Aus dem
CharitéCentrum für Grundlagenmedizin (CC 2)
Institut für Physiologie
Direktor: Prof. Dr. Wolfgang Kübler

Habilitationsschrift

Neurobehavioral Changes in Response to Long-Duration Bed Rest

zur Erlangung der Lehrbefähigung
für das Fach Physiologie

vorgelegt dem Fakultätsrat der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

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aus Berlin

Eingereicht: **06/2021**

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To Daria, Carlo, Luca, Antonio & Matteo

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Abbreviations

AG	artificial gravity
AGRESA	Artificial Gravity Bed Rest ESA
BOLD	blood oxygen level dependent
Cocktail	Effects of a nutritional cocktail consisting of antioxidant and anti-inflammatory supplements to prevent the deconditioning induced by 60 days of antiorthostatic bed rest
CSF	cerebrospinal fluid
DLR	Deutsches Zentrum für Luft- und Raumfahrt
EEG	electroencephalogram
ERP	event-related potential
ESA	European Space Agency
HDT	head-down tilt
HDBR	head-down tilt bed rest
IAPS	International Affective Picture System
ICC	Isolated and controlled confinement
ICE	Isolated confined and extreme environments
ISS	International Space Station
MEDES	Institut de Médecine et de Physiologie Spatiales
fMRI	functional magnetic resonance imaging
NASA	National Aeronautics and Space Administration
PFC	prefrontal cortex
RSL	Reactive jumps in a sledge jump system as a counter- measure during long-term bed rest

1 Introduction

1.1 Pushing the Boundaries of Human Exploration

"Flight is proceeding normally; I am well."

Yuri Alekseyevich Gagarin, April 12, 1961

Following Sputnik's launch in 1957, cosmonaut Yuri Alekseyevich Gagarin made history, becoming the first human in space, orbiting the Earth, and safely returning to Earth after about two hours in 1961. This success accelerated the *Space Race* between the USSR and the USA, reaching its next climax in 1969 when American Astronaut Neil Armstrong became the first man to walk on the Moon.

Today, the ambition of space-faring nations and private entities is fueling a new race that goes well beyond the Moon. On February 9, 2021, the United Arab Emirates' probe *Hope* entered Mars orbit, shortly followed by the Chinese *Tiawen-1* mission. Nine days later, NASA successfully landed their fifth rover, *Perseverance*, on Mars. *ExoMars 2022*, the integrated European/Russian mission sending a Rover to Mars, is expected to start in the fall of 2022. India is currently preparing their second interplanetary *Mars Orbiter Mission (MOM)*, and Japan is planning to launch the *Martian Moons eXploration (MMX)* mission in 2024 to return the first samples from Mars' largest Moon Phobos. As more nations are sending their probes and rovers to space, the world is pushing the boundaries of exploration, looking for extraterrestrial life, and sending the first human to Mars. Under current and notional NASA plans, crewed orbital missions to Mars could last as long as 1,100 days, and exploration campaigns could even span decades (National Aeronautics and Space Administration, 2015). These missions will be some of the most challenging, dangerous, and dynamic operations in history and considerably longer than current standard missions on the International Space Station (ISS) (Maluf et al., 2005).

1.2 Hazards and Health Risks associated with Exploration Class Missions

Space is a naturally hostile environment characterized by weightlessness or reduced gravity levels, galactic cosmic radiation, hypercapnia, hypoxia, and altered day and night cycles. These hazards can induce a series of cellular, molecular and physiological responses that can pose significant health risks, including, but not limited to cardiovascular deconditioning, muscle and bone loss, immune dysfunction, damage of the central nervous system, circadian disruptions, and ocular disorders (Afshinnekoo et al., 2020). In addition to the extreme environmental conditions, astronauts are also exposed to various operational and psychological stressors such as high workload, lack of fresh foods, crew conflicts, communication delays, little privacy, separation from friends and family, physical distance to Earth, sensory deprivation, and prolonged isolation and confinement (Palinkas, 2001). Some of these effects can be attributed to living in an isolated and confined environment for prolonged times, and have been shown to impair brain and cognitive plasticity (Stahn et al., 2019). Together, the environmental, operational, and psychological hazards during exploration class missions are an increasing concern and unmitigated risk for brain and behavioral health (Flynn-Evans et al., 2016).

1.3 Modeling Neurobehavioral Responses to Spaceflight on Earth

Space agencies have advocated various research platforms termed spaceflight analogs on Earth to simulate some of the risks associated with spaceflight. Novel low dose-rate neutron irradiation facilities have been used to assess the effects of space-relevant radiation exposures of solar and galactic cosmic rays on brain and behavior in animals (Acharya et al., 2019). Environmental test chambers allow simulating a range of atmospheric pressures and compositions to study the neurobehavioral effects of hypoxia and hypercapnia (Bacal et al., 2008). Parabolic flight maneuvers provide unique opportunities to study the acute effects of hypo- and hypergravity on cognitive performance and neurophysiological responses (e.g., Stahn, Riemer, et al., 2020). Long-duration head-down tilt bed rest (HDBR), i.e., bed rest with –6° head-down tilt (HDT) of one-month duration and longer, has been widely employed to simulate the upward fluid shift and body unloading associated with weightlessness (Hargens & Vico, 2016). Spaceflight programs have also advocated isolation studies investigating the psychological responses to social isolation and confinement for over 30 years. All these models still face Earth's gravity field (including parabolic flight, which simulates weightlessness by exposing the aircraft to a free fall around the apex of the parabola). However, (1) they can be

performed under highly controlled conditions; (2) are less confounded by crew activities; (3) can systematically focus on particular spaceflight stressors; and (4) allow testing the efficacy of interventions, i.e., countermeasures, to mitigate health and performance risks (Choukér & Stahn, 2020). To this aim, the present work leverages long-duration HDBR as a spaceflight analog to investigate the effects of prolonged periods of physical inactivity on brain function and cognitive performance and the efficacy of different mitigation strategies to minimize neurobehavioral impairments.

1.4 Bed Rest

For more than two decades, an extensive body of research has shown the beneficial effects of exercise on brain health, cognitive function, and mental well-being across the life span (Guiney & Machado, 2013; Heinze et al., 2021; Voss et al., 2011). The most potent effects in response to physical activity have been observed for executive control processes, involving scheduling, planning, working memory, multi-tasking, and dealing with ambiguity (Hillman et al., 2008). Imaging and electrophysiological data support the beneficial effects of exercise on the central nervous system and reveal consistent effects on brain regions critical for executive functions such as the frontal lobes (prefrontal and temporal cortices), anterior cingulate cortex (ACC) and the hippocampus (Domingos et al., 2021; Erickson et al., 2014; Firth et al., 2018; Ji et al., 2021). Despite the plethora of studies investigating the effects of exercise on brain and behavior, the role of the type of exercise, frequency, intensity, and duration remain to be determined. Further, most studies may have been confounded by various factors such as social interactions, sleep, and nutrition, all well known to affect brain plasticity.

Long-duration bed rest can be considered a unique opportunity to investigate the effects of physical inactivity by leveraging a highly standardized setting relative to work and rest schedules, nutrition, and daily activities. It also allows to systematically examine the efficacy of specific interventions such as exercise, diet, nutritional supplementation, or other training programs. Lipnicki & Gunga (2009) reviewed 17 studies investigating the effect of bed rest on cognitive performance, reporting detrimental effects, no effects, and even performance improvements. Since the seminal review, very few studies investigated the cognitive effects in response to long-duration bed rest (Lipnicki et al., 2009; Liu et al., 2012; Rao et al., 2014; Yuan et al., 2016), and only two of those studies used task functional imaging to assess the neural bases of cognitive changes (Rao et al., 2014; Yuan et al., 2016). Further, according to the

author's best knowledge, no study investigated the effects of different intervention strategies to mitigate any impairments in cognitive performance in response to bed rest.

1.5 Objectives and Hypotheses

The overarching objective of the work presented here was to investigate neurobehavioral changes in response to long-duration HDBR (>1 month), and how potential adverse effects can be mitigated by physical exercise, artificial gravity, or antioxidant supplementation. Data were collected in a total of n = 67 participants as part of the following ESA, DLR, and NASA sponsored studies:

1. *Effects of a nutritional cocktail comprising antioxidant and anti-inflammatory supplements to prevent the deconditioning induced by 60 days of antiorthostatic bed rest (Cocktail)*
2. *Reactive jumps in a sledge jump system as a countermeasure during long-term bed rest (RSL)*
3. *Artificial Gravity Bed Rest ESA (AGBRESA)*

The specific aims (SA) were as follows:

Investigate the effects of prolonged HDBR on…

- SA1: …cognitive performance and the use of artificial gravity as a countermeasure
- SA2: …resting electrocortical activity
- SA3: …neural indices of affective processing
- SA4: …neural indices of attentional processes and the use of antioxidant supplement as a countermeasure
- SA5: …hippocampal pattern separation and the use of exercise as a countermeasure

2 Original Articles

2.1 General Cognitive Performance

Artificial gravity (AG) has been proposed as an integrative countermeasure to minimize the risks of cardiovascular deconditioning, muscle and loss, and neurovestibular alterations associated with long-duration spaceflight (Clément et al., 2015). A critical milestone of future countermeasure candidates is whether they also mitigate potential adverse effects on cognition. The first study identified the time course of cognitive performance using NASA's *Cognition* battery (Basner et al., 2015) during 60 days of HDBR. It examined the efficacy of AG to mitigate potential cognitive impairments. Data were collected at the DLR :envihab as part of the ESA/NASA sponsored study *Artificial Gravity Bed Rest ESA* (AGBRESA).

The following abstract is from the original research article:

Basner M, Dinges DF, Howard K, Moore TM, Gur RC, Mühl C, Stahn, AC. Continuous and Intermittent Artificial Gravity as a Countermeasure to the Cognitive Effects of 60 Days of Head-Down Tilt Bed Rest. *Frontiers in Physiology.* (2021) 12: 643854. doi: https://doi.org/10.3389/fphys.2021.643854

> *"Environmental and psychological stressors can adversely affect astronaut cognitive performance in space. This study used a 6° head-down tilt bed rest (HDBR) paradigm to simulate some of the physiologic changes induced by microgravity. Twenty-four participants (mean ± SD age 33.3 ± 9.2 years, N = 16 men) spent 60 consecutive days in strict HDBR. They were studied in three groups of eight subjects each. One group served as Control, whereas the other two groups received either a continuous or intermittent artificial gravity (AG) countermeasure of 30 min centrifugation daily (1 g acceleration at the center of mass and 2 g at the feet). Participants performed all 10 tests of NASA's Cognition battery and a brief alertness and mood survey repeatedly before, during, and after the HDBR period. Test scores were adjusted for practice and stimulus set difficulty effects. A modest but statistically significant slowing*

across a range of cognitive domains was found in all three groups during HDBR compared to baseline, most consistently for sensorimotor speed, whereas accuracy was unaffected. These changes were observed early during HDBR and did not further worsen or improve with increasing time in HDBR, except for emotion recognition performance. With increasing time spent in HDBR, participants required longer time to decide which facial emotion was expressed. They were also more likely to select categories with negative valence over categories with neutral or positive valence. Except for workload, which was rated lower in the Control group, continuous or intermittent AG did not modify the effect of HDBR on cognitive performance or subjective responses. Participants expressed several negative survey responses during HDBR relative to baseline, and some of the responses further deteriorated during recovery, which highlights the importance of adequate medical and psychological support during extended duration HDBR studies. In conclusion, 60 days of HDBR were associated with moderate cognitive slowing and changes in emotion recognition performance, but these effects were not mitigated by either continuous or intermittent exposure to AG for 30 min daily."

frontiers in Physiology

ORIGINAL RESEARCH
published: 17 March 2021
doi: 10.3389/fphys.2021.643854

Continuous and Intermittent Artificial Gravity as a Countermeasure to the Cognitive Effects of 60 Days of Head-Down Tilt Bed Rest

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OPEN ACCESS

Edited by:
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Reviewed by:
Ajitkumar Mulavara, Universities Space Research Association (USRA), United States
Ana Diaz Artiles, Texas A&M University, United States

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Specialty section:
This article was submitted to Environmental, Aviation and Space Physiology, a section of the journal Frontiers in Physiology

Received: *18 December 2020*
Accepted: *18 February 2021*
Published: *17 March 2021*

Citation:
Basner M, Dinges DF, Howard K, Moore TM, Gur RC, Mühl C and Stahn AC (2021) Continuous and Intermittent Artificial Gravity as a Countermeasure to the Cognitive Effects of 60 Days of Head-Down Tilt Bed Rest. Front. Physiol. 12:643854. doi: 10.3389/fphys.2021.643854

Environmental and psychological stressors can adversely affect astronaut cognitive performance in space. This study used a 6° head-down tilt bed rest (HDBR) paradigm to simulate some of the physiologic changes induced by microgravity. Twenty-four participants (mean ± SD age 33.3 ± 9.2 years, *N* = 16 men) spent 60 consecutive days in strict HDBR. They were studied in three groups of eight subjects each. One group served as Control, whereas the other two groups received either a continuous or intermittent artificial gravity (AG) countermeasure of 30 min centrifugation daily (1 *g* acceleration at the center of mass and 2 *g* at the feet). Participants performed all 10 tests of NASA's Cognition battery and a brief alertness and mood survey repeatedly before, during, and after the HDBR period. Test scores were adjusted for practice and stimulus set difficulty effects. A modest but statistically significant slowing across a range of cognitive domains was found in all three groups during HDBR compared to baseline, most consistently for sensorimotor speed, whereas accuracy was unaffected. These changes were observed early during HDBR and did not further worsen or improve with increasing time in HDBR, except for emotion recognition performance. With increasing time spent in HDBR, participants required longer time to decide which facial emotion was expressed. They were also more likely to select categories with negative valence over categories with neutral or positive valence. Except for workload, which was rated lower in the Control group, continuous or intermittent AG did not modify the effect of HDBR on cognitive performance or subjective responses. Participants expressed several negative survey responses during HDBR relative to baseline, and some of the responses further deteriorated during recovery, which highlights the importance of adequate medical and psychological support during extended duration HDBR studies. In conclusion, 60 days of HDBR were associated with moderate cognitive slowing and changes in emotion recognition performance, but these effects were not mitigated by either continuous or intermittent exposure to AG for 30 min daily.

Keywords: cognition, spaceflight, performance, microgravity, bed rest, emotion recognition

INTRODUCTION

Sustained high levels of astronaut cognitive performance are a prerequisite of successful human spaceflight. Several environmental, operational, physiologic, and psychological stressors related to living in the isolated, confined, and extreme spaceflight environment may adversely affect cognitive performance, and thereby pose risks to astronaut safety and health (Strangman et al., 2014; Basner et al., 2015; Stahn et al., 2019). Microgravity is one prominent stressor that has been implicated in the development of ocular and vision changes in spaceflight [spaceflight-associated neuro-ocular syndrome (SANS); Laurie et al., 2019; Marshall-Goebel et al., 2019a; Roberts et al., 2020]. Microgravity induces a physical body unloading and a headward fluid shift (Marshall-Goebel et al., 2019b). Structural brain changes observed in astronauts after return from International Space Station (ISS) missions have included an upward shift of the brain (Roberts et al., 2017; Lee et al., 2019; Roberts et al., 2019), changes in gray matter volume (Koppelmans et al., 2016), increased white matter in the cerebellum (Jillings et al., 2020), and cerebrospinal fluid volume increases in the third and lateral ventricles (Alperin et al., 2017; Roberts et al., 2017; Van Ombergen et al., 2019; Jillings et al., 2020; Kramer et al., 2020). The functional consequences of these physiologic and anatomical changes remain largely unknown (Roberts et al., 2019).

In addition to research performed mostly in low Earth orbit, international space agencies use ground-based analogs to investigate the effects of common spaceflight stressors on human physiology and performance. Head-down tilt bed rest (HDBR) has been used for at least 50 years as a ground-based analog for microgravity-induced physiologic changes (Pavy-Le Traon et al., 2007). Findings of studies investigating the effects of HDBR on cognitive performance have been inconclusive thus far, likely due to the diversity of cognitive test batteries used, protocol differences [e.g., exposure to stressors, degrees of head-down tilt (HDT), and duration], practice effect confounds, circadian time of testing, low sample size, and inadequate control groups (Lipnicki and Gunga, 2009). A few studies have investigated changes in cognitive performance induced by HDBR since the seminal review of Lipnicki and Gunga (2009). Rao et al. (2014) found that, while risk-taking behavior was not affected by 45 days of HDBR, functional MRI (fMRI) task activation patterns changed. A missing control group complicates the interpretation of the findings. In a 70-day HDBR study, Yuan et al. (2016) found a lower counting accuracy on a dual task, as well as a brain activation increase for dual tasking in the HDBR group, which implies that more neurocognitive control was needed for dual task execution during HDBR. Lipnicki et al. (2009) found no difference in Iowa Gambling Task performance induced by 51 days of HDBR. However, in contrast to ambulatory controls, HDBR subjects failed to adapt their card selection strategy as the task progressed. A study by Friedl-Werner et al. (2020) found that, compared to a group of subjects that received high-intensity interval training five to six times weekly during a 60-day HDBR study, the non-exercise control group demonstrated an increased BOLD signal in the left hippocampus and parahippocampal gyrus, while mnemonic performance on an episodic memory task did not differ between groups. This was interpreted as higher neuronal efficiency in the training group during memory encoding and retrieval.

Artificial gravity (AG) has been proposed as a countermeasure to the adverse physiologic effects induced by microgravity (Clement et al., 2015). It can be achieved either by rotating a spacecraft or station, or by centrifugation of the astronaut. Whereas neither have been implemented in spaceflight to date, the concept and its benefits are intriguing. Research on the effects of AG countermeasures during HDBR is scarce. One study investigated the effects of an AG countermeasure on spatial orientation of eight subjects undergoing 21 days of 6° HDBR (Moore et al., 2010). This study found a significant increase in error on a subjective visual vertical task for 48 h post bed rest. Another study investigated the effects of artificial gravity on cognitive performance during 21 days of 6° HDBR in 15 subjects using NASA's WinSCAT tool (Seaton et al., 2007). These investigators found more off-nominal WinSCAT scores in the AG group (1 h centrifugation per day) relative to the control group, and accuracy tended to be more affected than speed.

To further elucidate the effects of AG on general cognitive performance in HDBR, 24 subjects underwent 60 days of 6° HDBR in this study, eight of them exposed to continuous AG for 30 min daily, eight of them exposed to intermittent AG for 30 min daily, while the remaining eight served as controls without AG countermeasure. Participants performed NASA's Cognition test battery and a brief alertness and mood survey repeatedly before, during, and after the HDBR period.

MATERIALS AND METHODS

Study Design and Participants

This report includes data from a study on the effects of continuous and intermittent artificial gravity on participants spending 60 consecutive days in strict 6° HDBR performed at the :envihab at the German Aerospace Center (DLR) in Cologne, Germany, a research facility that allows for investigating up to 12 subjects concurrently under controlled environmental conditions. The study was titled Artificial Gravity Bed Rest – European Space Agency (AGBRESA). Study participants were randomly assigned to one of three groups consisting of $N = 8$ participants each, all of them undergoing 60 days of strict 6° HDBR: (1) Control group: no Artificial Gravity intervention; (2) continuous Artificial Gravity (cAG) group: one continuous 30-min bout of centrifugation daily; and (3) intermittent Artificial Gravity (iAG) group: six 5-min bouts of centrifugation with 3 min rest between bouts daily (see centrifugation protocol section below for details). Participants were pseudo-randomly assigned to groups in the first campaign. Due to three women dropping out in campaign 2, subsequent replacement was based on demographic balancing particularly with regards to sex. The 24 participants had an average age of 33.3 ± 9.2 years (range 23–54 years) and 14 (66.7%) were male. The three experimental groups did not differ significantly on age or sex (**Supplementary Table S1**).

Participants were recruited by the DLR. Study eligibility criteria included age between 24 and 55 years, non-smokers, body mass index between 19 and 30 kg/m^2, no elevated risk of thrombosis, no recent history of bone fractures, and no history of chronic pain, hypertension, hyperlipidemia, arthritis, diabetes, obesity, hepatic disease, eye conditions, or a calcium/bone metabolism disorder. Subjects were screened to ensure that they were psychologically healthy before participation. They were empaneled 14 days before the start of the HDBR period (for baseline data acquisition) and discharged 14 days after the end of the HDBR period (for recovery phase data acquisition). The HDT position was continuously maintained throughout the course of the HDBR period. A specially designed neck support was allowed when subjects were on their sides during sleep, although many chose not to use it (see Laurie et al., 2020 for pictures). Subjects participated in several scientific investigations with a focus on SANS that were scheduled throughout the day, interrupted by meals that reflected a controlled diet. Participants were provided a daily 8-h sleep opportunity between 22:30 and 6:30. They were compensated for participating in the study, which was approved by the local ethics committee (Ärztekammer Nordrhein) and by the Institutional Review Board of NASA Johnson Space Center. Subjects provided written informed consent before participation and were allowed to discontinue the study at any time. The study was registered at the German Clinical Trials Register (DRKS) under #DRKS00015677.

Centrifugation Protocol

Participants remained at 6° HDT at all times during transport to and from the 3.8 m radius short-arm centrifuge without using their leg muscles. Subjects were oriented radially in supine position on the centrifuge arm with their head toward the center of rotation and feet resting against a force plate. The centrifugation protocol included: acceleration at 5°/s^2 for 32–33 s until target rotation speed was achieved followed by rotation at constant velocity (on average 30.5 rpm; with exposures of 1 *g* at participants' estimated center of mass and 2 *g* at the feet) for either 30 min (cAG) or 5 min, with a 3-min rest, repeated six times (iAG). Deceleration was at 5°/s^2. Participants were instructed not to move their head, relax their leg muscles, and to remain calm. All centrifugation runs were conducted between 09:00 h and 19:00 h, and the time of the day of the AG runs (morning vs. afternoon) were counterbalanced within subject. This was done to avoid any systematic effects of circadian variation on countermeasure tolerance and/or efficacy, but the timing of the centrifuge runs also necessarily changed daily to allow for scientific testing that needed to be scheduled before the commencing of centrifugation on any given bed rest day. Participant safety was guaranteed through continuous medical monitoring.

Cognition Test Battery and Cognition Outcome Variables

The following description of the Cognition battery was modified from Basner et al. (2015, 2017). Cognition contains a subset of tests from a widely used and validated neurocognitive battery, the Penn Computerized Neurocognitive Battery (PennCNB; Gur et al., 2010, 2012; Moore et al., 2014), and a number of additional tests. Cognition emphasizes tests that have either been used extensively in spaceflight or that assess cognitive domains of particular interest in spaceflight (such as spatial orientation, emotion recognition, and risk decision making; Lim and Dinges, 2008; Usui et al., 2009). The 10 Cognition tests were modified to reflect the high aptitude and motivation of astronauts. They assess a range of cognitive domains, and the brain regions primarily recruited by each test have been previously established. Importantly, Cognition has 15 unique stimulus sets (i.e., versions) that allow for repeated administration without the need to re-use stimulus sets. Six tests have unique stimulus sets, while the remaining four tests (Motor Praxis, Line Orientation, Digit Symbol Substitution, and Psychomotor Vigilance) randomly generate stimuli each time the test is administered. A detailed overview of Cognition can be found in Basner et al. (2015).

Analyses concentrated on one main accuracy and one main speed outcome for each Cognition test. Congruent with descriptions in Basner et al. (2020a), all accuracy outcomes ranged from 0 to 100% with 100% representing best possible performance. For all speed outcomes, lower values reflect shorter response times and thus higher speed. Average response time (milliseconds) was the speed outcome for all tests except the PVT (see below). Percentage correct was the accuracy outcome for five *Cognition* tests. The accuracy outcomes for the other tests are described for each test below. All outcomes were corrected for practice and stimulus set difficulty effects according to Basner et al. (2020a) based on an administration interval of 5 days or less before statistical analyses. All Cognition outcomes were also z-transformed based on the average and SD of baseline performance scores (administrations 9, 7, and 6 days before bed rest) across study subjects and conditions (i.e., the average and SD used for z-transformation were based on 3*24 = 72 scores). Summary scores for accuracy and speed were calculated by averaging across z-transformed scores within the accuracy and speed domain, respectively. Speed summary scores were multiplied by −1 so that higher scores reflected higher speed. An efficiency score was calculated by averaging the accuracy and speed summary (z) scores. In the following paragraphs, we provide a brief description of each of the 10 Cognition tests. The tests were always performed in the order listed below, starting with stimulus set 1 and sequentially progressing through the 15 stimulus sets.

The **Motor Praxis Task (MP)** was administered as the first test to ensure that participants had sufficient command of the computer interface. It is a measure of sensorimotor speed (Gur et al., 2001). Participants were instructed to click on squares that appear randomly on the screen, with each successive square smaller and thus more difficult to track. Performance was assessed by the speed with which participants click each square. For the MP accuracy outcome, the distance from the center of each square (in pixels) was averaged across all responses. The center of the square translated to 100% accuracy, 50 pixels or more away from the center translated to an accuracy score of 0%, with linear scaling between 0 and 50 pixels.

The **Visual Object Learning Test (VOLT)** assessed participant memory for complex figures (Glahn et al., 1997). Participants were asked to memorize 10 sequentially displayed

3D Euclidean shapes. Later, they were presented with 20 such objects, half of them from the learning set and half new. Participants were instructed to decide for each object whether they had seen it before or not, and how confident they were in their decision ("definitely" or "probably").

The **Fractal 2-Back (F2B)** is a nonverbal variant of the Letter 2-Back. *N*-back tasks have become standard probes of the working memory system and activate canonical working memory brain areas (Ragland et al., 2002). The F2B consists of the sequential presentation of a set of figures (fractals), each potentially repeated multiple times. Participants are instructed to respond when the current stimulus matches the stimulus displayed two figures ago. The current implementation used 62 consecutive stimuli. For the F2B accuracy outcome, the percentage correct for matches and non-matches was averaged to avoid subjects achieving good accuracy scores even if they never hit the spacebar.

The **Abstract Matching (AM)** test is a validated measure of the abstraction and flexibility components of executive function, including an ability to discern general rules from specific instances (Glahn et al., 2000). The test paradigm presents subjects with two pairs of objects at the bottom left and right of the screen, varied on perceptual dimensions (e.g., color and shape). Subjects are presented with a target object in the upper middle of the screen that they have to classify as belonging more with one of the two pairs, based on a set of implicit, abstract rules. The current implementation used 30 consecutive stimuli.

The **Line Orientation Test (LOT)** is a measure of spatial orientation and derived from the well-validated Judgment of Line Orientation Test (Benton et al., 1978). The LOT format consists of presenting two lines at a time, one stationary while the other could be rotated by clicking an arrow. Participants can rotate the movable line until they perceive it to be parallel to the stationary line. The implementation used in this study had 12 consecutive line pairs that varied in length and orientation. The LOT accuracy measure was calculated as 3 minus the average number of clicks off, which was then divided by 3 (lines are rotated with 2^0 per click on the LOT; subjects are on average ~0.8 clicks off). For tests with more than 3 clicks off on average, the accuracy score was set to 0%.

The **Emotion Recognition Task (ERT)** is a measure of facial emotion recognition (Gur et al., 2010). It presents subjects with photographs of professional actors (adults of varying age and ethnicity) portraying emotional facial expressions of varying types and intensities (biased toward lower intensities, and with the prevalence of emotion categories balanced within each version of the test). Subjects are given a set of emotion labels (happy, sad, angry, fearful, and no emotion) and have to select the label that correctly describes the expressed emotion. The implementation used in the study had 40 consecutive stimuli, with 8 stimuli each representing one of the five emotion categories. Stimuli that loaded negatively in an Item Response Theory (IRT) analysis were excluded for the calculation of both ERT speed and ERT accuracy (see Basner et al., 2020a for a list of excluded stimuli).

The **Matrix Reasoning Test (MRT)** is a measure of abstract reasoning and consists of increasingly difficult pattern matching tasks (Gur et al., 2001). It is analogous to Raven Progressive Matrices (Raven, 1965), recruits prefrontal, parietal, and temporal cortices (Perfetti et al., 2009) and is based on a well-known measure of general intelligence. The test consists of a series of patterns, overlaid on a grid. One element from the grid is missing and the participant has to select the element that fits the pattern from a set of alternative options. The implementation used in the study applied 12 consecutive stimuli. Stimuli that loaded negatively in an IRT analysis were excluded for the calculation of both MRT speed and MRT accuracy (see Basner et al., 2020a for a list of excluded stimuli).

The **Digit-Symbol Substitution Task (DSST)** is a computerized adaptation of a paradigm used in the Wechsler Adult Intelligence Scale (WAIS-III; Usui et al., 2009). The DSST required the participant to refer to a displayed legend relating each of the digits one through nine to specific symbols. One of the nine symbols appears on the screen and the participant has to select the corresponding number as quickly as possible. The test duration is fixed at 90 s, and the legend key is randomly re-assigned with each administration.

