect EEG recordings [14]. This was shown by simulation studies reporting that minute shifts in CSF concentration can significantly influence EEG signals ([48,49] as cited in [14]). Experimental studies have confirmed the impact of CSF on electrocortical power ([50] as cited in [14]). Rice and colleagues (2013) measured EEG in prone and supine position and reported an inverse relationship between CSF layer thickness and electrocortical power which they attributed to instant shifts in CSF thickness. A redistribution of CSF in response to the head-down tilt posture could therefore have resulted in the observed decreases in spectral power across all frequency bands [14]. Interestingly, the findings from the current experiment were replicated by Brauns and colleagues (2021a) in a second bed rest study assessing the efficacy of an exercise countermeasure and having the first resting EEG data collection in bed after 24 h of bed rest [14]. Data revealed a decrease of spectral power across all frequency bands with no further changes over the course of the two-month bed rest phase irrespective of the countermeasure. The timing of this response again argues for a postural effect. This is further supported by findings from various studies on the immediate effects of head-down tilt or supine posture showing decreases in EEG power within the alpha, beta, and gamma frequency bands [11–13]. Given these observations, it is likely that posture, rather than inactivity and prolonged immobilization, induced the changes in spectral power in the resting state experiment.

Unlike the resting state data, the task-based experiments revealed considerable changes in electrocortical activity in response to prolonged bed rest. As described in detail in Brauns et al. (2021b), data showed widespread fronto-central and parietal decreases in ERP amplitudes in response to targets and distractors presented as part of the oddball task [27]. These effects were paralleled by decreases in efficiency that were still evident eight days after completion of bed rest. Likewise, diminished ERP responses to affective stimuli, but not neutral pictures of the emotion task, were observed by Brauns and colleagues (2019) in participants tested after one month of bed rest when compared to an age-matched group tested before bed rest [26]. This inhibition was found to be localized in the precuneus, cingulate gyrus, and insula. Importantly, all data collections were performed in a -6 degrees head-down tilt position after at least 15 (oddball task) or even 30 minutes (emotion task) in this position. It is therefore assumed that the current findings are affected by the prolonged inactivity and semi-isolation associated with bed rest, rather than changes in body posture. Several mechanisms are likely to have contributed to the altered ERP responses. As stated before, MRI studies on the effects of prolonged bed rest revealed structural brain changes including but not limited to increases in gray matter volume in posterior parietal brain regions and decreases in the frontal lobes. Gray matter volume has been shown to correlate with the amplitudes of several ERP components ([51,52] as cited in [27]). For instance, Pergher et al. (2019) found significant positive correlations between P300 amplitudes at frontal, central and parietal areas and gray matter volume of the post-central gyrus, lingual gyrus, and the thalamus in the elderly. Similarly, Ford et al. (1994) reported correlations between P300 amplitudes at parietal sides and frontal gray matter volume as well as between P300 amplitudes at frontal sides and parietal gray matter volume. It is thus reasonable to assume that the changes in ERP responses detected as part of the behavioral experiments of this thesis could be the result of the cortical restructuring induced by bed rest. Another explanation for the current findings relates to changes in brain hemodynamics, i.e., decreases in cerebral blood flow (CBF) in response to bed rest ([53] as cited in [27]). There is some evidence that cerebral blood flow and neuroelectric processes are coupled [54,55]. Li and colleagues (2005) observed positive correlations between regional CBF at temporal and parietal regions and P300 amplitudes. Likewise, Higashima et al. (1996) reported that task-related increases in regional CBF positively correlated with P300 amplitudes in the right posterior superior temporal region. Thus, changes in cerebral blood flow induced by prolonged bed rest might have caused the electrocortical alterations observed for the oddball and emotion task. It is also reasonable to assume that the electrocortical alterations were driven by changes in neuroendocrine responses associated with bed rest. There is current evidence that inactivity and prolonged bed

rest affect various neurotransmitter systems, specifically the dopaminergic, noradrenergic, and serotonergic system ([56,57] as cited in [27]). Recent findings indicate that a dual-transmitter system is involved in generating the P300 sub-components of the three-stimulus oddball task. While dopamine is supposed to mediate the frontal P3a, the generation of the P3b is related to the locus coeruleus-norepinephrine system ([58] as cited in [27]). The monoaminergic system is also well-known for its critical role in controlling human behavior ([59] as cited in [27]). Several psychiatric disorders such as depression [60], and anxiety [61], as well as behavioral disturbances among people with dementia [62] have been shown to be associated with alterations in serotonin and norepinephrine concentrations. Modifications of the neurotransmitter level induced by long-duration bed rest could therefore have added to the altered ERP responses observed in the task-based experiments. Certainly, other factors related to prolonged bed rest such as elevated stress responses, circadian disruptions, and mood disturbances may also have influenced the outcome. Further research is thus needed to clarify the mechanisms behind the electrocortical changes.

In contrast to one of the hypotheses, there were no effects of the intervention on cognitive abilities and electrocortical activity. The antioxidant supplement, which included a considerable proportion of polyphenols, affected neither resting state spectral power [14] nor ERP responses or behavioral measures of the oddball paradigm [27]. Due to the experimental design used for the emotion task (half of the participants were tested before bed rest, half of the participants after 31 days of bed rest) no conclusions could be drawn regarding the impact of antioxidant supplementation on affective processing. There is a paucity of data regarding the effects of polyphenol-rich foods and beverages on electrocortical activity. As part of a feasibility study, Okello and colleagues (2016) assessed resting state electrocortical power in healthy adults one hour after the consumption of black and green tea [38] and reported significant increases in alpha, beta, and theta power albeit with considerable inter-individual differences. Likewise, Scholey and colleagues (2012) observed increases in resting state spectral power within the same frequency bands two hours after the administration of 300 mg of caffeine-free tea extract in young healthy men [36]. In contrast, resting state delta power slightly decreased 90 minutes after administration of mango leaf extract in adults [63]. In a task-based study, Saenghong and colleagues (2012) demonstrated that two months of supplementation with 400 or 800 mg of ginger extract lead to increases in P300 amplitudes during an auditory oddball paradigm in middle-aged women [39]. Similarly, in middle-aged participants a 30-d administration with 250 or 500 mg cocoa flavanols revealed modulations of steady-state visually evoked potentials during memory encoding and retrieval but no performance changes [64]. Apart from that, there is

ample evidence for a beneficial effect of dietary polyphenols on several cognitive aspects such as working memory, psychomotor vigilance, and attention. For instance, in a 90-day trial Falcone et al. (2019) reported improvements in complex and sustained attention assessed by the Stroop and Continuous Performance Task after a daily supplementation with 900 mg of proprietary spearmint extracts in young healthy adults [41]. In the elderly, a twelve-week trial of 250 mg daily grape seed extract supplementation revealed enhancements in general cognitive domains including attention and memory measured by a combination of neuropsychological tests and assessment tools [65]. Collectively, these data suggest that antioxidant-rich supplements and food positively affect neurobehavior. However, the generalizability of this assumption is questioned by some studies reporting no impact of polyphenols on cognitive performance [66,67]. Recently, a meta-analysis reviewed the influence of polyphenol-rich supplements on cognitive functions and neuroplasticity in young and middle-aged adults and suggested beneficial effects ([68] as cited in [27]). The authors stressed that the efficacy of the supplements highly depended upon the administration type (acute vs. chronic), and the protocols used (dose and bioavailability). The strongest effects were observed after acute supplementation. Chronic treatment with antioxidants required high doses (>500 mg/day) and/or high bioavailability of the phenolic content (>30%). The polyphenol dose of the supplement used in the current bed rest study was 741 mg (per day), which can thus be considered high. At this point, it is not clear why the countermeasure was not able to provide neuroprotective properties. Apparently, it is difficult to compare the findings from the current work with the aforementioned studies because of methodological differences, i.e., targeted population, treatment composition, doses, and duration as well as neuropsychological tasks used [27]. Bioavailability, i.e., the extent and rate at which an active compound enters systemic circulation, is individual and varies with sex and age [69]. It can be speculated that inter-individual differences in nutrient absorption might have accounted for the ineffectiveness of the supplement used in the present study. Notably, previous supplementation studies demonstrated the strongest effects in women, the aging population, and patients. The current study, however, targeted young, healthy men. At this point, it should also be stressed that the nutritional supplement that was evaluated as part of this study was initially intended to attenuate the inactivity-related metabolic alterations of the musculoskeletal system. In particular, the treatment has been shown to reduce deteriorations in lipid metabolism and muscle atrophy [70]. Future studies should therefore carefully revise the literature on nutritional supplements to reveal its potential for enhancing cognitive functioning during bed rest studies.

Given that head-down bed rest is a classical model to simulate some of the physiological adaptations to spaceflight, the current findings could be directly translated to astronauts. Just like bed rest, weightlessness induces a fluid shift towards the upper body. It is thus reasonable to assume that the concomitant redistribution of CSF might also significantly affect EEG data collected in space. The use of normalized measures of EEG like event-related spectral perturbations and event-related potentials or early inflight recordings as baseline measurements for follow-up data collections might help revealing the full potential of EEG recordings during human space exploration. This is particularly important as the task-based data imply significant cortical alterations in response to prolonged bed rest that could have serious consequences for astronauts as well. Any impairment in affective processing and emotion regulation may interfere with cognitive performance, impair mental well-being and lead to various forms of psychopathology, especially in the context of a stressful environment like deep space. The management of positive and negative emotions is fundamental to human behavior and directly relates to individual sociability and social interactions. During long-duration space missions, psychological issues do not only affect individual crewmembers, but also the entire crew, which could have serious implications for mission success. Equally important as the social component is, of course, the mental performance of astronauts. Several operational tasks require higher-level perceptual skills that rely on different cognitive aspects including memory, perception, and attention. Selective attention involves focusing on key information and suppressing irrelevant distractors, reducing visual overload during information processing. Thus, any impairment of attention could significantly influence performance of astronauts during several tasks, especially for mission-critical events like dockings and extravehicular activities.

In summary, head-down bed rest immediately induced considerable changes in spectral power likely evoked by the reallocation of CSF [14]. Prolonged bed rest caused changes in neuroelectric processing of affect [26] and selective attention [27]. The nutritional supplement that was supposed to counteract the neurobehavioral maladaptation did not have any effect. These findings highlight the pervasive consequences of physical inactivity and semi-isolation on cognitive function and behavior, which are not only significant for spaceflight but also have clinical relevance. Indeed, a sedentary lifestyle and physical inactivity are major health concerns in developed and developing countries. In addition, there is some evidence that physical inactivity promotes the occurrence of diseases, including neurodegenerative disorders such as depression, anxiety, and dementia. This is supported by the current results. The knowledge gained from this thesis could therefore be important for situations in which physical activity levels are markedly

limited, such as medical conditions with reduced physical activity levels, bed-confinement during hospitalized based care, people with sedentary lifestyles, and the aging population. Future research is needed to elucidate the neurophysiological mechanisms underlying these effects, to promote the development of efficient coping strategies and target-specific countermeasures for mitigating the adverse effects of reduced physical activity and semi-isolation associated with bed confinement on brain function and performance.

5 References

[1] E. Afshinnekoo *et al.*, “Fundamental Biological Features of Spaceflight: Advancing the Field to Enable Deep-Space Exploration,” *Cell*, vol. 183, no. 5, pp. 1162–1184, 2020, doi: 10.1016/j.cell.2020.10.050.

[2] A. Van Ombergen, A. Demertzi, E. Tomilovskaya, B. Jeurissen, J. Sijbers, I. B. Kozlovskaya, P. M. Parizel, P. H. Van de Heyning, S. Sunaert, S. Laureys, and F. L. Wuyts, “The effect of spaceflight and microgravity on the human brain,” *J. Neurol.*, vol. 264, no. s1, pp. 18–22, 2017, doi: 10.1007/s00415-017-8427-x.

[3] J. C. Mcphee and J. B. Charles, “Human health and performance risks of space exploration missions,” National Aeronautics and Space Administration For sale by the Supt. of Docs., Houston, Texas Washington, DC, 2009. Accessed: Mar. 29, 2021. [Online]. Available: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Human+Health+and+Performance+Risks+of+Space+Exploration+Missions#0.

[4] A. Pavy-Le Traon, M. Heer, M. V. Narici, J. Rittweger, and J. Vernikos, “From space to Earth: Advances in human physiology from 20 years of bed rest studies (1986-2006),” *European Journal of Applied Physiology*, vol. 101, no. 2. pp. 143–194, Sep. 2007, doi: 10.1007/s00421-007-0474-z.

[5] D. E. Watenpaugh, “Analogs of microgravity: Head-down tilt and water immersion,” *J. Appl. Physiol.*, vol. 120, no. 8, pp. 904–914, 2016, doi: 10.1152/japplphysiol.00986.2015.

[6] D. R. Roberts, X. Zhu, A. Tabesh, E. W. Duffy, D. A. Ramsey, and T. R. Brown, “Structural brain changes following long-term 6° head-down tilt bed rest as an analog for spaceflight,” *Am. J. Neuroradiol.*, vol. 36, no. 11, pp. 2048–2054, 2015, doi: 10.3174/ajnr.A4406.

[7] V. Koppelmans, J. J. Bloomberg, Y. E. De Dios, S. J. Wood, P. A. Reuter-Lorenz, I. S. Kofman, R. Riascos, A. P. Mulavara, and R. D. Seidler, “Brain plasticity and sensorimotor deterioration as a function of 70 days head down tilt bed rest,” *PLoS One*, vol. 12, no. 8, Aug. 2017, doi: 10.1371/journal.pone.0182236.

[8] L. L. Rao, Y. Zhou, Z. Y. Liang, H. Rao, R. Zheng, Y. Sun, C. Tan, Y. Xiao, Z. Q. Tian, X. P. Chen, C. H. Wang, Y. Q. Bai, S. G. Chen, and S. Li, “Decreasing ventromedial prefrontal cortex deactivation in risky decision making after simulated microgravity: Effects of -6° head-down tilt bed rest,” *Front. Behav. Neurosci.*, vol. 8, no. MAY, p. 187, May 2014, doi: 10.3389/fnbeh.2014.00187.

[9] P. Yuan, V. Koppelmans, P. Reuter-Lorenz, Y. De Dios, N. Gadd, S. Wood, R. Riascos, I. Kofman, J. Bloomberg, A. Mulavara, and R. Seidler, “Vestibular brain changes within 70 days of head down bed rest,” *Hum. Brain Mapp.*, vol. 39, no. 7, pp. 2753–2763, Jul. 2018, doi: 10.1002/hbm.24037.

[10] K. Li, X. Guo, Z. Jin, X. Ouyang, Y. Zeng, J. Feng, Y. Wang, L. Yao, and L. Ma, “Effect of simulatedmicrogravity on human brain graymatter and whitematter - Evidence from MRI,” *PLoS One*, vol. 10, no. 8, p. e0135835, Aug. 2015, doi: 10.1371/journal.pone.0135835.

[11] S. Schneider, V. Brümmer, H. Carnahan, A. Dubrowski, C. D. Askew, and H. K. Strüder, “What happens to the brain in weightlessness? A first approach by EEG tomography,”

Neuroimage, vol. 42, no. 4, pp. 1316–1323, 2008, doi: 10.1016/j.neuroimage.2008.06.010.

[12] L. J. Chang, J. F. Lin, C. F. Lin, K. T. Wu, Y. M. Wang, and C. D. Kuo, "Effect of body position on bilateral EEG alterations and their relationship with autonomic nervous modulation in normal subjects," *Neurosci. Lett.*, vol. 490, no. 2, pp. 96–100, Feb. 2011, doi: 10.1016/j.neulet.2010.12.034.

[13] C. Spironelli and A. Angrilli, "Posture used in fMRI-PET elicits reduced cortical activity and altered hemispheric asymmetry with respect to sitting position: An EEG resting state study," *Front. Hum. Neurosci.*, vol. 11, no. December, p. 621, 2017, doi: 10.3389/fnhum.2017.00621.

[14] K. Brauns, A. Friedl-Werner, M. A. Maggioni, H. C. Gunga, and A. C. Stahn, "Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity," *Front. Physiol.*, vol. 12, Feb. 2021, doi: 10.3389/fphys.2021.638669.

[15] C. H. Hillman, K. I. Erickson, and A. F. Kramer, "Be smart, exercise your heart: Exercise effects on brain and cognition," *Nature Reviews Neuroscience*. 2008, doi: 10.1038/nrn2298.

[16] A. F. Kramer and K. I. Erickson, "Capitalizing on cortical plasticity: influence of physical activity on cognition and brain function," *Trends Cogn. Sci.*, vol. 11, no. 8, pp. 342–348, 2007, doi: 10.1016/j.tics.2007.06.009.

[17] F. J. Penedo and J. R. Dahn, "Exercise and well-being: A review of mental and physical health benefits associated with physical activity," *Current Opinion in Psychiatry*. 2005, doi: 10.1097/00001504-200503000-00013.

[18] D. M. Lipnicki, H. C. Gunga, D. L. Belavỳ, and D. Felsenberg, "Bed rest and cognition: Effects on executive functioning and reaction time," *Aviat. Sp. Environ. Med.*, vol. 80, no. 12, pp. 1018–1024, 2009, doi: 10.3357/ASEM.2581.2009.

[19] P. Yuan, V. Koppelmans, P. A. Reuter-Lorenz, Y. E. De Dios, N. E. Gadd, S. J. Wood, R. Riascos, I. S. Kofman, J. J. Bloomberg, A. P. Mulavara, and R. D. Seidler, "Increased brain activation for dual tasking with 70-days head-down bed rest," *Front. Syst. Neurosci.*, vol. 10, no. AUG, p. 71, Aug. 2016, doi: 10.3389/fnsys.2016.00071.

[20] Q. Liu, R. Zhou, S. Chen, and C. Tan, "Effects of Head-Down Bed Rest on the Executive Functions and Emotional Response," *PLoS One*, vol. 7, no. 12, p. e52160, 2012, doi: 10.1371/journal.pone.0052160.

[21] C. W. DeRoshia and J. E. Greenleaf, "Performance and mood-state parameters during 30-day 6° head-down bed rest with exercise training," *Aviat. Sp. Environ. Med.*, 1993.

[22] A. P. le Traon, A. R. de Feneyrols, A. Cornac, R. Abdeseelam, D. N'uygen, M. Lazerges, A. Güell, and A. Bes, "Psychomotor performance during a 28 day head-down tilt with and without lower body negative pressure," *Acta Astronaut.*, vol. 32, no. 4, pp. 319–330, 1994, doi: 10.1016/0094-5765(94)90083-3.

[23] R. L. Shehab, R. E. Schlegel, S. G. Schiflett, and D. R. Eddy, "The nasa performance assessment workstation: Cognitive performance during head-down bed rest," *Acta Astronaut.*, vol. 43, no. 3–6, pp. 223–233, 1998, doi: 10.1016/S0094-5765(98)00156-8.

[24] Y. Ishizaki, H. Fukuoka, H. Tanaka, T. Ishizaki, Y. Fujii, Y. Hattori-Uchida, M. Nakamura, K. Ohkawa, H. Kobayashi, S. Taniuchi, and K. Kaneko, "Executive function on the 16-day of bed rest in young healthy men," *Acta Astronaut.*, 2009, doi:

10.1016/j.actaastro.2008.10.006.

[25] K. A. Seaton, K. J. Slack, W. A. Sipes, and K. E. Bowie, “Cognitive functioning in long-duration head-down bed rest,” *Aviat. Sp. Environ. Med.*, 2009, doi: 10.3357/ASEM.BR09.2009.

[26] K. Brauns, A. Werner, H.-C. Gunga, M. A. Maggioni, D. F. Dinges, and A. Stahn, “Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization,” *Sci. Rep.*, vol. 9, no. 1, p. 16610, Dec. 2019, doi: 10.1038/s41598-019-52555-1.

[27] K. Brauns, A. Friedl-Werner, H.-C. Gunga, and A. C. Stahn, “Effects of two months of bed rest and antioxidant supplementation on attentional processing,” *Cortex*, vol. 141, pp. 81–93, Apr. 2021, doi: 10.1016/j.cortex.2021.03.026.

[28] L. Ploutz-Snyder, “Evaluating countermeasures in spaceflight analogs,” *J. Appl. Physiol.*, vol. 120, no. 8, pp. 915–921, Apr. 2016, doi: 10.1152/japplphysiol.00860.2015.

[29] R. C. Burini, E. Anderson, J. L. Durstine, and J. A. Carson, “Inflammation, physical activity, and chronic disease: An evolutionary perspective,” *Sport. Med. Heal. Sci.*, vol. 2, no. 1, pp. 1–6, Mar. 2020, doi: 10.1016/j.smhs.2020.03.004.

[30] P. Hoff, D. L. Belavý, D. Huscher, A. Lang, M. Hahne, A. K. Kuhlmey, P. Maschmeyer, G. Armbrecht, R. Fitzner, F. H. Perschel, T. Gaber, G. R. Burmester, R. H. Straub, D. Felsenberg, and F. Buttgereit, “Effects of 60-day bed rest with and without exercise on cellular and humoral immunological parameters,” *Cell. Mol. Immunol.*, vol. 12, no. 4, pp. 483–492, Jul. 2015, doi: 10.1038/cmi.2014.106.

[31] A. L. Marsland, P. J. Gianaros, D. C. H. Kuan, L. K. Sheu, K. Krajina, and S. B. Manuck, “Brain morphology links systemic inflammation to cognitive function in midlife adults,” *Brain. Behav. Immun.*, vol. 48, pp. 195–204, Aug. 2015, doi: 10.1016/j.bbi.2015.03.015.

[32] K. N. Warren, L. L. Beason-Held, O. Carlson, J. M. Egan, Y. An, J. Doshi, C. Davatzikos, L. Ferrucci, and S. M. Resnick, “Elevated Markers of Inflammation Are Associated with Longitudinal Changes in Brain Function in Older Adults,” *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.*, vol. 73, no. 6, pp. 770–778, 2018, doi: 10.1093/gerona/glx199.

[33] D. Vauzour, “Dietary polyphenols as modulators of brain functions: Biological actions and molecular mechanisms underpinning their beneficial effects,” *Oxidative Medicine and Cellular Longevity*, vol. 2012. Hindawi Limited, p. 16, 2012, doi: 10.1155/2012/914273.

[34] D. J. Lamport, L. Dye, J. L. D. Wightman, and C. L. Lawton, “The effects of flavonoid and other polyphenol consumption on cognitive performance: A systematic research review of human experimental and epidemiological studies,” *Nutr. Aging*, vol. 1, no. 1, pp. 5–25, 2012, doi: 10.3233/NUA-2012-0002.

[35] D. Vauzour, “Polyphenols and brain health,” *OCL - Oilseeds fats, Crop. Lipids*, vol. 24, no. 2, 2017, doi: 10.1051/ocl/2017008.

[36] A. Scholey, L. A. Downey, J. Ciorciari, A. Pipingas, K. Nolidin, M. Finn, M. Wines, S. Catchlove, A. Terrens, E. Barlow, L. Gordon, and C. Stough, “Acute neurocognitive effects of epigallocatechin gallate (EGCG),” *Appetite*, vol. 58, no. 2, pp. 767–770, 2012, doi: 10.1016/j.appet.2011.11.016.

[37] A. W. Watson, E. J. Okello, H. J. Brooker, S. Lester, G. J. McDougall, and K. A. Wesnes, “The impact of blackcurrant juice on attention, mood and brain wave spectral activity in

young healthy volunteers," *Nutr. Neurosci.*, vol. 22, no. 8, pp. 596–606, Aug. 2019, doi: 10.1080/1028415X.2017.1420539.

[38] E. J. Okello, A. M. Abadi, and S. A. Abadi, "Effects of green and black tea consumption on brain wave activities in healthy volunteers as measured by a simplified Electroencephalogram (EEG): A feasibility study," *Nutr. Neurosci.*, vol. 19, no. 5, pp. 196–205, 2016, doi: 10.1179/1476830515Y.0000000008.

[39] N. Saenghong, J. Wattanathorn, S. Muchimapura, T. Tongun, N. Piyavhatkul, C. Banchonglikitkul, and T. Kajsongkram, "Zingiber officinale improves cognitive function of the middle-aged healthy women," *Evidence-based Complement. Altern. Med.*, vol. 2012, 2012, doi: 10.1155/2012/383062.

[40] P. Philip, P. Sagaspe, J. Taillard, C. Mandon, J. Constans, L. Pourtau, C. Pouchieu, D. Angelino, P. Mena, D. Martini, D. Del Rio, and D. Vauzour, "Acute intake of a grape and blueberry polyphenol-rich extract ameliorates cognitive performance in healthy young adults during a sustained cognitive effort," *Antioxidants*, vol. 8, no. 12, 2019, doi: 10.3390/antiox8120650.

[41] P. H. Falcone, K. M. Nieman, A. C. Tribby, R. M. Vogel, J. M. Joy, J. R. Moon, C. A. Slayton, M. M. Henigman, J. A. Lasrado, B. J. Lewis, B. A. Fonseca, and K. A. Herrlinger, "The attention-enhancing effects of spearmint extract supplementation in healthy men and women: a randomized, double-blind, placebo-controlled, parallel trial," *Nutr. Res.*, vol. 64, pp. 24–38, Apr. 2019, doi: 10.1016/j.nutres.2018.11.012.

[42] B. N. Lang, P. J., Bradley, M. M., & Cuthbert, "International affective picture system (IAPS): Affective ratings of pictures and instruction manual.," *Tech. Rep. A-8.*, p. 2008, 2008.

[43] R Core Team, "R: A Language and Environment for Statistical Computing," *R Foundation for Statistical Computing*. Vienna, Austria, 2018, doi: 10.16373/j.cnki.ahr.150049.

[44] R. D. Pascual-Marqui, "Discrete, 3D distributed, linear imaging methods of electric neuronal activity. Part 1: exact, zero error localization," 2007, [Online]. Available: http://arxiv.org/abs/0710.3341.

[45] E. C. Fields, "Factorial Mass Univariate ERP Toolbox [Computer software]. Available from: https://github.com/ericcfields/FMUT/releases." 2017, Accessed: May 25, 2020. [Online]. Available: https://github.com/ericcfields/FMUT/releases.

[46] D. M. Groppe, T. P. Urbach, and M. Kutas, "Mass univariate analysis of event-related brain potentials/fields I: A critical tutorial review," *Psychophysiology*. 2011, doi: 10.1111/j.1469-8986.2011.01273.x.

[47] V. Koppelmans, O. Pasternak, J. J. Bloomberg, Y. E. D. Dios, S. J. Wood, R. Riascos, P. A. Reuter-Lorenz, I. S. Kofman, A. P. Mulavara, and R. D. Seidler, "Intracranial Fluid Redistribution but No White Matter Microstructural Changes during a Spaceflight Analog," *Sci. Rep.*, vol. 7, no. 1, Dec. 2017, doi: 10.1038/s41598-017-03311-w.

[48] K. Wendel, N. G. Narra, M. Hannula, P. Kauppinen, and J. Malmivuo, "The influence of CSF on EEG sensitivity distributions of multilayered head models," *IEEE Trans. Biomed. Eng.*, vol. 55, no. 4, pp. 1454–1456, Apr. 2008, doi: 10.1109/TBME.2007.912427.

[49] Z. Akalin Acar and S. Makeig, "Effects of forward model errors on EEG source localization," *Brain Topogr.*, vol. 26, no. 3, pp. 378–396, Jul. 2013, doi: 10.1007/s10548-012-0274-6.

[50] J. K. Rice, C. Rorden, J. S. Little, and L. C. Parra, “Subject position affects EEG magnitudes,” *Neuroimage*, vol. 64, no. 1, pp. 476–484, Jan. 2013, doi: 10.1016/j.neuroimage.2012.09.041.

[51] J. M. Ford, E. V. Sullivan, L. Marsh, P. M. White, K. O. Lim, and A. Pfefferbaum, “The relationship between P300 amplitude and regional gray matter volumes depends upon the attentional system engaged,” *Electroencephalogr. Clin. Neurophysiol.*, vol. 90, no. 3, pp. 214–228, Mar. 1994, doi: 10.1016/0013-4694(94)90093-0.

[52] V. Pergher, J. Tournoy, B. Schoenmakers, and M. M. Van Hulle, “P300, gray matter volume and individual characteristics correlates in healthy elderly,” *Front. Aging Neurosci.*, vol. 11, no. MAY, p. 104, May 2019, doi: 10.3389/fnagi.2019.00104.

[53] L. A. Kramer, K. M. Hasan, A. E. Sargsyan, K. Marshall-Goebel, J. Rittweger, D. Donoviel, S. Higashi, B. Mwangi, D. A. Gerlach, E. M. Bershad, and SPACECOT Investigators Group, “Quantitative MRI volumetry, diffusivity, cerebrovascular flow, and cranial hydrodynamics during head-down tilt and hypercapnia: The SPACECOT study,” *J. Appl. Physiol.*, vol. 122, no. 5, pp. 1155–1166, May 2017, doi: 10.1152/japplphysiol.00887.2016.

[54] M. Higashima, Y. Kawasaki, K. Urata, Y. Maeda, N. Sakai, C. Mizukoshi, T. Nagasawa, T. Kamiya, N. Yamaguchi, Y. Koshino, H. Matsuda, S. Tsuji, H. Sumiya, and K. Hisasda, “Simultaneous observation of regional cerebral blood flow and event-related potential during performance of an auditory task,” *Cogn. Brain Res.*, vol. 4, no. 4, pp. 289–296, Nov. 1996, doi: 10.1016/S0926-6410(96)00065-1.

[55] M. Li, Y. Kuroiwa, L. Wang, T. Kamitani, S. Omoto, E. Hayashi, T. Takahashi, Y. Suzuki, S. Koyano, T. Ikegami, and S. Matsubara, “Visual event-related potentials under different interstimulus intervals in Parkinson’s disease: Relation to motor disability, WAIS-R, and regional cerebral blood flow,” *Park. Relat. Disord.*, vol. 11, no. 4, pp. 209–219, Jun. 2005, doi: 10.1016/j.parkreldis.2004.11.004.

[56] P. J. Corcoran, “Use it or lose it - The hazards of bed rest and inactivity,” *Western Journal of Medicine*, vol. 154, no. 5. BMJ Publishing Group, pp. 536–538, 1991, [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1002823/pdf/westjmed00105-0054.pdf.

[57] D. S. Goldstein, J. Vernikos, C. Holmes, and V. A. Convertino, “Catecholaminergic effects of prolonged head-down bed rest,” *J. Appl. Physiol.*, vol. 78, no. 3, pp. 1023–1029, 1995, doi: 10.1152/jappl.1995.78.3.1023.

[58] J. Polich, “Updating P300: An integrative theory of P3a and P3b,” *Clin. Neurophysiol.*, vol. 118, no. 10, pp. 2128–2148, 2007, doi: 10.1016/j.clinph.2007.04.019.

[59] G. Sedvall, B. Fyrö, B. Gullberg, H. Nybäck, F.-A. Wiesel, and B. Wode-Helgodt, “Relationships in healthy volunteers between concentrations of monoamine metabolites in cerebrospinal fluid and family history of psychiatric morbidity,” *Br. J. Psychiatry*, vol. 136, no. 4, pp. 366–374, 1980, doi: 10.1192/bjp.136.4.366.

[60] S. I. Sharp, C. G. Ballard, I. Ziabreva, M. A. Piggott, R. H. Perry, E. K. Perry, D. Aarsland, U. Ehrt, J. P. Larsen, and P. T. Francis, “Cortical serotonin 1A receptor levels are associated with depression in patients with dementia with lewy bodies and Parkinson’s disease dementia,” *Dement. Geriatr. Cogn. Disord.*, vol. 26, no. 4, pp. 330–338, Oct. 2008, doi: 10.1159/000161058.

[61] D. J. Nutt, “The pharmacology of human anxiety,” *Pharmacology and Therapeutics*, vol.

47, no. 2. pp. 233–266, 1990, doi: 10.1016/0163-7258(90)90089-K.

[62] M. K. P. Lai, S. W. Y. Tsang, P. T. Francis, M. M. Esiri, J. Keene, T. Hope, and C. P. L. H. Chen, "Reduced serotonin 5-HT1A receptor binding in the temporal cortex correlates with aggressive behavior in Alzheimer disease," *Brain Res.*, vol. 974, no. 1–2, pp. 82–87, 2003, doi: 10.1016/S0006-8993(03)02554-X.

[63] L. López-Ríos, J. C. Wiebe, T. Vega-Morales, and N. Gericke, "Central nervous system activities of extract Mangifera indica L.," *J. Ethnopharmacol.*, vol. 260, p. 112996, Oct. 2020, doi: 10.1016/j.jep.2020.112996.

[64] D. A. Camfield, A. Scholey, A. Pipingas, R. Silberstein, M. Kras, K. Nolidin, K. Wesnes, M. Pase, and C. Stough, "Steady state visually evoked potential (SSVEP) topography changes associated with cocoa flavanol consumption," *Physiol. Behav.*, vol. 105, no. 4, pp. 948–957, Feb. 2012, doi: 10.1016/j.physbeh.2011.11.013.

[65] G. Calapai, F. Bonina, A. Bonina, L. Rizza, C. Mannucci, V. Arcoraci, G. Laganà, A. Alibrandi, C. Pollicino, S. Inferrera, and U. Alecci, "A randomized, double-blinded, clinical trial on effects of a Vitis vinifera extract on cognitive function in healthy older adults," *Front. Pharmacol.*, vol. 8, no. OCT, 2017, doi: 10.3389/fphar.2017.00776.

[66] L. Bell, A. R. Whyte, D. J. Lamport, J. P. E. Spencer, L. T. Butler, and C. M. Williams, "Grape seed polyphenol extract and cognitive function in healthy young adults: a randomised, placebo-controlled, parallel-groups acute-on-chronic trial," *Nutr. Neurosci.*, vol. 0, no. 0, pp. 1–10, 2020, doi: 10.1080/1028415X.2019.1708033.

[67] M. P. Pase, A. B. Scholey, A. Pipingas, M. Kras, K. Nolidin, A. Gibbs, K. Wesnes, and C. Stough, "Cocoa polyphenols enhance positive mood states but not cognitive performance: A randomized, placebo-controlled trial," *J. Psychopharmacol.*, vol. 27, no. 5, pp. 451–458, May 2013, doi: 10.1177/0269881112473791.

[68] A. Ammar, K. Trabelsi, O. Boukhris, B. Bouaziz, P. Müller, J. M Glenn, N. T. Bott, N. Müller, H. Chtourou, T. Driss, and A. Hökelmann, "Effects of Polyphenol-Rich Interventions on Cognition and Brain Health in Healthy Young and Middle-Aged Adults: Systematic Review and Meta-Analysis," *J. Clin. Med.*, vol. 9, no. 5, p. 1598, May 2020, doi: 10.3390/jcm9051598.

[69] R. S. Gibson, "The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates," *Food Nutr. Bull.*, 2007, doi: 10.1177/15648265070281s108.

[70] A. Damiot *et al.*, "A nutrient cocktail prevents lipid metabolism alterations induced by 20 days of daily steps reduction and fructose overfeeding: Result from a randomized study," *J. Appl. Physiol.*, vol. 126, no. 1, pp. 88–101, Jan. 2019, doi: 10.1152/japplphysiol.00018.2018.

6 Statutory Declaration

"I, Katharina Brauns, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic *"Effect of Head-Down Tilt Bed Rest on Brain Structure and Function (Auswirkung von Bettruhe in Kopftieflage auf Gehirnstruktur und -funktion)"*, independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

7 Declaration of Your Own Contribution to the Publications

Katharina Brauns had the following share in the publications listed below:

Publication I

K. Brauns, A. Friedl-Werner, M. A. Maggioni, H. C. Gunga, and A. C. Stahn, "Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity," *Front. Physiol.*, vol. 12, Feb. 2021, doi: 10.3389/fphys.2021.638669.

Contribution in detail

I was partly involved in preparing the study, i.e., writing of the ethical documents, designing of the experimental schedule, and requesting of quotes for purchasing the equipment. In addition, I run some pre-test before study start. I supervised all experiments of one of the two bed rest studies (RSL) and performed half of the data collections including preparation of EEG recordings. Before doing the analyses, I implemented the code for pre-processing and analyzing the data. I did all statistical analyses using self-written R code and created all tables and figures shown in the article. I wrote the first draft of the manuscript and edited the manuscript after getting feedback from my co-authors. I submitted the article to the journal and corresponded with the editor and reviewers. I answered all reviewer comments and edited the manuscript accordingly during the review process. Before re-submitting the manuscript, I included any changes from my co-authors as well.

Publication 2

K. Brauns, A. Werner, H.-C. Gunga, M. A. Maggioni, D. F. Dinges, and A. Stahn, "Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization," *Sci. Rep.*, vol. 9, no. 1, p. 16610, Dec. 2019, doi: 10.1038/s41598-019-52555-1.

Contribution in detail

I was partly involved in preparing the study, i.e., writing of the ethical documents, designing of the experimental schedule, and requesting of quotes for purchasing the equipment. In addition, I run some pre-test before study start. I designed the task in accordance with current literature and implemented the computer code. Before doing the analyses, I implemented the code for pre-processing and analyzing the data. I did all statistical analyses using self-written R code and created all tables and figures shown in the article. I wrote the first draft of the manuscript and edited the manuscript after getting feedback from my co-authors. I prepared all answers to

the reviewer comments, edited the manuscript accordingly, and thereafter incorporated also potential changes from my co-authors.

Publication 3

K. Brauns, A. Friedl-Werner, H.-C. Gunga, and A. C. Stahn, "Effects of two months of bed rest and antioxidant supplementation on attentional processing," *Cortex*, vol. 141, pp. 81–93, Apr. 2021, doi: 10.1016/j.cortex.2021.03.026.

Contribution in detail

I was partly involved in preparing the study, i.e., writing of the ethical documents, designing of the experimental schedule, and requesting of quotes for purchasing the equipment. In addition, I run some pre-test before study start. I designed the task in accordance with current literature and implemented the computer code. Before doing the analyses, I implemented the code for pre-processing and analyzing the data. I did all statistical analyses using self-written R and MATLAB code and created all tables and figures shown in the article except for the graphical abstract. I wrote the first draft of the manuscript and edited the manuscript after getting feedback from my co-authors. I prepared all answers to the reviewer comments, edited the manuscript accordingly, and thereafter incorporated also potential changes from my co-authors.

_____

Signature, date and stamp of first supervising university professor

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Signature of doctoral candidate

8 Printed Copies of the Selected Publications

Experiment I

Impact of 60-Day Bed Rest on Resting State Electrocortical Activity

K. Brauns, A. Friedl-Werner, M. A. Maggioni, H. C. Gunga, and A. C. Stahn, "Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity," *Front. Physiol.*, vol. 12, Feb. 2021, doi: 10.3389/fphys.2021.638669.

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ORIGINAL RESEARCH
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Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity

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Adverse cognitive and behavioral conditions and psychiatric disorders are considered a critical and unmitigated risk during future long-duration space missions (LDSM). Monitoring and mitigating crew health and performance risks during these missions will require tools and technologies that allow to reliably assess cognitive performance and mental well-being. Electroencephalography (EEG) has the potential to meet the technical requirements for the non-invasive and objective monitoring of neurobehavioral conditions during LDSM. Weightlessness is associated with fluid and brain shifts, and these effects could potentially challenge the interpretation of resting state EEG recordings. Head-down tilt bed rest (HDBR) provides a unique spaceflight analog to study these effects on Earth. Here, we present data from two long-duration HDBR experiments, which were used to systematically investigate the time course of resting state electrocortical activity during prolonged HDBR. EEG spectral power significantly reduced within the delta, theta, alpha, and beta frequency bands. Likewise, EEG source localization revealed significantly lower activity in a broad range of centroparietal and occipital areas within the alpha and beta frequency domains. These changes were observed shortly after the onset of HDBR, did not change throughout HDBR, and returned to baseline after the cessation of bed rest. EEG resting state functional connectivity was not affected by HDBR. The results provide evidence for a postural effect on resting state brain activity that persists throughout long-duration HDBR, indicating that immobilization and inactivity *per se* do not affect resting state electrocortical activity during HDBR. Our findings raise an important issue on the validity of EEG to identify the time course of changes in brain function during prolonged HBDR, and highlight the importance to maintain a consistent body posture during all testing sessions, including data collections at baseline and recovery.

Keywords: spaceflight, bed rest, brain, EEG, cognition, fluid shift

INTRODUCTION

Future long-duration spaceflight missions (LDSM) will be much longer than current standard missions on the International Space Station (ISS). They will be characterized by increased physiological, environmental, and psychosocial stressors, including, but not limited to weightlessness, hypokinesia, isolation and confinement, radiation, increased CO_2 levels, and sleep

disruptions. Adverse cognitive and behavioral conditions and psychiatric disorders are considered a critical and unmitigated risk during such missions (McPhee and Charles, 2009). Monitoring and mitigating crew health and performance risks during LDSM will require tools and technologies that allow to reliably assess cognitive performance and behavioral health. Functional resting state magnetic resonance imaging (rsfMRI) has considerable potential to predict human behavior. Using rsfMRI, resting state functional connectivity was shown to be associated with a variety of cognitive performance tasks, such as attention (Clare Kelly et al., 2008), working memory (Gordon et al., 2012) and fluid intelligence (Cole et al., 2012), as well as emotional states (Bush et al., 2018; Kim et al., 2019), and stress (Chen et al., 2019; Nowak et al., 2020; Sato et al., 2020). Currently, no MRI system is available on the ISS. Due to size as well as technical and operational requirements, it is rather unlikely that any such system featuring neuroimaging capabilities will be deployed on spacecrafts in the near future. Electroencephalography (EEG) has the potential to meet the technical requirements for the non-invasive and objective monitoring of neurobehavioral conditions during LDSM. Some technologies are readily commercially available that are lightweight, highly mobile, battery-operated, and allow non-invasive recordings of electrical cortical activity (Amaral et al., 2017; Casson, 2019; He et al., 2019). EEG recordings in orbit have been successfully employed as part of the Shuttle mission 'Neurolab' (Witten, 2005) and the experiment 'NeuroSpat' on the ISS (Cheron et al., 2006, 2014; Cebolla et al., 2016; Petit et al., 2019). Weightlessness induces a considerable fluid shift to the upper body (Thornton et al., 1987). Furthermore, Roberts et al. (2017) reported an upward shift of the brain in response to long-duration spaceflight (Roberts et al., 2017). Head-down tilt bed rest (HDBR) also causes a cephalic fluid shift (Hargens and Vico, 2016). Likewise, HDBR has been associated with upward and posterior brain shifts, increased density of brain tissue at the vertex, contraction of adjacent cerebrospinal fluid (CSF) spaces, and increased ventricular volume (Roberts et al., 2015). Collectively, these data suggest that weightlessness provokes fluid and brain shifts, which could be expected to systematically affect EEG recordings.

Here, we present data from two long-duration bed rest studies to identify the time course of resting state electrocortical activity, and the effects of exercise and antioxidant supplementation as countermeasures. The experiments were conducted as part of the European Space Agency (ESA) sponsored 60-days bed rest studies 'RSL' and 'COCKTAIL'. Previous studies investigating the effects of immediate postural changes or short-term head-down tilt (HDT) of up to 2 hours reported decreases in EEG power of the alpha, beta, and gamma frequency bands (Schneider et al., 2008; Chang et al., 2011; Spironelli and Angrilli, 2017). In line with the acute postural effects of HDT, we hypothesized that resting state EEG spectral power would decrease with the onset of the first day of HDBR. Second, we anticipated that functional and structural brain changes observed in response to prolonged HDBR (Zhou et al., 2014; Liao et al., 2015; Yuan et al., 2016, 2018; Friedl-Werner et al., 2020) would result in further changes in EEG spectral power and affect resting state functional connectivity, and that these effects would be moderated by exercise and antioxidant supplementation.

METHODS

Study Design

Experiment 1: Long-Term Effects of HDBR With and Without Exercise as a Countermeasure (RSL)

As part of the ESA sponsored bed rest study 'Reactive jumps in a Sledge jump system as a countermeasure during Long-term bed rest – RSL Study' (RSL) we acquired resting state EEG data once before, three times during, and once after 60 days of HDBR to identify the time course of electrocortical activity in response to prolonged HDBR with and without exercise as a countermeasure. The study was carried out at the :envihab facility of the German Aerospace Agency (DLR) in Cologne, Germany in 2015/2016. Details on the general study design and exercise program are described elsewhere (Kramer et al., 2017). Briefly, twenty-three young, healthy right-handed men [age: 29 $\pm$ 6 years, height: 181 $\pm$ 6 cm, body mass: 77 $\pm$ 7 kg (mean $\pm$ SD)] with no personal history of neurological or psychiatric illness, drug or alcohol abuse, and normal or corrected-to-normal vision were enrolled in the study. All participants underwent 15 days of baseline data collection (BDC-15 through BDC-1), 60 days of −6 degrees HDBR (HDBR1 through HDBR60) and 15 days of recovery (R+0 through R+14). On the first day of bed rest, participants were randomly assigned to either an exercise group (RSL-TRAIN, n = 12) that performed a high-intensity interval training during HDBR or a control group (RSL-CTRL, n = 11) that did not perform any physical training. Each training session consisted of repetitive jumps and different series of countermovement jumps with an average load $\geq$ 80% of the participant's body weight. RSL-TRAIN performed a total of 48 exercise sessions during the 60-day bed rest phase (5× per week during the first two weeks of HDBR, and 6× per week for the following six weeks). The sessions were scheduled in the afternoon between 2 pm and 6 pm. Each training had a total duration of 20 min including preparation. Because of medical reasons two participants (one from each group) started their recovery after HDBR49 and HDBR50, respectively (instead of HDBR60). A comparison of the subgroup demographics is displayed in **Table 1**. There were no significant differences in any subject characteristics (all ps > 0.35).