The **Balloon Analog Risk Test (BART)** is a validated assessment of risk-taking behavior (Lejuez et al., 2002). The BART requires participants to either inflate an animated balloon or stop inflating and collect a reward. Participants are rewarded in proportion to the final size of each balloon, but a balloon pops after a hidden number of pumps that changes across stimuli, in which case the reward is voided. The implementation used in the study had 30 consecutive stimuli. The average tendency of balloons to pop is varied systematically between test administrations. This variation requires subjects to adjust the level of risk based on the behavior of the balloons. It prevents subjects from identifying a strategy during the first administrations of the battery and carrying it through to later administrations. For each pump on the BART, a value of 1 divided by the number of possible pumps across all 30 balloons was added to the BART Risk Score. This Risk Score, therefore, takes into account that different sets of balloons popped at different inflation rates. We list BART risk-taking as an accuracy outcome despite the fact that it inherently measures risk-taking. For this reason, it was not included in calculating the accuracy summary score across cognitive domains (see above).

The **Psychomotor Vigilance Test (PVT)** records reaction times (RT) to visual stimuli that occur at random inter-stimulus intervals (Basner and Dinges, 2011). The PVT is a sensitive measure of vigilant attention, and sensitive to acute and chronic sleep deprivation as well as circadian misalignment (Barger et al., 2014). Subjects are instructed to monitor a box on the screen, and press the space bar once a millisecond counter appears in the box and starts incrementing. The reaction time is displayed for 1 s. Subjects are instructed to be as fast as possible without hitting the spacebar in the absence of a stimulus (i.e., false starts or errors of commission). In the current study, Cognition contained a validated 3-min brief PVT-B with 2–5 s inter-stimulus intervals and a 355 ms lapse threshold (Basner et al., 2011). For the PVT, 10 minus reciprocal

response time (1/RT) was used as the speed outcome, as this metric was shown to be a superior outcome for the PVT relative to average RT (Basner and Dinges, 2011). The PVT accuracy score was calculated as 1 − [(# of Lapses + # of False Starts)/(Number of Stimuli + # of False Starts)]. Any response time not falling between the false start threshold (100 ms) and the lapse threshold (355 ms) thus decreased accuracy on the PVT.

The Cognition software administered a brief survey before each administration of the test battery. Participants entered the time they tried to fall asleep and woke up, which was used as an estimate of their sleep duration. They then indicated their current status on the following 13 11-point Likert scales (anchors are provided in parenthesis after each question; the middle point was labeled neutral): (1) What was the quality of your sleep? (good-poor); (2) What was today's workload? (very high-very low); How are you feeling right now? (3; not sleepy at all-very sleepy); (4; happy-unhappy); (5; sick-healthy); (6; energetic-physically exhausted); (7; mentally sharp-mentally fatigued); (8; not stressed at all-very stressed); (9; tired-fresh, ready to go); (10; very depressed-not depressed at all); (11; very bored-not bored at all); (12; not lonely at all-very lonely); and (13) What is your current everyday life like? (very monotonous-not monotonous at all). For analysis purposes, items 2, 5, 9, 10, 11, and 13 were inverted so that high scores always reflected more negative responses.

Cognition Procedures

Participants first watched a standardized familiarization video. They then performed the full Cognition battery twice for task familiarization 13 and 11 days before the start for the HDBR period. They were required to perform a brief practice version immediately before each test during the first familiarization bout (except for the VOLT and BART, which do not have practice versions). Cognition was performed three more times on days 9, 7, and 6 before bed rest. These administrations served as baseline. Cognition then was performed on days 1, 3, 5, 14, 28, 42, and 57 after the initiation of the bed rest period. Finally, Cognition was administered on days 1, 5, and 12 during the recovery period following bed rest.

Cognition (version 3.0.9, using the version 2 ERT with 40 stimuli) was administered on Dell laptop computers (12.5″ screen diagonal, 1,366 × 768 resolution) calibrated for timing accuracy in the afternoon (mean ± SD of administration time across all study periods: 17:24 ± 0:33). It was performed in the seated upright position before and after the bed rest period. For testing in the HDT position, laptops were mounted vertically on an adjustable swivel arm and positioned in chest-height in front of the participants (see **Supplementary Figure S1**). Participants used the laptop's track pad and integrated mouse button to operate the arrow on the screen.

Statistical Analyses

Data were analyzed with SAS (SAS Institute, Carey, NC, United States; version 9.4). Linear mixed effect models with random intercepts were used to account for the fact that test data were clustered within participants. Survey data were treated as continuous for analysis purposes (Leung, 2011). All models were adjusted for sex and age (continuous variable). Furthermore, all models were adjusted for baseline performance, with the exception of models for subjective outcomes and sleep duration (unless otherwise noted). Values of p were adjusted for multiple testing according to the false discovery rate method (Benjamini and Hochberg, 1995) for the 23 Cognition outcomes (one standard speed and accuracy outcome for each test plus accuracy, speed, and efficiency across tests; i.e., $N = 23$ comparisons) and for the 13 subjective outcomes and sleep duration (i.e., $N = 13$ comparisons). We provide both unadjusted values of p and confidence intervals as well as the alpha level that survived adjustment (i.e., $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$).

The following analyses were performed on both cognitive performance and self-report outcomes: (1) The difference in baseline assessments between the three experimental groups was assessed; (2) marginal means were estimated for the Control and AG groups during the HDT phase and the recovery phase using observed marginal means for sex, age, and baseline performance. As z-transformation was performed using baseline data only, estimated marginal means reflect the difference of cognitive test scores during HDT/recovery to baseline cognitive test scores, adjusted for potential differences in baseline performance between the three groups; (3) the cAG group and the iAG group were contrasted to the Control group separately for the bed rest and the recovery phase; (4) it was investigated whether assessments changed linearly with time in HDBR, and whether the slope differed significantly between groups (i.e., group*time interaction). Model 4 was the only model that allowed for random intercepts and random slopes (unstructured covariance).

RESULTS

Data were extracted and visualized for each subject. Seven out of 3,600 expected test bouts (data 99.8% complete) were excluded from data analysis, six due to subject non-compliance, and one due to technical difficulties. The three experimental groups did not differ significantly at baseline in terms of cognitive performance, sleep duration, or survey responses (**Supplementary Table S1**). Self-reported sleep duration averaged 7.54 h in the Control group, 7.61 h in the cAG group, and 7.55 h in the iAG group, respectively. Subjects in all three groups reported moderate levels of tiredness, sleepiness, sleep quality, mental fatigue, physical exhaustion, and workload; low levels of monotony, boredom, loneliness, depression, and stress; and high levels of health and happiness at baseline.

Head-Down Tilt Bed Rest Effects

Compared to baseline performance, there was a small but statistically significant decrease in speed across cognitive domains observed in all experimental groups (Control −0.23 SD, adjusted $p < 0.05$; cAG −0.31 SD, adjusted $p < 0.001$; iAG −0.25 SD,

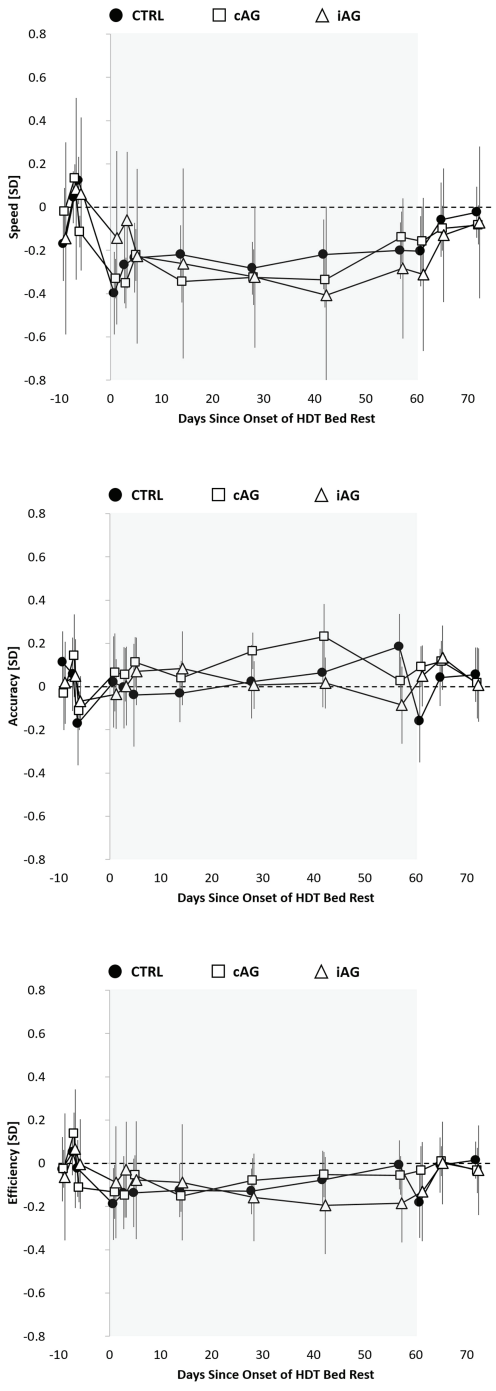

FIGURE 1 | Cognitive speed, accuracy, and efficiency across cognitive domains relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for

(Continued)

FIGURE 1 | the Control group (black circles), continuous artificial gravity group (cAG; white squares), and intermittent artificial gravity group (iAG; white triangles). Estimates reflect unadjusted means z-transformed based on baseline (pre-HDT) performance within each of the 10 Cognition tests and then averaged across tests. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).

adjusted $p < 0.01$; **Figures 1, 2**; **Supplementary Table S2**). Accuracy across cognitive domains did not differ from baseline in any of the three groups (effect sizes <0.1 SD; all $p > 0.22$). Cognitive efficiency also did not differ relative to baseline in any of the groups after adjustment for multiple testing. Neither cognitive speed, accuracy nor efficiency differed significantly between the three groups (effect sizes ≤0.08; all $p > 0.34$; **Supplementary Table S2**).

Focusing on individual tests, speed was significantly slower on the MP in all three groups, BART speed was significantly lower in the cAG group only, and ERT and MRT speed was significantly lower in the iAG group only (**Figure 2A**; **Supplementary Table S2**). Accuracy did not differ significantly from baseline for any of the 10 tests in any of the three groups. Also, none of the speed or accuracy outcomes differed between the three groups for any of the 10 tests after adjusting for multiple testing (**Figure 2B**; **Supplementary Table S2**).

Self-reported sleep duration did not differ significantly between bed rest and baseline periods for any of the three experimental groups (**Supplementary Table S2**). Analyses of the survey responses showed significantly lower levels of happiness in the iAG group, significantly higher levels of sickness in the Control group and iAG group, significantly higher levels of mental fatigue and stress in the cAG group, significantly higher levels of depression, boredom, and loneliness in the cAG group and iAG group, and significantly higher levels of monotony in all three groups during the HDBR period compared to baseline (**Figure 3A**; **Supplementary Table S2**). However, the only reliable difference between the three groups was a significantly higher rating of workload in the cAG and iAG groups relative to Control (**Figure 3A**; **Supplementary Table S2**).

Except for ERT speed, none of the other cognitive test outcomes, survey responses, or sleep duration changed significantly with days in HDBR (all adjusted $p > 0.05$; **Supplementary Table S4**; **Supplementary Figures S2–S10**). Also, time in HDBR slopes did not differ significantly between groups (all adjusted $p > 0.05$ for time*group interaction; **Supplementary Table S4**). Speed on the ERT decreased significantly with time in HDBR ($\beta = -0.009$ SD per day in HDBR; adjusted $p < 0.01$; **Supplementary Table S4**; **Figure 4**). The decline in response speed on the ERT was consistently observed across the three experimental groups (unadjusted $p = 0.2580$ for time*group interaction). ERT accuracy did not change significantly with days in HDBR. An in-depth analysis of ERT responses showed that, with increasing time spent in HDBR, subjects were significantly more likely to rate faces angry (adjusted $p < 0.01$) and significantly less likely to rate them happy (adjusted $p < 0.05$) or neutral (adjusted $p < 0.05$; **Supplementary Table S4**).

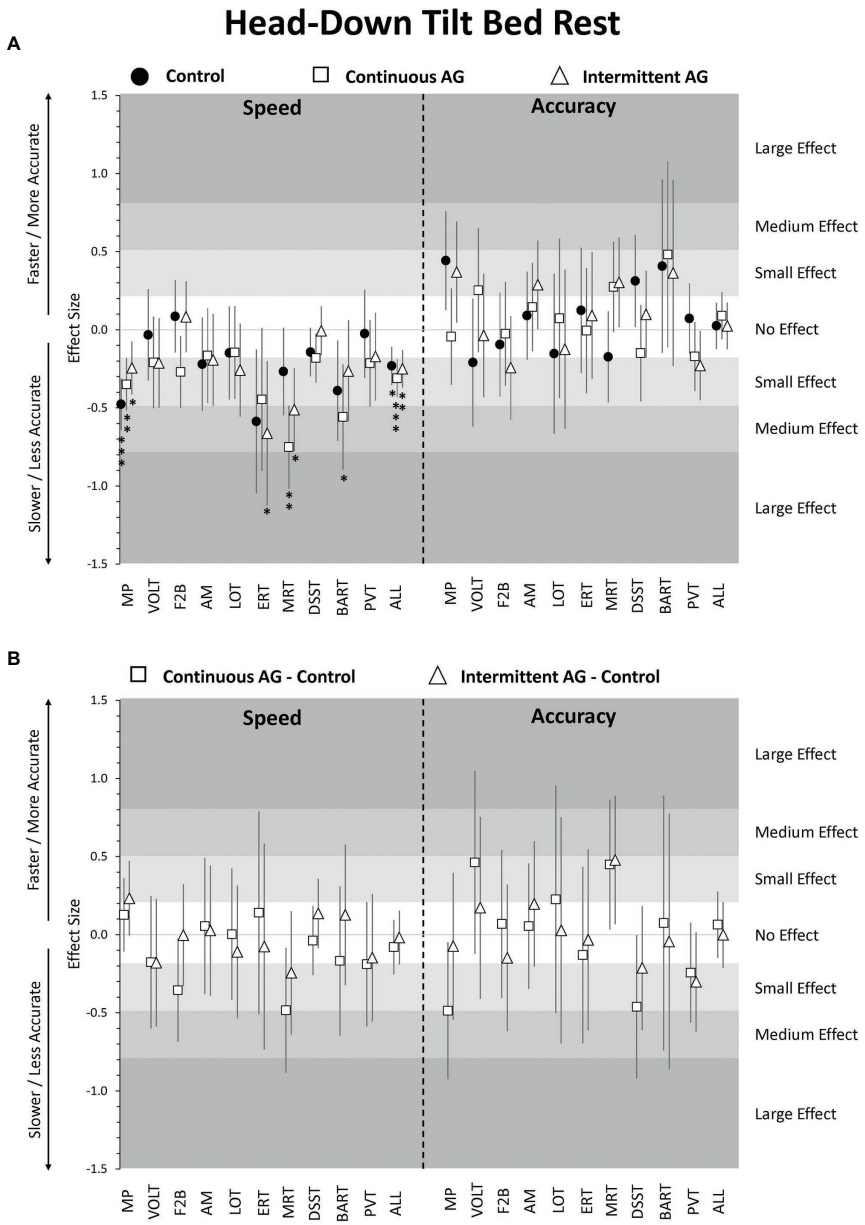

FIGURE 2 | Change in cognitive performance in the head-down tilt (HDT) bed rest period relative to pre-HDT baseline. Estimates reflect z-scores based on the mean and SD of pre-HDT baseline performance. Error bars reflect unadjusted 95% confidence intervals. **(A)** Estimates for the Control group (black circles), cAG group (white squares), and iAG group (white triangles); **(B)** Estimates for the difference cAG-Control (squares) and iAG-Control (triangles); *adjusted $p < 0.05$; **adjusted $p < 0.01$; ***adjusted $p < 0.001$; MP, Motor Praxis; VOLT, Visual Object Learning Test; F2B, Fractal 2-Back; AM, Abstract Matching; LOT, Line Orientation Test; ERT, Emotion Recognition Test; MRT, Matrix Reasoning Test; DSST, Digit Symbol Substitution Test; BART, Balloon Analog Risk Test; PVT, Psychomotor Vigilance Test; ALL, scores averaged across cognitive domains.

Recovery Effects

Point estimates for cognitive speed across cognitive domains were negative for the three experimental groups during the recovery period relative to baseline, but effect sizes were <0.2 SD and non-significant after adjustment for multiple testing. Raw data plots (**Figure 1**) suggest a gradual return of cognitive

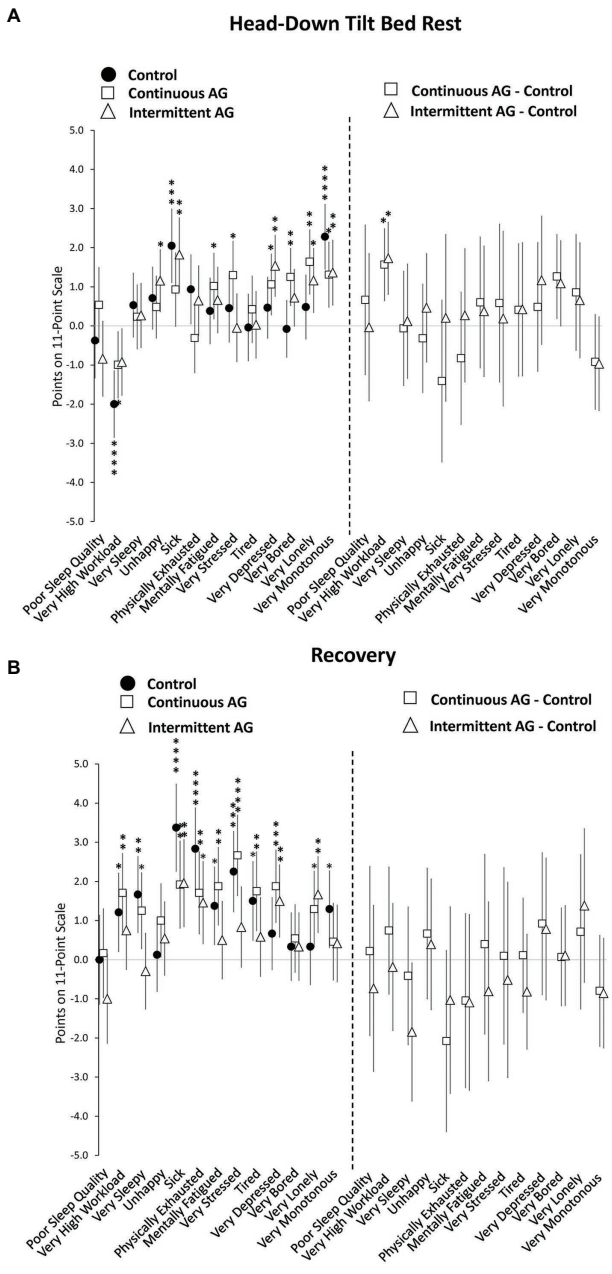

FIGURE 3 | Change in survey responses during head-down tilt (HDT) bed rest **(A)** and post-HDT recovery **(B)** relative to pre-HDT baseline for the Control group (black circles), cAG group (white squares), and iAG group (white triangles). Estimates reflect points on an 11-point scale. For each variable, the negative response anchor is shown (e.g., "unhappy" and "very sleepy"). Positive scores reflect more negative assessments relative to baseline (graphs on the left) or control (graphs on the right). Error bars reflect unadjusted 95% confidence intervals. *adjusted $p < 0.05$; **adjusted $p < 0.01$; ***adjusted $p < 0.001$; ****adjusted $p < 0.0001$.

speed to baseline, with similar or even slower performance on the first ambulatory day during recovery relative to the last test administration on HDBR day 57. Accuracy and efficiency across cognitive domains did not differ during recovery from baseline for any of the three groups. Neither cognitive speed, accuracy, nor efficiency across cognitive domains differed

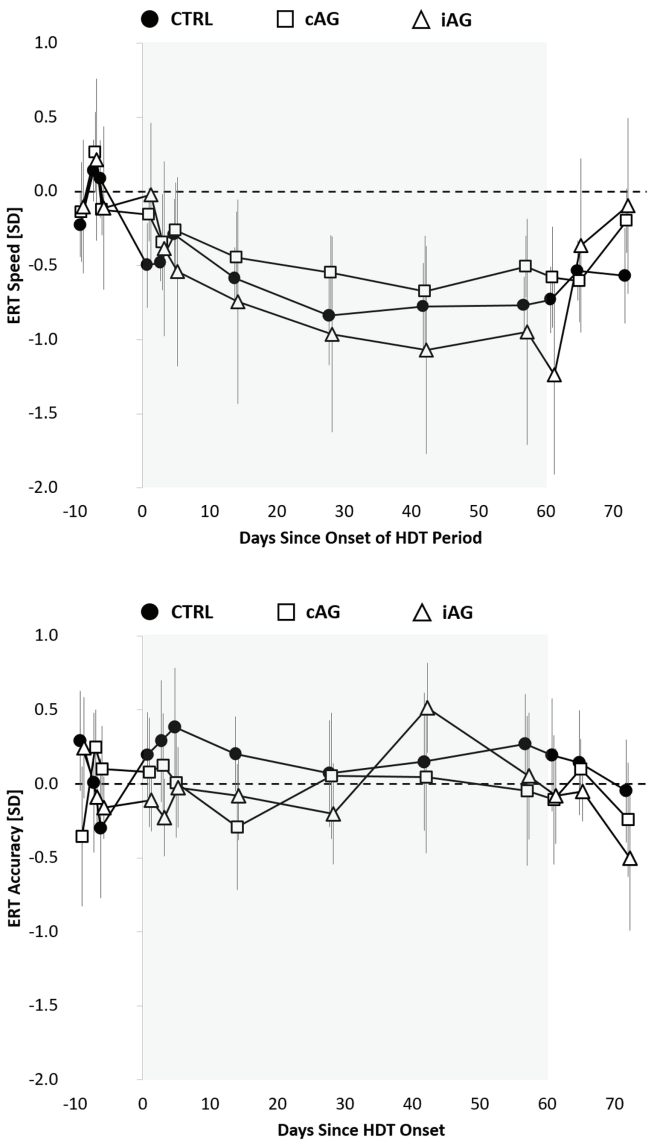

FIGURE 4 | Speed and accuracy on the Emotion Recognition Test (ERT) relative to the 60-day HDT bed rest period (gray background) for the control group (black circles), cAG group (white squares), and iAG group (white triangles). Estimates reflect unadjusted means (SEs) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).

significantly between any of the three groups during the recovery phase (**Figures 1, 5**; **Supplementary Table S3**).

Focusing on individual tests, ERT speed was significantly lower in all three groups relative to baseline with effect sizes ranging from −0.59 to −0.48 (all adjusted $p < 0.05$; **Figure 5A**; **Supplementary Table S3**). In addition, VOLT speed was significantly lower in the iAG group, while BART speed was significantly lower in the cAG group. None of the other tests differed from baseline in either of the three groups for both speed and accuracy. Also, none of the speed and accuracy outcomes differed between the three groups for any of the 10 Cognition tests in the recovery phase after adjustment for multiple testing (**Figure 5B**; **Supplementary Table S3**).

Sleep duration did not differ significantly between recovery and baseline periods for the Control group or the cAG group, but was 0.57 h shorter during recovery in the iAG group

(adjusted $p < 0.01$; **Supplementary Table S3**). Several subjective ratings differed significantly from baseline during recovery. All three groups rated themselves significantly less healthy and significantly more physically exhausted (**Figure 3A**; **Supplementary Table S3**). Workload, sleepiness, mental fatigue, stress, and tiredness were rated significantly higher in the Control group and cAG group only. Depression and loneliness were rated significantly higher in the cAG group and iAG group only. Finally, only the Control group rated higher levels of monotony compared to baseline. However, neither sleep duration nor survey responses differed significantly between the three groups during the recovery period (all adjusted $p > 0.05$; **Figure 3B**; **Supplementary Table S3**).

DISCUSSION

This study investigated the effects of a 60-day 6° HDBR period with and without an artificial gravity countermeasure on cognitive performance across a range of cognitive performance domains. A small but reliable slowing of cognitive speed across a range of cognitive domains was found in all three experimental groups with the onset of HDBR. Twenty-eight out of 30 (i.e., 93.3%) individual test speed point estimates across the three groups were negative. The slowing was most consistently observed for MP, the only Cognition test with significantly slower response speed during HDT relative to baseline in each of the three experimental groups. MP is a measure of sensorimotor speed that probes the sensorimotor cortex. Both spaceflight (Roberts et al., 2017; Lee et al., 2019; Roberts et al., 2019) and bed rest studies (Roberts et al., 2015) have demonstrated an upward shift of the brain with increased brain tissue density at the vertex, which includes the somatosensory cortex and could be the cause for the observed slowing. Some of the reductions seen in the other nine tests also may be explained by reduced sensorimotor speed, because all Cognition tests have a sensorimotor component.

Other HDBR studies have likewise observed a response slowing for selected cognitive domains (Lipnicki and Gunga, 2009; Liu et al., 2012), which could be related to an increase in delta and theta EEG frequencies induced by HDBR and interpreted as signs of cortical inhibition (Vaitl et al., 1996). This finding is also consistent with a 30-day HDBR study (titled VaPER) performed at DLR :envihab, where CO_2 levels were increased to ~3.73 mmHg during the bed rest period (Basner et al., 2021). The fact that response slowing is not a more consistent finding across HDBR studies may be attributed to practice effect confounds or missing ambulatory controls. In addition, to our knowledge, strict HDT has rarely been enforced in past HDBR studies, which could have played a role.

A recent study investigating associations between Cognition performance and complex 6° of freedom docking performance found that speed on AM, LOT, and especially DSST were associated with high docking performance (Basner et al., 2020b). While point estimates indicate an HDBR-induced response slowing on all three tests, effect sizes were small and did not differ significantly from baseline, suggesting that the observed changes may have had limited impact on operationally relevant performance. However, additional HDBR studies with operationally relevant tasks need to verify that operational performance is not affected relevantly.

Accuracy was unaffected during HDBR, both across domains and for the 10 individual Cognition tests. This finding suggests that participants were able to maintain stable accuracy levels by slowing down. Accordingly, cognitive efficiency across tests also did not differ during HDBR from baseline.

In comparisons between the three experimental groups, there was no evidence for an effect of either continuous or intermittent artificial gravity on cognitive speed or accuracy. Indeed, the consistency in performance among the three groups was remarkable. While the study may have been underpowered to detect small differences between groups, none of the point estimates indicated even a medium effect size (>0.5 SD) for any difference between groups. This finding, therefore, suggests that a daily 30-min centrifugation protocol with exposures of 1 g at participants' estimated center of mass and 2 g at the feet was not sufficient to mitigate the HDBR-induced effects on cognitive speed. Furthermore, it suggests that the exposure modality (continuous vs. intermittent AG) does not play a relevant role, at least, at this centrifugation intensity and duration. Ultimately, 30 min of centrifugation translate to only 2.1% of the 24-h day, and this exposure duration may simply be too short to mitigate the cognitive effects caused by prolonged HDBR. Future studies will be needed to determine whether different modes or longer durations of centrifugation are more effective in reducing the effects of HDBR on cognitive slowing.

We are aware of only a single other study that investigated the effects of artificial gravity on general cognitive performance during 21 days of 6° HDBR in 15 subjects using NASA's WinSCAT tool (Seaton et al., 2007). These investigators found more off-nominal WinSCAT scores in the AG group (1 h centrifugation per day) relative to the control group, and accuracy tended to be more affected than speed. Comparable to our study, the length of time spent in bed rest was not associated with a change in cognitive function (WinSCAT does not probe emotion recognition).