Resting state eyes-closed EEG data were collected for 3 min seven days prior to bed rest (BDC-7), on the second day of HDBR (HDBR2), on the 28th day of HDBR (HDBR28), on the 56th day of HDBR (HDBR56), and after 11 days of recovery (R+10, the first day of recovery was R+0). For the two participants that started their recovery earlier, data collection was performed on the last day of their bed rest phase (i.e., HDBR49 and HDBR50, respectively). During the baseline and recovery period data were collected in seated position. During HDBR participants remained in supine position at −6 degrees head-down tilt.

The project was registered with the German Clinical Trials Register (DRKS, registration number DRKS00012946), and approved by the Ethics Committee of the Northern Rhine

TABLE 1 | Demographic characteristics for RSL and COCKTAIL subgroups at baseline.*

	RSL		COCKTAIL	
	CTRL	TRAIN	CTRL	TREAT
N	11	12	10	10
Age [years]	28.3 ± 5.5	29.9 ± 6.6	33.5 ± 8.3	34.8 ± 7.5
Height [cm]	179.6 ± 6.5	182.0 ± 5.4	176.1 ± 4.6	176.1 ± 4.7
Body Mass [kg]	77.6 ± 7.5	71.9 ± 5.1	74.9 ± 9.1	73.1 ± 5.7
BMI [kg/m^2]	23.5 ± 2.1	23.4 ± 1.7	24.1 ± 2.2	23.6 ± 1.6

Data are means and standard deviations; N, sample size; BMI, Body Mass Index. RSL, 60-day head-down tilt bed rest study investigating the effect of bed rest with (TRAIN) and without exercise (CTRL) as a countermeasure (Experiment 1); COCKTAIL, 60-day head-down tilt bed rest study investigating the effect of bed rest with (TREAT) and without (CTRL) antioxidant supplementation as a countermeasure (Experiment 2). There were no significant differences between subgroups within the RSL and Cocktail experiment (all ps > 0.35). Participants of the RSL study were slightly but significantly older and taller than participants of the COCKTAIL study (p = 0.026 for age and p = 0.006 for height).

Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany, and the local Ethics Committee of Charité – Universitätsmedizin Berlin, Germany. The study conformed to all standards and ethical principles for medical research on human subjects set out in the Declaration of Helsinki by the World Medical Association. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent to participate in the experiment.

Experiment 2: Long-Term Effects of HDBR With and Without Antioxidant/Anti-Inflammatory Supplementation as a Countermeasure (COCKTAIL)

The second experiment was carried out as part of the ESA sponsored bed rest study 'Effects of a Nutritional Cocktail Consisting of Antioxidant and Anti-inflammatory Supplements to Prevent the Deconditioning Induced by 60 Days of Antiorthostatic Bed Rest'. Resting state EEG data were collected once before, three times during, and once after 60 days of HDBR to identify the time course of electrocortical activity in response to prolonged HDBR with and without an antioxidant/anti-inflammatory nutritional supplement as a countermeasure. The study was carried out at the French Institute for Space Medicine and Physiology (MEDES), Toulouse, France in 2017. Details of the general study design and nutritional supplement are reported elsewhere (Arc-Chagnaud et al., 2020). Briefly, twenty young healthy men (mean age: 34 ± 8 years; mean height: 176 ± 5 cm; mean body mass: 74 ± 7 kg; n = 17 right-handed) with no personal history of neurological or psychiatric illness, drug or alcohol abuse, and normal or corrected-to-normal vision were enrolled in the study. The experiment comprised 15 days of baseline data collection (BDC-15 through BDC-1), 60 days of −6 degrees HDBR (HDBR1 through HDBR60) and 15 days of recovery (R+0 through R+14). On the first day of HDBR, the subjects were randomly allocated to one of two groups. The participants of the treatment group (COCKTAIL-TREAT, n = 10) received an antioxidant cocktail, consisting of 741 mg of a bioactive polyphenol compound mix (XXS-2A-BR2 mix, Spiral Company, Dijon, France), 2.1 g omega-3 fatty acids (Omacor, Pierre Fabre Laboratories, Toulouse France), and 138 mg vitamin E coupled with 80 μg of selenium (Solgar, Marne la Vallée, France) during the bed rest phase. The control group (COCKTAIL-CTRL, n = 10) did not receive any supplement or other countermeasure. A comparison of demographic group characteristics is displayed in **Table 1**. There were no significant differences in any subject characteristics (all ps > 0.54).

Resting state eyes-closed EEG data were collected for 3 min eight days prior to bed rest (BDC-8), on the seventh day of HDBR (HDBR7), on the 31st day of HDBR (HDBR31), on the 60th day of HDBR (HDBR60), and on the eighth day of the recovery period (R+7, the first day of recovery was R+0). During baseline and recovery, EEG was recorded in seated position. During HDBR data were collected in supine posture at −6 degrees head-down tilt.

The experiment was registered with the Clinical Trial.gov database under NCT03594799 and approved by the Comité de Protection des Personnes (CPP Sud-Ouest Outre-Mer I), the French Health Authorities (Agence Française de Sécurité Sanitaire des Produits de Santé), and the local Ethics Committee at Charité – Universitätsmedizin Berlin, Germany. The study conformed to all standards and ethical principles for medical research on human subjects set out in the Declaration of Helsinki by the World Medical Association. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent to participate in the experiment.

Data Acquisition

The measurements from both experiments, i.e., RSL and COCKTAIL, were performed in dimly lit and sound-attenuated rooms in the morning between 8.30 am and 1.30 pm. EEG data were acquired with a 32-channel amplifier (actiCHamp, Brain Products GmbH, Germany). Electrodes were attached to an EEG cap (actiCap, Brain Products GmbH, Germany) at positions Fp1, Fp2, F7, F3, Oz, Fz, F4, F8, FT9, FC5, FC1, TP9, CP5, CP1, TP10, CP6, CP2, FT10, FC6, FC2, FC3, C3, Cz, C4, T7, T8, P7, P3, Pz, P4, P8, O1, and O2, according to the International 10/20 System (Jasper, 1958). Signals were referenced to Fz. Electrode impedance was checked for each subject before data collection and maintained at less than 5 kΩ. Eye movements and eye blinks were monitored via tin electrooculogram (EOG) electrodes (B18 Multitrodes, EASYCAP GmbH, Germany) placed above and below the left eye as well as at the outer canthi of both eyes. EEG and EOG signals were amplified by a multi-channel bio-signal amplifier and A/D converted at 1000 Hz per channel with 24-bit resolution. During the bed rest phase, i.e., when participants were tested in a −6 degrees HDT posture, participants' heads were placed on a memory foam to minimize discomfort while wearing the EEG cap.

Data Processing

All data were analyzed offline using EEGLAB (version 2019.1.0), a toolbox embedded in Matlab (version R2015b, The MathWorks, Inc., Natick, Massachusetts, United States). First, the EEG signals

were filtered with a 0.5 to 65 Hz bandpass filter. Sinusoidal artifacts (50 Hz line noise) were removed using the CleanLine function (Mullen, 2012). Next, recordings were visually inspected to allow for the interpolation of bad channels. Data from electrodes with poor signal quality were replaced using spherical spline interpolation. On average, less than 2% of the channels had to be interpolated. After re-referencing to average reference, data were segmented into 4096-ms-epochs with an overlap of 10% between consecutive segments. To exclude the possibility that EEG modifications were due to eye movement artifacts or other transient effects related to opening and closing of the eyes, the first and last 5 s of each recording were excluded for the successive analysis. EOG artifacts were removed using vertical and horizontal EOG regression channels (Gómez-Herrero et al., 2007). Muscle artifacts were removed using a spatial filtering framework with defaults (De Clercq et al., 2006). After baseline removal, an automated exclusion procedure was used, rejecting epochs which exceed a gradient threshold of 100 μV, or a maximum and minimum amplitude of ± 200 μV. On average, 89% of the epochs were accepted for further analysis.

Segmented data were analyzed by fast Fourier transform spectral analysis with 0.244 Hz resolution and averaged over all artifact-free epochs to calculate absolute (μV^2/Hz) power density. For each electrode, the absolute theta (0.5 to 4 Hz), delta (4 to 7.5 Hz), alpha (7.5 to 12.5 Hz), and beta (12.5 to 35.0 Hz) power were exported as the mean of activity values within each frequency band. In agreement with previous work on resting state spectral power (Spironelli and Angrilli, 2017) we clustered the electrodes into four regions of interest with two spatial factors (laterality, region) consisting of two levels each (left/right and anterior/posterior, respectively). Each region comprised the averaged absolute spectral power of six electrodes as follows: (anterior-left) Fp1, F3, FC5, FC1, F7, FT9; (anterior-right) Fp2, F4, FC6, FC2, F8, FT10; (posterior-left) P3, P7, TP9, O1, CP1, CP5; (posterior-right) P4, P8, TP10, O2, CP6, CP2.

Next, we identified the neural sources of resting state electrocortical activity using exact low-resolution brain electromagnetic tomography (eLORETA) (Pascual-Marqui, 2007). eLORETA enables the spatial identification of cortical activity by employing a discrete, three-dimensional distributed, linear, weighted minimum norm inverse solution method that allows for an exact localization to test point sources. We used a three-dimensional head model based on the MNI152 template registered to the Talairach brain atlas and digitized at the Montreal Neurologic Institute (MNI) brain imaging center (Mazziotta et al., 2001). The solution space was limited to the cortical gray matter, including 6239 voxels of 5 mm spatial resolution. All artifact-free EEG epochs were used to calculate the cortical current source density for each of our four frequency bands. The transformed data, containing the corresponding 3D cortical distribution of the electrical neuronal generators were used for further statistical analysis.

We then used eLORETA to analyze the effects of bed rest on source-based functional connectivity of electrocortical activity. In line with previous research on EEG resting state functional connectivity we selected 19 seeds from key regions of the default mode network (DMN) and the fronto-parietal network (Thatcher et al., 2014; Whitton et al., 2018). The MNI coordinates for the seeds are provided in **Supplementary Table 1**. Given the low spatial resolution of eLORETA (voxel dimension: 5 mm^3), single voxels that were closest to the seed point were defined as the centroid of each region of interest (ROI). The use of a single ROI voxel reduced the potential bias associated with high correlations among neighboring voxels generated because of the relatively low spatial resolution and inherent smoothness of the eLORETA inverse solution. Connectivity between pairs of all 19 ROIs was then defined as the lagged phase synchronization between the intracortical EEG-source estimates, which is expected to minimize artifacts related to volume conduction and maximize physiological connectivity information (Pascual-Marqui et al., 2011).

Statistical Analysis

To assess the effects of bed rest and the interventions on EEG spectral power, we performed linear mixed models with participants as random effects (random intercepts), and *Time* (sessions before, during, and after HDBR), *Group* (intervention, control), *Laterality* (left, right), *Region* (anterior, posterior), and their interactions as fixed factors for each frequency band (delta, theta, alpha, beta) and experiment (RSL, COCKTAIL). Covariance matrices were determined by restricted maximum likelihood (REML) estimation. *P*-values were obtained using Satterthwaite's approximation for denominator degrees of freedom. To assess changes from baseline simple comparisons were performed between the baseline recording before HDBR and all subsequent time points using pre-planned contrasts corrected for multiple comparisons (Hochberg, 1988). Effect sizes were reported as Cohen's *d* and 95% confidence intervals. The level of significance was set at $\alpha = 0.05$ (two-sided) for all testing. All statistical analyses and graphical illustrations were carried out using the software package R (version 3.5.1, R Core Team, 2018). Mixed models were run using the packages lme4 and lmerTest (Bates et al., 2015). Estimated marginal means were calculated using emmeans (Lenth, 2016). Figures were created using ggplot2 (Wickham, 2016).

The eLORETA software was used to assess changes in the neural sources of electrocortical activity by performing dependent *t*-tests for log-transformed estimated cortical current density between baseline (before HDBR) and all subsequent time points. Statistical significance was assessed for all frequency bands using a non-parametric randomization procedure with 5000 randomizations that determined the critical probability threshold ($t_{critical}$) with corrections for multiple testing (Nichols and Holmes, 2002).

To assess changes in EEG resting state functional connectivity between the pairs of the nineteen ROIs in each frequency band, eLORETA was used to perform dependent sample *t*-tests comparing the connectivity values from baseline (before HDBR) with all subsequent points in time. For each of these *t*-tests a total of 684 tests were performed (171 ROI connections x 4 frequency bands). A non-parametric randomization procedure with 5000 randomizations and corrections for multiple testing was used to determine statistical significance (Nichols and Holmes, 2002).

The level of significance was set at $\alpha = 0.05$ (two-sided) for all testing performed using the eLORETA software package.

RESULTS

EEG Spectral Power

The mean changes in EEG spectral power were very similar between groups (RSL-TRAIN, RSL-CTRL, COCKTAIL-TREAT, and COCKTAIL-CTRL) for all frequency domains (**Figure 1**). The similarity across studies and intervention and control groups was confirmed by mixed model analyses (see **Supplementary Tables 2, 3** for the RSL and COCKTAIL experiment, respectively). There were neither significant main effects for *Group* (all *ps* > 0.441) or *Laterality* (all *ps* > 0.125) nor significant interactions between *Group* and *Laterality, Time,* or *Region* (all *ps* > 0.075) for any frequency band and experiment.

Irrespective of the experiment and subgroup mean EEG spectral power decreased after the onset of HDBR, remained decreased during HDBR, and returned to baseline levels after the cessation of HDBR. This pattern was quantified by significant main effects for *Time* and *Region,* and a significant interaction between *Time* and *Region*. These effects were observed for all frequency bands except for delta power in the COCKTAIL study (*Time* x *Region*: $F_{4,342} = 0.10$, $p = 0.983$). **Figure 2** shows the time courses of absolute EEG spectral power by *Region* (Anterior, Posterior) within the theta, delta, alpha, and beta frequency band for both experiments (RSL, COCKTAIL). Details on the effects of *Time* by *Region* (Anterior, Posterior) for each frequency band (theta, delta, alpha, beta) and experiment (RSL, COCKTAIL) are provided in **Supplementary Tables 4, 5**. Briefly, contrasts (*Time* by *Region*) revealed that all power indices significantly decreased during the bed rest period at posterior sites, reaching a plateau as early as at the first measurement during HDBR, i.e., 24 h of bed rest for RSL and 7 days of bed rest for COCKTAIL. A similar though less pronounced pattern was observed for the anterior region. Spectral power significantly decreased during HDBR within the delta, theta, and alpha frequency ranges for RSL, and within the delta, theta, and beta frequency domain for COCKTAIL. In both regions the reductions in EEG spectral power returned to baseline after the cessation of bed rest. **Figure 3** displays the topographical distributions pooled for both experiments (RSL and COCKTAIL). The topographical maps indicate that the decrease in absolute power during HDBR was related to a decrease in spectral power across all electrode sites with larger reductions at posterior areas of the brain. Visual inspection did not reveal any effect of *Time* between short-, mid- and long-term HDBR. This was confirmed by a mixed model ANOVA yielding no significant interaction of *Time* and *Region* for any of the investigated frequency bands (all *ps* > 0.582 for RSL; all *ps* > 0.615 for COCKTAIL) when including HDBR data only. To account for inter-individual and intra-individual

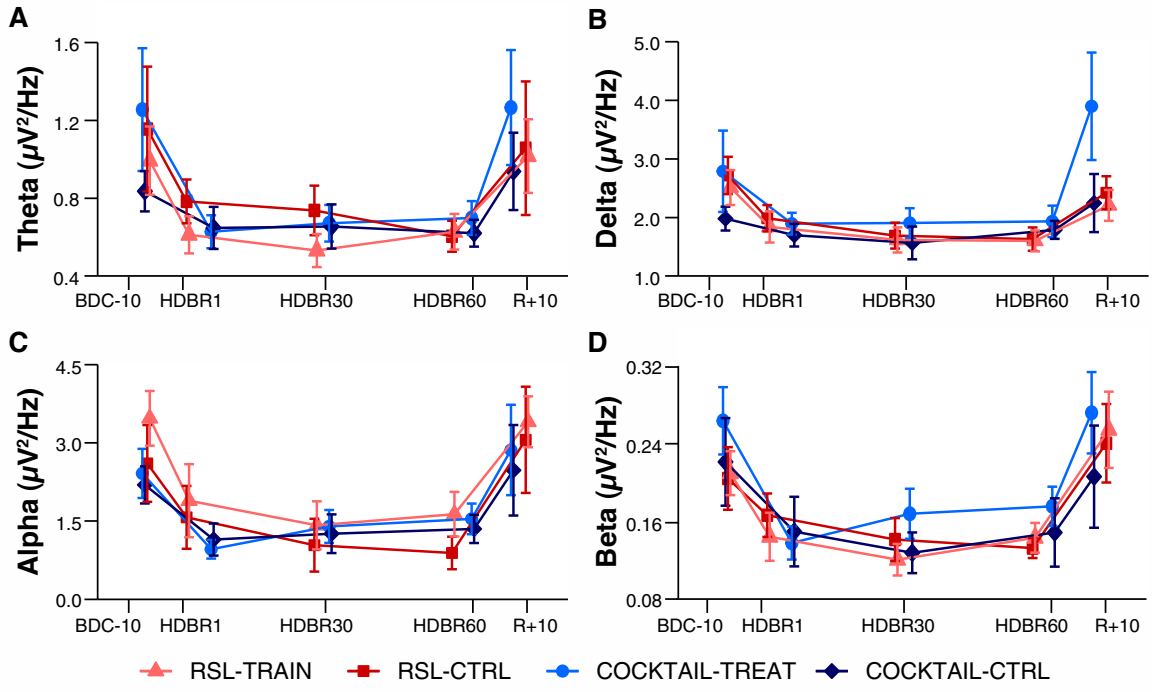

FIGURE 1 | Changes in electrocortical activity during long-duration head-down tilt bed rest of intervention and treatment groups of the RSL and COCKTAIL study. EEG power in **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency bands are averaged over all electrodes ($n = 31$) for RSL-TRAIN ($n = 11$, light red triangle), RSL-CTRL ($n = 11$, dark red square), COCKTAIL-TREAT ($n = 10$, light blue circle), and COCKTAIL-CTRL ($n = 10$, dark blue diamond), respectively. BDC-10 to BDC-1 refer to baseline data collection. HDBR1, HDBR30, and HDBR60 indicate first, 30th, and 60th day of HDBR. R+0 to R+10 correspond to the first and 11th day after HDBR. For RSL data were collected at BDC-7, HDBR2, HDBR28, HDBR56, and R+10. For COCKTAIL data were collected at BDC-8, HDBR7, HDBR30, HDBR60, and R+7. Data are presented as mean and standard errors.

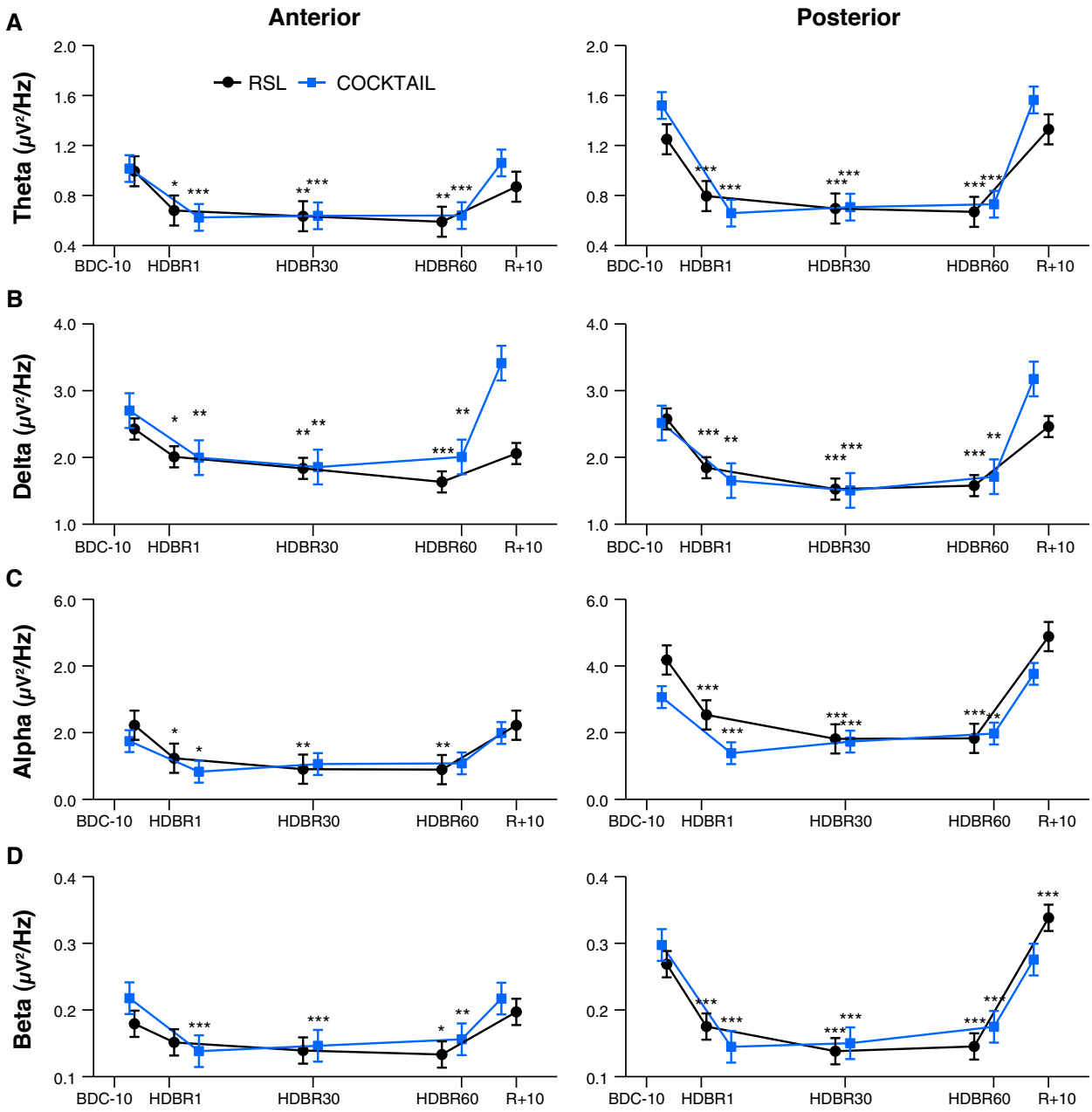

FIGURE 2 | Effect of long-duration head-down tilt bed rest on electrocortical activity. Time courses show changes of EEG spectral power by *Region* (Anterior, Posterior) within the **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency band for RSL (n = 23, blue circle), and COCKTAIL (n = 20, black square), respectively. Data are presented for each time point as estimated marginal means and standard errors. Significant levels with respect to baseline are indicated by asterisks. BDC-10 to BDC-1 refers to baseline data collection. HDBR1, HDBR30, and HDBR60 indicate first, 30th, and 60th day of HDBR. R+0 to R+10 correspond to the first and 11th day after HDBR. For RSL data were collected at BDC-7, HDBR2, HDBR28, HDBR56, and R+10. For COCKTAIL data were collected at BDC-8, HDBR7, HDBR30, HDBR60, and R+7. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to baseline.

differences we z-transformed the absolute power values across participants and testing days and re-analyzed the data set. The analyses confirmed the previous findings. EEG spectral power significantly decreased after the beginning of HDBR, remained decreased during HDBR, and reached baseline levels after the completion of bed rest (see **Supplementary Figure 1**).

eLORETA Source Localization

Table 2 summarizes the results for the analyses of the neural bases of electrocortical activity in response to HDBR. In line with the analyses performed on spectral power, we assessed changes in cortical current density between testing days irrespective of the subgroups (intervention and control) of each experiment.