With the exception of speed on the ERT, cognitive speed and accuracy did not change significantly with time in HDBR on any of the 10 Cognition tests – thus, any change observed initially during HDBR remained stable until HDBR day 57. This stability not only suggests that the changes induced by HDBR were instantaneous, but also that they neither ameliorated nor further deteriorated over a period of 60 days. Speed on the ERT decreased significantly with time in HDBR and the slope of change did not differ between the three experimental groups. In contrast, ERT accuracy did not change significantly with time in HDT. An in-depth analysis showed that subjects were also significantly less likely to rate faces as happy or neutral and more likely to rate them as angry with increasing time spent in HDT. These findings suggest that participants not only needed significantly more time with increasing time spent in HDBR to identify the correct emotion, but they also developed a response bias from responses of neutral or positive valence to responses of negative valence. The spaceflight relevance of a deterioration of emotional processing with increasing time in mission cannot be overstated, especially for exploration space

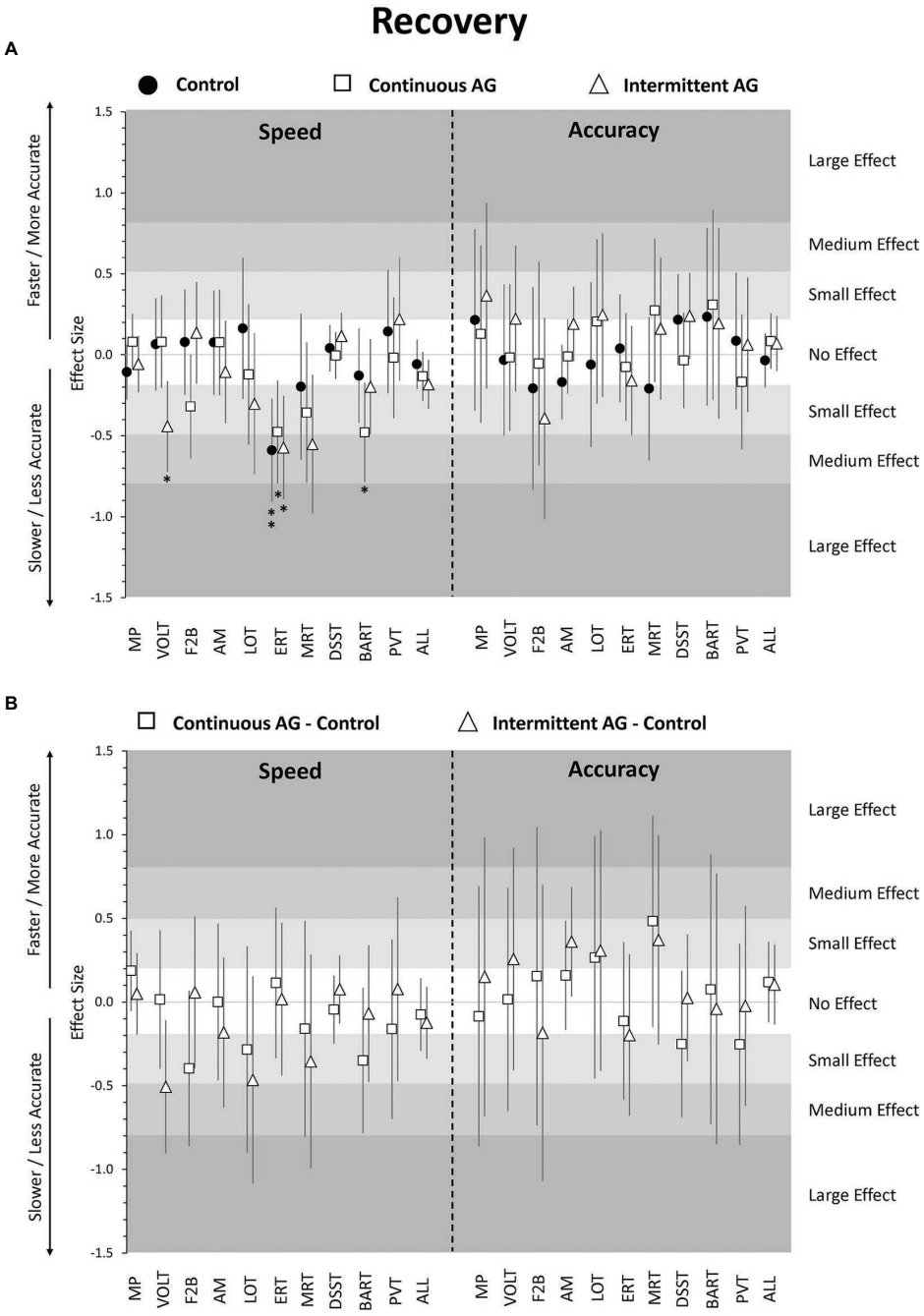

FIGURE 5 | Change in cognitive performance in post-HDT recovery period relative to pre-HDT baseline. Estimates reflect z-scores based on the mean and SD of pre-HDT baseline performance. Error bars reflect unadjusted 95% confidence intervals. **(A)** Estimates for the Control group (black circles), cAG group (white squares), and iAG group (white triangles); **(B)** Estimates for the difference cAG-Control (white squares) and iAG-Control (white triangles); *adjusted $p < 0.05$; **adjusted $p < 0.01$; MP, Motor Praxis; VOLT, Visual Object Learning Test; F2B, Fractal 2-Back; AM, Abstract Matching; LOT, Line Orientation Test; ERT, Emotion Recognition Test; MRT, Matrix Reasoning Test; DSST, Digit Symbol Substitution Test; BART, Balloon Analog Risk Test; PVT, Psychomotor Vigilance Test; ALL, scores averaged across cognitive domains.

missions, where astronauts will be confined to a small space with a small group of peers for a period of up to 3 years.

Previous research also found evidence for changes in emotional processing during a 30-day 6° HDBR study using event-related potentials (ERP), with an inhibition of P300 and late positive potential (LPP) components for emotional stimuli, but not neutral pictures, suggestive of emotional blunting (Brauns et al., 2019). Messerotti Benvenuti et al. (2013) likewise found emotional blunting in P300 and LPP components after only 3 h of 6° HDBR. In our study, participants stayed in separate rooms and had only sporadic contact with the study team. It is therefore, unclear whether the changes in emotional processing observed in this study were caused by prolonged periods of HDBR, low levels of human interaction, or both. Interestingly, neither ERT speed nor ERT accuracy declined during a 30-day HDBR study with elevated levels of ambient CO_2 (~3.73 mmHg) performed by the same study team in the same research facility (Basner et al., 2021). It is, however, unclear whether the elevated CO_2, the shorter HDBR duration, or other factors that differed from the study discussed here can explain this finding. Future HDBR studies should consider varying the degree of social isolation to disentangle the mechanisms involved in altered emotional processing.

The cognitive slowing observed in the bed rest period did not immediately return to baseline levels during recovery. Cognitive speed across domains was similar or even slightly lower on recovery day 1 compared to HDBR day 57, and then gradually recovered on recovery days 5 and 12. No evidence was found for a significant difference among the three experimental groups during the recovery period.

All study participants showed healthy survey responses before the HDBR period. During the HDBR period, several negative survey responses were observed, with participants feeling less healthy and expressing higher levels of depression, boredom, loneliness, and monotony. Subjective assessments indicated lower levels of workload in all three groups, but significantly more so in the Control group. Many of these negative survey responses further deteriorated during the recovery phase, especially ratings of sickness, physical exhaustion, mental fatigue, and stress, without a significant difference among experimental groups. These findings suggest a considerable psychological toll of spending 60 days in HDBR, including difficulties re-adapting to ambulatory conditions.

Strengths and Limitations

To our knowledge, this is the first study investigating the effects on cognitive performance of a continuous and intermittent artificial gravity countermeasure during and after a 60-day HDBR period. The breadth of the Cognition test battery, the near completeness of the data, and the ability to adjust for practice and stimulus set effects are strengths of this study (Basner et al., 2020a). Practice effects in the absence of proper ambulatory controls may have restricted the interpretability of cognitive test data obtained in most bed rest studies performed to date (Lipnicki and Gunga, 2009). That HDT was strictly enforced is another strength of this study. Strict HDBR accurately replicates the sustained head-ward fluid shift that occurs in weightlessness and creates a consistent and uniform stimulus. This consistency seems especially relevant for studies that investigate neurostructural and functional effects of HDBR.

However, the study also had several limitations. First and foremost, HDBR is a spaceflight analog, and as such an imperfect replication of the conditions caused by microgravity and other stressors in spaceflight. Whereas the change in gravity vector is the most plausible explanation for the observed effects, we cannot rule out other contributing factors (e.g., performing the cognitive tests in an unusual body position). However, it was not possible to quantify the contribution of individual factors as they were perfectly confounded with the HDBR intervention. Similar limitations apply to cognitive testing in spaceflight. Also, as evidenced by the large 95% confidence intervals in **Figure 2B**, we were likely underpowered to find small or even medium effect sizes statistically significant in this between-subject design with a group size of $N = 8$. Larger studies are needed to more conclusively eliminate artificial gravity as an effective countermeasure for cognitive performance deficits induced by HDBR.

Conclusion

This study found a small but statistically reliable slowing of cognitive performance across a range of cognitive functions induced by 60 days of 6° HDBR, most consistently for sensorimotor speed, whereas accuracy was unaffected. These changes were observed early during HDBR and neither deteriorated further nor improved with increasing time in HDBR. The only exception was the Emotion Recognition Test. After an initial drop in speed on HDBR day 1, subjects needed increasingly more time with longer time spent in HDBR to decide which facial emotion was displayed, and they also favored categories with negative valence over categories with neutral or positive valence. The success of long-duration space missions will critically depend on astronaut emotional health, and correctly reading each other's facial expressions is an important part of this domain. Except for workload, which was assessed lower in the Control group relative to both artificial gravity groups, this study found no evidence for an effect of either continuous or intermittent artificial gravity on either cognitive performance or subjective responses. Participants expressed several negative survey responses during HDBR and some of them further deteriorated during the recovery phase, stressing the importance of adequate medical and psychological support during extended duration bed rest studies.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found by request at the NASA Life Science Data Archive (https://lsda.jsc.nasa.gov/).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ärztekammer Nordrhein and the Institutional

Review Board of Johnson Space Center. Participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB and AS: study design. CM, MB, KH, and AS: data collection. KH and MB: database management. MB: data analysis, manuscript draft, and figures. MB, AS, DD, TM, RG, and CM: results interpretation. MB, AS, KH, DD, TM, RG, and CM: manuscript revisions. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by NASA through 80NSSC18K0765 and NNJ14ZSA001N. The study design was refined during a definition phase of the project with feedback from NASA Human Research Program scientists. The funders had no role in data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

We would like to thank the German Aerospace Center (DLR), Institute of Aerospace Medicine and the European Space Agency for administration of conducting the bed rest protocols and their support in collecting the data.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.643854/full#supplementary-material

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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 Materials

Continuous and Intermittent Artificial Gravity as a Countermeasure to the Cognitive Effects of 60 Days of Head-Down Tilt Bed Rest

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Figure S1: For testing in the HDT position, laptops were mounted vertically on an adjustable swivel arm and positioned in chest-height in front of the participants (source: DLR, with permission).

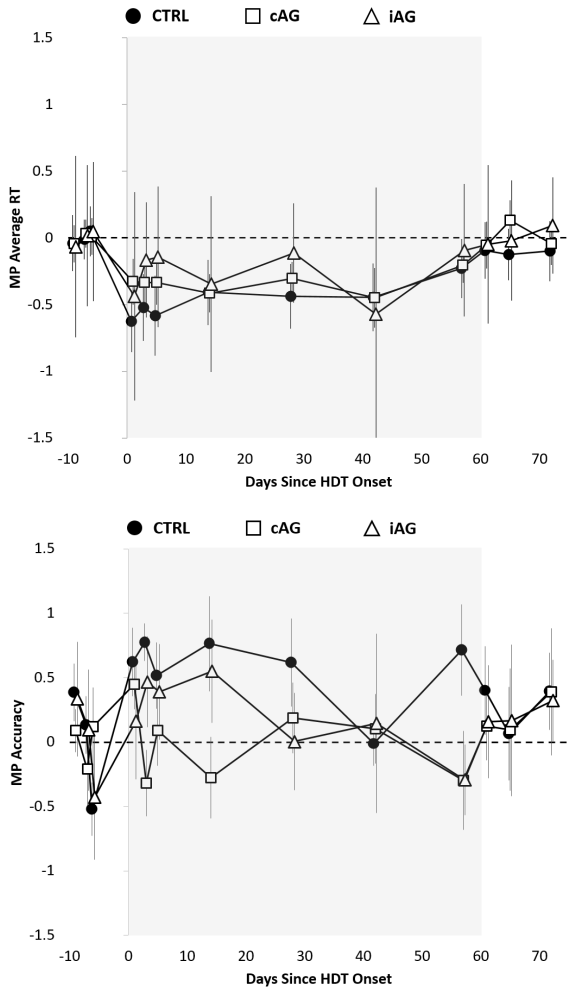
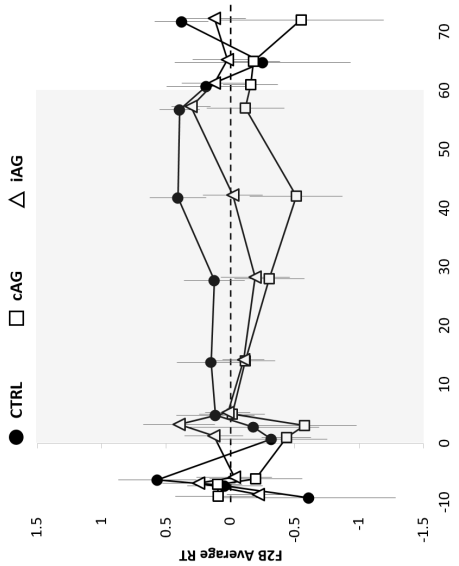


Figure S2: Speed and accuracy on the Motor Praxis (MP) test relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



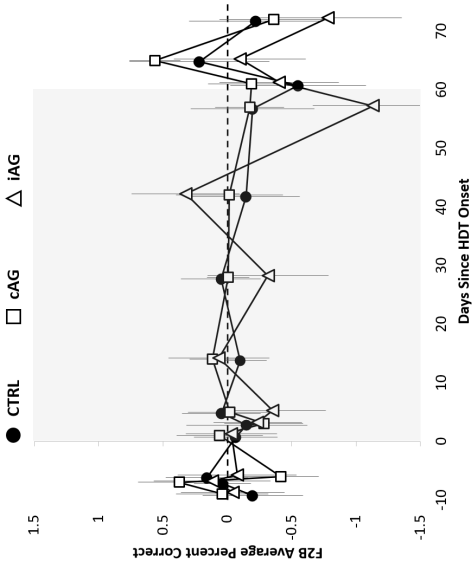
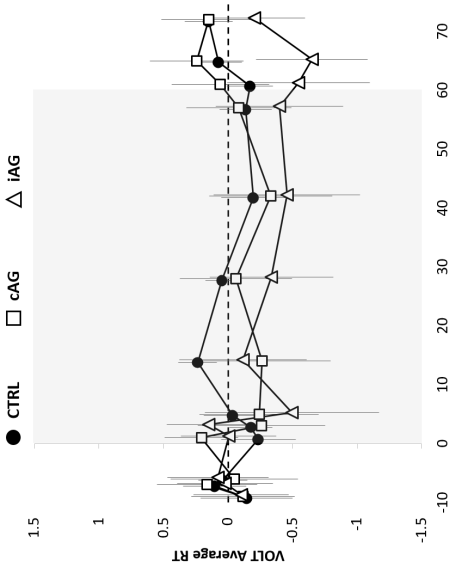


Figure S4: Speed and accuracy on the Fractal 2-Back (F2B) test relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



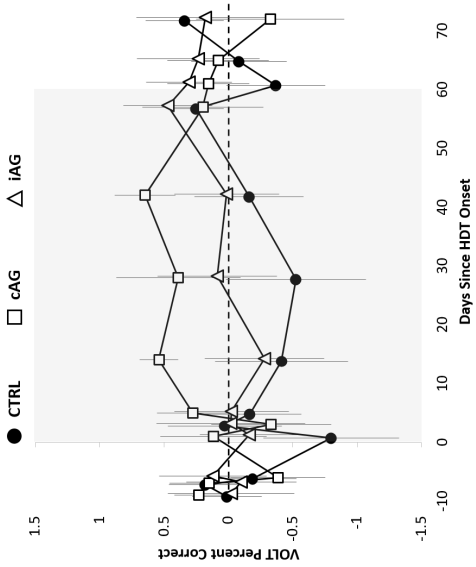
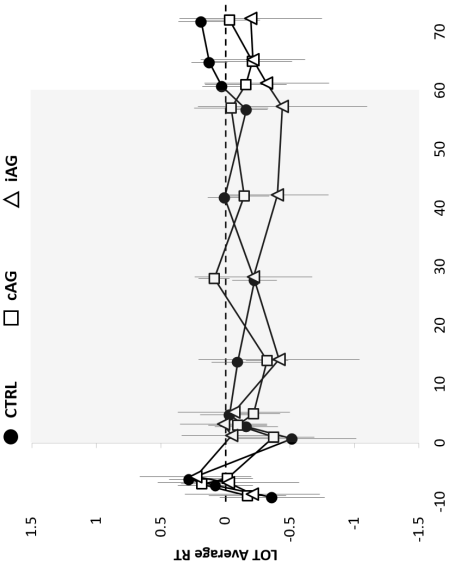


Figure S3: Speed and accuracy on the Visual Object learning Test (VOLT) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



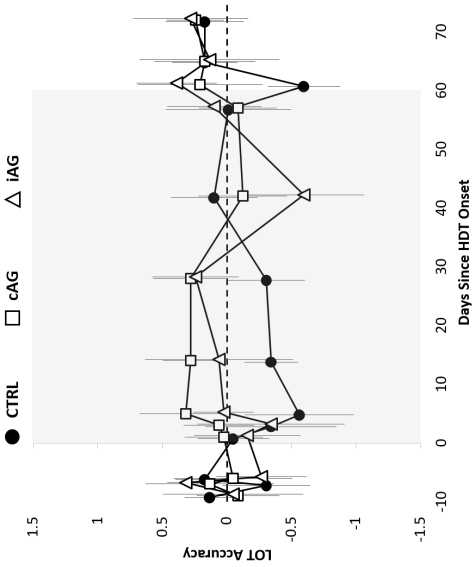
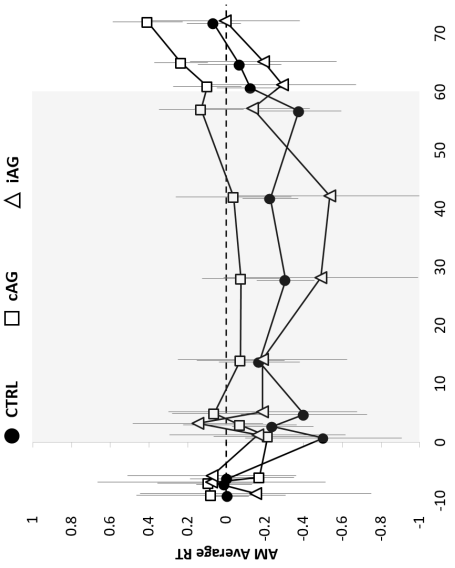


Figure S6: Speed and accuracy on the Line Orientation Test (LOT) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



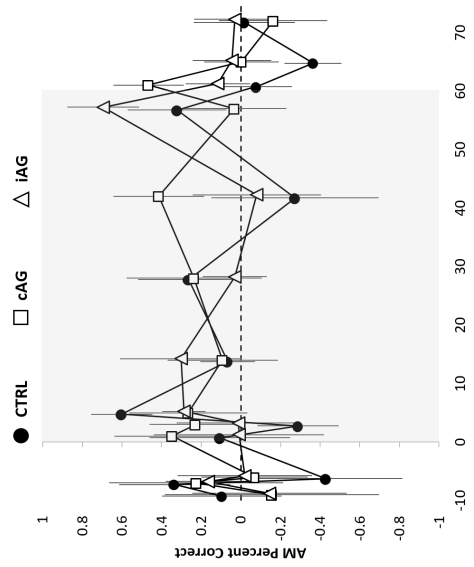
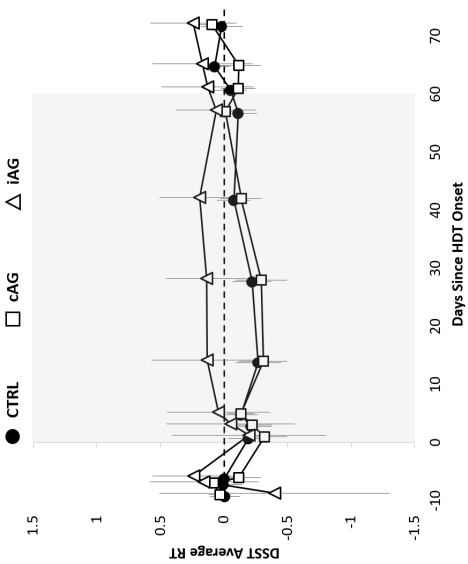


Figure S5: Speed and accuracy on the Abstract Matching (AM) test relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



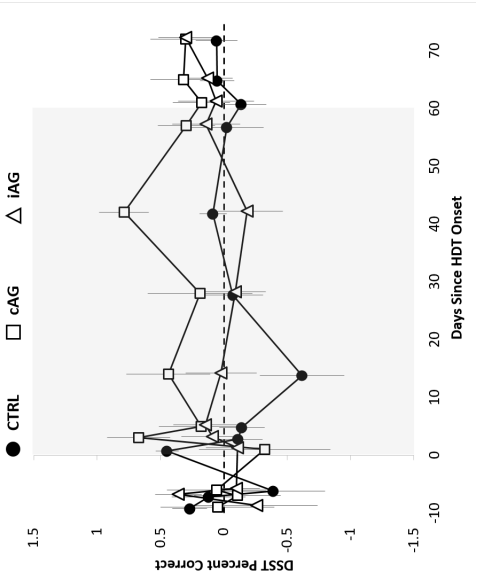
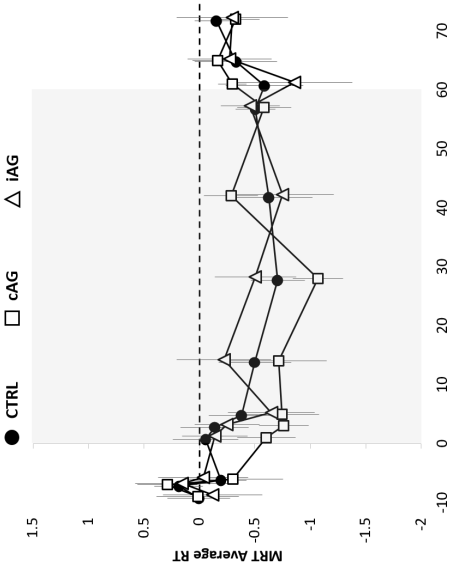


Figure S8: Speed and accuracy on the Digit Symbol Substitution Test (DSST) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



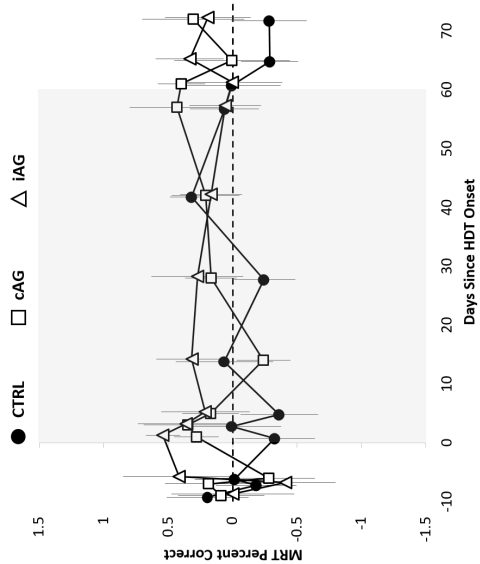
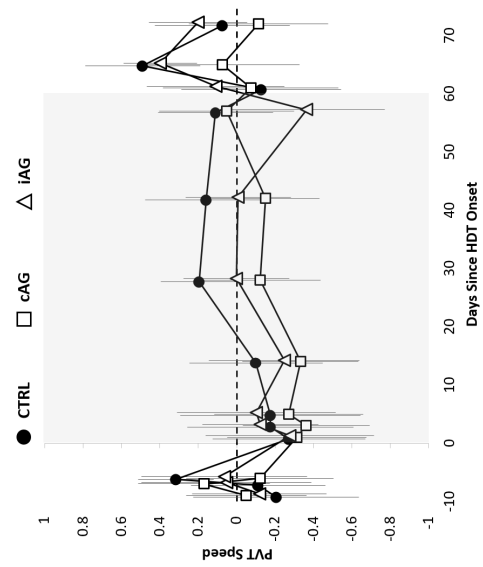


Figure S7: Speed and accuracy on the Matrix Reasoning Test (MRT) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



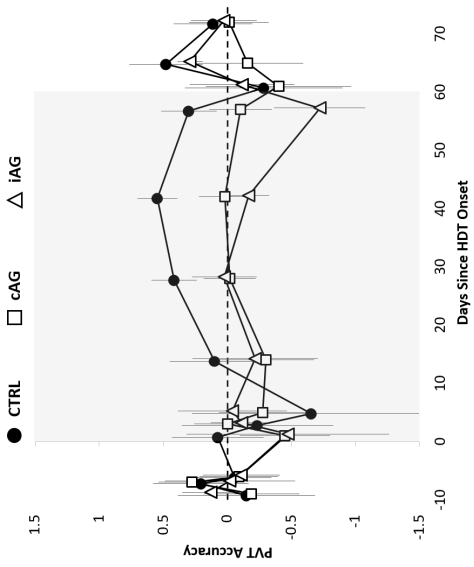
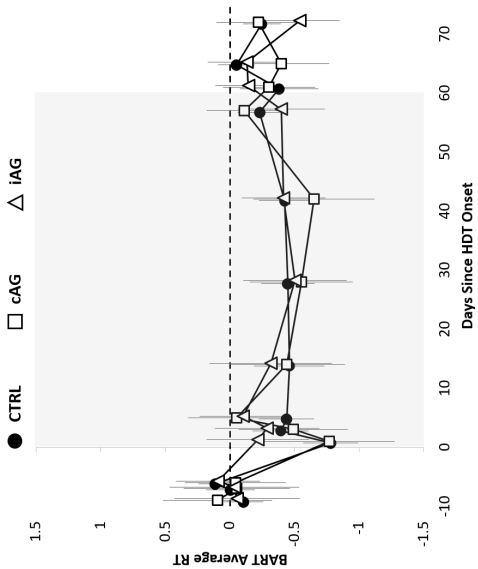


Figure S10: Speed and accuracy on the Psychomotor Vigilance Test (PVT) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).



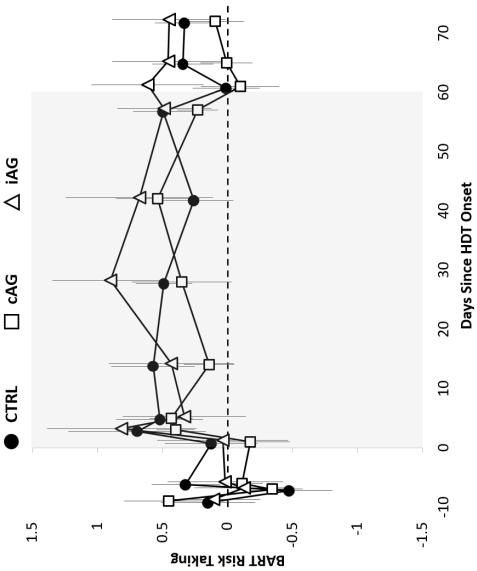


Figure S9: Speed and accuracy on the Balloon Analog Risk Test (BART) relative to the 60-day head-down tilt (HDT) bed rest period (gray background) for the control group (black circles), continuous artificial gravity group (cAG; white squares) and intermittent artificial gravity group (iAG, white triangles). Estimates reflect unadjusted means (standard errors) z-transformed based on baseline (pre-HDT) performance. To reflect the analytical approach (adjusting for baseline performance), means were shifted within groups to reflect a pre-HDT baseline performance of 0 (zero).

Table S1: Comparison of baseline assessments

	Baseline Performance L-9/-7/-6				
Variable	**Control**	**Continuous AG**	**Intermittent AG**	**Test III**	**Adj. Test III**
MP Average RT [ms]	892.0 (725.5; 1058.5)	835.7 (669.2; 1058.5)	991.0 (824.5; 1058.5)	0.3973	0.3517
VOLT Average RT [ms]	1491.6 (1081.7; 1901.4)	1759.4 (1349.5; 1901.4)	1597.9 (1188.0; 1901.4)	0.6325	0.4726
F2B Average RT [ms]	621.7 (540.8; 702.5)	575.3 (494.9; 702.5)	590.7 (510.4; 702.5)	0.6944	0.7091
AM Average RT [ms]	1737.8 (1081.4; 2394.1)	2128.7 (1472.4; 2394.1)	1834.9 (1178.6; 2394.1)	0.6650	0.4149
LOT Average RT [ms]	5093.6 (3752.7; 6434.6)	5117.7 (3776.7; 6434.6)	5719.5 (4378.6; 6434.6)	0.7421	0.7814
ERT Average RT [ms]	1495.6 (1120.0; 1871.3)	1529.7 (1154.1; 1871.3)	1747.9 (1372.2; 1871.3)	0.5719	0.6172
MRT Average RT [ms]	6520.0 (4425.6; 8614.4)	8446.8 (6352.4; 8614.4)	8547.3 (6447.7; 8614.4)	0.2973	0.2073
DSST Average RT [ms]	1332.3 (965.9; 1698.6)	1296.5 (930.1; 1698.6)	1471.3 (1104.9; 1698.6)	0.7625	0.8162
BART Average RT [ms]	1286.7 (738.9; 1834.5)	1742.6 (1194.8; 1834.5)	1307.9 (760.0; 1834.5)	0.4012	0.1950
PVT Slowness [10 - 1/s]	5.40 (5.00; 5.80)	5.33 (4.92; 5.80)	5.23 (4.83; 5.80)	0.8125	0.4667
MP Accuracy [%]	25.4 (19.0; 31.7)	28.0 (21.7; 31.7)	33.5 (27.2; 31.7)	0.1813	0.1918
VOLT Percent Correct	91.7 (86.0; 97.3)	87.1 (81.5; 97.3)	86.4 (80.8; 97.3)	0.3509	0.3055
F2B Average Percent Correct	85.0 (78.5; 91.6)	87.1 (80.6; 91.6)	85.6 (79.1; 91.6)	0.8917	0.9872
AM Percent Correct	78.7 (72.1; 85.3)	77.4 (70.8; 85.3)	80.6 (74.0; 85.3)	0.7761	0.7792
LOT Accuracy [%]	78.2 (72.5; 84.0)	76.5 (70.7; 84.0)	76.5 (70.7; 84.0)	0.8781	0.9507
ERT Percent Correct	68.9 (62.4; 75.3)	70.8 (64.3; 75.3)	73.7 (67.2; 75.3)	0.5481	0.4748
MRT Percent Correct	73.4 (65.3; 81.4)	77.1 (69.1; 81.4)	75.4 (67.3; 81.4)	0.7871	0.8370
DSST Percent Correct	98.4 (96.7; 100.0)	95.9 (94.2; 100.0)	97.7 (96.0; 100.0)	0.0917	**0.0495**
BART Risk Score P [%]	65.9 (59.3; 72.5)	71.1 (64.5; 72.5)	60.4 (53.8; 72.5)	0.0816	0.0708
PVT Accuracy [%]	92.1 (87.6; 96.5)	94.4 (90.0; 96.5)	94.2 (89.7; 96.5)	0.6988	0.6365
Speed	0.11 (-0.40; 0.62)	-0.03 (-0.54; 0.62)	-0.08 (-0.59; 0.62)	0.8549	0.7974
Accuracy	-0.02 (-0.31; 0.28)	-0.06 (-0.36; 0.28)	0.08 (-0.21; 0.28)	0.7687	0.4830
Efficiency	0.05 (-0.30; 0.40)	-0.05 (-0.40; 0.40)	0.00 (-0.35; 0.40)	0.9270	0.7005
Sleep Duration [h]	7.54 (7.31; 7.76)	7.61 (7.39; 7.76)	7.55 (7.33; 7.76)	0.8756	0.7553
Poor Sleep Quality	4.6 (3.4; 5.8)	3.9 (2.7; 5.8)	5.4 (4.2; 5.8)	0.2209	0.1980
Low Workload	5.0 (3.9; 6.1)	4.0 (3.0; 6.1)	3.9 (2.8; 6.1)	0.2934	0.3350
Sleepy	4.4 (3.2; 5.6)	5.0 (3.8; 5.6)	5.1 (3.9; 5.6)	0.6608	0.7496
Unhappy	2.7 (1.6; 3.7)	2.9 (1.8; 3.7)	3.0 (2.0; 3.7)	0.8700	0.8887
Healthy	8.5 (7.1; 9.8)	8.5 (7.2; 9.8)	7.4 (6.1; 9.8)	0.3811	0.3829
Physically Exhausted	3.5 (2.2; 4.7)	4.7 (3.4; 4.7)	4.8 (3.6; 4.7)	0.2171	0.2240
Mentally Fatigued	4.0 (2.5; 5.4)	4.0 (2.5; 5.4)	4.3 (2.8; 5.4)	0.9516	0.9626
Stressed	2.2 (1.0; 3.5)	2.2 (0.9; 3.5)	4.0 (2.7; 3.5)	0.0777	0.0803
Fresh	5.0 (3.7; 6.2)	5.1 (3.8; 6.2)	4.2 (2.9; 6.2)	0.5556	0.5976
Not Depressed	9.3 (8.2; 10.3)	8.9 (7.9; 10.3)	8.9 (7.9; 10.3)	0.8414	0.8505
Not Bored	8.3 (6.9; 9.8)	8.3 (6.8; 9.8)	7.7 (6.2; 9.8)	0.7611	0.5699
Lonely	1.5 (0.0; 3.0)	1.5 (0.1; 3.0)	1.6 (0.2; 3.0)	0.9918	0.9578
Not Monotonous	7.8 (6.2; 9.5)	7.4 (5.8; 9.5)	7.8 (6.2; 9.5)	0.9172	0.8925
Age (years ±SD)	34.3 (7.9)	31.9 (9.7)	33.8 (10.8)	0.872	N/A
Male N (%)	6 (75%)	5 (62.5%)	5 (62.5%)	0.829	N/A

Mixed effect model estimates (shown with 95% confidence limits) are based on data of test bouts performed 9, 7 and 6 days prior to initiation of the head-down tilt bed rest period. Adjusted p-values reflect models adjusted for age and sex. Estimates for self-report data reflect points on an 11-point scale (variables are listed by anchors for high values). For Age, values in parenthesis reflect standard deviation. For Male, the p-value is based on a χ^2 test. MP: Motor Praxis; VOLT: Visual Object Learning Test; F2B: Fractal 2-Back; AM: Abstract Matching; LOT: Line Orientation Test; ERT: Emotion Recognition Test; MRT: Matrix Reasoning Test; DSST: Digit Symbol Substitution Test; BART: Balloon Analog Risk Test; PVT: Psychomotor Vigilance Test; ms: milliseconds; AG: artificial gravity; Adj.: adjusted

Table S2: Mixed effect model results for the head-down tilt bed rest period

	Main Effects						Contrasts						
Variable	CTRL	cAG	iAG	p(CTRL)	p(cAG)	p(iAG)	cAG-CTRL	iAG-CTRL	cAG-iAG	p(cAG-CTRL)	p(iAG-CTRL)	p(cAG-iAG)	Test III
MP Speed	-0.48 (-0.64; -0.31)	-0.35 (-0.52; -0.18)	-0.24 (-0.41; -0.07)	<.0001***	0.0004**	0.0073*	0.13 (-0.11; 0.36)	0.23 (-0.01; 0.47)	-0.11 (-0.35; 0.14)	0.2717	0.0551	0.3729	0.1487
VOLT Speed	-0.03 (-0.32; 0.26)	-0.21 (-0.50; 0.08)	-0.21 (-0.50; 0.07)	0.8172	0.1503	0.1368	-0.18 (-0.60; 0.25)	-0.18 (-0.59; 0.23)	0.00 (-0.41; 0.41)	0.3926	0.3688	0.9895	0.5951
F2B Speed	0.09 (-0.14; 0.32)	-0.27 (-0.50; -0.04)	0.08 (-0.14; 0.31)	0.4448	0.0232	0.4521	-0.36 (-0.69; -0.03)	0.00 (-0.33; 0.32)	-0.35 (-0.67; -0.03)	0.0357	0.9856	0.0330	0.0521
AM Speed	-0.22 (-0.52; 0.08)	-0.17 (-0.47; 0.14)	-0.19 (-0.49; 0.10)	0.1380	0.2661	0.1837	0.05 (-0.38; 0.49)	0.03 (-0.39; 0.44)	0.03 (-0.40; 0.45)	0.7944	0.8928	0.8927	0.9657
LOT Speed	-0.15 (-0.45; 0.15)	-0.15 (-0.44; 0.15)	-0.26 (-0.55; 0.04)	0.3077	0.3152	0.0844	0.00 (-0.42; 0.42)	-0.11 (-0.53; 0.31)	0.11 (-0.31; 0.53)	0.9883	0.5929	0.5805	0.8172
ERT Speed	-0.59 (-1.05; -0.13)	-0.45 (-0.90; 0.01)	-0.66 (-1.12; -0.20)	0.0152	0.0549	0.0074*	0.14 (-0.51; 0.79)	-0.08 (-0.73; 0.58)	0.22 (-0.44; 0.87)	0.6553	0.8123	0.4962	0.7815
MRT Speed	-0.27 (-0.55; 0.01)	-0.75 (-1.02; -0.49)	-0.51 (-0.78; -0.25)	0.0593	<.0001	0.0007**	-0.48 (-0.88; -0.09)	-0.24 (-0.64; 0.15)	-0.24 (-0.61; 0.13)	0.0200	0.2096	0.1901	0.0614
DSST Speed	-0.14 (-0.30; 0.01)	-0.18 (-0.34; -0.03)	-0.01 (-0.16; 0.15)	0.0700	0.0252	0.9298	-0.04 (-0.26; 0.18)	0.14 (-0.09; 0.36)	-0.17 (-0.40; 0.05)	0.7213	0.2123	0.1153	0.2468
BART Speed	-0.39 (-0.71; -0.07)	-0.56 (-0.89; -0.22)	-0.26 (-0.59; 0.06)	0.0199	0.0026*	0.1034	-0.17 (-0.65; 0.31)	0.13 (-0.32; 0.58)	-0.29 (-0.78; 0.19)	0.4693	0.5626	0.2162	0.4555
PVT Speed	-0.02 (-0.31; 0.26)	-0.21 (-0.49; 0.06)	-0.17 (-0.45; 0.11)	0.8547	0.1205	0.2152	-0.19 (-0.59; 0.21)	-0.15 (-0.55; 0.26)	-0.04 (-0.44; 0.35)	0.3297	0.4581	0.8233	0.5916
MP Accuracy	0.44 (0.13; 0.76)	-0.04 (-0.35; 0.26)	0.37 (0.05; 0.69)	0.0087	0.7678	0.0273	-0.49 (-0.92; -0.05)	-0.07 (-0.54; 0.40)	-0.41 (-0.87; 0.04)	0.0314	0.7467	0.0716	0.0648
VOLT Accuracy	-0.21 (-0.62; 0.20)	0.25 (-0.14; 0.65)	-0.04 (-0.43; 0.36)	0.2964	0.1966	0.8481	0.46 (-0.12; 1.05)	0.17 (-0.41; 0.75)	0.29 (-0.26; 0.84)	0.1140	0.5410	0.2865	0.2627
F2B Accuracy	-0.09 (-0.43; 0.24)	-0.03 (-0.36; 0.31)	-0.24 (-0.57; 0.09)	0.5595	0.8749	0.1404	0.07 (-0.40; 0.54)	-0.15 (-0.62; 0.32)	0.22 (-0.25; 0.69)	0.7630	0.5149	0.3426	0.6161
AM Accuracy	0.09 (-0.19; 0.37)	0.15 (-0.14; 0.43)	0.29 (0.01; 0.57)	0.5071	0.2946	0.0460	0.05 (-0.35; 0.46)	0.20 (-0.20; 0.60)	-0.14 (-0.54; 0.26)	0.7790	0.3146	0.4646	0.5754
LOT Accuracy	-0.15 (-0.66; 0.36)	0.07 (-0.44; 0.58)	-0.12 (-0.63; 0.38)	0.5394	0.7671	0.6127	0.23 (-0.50; 0.95)	0.03 (-0.70; 0.75)	0.20 (-0.52; 0.92)	0.5232	0.9364	0.5712	0.7785
ERT Accuracy	0.12 (-0.28; 0.53)	-0.01 (-0.41; 0.39)	0.09 (-0.31; 0.50)	0.5222	0.9775	0.6413	-0.13 (-0.69; 0.44)	-0.03 (-0.61; 0.55)	-0.10 (-0.67; 0.48)	0.6350	0.9057	0.7280	0.8826
MRT Accuracy	-0.17 (-0.47; 0.12)	0.27 (-0.01; 0.56)	0.30 (0.02; 0.59)	0.2259	0.0607	0.0393	0.45 (0.03; 0.86)	0.48 (0.07; 0.89)	-0.03 (-0.44; 0.38)	0.0351	0.0248	0.8842	0.0449
DSST Accuracy	0.31 (0.02; 0.61)	-0.15 (-0.46; 0.16)	0.10 (-0.18; 0.38)	0.0389	0.3216	0.4637	-0.46 (-0.92; 0.00)	-0.21 (-0.61; 0.18)	-0.25 (-0.67; 0.18)	0.0483	0.2743	0.2364	0.1348
BART Risk Taking	0.41 (-0.15; 0.96)	0.48 (-0.11; 1.08)	0.36 (-0.23; 0.96)	0.1402	0.1059	0.2161	0.07 (-0.74; 0.89)	-0.04 (-0.86; 0.77)	0.12 (-0.78; 1.02)	0.8502	0.9120	0.7857	0.9616
PVT Accuracy	0.07 (-0.15; 0.30)	-0.17 (-0.39; 0.05)	-0.23 (-0.45; -0.01)	0.5255	0.1289	0.0414	-0.24 (-0.56; 0.08)	-0.30 (-0.62; 0.02)	0.06 (-0.25; 0.37)	0.1343	0.0625	0.7101	0.1459
Speed	-0.23 (-0.35; -0.11)	-0.31 (-0.43; -0.19)	-0.25 (-0.37; -0.13)	0.0009*	<.0001***	0.0004**	-0.08 (-0.25; 0.09)	-0.02 (-0.19; 0.15)	-0.06 (-0.23; 0.11)	0.3476	0.8201	0.4634	0.6073
Accuracy	0.03 (-0.12; 0.17)	0.09 (-0.06; 0.24)	0.02 (-0.12; 0.17)	0.7189	0.2261	0.7321	0.06 (-0.15; 0.28)	0.00 (-0.21; 0.21)	0.06 (-0.15; 0.28)	0.5339	0.9926	0.5357	0.7710
Efficiency	-0.10 (-0.20; -0.01)	-0.11 (-0.20; -0.01)	-0.12 (-0.21; -0.02)	0.0328	0.0280	0.0176	0.00 (-0.14; 0.13)	-0.01 (-0.14; 0.12)	0.01 (-0.12; 0.14)	0.9519	0.8478	0.8970	0.9803
Sleep Duration [h]	-0.18 (-0.47; 0.10)	-0.06 (-0.34; 0.23)	-0.09 (-0.38; 0.19)	0.2131	0.6917	0.5151	0.25 (-0.19; 0.69)	0.13 (-0.30; 0.57)	0.12 (-0.32; 0.56)	0.2495	0.5350	0.5702	0.5055
Poor Sleep Quality	-0.4 (-1.3; 0.6)	0.5 (-0.4; 1.5)	-0.8 (-1.8; 0.1)	0.4456	0.2761	0.0885	0.7 (-1.3; 2.6)	0.0 (-1.9; 1.9)	0.7 (-1.3; 2.7)	0.4764	0.9688	0.4746	0.7130
Low Workload	2.0 (1.1; 2.9)	1.0 (0.1; 1.8)	0.9 (0.1; 1.8)	<.0001****	0.0229*	0.0346	-1.6 (-2.5; -0.6)	-1.7 (-2.7; -0.8)	0.2 (-0.7; 1.0)	0.0023*	0.0010*	0.7040	0.0019*
Sleepy	0.5 (-0.3; 1.4)	0.2 (-0.6; 1.1)	0.3 (-0.6; 1.1)	0.2075	0.5803	0.5235	-0.1 (-1.5; 1.4)	0.1 (-1.4; 1.6)	-0.2 (-1.6; 1.3)	0.9293	0.8678	0.7957	0.9651
Unhappy	0.7 (-0.1; 1.5)	0.5 (-0.3; 1.3)	1.2 (0.4; 2.0)	0.0822	0.2361	0.0048*	-0.3 (-1.7; 1.1)	0.5 (-0.9; 1.9)	-0.8 (-2.2; 0.6)	0.6371	0.4930	0.2507	0.5038
Healthy	-2.0 (-3.0; -1.1)	-0.9 (-1.9; 0.0)	-1.8 (-2.8; -0.9)	<.0001***	0.0548	0.0002**	1.4 (-0.7; 3.5)	-0.2 (-2.3; 1.9)	1.6 (-0.5; 3.8)	0.1711	0.8419	0.1306	0.2421
Physically Exhausted	0.9 (0.0; 1.8)	-0.3 (-1.2; 0.6)	0.6 (-0.2; 1.5)	0.0404	0.4959	0.1540	-0.8 (-2.5; 0.9)	0.3 (-1.4; 2.0)	-1.1 (-2.7; 0.5)	0.3203	0.7448	0.1672	0.3488
Mentally Fatigued	0.4 (-0.5; 1.2)	1.0 (0.2; 1.9)	0.7 (-0.2; 1.5)	0.3757	0.0184*	0.1250	0.6 (-1.1; 2.3)	0.4 (-1.3; 2.0)	0.2 (-1.4; 1.9)	0.4634	0.6448	0.7792	0.7545
Stressed	0.5 (-0.4; 1.3)	1.3 (0.4; 2.2)	0.0 (-0.9; 0.8)	0.3095	0.0038*	0.9147	0.6 (-1.4; 2.6)	0.2 (-2.1; 2.4)	0.4 (-1.8; 2.6)	0.5529	0.8628	0.7109	0.8284
Fresh	0.0 (-0.8; 0.9)	-0.4 (-1.3; 0.4)	0.0 (-0.9; 0.8)	0.9241	0.3340	0.9457	-0.4 (-2.1; 1.3)	-0.4 (-2.1; 1.3)	0.0 (-1.7; 1.7)	0.6207	0.6088	0.9843	0.8396
Not Depressed	-0.5 (-1.3; 0.3)	-1.1 (-1.8; -0.3)	-1.5 (-2.3; -0.7)	0.2460	0.0084*	0.0001**	-0.5 (-2.1; 1.2)	-1.2 (-2.8; 0.5)	0.7 (-1.0; 2.3)	0.5457	0.1548	0.3920	0.3491
Not Bored	0.1 (-0.7; 0.8)	-1.3 (-2.0; -0.5)	-0.7 (-1.5; 0.0)	0.8362	0.0009**	0.0551	-1.3 (-2.3; -0.2)	-1.1 (-2.2; 0.0)	-0.2 (-1.3; 0.9)	0.0246	0.0523	0.7425	0.0520
Lonely	0.5 (-0.3; 1.3)	1.6 (0.8; 2.5)	1.2 (0.3; 2.0)	0.2519	0.0001**	0.0061*	0.9 (-0.6; 2.3)	0.7 (-0.8; 2.1)	0.2 (-1.3; 1.7)	0.2428	0.3642	0.7797	0.4651
Not Monotonous	-2.3 (-3.1; -1.4)	-1.3 (-2.1; -0.5)	-1.4 (-2.2; -0.5)	<.0001****	0.0022*	0.0015**	0.9 (-0.3; 2.1)	1.0 (-0.2; 2.2)	0.0 (-1.3; 1.2)	0.1301	0.1093	0.9355	0.1973

All models were adjusted for sex and age. Models with cognitive outcomes were additionally adjusted for baseline performance. Estimates for cognitive tests reflect z-scores. As z-transformation was based on baseline performance, an estimate of 0 (zero) reflects baseline performance. Estimates for self-report data reflect points on an 11-point scale (variables are listed by anchors for high values). As sleep and subjective outcomes were not z-transformed, estimates for these variables were not adjusted for baseline values but are based on a direct contrast between the head-down tilt and baseline period instead. Est.: Estimate; CI: Confidence Interval; CTRL: Control; cAG: continuous Artificial Gravity; iAG: intermittent Artificial Gravity; *adjusted p<0.05; **adjusted p<0.01; ***adjusted p<0.001; ****adjusted p<0.0001; MP: Motor Praxis; VOLT: Visual Object Learning Test; F2B: Fractal 2-Back; AM: Abstract Matching; LOT: Line Orientation Test; ERT: Emotion Recognition Test; MRT: Matrix Reasoning Test; DSST: Digit Symbol Substitution Test; BART: Balloon Analog Risk Test; PVT: Psychomotor Vigilance Test

Table S3: Mixed effect model results for the recovery period

	Main Effects						Contrasts						
Variable	CTRL	cAG	iAG	p(CTRL)	p(cAG)	p(iAG)	cAG-CTRL	iAG-CTRL	cAG-iAG	p(cAG-CTRL)	p(iAG-CTRL)	p(cAG-iAG)	Test III
MP Speed	-0.11 (-0.28; 0.06)	0.08 (-0.09; 0.25)	-0.06 (-0.23; 0.11)	0.1984	0.3402	0.4881	0.19 (-0.05; 0.43)	0.05 (-0.20; 0.29)	0.14 (-0.11; 0.39)	0.1192	0.6791	0.2600	0.2687
VOLT Speed	0.06 (-0.22; 0.35)	0.08 (-0.21; 0.36)	-0.44 (-0.72; -0.16)	0.6389	0.5656	0.0036*	0.01 (-0.40; 0.43)	-0.51 (-0.90; -0.11)	0.52 (0.12; 0.92)	0.9407	0.0154	0.0133	0.0185
F2B Speed	0.08 (-0.25; 0.40)	-0.32 (-0.64; 0.00)	0.14 (-0.18; 0.45)	0.6166	0.0512	0.3719	-0.40 (-0.86; 0.07)	0.06 (-0.40; 0.51)	-0.45 (-0.90; -0.01)	0.0883	0.7927	0.0472	0.1005
AM Speed	0.08 (-0.24; 0.40)	0.08 (-0.25; 0.40)	-0.11 (-0.42; 0.21)	0.6255	0.6300	0.4893	0.00 (-0.47; 0.47)	-0.18 (-0.63; 0.27)	0.18 (-0.28; 0.64)	0.9993	0.4045	0.4150	0.6228
LOT Speed	0.16 (-0.27; 0.60)	-0.12 (-0.55; 0.31)	-0.30 (-0.74; 0.13)	0.4414	0.5623	0.1602	-0.28 (-0.90; 0.33)	-0.47 (-1.09; 0.15)	0.18 (-0.43; 0.80)	0.3446	0.1313	0.5435	0.3048
ERT Speed	-0.59 (-0.91; -0.27)	-0.48 (-0.79; -0.16)	-0.57 (-0.89; -0.25)	0.0004**	0.0037*	0.0006*	0.11 (-0.33; 0.56)	0.02 (-0.44; 0.47)	0.10 (-0.35; 0.55)	0.6123	0.9401	0.6686	0.8598
MRT Speed	-0.20 (-0.65; 0.25)	-0.36 (-0.79; 0.07)	-0.55 (-0.98; -0.12)	0.3692	0.0989	0.0143	-0.16 (-0.80; 0.48)	-0.36 (-0.99; 0.28)	0.20 (-0.40; 0.79)	0.6082	0.2575	0.5023	0.5108
DSST Speed	0.04 (-0.10; 0.18)	-0.01 (-0.15; 0.14)	0.12 (-0.03; 0.26)	0.5605	0.9397	0.1062	-0.05 (-0.25; 0.16)	0.08 (-0.13; 0.28)	-0.12 (-0.32; 0.08)	0.6429	0.4454	0.2268	0.4652
BART Speed	-0.13 (-0.42; 0.16)	-0.48 (-0.78; -0.17)	-0.20 (-0.49; 0.10)	0.3638	0.0041*	0.1740	-0.35 (-0.78; 0.08)	-0.07 (-0.48; 0.34)	-0.28 (-0.72; 0.16)	0.1080	0.7285	0.1951	0.2403
PVT Speed	0.14 (-0.24; 0.52)	-0.02 (-0.39; 0.35)	0.22 (-0.16; 0.60)	0.4396	0.9182	0.2380	-0.16 (-0.70; 0.37)	0.08 (-0.47; 0.63)	-0.24 (-0.77; 0.29)	0.5337	0.7719	0.3581	0.6337
MP Accuracy	0.21 (-0.35; 0.77)	0.13 (-0.42; 0.67)	0.37 (-0.21; 0.94)	0.4310	0.6268	0.1972	-0.09 (-0.86; 0.69)	0.15 (-0.68; 0.98)	-0.24 (-1.04; 0.57)	0.8185	0.7089	0.5446	0.8271
VOLT Accuracy	-0.03 (-0.50; 0.43)	-0.02 (-0.47; 0.43)	0.22 (-0.23; 0.67)	0.8840	0.9379	0.3115	0.02 (-0.65; 0.68)	0.26 (-0.41; 0.92)	-0.24 (-0.87; 0.39)	0.9605	0.4283	0.4341	0.6502
F2B Accuracy	-0.21 (-0.83; 0.42)	-0.05 (-0.68; 0.57)	-0.39 (-1.01; 0.23)	0.4932	0.8583	0.2007	0.15 (-0.74; 1.04)	-0.18 (-1.07; 0.70)	0.34 (-0.54; 1.22)	0.7210	0.6652	0.4309	0.7260
AM Accuracy	-0.17 (-0.40; 0.06)	-0.01 (-0.24; 0.22)	0.19 (-0.04; 0.42)	0.1453	0.9255	0.1027	0.16 (-0.17; 0.48)	0.36 (0.03; 0.69)	-0.20 (-0.53; 0.13)	0.3353	0.0310	0.2233	0.0950
LOT Accuracy	-0.06 (-0.57; 0.45)	0.20 (-0.30; 0.71)	0.25 (-0.26; 0.75)	0.8047	0.4070	0.3183	0.27 (-0.46; 0.99)	0.31 (-0.41; 1.03)	-0.04 (-0.76; 0.67)	0.4501	0.3805	0.9036	0.6317
ERT Accuracy	0.04 (-0.29; 0.37)	-0.08 (-0.41; 0.26)	-0.16 (-0.50; 0.18)	0.8119	0.6371	0.3344	-0.11 (-0.58; 0.36)	-0.20 (-0.68; 0.28)	0.08 (-0.40; 0.56)	0.6161	0.4001	0.7183	0.6918
MRT Accuracy	-0.21 (-0.65; 0.23)	0.27 (-0.17; 0.71)	0.16 (-0.28; 0.60)	0.3330	0.2089	0.4503	0.48 (-0.15; 1.11)	0.37 (-0.25; 0.99)	0.11 (-0.51; 0.73)	0.1250	0.2286	0.7073	0.2684
DSST Accuracy	0.22 (-0.06; 0.50)	-0.04 (-0.33; 0.26)	0.24 (-0.02; 0.51)	0.1230	0.8034	0.0732	-0.25 (-0.69; 0.18)	0.02 (-0.35; 0.40)	-0.28 (-0.68; 0.13)	0.2416	0.8957	0.1712	0.3494
BART Risk Taking	0.23 (-0.31; 0.78)	0.31 (-0.28; 0.90)	0.19 (-0.40; 0.78)	0.3815	0.2837	0.4985	0.07 (-0.73; 0.88)	-0.04 (-0.85; 0.77)	0.12 (-0.77; 1.00)	0.8473	0.9175	0.7880	0.9621
PVT Accuracy	0.09 (-0.34; 0.51)	-0.17 (-0.58; 0.25)	0.06 (-0.35; 0.48)	0.6749	0.4072	0.7561	-0.25 (-0.85; 0.35)	-0.02 (-0.62; 0.57)	-0.23 (-0.81; 0.35)	0.3864	0.9355	0.4190	0.6178
Speed	-0.06 (-0.21; 0.09)	-0.13 (-0.28; 0.02)	-0.18 (-0.33; -0.03)	0.4239	0.0786	0.0197	-0.07 (-0.29; 0.14)	-0.12 (-0.34; 0.09)	0.05 (-0.16; 0.26)	0.4772	0.2416	0.6344	0.4909
Accuracy	-0.03 (-0.20; 0.13)	0.08 (-0.09; 0.25)	0.07 (-0.10; 0.24)	0.6667	0.3160	0.3995	0.12 (-0.12; 0.36)	0.10 (-0.13; 0.34)	0.01 (-0.23; 0.26)	0.3139	0.3693	0.9056	0.5315
Efficiency	-0.05 (-0.16; 0.06)	-0.03 (-0.14; 0.08)	-0.05 (-0.16; 0.06)	0.3319	0.6291	0.3356	0.03 (-0.13; 0.18)	0.00 (-0.15; 0.15)	0.03 (-0.13; 0.18)	0.7301	0.9899	0.7355	0.9240
Sleep Duration [h]	-0.07 (-0.41; 0.27)	-0.15 (-0.49; 0.19)	-0.57 (-0.91; -0.24)	0.6769	0.3869	0.0009**	0.03 (-0.50; 0.57)	-0.47 (-1.00; 0.05)	0.51 (-0.02; 1.03)	0.8950	0.0748	0.0583	0.1026
Poor Sleep Quality	0.0 (-1.1; 1.1)	0.2 (-1.0; 1.3)	-1.0 (-2.1; 0.1)	1.0000	0.7744	0.0863	0.2 (-2.0; 2.4)	-0.7 (-2.9; 1.4)	1.0 (-1.3; 3.2)	0.8352	0.4784	0.3907	0.6532
Low Workload	-1.2 (-2.2; -0.2)	-1.7 (-2.7; -0.7)	-0.8 (-1.8; 0.3)	0.0194*	0.0010**	0.1457	-0.7 (-2.4; 0.9)	0.2 (-1.4; 1.8)	-0.9 (-2.5; 0.6)	0.3501	0.8097	0.2223	0.4311
Sleepy	1.7 (0.7; 2.6)	1.3 (0.3; 2.2)	-0.3 (-1.3; 0.7)	0.0009**	0.0123*	0.5571	-0.4 (-2.2; 1.4)	-1.8 (-3.6; -0.1)	1.4 (-0.3; 3.2)	0.6298	0.0421	0.1015	0.0964
Unhappy	0.1 (-0.8; 1.1)	1.0 (0.1; 1.9)	0.5 (-0.4; 1.5)	0.7949	0.0383	0.2605	0.7 (-1.0; 2.3)	0.4 (-1.3; 2.1)	0.3 (-1.4; 1.9)	0.4156	0.6292	0.7344	0.7091
Healthy	-3.4 (-4.5; -2.3)	-1.9 (-3.0; -0.8)	-2.0 (-3.1; -0.8)	<.0001****	0.0009**	0.0007**	2.1 (-0.2; 4.4)	1.0 (-1.4; 3.4)	1.0 (-1.3; 3.4)	0.0761	0.3771	0.3700	0.1985
Physically Exhausted	2.8 (1.8; 3.9)	1.7 (0.7; 2.8)	1.5 (0.4; 2.5)	<.0001****	0.0016**	0.0070*	-1.0 (-3.3; 1.2)	-1.1 (-3.3; 1.2)	0.0 (-2.1; 2.1)	0.3382	0.3220	0.9638	0.5374
Mentally Fatigued	1.4 (0.4; 2.4)	1.9 (0.9; 2.9)	0.5 (-0.5; 1.5)	0.0072*	0.0003**	0.3259	0.4 (-1.9; 2.7)	-0.8 (-3.1; 1.5)	1.2 (-1.1; 3.5)	0.7226	0.4688	0.2839	0.5414
Stressed	2.3 (1.2; 3.3)	2.7 (1.6; 3.7)	0.8 (-0.2; 1.9)	<.0001***	<.0001****	0.1140	0.1 (-2.2; 2.4)	-0.5 (-3.0; 2.0)	0.6 (-1.9; 3.1)	0.9280	0.6694	0.6063	0.8619
Fresh	-1.5 (-2.5; -0.5)	-1.8 (-2.8; -0.7)	-0.6 (-1.6; 0.4)	0.0040*	0.0008**	0.2599	-0.1 (-1.6; 1.4)	0.8 (-0.7; 2.3)	-0.9 (-2.4; 0.6)	0.8750	0.2595	0.2064	0.3787
Not Depressed	-0.7 (-1.6; 0.3)	-1.9 (-2.8; -0.9)	-1.5 (-2.4; -0.6)	0.1594	0.0001***	0.0017**	-0.9 (-2.7; 0.9)	-0.8 (-2.6; 1.0)	-0.1 (-1.9; 1.7)	0.3023	0.3777	0.8745	0.5325
Not Bored	-0.3 (-1.2; 0.5)	-0.5 (-1.4; 0.3)	-0.3 (-1.2; 0.5)	0.4519	0.2219	0.4519	-0.1 (-1.3; 1.2)	-0.1 (-1.4; 1.2)	0.0 (-1.2; 1.3)	0.9094	0.8622	0.9506	0.9841
Lonely	0.3 (-0.6; 1.3)	1.3 (0.3; 2.3)	1.7 (0.7; 2.6)	0.5029	0.0098*	0.0009**	0.7 (-1.3; 2.7)	1.4 (-0.6; 3.4)	-0.7 (-2.6; 1.3)	0.4597	0.1577	0.4835	0.3584
Not Monotonous	-1.3 (-2.3; -0.3)	-0.5 (-1.4; 0.5)	-0.4 (-1.4; 0.6)	0.0106*	0.3619	0.4072	0.8 (-0.6; 2.2)	0.9 (-0.6; 2.3)	-0.1 (-1.5; 1.3)	0.2550	0.2180	0.9302	0.3867