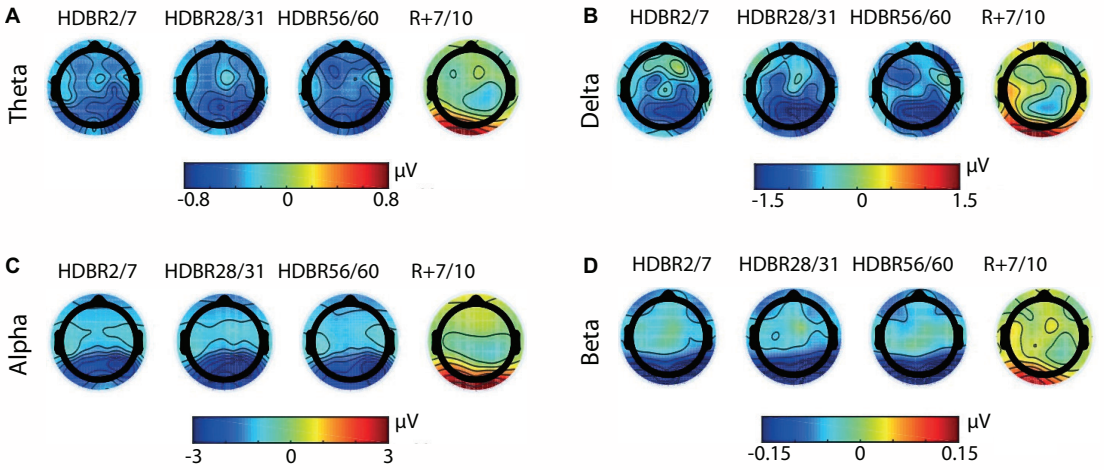

FIGURE 3 | Grand-averaged topographical maps of absolute spectral power within the **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency band. Scalp maps show changes from baseline (BDC-8/-7) to short-term (HDBR2/7), mid-term (HDBR28/31), and long-term (HDBR56/60) head-down tilt bed rest, and recovery (R+7/+10) averaged for all groups (RSL+COCKTAIL, n = 43). Absolute power increases are shown in red and decreases in blue. For RSL data were collected at BDC-7, HDBR2, HDBR28, HDBR56, and R+10. For COCKTAIL data were collected at BDC-8, HDBR7, HDBR30, HDBR60, and R+7. BDC, baseline data collection; HDBR, head-down tilt bed rest; R, recovery.

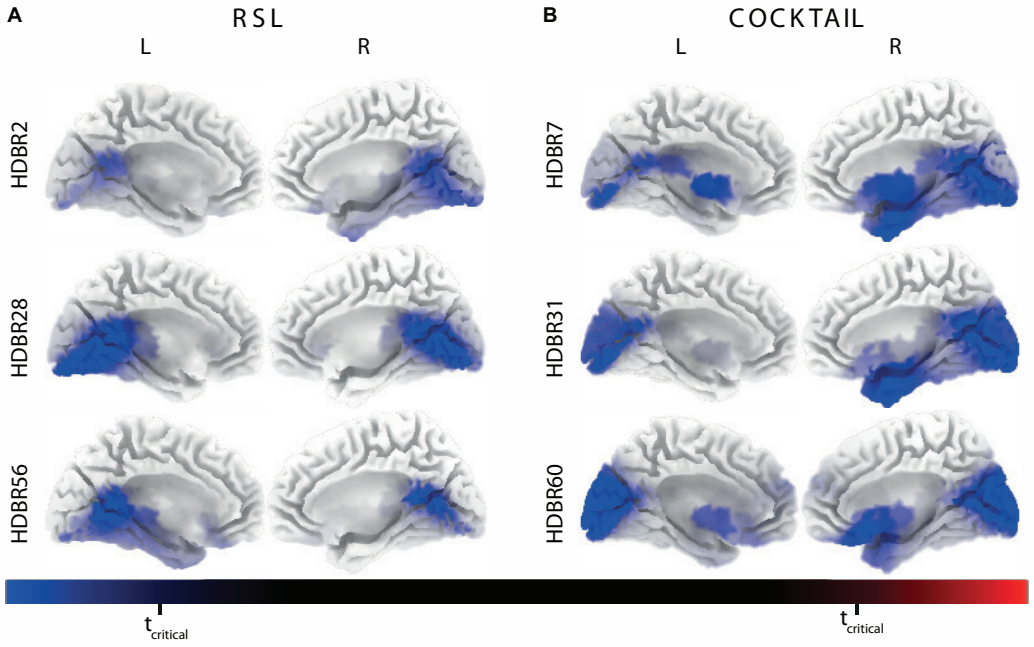

FIGURE 4 | Statistical parametric maps (SPMs) indicating significant effects of *Time* using the baseline (before bed rest) current source density distribution as a reference on brain source localization for **(A)** RSL (n = 23) and **(B)** COCKTAIL (n = 20) within the beta frequency domain. The color scale displays t-values with blue colors indicating decreased activity during head-down tilt bed rest (HDBR) compared to baseline and red colors indicating increased activity. All cortical regions showing significant effects are listed in **Supplementary Table 6**. L, left hemisphere; R, right hemisphere; $t_{critical}$, critical probability threshold of non-parametric randomization tests with 5000 randomizations corrected for multiple comparisons.

We found significantly lower cortical activations within the alpha and beta frequency band on HDBR2, HDBR28, and HDBR56 compared to BDC-7 in the RSL experiment (all $ts > 4.48$, all $ps < 0.05$). Likewise, we observed statistically lower cortical activations within the alpha and beta frequency ranges between BDC-8 and HDBR7, HDBR31, and HDBR60 in the COCKTAIL study (all $ts > 4.50$, all $ps < 0.05$). As shown in **Figure 4** the inhibition of electrocortical activity during HDBR was localized in a broad cluster of voxels, including but not limited to the bilateral precuneus, posterior cingulate

TABLE 2 | Contrasts indicating differences in eLORETA cortical current density between baseline (BDC-8 for COCKTAIL and BDC-7 for RSL) and all subsequent points in time.*

Experiment	Study Day	$t_{critical}$	Alpha		Beta	
			t_{max}	x y z	t_{max}	x y z
RSL	HDBR2	4.48	−4.96	30 −85 15	−5.83	15 −65 10
	HDBR28	4.57	−5.77	20 −65 30	−7.96	−5 −65 5
	HDBR56	4.57	−4.70	45 −45 35	−6.989	5 −60 15
	R+10	4.55	3.84	−15 −10. −15	3.24	−45 −65 45
COCKTAIL	HDBR7	4.50	−6.18	45 −35 35	−7.54	20 −70 20
	HDBR31	4.65	−6.41	45 −5 35	−8.11	10 −65 20
	HDBR60	4.58	−5.04	55 −5 35	−9.60	20 −80 20
	R+7	4.67	4.22	−40 −5 0	−2.49	35 15 0

RSL, 60-day head-down tilt bed rest study investigating the effect of bed rest with and without exercise as a countermeasure (n = 23, Experiment 1); COCKTAIL, 60-day head-down tilt bed rest study investigating the effect of bed rest with and without antioxidant supplementation as a countermeasure (n = 20, Experiment 2); HDBR, head down-tilt bed rest; R, recovery; $t_{critical}$, critical probability threshold of non-parametric randomization test with 5000 randomizations corrected for multiple comparisons; t_{max}, maximal t-statistic; x, y, z, MNI coordinates of peak voxel.

gyrus, and lingual gyrus. This effect was very similar in both studies, frequency domains, and across time points. A list of cortical regions showing significant effects is provided in **Supplementary Table 6**.

Visual inspection also revealed reductions in cortical current density during HDBR within the delta and theta domain, but these effects did not reach statistical significance. We also did not find any significant differences between data collected during the baseline and recovery periods (t_{max} = 3.84, p = 0.163 for RSL; t_{max} = 4.22, p = 0.099 for COCKTAIL) and between data collected during the different HDBR testing sessions (all ts < 3.98, all ps > 0.167).

eLORETA Functional Connectivity

The results of the functional connectivity analyses are summarized in **Supplementary Table 7**. Briefly, we did not observe any changes in functional connectivity between baseline (before bed rest) and all subsequent time points for any of the frequency bands and experiments (all ts < 3.86 and all ps > 0.141). We also did not find a significant difference between data collected during the HDBR sessions (all ts < 3.95 and all ps > 0.148).

DISCUSSION

This study aimed to identify the time course of resting state electrocortical activity in response to prolonged HDBR using data from two 60-day bed rest studies conducted at two different sites. Our data revealed a considerable and significant decrease in EEG spectral power across all frequency bands during HDBR. Likewise, we demonstrated significantly lower activity of the neural sources of electrocortical activity within the alpha and beta frequency domain over a wide range of brain regions. These changes occurred immediately after the onset of HDBR, i.e., after 24 h of bed rest, and were completely uncoupled from the duration of bed rest. Prolonged bed rest did not induce any further changes in resting EEG spectral power or cortical source distribution of resting state EEG. After the cessation of HDBR electrocortical activity was not significantly different from baseline levels recorded before HDBR. The time courses of EEG spectral power and cortical source distribution were highly comparable between the RSL and COCKTAIL study, and neither exercise nor antioxidant supplementation as a countermeasure affected this response.

Our results of the immediate effects of HDBR on EEG spectral power are in line with various data previously published on the acute effects of supine or HDT position on absolute power and cortical source distribution of resting state EEG, reporting decreases within high-frequency domains including alpha and beta power (Schneider et al., 2008; Chang et al., 2011; Thibault et al., 2014; Spironelli and Angrilli, 2017). Based on MRI studies accounting functional and structural brain changes in response to prolonged bed rest (Rao et al., 2014; Zhou et al., 2014; Roberts et al., 2015; Yuan et al., 2016; Friedl-Werner et al., 2020), we also expected alterations in resting state EEG after 30 days and 60 days of bedrest. In contrast to this hypothesis, we could not demonstrate any changes in EEG spectral power or EEG resting state functional connectivity with increasing duration of HDBR. These findings suggest that the reductions in electrocortical activity observed in our study can be attributed to postural changes rather than the immobilization associated with long-term bed rest.

Several mechanisms are likely to have contributed to the effects of HDT on electrical scalp activity. HDT has been shown to modulate brain hemodynamics by increasing cerebral blood flow. Kawai et al. (2003) and Kurihara et al. (2003) reported elevations in brain oxygenation and hemoglobin concentrations in HDT position. The relationship between local neural activity and changes in cerebral blood flow has been well established (Shibasaki, 2008; Chiarelli et al., 2017). A number of studies have shown that alterations in cerebral oxygenation are associated with changes in electrocortical power (Pfurtscheller et al., 2012; Lachert et al., 2017; Dravida et al., 2019; Lin et al., 2020). For instance, Pfurtscheller et al. (2012) reported a coupling between prefrontal oxyhemoglobin (HbO_2) and central EEG alpha and beta power. Further evidence comes from Lachert et al. (2017) showing that increases in cortical HbO_2 concentration are related to decreases in alpha and beta power during a motor task. It is therefore possible that changes in brain hemodynamics during HDT demonstrated by Kawai et al. (2003) and Kurihara et al. (2003) are accompanied by a modulation of electrocortical activity. Similar conclusions were also reached by Schneider et al. (2008) who attributed decreases in alpha and beta power during supine and −6 degrees HDT position to an increase in brain oxygenation and hemoglobin saturation (Schneider et al., 2008). These assumptions were questioned by Lipnicki (2009) who proposed an alternative explanation associated with the interaction between the brain and the autonomous control of the cardiovascular system (Lipnicki, 2009). Postural changes induce a cephalic fluid shift that leads to increases in thoracic blood volume and hydrostatic pressure, stimulating cardiopulmonary and arterial baroreceptors, which in turn reduce sympathetic

system activation (Mohrman and Heller, 2018). There is ample evidence that arterial baroreceptor stimulation inhibits cortical activity (Rau et al., 1993). This effect seems to be mediated by decreasing locus coeruleus activity and cortical noradrenaline turnover (Murase et al., 1994; Berridge and Waterhouse, 2003), which are key modulators of arousal and wakefulness. As noted by Elam et al. (1984) postural changes from upright to supine may dampen arousal via reduced locus coeruleus-noradrenergic system activity in response to increased baroreceptor stimulation.

Another explanation for the global decrease in electrocortical activity seen in the present study may be attributed to the HDT-induced shift of the brain together with a change in cerebrospinal fluid (CSF) layer thickness and a redistribution of CSF. Alperin et al. (2005) investigated the acute effects of postural changes from sitting upright to supine on CSF using CSF flow imaging (Alperin et al., 2005). They observed that intracranial CSF volume increases from sitting to supine position. Simulation studies have shown that minute shifts in CSF concentration can have considerable effects on EEG signals (Ramon et al., 2004; Wendel et al., 2008; Akalin Acar and Makeig, 2013). CSF is up to ten times more conductive than white or gray matter, and up to 100 times more conductive than bone (Ramon et al., 2006). Despite CSF being highly conductive, it weakens the electric field and current density in the scalp (Stenroos and Nummenmaa, 2016). The role of CSF on electrocortical activity has also been illustrated experimentally by measuring EEG in prone and supine position (Rice et al., 2013). Rice et al. (2013) reported an inverse relationship between CSF layer thickness and electrocortical power. They attributed these changes to instant shifts in CSF thickness associated with reallocations of the brain within the skull as a result of the different head orientations. Results from prolonged bed rest studies do not show indications for an overall fluid increase within the intracranial compartment, but rather a redistribution of existing CSF (Roberts et al., 2015; Koppelmans et al., 2017). This was shown by Roberts et al. (2015) who observed an upward shifting of the center's brain mass concomitant with a posterior rotation of the brain relative to the skull of less than 1 mm during HDBR. Such a brain shift is considerably smaller than the electrode location precision typically obtained for EEG recordings. The uncertainty of electrode positions relative to the cortex can be expected to be within several millimeters for highly trained operators. Notably, even small variations in electrode positions can lead to significant shifts in estimated source localizations. For instance, a change in electrode position of 1 cm could lead to a shift of a single dipole by more than 2 cm (Shirazi and Huang, 2019). However, even considering such uncertainties, they would not contradict the possibility that the upward brain shift and the reallocation of CSF associated with HDBR could have caused the attenuation of the EEG signal observed in the present study.

Our findings are also consistent with observations made during acute exposure to microgravity, showing a global decrease of electrocortical activity with the onset of weightlessness (Schneider et al., 2008; Klein et al., 2019). In contrast, early studies on EEG recordings during spaceflight reported no changes (Maulsby, 1966) or increases in alpha, theta and beta power (Frost et al., 1975, 1977). Cheron et al. (2006) used one-minute resting state recordings alternating between eyes opened and eyes closed every ten seconds to assess the impact of long-duration spaceflight on event-related spectral perturbations. They reported increases in alpha power during the arrest reaction in eyes-closed state (Cheron et al., 2006) compared to pre-mission levels. Recently, Cebolla et al. (2016) employed a normalized measure on resting EEG data collected before administering a visuo-attention task to investigate the effect of microgravity, and observed decreases in alpha power desynchronization (Cebolla et al., 2016) in relation to pre-mission. According to the authors the changes observed during/after long-term space missions can be explained by an increased demand for the integration and processing of vestibular information due to the decreased gravitational reference frame in space as well as by the reduction of support related proprioceptive afferents. As bed rest is not a simulation of microgravity but mimics some of the physiological responses to weightlessness these data may not directly translate the data of the present experiments. Additionally, the use of different methodologies could account for the observed discrepancies. Similar to a recent proposition for standardizing brain imaging protocols for spaceflight (Roberts et al., 2020), normative data on EEG recordings using a set of standardized procedures and analyses could help elucidating the effects related to fluid and brain shifts vs. possible structural and functional reorganization of the brain during prolonged spaceflight and spaceflight analogs.

Although the study was highly controlled, our findings are subject to a few limitations. First, EEG signals are prone to physiological (e.g., ocular and muscle activity) and non-physiological artifacts (e.g., electromagnetic interferences and electrode artifacts) that may affect the reliability of the data (Tandle et al., 2015). By using hardware equipped with active noise cancellation and electrodes that amplify the signal directly at the recording site (active circuits for impedance conversion were integrated directly in the electrodes); standardizing the data collection procedures; instructing participants not to move; recording EOG data; and employing rigorous and robust pre-processing pipelines including visual inspection, filtering, and artifact rejection, we minimized the impact of noise on EEG recordings. However, even under consideration of excellent signal-to-noise ratios, cephalic fluid shifts associated with postural changes (or changing gravity levels) raise caution regarding the interpretability of EEG recordings in these conditions. Specifically, the electric field and current flow in the scalp are considerably attenuated by the CSF and the resistive properties of the skull (Van Den Broek et al., 1998). We also acknowledge that EEG lacks the spatial resolution for identifying sub-cortical structures that could be critical for operational performance during spaceflight. A mathematical approach for representing current source density of EEG recordings in 3D space is eLORETA, which we also employed in the current study. Several studies have confirmed that eLORETA has zero localization error in the presence of measurement and structured biological noise (Dattola et al., 2020). It should be noted though that eLORETA relies on a standard head model that does not account for interindividual variability in brain size and shape as well as tissue conductivity that can affect localization accuracy. In addition, EEG measures of brain connectivity

can be confounded by volume conduction effects (He et al., 2019). We tried to minimize these effects by employing source localization-based connectivity measure. However, due to the high correlation of adjacent voxels and the relatively low spatial resolution and inherent smoothness of the eLORETA inverse solution our findings should be interpreted with caution. Finally, data were collected in −6 degrees HDT during the bed rest phase. In contrast, participants were tested in seated position during the baseline and recovery period, resulting in global reductions of electrocortical power. It is therefore possible that the filtering effects associated with postural changes have masked underlying bed rest related alterations. Future studies should therefore employ the same position on all measurement days to discriminate the impact of bed rest from the effects of posture.

Taken together, our data show that HDBR reduces electrocortical activity and its neural sources within a broad range of brain regions. These changes occur as early as after 24 h of HDBR and can be expected to onset immediately after bed rest commencement. The reductions in EEG spectral power and cortical source distribution persist until returning to an upright position again after the cessation of bed rest. Considering previous studies using structural brain imaging we attribute the alterations in EEG power to a brain shift and redistribution of CSF in response to the postural change to HDT. Our findings offer a plausible mechanism for EEG changes observed during bed rest, and should be taken into consideration in the presence of cephalic fluid shifts. Furthermore, prolonged bed rest, i.e., increasing time in HDBR did not result in further changes of EEG spectral power and cortical source distribution, suggesting that immobilization and inactivity *per se* do not affect resting state electrocortical activity during HDBR. These findings raise some caution about the use of resting state EEG recordings to identify the time course of changes in brain function during prolonged HBDR. Future bed rest studies employing EEG should consider the use of −6 degrees supine position for all recordings, i.e., including the baseline and recovery period, allow sufficient time to adapt to the postural change minimizing the effects associated with fluid shifts, and also acquire event-related task data or event-related spectral perturbations to identify the effects of prolonged bed rest on electrocortical changes and performance. Likewise, our findings could also have important implications for EEG resting state data collections performed during spaceflight or altered gravity conditions. For instance, to determine the time course of resting state EEG during spaceflight, early inflight recordings could serve as a baseline for follow-up data collections. Future studies may also be able to systematically validate the effects of brain shifts and redistribution of CSF at varying levels of gravity on resting EEG recordings, which could provide the basis to apply normalization techniques to EEG recordings performed under microgravity conditions. Collectively, such approaches could help to disentangle the neurobehavioral impact of spaceflight stressors from cephalic fluid and brain shifts, and reveal the full potential of resting state EEG recordings during human space exploration.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: http://doi.org/10.6084/m9.figshare.12148359.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the Northern Rhine Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany, the Comité de Protection des Personnes (CPP Sud-Ouest Outre-Mer I), the French Health Authorities (Agence Française de Sécurité Sanitaire des Produits de Santé), and the Ethics Committee of Charité – Universitätsmedizin Berlin, Germany. The participants provided their written informed consent to participate in this study.