All models were adjusted for sex and age. Models with cognitive outcomes were additionally adjusted for baseline performance. Estimates for cognitive tests reflect z-scores. As z-transformation was based on baseline performance, an estimate of 0 (zero) reflects baseline performance. Estimates for self-report data reflect points on an 11-point scale (variables are listed by anchors for high values). As sleep and subjective outcomes were not z-transformed, estimates for these variables were not adjusted for baseline values but are based on a direct contrast between the head-down tilt and baseline period instead. Est.: Estimate; CI: Confidence Interval; CTRL: Control; cAG: continuous Artificial Gravity; iAG: intermittent Artificial Gravity; *adjusted p<0.05; **adjusted p<0.01; ***adjusted p<0.001; ****adjusted p<0.0001; MP: Motor Praxis; VOLT: Visual Object Learning Test; F2B: Fractal 2-Back; AM: Abstract Matching; LOT: Line Orientation Test; ERT: Emotion Recognition Test; MRT: Matrix Reasoning Test; DSST: Digit Symbol Substitution Test; BART: Balloon Analog Risk Test; PVT: Psychomotor Vigilance Test

Table S4: Mixed effect model results for time in head-down tilt bed rest analyses

	Pooled		Control		Continuous AG		Intermittent AG		DiHDT*Intervention
Variable	Change per DiHDT	p-value	Change per DiHDT	p-value	Change per DiHDT	p-value	Change per DiHDT	p-value	p-value
MP Speed	0.002 (0.002)	0.1853	0.005 (0.002)	0.0061	0.001 (0.002)	0.5782	0.000 (0.004)	0.9276	0.4307
VOLT Speed	-0.003 (0.002)	0.2041	-0.001 (0.003)	0.8527	-0.001 (0.004)	0.7575	-0.006 (0.004)	0.1569	0.4993
F2B Speed	0.004 (0.003)	0.1365	0.011 (0.006)	0.1072	0.001 (0.004)	0.7558	0.000 (0.003)	0.9737	0.1847
AM Speed	0.000 (0.003)	0.8968	0.001 (0.006)	0.8933	0.003 (0.004)	0.4947	-0.005 (0.003)	0.0917	0.4573
LOT Speed	0.000 (0.003)	0.9470	0.003 (0.005)	0.6025	0.004 (0.003)	0.2132	-0.007 (0.006)	0.2730	0.2217
ERT Speed	-0.009 (0.002)	0.0001**	-0.007 (0.003)	0.0138	-0.007 (0.003)	0.0205	-0.014 (0.005)	0.0185	0.2580
MRT Speed	-0.003 (0.003)	0.2756	-0.007 (0.003)	0.0135	0.004 (0.006)	0.5882	-0.005 (0.004)	0.2171	0.2234
DSST Speed	0.003 (0.002)	0.0710	0.002 (0.001)	0.1238	0.004 (0.002)	0.0788	0.004 (0.005)	0.4457	0.8726
BART Speed	0.001 (0.003)	0.6513	0.005 (0.003)	0.1013	0.003 (0.006)	0.6536	-0.004 (0.003)	0.2420	0.2958
PVT Speed	0.004 (0.003)	0.1162	0.007 (0.005)	0.2035	0.006 (0.003)	0.0459	-0.001 (0.006)	0.9192	0.4382
MP Accuracy	-0.006 (0.003)	0.0331	-0.004 (0.005)	0.4174	-0.004 (0.006)	0.5650	-0.011 (0.005)	0.0477	0.5379
VOLT Accuracy	0.007 (0.003)	0.0318	0.008 (0.007)	0.2272	0.006 (0.006)	0.3033	0.007 (0.005)	0.2197	0.9584
F2B Accuracy	-0.004 (0.003)	0.1744	-0.002 (0.004)	0.6382	-0.001 (0.007)	0.8722	-0.009 (0.005)	0.0718	0.4908
AM Accuracy	0.001 (0.002)	0.5794	0.000 (0.005)	0.9825	-0.002 (0.003)	0.5665	0.006 (0.005)	0.2567	0.4108
LOT Accuracy	0.001 (0.003)	0.7726	0.007 (0.006)	0.3025	-0.004 (0.004)	0.2915	0.001 (0.006)	0.9334	0.3329
ERT Accuracy	0.001 (0.003)	0.7430	-0.001 (0.006)	0.8041	-0.002 (0.005)	0.7302	0.007 (0.007)	0.3536	0.5131
MRT Accuracy	0.001 (0.004)	0.7373	0.007 (0.005)	0.1743	0.003 (0.008)	0.7396	-0.006 (0.006)	0.3370	0.3527
DSST Accuracy	0.002 (0.003)	0.5319	0.000 (0.004)	0.9791	0.006 (0.006)	0.3721	-0.001 (0.004)	0.8944	0.6093
BART Risk Taking	0.002 (0.003)	0.4592	-0.001 (0.006)	0.9089	0.003 (0.004)	0.3632	0.005 (0.007)	0.5433	0.7911
PVT Accuracy	0.004 (0.005)	0.4553	0.013 (0.012)	0.2882	0.004 (0.004)	0.2798	-0.005 (0.011)	0.6465	0.3994
Speed	0.000 (0.001)	0.9538	0.002 (0.001)	0.1188	0.002 (0.002)	0.3315	-0.004 (0.002)	0.0482	0.0227
Accuracy	0.001 (0.001)	0.5039	0.003 (0.002)	0.2075	0.001 (0.002)	0.5963	-0.001 (0.003)	0.6554	0.3719
Efficiency	0.000 (0.001)	0.6661	0.002 (0.001)	0.1017	0.001 (0.001)	0.1385	-0.003 (0.002)	0.2110	0.0488
Sleep Duration [h]	0.001 (0.002)	0.6458	0.002 (0.007)	0.7911	0.003 (0.003)	0.3077	-0.002 (0.003)	0.5654	0.6995
Poor Sleep Quality	0.011 (0.008)	0.1819	0.022 (0.015)	0.1812	-0.003 (0.012)	0.7790	0.014 (0.014)	0.3608	0.4159
Low Workload	0.01 (0.007)	0.1546	-0.001 (0.012)	0.9458	0.011 (0.015)	0.4897	0.021 (0.012)	0.0892	0.4656
Sleepy	-0.001 (0.007)	0.8429	0.002 (0.014)	0.8732	-0.007 (0.011)	0.5712	0.000 (0.012)	0.9718	0.8598
Unhappy	0.009 (0.008)	0.2635	0.003 (0.018)	0.8659	0.004 (0.01)	0.6780	0.019 (0.013)	0.2026	0.6528
Healthy	-0.005 (0.007)	0.4379	-0.015 (0.014)	0.3028	-0.003 (0.012)	0.8022	0.001 (0.01)	0.8872	0.6186
Physically Exhausted	0.005 (0.007)	0.4556	0.007 (0.015)	0.6302	-0.002 (0.014)	0.8685	0.011 (0.01)	0.2735	0.7437
Mentally Fatigued	0.004 (0.007)	0.5461	-0.007 (0.01)	0.4912	0.002 (0.015)	0.8879	0.018 (0.009)	0.1000	0.3480
Stressed	0.004 (0.006)	0.4758	0.012 (0.012)	0.3203	-0.006 (0.009)	0.5255	0.006 (0.008)	0.4567	0.4329
Fresh	0.000 (0.007)	0.9553	0.004 (0.016)	0.7893	0.001 (0.014)	0.9681	-0.006 (0.01)	0.5258	0.8526
Not Depressed	-0.012 (0.008)	0.1346	0.006 (0.011)	0.5718	-0.016 (0.012)	0.2320	-0.026 (0.016)	0.1417	0.2233
Not Bored	-0.008 (0.007)	0.2667	-0.011 (0.01)	0.3083	-0.006 (0.019)	0.7463	-0.007 (0.015)	0.6274	0.9726
Lonely	0.006 (0.008)	0.4242	0.007 (0.016)	0.6490	-0.004 (0.009)	0.6983	0.016 (0.016)	0.3583	0.6235
Not Monotonous	-0.022 (0.009)	0.0256	-0.024 (0.017)	0.1931	-0.009 (0.011)	0.4484	-0.033 (0.02)	0.1447	0.5818
ERT Happy	-0.01 (0.004)	0.0163*	-0.015 (0.007)	0.0812	-0.013 (0.007)	0.1687	-0.003 (0.007)	0.7111	0.4259
ERT Sad	0.004 (0.003)	0.0915	0.011 (0.005)	0.0830	0.002 (0.003)	0.6280	0.001 (0.006)	0.8910	0.2516
ERT Angry	0.012 (0.003)	0.0006**	0.01 (0.005)	0.0363	0.004 (0.004)	0.3927	0.022 (0.006)	0.0010*	0.0306
ERT Fear	0.002 (0.004)	0.5808	-0.005 (0.006)	0.3971	0.003 (0.005)	0.6309	0.012 (0.008)	0.1715	0.1940
ERT Neutral	-0.008 (0.003)	0.0074*	-0.006 (0.003)	0.0999	0.000 (0.004)	0.9356	-0.016 (0.005)	0.0010*	0.0243

All models were adjusted for sex, age and baseline values. Estimates for cognitive tests reflect z-scores. Estimates for self-report data reflect points on an 11-point scale (variables are listed by anchors for high values). DiHDT: Day in head-down tilt; Change per DiHDT reflects estimate (standard error). DiHDT*Intervention reflects a test for the interaction between DiHDT (continuous) and the three intervention groups (Control, cAG, iAG). "Pooled" reflects an analysis with data pooled across the three experimental groups. ERT Happy/Sad/Angry/Fear/Neutral expresses the tendency to rate an item in the respective category based on comparisons to responses of a normative group of subjects. Adjustments for ERT tendency p-values were based on N=5 comparisons. *adjusted $p<0.05$; **adjusted $p<0.01$; AG: Artificial Gravity; MP: Motor Praxis; VOLT: Visual Object Learning Test; F2B: Fractal 2-Back; AM: Abstract Matching; LOT: Line Orientation Test; ERT: Emotion Recognition Test; MRT: Matrix Reasoning Test; DSST: Digit Symbol Substitution Test; BART: Balloon Analog Risk Test; PVT: Psychomotor Vigilance Test

2.2 Resting State Brain Activity

To better understand the neural bases of the cognitive impairments observed in the previous study, a series of neuroimaging experiments were performed to assess changes in resting state brain activity, emotion processing, selective attention, and episodic memory formation. The first study used resting state electrocortical activity recordings before, during, and after 60 days of HDBR. Data were combined from two HDBR experiments (*RSL* and *Cocktail*), which allowed identifying the time course of brain activity in response to HDBR and assessing whether a structured exercise program or an antioxidant supplementation affected resting state brain activity during HDBR.

The following abstract is from the original research article:

Brauns K, Friedl-Werner A, Maggioni, MA, Gunga HC, Stahn, AC. Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity. *Frontiers in Physiology* 12: 638669 (2021). doi: https://doi.org/10.3389/fphys.2021.638669

> *"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."

frontiers in Physiology

ORIGINAL RESEARCH
published: 24 February 2021
doi: 10.3389/fphys.2021.638669

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

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OPEN ACCESS

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Specialty section:
This article was submitted to Environmental, Aviation and Space Physiology, a section of the journal Frontiers in Physiology

Received: 07 December 2020
Accepted: 28 January 2021
Published: 24 February 2021

Citation:
Brauns K, Friedl-Werner A, Maggioni MA, Gunga H-C and Stahn AC (2021) Head-Down Tilt Position, but Not the Duration of Bed Rest Affects Resting State Electrocortical Activity. Front. Physiol. 12:638669. doi: 10.3389/fphys.2021.638669

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

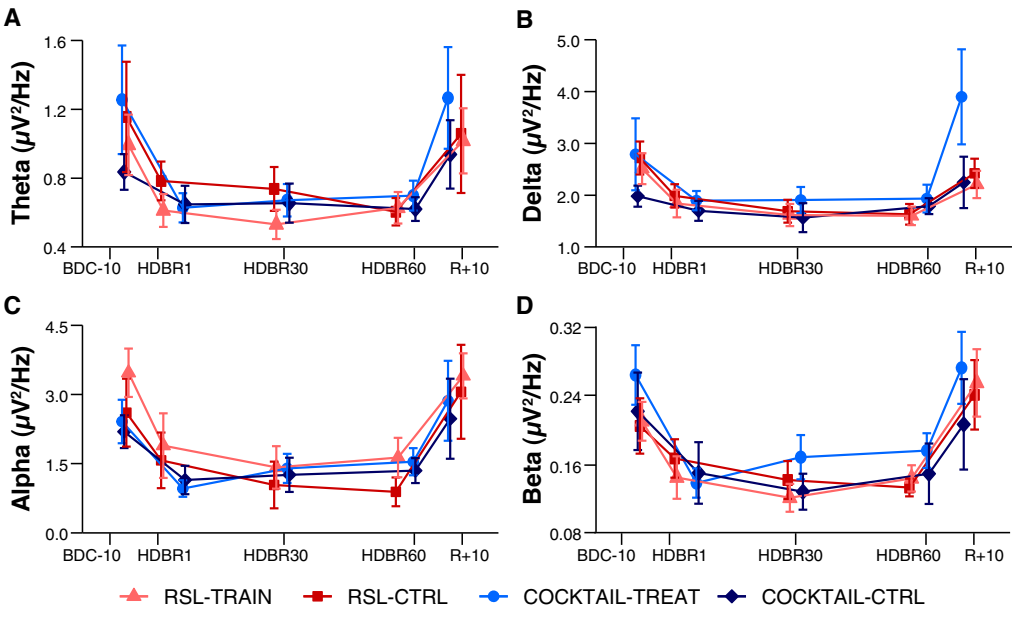

FIGURE 1 | Changes in electrocortical activity during long-duration head-down tilt bed rest of intervention and treatment groups of the RSL and COCKTAIL study. EEG power in **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency bands are averaged over all electrodes ($n = 31$) for RSL-TRAIN ($n = 11$, 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.

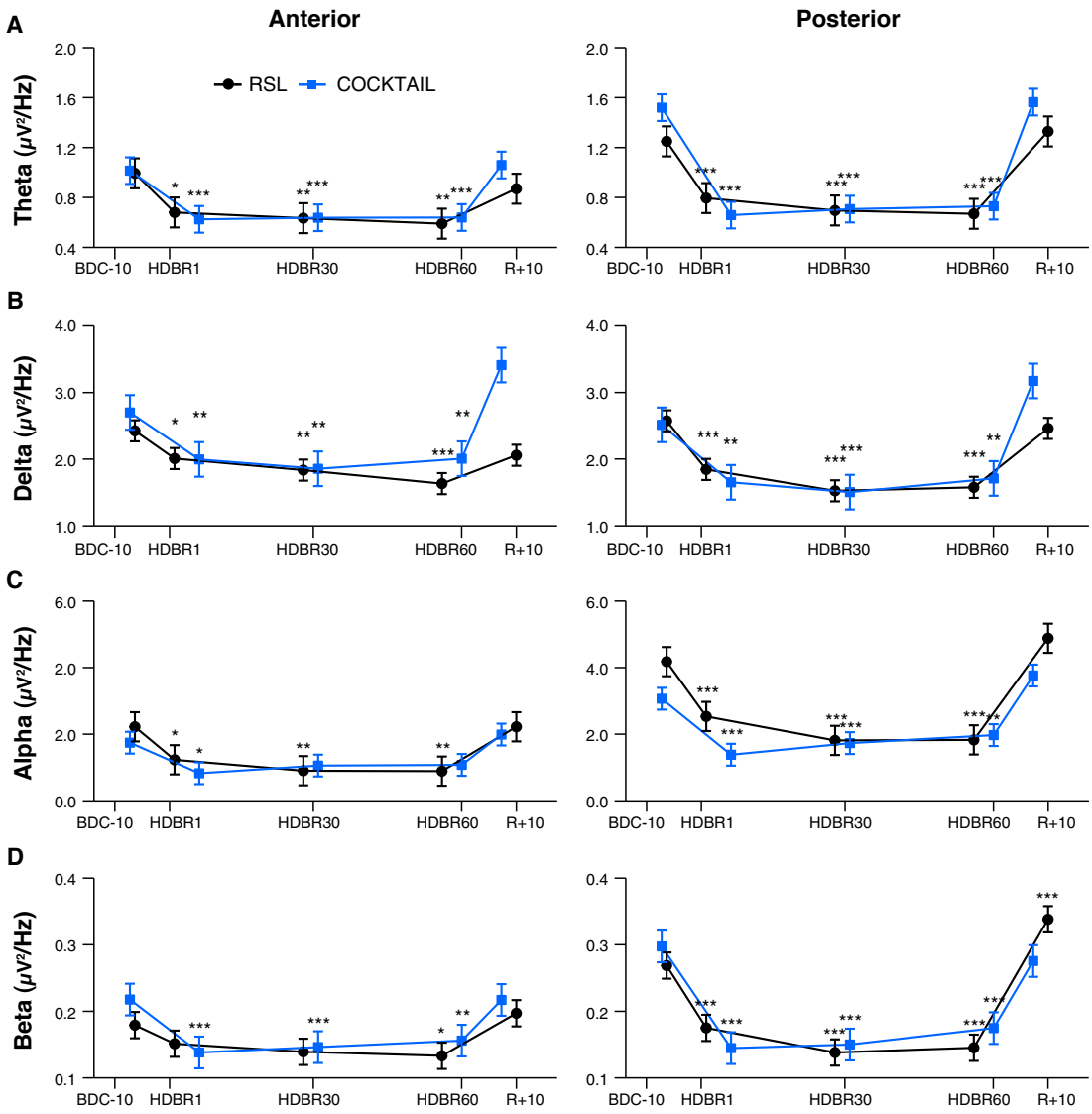

FIGURE 2 | Effect of long-duration head-down tilt bed rest on electrocortical activity. Time courses show changes of EEG spectral power by *Region* (Anterior, Posterior) within the **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency band for RSL (n = 23, blue circle), and COCKTAIL (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.

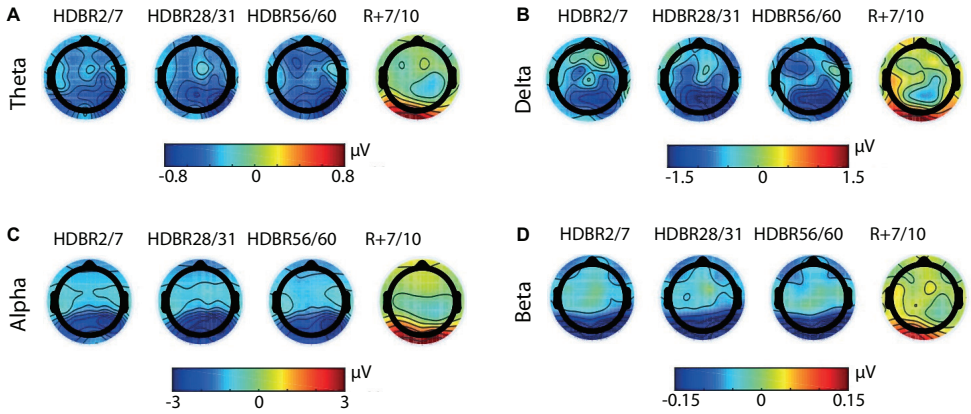

FIGURE 3 | Grand-averaged topographical maps of absolute spectral power within the **(A)** theta, **(B)** delta, **(C)** alpha, and **(D)** beta frequency band. Scalp maps show changes from baseline (BDC-8/-7) to short-term (HDBR2/7), mid-term (HDBR28/31), and long-term (HDBR56/60) head-down tilt bed 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.

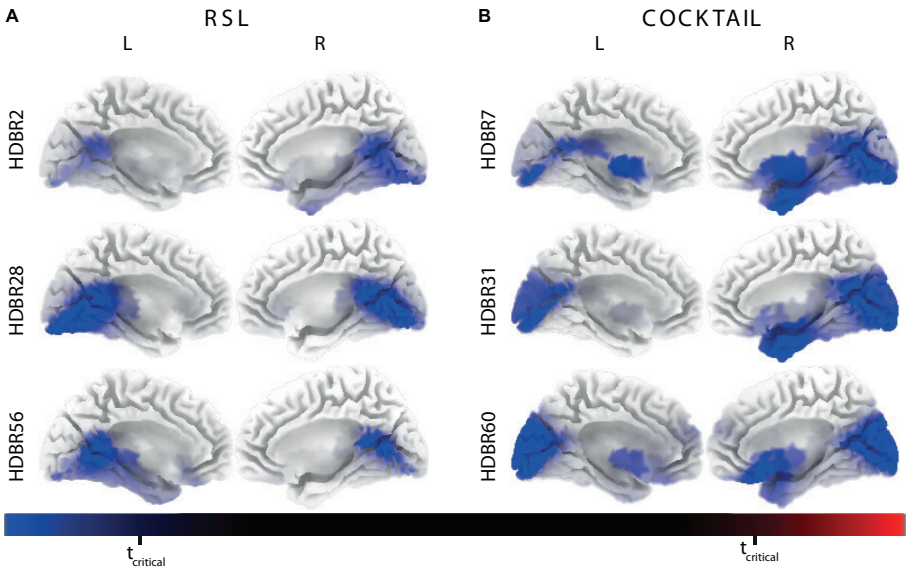

FIGURE 4 | Statistical parametric maps (SPMs) indicating significant effects of *Time* using the baseline (before bed rest) current source density distribution as a reference on brain source localization for **(A)** RSL (n = 23) and **(B)** COCKTAIL (n = 20) within the beta frequency domain. The color 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.

FUNDING

This investigation was supported by the European Space Agency and by the German Aerospace Center (DLR, Deutsches Zentrum für Luft- und Raumfahrt) through grant 50WB1525.

ACKNOWLEDGMENTS

We thank Edwin Mulder, Melanie von der Wiesche, Alexandra Noppe, and Ulrich Limper from DLR, and Marie-Pierre Bareille and Arnaud Beck from MEDES for their medical, technical, and administrative support in implementing the study protocol, and all volunteers whose participation and dedication made this study possible. We also acknowledge the support from the German Research Foundation (DFG) and the Open Access Publication Fund of Charité – Universitätsmedizin Berlin.

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

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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 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.*

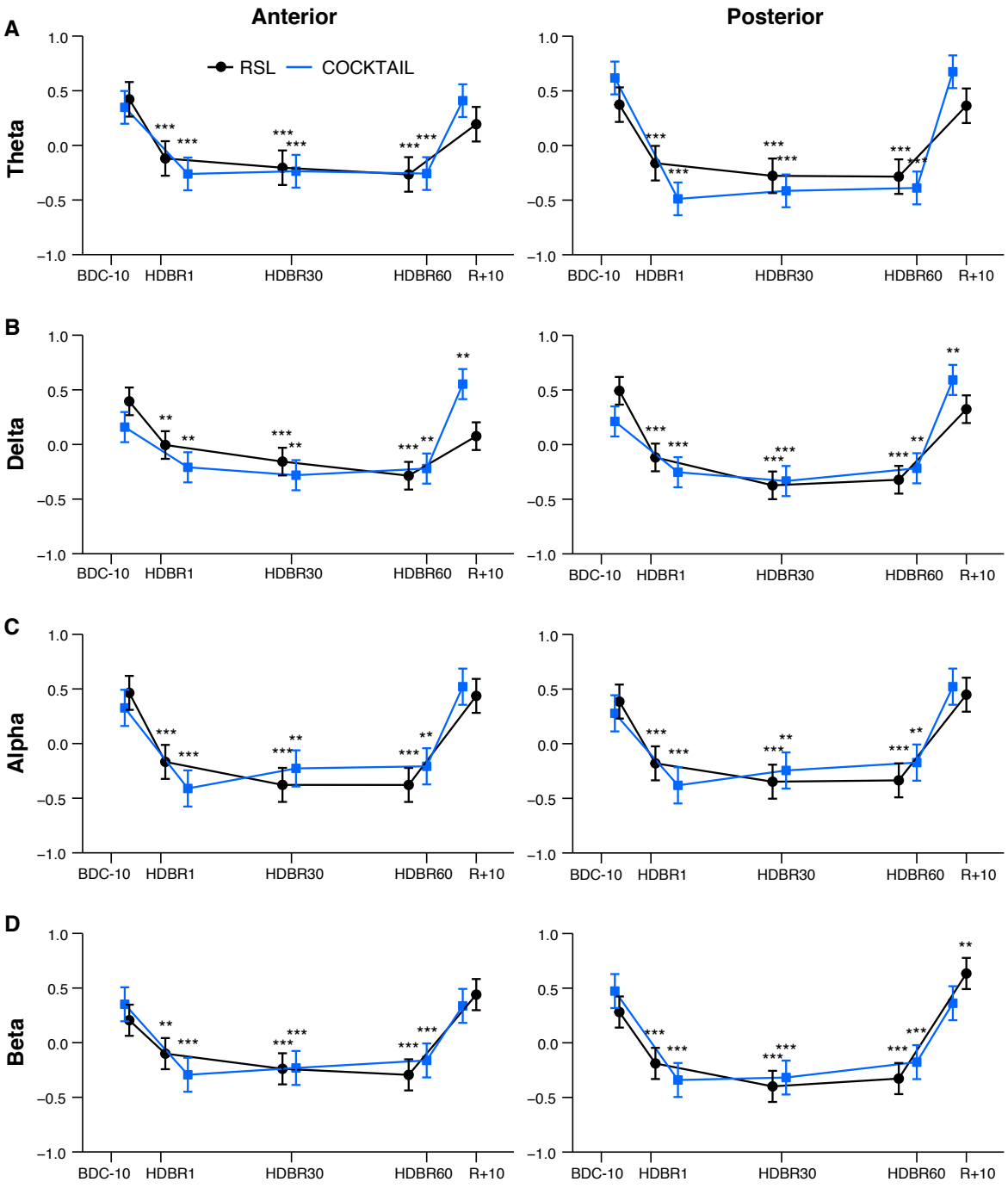
	Brain Region	Short-term				Mid-term				Long-term			
		Alpha		Beta		Alpha		Beta		Alpha		Beta	
		L	R	L	R	L	R	L	R	L	R	L	R
RSL	Frontal lobe				4	1	1					7	
	Limbic Lobe	10	20	32	74	74	61	109	59	3	2	110	36
	Occipital Lobe	10	100	22	207	217	163	268	236	1	9	78	49
	Parietal lobe	39	53	7	11	117	123	20	23	12	26	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.