AUTHOR CNTRIBUTIONS

AS conceived, designed, planned, and supervised the experiments. KB collected the data for Experiment 1, and AF-W for Experiment 2. KB processed the data. KB and AS performed statistical analysis and wrote the manuscript. AF-W, H-CG, and MM provided critical feedback and contributed to the interpretation of the results. All authors discussed the results and reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.638669/full#supplementary-material

REFERENCES

Akalin Acar, Z., and Makeig, S. (2013). Effects of forward model errors on EEG source localization. *Brain Topogr.* 26, 378–396. doi: 10.1007/s10548-012-0274-6

Alperin, N., Lee, S. H., Sivaramakrishnan, A., and Hushek, S. G. (2005). Quantifying the effect of posture on intracranial physiology in humans by MRI flow studies. *J. Magn. Reson. Imaging* 22, 591–596. doi: 10.1002/jmri.20427

Amaral, C. P., Simões, M. A., Mouga, S., Andrade, J., and Castelo-Branco, M. (2017). A novel brain computer interface for classification of social joint attention in autism and comparison of 3 experimental setups: a feasibility study. *J. Neurosci. Methods* 290, 105–115. doi: 10.1016/j.jneumeth.2017.07.029

Arc-Chagnaud, C., Py, G., Fovet, T., Roumanille, R., Demangel, R., Pagano, A. F., et al. (2020). Evaluation of an antioxidant and anti-inflammatory cocktail against human hypoactivity-induced skeletal muscle deconditioning. *Front. Physiol.* 11:71. doi: 10.3389/fphys.2020.00071

Bates, D., Mächler, M., Bolker, B. M., and Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Berridge, C. W., and Waterhouse, B. D. (2003). The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Rev.* 42, 33–84. doi: 10.1016/S0165-0173(03)00143-7

Bush, K. A., Privratsky, A., Gardner, J., Zielinski, M. J., and Kilts, C. D. (2018). Common functional brain states encode both perceived emotion and the psychophysiological response to affective stimuli. *Sci. Rep.* 8:15444. doi: 10.1038/s41598-018-33621-6

Casson, A. J. (2019). Wearable EEG and beyond. *Biomed. Eng. Lett.* 9, 53–71. doi: 10.1007/s13534-018-00093-6

Cebolla, A. M., Petieau, M., Dan, B., Balazs, L., McIntyre, J., and Cheron, G. (2016). Cerebellar contribution to visuo-attentional alpha rhythm: insights from weightlessness. *Sci. Rep.* 6:37824. doi: 10.1038/srep37824

Chang, L. J., Lin, J. F., Lin, C. F., Wu, K. T., Wang, Y. M., and Kuo, C. D. (2011). Effect of body position on bilateral EEG alterations and their relationship with autonomic nervous modulation in normal subjects. *Neurosci. Lett.* 490, 96–100. doi: 10.1016/j.neulet.2010.12.034

Chen, J., Wei, Z., Han, H., Jin, L., Xu, C., Dong, D., et al. (2019). An effect of chronic stress on prospective memory via alteration of resting-state hippocampal subregion functional connectivity. *Sci. Rep.* 9:19698. doi: 10.1038/s41598-019-56111-9

Cheron, G., Leroy, A., De Saedeleer, C., Bengoetxea, A., Lipshits, M., Cebolla, A., et al. (2006). Effect of gravity on human spontaneous 10-Hz electroencephalographic oscillations during the arrest reaction. *Brain Res.* 1121, 104–116. doi: 10.1016/j.brainres.2006.08.098

Cheron, G., Leroy, A., Palmero-Soler, E., De Saedeleer, C., Bengoetxea, A., Cebolla, A. M., et al. (2014). Gravity influences top-down signals in visual processing. *PLoS One* 9:e82371. doi: 10.1371/journal.pone.0082371

Chiarelli, A. M., Zappasodi, F., Di Pompeo, F., and Merla, A. (2017). Simultaneous functional near-infrared spectroscopy and electroencephalography for monitoring of human brain activity and oxygenation: a review. *Neurophotonics* 4:041411. doi: 10.1117/1.nph.4.4.041411

Clare Kelly, A. M., Uddin, L. Q., Biswal, B. B., Castellanos, F. X., and Milham, M. P. (2008). Competition between functional brain networks mediates behavioral variability. *Neuroimage* 39, 527–537. doi: 10.1016/j.neuroimage.2007.08.008

Cole, M. W., Yarkoni, T., Repovš, G., Anticevic, A., and Braver, T. S. (2012). Global connectivity of prefrontal cortex predicts cognitive control and intelligence. *J. Neurosci.* 32, 8988–8999. doi: 10.1523/JNEUROSCI.0536-12.2012

Dattola, S., Morabito, F. C., Mammone, N., and La Foresta, F. (2020). Findings about loreta applied to high-density eeg—a review. *Electron* 9:660. doi: 10.3390/electronics9040660

De Clercq, W., Vergult, A., Vanrumste, B., Van Paesschen, W., and Van Huffel, S. (2006). Canonical correlation analysis applied to remove muscle artifacts from the electroencephalogram. *IEEE Trans. Biomed. Eng.* 53, 2583–2587. doi: 10.1109/TBME.2006.879459

Dravida, S., Ono, Y., Noah, J. A., Zhang, X., and Hirsch, J. (2019). Co-localization of theta-band activity and hemodynamic responses during face perception: simultaneous electroencephalography and functional near-infrared spectroscopy recordings. *Neurophotonics* 6:045002. doi: 10.1117/1.nph.6.4.045002

Elam, M., Yoa, T., Svensson, T. H., and Thoren, P. (1984). Regulation of locus coeruleus neurons and splanchnic, sympathetic nerves by cardiovascular afferents. *Brain Res.* 290, 281–287. doi: 10.1016/0006-8993(84)90945-4

Friedl-Werner, A., Brauns, K., Gunga, H. C., Kühn, S., and Stahn, A. C. (2020). Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest. *Neuroimage* 223:117359. doi: 10.1016/j.neuroimage.2020.117359

Frost, J. D., Shumate, W. H., Booher, C. R., and DeLucchi, M. R. (1975). The skylab sleep monitoring experiment: methodology and initial results. *Acta Astronaut.* 2, 319–336. doi: 10.1016/0094-5765(75)90099-5

Frost, J. D. Jr., Shumate, W. H., Salamy, J. G., and Booher, C. R. (1977). "Experiment M133. Sleep monitoring on Skylab," in *Biomedical Results from Skylab*, eds R. S. Johnston and L. F. Dietlein (Washington, DC: NASA Headquarters), NASA SP-377.

Gómez-Herrero, G., De Clercq, W., Anwar, H., Kara, O., Egiazarian, K., Van Huffel, S., et al. (2007). "Automatic removal of ocular artifacts in the EEG without an EOG reference channel," *Proceedings of the 7th Nordic Signal Processing Symposium – NORSIG 2006* (Reykjavik: IEEE), 130–133. doi: 10.1109/NORSIG.2006.275210

Gordon, E. M., Stollstorff, M., and Vaidya, C. J. (2012). Using spatial multiple regression to identify intrinsic connectivity networks involved in working memory performance. *Hum. Brain Mapp.* 33, 1536–1552. doi: 10.1002/hbm.21306

Hargens, A. R., and Vico, L. (2016). Long-duration bed rest as an analog to microgravity. *J. Appl. Physiol.* 120, 891–903. doi: 10.1152/japplphysiol.00935.2015

He, B., Astolfi, L., Valdes-Sosa, P. A., Marinazzo, D., Palva, S. O., Benar, C. G., et al. (2019). Electrophysiological brain connectivity: theory and implementation. *IEEE Trans. Biomed. Eng.* doi: 10.1109/TBME.2019.2913928 [Epub ahead of print].

Hochberg, Y. (1988). A sharper bonferroni procedure for multiple tests of significance. *Biometrika* 75, 800–802. doi: 10.1093/biomet/75.4.800

Jasper, H. (1958). The ten twenty electrode system of the international federation. *Electroencephalogr. Clin. Neurophysiol.* 52, 3–6.

Kawai, Y., Doi, M., Setogawa, A., Shimoyama, R., Ueda, K., Asai, Y., et al. (2003). Effects of microgravity on cerebral hemodynamics. *Yonago Acta Med.* 46, 1–8.

Kim, H. C., Bandettini, P. A., and Lee, J. H. (2019). Deep neural network predicts emotional responses of the human brain from functional magnetic resonance imaging. *Neuroimage* 186, 607–627. doi: 10.1016/j.neuroimage.2018.10.054

Klein, T., Wollseiffen, P., Sanders, M., Claassen, J., Carnahan, H., Abeln, V., et al. (2019). The influence of microgravity on cerebral blood flow and electrocortical activity. *Exp. Brain Res.* 237, 1057–1062. doi: 10.1007/s00221-019-05490-6

Koppelmans, V., Pasternak, O., Bloomberg, J. J., Dios, Y. E. D., Wood, S. J., Riascos, R., et al. (2017). Intracranial fluid redistribution but no white matter microstructural changes during a spaceflight analog. *Sci. Rep.* 7:3154. doi: 10.1038/s41598-017-03311-w

Kramer, A., Kuemmel, J., Mulder, E., Gollhofer, A., Frings-Meuthen, P., Gruber, M., et al. (2017). High-intensity jump training is tolerated during 60 days of bed rest and is very effective in preserving leg power and lean body mass: an overview of the cologne RSL study. *PLoS One* 12:e0169793. doi: 10.1371/journal.pone.0169793

Kurihara, K., Kikukawa, A., and Kobayashi, A. (2003). Cerebral oxygenation monitor during head-up and -down tilt using near-infrared spatially resolved spectroscopy. *Clin. Physiol. Funct. Imaging* 23, 177–181. doi: 10.1046/j.1475-097X.2003.00488.x

Lachert, P., Janusek, D., Pulawski, P., Liebert, A., Milej, D., and Blinowska, K. J. (2017). Coupling of oxy- and deoxyhemoglobin concentrations with EEG rhythms during motor task. *Sci. Rep.* 7:15414. doi: 10.1038/s41598-017-15770-2

Lenth, R. V. (2016). Least-squares means: the R package lsmeans. *J. Stat. Softw.* 69, 1–33. doi: 10.18637/jss.v069.i01

Liao, Y., Lei, M., Huang, H., Wang, C., Duan, J., Li, H., et al. (2015). The time course of altered brain activity during 7-day simulated microgravity. *Front. Behav. Neurosci.* 9:124. doi: 10.3389/fnbeh.2015.00124

Lin, C. T., King, J. T., Chuang, C. H., Ding, W., Chuang, W. Y., De Liao, L., et al. (2020). Exploring the brain responses to driving fatigue through simultaneous EEG and fNIRS measurements. *Int. J. Neural. Syst.* 30:1950018. doi: 10.1142/S0129065719500187

Lipnicki, D. M. (2009). Baroreceptor activity potentially facilitates cortical inhibition in zero gravity. *Neuroimage* 46, 10–11. doi: 10.1016/j.neuroimage.2009.01.039

Maulsby, R. L. (1966). Electroencephalogram during orbital flight. *Aerosp. Med.* 37, 1022–1026.

Mazziotta, J., Toga, A., Evans, A., Fox, P., Lancaster, J., Zilles, K., et al. (2001). A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). *Philos. Trans. R. Soc. B Biol. Sci.* 356, 1293–1322. doi: 10.1098/rstb.2001.0915

McPhee, J., and Charles, J. (2009). *Human Health and Performance Risks of Space Exploration Missions: Evidence Reviewed by the NASA Human Research Program*. Washington, DC: US National Aeronautics and Space Admin.

Mohrman, D. E., and Heller, L. J. (2018). *Cardiovascular Physiology*. New York, NY: McGraw-Hill Education Ltd.

Mullen, T. (2012). *NITRC: CleanLine: Tool/Resource Info*. Available online at: https://www.nitrc.org/projects/cleanline (accessed January 14, 2020)

Murase, S., Inui, K., and Nosaka, S. (1994). Baroreceptor inhibition of the locus coeruleus noradrenergic neurons. *Neuroscience* 61, 635–643. doi: 10.1016/0306-4522(94)90440-5

Nichols, T. E., and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25. doi: 10.1002/hbm.1058

Nowak, J., Dimitrov, A., Oei, N. Y. L., Walter, H., Adli, M., and Veer, I. M. (2020). Association of naturally occurring sleep loss with reduced amygdala resting-state functional connectivity following psychosocial stress. *Psychoneuroendocrinology* 114:104585. doi: 10.1016/j.psyneuen.2020.104585

Pascual-Marqui, R. D. (2007). Discrete, 3D distributed, linear imaging methods of electric neuronal activity. Part 1: exact, zero error localization. arXiv [Preprint]. Available online at: http://arxiv.org/abs/0710.3341 (accessed September 28, 2019).

Pascual-Marqui, R. D., Lehmann, D., Koukkou, M., Kochi, K., Anderer, P., Saletu, B., et al. (2011). Assessing interactions in the brain with exact low-resolution electromagnetic tomography. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 369, 3768–3784. doi: 10.1098/rsta.2011.0081

Petit, G., Cebolla, A. M., Fattinger, S., Petieau, M., Summerer, L., Cheron, G., et al. (2019). Local sleep-like events during wakefulness and their relationship to decreased alertness in astronauts on ISS. *NPJ Microgravity* 5:10. doi: 10.1038/s41526-019-0069-0

Pfurtscheller, G., Daly, I., Bauernfeind, G., and Müller-Putz, G. R. (2012). Coupling between intrinsic prefrontal Hbo2 and central EEG beta power oscillations in the resting brain. *PLoS One* 7:e43640. doi: 10.1371/journal.pone.0043640

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Found. Stat. Comput. doi: 10.16373/j.cnki.ahr.150049

Ramon, C., Schimpf, P., Haueisen, J., Holmes, M., and Ishimaru, A. (2004). Role of soft bone, CSF and gray matter in EEG simulations. *Brain Topogr.* 16, 245–248. doi: 10.1023/B:BRAT.0000032859.68959.76

Ramon, C., Schimpf, P. H., and Haueisen, J. (2006). Influence of head models on EEG simulations and inverse source localizations. *Biomed. Eng. Online* 5:10. doi: 10.1186/1475-925X-5-10

Rao, L. L., Zhou, Y., Liang, Z. Y., Rao, H., Zheng, R., Sun, Y., et al. (2014). Decreasing ventromedial prefrontal cortex deactivation in risky decision making after simulated microgravity: effects of -6° head-down tilt bed rest. *Front. Behav. Neurosci.* 8:187. doi: 10.3389/fnbeh.2014.00187

Rau, H., Brody, S., Elbert, T., and Birbaumer, N. (1993). Baroreceptor stimulation alters cortical activity. *Psychophysiology* 30, 322–325. doi: 10.1111/j.1469-8986.1993.tb03359.x

Rice, J. K., Rorden, C., Little, J. S., and Parra, L. C. (2013). Subject position affects EEG magnitudes. *Neuroimage* 64, 476–484. doi: 10.1016/j.neuroimage.2012.09.041

Roberts, D. R., Albrecht, M. H., Collins, H. R., Asemani, D., Chatterjee, A. R., Spampinato, M. V., et al. (2017). Effects of spaceflight on astronaut brain structure as indicated on MRI. *N. Engl. J. Med.* 377, 1746–1753. doi: 10.1056/NEJMoa1705129

Roberts, D. R., Stahn, A. C., Seidler, R. D., and Wuyts, F. L. (2020). Towards understanding the effects of spaceflight on the brain. *Lancet Neurol.* 19:808. doi: 10.1016/S1474-4422(20)30304-5

Roberts, D. R., Zhu, X., Tabesh, A., Duffy, E. W., Ramsey, D. A., and Brown, T. R. (2015). Structural brain changes following long-term 6° head-down tilt bed rest as an analog for spaceflight. *Am. J. Neuroradiol.* 36, 2048–2054. doi: 10.3174/ajnr.A4406

Sato, W., Kochiyama, T., Uono, S., Sawada, R., and Yoshikawa, S. (2020). Amygdala activity related to perceived social support. *Sci. Rep.* 10:2951. doi: 10.1038/s41598-020-59758-x

Schneider, S., Brümmer, V., Carnahan, H., Dubrowski, A., Askew, C. D., and Strüder, H. K. (2008). What happens to the brain in weightlessness? A first approach by EEG tomography. *Neuroimage* 42, 1316–1323. doi: 10.1016/j.neuroimage.2008.06.010

Shibasaki, H. (2008). Human brain mapping: hemodynamic response and electrophysiology. *Clin. Neurophysiol.* 119, 731–743. doi: 10.1016/j.clinph.2007.10.026

Shirazi, S. Y., and Huang, H. J. (2019). More reliable EEG electrode digitizing methods can reduce source estimation uncertainty, but current methods already accurately identify brodmann areas. *Front. Neurosci.* 13:1159. doi: 10.3389/fnins.2019.01159

Spironelli, C., and Angrilli, A. (2017). Posture used in fMRI-PET elicits reduced cortical activity and altered hemispheric asymmetry with respect to sitting position: an EEG resting state study. *Front. Hum. Neurosci.* 11:621. doi: 10.3389/fnhum.2017.00621

Stenroos, M., and Nummenmaa, A. (2016). Incorporating and compensating cerebrospinal fluid in surface-based forward models of magneto- and electroencephalography. *PLoS One* 11:e0159595. doi: 10.1371/journal.pone.0159595

Tandle, A., Jog, N., D'cunha, P., and Chheta, M. (2015). Classification of artefacts in EEG signal recordings and overview of removing techniques. *Int. J. Comput. Appl.* 4, 12–19. doi: 10.5120/cae2016651997

Thatcher, R. W., North, D. M., and Bive, C. J. (2014). LORETA EEG phase reset of the default mode network. *Front. Hum. Neurosci.* 8:529. doi: 10.3389/fnhum.2014.00529

Thibault, R. T., Lifshitz, M., Jones, J. M., and Raz, A. (2014). Posture alters human resting-state. *Cortex* 58, 199–205. doi: 10.1016/j.cortex.2014.06.014

Thornton, W. E., Moore, T. P., and Pool, S. L. (1987). Fluid shifts in weightlessness. *Aviat. Space Environ. Med.* 58(9 Pt 2), A86–A90.

Van Den Broek, S. P., Reinders, F., Donderwinkel, M., and Peters, M. J. (1998). Volume conduction effects in EEG and MEG. *Electroencephalogr. Clin. Neurophysiol.* 106, 522–534. doi: 10.1016/S0013-4694(97)00147-8

Wendel, K., Narra, N. G., Hannula, M., Kauppinen, P., and Malmivuo, J. (2008). The influence of CSF on EEG sensitivity distributions of multilayered head models. *IEEE Trans. Biomed. Eng.* 55, 1454–1456. doi: 10.1109/TBME.2007.912427

Whitton, A. E., Deccy, S., Ironside, M. L., Kumar, P., Beltzer, M., and Pizzagalli, D. A. (2018). Electroencephalography source functional connectivity reveals abnormal high-frequency communication among large-scale functional networks in depression. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 3, 50–58. doi: 10.1016/j.bpsc.2017.07.001

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag New York.

Witten, M. L. (2005). The neurolab spacelab mission: neuroscience research in space. *J. Clin. Sleep Med.* 1:211. doi: 10.5664/jcsm.26324

Yuan, P., Koppelmans, V., Reuter-Lorenz, P., De Dios, Y., Gadd, N., Wood, S., et al. (2018). Vestibular brain changes within 70 days of head down bed rest. *Hum. Brain Mapp.* 39, 2753–2763. doi: 10.1002/hbm.24037

Yuan, P., Koppelmans, V., Reuter-Lorenz, P. A., De Dios, Y. E., Gadd, N. E., Wood, S. J., et al. (2016). Increased brain activation for dual tasking with 70-days head-down bed rest. *Front. Syst. Neurosci.* 10:71. doi: 10.3389/fnsys.2016.00071

Zhou, Y., Wang, Y., Rao, L. L., Liang, Z. Y., Chen, X. P., Zheng, D., et al. (2014). Disrutpted resting-state functional architecture of the brain after 45-day simulated microgravity. *Front. Behav. Neurosci.* 8:200. doi: 10.3389/fnbeh.2014.00200

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary Table 1: Seed coordinates used for functional connectivity analysis.*

Anatomical Structure	**MNI coordinates**		
	x	y	z
Left posterior inferior parietal lobule	-50	-70	30
Right posterior inferior parietal lobule	50	-70	30
Left anterior inferior parietal lobule	-50	-50	45
Right anterior inferior parietal lobule	50	-50	45
Left hippocampal formation	-20	-20	-20
Right hippocampal formation	20	-20	-20
Left superior parietal lobule	-25	-50	60
Right superior parietal lobule	25	-50	60
Anterior cingulate cortex	5	30	25
Left anterior insula	-30	20	5
Right anterior insula	30	20	5
Posterior cingulate cortex	0	-55	15
Left medial frontal cortex	-35	55	5
Right medial frontal cortex	35	55	5
Left parahippocampal gyrus	-25	-25	20
Right parahippocampal gyrus	25	-25	20
Precuneus	0	-75	45
Left middle temporal gyrus	-65	-20	-10
Right middle temporal gyrus	65	-20	-10

*The eLORETA solution space was restricted to the cortical gray matter of a realistic head model (MNI152), co-registered to the Talairach brain atlas and digitized at the Montreal Neurologic Institute (MNI) brain imaging center. A single voxel that was closest to the seed point was defined as the centroid of each region of interest (ROI).

Supplementary Table 2: Mixed-models of spectral analysis assessing the effects of *Time*, *Group, Region, Laterality,* and their interaction for the RSL study (n = 23).*

Effect	Theta				Delta				Alpha				Beta			
	df_1	df_2	F	p	df_1	df_2	F	p	df_1	df_2	F	p	df_1	df_2	F	p
Time	4	399	20.54	<0.001	4	399	23.50	< 0.001	4	399	31.38	< 0.001	4	399	40.58	< 0.001
Group	1	21	0.41	0.530	1	21	0.40	0.533	1	21	0.59	0.891	1	21	0.02	0.891
Region	1	399	16.69	<0.001	1	399	0.01	0.967	1	399	87.62	< 0.001	1	399	43.80	< 0.001
Laterality	1	399	0.01	0.936	1	399	0.01	0.974	1	399	0.68	0.206	1	399	1.61	0.206
Time x Group	4	399	1.04	0.383	4	399	0.18	0.949	4	399	0.46	0.375	4	399	1.06	0.375
Time x Region	4	399	2.45	0.046	4	399	3.15	0.014	4	399	0.46	< 0.001	4	399	11.29	< 0.001
Group x Region	4	399	0.05	0.821	4	399	4.45	0.035	4	399	1.67	0.447	4	399	0.58	0.447
Time x Laterality	1	399	0.24	0.916	1	399	0.31	0.870	1	399	0.09	0.931	1	399	0.25	0.931
Group x Laterality	1	399	0.26	0.611	1	399	0.89	0.345	1	399	0.79	0.643	1	399	0.21	0.643
Region x Laterality	1	399	0.71	0.399	1	399	0.21	0.647	1	399	3.49	0.484	1	399	0.49	0.484
Time x Group x Region	4	399	0.07	0.992	4	399	0.38	0.821	4	399	0.22	0.819	4	399	0.39	0.819
Time x Group x Laterality	4	399	0.01	0.999	4	399	0.26	0.905	4	399	0.02	0.963	4	399	0.15	0.963
Time x Region x Laterality	4	399	0.51	0.729	4	399	0.44	0.781	4	399	0.10	0.881	4	399	0.30	0.881
Group x Region x Laterality	1	399	0.10	0.755	1	399	0.01	0.942	1	399	0.91	0.737	1	399	0.11	0.737
Time x Region x Group x Laterality	4	399	0.17	0.952	4	399	0.25	0.910	4	399	0.09	0.988	4	342	0.08	0.988

*Mixed-models were performed separate for each frequency band using *Time*, *Group*, *Region*, and *Laterality* as fixed factors and subject as a random factor. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; F, F-statistics, p, p-value.