2.3 Emotion Processing

Following up on the previous finding that functional brain connectivity did not reveal any characteristic responses with increasing duration of HDBR, it was hypothesized that task based neural indices could be more sensitive to detect changes in brain function. Based on the first study, it was expected that event-related potentials associated with emotional processing could be particularly vulnerable to HDBR. The International Affective Picture System (IAPS) was used to elicit neural responses to negative, positive, and neutral emotions. To avoid sensitization or habituation with repeated exposures of the type of visual stimuli (Larson et al., 2000), data were collected once after 30 days of HDBR and compared to a control group that also comprised bed rest subjects but was tested before the start of the bed rest intervention.

The following abstract is from the original research article:

Brauns K, Werner A, Gunga HC, Maggioni MA, Dinges DF, Stahn, AC. Electrocortical Evidence for Impaired Affective Picture Processing after Long-term Immobilization. *Scientific Reports* (2019) 9:16610. doi: https://doi.org/10.1038/s41598-019-52555-1

"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."

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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

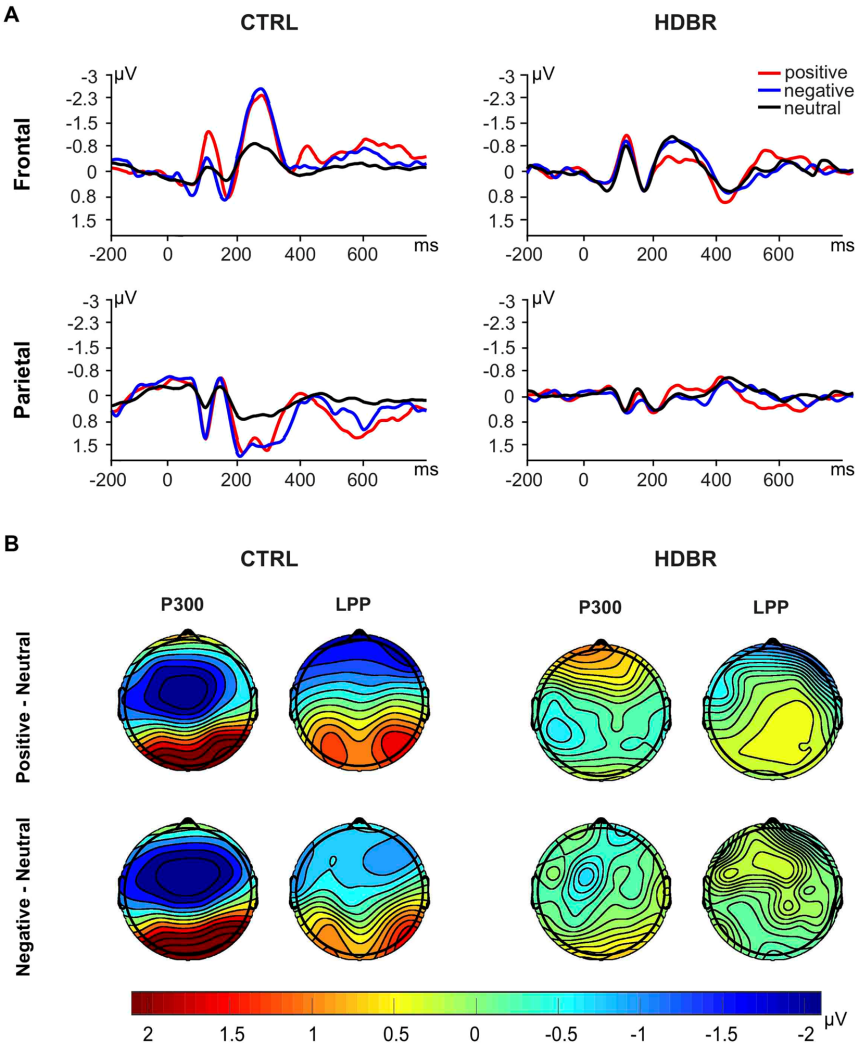

Figure 1. Event-related potential (ERP) results. (**A**) Grand average ERP waveforms at selected electrode clusters (frontal: F3, F4; parietal: P3, P4, Pz) for positive. (n = 25), negative (n = 25) and neutral (n = 25) stimuli in a control group (CTRL, n = 10) and a bed rest group (HDBR, n = 10). (**B**) 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. *$p < 0.05$. **$p < 0.01$. ***$p < 0.0001$.

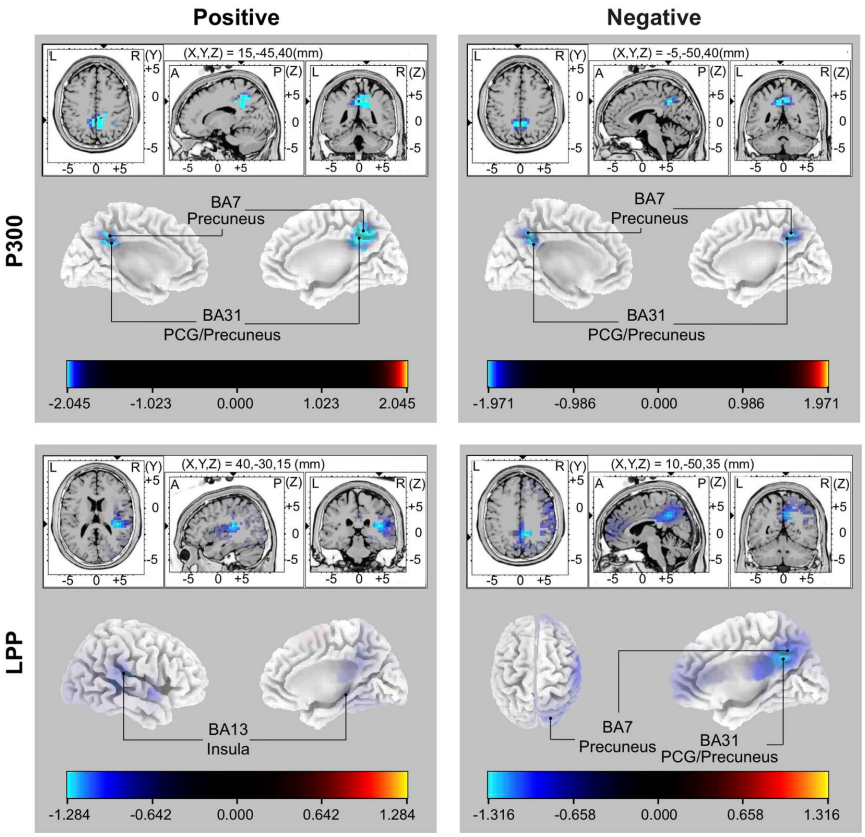

Figure 2. Statistical parametric maps (SPMs) indicating the differences in brain source localization between control (CTRL, n = 10) and head-down-tiltbed rest group (HDBR, n = 10). Data for positive and negative stimuli are shown on the left and right panels, respectively. Results for the P300 and LPP 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.

Received: 15 September 2019; Accepted: 18 October 2019;
Published online: 12 November 2019

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Acknowledgements
This investigation was supported by the ESA (European Space Agency) and by the German Aerospace Center (DLR, Deutsches Zentrum für Luft- und Raumfahrt) through grant 50WB1525. We thank the team of MEDES for their technical and logistical support, and all volunteers whose participation and dedication made this study possible.

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
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-019-52555-1.

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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.

2.4 Selective Attention

The second study assessing neural indices of electrocortical activity examined whether neural indices elicited by an Oddball paradigm are reduced after long-duration bed rest and whether these changes are affected by a daily nutritional supplement consisting of polyphenols, omega-3 fatty acids, vitamin E, and selenium.

The following abstract is from the original research article:

Brauns K, Friedl-Werner A, Gunga HC, Stahn, AC. Electrocortical Effects of two months of bed rest and antioxidant supplementation on attentional processing. *Cortex* (2021), 141, 81-93. doi: https://doi.org/10.1016/j.cortex.2021.03.026

"Physical inactivity across the lifespan is a growing public health concern affecting the cardiovascular, musculoskeletal, and central nervous system. Data on the effects of dietary antioxidants as neuroprotective treatments when physical activity levels are impaired are lacking. In this randomized controlled study twenty young healthy men underwent 60 days of bed rest. Participants were randomly assigned to a treatment group (N=10) receiving a daily antioxidant supplement comprising polyphenols, omega-3 fatty acids, vitamin E, and selenium or a control group (N=10). Event-related potentials (ERPs) and behavioral data from a three-stimulus oddball paradigm were collected eight days before bed rest, after 60 days of immobilization, and after eight days of recovery. After two months of bed rest, we found a significant decrease in task efficiency irrespective of the treatment that was corroborated by lower ERPs in fronto-central and parietal brain regions. Neither behavioral nor electrocortical data returned to baseline values after eight days of recovery. Our results provide support for the adverse and persistent neurobehavioral effects of prolonged bed rest, which could not be mitigated by antioxidant supplementation. These findings raise important implications for situations in which physical activity levels become severely restricted such as medical conditions or sedentary lifestyles."

2.5 Memory Formation and Hippocampal Activation

Given the increasing evidence of physical activity on the temporal lobe and hippocampus (Domingos et al., 2021; Erickson et al., 2014; Firth et al., 2018; Ji et al., 2021), a third study employed task functional magnetic resonance imaging (fMRI) using a paradigm that has been shown to reliably activate the hippocampus (Kirwan & Stark, 2007). The study examined the effects of 60 days of HDBR on pattern separation and completion and their neural bases, and whether any changes were affected by a structured exercise program.

The following abstract is from the original research article:

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

> *"Episodic memory depends decisively on the hippocampus and the parahippocampal gyrus, brain structures that are also prone to exercise-induced neuroplasticity and cognitive improvement. We conducted a randomized controlled trial to investigate the effects of a high-intensity exercise program in twenty-two men resting in bed for 60 days on episodic memory and its neuronal basis. All participants were exposed to 60 days of uninterrupted bed rest. Eleven participants were additionally assigned to a high-intensity interval training that was performed five to six times weekly for 60 days. Episodic memory and its neural basis were determined four days prior to and on the 58th day of bed rest using functional magnetic resonance imaging (fMRI). We found increased BOLD signal in the left hippocampus and parahippocampal gyrus in the non-exercising group compared to the exercising bed rest group whereas the mnemonic performance did not differ significantly. These findings indicate a higher neuronal efficiency in the training group during memory encoding and retrieval and may suggest a dysfunctional mechanism in the non-exercising bed rest group induced by two months of physical inactivity. Our results provide further support for the modulating effects of physical exercise and adverse implications of a sedentary lifestyle and bedridden patients."*

NeuroImage 223 (2020) 117359

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Exercise-induced changes in brain activity during memory encoding and retrieval after long-term bed rest

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ARTICLE INFO

Keywords:
Exercise
Bed rest
Pattern separation
Episodic memory

ABSTRACT

Episodic memory depends decisively on the hippocampus and the parahippocampal gyrus, brain structures that are also prone to exercise-induced neuroplasticity and cognitive improvement. We conducted a randomized controlled trial to investigate the effects of a high-intensity exercise program in twenty-two men resting in bed for 60 days on episodic memory and its neuronal basis. All participants were exposed to 60 days of uninterrupted bed rest. Eleven participants were additionally assigned to a high-intensity interval training that was performed five to six times weekly for 60 days. Episodic memory and its neural basis were determined four days prior to and on the 58th day of bed rest using functional magnetic resonance imaging (fMRI). We found increased BOLD signal in the left hippocampus and parahippocampal gyrus in the non-exercising group compared to the exercising bed rest group whereas the mnemonic performance did not differ significantly. These findings indicate a higher neuronal efficiency in the training group during memory encoding and retrieval and may suggest a dysfunctional mechanism in the non-exercising bed rest group induced by two months of physical inactivity. Our results provide further support for the modulating effects of physical exercise and adverse implications of a sedentary lifestyle and bedridden patients.

1. Introduction

Physical exercise is widely suggested as an effective, low-cost, non-pharmacological strategy for maintaining and improving physical and psychological health and well-being. There is increasing evidence that regular physical activity has also considerable neurobehavioral benefits across the lifespan. Recently, particular attention has been paid to the prefrontal cortex and the hippocampal formation and their associated cognitive functions such as executive control and declarative memory (Erickson, Leckie, & Weinstein, 2014; Firth et al. 2018). For instance, cross-sectional studies in preadolescents revealed that more physically fit children showed greater hippocampal volume and greater volume was accompanied with better performance in relational memory tasks (Chaddock et al., 2010; Chaddock, Hillman, Buck, & Cohen, 2011). Likewise, there is cross-sectional evidence suggesting that elderly people engaged in habitual exercise show less degenerative symptoms and perform better in spatial memory tasks than persons of the same age not engaged in exercise (Erickson et al., 2009; Erickson et al., 2010; Bugg & Head, 2011; Szabo et al., 2011). Longitudinal training studies in rodents (van Praag, Shubert, Zhao, & Gage, 2005; Lafenetre, 2010; (Wrann et al., 2013) and humans (Erickson et al., 2011) showed that regular physical activity can increase the concentrations of (neurotrophic) growth hormones, which are critical for hippocampal plasticity, learning, and memory formation. Changes in hippocampal volume were associated with changes in brain-derived neurotrophic factor (BDNF) (Erickson et al., 2011) as well as with changes in spatial (Erickson et al., 2011) and episodic memory (Hötting et al., 2012). These data highlight the positive neurobehavioral effects associated with regular physical activity.

Likewise, it can be speculated that a sedentary life-style may have adverse effects on hippocampal plasticity, learning, and memory formation. However, previous work investigating the effects of physical inactivity or immobilization has mainly been limited to structural brain changes in patients and the elderly (Liepert, Tegenthoff, & Malin, 1995; Lissek et al., 2009; Langer, Hänggi, Müller, Simmen, & Jäncke, 2012) or focused on functional changes in other brain regions such as the frontal and parietal lobes (Yuan et al., 2016) and on the (somatosensory) motor cortex (Cassady et al., 2016; Koppelmans et al., 2018). The majority of these studies have been cross-sectional in nature, questioning the causal relationship between physical inactivity and brain plasticity. For instance, it is well-known from animal studies that

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https://doi.org/10.1016/j.neuroimage.2020.117359
Received 7 May 2020; Received in revised form 17 July 2020; Accepted 3 September 2020
Available online 10 September 2020

environmental enrichment (Fabel et al., 2009) and social interaction (Djordjevic, Adzic, Djordjevic, & Radojcic, 2009; Murínová, Hlaváčová, Chmelová, & Riečanský, 2017) can also reinforce brain plasticity and memory function. These and other potential confounding factors have not been controlled for in human studies investigating the effects of physical (in)activity on the hippocampus and episodic memory formation.

To address this gap, we investigated the effects of an exercise program during two months of strict bed rest on memory-specific neuronal activity in humans using a randomized controlled trial study that standardized food, wake/sleep cycles, social interaction, and environmental enrichment. Healthy men undergoing two months of bed rest were randomly allocated to a control group and an exercise group that performed a high-intensity interval training. Both groups remained in bed rest throughout the entire study period. Using functional magnetic resonance imaging (fMRI) we determined neuronal activity during an episodic memory task that specifically targets the hippocampus and parahippocampal gyrus before and after two months of bed rest. Prolonged bed rest can be considered as a model to mimic accelerated physiological aging processes (Pavy-Le Traon, Heer, Narici, Rittweger, & Vernikos, 2007). Previous studies have shown that aging and cognitive decline are associated with hyperactive signaling of the hippocampus during episodic memory formation, which has been interpreted as a compensatory response (Hämäläinen et al., 2007; Miller et al., 2008a; Miller et al., 2008b; Nyberg et al. 2019; Yassa et al., 2010; Yassa, Mattfeld, Stark, & Stark, 2011). Consequently, we hypothesized that two months of bed rest would affect behavioral mnemonic performance and its neural basis, and that these changes were counteracted by the structured high-intensity interval training. In line with the above-mentioned studies assessing the effects of aging and cognitive decline, we expected that the maladaptive effects of prolonged physical inactivity would be evident as increases in hippocampal and parahippocampal neuronal signaling during mnemonic processing in the bed rest control group compared to the bed rest group that additionally performed the exercise program.

2. Methods

2.1. Study design

The experiment was performed as part of the European Space Agency (ESA) study “Reactive jumps in a sledge jump system as a countermeasure during long-term bed rest” (RSL). The study was conducted at the:envihab facility of the German Aerospace Center (DLR) in Cologne, Germany, and recorded on the German Clinical Trials Register (DRKS, registration number DRKS00012946, 18th of September 2017). The general study design is described elsewhere (Kramer et al., 2017a). Briefly, 23 young, healthy men underwent 60 days of six-degree head-down tilt bed rest (HDT). The baseline data collection (BDC-15 through BDC- 1) and subsequent recovery period (R+0 through R+14) lasted 15 days. On the first day of bed rest, eleven participants were randomly assigned to a high-intensity interval training (TRAIN). All participants underwent MRI scans four days prior to the bed rest commencing (BDC-4) and after 58 days of head-down tilt bed rest (HDT58). The experiment was approved by the Ethics Committee of the Northern Rhine Medical Association (Ärztekammer Nordrhein) in Düsseldorf, Germany and by the local Ethics Committee of the Charité - Universitätsmedizin Berlin, Germany. The study conformed to all standards of human research set out in the declaration of Helsinki. All participants were informed about the purpose, experimental procedures, and risks before giving their verbal and written informed consent.

2.2. Exercise training

The exercise training was performed in a supine position on a custom-built sledge jump system (Novotec Medical GmbH, Pforzheim, Germany) (Kramer et al., 2017a). All participants were familiarized with the correct jumping technique in nine 30 min sessions during the baseline data collection period. Four different training sessions consisting of varying numbers of countermovement jumps and hops were designed, and applied to TRAIN on 48 out of 60 days during the HDT phase, resulting in five to six training sessions per week (five sessions during the first two weeks of HDT and six sessions per week for the following six weeks of HDT). The force in the training device was increased gradually from 50% to 100% of participants’ body weight. The total training duration of one session did not exceed more than 17 min using an average training load between 80% and 90% of the body weight. Thus, the plyometric jump training can be considered as a short-duration high-intensity training that has been shown to successfully prevent musculoskeletal and cardiovascular deconditioning caused by 60 days of bed rest (Kramer et al., 2017b; Maggioni et al., 2018). All training sessions were scheduled in the afternoon from 2 pm to 6 pm. The timing of exercise sessions was kept constant within subjects. Visual feedback for jump height and peak force were provided via a monitor. Verbal feedback was given to the participants by an exercise physiologist to ensure correct execution during each session. All participants completed all scheduled training sessions. Maximum effort was not achieved in about 6% of all familiarization and training sessions due to headache, indisposition or minor discomfort (Kramer et al., 2017a). Further details about the training protocol and adherence are provided elsewhere (Kramer et al., 2017a).

2.3. Participants and recruitment process

Subject recruitment was supported by announcements in local and nationwide newspapers, internet, radio, poster advertisement, and DLR’s test participant archive. Short information was sent to all interested candidates, followed by a telephone screening. If interested, eligible participants received detailed information by email. Qualifying volunteers were invited to an information session, where the objectives, content, and risks of the experiment were explained in detail. Next, interested participants were medically and psychologically screened to ensure their compliance with all inclusion and exclusion criteria (Kramer et al., 2017a). The medical screening comprised a comprehensive anamnesis and physical examination, including an assessment of resting electrocardiogram (ECG), orthostatic tolerance, thrombosis risk, nicotine and substance abuse, the prevalence of infectious diseases, and cardiopulmonary fitness using graded exercise testing. The psychological screening was performed by a psychologist using questionnaires and a personal interview. Eligible participants underwent a dual energy X-ray absorptiometry (DEXA) scan to evaluate the bone mineral density of the femur and the lumbar vertebra column as a final criterion to be included in the study. Finally, twenty-seven volunteers passed the entire screening process and twenty-four of them were enrolled in the study. Twenty-three participants (29 ± 6 years, height: 181 ± 6 cm, weight: 77 ± 7 kg) completed the study. A detailed CONSORT flow diagram is displayed in Figure 1.

Because of medical reasons two subjects (one from each group) started their recovery on HDT 49 and HDT 50, respectively (instead of HDT60). MRI scanning for these participants was performed on the last day of their bed rest (i.e., HDT48 and HDT49 instead of HDT58). Due to an incomplete log file data set, one subject (TRAIN) had to be excluded from the data analysis. Both groups did not differ with respect to their age, height, weight, and BMI at baseline (two-tailed Student’s t-test: all $Ps > 0.3$). An overview of subjects’ group characteristics is displayed in Table 1.

2.4. MRI Scanning procedure

Magnetic resonance imaging was conducted on a 3 Tesla Siemens Biograph mMR (Siemens Healthcare, Erlangen, Germany), equipped with a 16-channel head coil. Functional images were acquired using a T2-weighted echo planar imaging (EPI) sequence sensitive to blood oxygen

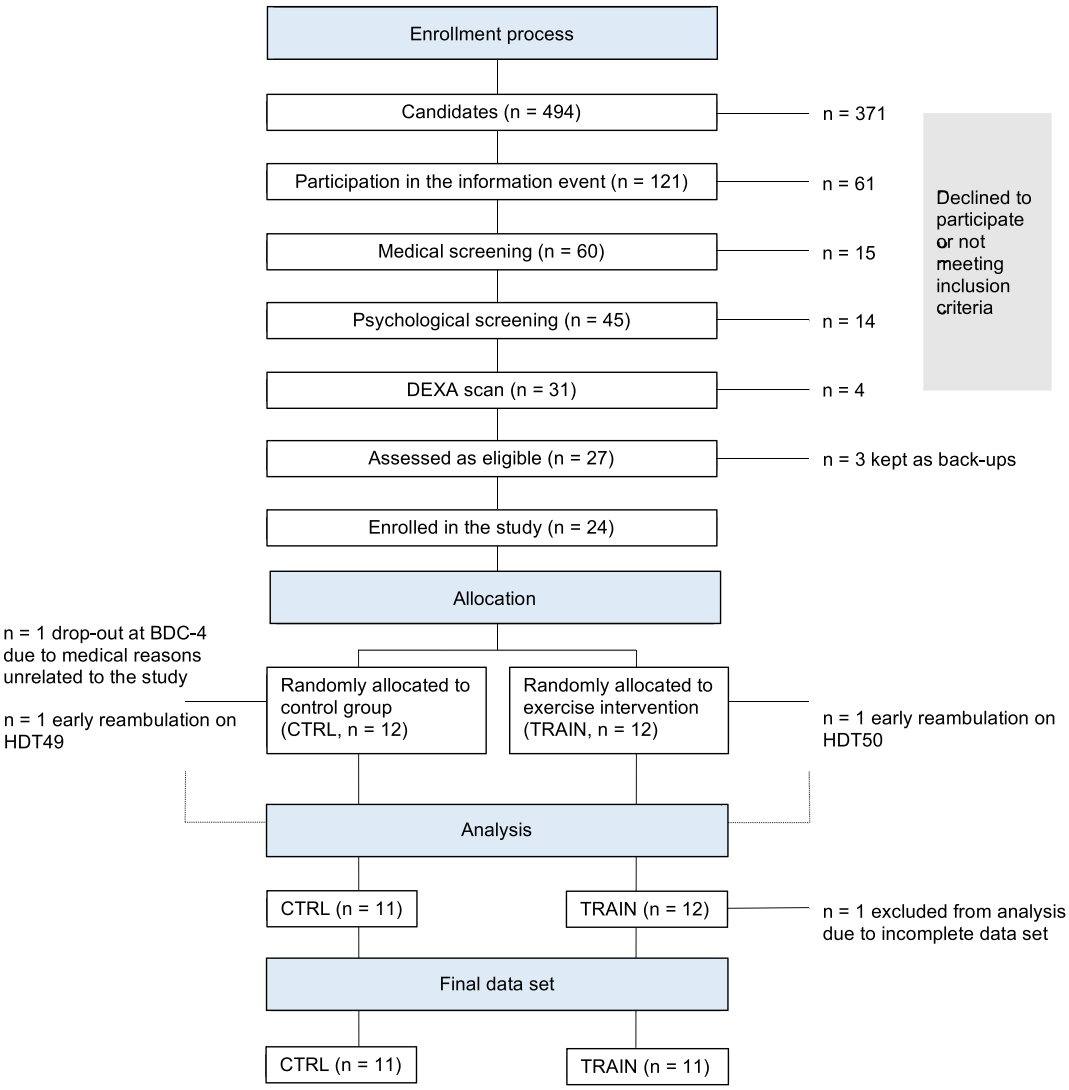

Fig. 1. CONSORT flow diagram. Overview of recruiting, enrollment, and analysis process.

Table 1
Subjects' group characteristics at baseline.*

Characteristic	CTRL (n = 11)	TRAIN (n = 11)	t_{20}	*P*
Age [years]	28.2 (5.7)	30.8 (6.2)	1.02	0.318
Height [cm]	180.6 (5.0)	181.6 (6.8)	0.39	0.699
Body Mass [kg]	76.2 (8.0)	77.9 (6.6)	0.54	0.593
BMI [kg/m^2]	23.4 (2.0)	23.6 (1.9)	0.24	0.812

* Data are means (SD); BMI, Body Mass Index; CTRL, bed rest control group; TRAIN, exercising bed rest group; *t*, t-statistics; *P*, p-value.

level dependent (BOLD) contrast (36 axial slices, interleaved slice order, time to repeat (TR) = 2000 ms, time to echo (TE) = 30 ms, field of view (FoV) read = 216 mm, FoV phase = 100%, flip angle = 80°, slice thickness = 3 mm, distance factor = 20%, voxel size: 3 mm × 3 mm × 3 mm). For anatomical reference, a three-dimensional volumetric T1-weighted Magnetization Prepared Rapid Acquisition of Gradient Echo (MPRAGE) sequence was acquired in a sagittal plane with the following parameters: voxel size: 1 mm × 1 mm × 1 mm; TR = 2500 ms, TE = 4.82 ms, inversion time = 1100 ms, FoV read = 256 mm, FoV phase = 100%, flip angle = 7°, and bandwidth = 140 Hz/Px. Scanning was always performed before noon between 8 am and 12 pm.

2.5. *fMRI paradigm*

Episodic memory was assessed using a continuous memory recognition task that was originally proposed by Kirwan & Stark (2007) and that was shown to reliably target the hippocampus (Bakker, Kirwan, Miller, & Stark, 2008; Kirwan et al., 2012; Ally, Hussey, Ko, & Molitor, 2013). A schematic overview of the fMRI paradigm is displayed in Fig. 2. Images of different objects were presented to the participants via a mirror system mounted on top of the head coil. Each picture was shown for 2000 ms with a randomized intertrial interval (ITI) of 2000 to 4000 ms. For each picture, participants had to indicate via a button press whether

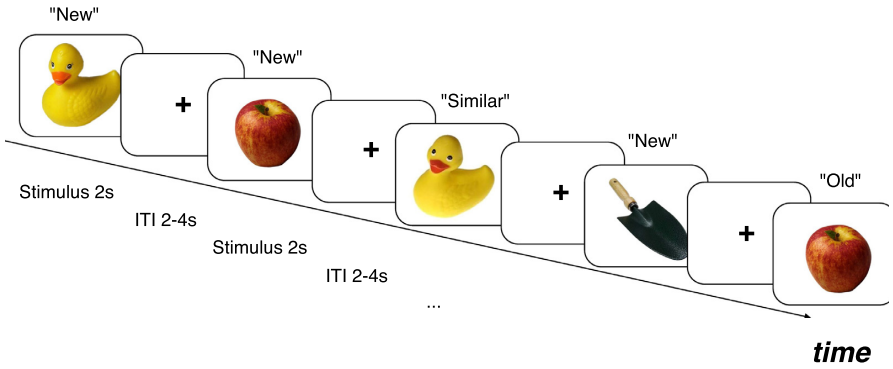

Fig. 2. fMRI paradigm for pattern separation task. A picture was presented for 2 s followed by an intertrial interval (ITI) of 2 to 4 s. Quotation marks above the picture indicate the correct response.

the presented object was "new" (novel condition), "old" (repetitive condition), or "similar", though not identical to an object shown earlier in the run (lure condition). The experiment consisted of 216 trials administered in two blocks, each containing 108 items (16 similar pairs, 16 identical pairs and 44 unrelated novel stimuli) with an overall duration of approximately six minutes per run. To avoid that participants remembered the objects from pre- to posttest, one of two sets of stimuli were either presented on BDC-4 or HDT58. The order of the two sets was selected in a randomized counterbalanced fashion. Picture presentation and timing was controlled using Presentation® software (Version 18.1, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com).

2.6. Behavioral analysis

For each group and time point separately, the number of each stimulus type (novel, lure, repetition) in accordance with participants' individual response (new, similar, old) was extracted from the data log file. We then calculated the percentage for each response type relative to the total number of trial type (i.e., number of correctly identified repeated stimuli as old relative to the total number of repeated stimuli). In order to distinguish between signal and noise that corrects for a possible response bias, we also determined separation bias (Yassa et al., 2010), also referred to as the lure discrimination index (Stark, Stevenson, Wu, Rutledge, & Stark, 2015) and recognition memory performance (Stark, Yassa, Lacy, & Stark, 2013).[1] The separation bias score was assessed as the percent of lure trials correctly identified as "similar" (Lure Correct Rejection) minus the percent of novel trials endorsed as "similar". This approach corrects for a possible response bias toward exhibiting a tendency for the use of similar responses (Yassa et al., 2010). Recognition memory performance was operationalized as the percent of repetition trials correctly identified as "old" (Hits) minus the percent of novel trials endorsed as "old" (Stark et al., 2013). The behavioral response according to each trial type as well as the separation bias and recognition memory scores were subjected to mixed linear models to quantify the effects of *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN), and their interaction. Details of the statistical model are provided in section 2.8 below.

[1] Typically, d' prime is used as a sensitivity index to distinguish between signal and noise that corrects for a possible response bias in two-item response paradigms. For instance, in a yes/no discrimination paradigm giving the same answer for all trials yields 100% correct for one item, and 0% for the other (Pallier, 2002). The current task, however, was characterized by a three-choice serial reaction time task, requiring a slightly different analysis approach, taking into account three alternative responses. We here follow the suggestions to determine separation bias and recognition memory score for the pattern separation paradigm as outlined by Stark and colleagues (Stark, Yassa, Lacy, & Stark, 2013; Stark, Stevenson, Wu, Rutledge, & Stark, 2015; Yassa et al., 2010).

2.7. fMRI Analyses

2.7.1. Image preprocessing

fMRI data were preprocessed and analyzed using SPM12 software (Wellcome Department of Cognitive Neurology, London, UK) running on Matlab R2015b. The first four volumes of all EPI series were excluded from the analysis to allow the magnetization to reach a dynamic equilibrium. Data processing started with slice time correction and realignment of the EPI datasets. A mean image of EPI volumes was created to which individual volumes were spatially realigned by means of rigid body transformations. The individual structural image of each subject was co-registered with the mean image of the respective EPI series. The structural images were normalized to the Montreal Neurological Institute (MNI) template and normalization parameters were applied to the EPI images to ensure an anatomically informed normalization. A commonly applied filter of 8 mm FWHM (full-width at half maximum) was used to smooth the images. Low-frequency drifts in the time domain were removed by modelling the time series for each voxel by a set of discrete cosine functions to which a cut-off of 128 seconds was applied.

2.7.2. Subject-level analyses

Region of interest model. The subject-level statistical analyses were performed using general linear models (GLM) within the SPM-framework. The model was based on the first-level model reported by Kirwan and Stark (2007) using the following conditions: 1) *Hits* (repeated stimuli correctly called "old"; 2) *Lure Correct Rejection* (lure stimuli correctly identified as "similar"); 3) *Lure False Alarms* (lure stimuli called "old"); 4) *Miss* (repeated or lure stimuli called "new"); 5) *Subsequent Hits* (first time presentation of a repetition stimuli that was later correctly identified as "old"); 6) *Subsequent Lure Correct Rejection* (first time presentation of a lure stimuli that was later correctly identified as "similar"); 7) *Subsequent Lure False Alarms* (first time presentation of a lure stimuli that was later incorrectly identified as "old"); 8) *Subsequent Misses* (first time presentation of a repetition or lure stimuli that was later incorrectly labeled as "new"); 9) *Foils* (stimuli that have only been shown once and not in the same or similar way again and have been classified as "new"); and 10) *Other* (foils and first presentations that were incorrectly labeled as "old" or "similar"). Vectors of onsets for each of the above-mentioned event types for each participant and each point in time were convolved with the canonical hemodynamic response function (HRF) and the temporal derivative. Both session runs that have been administered at one point in time were modeled in the same GLM. Furthermore, the six movement regressors obtained from the realignment step were

entered into the GLM. After model estimation, this model was used for the region of interest analysis (ROI).

To verify if the regions of the medial temporal lobe were engaged in the task, we additionally computed a contrast on our first-level GLM from the ROI analysis were the average activity in *Hits* is greater than the average in *Lure Correct Rejection* and *Foils* (*Hits* > *Lure CR* = *Foils*) and a contrast where average activity in *Lure Correct Rejection* is greater than the average activity in *Hits* and *Foils* (*Lure CR* > *Hits* = *Foils)*. These contrasts were computed for all participants during their baseline assessment (BDC-4) before they were assigned to any intervention. The resulting contrast images were used for group-level statistics within the SPM-framework.

Whole-brain model. For whole-brain data we modeled a second GLM on a subject-level, but limited the analyses to conditions with correct responses, i.e., *Foils, Hits,* and *Lure Correct Rejections* (Pidgeon & Morcom, 2016). We computed *t*-contrasts for pattern separation and pattern completion as follows: Pattern separation was defined as the contrast where average activity in *Lure Correct Rejection* is the same as in *Foils* and greater than the average activity in *Hits* (*Lure CR* = *Foils* > *Hits*), and pattern completion was defined as the contrast where average activity in *Foils* was greater than in *Hits* and *Lure Correct Rejection* (*Foils* > *Hits* = *Lure CR*) (Pidgeon & Morcom, 2016). The resulting contrast images were used for further whole brain analysis on a group level.

2.7.3. Group-level analyses

Region of interest analysis. Anatomical regions of interests were determined a priori based on 1) regions that have been shown to be vulnerable to physical exercise, and 2) regions that have been reported previously to be involved in the task that we used. Previous work on the effects of physical exercise suggests that the hippocampus is significantly affected by regular exercise (Erickson et al., 2011; Firth et al., 2018). The pattern separation paradigm used in the present study distinctly activates the hippocampus and parahippocampal gyri (Kirwan & Stark, 2007; Bakker et al., 2008). Hence, we retrieved BOLD signal change in bilateral hippocampus and parahippocampal gyrus from the Automated Anatomical Labeling (AAL) Atlas (Tzourio-Mazoyer et al., 2002). This subject-level design matrix was used to extract the mean percent signal changes for each subject, each condition, and each point in time. Given that blood oxygenation peaks 4 to 6 seconds post-stimulus (Poldrack, Mumford & Nichols, 2011; Chen, Shen & Truong, 2016), we extracted the mean percent signal change covering a time window of 4 to 6 seconds after stimulus onset for each of the four regions of interest (left and right hippocampus and left and right parahippocampal gyrus) using the MarsBaR toolbox (http://marsbar.sourceforge.net/) (Brett, Anton, Valabregue, & Poline, 2002). Differences in mean percent signal change of hippocampal and parahippocampal activation were analyzed using mixed linear models (see also statistical models in section 2.8 for details). To validate the paradigm on the group-level, the contrast images for the contrasts *Hits* > *Lure CR* = *Foils* and *Lure CR* > *Hits* = *Foils* that had been computed across all participants during the baseline assessment prior to the intervention (BDC-4) were subjected to a one-sample *t*-test within the SPM-framework to verify which regions were engaged during the retrieval of repetitive and lure stimuli. The results were corrected using a family wise error rate (FWE).

Whole-brain analyses. The contrast images of the pattern separation and completion contrasts that had been acquired on a subject-level were used in a flexible factorial design within the SPM framework with *Subject* as a random factor, and *Time* and *Group* as fixed factors. Contrasts for main and interaction effects were computed and results were corrected for multiple comparisons using a family wise error rate (FWE).

2.8. Statistical analysis

Behavioral data and mean percent BOLD signal change for our *a priori* regions of interest, i.e., bilateral hippocampus and parahippocampal gyrus were analyzed by linear mixed models. To assess the effects of bed rest and the exercise intervention on percent responses classified as new, similar or old, we performed mixed models with *Group* (CTRL, TRAIN), *Time* (BDC-4, HDT58), and their interaction as fixed factors, and *Subject* as a random factor. The inclusion of *Stimulus Type* (novel, lure, repetition) as an additional factor did not allow precise estimations of the variance-covariance matrices. Hence, we ran separate mixed models for each stimulus (novel, lure, repetition) and response type (new, similar, old). Mean percent BOLD signal change was assessed for hippocampus and parahippocampal gyrus in two separate models. In each model, we entered *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterality* (Left, Right), *Condition* (Encoding, Retrieval), and *Stimulus Type* (Lure, Repetition). Covariance matrices were determined by restricted maximum likelihood (REML) estimation. P-values were obtained by using Satterthwaite's approximation for denominator degrees of freedom. Pre-planned contrasts were used to quantify the interaction between *Time* and *Group* by *Laterality* crossed with *Condition* and *Stimulus Type* for BOLD signal change. Contrasts were adjusted using a false discovery rate (FDR) procedure treating each region of interest (left and right hippocampus and parahippocampal gyrus) as one family of four comparisons each (two comparisons for *Condition* x two comparisons for *Stimulus Type*) (Benjamini & Hochberg, 1995). Effect sizes were reported as Cohen's *d* and 95% confidence intervals. For imaging data, a false coverage statement rate (FCR) was computed using a Matlab script (Groppe, 2020) to construct multiple comparison corrected confidence intervals that correspond to the FDR-adjusted p-values (Benjamini & Yekutieli, 2005). The level of significance was set at = 0.05 (two-sided) for all tests. All statistical analyses and graphical illustrations were carried out using the software package R version 3.5.2 (R Core Team, 2018).

3. Results

3.1. Validation of the fMRI paradigm

All participants demonstrated high accuracy in correctly identifying novel (> 93%) and repetitive stimuli (> 90%), whereas lure stimuli were harder to classify correctly (between 43% to 51% for BDC-4). A detailed summary of the descriptive statistics is provided in the Supplementary Material (Table S1). To verify which regions were engaged in the paradigm, we assessed brain activations during the contrasts *Hits* > *Lure CR* = *Foils* and *Lure CR* > *Hits* = *Foils* at the first point in time (BDC-4) for all participants (n = 22). Clusters that were significantly activated for the contrast *Hits* > *Lure CR* = *Foils* were the left fusiform gyrus, left hippocampus, left MTG (for all 3 clusters $P_{FWE\text{-}corr.}$ = 0.033), and bilateral precuneus ($P_{FWE\text{-}corr.}$ < 0.001) as well as bilateral cerebellum ($P_{FWE\text{-}corr.}$ = 0.029 for right and $P_{FWE\text{-}corr.}$ = 0.002 for left cerebellum). For the contrast *Lure CR* > *Hits* = *Foils*, we observed significant activations in bilateral precuneus ($P_{FWE\text{-}corr.}$ < 0.001), left hippocampus ($P_{FWE\text{-}corr.}$ = 0.042), and bilateral cerebellum ($P_{FWE\text{-}corr.}$ = 0.027 for right and $P_{FWE\text{-}corr.}$ = 0.026 for left cerebellum). Both, the behavioral performance and the regions activated at BDC-4 are very similar to the results reported previously confirming the validity of the paradigm (Hämäläinen et al., 2007; Kirwan & Stark, 2007; Pidgeon & Morcom, 2011).

3.2. Behavioral findings

Figure 3 shows frequency of the responses given to each stimulus type for the contrast between HDT58 and BDC-4. Both groups showed a significant improvement in the discrimination of lure items by identifying them more often as "similar" and less as "old" on HDT58 (all *P*s < 0.05). Numerically, TRAIN also improved in the recognition of repetition trials (t_{20} = 0.46, *P* = 0.654, *d* = 0.19, [-0.65, 1.03]), whereas CTRL showed a small decrease from HDT58 to BDC-4 (t_{20} = -0.76, *P* = 0.457, *d* = -0.32, [-1.16, 0.52]). There was neither a significant main effect for *Group* nor a *Group* x *Time* interaction in any of the behavioral

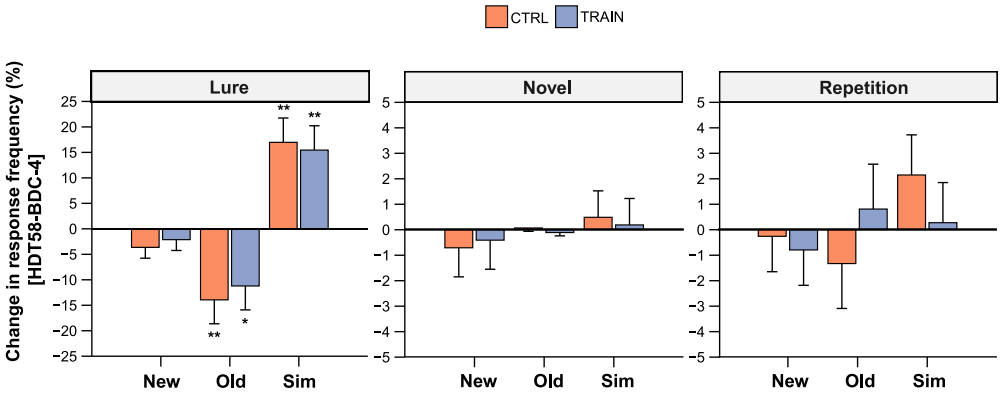

Fig. 3. Changes in response frequency from baseline (HDT58 vs. BDC-4) by *Stimulus Type* (lure, novel and repeated stimuli), *Group* (TRAIN, CTRL), and *Response* (items classified as new, old, or similar). Data are marginal means and standard errors. CTRL, bed rest control group; TRAIN, exercising bed rest group. N = 11 for each group respectively. * $P < 0.05$, ** $P < 0.01$ compared to BDC-4.

conditions (all *Ps* > 0.124). Detailed statistical results are provided in Supplementary Material (Table S2 and S3).

Results for the separation bias and the recognition memory score were similar to changes observed for discriminating lure and repetition trials (see Supplementary Material Figure S1). We also observed an increase in the separation bias score in both groups ($F_{1,20} = 26.16$, $P < 0.001$ for *Time*). This is in line with the improvement in discriminating lure trials, given that the separation bias score was calculated as the difference between the probability of identifying a lure item as "similar" and the probability of identifying a novel foil item as "similar". Numerically, TRAIN showed a higher and CTRL a lower recognition memory score that, however, did not reach statistical significance (*Group* x *Time*, $F_{1,20} = 0.89$, $P = 0.358$). Detailed statistical results are provided in Supplementary Material Table S4.

3.3. fMRI results

3.3.1. Region of interest analysis

After two months of bed rest, increases in BOLD signal were observed in CTRL for all mnemonic conditions, whereas the signal in TRAIN decreased (Fig. 4). Pre-planned contrasts revealed that the decrease in BOLD signal within TRAIN was significant for five out of eight conditions in the left hemisphere (all *Ps* < 0.05). A detailed summary of the simple effects of *Time* by *Laterality* (Left, Right) and *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition) for each group separately and for all main and interaction effects of the multilevel analysis is provided in Supplementary Material Table S5, S6, and S7. We also observed a significant interaction between *Group* and *Time* in the left hemisphere for the retrieval process of lure stimuli (hippocampus: $P = 0.035$, $d = -1.12$ [-2.27, 0.04]); parahippocampal gyrus: $P = 0.012$, $d = -1.23$ [(-2.14, -0.30]). Furthermore, a significant interaction of *Group* x *Time* during correct encoding and retrieval of repetition stimuli was found in the left parahippocampal gyrus ($P = 0.019$, $d = -1.01$[-1.89, -0.11]; $P = 0.018$, $d = -1.05$ [-1.93, -0.14], respectively). A nearly significant interaction was observed for the left hippocampus during encoding of lure items ($P = 0.054$, $d = -0.95$ [-2.07, 0.19]) and for the right parahippocampal gyrus during retrieval of lures ($P = 0.056$, $d = -1.05$). There were no other significant main or interaction effects (all *Ps* > 0.215). Details of these analysis are provided in Supplementary Material Table S8.

3.3.2. Whole-brain analysis

Greater activation on HDT58 compared to BDC-4 for the pattern separation contrast [BDC-4 (*Lure CR* = *Foils* > *Hits*)] vs. [HDT58 (*Lure CR* = *Foils* > *Hits*)] was observed in the right occipital pole (R OCP), right middle temporal gyrus (R MTG), frontal pole, right fusiform gyrus (R FuG), and left inferior temporal gyrus (L ITG) for CTRL ($P < 0.001$, clusterwise FWE-corrected ($P < 0.05$)). Notably, only the right OCP survived the FWE-cluster correction (Fig. 5). Higher activation was also seen in the right superior parietal lobule in TRAIN, but did not reach the level of significance. There was no *Group* x *Time* interaction for the pattern separation contrast.

Regions showing decreased activation for the pattern completion contrast [BDC-4 (*Foils* > *Hits* = *Lure CR*)] vs. [HDT58 (*Foils* > *Hits* = *Lure CR*)] involved bilateral precentral gyrus, bilateral insular cortex, and right middle temporal gyrus for CTRL and left superior temporal gyrus (L STG) and left occipital fusiform gyrus (L OFuG) for TRAIN. Only the bilateral precentral gyrus survived FWE-cluster correction. A *Group* x *Time* interaction that did not reached statistical significance was observed in the right precentral gyrus. A detailed overview of the regions that showed changes in the pattern separation and completion contrast from BDC-4 to HDT58 and exceeded the threshold of 20 voxels is provided in the Supplementary Material Table S9 and S10.

4. Discussion

We investigated the effects of long-term bed rest on episodic memory performance and its neural basis and whether a high-intensity jump training can mitigate the effects of physical inactivity on the neural underpinnings of memory functioning. After two months of bed rest, we found increases in BOLD signal during memory encoding and retrieval in the hippocampal formation in CTRL compared to TRAIN, suggesting a modulating effect of the exercise intervention. The strongest effects of exercise were observed in the left hemisphere. This is in line with recent research summarizing the effects of regular physical activity on the hippocampus. In a meta-analysis of 14 longitudinal studies, Firth et al. (2018) reported significantly larger effects for the left hippocampus (Hedge's *g* [95% CI] = 0.265 [0.090, 0.441], $P = 0.003$) compared to the right hippocampus (Hedge's *g* [95% CI] = 0.164, [-0.010, 0.339], $P = 0.065$).

Increased brain activity during pattern separation has been observed in the elderly compared to young adults (Yassa et al., 2011), as well as in patients with mild cognitive impairment (MCI) compared to healthy controls (Hämäläinen et al., 2007; Yassa et al., 2010). Bed rest is a classical model to simulate some of the physiological adaptations associated with spaceflight (Pavy-Le Traon et al., 2007). Given that the physiological responses to spaceflight reflect an accelerated aging process (McGuire et al., 2001; Vernikos & Schneider, 2010), bed

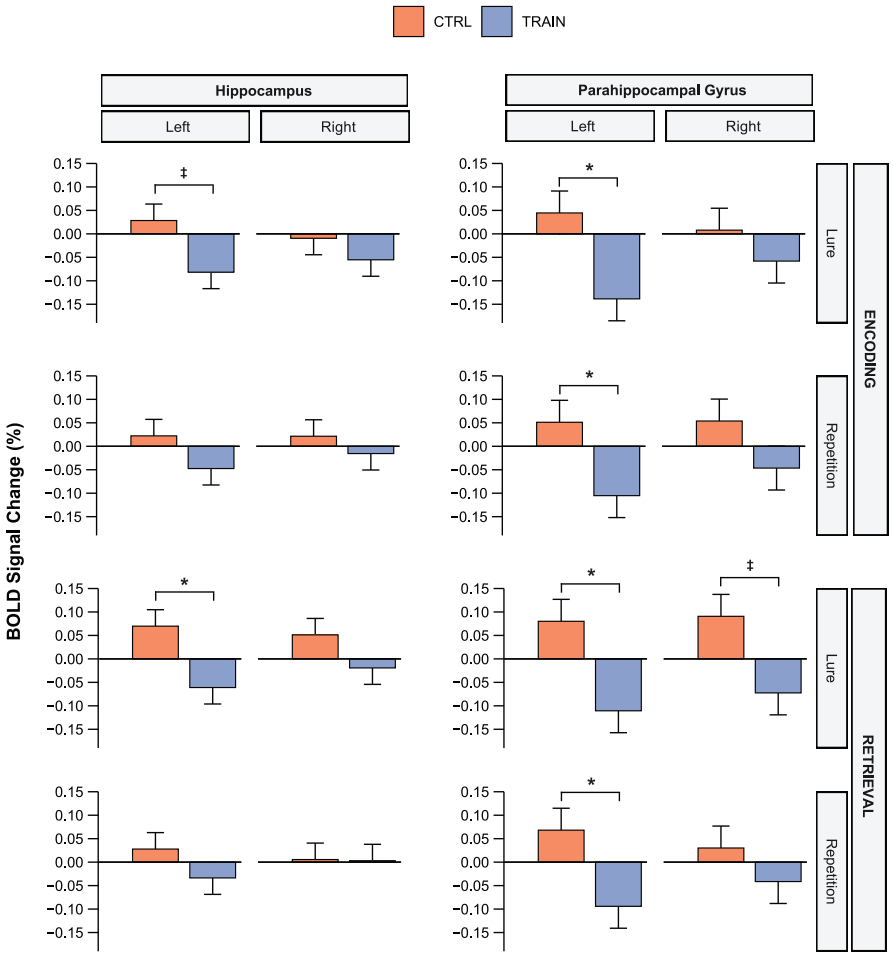

Fig. 4. BOLD signal change during memory encoding and retrieval of lure and repetition stimuli for bilateral hippocampus and parahippocampal gyrus. Data are marginal means and standard errors for the interaction of *Group* (TRAIN, CTRL) x *Time* (BDC-4, HDT58) by *Laterality* (Left, Right) crossed with *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition). CTRL, bed rest control group; TRAIN, exercising bed rest group. N = 11 for each group respectively. * $P < 0.05$, ‡ interaction was close to statistical significance with $P = 0.054$ for left hippocampus during encoding of lure stimuli and $P = 0.056$ for right parahippocampal gyrus during retrieval of lure stimuli.

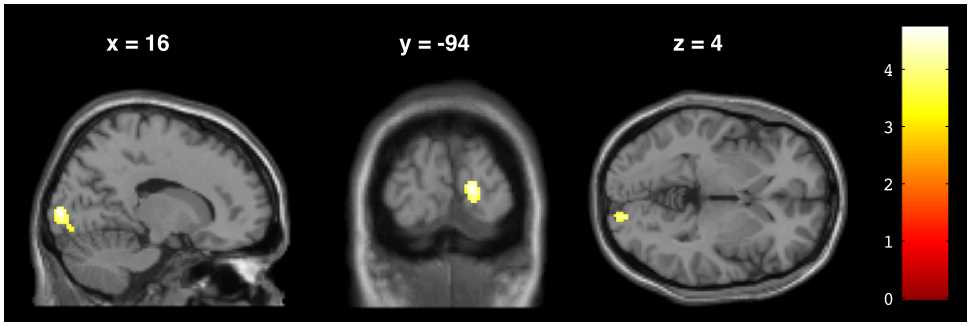

Fig. 5. Right occipital pole showing increased activation during pattern separation in CTRL. SPMs for the contrast [BDC-4 (*Lure CR = Foils > Hits*)] vs. [HDT58 (*Lure CR = Foils > Hits*)] averaged over 11 CTRL subjects mapped onto an MNI template ($P < 0.001$, clusterwise FWE-corrected ($P < 0.05$)).

rest can also be considered as a unique model to better understand the effects of premature physiological aging. In this regard, our findings of increased brain activation in CTRL could be interpreted as a consequence of bed rest-induced accelerated aging. This is also supported by findings from Miller and colleagues (Miller et al., 2008a), showing a very similar response in the elderly. Specifically, they observed greater hippocampal activity during the encoding process in low-performing older adults and explained this phenomenon as a compensatory response (Miller et al., 2008a). In another study Miller and colleagues also demonstrated that greater hippocampal activation during memory encoding predicted the rate of cognitive decline (Miller et al., 2008b). Additional evidence comes from Yassa et al. (2010) who found an inverse relationship between hyperactivity in the dentate gyrus and CA3 region and behavioral performance, suggesting that the increase in brain activity resulted from dysfunctional encoding mechanisms. Conflicting results have been reported in a precedent study by Small and colleagues (Small, Perera, DeLaPaz, Mayeux, & Stern, 1999) who found reduced activation in all hippocampal subfields in Alzheimer patients compared to healthy elder controls. Sperling speculated that hyperactivation occurs in very early stages of MCI as a compensatory response to maintain memory performance but at a later stage the hippocampus fails, resulting in decreased activation (Sperling, 2007). In a longitudinal study Nyberg and colleagues (2019) have observed both during encoding, an age-related hypoactivity in the anterior hippocampus, and hyperactivity in the anterior and posterior hippocampus in elderly having lower memory performance and a higher dementia risk. The authors suggested that hippocampal hyperactivity is not a response per se to normal aging but rather a pathological sign of neurocognitive disorders (Nyberg et al. 2019). The same group also assumed that hippocampal atrophy and memory decline induces functional reorganization in the prefrontal cortex (Pudas et al., 2018) and elevated functional connectivity between PFC and anterior hippocampus (Nyberg et al. 2019).

In the present study, differences in brain activity between groups were not paralleled by changes in behavioral performance. Participants most often identified novel, lure, and repetition stimuli correctly as new, similar, and old showing the highest accuracy in novel and repetition items. Lure items were harder to classify and only correctly identified in 43% to 66% of all lure trials. Very similar performances on novel, lure and repetitive trials have been reported in previous studies (Hämäläinen et al., 2007; Kirwan & Stark, 2007) confirming the validity of the paradigm in the present study. Notably, we observed an improvement in the ability of discriminating lure trials on HDT58 compared to BDC-4 in both groups that may be the result of additional test-enhanced learning effects that occurred independently of the intervention. It can only be speculated whether the discrepancy between neuronal activity and mnemonic performance is due to a reduced neuronal efficiency in CTRL (i.e., that maintaining behavioral performance requires a higher neural demand) or that bed rest induced dysfunctional mechanisms in neuronal coupling, which were counteracted by the exercise intervention. In addition to our *a priori* hypothesis anticipating changes in hippocampus and parahippocampal gyrus, we also explored whole-brain BOLD signal changes during pattern separation and completion from BDC-4 to HDT58. We observed a significant increase in the right occipital pole during pattern separation in participants that did not undergo the training intervention. Occipital, parietal, and temporal regions have been reported previously to contribute to pattern separation (Pidgeon & Morcom, 2016) and memory retrieval (Jonker et al., 2018). It is also possible that these activations reflect other processes associated with the encoding of the stimuli. For instance, Sestieri, Shulman, & Corbetta (2017) noted that such activations could be related to perceptual attention. Our data also suggested a stronger decrease in CTRL for bilateral precentral gyrus. However, the interaction between *Group* and *Time* was not significant. It is possible that this activation is likely to be attributed to bed rest (Cassady et al., 2016). Cassady and colleagues reported that the intrinsic connectivity contrast (ICC) of the precentral gyrus is increased after 70 days of bed rest (Cassady et al., 2016). We therefore assume that the observed cluster is unrelated to our fMRI paradigm and a result of prolonged bed rest per se.