Supplementary Table 3: Mixed-models of spectral analysis assessing the effects of *Time*, *Group, Region, Laterality,* and their interaction for the COCKTAIL study ($n = 20$).*

Effect	Theta				Delta				Alpha				Beta			
	df_1	df_2	F	p	df_1	df_2	F	p	df_1	df_2	F	p	df_1	df_2	F	p
Time	4	342	60.89	< 0.001	4	342	36.61	< 0.001	4	342	21.45	< 0.001	4	18	41.21	< 0.001
Group	1	18	0.35	0.562	1	18	0.23	0.636	1	18	0.09	0.762	1	342	0.62	0.441
Region	1	342	37.53	< 0.001	1	342	7.88	0.005	1	342	52.44	< 0.001	1	342	18.60	< 0.001
Laterality	1	342	0.28	0.597	1	342	0.61	0.436	1	342	0.24	0.623	1	342	0.13	0.719
Time x Group	4	342	1.49	0.204	4	342	1.20	0.310	4	342	0.49	0.742	4	342	2.37	0.092
Time x Region	4	342	7.58	< 0.001	4	342	0.10	0.983	4	342	2.42	0.048	4	342	3.80	0.005
Group x Region	4	342	0.46	0.497	4	342	3.20	0.075	4	342	0.06	0.808	4	342	0.01	0.928
Time x Laterality	1	342	0.16	0.957	1	342	0.31	0.862	1	342	0.14	0.970	1	342	0.76	0.551
Group x Laterality	1	342	0.35	0.553	1	342	0.94	0.333	1	342	0.35	0.554	1	342	2.36	0.125
Region x Laterality	1	342	0.23	0.629	1	342	0.01	0.938	1	342	2.96	0.086	1	342	0.86	0.353
Time x Group x Region	4	342	0.14	0.967	4	342	0.39	0.813	4	342	0.06	0.994	4	342	0.29	0.886
Time x Group x Laterality	4	342	0.62	0.648	4	342	0.68	0.606	4	342	0.08	0.988	4	342	0.81	0.518
Time x Region x Laterality	4	342	0.10	0.982	4	342	0.22	0.927	4	342	0.29	0.881	4	342	0.40	0.812
Group x Region x Laterality	1	342	0.25	0.617	1	342	1.95	0.163	1	342	0.71	0.398	1	342	0.25	0.615
Time x Region x Group x Laterality	4	342	0.25	0.909	4	342	0.16	0.958	4	342	0.15	0.962	4	342	0.40	0.805

*Mixed-models were performed separate for each frequency band using *Time*, *Group*, *Region*, and *Laterality* as fixed factors and subject as a random factor. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; F, F-statistics, p, p-value.

Supplementary Table 4. Contrasts examining the effect of *Time* on spectral power for the RSL study (n = 23) using baseline as a reference level.*

Frequency Band	Time	Region	*df*	*t*	p_{corr}	*Effect Size d (95% CI)*
Theta	HDBR2	Anterior	399	-2.96	0.013	-1.23 (-2.12, -0.32)
	HDBR2	Posterior	399	-4.27	< 0.001	-1.78 (-2.75, -0.79)
	HDBR28	Anterior	399	-3.38	0.003	-1.41 (-2.32, -0.48)
	HDBR28	Posterior	399	-5.21	< 0.001	-1.78 (-3.2, -1.11)
	HDBR56	Anterior	399	-3.8	0.001	-1.59 (-2.52, -0.62)
	HDBR56	Posterior	399	-5.46	< 0.001	-2.28 (-3.33, -1.2)
	R+10	Anterior	399	-1.16	0.981	0.49 (-1.31, 0.35)
	R+10	Posterior	399	0.75	1	0.31 (-0.52, 1.13)
Delta	HDBR2	Anterior	399	-2.64	0.034	-1.1 (-1.97, -0.21)
	HDBR2	Posterior	399	-4.64	< 0.001	-1.94 (-2.92, -0.92)
	HDBR28	Anterior	399	-3.75	0.001	-1.56 (-2.49, -0.61)
	HDBR28	Posterior	399	-6.68	< 0.001	-2.79 (-3.94, -1.6)
	HDBR56	Anterior	399	-5.03	< 0.001	-2.1 (-3.12, -1.05)
	HDBR56	Posterior	399	-6.35	< 0.001	-2.65 (-3.77, -1.49)
	R+10	Anterior	399	-2.33	0.081	-0.97 (-1.83, -0.09)
	R+10	Posterior	399	-0.72	1	-0.3 (-1.12, 0.52)
Alpha	HDBR2	Anterior	399	-2.67	0.032	-1.11 (-1.98, -0.22)
	HDBR2	Posterior	399	-4.44	< 0.001	-1.85 (-2.82, -0.85)
	HDBR28	Anterior	399	-3.55	0.002	-1.48 (-2.4, -0.54)
	HDBR28	Posterior	399	-6.38	< 0.001	-2.66 (-3.79, -1.5)
	HDBR56	Anterior	399	-3.59	0.002	-1.5 (-2.41, -0.55)
	HDBR56	Posterior	399	-6.34	< 0.001	-2.64 (-3.77, -1.49)
	R+10	Anterior	399	0.00	1	0 (-0.82, 0.82)
	R+10	Posterior	399	1.89	0.236	0.79 (-0.07, 1.63)
Beta	HDBR2	Anterior	399	-1.56	0.482	-0.65 (-1.48, 0.2)
	HDBR2	Posterior	399	-5.22	< 0.001	-2.18 (-3.21, -1.11)
	HDBR28	Anterior	399	-2.24	0.103	-0.93 (-1.79, -0.06)
	HDBR28	Posterior	399	-7.28	< 0.001	-2.04 (-4.24, -1.79)
	HDBR56	Anterior	399	-2.57	0.042	-1.07 (-1.94, -0.18)
	HDBR56	Posterior	399	-6.88	< 0.001	-2.87 (-4.04, -1.66)
	R+10	Anterior	399	0.99	1	0.41 (-0.42, 1,24)
	R+10	Posterior	399	3.88	< 0.001	1.62 (0.65, 2.55)

*Data show effects of *Time* (HDBR2, HDBR28, HDBR56, R+10) by *Region* (Anterior, Posterior) using baseline (BDC-7) as a reference. *df*, degrees of freedom; $p_{corr.}$, *p*-value corrected for multiple comparisons using the Bonferroni correction for each main effect (theta, delta, alpha, and beta power); Effect Size is Cohen's d; 95% CI, 95% confidence interval.

Supplementary Table 5. Contrasts examining the effect of *Time* on spectral power for the COCKTAIL study (n = 20) using baseline as a reference level.*

Frequency Band	Time	Region	*df*	*t*	p_{corr}	Effect Size *d* (95% CI)
Theta	HDBR7	Anterior	342	-4.45	< 0.001	-1.86 (-2.83, -0.85)
	HDBR7	Posterior	342	-9.82	< 0.001	-4.09 (-5.55, -2.6)
	HDBR31	Anterior	342	-4.31	< 0.001	-1.80 (-2.76, -0.8)
	HDBR31	Posterior	342	-9.26	< 0.001	-3.86 (-5.27, -2.43)
	HDBR60	Anterior	342	-4.28	< 0.001	-1.79 (-2.75, -0.79)
	HDBR60	Posterior	342	-9.00	< 0.001	-3.75 (-5.13, -2.35)
	R+7	Anterior	342	0.51	1	0.21 (-0.61, 1.03)
	R+7	Posterior	342	0.50	1	0.21 (-0.61, 1.03)
Delta	HDBR7	Anterior	342	-3.11	0.008	-1.30 (-2.19, -0.38)
	HDBR7	Posterior	342	-3.81	0.001	-1.59 (-2.52, -0.63)
	HDBR31	Anterior	342	-3.73	0.001	-1.56 (-2.49, -0.6)
	HDBR31	Posterior	342	-4.46	< 0.001	-1.86 (-2.84, -0.86)
	HDBR60	Anterior	342	-3.81	0.009	-1.28 (-2.17, -0.36)
	HDBR60	Posterior	342	-3.56	0.002	-1.48 (-2.40, -0.54)
	R+7	Anterior	342	3.14	0.007	1.31 (0.39, 2.2)
	R+7	Posterior	342	2.91	0.015	1.21 (0.31, 2.10)
Alpha	HDBR7	Anterior	342	-2.85	0.019	-1.19 (-2.07, -0.28)
	HDBR7	Posterior	342	-5.22	< 0.001	-2.18 (-3.21, -1.11)
	HDBR31	Anterior	342	-2.13	0.135	-0.89 (-1.74, -0.02)
	HDBR31	Posterior	342	-4.14	< 0.001	-1.73 (-2.68, -0.74)
	HDBR60	Anterior	342	-2.07	0.157	-0.86 (-1.71, -0.01)
	HDBR60	Posterior	342	-3.39	0.003	-1.41 (-2.32, -0.48)
	R+7	Anterior	342	0.76	1	0.32 (-0.51, 1.13)
	R+7	Posterior	342	2.17	0.124	0.9 (0.03, 1.76)
Beta	HDBR7	Anterior	342	-4.58	< 0.001	-1.91 (-2.89, -0.89)
	HDBR7	Posterior	342	-8.79	< 0.001	-3.67 (-5.02, -2.28)
	HDBR31	Anterior	342	-4.11	< 0.001	-1.71 (-2.67, -0.73)
	HDBR31	Posterior	342	-8.48	< 0.001	-3.54 (-4.86, -2.18)
	HDBR60	Anterior	342	-3.55	0.002	-1.48 (-2.4, -0.54)
	HDBR60	Posterior	342	-7.06	< 0.001	-2.95 (-4.13, -1.72)
	R+7	Anterior	342	-0.04	1	-0.01 (-0.83, 0.8)
	R+7	Posterior	342	-1.26	0.835	-0.53 (-1.35, 0.31)

*Data show effects of *Time* (HDBR7, HDBR31, HDBR60, R+7) by *Region* (Anterior, Posterior) using baseline (BDC-8) as a reference. *df*, degrees of freedom; $p_{corr.}$, *p*-value corrected for multiple comparisons using the Bonferroni correction for each main effect (theta, delta, alpha, and beta power); Effect Size is Cohen's *d*; 95% CI, 95% confidence interval.

Supplementary Table 6. Number of significant voxels ($p < 0.05$) per lobe and hemisphere revealed by eLORETA analysis for the RSL (N = 23) and COCKTAIL study ($n = 20$) showing decreases in alpha and beta cortical current density with respect to baseline.*

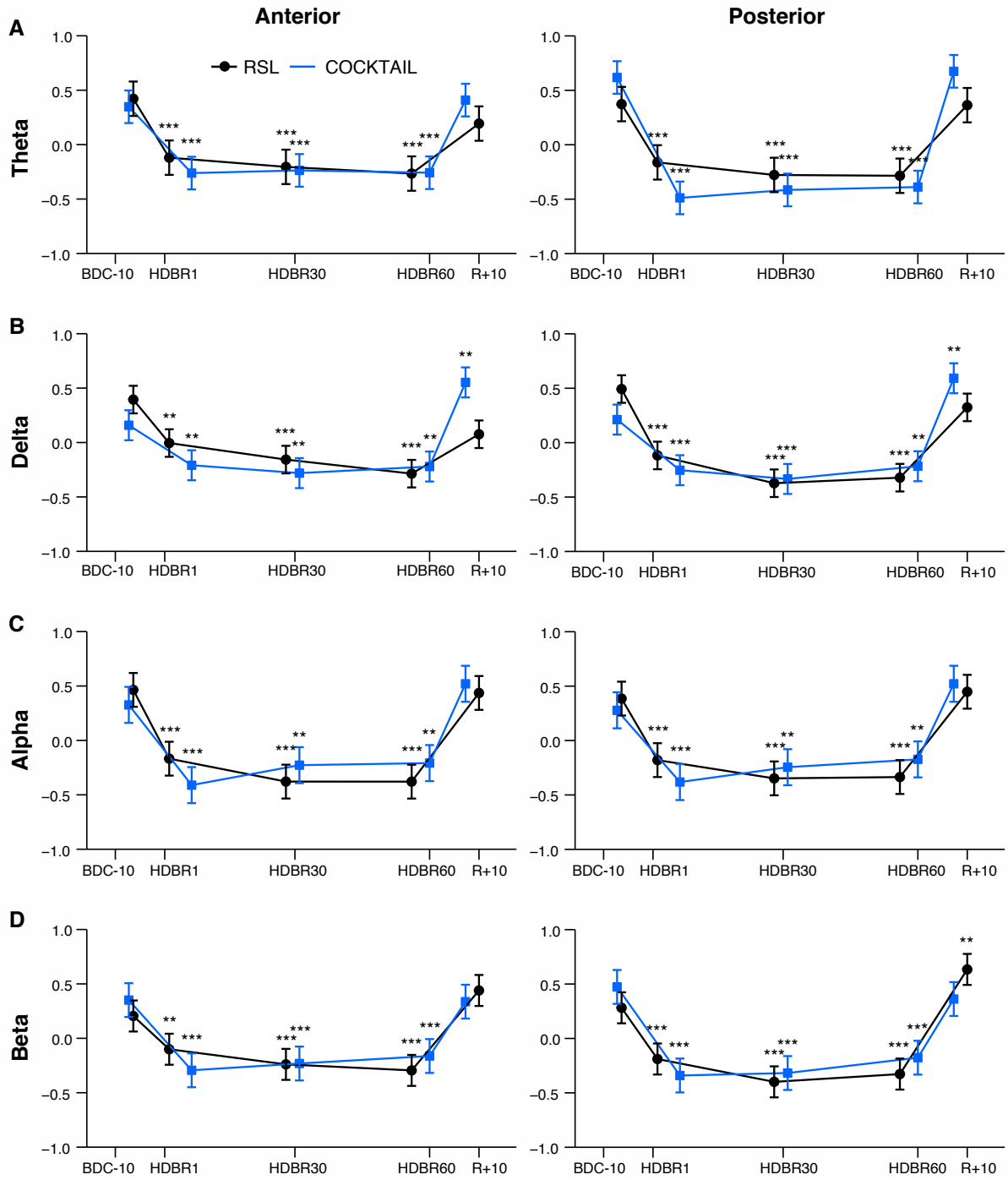
	Brain Region	Short-term				Mid-term				Long-term			
		Alpha		Beta		Alpha		Beta		Alpha		Beta	
		L	R	L	R	L	R	L	R	L	R	L	R
RSL	Frontal lobe				4	1	1					7	
	Limbic Lobe	10	20	32	74	74	61	109	59	3	2	110	36
	Occipital Lobe	10	100	22	207	217	163	268	236	1	9	78	49
	Parietal lobe	39	53	7	11	117	123	20	23	12	26	2	3
	Sub-lobar				3	1	1	13	5	2	14	5	2
	Temporal lobe		8		108	8	27	109	11	10	1	27	
COCKTAIL	Frontal lobe		1	3	192				166			23	172
	Limbic Lobe	24	23	49	187	2	6	18	136	8	18	31	78
	Occipital Lobe			59	336	11	27	114	351	6	11	207	259
	Parietal lobe		146	9	90	27	245	10	65	23	155	27	57
	Sub-lobar		12		100		19		48		15		26
	Temporal lobe		4		557		50		505		60	1	152

*The eLORETA solution space was restricted to the cortical gray matter of a realistic head model (MNI152) registered to the Talairach brain atlas. Short-term, mid-term, and long-term refer to HDBR2/HDBR7, HDBR28/HDBR31, and HDBR56/HDBR60 for the RSL and COCKTAIL study, respectively. L, left hemisphere; R, right hemisphere.

Supplementary Table 7. Contrasts examining the effect of *Time* on eLORETA resting state functional connectivity for the RSL (n = 23) and COCKTAIL (n = 20) experiment.*

Experiment	Contrast	$t_{critical}$	t_{max}	p
RSL	HDBR2 vs BDC-7	4.21	3.86	0.142
	HDBR28 vs BDC-7	4.23	3.20	0.597
	HDBR56 vs BDC-7	4.19	3.41	0.414
	R+10 vs BDC-7	4.26	2.88	0.872
	HDBR28 vs HDBR2	4.23	3.16	0.668
	HDBR56 vs HDBR2	4.21	3.07	0.756
	HDBR56 vs HDBR28	4.21	2.64	0.989
COCKTAIL	HDBR7 vs BDC-8	4.49	3.77	0.281
	HDBR31 vs BDC-8	4.42	3.81	0.224
	HDBR60 vs BDC-8	4.37	3.14	0.757
	R+7 vs BDC-8	4.42	3.16	0.738
	HDBR31 vs HDBR7	4.32	3.95	0.149
	HDBR60vs HDBR7	4.38	3.85	0.199
	HDBR60 vs HDBR31	4.31	3.33	0.533

*Connectivity was defined as the lagged phase synchronization between the intracortical EEG-source estimates of the regions of interest. $t_{critical}$, critical probability threshold of non-parametric randomization test with 5000 randomizations corrected for multiple comparisons; t_{max}, maximal t-statistic; $p_{corr.}$, p-value.



Supplementary Figure 1. Impact of long-duration head-down tilt bed rest on normalized electrocortical activity for the RSL study (n = 23, blue circle), and the COCKTAIL study (N = 20, black square). Time courses show changes of EEG spectral power after z-transforming across study participants and testing days for anterior and posterior sites, within the **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency. Data are presented for each time point as estimated marginal means and standard errors. Significant levels with respect to baseline are indicated by asterisks. BDC-10 to BDC-1 refers to baseline data collection. HDBR1, HDBR30, and HDBR60 indicate first, 30th, and 60th day of HDBR. R+0 to R+10 correspond to the first and 11th day after HDBR. For RSL data were collected at BDC-7, HDBR2, HDBR28, HDBR56, and R+10. For COCKTAIL data were collected at BDC-8, HDBR7, HDBR30, HDBR60, and R+7. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to baseline.

Experiment II

Impact of 30-Day Immobilization on Affective Picture Processing

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Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization

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The neurobehavioral risks associated with spaceflight are not well understood. In particular, little attention has been paid on the role of resilience, social processes and emotion regulation during long-duration spaceflight. Bed rest is a well-established spaceflight analogue that combines the adaptations associated with physical inactivity and semi-isolation and confinement. We here investigated the effects of 30 days of 6 degrees head-down tilt bed rest on affective picture processing using event-related potentials (ERP) in healthy men. Compared to a control group, bed rest participants showed significantly decreased P300 and LPP amplitudes to pleasant and unpleasant stimuli, especially in centroparietal regions, after 30 days of bed rest. Source localization revealed a bilateral lower activity in the posterior cingulate gyrus, insula and precuneus in the bed rest group in both ERP time frames for emotional, but not neutral stimuli.

Affective processing and emotion regulation are fundamental to human behaviour. They facilitate decision making, have significant influences on learning and memory and provide the motivation for critical action in the face of environmental incentives. The management of positive and negative emotions also directly relates to individual sociability and social interactions. Any emotional alteration may interfere with cognitive performance, impair mental well-being and lead to various forms of psychopathology, especially in the context of a stressful environment[1]. When living and working in an isolated, confined and hostile environment like deep space for prolonged durations, astronauts are exposed to numerous stressors including social isolation, confinement and weightlessness. Currently, the neurobehavioral risks associated with these stressors are not fully understood. In particular, the role of resilience, social processes and emotion regulation during long-duration spaceflight has received little attention so far. Head-down tilt bedrest (HDT) is a well-established model to simulate physical deconditioning and cephalic fluid shifts during standard space missions on the International Space Station (ISS)[2]. Bed rest also comprises a degree of sensory deprivation, isolation, and confinement[3]. Previous studies suggest that long-duration bed rest increases the risk for mood disorders[4], and impairs emotion recognition processing during a Flanker task[5]. According to the authors' knowledge no study has investigated the effects of long-duration bed rest on the neural correlates of emotional processing. The current study aimed to address this gap by investigating the effects of 30 days of -6 degrees HDT bed rest on cortical emotional modulation using event-related brain potentials from a standardized and well-established paradigm[6]. We hypothesized that long-term bed rest would lead to a cortical inhibition of affective processes as indicated by reduced event-related potentials.

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Group	Stimulus	Valence Rating	Arousal Rating
CTRL	pleasant	7.6 (0.6)	5.7 (1.2)
	neutral	4.6 (0.9)	2.4 (1.2)
	unpleasant	2.6 (0.5)	5.7 (1.0)
HDBR	pleasant	7.6 (0.9)	5.7 (1.6)
	neutral	5.2 (0.8)	2.2 (1.2)
	unpleasant	2.6 (0.7)	5.7 (2.2)

Table 1. Subjective ratings for pleasant, neutral and unpleasant IAPS pictures in CTRL and HDBR group. Note: Subjective ratings are based on 9-point Likert scales, ranging from very unpleasant/not arousing at all to very pleasant/very arousing. Data are means and standard deviations.

Results

Emotional self-reports. Table 1 illustrates the self-reported evaluations of each picture category for the control (CTRL) group tested before bed rest, and the intervention group tested after 30 days of head-down tilt bed rest (HDBR). The ratings for all three picture categories were consistent with IAPS normative data[6], confirming the validity of the paradigm in the present experimental setup. In both groups, positive pictures were rated as more arousing and got greater scores for valence than neutral ones (Table 1). Additionally, unpleasant slides received a lower scoring than neutral pictures for valence and were evaluated as more arousing (Table 1). This was confirmed by mixed model analyses, showing a significant main effect of stimulus condition on arousal ($F(2,36) = 76.78$, $p < 0.001$) and valence ($F(2,36) = 309.20$, $p < 0.001$).

However, statistical analyses neither revealed a significant stimulus x group interaction, nor a significant group effect for valence ($F(2,36) = 0.05$, $p = 0.948$ and $F(1,18) = 0.02$, $p = 0.879$, respectively) or arousal ($F(2,36) = 0.10$, $p = 0.909$ and $F(1,18) = 0.08$, $p = 0.928$, respectively). Planned contrasts revealed similar ratings for valence and arousal for all picture categories between groups (all $ps > 0.728$).

Electrophysiological data. Figure 1A depicts the grand average ERP waveforms for CTRL and HDBR subjects in frontal and parietal regions, respectively. While neutral pictures elicited similar responses in CTRL and HDBR participants, the ERP waveforms of emotional stimuli were inhibited in the HDBR group compared to the CTRL group. As shown in Table 2, the mixed ANOVA analysis of mean P300 amplitude revealed a significant interaction of group and stimulus in the frontal ($p = 0.002$) and parietal sites ($p = 0.002$). Mean LPP amplitude showed a significant effect of group in frontal ($p = 0.048$) and parietal sites ($p = 0.026$) and a significant effect of stimulus in parietal site ($p < 0.001$). Simple comparisons are shown in Table S1 and Table S2 that can be found in the Supplementary Information.

Planned contrasts (Table S1) confirmed that emotional pictures induced enhanced electrocortical responses in CTRL compared to HDBR participants in both regions and time frames (all $ps < 0.029$) except for the frontal LPP which was not significant between groups for positive pictures ($p = 0.074$). For the neutral stimuli, no differences in LPP and P300 amplitudes between groups were observed (all $ps > 0.314$). The ERP difference topography between emotional and neutral stimuli for both components and both groups is illustrated in Fig. 1B. While CTRL participants showed enhanced P300 and LPP amplitudes for emotional stimuli relative to neutral pictures, there was no visible difference in the HDBR group. A follow-up analysis using pre-planned contrasts (Table S2) revealed that positive and negative stimuli evoked significantly increased P300 components compared to neutral stimuli in the CTRL (all $ps < 0.003$), but not the HDBR group (all $ps > 0.414$). We also observed significant differences between LPP components induced by positive stimuli and neutral stimuli in both regions (all $ps < 0.037$) and a significantly smaller LPP amplitude in the frontal area induced by negative pictures compared to neutral pictures ($p < 0.001$) in CTRL participants only.

eLORETA data. For the averaged LPP evoked by positive pictures, a significantly lower cortical activation for HDBR compared to CTRL participants was found in the right insula (BA 13, $p < 0.05$, Fig. 2). The P300 comparison between CTRL and HDBR group revealed statistically lower cortical activations in the bilateral precuneus and the bilateral cingulate gyrus (BA 31/7, $p < 0.05$, Fig. 2). Moreover, analysis of P300 and LPP showed a decrease in cortical activity at the same locations (BA 31/7; all $ps < 0.05$, Fig. 2) when processing negative pictures, as compared to CTRL group. No significant differences were found comparing CTRL and HDBR group for mean P300 and LPP amplitudes evoked by neutral stimuli (see $F_{critical}$ in Table 3).

Discussion

The present study investigated the effects of 30 days of immobilization on affective picture processing in young healthy men. To evaluate the impact of long-term bed rest on emotional processing we employed a well-established ERP paradigm using standardized affective stimuli. Our main findings include an inhibition of P300 and LPP components for emotional stimuli, but not neutral pictures in HDBR participants when compared to a sex- and age-matched control group. This inhibition was found to be localized in the precuneus, cingulate gyrus, and insula.

The CTRL group exhibited larger P300 and LPP components when viewing pleasant and unpleasant pictures as compared to neutral slides. This result is well in line with previous research investigating affective picture processing in young healthy adults[7,8]. Larger evoked potentials are thought to reflect increased attention towards biologically relevant emotional stimuli[9]. Particularly, the P300 has been hypothesized to be an index of

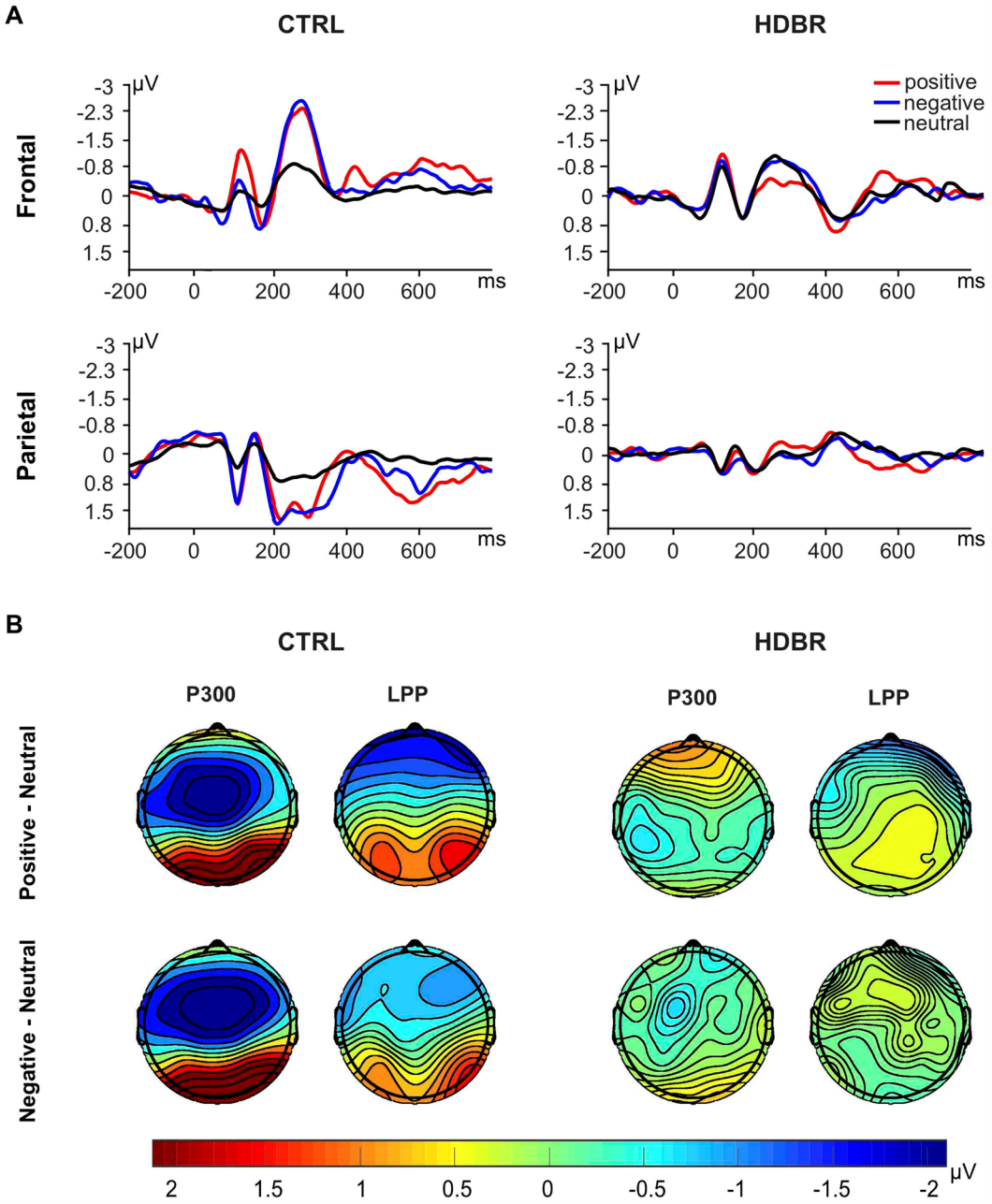

Figure 1. Event-related potential (ERP) results. (**A**) Grand average ERP waveforms at selected electrode clusters (frontal: F3, F4; parietal: P3, P4, Pz) for positive. (n = 25), negative (n = 25) and neutral (n = 25) stimuli in a control group (CTRL, n = 10) and a bed rest group (HDBR, n = 10). (**B**) Topographical maps depicting mean voltage differences between positive and neutral, and between negative and neutral stimuli averaged for the CTRL group and HDBR group for each ERP component (i.e., P300, and LPP).

initial memory storage and attention[10], whereas the LPP is supposed to be a cortical correlate that is associated with encoding and memory processes[11]. Additionally, emotional stimuli are better perceived, encoded, consolidated and retrieved than neutral stimuli[12]. In contrast, we did not observe the expected difference between brain potentials in HDBR participants, immobilized for 30 days in -6 degrees head-down tilt position. We found that long-term immobilization resulted in emotional blunting as evidenced by reduced LPP and P300 amplitudes in response to affective images, i.e., pleasant and unpleasant stimuli elicited a similar flattened response as neutral ones. The emotional blunting indicates dysfunctional modulations in the processing of emotional information.

A source localization revealed a cortical inhibition of distinct brain regions. Specifically, long-term bed rest was found to be associated with a lower activation within the right insula, the bilateral precuneus, and the bilateral posterior cingulate gyrus (PCG) when processing pleasant and unpleasant stimuli. Electrophysiological recordings and neuroimaging have supported key positions of the amygdala, cingulate gyrus and insula in response to emotional stimuli[13]. Moreover, past studies reported a similar role in emotional information processing for

Factor	P300	LLP
Frontal electrode cluster		
Group	$F(1, 18) = 2.84$	$F(1, 18) = 4.50$*
Stimulus	$F(2, 36) = 10.12$***	$F(2, 36) = 1.33$
Group x Stimulus	$F(2, 36) = 7.22$**	$F(2, 36) = 3.03$
Parietal electrode cluster		
Group	$F(1, 18) = 11.16$**	$F(1, 18) = 5.86$*
Stimulus	$F(2, 3\,6) = 3.72$*	$F(2, 36) = 16.48$***
Group x Stimulus	$F(2, 36) = 7.65$**	$F(2, 36) = 2.21$

Table 2. Mixed-model analyses assessing the effects of group (HDBR, CTRL) and stimuli (negative, postive, neutral) on P300 and LLP components. *$p < 0.05$. **$p < 0.01$. ***$p < 0.0001$.

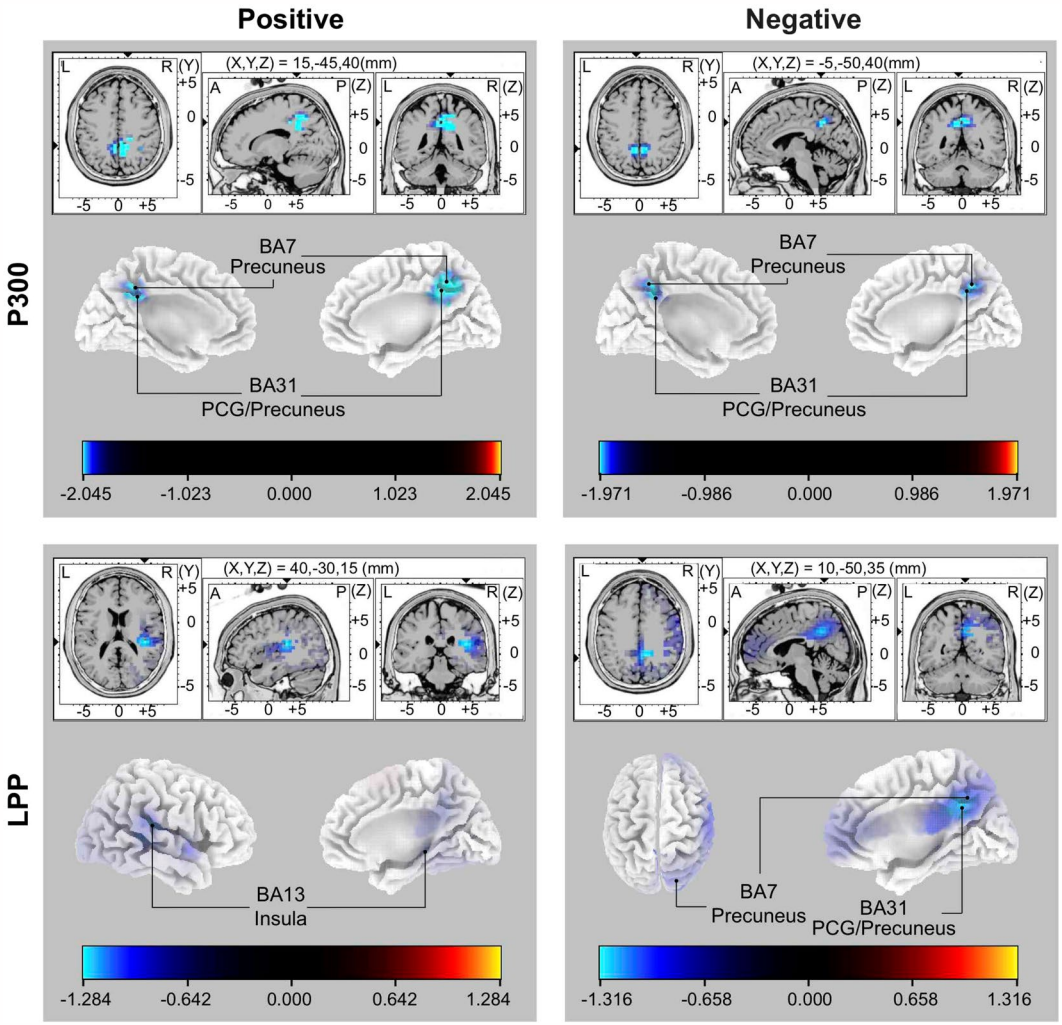

Figure 2. Statistical parametric maps (SPMs) indicating the differences in brain source localization between control (CTRL, n = 10) and head-down-tiltbed rest group (HDBR, n = 10). Data for positive and negative stimuli are shown on the left and right panels, respectively. Results for the P300 and LPP components are provided in the upper and lower panels, respectively. Blue colours indicate decreased activity in the HDBR compared to the CTRL group. The color scale indicates F-values for group differences of brain activity. L left, R right, A anterior, P posterior, PCG posterior cingulate gyrus, BA Brodmann area.

PCG and precuneus due to their structural and functional similarities[14]. There is current evidence, that PCG and precuneus are activated during the evaluation of emotional words[15], the retrieval of emotional memories[16] and the processing of self-relevant affect[17]. The insula, however, plays an important role in pain processing[18]

		Positive	Neutral	Negative
P300	$F_{critical}$ for $p < 0.05$	1.85	1.71	1.78
	$F_{critical}$ for $p < 0.01$	2.05	1.91	1.97
	Statistical Threshold	−2.48	−1.53	−2.02
LPP	$F_{critical}$ for $p < 0.05$	1.24	1.31	1.23
	$F_{critical}$ for $p < 0.01$	1.37	1.51	1.39
	F_{max}	−1.28	−0.88	−1.32

Table 3. Loreta critical thresholds ($F_{critical}$) and maximal *F*-statistics (F_{max}) for ERP components and each stimulus type.

and, additionally, has been shown to be instrumental in the detection, interpretation, and regulation of internal bodily states[19], therefore serving as a critical bridge between affective and cognitive processes. Moreover, precuneus, PCG and insula are reciprocally connected to areas involved in emotional processing such as the anterior cingulate and the orbital frontal cortices, as well as the amygdala[20,21]. Considering these findings, it is reasonable that PCG, precuneus and insula carry emotion-specific information. Interestingly, Zou and colleagues recently showed that 45 days of bed rest altered the resting-state functional architecture of a similar region including the insula and cingulate cortex and hypothesized that these effects might influence the processing of salient information[22]. These data support the vulnerability of these structures to the detrimental neurocognitive effects of prolonged immobilization.

Notably, the self-evaluation of valence and arousal did not differentiate our two groups. The absence of any differences indicates that physiological data may be more objective than behavioural measures as they do not underlie cognitive-social control and are therefore less sensitive to experimental manipulations. Participants possibly tend to respond to self-evaluation in a stereotyped fashion. In line with this, Messerotti and colleagues have shown that acute HDT can suppress cortical emotional responses[23], without affecting behavioural responses. They attribute the electrocortical changes to an altered body position. Recent research performed by the same group has demonstrated that these postural effects on electrocortical activity are immediately observed after changing from sitting to the supine position[24]. To account for postural effects in the present experiment, both groups were tested in the same position, i.e., at -6 degrees HDT, providing sufficient time to account for the cephalic fluid shifts[25]. We therefore assume that the present findings are explained by mechanisms other than acute postural effects.

HDT leads to alterations in brain hemodynamics including an increase in cerebral blood flow (CBF), intracranial pressure, and oxygenated haemoglobin[26], which are hypothesized to trigger cortical inhibition[27]. Additionally, HDT is associated with a cephalic fluid shift leading to increases in thoracic blood volume and hydrostatic pressure, stimulating cardiopulmonary and arterial baroreceptors[2]. These cardiovascular dynamics have been shown to affect cortical activation. Arterial baroreceptors can inhibit cortical activity[28] by decreasing locus coeruleus activity and cortical noradrenaline turnover[29]. Likewise, the blunted responses in HDBR subjects might also be explained by neuroendocrine changes associated with bed rest. Several neurotransmitters are known to be decreased by inactivity including serotonin and norepinephrine[30]. The monoaminergic system which includes norepinephrine and serotonin is well-known for its critical role in controlling human behaviour[31] and in several psychiatric disorders such as depression[32], anxiety[33], and behavioural disturbances among people with dementia[34]. A change in monoamine concentrations associated with long-duration immobilization[35] could therefore also contribute to the changes in visual affective processing observed in the present study. Future studies should therefore also combine behavioural, brain functional, cardiovascular and neuroendocrine measures that will allow to better understand such mechanisms. We also acknowledge that we chose a between-subjects design to exclude any learning effects. Direct between-subject comparisons can be biased by various factors associated with the heterogeneity of the two groups. However, all participants underwent intensive psychological and medical screening for their inclusion in the bed rest study, and they were carefully matched and randomly assigned to one of the two groups. Resting state EEG measured eight days before the intervention, confirmed that EEG spectral power did not differ between the two groups. However, future studies are certainly needed to verify these findings using a within-subjects design in a larger cohort.

Taken together, our data show that head-down tilt bed rest can have adverse neurobehavioral effects associated with negative and positive valence. Impaired affective picture processing following prolonged bed rest was evidenced by a reduction in LPP and P300 in specific brain areas including the insula, precuneus and cingulate gyrus. These results highlight the pervasive effects of physical inactivity that go beyond cardiovascular and musculoskeletal deconditioning. They could have important implications for situations, in which physical activity levels are markedly limited such as during long-duration spaceflight, the aging population, in bed-confinement during hospitalized based care, and people with sedentary lifestyles. Future research needs to elucidate the mechanisms underlying the effects of physical inactivity, examine inter- and intraindividual vulnerabilities relative to emotional regulation, and identify the interaction of physical inactivity and other stressors.

Methods

The present experiment was part of a European Space Agency (ESA) sponsored bed rest study performed at the facilities of the French Institute for Space Medicine and Physiology (MEDES), Toulouse, France in 2017. The project has been registered in the Clinical Trial.gov database under NCT03594799. It comprised 15 days of baseline data collection, 60 days of -6 degrees HDT bed rest and 15 days of recovery. It was conducted following

the Declaration of Helsinki for Medical Research Involving Human Subjects and approved by the Comité de Protection des Personnes (CPP Sud-Ouest Outre-Mer I), the French Health Authorities (Agence Française de Sécurité Sanitaire des Produits de Santé) and the Ethics Committee at Charité–Universitätsmedizin Berlin. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent.

Participants. Data was collected from 20 young healthy male participants (mean age = 34 years, SD = 8; mean height = 176 cm, SD = 4.7; mean weight = 74.0 kg, SD = 7.1; n = 17 right-handed). Handedness was assessed using the Edinburgh Handedness Inventory[36]. Sample sizes were based on previous bed rest studies, suggesting neurobehavioral effects for bed rest[4,5]. We also performed sensitivity analyses for our main outcome, i.e., the comparison of ERP between the bed rest (HDBR) and the control (CTRL) group. For a two-sided independent t-test, a level of significance of 0.05, and a power of 80%, a significant difference corresponding to a Cohen's d of 1.32 should be detectable. This effect is much larger than in a previously reported study using the identical paradigm to assess the acute effects of head-down tilt bed rest[23]. We were therefore confident that the current sample size would be sufficient to reveal a significant between-subjects effect for our primary outcome. All volunteers had no personal history of neurological or psychiatric illness, drug or alcohol abuse, or current medication, and they had a normal or corrected-to-normal vision. The subjects were randomly assigned to one of two groups in a counterbalanced fashion. One of the group served as a control (CTRL: mean age = 34 years, SD = 7; mean height = 176 cm, SD = 3.5; mean weight = 73.1 kg, SD = 5.4) and was tested 8 days prior to bed rest in a -6 degrees HDT position after an adaptational period of 30 minutes of rest. The experimental group (HDBR: mean age: 34 years, SD = 8; mean height = 176 cm, SD = 5.6; mean weight = 74.9 kg, SD = 6.5) was tested after 30 days of (-6 degrees HDT) immobilization. Study cohorts did not differ in age and anthropometric factors (all $ps > 0.740$). Moreover, spectral power analysis of resting state EEG data collected eight days before bed rest revealed no significant difference between groups (data not shown, $p = 0.420$).

Stimuli. Seventy-five standardized stimuli were selected from the IAPS dataset[6] including unpleasant (n = 25, e.g., scenes of violence, threat and injuries), pleasant (n = 25, e.g., sporting events, erotic scenes) and neutral pictures (n = 25, e.g., household objects, landscapes) and presented in a random order. The normative valence ratings (mean (SD)) for each picture category were 7.55 (0.40), 4.99 (0.26), and 3.00 (0.81), and the normative arousal levels (mean (SD)) for each stimulus type were 6.31 (1.10), 2.63 (0.52) and 5.19 (0.61) for positive, neutral and negative images, respectively. The catalogue numbers of pictures from the IAPS dataset used in this study can be found in Supplementary Information.

Procedure. Subjects were positioned in -6 degrees HDT in a dimly lit sound-attenuated room. Testing was performed using a desktop computer (PCGH-Supreme-PC, Alternate), with a 21.5-in monitor (Iiyama ProLite, 1 ms response time, 55–75 Hz refresh rate, luminance 250 cd/m2) installed approximately 60 cm apart from the participant. Before each trial, a central fixation cross appeared for 500 ms. Pictures were displayed on the screen for 2000 ms. After each picture presentation participants were asked to rate the arousal and valence of their emotional perception using two independent 9-point self-assessment Likert scales (SAM) that ranged from very unpleasant/not arousing at all to very pleasant/very arousing[37]. The rating was performed using a computer mouse without any time constraints. The accuracy was emphasized to ensure response reliability and maximal attention from the subjects to their feelings.

EEG recording. The electrocortical activity was continuously recorded and synchronized with the stimuli using an active electrode 32-channel amplifier (actiCHamp, Brain Products GmbH, Germany). Picture presentation and timing were controlled through the use of Presentation software version 18.1 (Neurobehavioral Systems, Inc., USA). Electrodes were attached to an EEG cap (actiCap, Brain Products GmbH, Germany) and placed at positions Fp1, F3, FT9, FC5, FC1, T7, TP9, CP5, CP1, P7, P8, TP10, CP6, CP2, T8, FT10, FC6, FC2, Fp2, F7, F8, F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1 and O2 in accordance with the International 10–20 System. Signals were referenced to Fz. Electrode impedance was checked for each subject before data collection and maintained at less than 5 kΩ. Eye movements and eye blinks were monitored via tin electrooculogram (EOG) electrodes (B18 Multitrodes, EASYCAP GmbH, Germany) placed above and below the left eye as well as at the outer canthi of both eyes. EEG and EOG signals were amplified by a multi-channel bio-signal amplifier and A/D converted at 1000 Hz per channel with 24-bit resolution.

EEG data processing. The data were analysed offline employing EEGLAB 14.0.0[38], a toolbox embedded in Matlab R2015b (The MathWorks, Inc., Natick, Massachusetts, United States). First, data were filtered using a 0.1 to 40 Hz band pass filter. Then, recordings were visually inspected allowing also an interpolation of bad channels. After re-referencing to average reference, EEG data were epoched to the respective stimulus presentation including 200 ms of pre-stimulus baseline and 800 ms of stimulus-dependent data. EOG artefacts were removed using vertical and horizontal EOG regression channels[39]. Muscle artefacts were removed using a spatial filtering framework with defaults[40]. After baseline removal, ERPlab 6.1.3[41] was used to run an additional automated exclusion procedure, rejecting epochs which exceed a gradient threshold of 50 μV, or a maximum and minimum amplitude of ± 100 μV. A total of 2.1% of the trials were excluded in the CTRL group, while 1.1% of the trials had to be excluded for the HDBR group. Average ERPs were computed separately for each subject and each condition. Further, the waveforms were transformed into topographic maps of the ERP potential distributions. The LPP was measured as the average voltage of 400 to 700 ms following picture onset. The P300 was measured as the average voltage of 280 to 350 ms after stimulus presentation. Mean P300 and LPP amplitude was averaged for F3 and F4 as well as P3, P4 and Pz to assess frontal and parietal activity, respectively. A digital 12 Hz low-pass filter was applied offline for plotting grand-averaged waveforms while electrophysiological activity using original filter settings was used for all statistical analyses.

Time-dependent cortical localization of EEG activity. Source analysis was performed by exact low-resolution brain electromagnetic tomography (eLORETA, http://www.uzh.ch/keyinst/loreta.htm), enabling the spatial identification of the cortical activity. The eLORETA software employs a discrete, three-dimensional distributed, linear, weighted minimum norm inverse solution method. The particular weights used in eLORETA allow for an exact localization to test point sources and provide better localization of highly correlated point sources with low signal to noise ratio data[42]. Three-dimensional solution space is restricted to cortical gray matter, as determined by the probabilistic Talairach Atlas. The brain compartment includes 6239 voxels with 5 mm spatial resolution. Anatomical labels, i.e., Brodmann areas (BA) are reported using MNI space, with correction to Talairach space.

In order to receive the 3D cortical distribution of the electrical neuronal generators, the electrode positions were applied to a probabilistic anatomical template of the Talairach Atlas. The Talairach coordinates were used to compute the eLORETA transformation matrix. The eLORETA files were obtained, using the transformation matrix and the ERP data of each subject for each stimuli type. The transformed eLORETA files, containing the corresponding 3D cortical distribution of the electrical neuronal generators, were used for further statistical analysis.

Statistical analysis. *Differences in the temporal dynamics of ERP maps.* Descriptive statistics are reported as means and standard deviations (SD). To test for differences in self-reported evaluations of emotional valence and arousal we performed two-factorial mixed linear models. Subjects were entered as random factors and group (CTRL, HDBR) and stimulus type (positive, neutral, negative) were included as fixed factors, respectively. Further, a mixed-model design was employed to compare the ERP components between groups (CTRL, HDBR) and stimulus type (positive, negative, neutral). Separate mixed model ANOVAs were run for each combination of region (frontal, parietal) and ERP component (P300, LPP). Stimulus type and group were entered as fixed factors and subjects as random effects. Simple comparisons for each condition were performed using pre-planned contrasts with corrections for multiple comparisons[43]. Effect sizes were reported as Cohen's *d*. Confidence intervals of effect sizes were bootstrapped using 2000 resamples[44]. All statistical analyses were carried out using the software package R version 3.5.1[45]. Mixed models were run using the packages lme4[46] and lmerTest2[47]. The level of significance was set at $\alpha = 0.05$ (two-sided) for all testing.

Time-dependent localization of significant differences in temporal dynamics. Independent sampled F-tests were used to test for differences in estimated cortical current density between CTRL and HDBR in all emotional conditions and both time frames. Statistical significance was assessed using a non-parametric randomization test with 5000 randomizations that determined the critical probability threshold ($F_{critical}$) with corrections for multiple testing[48]. As a result, each voxel was assigned a F-value. Voxel-by-voxel F-values are displayed as statistical parametric maps (SPMs).

Data availability

The datasets that support the findings of the current study are available from the corresponding author on reasonable request.

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References

1. Davidson, R. J. & McEwen, B. S. Social influences on neuroplasticity: stress and interventions to promote well-being. *Nature neuroscience* **15**, 689 (2012).
2. Pavy-Le Traon, A., Heer, M., Narici, M. V., Rittweger, J. & Vernikos, J. From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *European journal of applied physiology* **101**, 143–194 (2007).
3. Sandler, H. *Inactivity: physiological effects* (Elsevier, 2012).
4. Ishizaki, Y. *et al*. Changes in mood status and neurotic levels during a 20-day bed rest. *Acta astronautica* **50**, 453–459 (2002).
5. Liu, Q., Zhou, R., Chen, S. & Tan, C. Effects of head-down bed rest on the executive functions and emotional response. *PLoS One* **7**, e52160 (2012).
6. Lang, P. J., Bradley, M.M., & Cuthbert, B.N. International affective picture system (IAPS): Affective ratings of pictures and instruction manual. *Technical Report A-8*. University of Florida, Gainesville, FL. (2008).
7. Cuthbert, B. N., Schupp, H. T., Bradley, M. M., Birbaumer, N. & Lang, P. J. Brain potentials in affective picture processing: covariation with autonomic arousal and affective report. *Biological psychology* **52**, 95–111 (2000).
8. Schupp, H. T., Junghöfer, M., Weike, A. I. & Hamm, A. O. The selective processing of briefly presented affective pictures: an ERP analysis. *Psychophysiology* **41**, 441–449 (2004).
9. Schupp, H. *et al*. Brain processes in emotional perception: Motivated attention. *Cognition and emotion* **18**, 593–611 (2004).
10. Polich, J. Updating P300: an integrative theory of P3a and P3b. *Clin Neurophysiol* **118**, 2128–2148 (2007).
11. Hajcak, G., Weinberg, A., MacNamara, A. & Foti, D. ERPs and the study of emotion. *The Oxford handbook of event-related potential components* **441**, 474 (2012).
12. Mackay, D. G. *et al*. Relations between emotion, memory, and attention: Evidence from taboo Stroop, lexical decision, and immediate memory tasks. *Memory & Cognition* **32**, 474–488 (2004).
13. Urry, H. L. *et al*. Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *Journal of Neuroscience* **26**, 4415–4425 (2006).
14. Ochsner, K. N. *et al*. Reflecting upon feelings: an fMRI study of neural systems supporting the attribution of emotion to self and other. *Journal of cognitive neuroscience* **16**, 1746–1772 (2004).
15. Maddock, R. J., Garrett, A. S. & Buonocore, M. H. Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. *Human brain mapping* **18**, 30–41 (2003).
16. Andreasen, N. C., O'leary, D. S. & Cizadlo, T. Remembering the past: two facets of episodic memory explored with positron emission tomography. *American Journal of Psychiatry* **152**, 1576–1584 (1995).
17. Cabanis, M. *et al*. The precuneus and the insula in self-attributional processes. *Cognitive, Affective, & Behavioral Neuroscience* **13**, 330–345 (2013).

18. Orenius, T. I. *et al*. The interaction of emotion and pain in the insula and secondary somatosensory cortex. *Neuroscience* **349**, 185–194 (2017).
19. Critchley, H. D. *et al*. Activity in the human brain predicting differential heart rate responses to emotional facial expressions. *Neuroimage* **24**, 751–762 (2005).
20. Baleydier, C. & Mauguiere, F. The duality of the cingulate gyrus in monkey. Neuroanatomical study and functional hypothesis. *Brain: a journal of neurology* **103**, 525–554 (1980).
21. Mufson, E. J., Mesulam, M.-M. & Pandya, D. N. Insular interconnections with the amygdala in the rhesus monkey. *Neuroscience* **6**, 1231–1248 (1981).
22. Zhou, Y. *et al*. Disrupted resting-state functional architecture of the brain after 45-day simulated microgravity. *Frontiers in behavioral neuroscience* **8**, 200 (2014).
23. Messerotti Benvenuti, S., Bianchin, M. & Angrilli, A. Posture affects emotional responses: a Head Down Bed Rest and ERP study. *Brain Cogn* **82**, 313–318 (2013).
24. Spironelli, C. & Angrilli, A. Posture Used in fMRI-PET Elicits Reduced Cortical Activity and Altered Hemispheric Asymmetry with Respect to Sitting Position: An EEG Resting State Study. *Front Hum Neurosci* **11**, 621 (2017).
25. Shirreffs, S. M. & Maughan, R. J. The effect of posture change on blood volume, serum potassium and whole body electrical impedance. *European journal of applied physiology and occupational physiology* **69**, 461–463 (1994).
26. Yasumasa, A., Inoue, S., Tatebayashi, K., Shiraishi, Y. & Kawai, Y. Effects of head-down tilt on cerebral blood flow in humans and rabbits. *Journal of gravitational physiology: a journal of the International Society for Gravitational Physiology* **9**, P89–90 (2002).
27. Vaitl, D., Gruppe, H., Stark, R. & Pössel, P. Simulated micro-gravity and cortical inhibition: a study of the hemodynamic-brain interaction. *Biological psychology* **42**, 87–103 (1996).
28. Rau, H., Pauli, P., Brody, S., Elbert, T. & Birbaumer, N. Baroreceptor stimulation alters cortical activity. *Psychophysiology* **30**, 322–325 (1993).
29. Murase, S., Inui, K. & Nosaka, S. Baroreceptor inhibition of the locus coeruleus noradrenergic neurons. *Neuroscience* **61**, 635–643 (1994).
30. Corcoran, P. J. Use it or lose it–the hazards of bed rest and inactivity. *Western Journal of Medicine* **154**, 536 (1991).
31. Sedvall, G. *et al*. Relationships in healthy volunteers between concentrations of monoamine metabolites in cerebrospinal fluid and family history of psychiatric morbidity. *The British Journal of Psychiatry* **136**, 366–374 (1980).
32. Sharp, S. I. *et al*. Cortical serotonin 1A receptor levels are associated with depression in patients with dementia with Lewy bodies and Parkinson's disease dementia. *Dementia and geriatric cognitive disorders* **26**, 330–338 (2008).
33. Nutt, D. J. The pharmacology of human anxiety. *Pharmacology & therapeutics* **47**, 233–266 (1990).
34. Lai, M. K. P. *et al*. Reduced serotonin 5-HT1A receptor binding in the temporal cortex correlates with aggressive behavior in Alzheimer disease. *Brain research* **974**, 82–87 (2003).
35. Goldstein, D. A. V. I. D. S., Vernikos, J. O. A. N., Holmes, C. O. U. R. T. N. E. Y. & Convertino, V. I. C. T. O. R. A. Catecholaminergic effects of prolonged head-down bed rest. *Journal of Applied Physiology* **78**, 1023–1029 (1995).
36. Oldfield, R. C. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113 (1971).
37. Bradley, M. M. & Lang, P. J. Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. *J Behav Ther Exp Psychiatry* **25**, 49–59 (1994).
38. Delorme, A. & Makeig, S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* **134**, 9–21 (2004).
39. Gómez-Herrero, G. *et al*. Automatic removal of ocular artifacts in the EEG without an EOG reference channel. Signal Processing Symposium, 2006. NORSIG 2006. Proceedings of the 7th Nordic, 130-133 (2006).
40. De Clercq, W., Vergult, A., Vanrumste, B., Van Paesschen, W. & Van Huffel, S. Canonical correlation analysis applied to remove muscle artifacts from the electroencephalogram. *IEEE transactions on Biomedical Engineering* **53**, 2583–2587 (2006).
41. Lopez-Calderon, J. & Luck, S. J. ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Front Hum Neurosci* **8**, 213 (2014).
42. Pascual-Marqui, R. D. Discrete, 3D distributed, linear imaging methods of electric neuronal activity. Part 1: exact, zero error localization. *arXiv* **0710**, 3341 (2007).
43. Hochberg, Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika* **75**, 800–802 (1988).
44. Kirby, K. N. & Gerlanc, D. BootES: An R package for bootstrap confidence intervals on effect sizes. *Behavior research methods* **45**, 905–927 (2013).
45. Development Core Team, R. R*: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2008).
46. Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823* (2014).
47. Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest: tests in linear mixed effects models. R package version 2.0-20. *Vienna: R Foundation for Statistical Computing* (2015).
48. Nichols, T. E. & Holmes, A. P. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Human brain mapping* **15**, 1–25 (2002).

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Author contributions

A.S. conceived, designed, planned, and supervised the experiment. K.B. drafted the manuscript and processed the data. A.W. performed data collections with support from K.B. A.W., M.A.M. D.F.D. and H.C.G. provided critical feedback and contributed to the interpretation of the results. All authors discussed the results and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization

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Supplementary Information

Supplementary Study Materials

Study stimuli

The following pictures from the International Affective Picture System, listed by catalog number, were used in the current study: negative - 1114, 1205, 1110, 3064, 3140, 3168, 3250, 3261, 9140, 9265, 9301, 9419, 9420, 9433, 9520, 9592, 9800, 9925, 9926, 9561, 9341, 9342, 9340, 3230, 9584; neutral - 2235, 7057, 5390, 5731, 5740, 7002, 7004, 7006, 7009, 7010, 7056, 7025, 7041, 7050, 7052, 7053, 7055, 7059, 7060, 7080, 7090, 7150, 7175, 7233, 7235; positive - 1440, 8185, 1460, 5629, 1710, 1722, 1750, 2071, 8260, 2311, 8185, 4002, 4006, 4141, 4142, 4180, 4225, 4232, 4250, 4255, 4652, 4659, 4694, 4695.

Supplemental Results

Supplemental Study Table

Table S1. Contrasts comparing ERPs for negative, neutral and positive stimuli between control (CTRL) and bed rest (HDBR) groups.*

ERP	Stimulus	*df*	*t*	*p*	*d* [95%CI]
P300 frontal	negative	30	2.52	0.017	-1.15 [-1.98, -0.31]
	neutral	30	-0.45	0.659	0.30 [-0.65, 1.37]
	positive	30	2.29	0.029	-0.81[-1.70, -0.06]
P300 parietal	negative	30	-3.81	<0.001	1.56 [0.69, 2.22]
	neutral	30	-1.03	0.314	0.78 [-0.11, 1.63]
	positive	30	-3.93	<0.001	1.45 [0.75, 2.04]
LPP frontal	negative	39	2.90	0.006	-1.25 [-1.09, 0.73]
	neutral	39	0.33	0.745	-0.25 [-0.65, 1.37]
	positive	39	1.83	0.074	-0.65 [-1.69, 0.35]
LPP parietal	negative	44	-2.37	0.022	0.98 [0.13, 1.96]
	neutral	44	-0.44	0.659	0.21 [-0.83, 1.01]
	positive	44	-2.65	0.011	1.21 [-0.10, 2.19]

*_df_, degrees of freedom; _d_, effect size (Cohen's _d_) and 95% confidence intervals (CI). CIs are bootstrapped using 2000 resamples.

Supplemental Study Table

Table S2. Contrasts comparing ERPs between stimuli conditions (negative vs. neutral and positive vs. neutral) in control (CTRL) and bed rest (HDBR) groups.*

ERP	Group	Stimulus	*df*	*t*	*p*	*d* [95% CI]
P300 frontal	CTRL	negative - neutral	36	-5.52	<0.001	-1.76 [-2.76, -1.02]
		positive - neutral	36	3.17	0.003	-0.71 [-1.39, -0.06]
	HDBR	negative - neutral	36	-0.69	0.497	-0.21 [-0.86, 0.54]
		positive - neutral	36	1.29	0.414	0.48 [-0.35, 1.26]
P300 parietal	CTRL	negative - neutral	36	4.25	<0.001	1.19 [0.58, 1.75]
		positive - neutral	36	3.62	<0.001	0.82 [0.19, 1.43]
	HDBR	negative - neutral	36	-0.43	0.669	-0.12 [-0.82, 0.56]
		positive - neutral	36	-1.27	0.424	-0.48 [-1.17, 0.18]
LPP frontal	CTRL	negative - neutral	36	-2.36	0.037	-0.88 [-1.53, -0.19]
		positive - neutral	36	-2.17	0.037	-0.65 [-1.48, -0.06]
	HDBR	negative - neutral	36	1.11	0.552	0.45 [-0.26, 1.36]
		positive - neutral	36	-0.14	0.891	-0.04 [-0.74, 0.69]
LPP parietal	CTRL	negative - neutral	36	1.22	0.230	0.42 [-0.26, 1.23]
		positive - neutral	36	4.89	<0.001	1.88 [0.62, 3.98]
	HDBR	negative - neutral	36	-1.16	0.252	-0.30 [0.62, 3.98]
		positive - neutral	36	2.17	0.074	0.57 [-0.02, 1.15]

**df*, degrees of freedom; *d*, effect size (Cohen's *d*) and 95% confidence intervals (CI).
CIs are bootstrapped using 2000 resamples.

Experiment III

Impact of 60-Day Bed Rest on Attention and its Neuroelectric Basis

K. Brauns, A. Friedl-Werner, H.-C. Gunga, and A. C. Stahn, "Effects of two months of bed rest and antioxidant supplementation on attentional processing," *Cortex*, vol. 141, pp. 81–93, Apr. 2021, doi: 10.1016/j.cortex.2021.03.026.

IF: 4.009

https://doi.org/10.1016/j.cortex.2021.03.026

9 Curriculum Vitae

My curriculum vitae will not be published in the electronic version of my work for data protection reasons.

10 List of Publications

Research Articles

M. Nordine, M. A. Maggioni, A. C. Stahn, S. Mendt, **K. Brauns**, H. C. Gunga, H. Habazettl, A. Nitsche, O. Opatz, "Form influences function: Anthropometry and orthostatic stability during sustained acceleration in a short arm human centrifuge," *Acta Astronaut.*, vol. 115, pp. 138–146, 2015, doi: 10.1016/j.actaastro.2015.05.025.

Z. Masatli, M. Nordine, M. A. Maggioni, S. Mendt, B. Hilmer, **K. Brauns**, A. Werner, A. Schwarz, H. Habazettl, H. C. Gunga, Opatz, "Gender-specific cardiovascular reactions to +GZ interval training on a short arm human centrifuge," *Front. Physiol.*, vol. 9, 2018, doi: 10.3389/fphys.2018.01028.

M. A. Maggioni, P. Castiglioni, G. Merati, **K. Brauns**, H. C. Gunga, S. Mendt, O. Opatz, L. C. Rundfeldt, M. Steinach, A. Werner, A. C. Stahn, "High-intensity exercise mitigates cardiovascular deconditioning during long-duration bed rest," *Front. Physiol.*, vol. 9, no. NOV, Nov. 2018, doi: 10.3389/fphys.2018.01553.

K. Brauns, A. Werner, H.-C. Gunga, M. A. Maggioni, D. F. Dinges, and A. Stahn, "Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization," *Sci. Rep.*, vol. 9, no. 1, p. 16610, Dec. 2019, doi: 10.1038/s41598-019-52555-1.

A. C. Stahn, Stahn, M. Riemer, T. Wolbers, A. Werner, **K. Brauns**, S. Besnard, P. Denise, S. Kühn, H. C. Gunga, "Spatial Updating Depends on Gravity," *Front. Neural Circuits*, vol. 14, 2020, doi: 10.3389/fncir.2020.00020.

A. Friedl-Werner, **K. Brauns**, H. C. Gunga, S. Kühn, and A. C. Stahn, "Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest," *Neuroimage*, vol. 223, Dec. 2020, doi: 10.1016/j.neuroimage.2020.117359.

K. Brauns, A. Friedl-Werner, M. A. Maggioni, H. C. Gunga, and A. C. Stahn, "Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity," *Front. Physiol.*, vol. 12, Feb. 2021, doi: 10.3389/fphys.2021.638669.

K. Brauns, A. Friedl-Werner, H.-C. Gunga, and A. C. Stahn, "Effects of two months of bed rest and antioxidant supplementation on attentional processing," *Cortex*, vol. 141, pp. 81–93, Apr. 2021, doi: 10.1016/j.cortex.2021.03.026.

S. Mendt, **K. Brauns**, A. Friedl-Werner, D. Belavy, M. Steinach, T. Schlabs, A. Werner, H.-C. Gunga, and A. C. Stahn, "Long-term Bed Rest Delays the Circadian Phase of Core Body Temperature," *Front. Physiol.*, vol. 12, p. 564, 2021, doi: 10.3389/FPHYS.2021.658707.

A. Friedl-Werner, M.-L. Machado, C. Balestra, Y. Liegard, B. Philoxene, **K. Brauns**, A. C. Stahn, M. Hitier, and S. Besnard, "Impaired attentional processing during parabolic flight," *Front. Physiol.*, vol. 12, p. 597, 2021, doi: 10.3389/FPHYS.2021.675426.

Conference Contributions (Talks)

39th International Society for Gravitational Physiology Meeting, 2018

- Alterations in resting state electrocortical activity after 60 days of bed rest
- Effects of long-term immobilization on affective picture processing – an ERP study

69th International Astronautical Congress, 2018

- Cortical Sources of Resting State EEG During Bed Rest
- Electrocortical Evidence for Impaired Affective Picture Processing after Long-Term Immobilization Stress

23rd IAA Humans in Space Symposium, 2021

- Head-down tilt position, but not the duration of bed rest affects resting state electrocortical activity
- Effects of two months of bed rest and antioxidant supplementation on attentional processing
- Effects of short-term isolation and chronic sleep deprivation on cognitive performance

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