Key strengths of this study are the highly-standardized conditions and environment of the experimental and control group. In contrast to previous ambulatory training studies (Erickson et al., 2011; Ruscheweyh et al., 2011; Hötting et al., 2012), we were able to standardize various critical factors that are known to affect neurobehavioral measures, including the social environment, leisure time activities, nutrition, sleep, and day and night cycles. Over three months, including the 60 days of bed rest, sleep, diet, light exposure, environmental conditions, and physical activity were strictly regulated and standardized (Kramer et al., 2017a). Social interactions were limited to staff, other participants, and individual personal phone calls only. Thus, the differences in neuronal activity observed in the study can likely be exclusively attributed to the result of the exercise intervention. It should also be noted that the exercise intensity and target population of the current project differed compared to previous studies.

Our exercise group followed a short but intensive jump training protocol and completed 48 sessions within two months. For example, this was the same amount of sessions over a course of six months in the study by Hötting et al. (2012). Moreover, a large part of the existing body of research focuses on older adults with a mean age >60 years (e.g., Erickson et al., 2011; Ruscheweyh et al., 2011; Jonasson et al., 2016). Only very few studies with a similar cohort following a comparable study design have been reported so far and none of these studies particularly targeted the function of the hippocampus (Koppelmans et al., 2013; Rao et al., 2014; Zhou et al., 2014; Yuan et al., 2016). In these studies, 45 days of bed rest led to altered functional connectivity in the left anterior insula and dorsal anterior cingulate cortex (Zhou et al., 2014) as well as to greater activation in the ventromedial prefrontal cortex during risky decision making (Rao et al., 2014). Another research group reported increased brain activity in frontal and parietal regions that were accompanied with slower reaction times (Yuan et al., 2016) and detrimental effects on functional connectivity in motor and somatosensory brain areas after 70 days of bed rest (Cassady et al., 2016; Koppelmans et al., 2017; Koppelmans et al., 2018). The authors concluded from the observed increased brain activation that more neurocognitive control is required during bed rest for dual-task execution (Yuan et al., 2016). The results of the present study confirm these findings for episodic memory and its neural basis, and provide novel insights into the effects of physical activity on mitigating the effects of bed rest on brain function.

4.1. Limitations

Although the study was highly standardized, our findings are subject to a few limitations. The overall experimental protocol was defined by the study sponsor, including inclusion and exclusion criteria as well as sample size. These restricted criteria have resulted in a highly selective sample of young, healthy men. Accordingly, caution must be applied with respect to the generalizability of the findings to women, the aging population, and patients. Further research is needed to investigate bed rest induced changes in mnemonic processing and their neural basis in different populations. We also acknowledge that the present study lacks a non-resting control group. Albeit, the challenges in controlling for diet, sleep, social contacts, and physical activity levels in ambulatory controls, such data could also provide important information to better understand the effects of long-duration immobilization.

5. Conclusion

The current study assessed the effects of long-term bed rest on episodic memory and its neural correlates and the efficacy of a regular high-intensity exercise to mitigate adverse neurobehavioral effects. With the same mnemonic performance, we found an elevated BOLD

signal in the non-exercising bed rest group compared to the exercising bed rest group. It cannot be conclusively decided whether this is a compensatory response or the result of an underlying dysfunctional mechanism. Our findings show, however, that high-intensity exercise modulates neuronal activity and may counteract hyperactive signaling in the hippocampal formation. Further research is needed to elucidate sex-specific effects of the high-intensity exercise program on hippocampal activation, and explore the potential of these programs to preserve brain function in the aging population.

Funding

The project was supported by the European Space Agency (ESA) and by the German Aerospace Center (Deutsches Zentrum für Luft- und Raumfahrt; DLR) through grants 50WB1525 and 50WB1519 awarded to ACS.

CRediT authorship contribution statement

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Acknowledgements

The authors thank the European Space Agency for providing the opportunity to participate in this study, Ulrich Limper, Edwin Mulder, and Alexandra Noppe from DLR for their medical, technical, and administrative support in implementing the study protocol as well as Darius Gerlach, Kerstin Kempter, and Annette von Wächter for MRI acquisition.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2020.117359.

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1 Supplementary Tables

Table S1. Descriptive statistics of the behavioral response by stimulus type.*

Stimuli	Response	BDC-4		HDT58	
		CTRL	TRAIN	CTRL	TRAIN
Novel	New	96.77 (3.8)	94.32 (7.5)	96.05 (3.9)	93.90 (6.8)
Novel	Similar	2.75 (3.6)	4.96 (7.5)	3.23 (3.9)	5.14 (6.4)
Novel	Old	0.30 (0.5)	0.36 (0.5)	0.36 (0.5)	0.24 (0.6)
Lure	New	12.73 (8.9)	10.00 (6.3)	9.09 (10.0)	7.88 (4.0)
Lure	Similar	43.33 (25.6)	50.91 (20.1)	60.30 (18.0)	66.36 (13.8)
Lure	Old	42.12 (21.4)	36.67 (16.7)	28.18 (18.9)	25.45 (13.4)
Repetition	New	4.01 (6.9)	2.14 (1.4)	3.74 (3.0)	1.34 (1.5)
Repetition	Similar	2.94 (4.4)	5.08 (3.5)	5.08 (4.8)	5.35 (5.4)
Repetition	Old	92.24 (9.5)	92.5 (4.4)	90.91 (7.3)	93.32 (6.0)
Missed responses		0.51 (0.6)	0.63 (0.9)	0.63 (1.1)	0.55 (1.2)

*Data are means and their standard deviation. BDC-4, baseline data collection 4 days prior to bed rest; HDT58, 58 days of head-down tilt bed rest, CTRL, bed rest control group; TRAIN, exercising bed rest group.

Table S2. Mixed-models assessing the effects of *Group*, *Time*, and their interaction on behavioral performance (new, similar, old) according to each stimulus type (novel, lure, repetition).*

Effect	Stimuli	Response	df_1	df_2	*F*	*P*
Group	Novel	New	1	20	0.98	0.333
Time	Novel	New	1	20	0.50	0.489
Group x Time	Novel	New	1	20	0.03	0.855
Group	Novel	Similar	1	20	0.83	0.374
Time	Novel	Similar	1	20	0.20	0.659
Group x Time	Novel	Similar	1	20	0.04	0.841
Group	Novel	Old	1	20	0.02	0.877
Time	Novel	Old	1	20	0.11	0.748
Group x Time	Novel	Old	1	20	0.96	0.340
Group	Lure	New	1	20	0.46	0.506
Time	Lure	New	1	20	3.67	0.070
Group x Time	Lure	New	1	20	0.25	0.620
Group	Lure	Similar	1	20	0..77	0.390
Time	Lure	Similar	1	20	23.05	<0.001
Group x Time	Lure	Similar	1	20	0.05	0.825
Group	Lure	Old	1	20	0.36	0.557
Time	Lure	Old	1	20	14.36	0.001
Group x Time	Lure	Old	1	20	0.17	0.689
Group	Repetition	New	1	20	2.58	0.124
Time	Repetition	New	1	20	0.30	0.592
Group x Time	Repetition	New	1	20	0.07	0.788
Group	Repetition	Similar	1	20	0.57	0.459
Time	Repetition	Similar	1	20	1.17	0.292
Group x Time	Repetition	Similar	1	20	0.71	0.411
Group	Repetition	Old	1	20	0.24	0.631
Time	Repetition	Old	1	20	0.05	0.832
Group x Time	Repetition	Old	1	20	0.74	0.401

*Mixed-models were performed separately for each stimulus type and behavioral response. *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN) were treated a fixed factors and *Subject* as a random factor. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom;

Table S3. Contrasts comparing the effects of *Time* on behavioral performance (new, similar, old) according to each stimulus type (novel, lure, repetition) and each group.*

Group	Stimuli	Response	*df*	*t-ratio*	*P*	*Effect Size (95% CI)*
CTRL	Novel	New	20	-0.63	0.536	-0.27 (-1.10, 0.57)
TRAIN	Novel	New	20	-0.37	0.717	-0.16 (-0.99, 0.68)
CTRL	Novel	Similar	20	0.46	0.65	0.20 (-0.64, 1.03)
TRAIN	Novel	Similar	20	0.17	0.865	0.07 (-0.76, 0.91)
CTRL	Novel	Old	20	0.46	0.650	0.20 (-0.64, 1.03)
TRAIN	Novel	Old	20	-0.92	0.367	-0.39 (-1.23, 0.46)
CTRL	Lure	New	20	-1.71	0.103	-0.73 (-1.59, 0.14)
TRAIN	Lure	New	20	-1.10	0.330	-0.43 (-1.27, 0.43)
CTRL	Lure	Similar	20	3.55	0.002	1.52 (0.54, 2.46)
TRAIN	Lure	Similar	20	3.24	0.004	1.38 (0.43, 2.30)
CTRL	Lure	Old	20	-2.97	0.008	-1.27 (-2.18, -0.33)
TRAIN	Lure	Old	20	-2.39	0.027	-1.02 (-1.90, -0.11)
CTRL	Repetition	New	20	-0.19	0.849	-0.08 (-0.92, 0.76)
TRAIN	Repetition	New	20	-0.58	0.570	-0.25 (-1.08, 0.60)
CTRL	Repetition	Similar	20	1.36	0.190	0.58 (-0.28, 1.43)
TRAIN	Repetition	Similar	20	0.17	0.867	0.07 (-0.76, 0.91)
CTRL	Repetition	Old	20	-0.76	0.457	-0.32 (-1.16, 0.52)
TRAIN	Repetition	Old	20	0.46	0.654	0.19 (-0.65, 1.03)

*_Time_ (HDT58, BDC-4). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. *df*, degrees of freedom, *P*, p-value; Effect size is Cohen's *d* and the corresponding 95% confidence interval (95% CI).

Table S4. Mixed-models assessing the effects of *Group*, *Time*, and their interaction on separation bias and recognition memory score.*

Score	Effect	*df1*	*df2*	*F*	*P*
Separation bias	Group	1	20	0.33	0.570
	Time	1	20	26.16	< 0.001
	Group x Time	1	20	0.04	0.847
Recognition memory	Group	1	20	0.25	0.624
	Time	1	20	0.04	0.850
	Group x Time	1	20	0.89	0.358

*Mixed-models were performed separately for each score. *Time* (BDC-4, HDT58) and *Group* (CTRL, TRAIN) were treated a fixed factor and *Subject* as a random factor. n = 11 for each group respectively. df_1, numerator degrees of freedom; df_2, denominator degrees of freedom; *F*, F-statistics, *P*, p-value.

Table S5. Contrasts of ROI analyses assessing the effects of *Time* (BDC-4 vs. HDT58) on BOLD signal change in bilateral hippocampus and parahippocampal gyrus within each group.*

Group	Region	Laterality	Condition	Stimulus Type	*df*	*t-ratio*	*P*	*Effect size (95% CI)*
CTRL	Hipp	L	Encoding	Lure	300	0.81	0.418	0.35 (-0.50, 1.18)
TRAIN	Hipp	L	Encoding	Lure	300	-2.33	0.021	-0.99 (-1.87, -0.09)
CTRL	Hipp	R	Encoding	Lure	300	-0.27	0.788	-0.11 (-0.95, 0.72)
TRAIN	Hipp	R	Encoding	Lure	300	-1.58	0.116	-0.67 (-1.53, 0.20)
CTRL	Hipp	L	Retrieval	Lure	300	1.99	0.048	0.85 (-0.04, 1.71)
TRAIN	Hipp	L	Retrieval	Lure	300	-1.74	0.083	-0.74 (-1.60, 0.13)
CTRL	Hipp	R	Retrieval	Lure	300	1.46	0.146	0.62 (-0.24, 1.47)
TRAIN	Hipp	R	Retrieval	Lure	300	-0.55	0.583	-0.23 (-1.07, 0.61)
CTRL	Hipp	L	Encoding	Repetition	300	0.63	0.526	0.27 (-0.57, 1.11)
TRAIN	Hipp	L	Encoding	Repetition	300	-1.35	0.177	-0.58 (-1.42, 0.28)
CTRL	Hipp	R	Encoding	Repetition	300	0.61	0.541	0.26 (-0.58, 1.10)
TRAIN	Hipp	R	Encoding	Repetition	300	-0.44	0.659	-0.19 (-1.02, 0.65)
CTRL	Hipp	L	Retrieval	Repetition	300	0.79	0.427	0.34 (-0.51, 1.18)
TRAIN	Hipp	L	Retrieval	Repetition	300	-0.96	0.338	-0.41 (-1.25, 0.44)
CTRL	Hipp	R	Retrieval	Repetition	300	0.16	0.874	0.07 (-0.77, 0.90)
TRAIN	Hipp	R	Retrieval	Repetition	300	0.08	0.933	0.04 (-0.80, 0.87)
CTRL	PH	L	Encoding	Lure	300	0.95	0.341	0.41 (-0.44, 1.25)
TRAIN	PH	L	Encoding	Lure	300	-2.97	0.003	-1.26 (-2.17, -0.33)
CTRL	PH	R	Encoding	Lure	300	0.17	0.866	0.07 (-0.77, 0.91)
TRAIN	PH	R	Encoding	Lure	300	-1.24	0.215	-0.53 (-1.38, 0.33)
CTRL	PH	L	Retrieval	Lure	300	1.72	0.087	0.73 (-0.14, 1.59)
TRAIN	PH	L	Retrieval	Lure	300	-2.37	0.019	-1.01 (-1.89, -0.11)
CTRL	PH	R	Retrieval	Lure	300	1.94	0.053	0.83 (-0.06, 1.69)
TRAIN	PH	R	Retrieval	Lure	300	-1.55	0.122	-0.66 (-1.51, 0.21)
CTRL	PH	L	Encoding	Repetition	300	1.10	0.274	0.47 (-0.39, 1.31)
TRAIN	PH	L	Encoding	Repetition	300	-2,25	0.025	-0.96 (-1.84, -0.06)
CTRL	PH	R	Encoding	Repetition	300	1.15	0.250	0.49 (-0.36, 1.33)
TRAIN	PH	R	Encoding	Repetition	300	-0.99	0.321	-0.42 (-1.26, 0.43)
CTRL	PH	L	Retrieval	Repetition	300	1.46	0.146	0.62 (-0.24, 1.47)
TRAIN	PH	L	Retrieval	Repetition	300	-2.01	0.045	-0.86 (-1.73, 0.03)
CTRL	PH	R	Retrieval	Repetition	300	0.65	0.519	0.28 (-0.57, 1.11)
TRAIN	PH	R	Retrieval	Repetition	300	-0.89	0.376	-0.38 (-1.22, 0.47)

*CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. Hipp, hippocampus; PH, parahippocampal gyrus, R, right, L, left, *df*, degrees of freedom, *P*, p-value; Effect size is Cohen's *d* and the corresponding 95% confidence interval (95% CI).

Table S6. Mixed-models of ROI analysis assessing the effects of *Time*, *Group, Laterality, Condition, Stimulus type* and their interaction in the hippocampus.*

Effect	*df1*	*df2*	*F*	*P*
Time	1	300	0.45	0.504
Group	1	20	0.47	0.500
Laterality	1	300	2.04	0.154
Condition	1	300	0.60	0.440
Stimulus Type	1	300	3.87	0.050
Time x Group	1	300	14.17	< 0.001
Time x Laterality	1	300	0.17	0.684
Group x Laterality	1	300	0.01	0.933
Time x Condition	1	300	1.66	0.199
Group x Condition	1	300	0.01	0.925
Laterality x Condition	1	300	0.08	0.778
Time x Stimulus Type	1	300	0.19	0.665
Group x Stimulus Type	1	300	1.74	0.188
Laterality x Stimulus Type	1	300	0.14	0.711
Condition x Stimulus Type	1	300	0.62	0.431
Time x Group x Laterality	1	300	2.38	0.124
Time x Group x Condition	1	300	0.00	0.984
Time x Laterality x Condition	1	300	0.02	0.899
Group x Laterality x Condition	1	300	0.20	0.653
Time x Group x Stimulus Type	1	300	1.77	0.185
Time x Laterality x Stimulus Type	1	300	0.06	0.810
Group x Laterality x Stimulus Type	1	300	0.00	0.988
Time x Condition x Stimulus Type	1	300	0.95	0.331
Group x Condition x Stimulus Type	1	300	0.31	0.581
Laterality x Condition x Stimulus Type	1	300	0.00	0.986
Time x Group x Laterality x Condition	1	300	0.03	0.874
Time x Group x Laterality x Stimulus Type	1	300	0.06	0.814
Time x Group x Condition x Stimulus Type	1	300	0.39	0.532
Time x Laterality x Condition x Stimulus Type	1	300	0.13	0.714
Group x Laterality x Condition x Stimulus Type	1	300	0.38	0.540
Time x Group x Laterality x Condition x Stimulus Type	1	300	0.05	0.831

*Mixed-models were performed using *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterality* (Left, Right), *Condition* (Encoding, Retrieval), and *Stimulus Type* (Lure, Repetition) 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.

Table S7. Mixed-models of ROI analysis assessing the effects of *Time*, *Group, Laterality, Condition, Stimulus type* and their interaction in the parahippocampal gyrus.*

Effect	*df1*	*df2*	*F*	*P*
Time	1	300	1.65	0.200
Group	1	20	0.04	0.841
Laterality	1	300	1.41	0.236
Condition	1	300	0.22	0.643
Stimulus Type	1	300	0.28	0.597
Time x Group	1	300	34.23	< 0.001
Time x Laterality	1	300	0.81	0.368
Group x Laterality	1	300	0.03	0.852
Time x Condition	1	300	0.57	0.450
Group x Condition	1	300	0.01	0.903
Laterality x Condition	1	300	0.00	0.977
Time x Stimulus Type	1	300	0.15	0.698
Group x Stimulus Type	1	300	1.53	0.217
Laterality x Stimulus Type	1	300	0.00	0.983
Condition x Stimulus Type	1	300	0.02	0.875
Time x Group x Laterality	1	300	2.43	0.120
Time x Group x Condition	1	300	0.19	0.661
Time x Laterality x Condition	1	300	0.05	0.823
Group x Laterality x Condition	1	300	0.03	0.853
Time x Group x Stimulus Type	1	300	0.36	0.548
Time x Laterality x Stimulus Type	1	300	0.01	0.930
Group x Laterality x Stimulus Type	1	300	0.03	0.851
Time x Condition x Stimulus Type	1	300	0.43	0.513
Group x Condition x Stimulus Type	1	300	0.06	0.806
Laterality x Condition x Stimulus Type	1	300	0.02	0.888
Time x Group x Laterality x Condition	1	300	0.09	0.769
Time x Group x Laterality x Stimulus Type	1	300	0.00	0.991
Time x Group x Condition x Stimulus Type	1	300	0.47	0.495
Time x Laterality x Condition x Stimulus Type	1	300	0.08	0.783
Group x Laterality x Condition x Stimulus Type	1	300	0.37	0.545
Time x Group x Laterality x Condition x Stimulus Type	1	300	0.44	0.507

*Mixed-models were performed using *Time* (BDC-4, HDT58), *Group* (TRAIN, CTRL), *Laterali* (Left, Right), *Condition* (Encoding, Retrieval), and *Stimulus Type* (Lure, Repetition) as fix factors and *Subject* as a random factor. df_1, numerator degrees of freedom; df_2, denominat degrees of freedom; *F*, F-statistics, *P*, p-value.

Table S8. Contrasts of ROI analysis comparing the BOLD signal change from BDC-4 to HDT58 between groups for bilateral hippocampus and parahippocampal gyrus.*

Region	Laterality	Condition	Stimulus Type	*df*	*t-ratio*	$P_{FDR\text{-}corr.}$	Effect size (95% CI)
Hipp	L	Encoding	Lure	300	-2.22	0.054	-0.95 (-2.07, 0.19)
Hipp	R	Encoding	Lure	300	-0.02	0.609	-0.39
Hipp	L	Retrieval	Lure	300	-2.64	0.035	-1.12 (-2.27, 0.04)
Hipp	R	Retrieval	Lure	300	-1.42	0.609	-0.61
Hipp	L	Encoding	Repetition	300	-1.41	0.215	-0.60 (-1.68, 0.50)
Hipp	R	Encoding	Repetition	300	-0.74	0.609	-0.32
Hipp	L	Retrieval	Repetition	300	-1.24	0.216	-0.53 (-1.61, 0.56)
Hipp	R	Retrieval	Repetition	300	-0.05	0.958	-0.02
PH	L	Encoding	Lure	300	-2.77	0.012	-1.18 (-2.08, -0.26)
PH	R	Encoding	Lure	300	-1.00	0.319	-0.43
PH	L	Retrieval	Lure	300	-2.89	0.012	-1.23 (-2.14, -0.30)
PH	R	Retrieval	Lure	300	-2.47	0.056	-1.05
PH	L	Encoding	Repetition	300	-2.37	0.019	-1.01 (-1.89, -0.11)
PH	R	Encoding	Repetition	300	-1.52	0.260	-0.65
PH	L	Retrieval	Repetition	300	-2.46	0.018	-1.05 (-1.93, -0.14)
PH	R	Retrieval	Repetition	300	-1.08	0.319	-0.46

*Data show effects of the interaction between *Group* (TRAIN, CTRL) and *Time* (HDT58, BDC-4) by *Laterality* (Left, Right) crossed with *Condition* (Encoding, Retrieval) and *Stimulus Type* (Lure, Repetition). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. Hipp, hippocampus; PH, parahippocampal gyrus, R, right, L, left, *df*, degrees of freedom, $P_{FDR\text{-}corr.}$, p-value corrected for multiple comparisons using the Benjamini and Hochberg (BH) false discovery rate procedure; Effect size is Cohen's *d*. 95% CI, 95% confidence interval corrected for multiple comparisons using the false coverage-statement rate (FCR). Note that FCR-adjusted CIs were derived after BH-selection and can only be computed for families containing significant p-values.

Table S9. Contrasts of whole-brain analyses indicating the BOLD signal change from BDC-4 to HDT58 during pattern separation by *Group*.*

	Region	***Direction of effect***	***x y z***	***k***	***Peak Z***	$P_{FWE\text{-}corr.}$
TRAIN	R SPL	Increase	-32 -58 42	25	3.41	0.897
	R OCP	Increase	16 -94 4	135	4.18	0.042
	R MTG	Increase	54 -12 -18	25	3.86	0.897
CTRL	Frontal Pole	Increase	38 36 6	31	3.86	0.815
	R FuG	Increase	28 -56 -18	74	3.83	0.257
	L ITG	Increase	-46 -50 -14	32	3.71	0.800

*Contrasts refer to comparison between BDC-4 (*Lure CR = Foils > Hits*) and HDT58 (*Lure CR = Foils > Hits*). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. R, right; L, left; SPL, superior parietal lobe; OCP, occipital pole; MTG, middle temporal gyrus; FuG, fusiform gyrus; ITG, inferior temporal gyrus; *x, y, z*, MNI coordinates of peak voxel; *k*, cluster size; $P_{FWE\text{-}corr.}$, p-value family-wise error corrected.

Table S10. Contrasts indicating the BOLD signal change from BDC-4 to HDT58 during pattern completion.*

	Region	***Direction of effect***	***x y z***	***k***	***Peak Z***	$P_{FWE\text{-}corr.}$
TRAIN	R SPL	Increase	-32 -58 42	25	3.41	0.897
	R OCP	Increase	16 -94 4	135	4.18	0.042
CTRL	L PrG	Decrease	-38 -16 54	264	4.77	0.002
	R MTG	Decrease	50 -18 -16	29	4.52	0.855
	R PrG	Decrease	30 -6 38	231	4.50	0.005
	R Insular Cortex	Decrease	36 -2 -10	53	4.18	0.512
	L Insular Cortex	Decrease	-34 -10 14	30	4.13	0.841
	L PrG	Decrease	-24 -12 70	37	3.72	0.742
	R PT	Decrease	58 -20 8	24	3.65	0.913
	L PoG	Decrease	-34 -34 62	47	3.64	0.595
Group* x *Time	R PrG		30 -4 42	28	4.12	0.838

* Contrasts refer to comparison between BDC-4 (*Foils > Hits = Lure CR*) and HDT58 (*Foils > Hits = Lure CR*). CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. L, left; R, right; STG, superior temporal gyrus; OFuG, occipital fusiform gyrus; PrG, precentral gyrus; MTG, middle temporal gyrus; PT, planum temporale; PoG, postcentral gyrus; *x, y, z*, MNI coordinates of peak voxel; *k*, cluster size; $P_{FWE\text{-}corr.}$, p-value family-wise error corrected.

2 Supplementary Figure

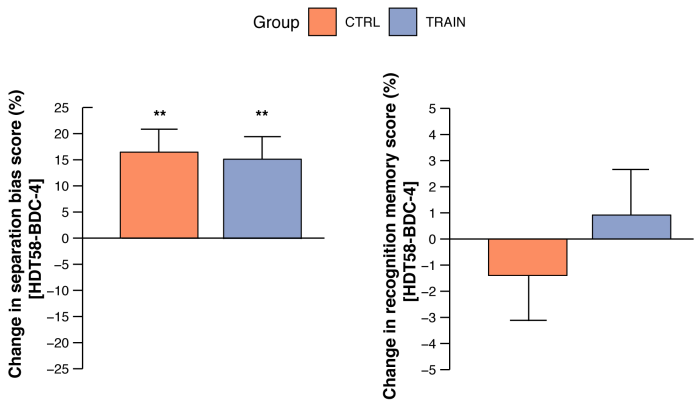

Figure S1. Changes in separation bias and recognition memory score between HDT58 and BDC-4. Data are marginal means and standard errors. CTRL, bed rest control group; TRAIN, exercising bed rest group. n = 11 for each group respectively. ** $P < 0.01$ compared to BDC-4.

3 Discussion

The present work investigated the neurobehavioral responses to long-duration HDBR and potential interventions to mitigate any adverse effects. Given that physical activity has been established as a very potent stimulus for cognitive and brain plasticity, it was hypothesized that prolonged HDBR would impair cognitive performance and brain function. In a series of five ESA and NASA sponsored studies conducted at two sites (at DLR :envihab in Cologne and at MEDES in Toulouse) neurobehavioral data were collected and analyzed in a total of n = 67 healthy adults (10 women). Taken together, these data confirm the initial hypothesis that prolonged HDBR induces significant adverse neurobehavioral effects, some of which do not return to baseline levels after one week of recovery.

The first study used a range of well-established paradigms implemented in NASA's test battery *Cognition* to identify the time course of HDBR on a range of cognitive domains (Basner et al., 2015). A unique feature of this battery is that it allows to statistically adjust for learning effects associated with repeated administrations of the tasks, which can mask potential effects related to the intervention (Basner et al., 2020). The main finding of the study was a decrease in response speed and efficiency across all cognitive tasks. The response slowing could be observed as early as the first day of bed rest. However, except for the emotion recognition task (assessing the ability to identify facial expressions correctly), none of the other nine tests suggested a systematic variation as a function of time in bed, i.e., performance neither improved nor further deteriorated during bed rest (Basner, Stahn, et al., 2021).

Given that the data were recorded in a sitting position before and after bed rest and in –6° HDT during bed rest, these findings suggest that posture rather than the duration of bed rest may account for the changes in cognitive performance of the majority of tasks of the Cognition battery. Posture has been reported to significantly affect brain activity (Shoemaker et al., 2012; Spironelli & Angrilli, 2017; Thibault et al., 2014; Vaitl et al., 1996), visual perception (Harris et al., 2015); pain perception (Fardo et al., 2013), startle reflexes (Messerotti Benvenuti et al., 2011), and emotional processing (Messerotti Benvenuti et al., 2013). These effects may be related to interactions between cognition and the vestibular system (Smith, 2017). Changes in body posture have been shown to induce rapid modifications of the neural circuitry associated

with baroreflex-mediated cardiac control. These brain regions include the anterior cingulate cortex and insula regions (Shoemaker et al., 2012), have close functional connectivity, and are engaged in various cognitive, affective, and behavioral contexts (Medford & Critchley, 2010).

To address this question, electrocortical activity was recorded before and after 60 days of HDBR in sitting position and during bed rest in HDT position in two further bed rest studies (*RSL* and *Cocktail* study). These data revealed an immediate drop in EEG spectral power with the onset of HDBR (as of the first measurement performed in the morning after the first day of HDBR). Source localization analyses revealed significant decreases of electrocortical activity in the alpha and beta frequency bands for a broad cluster of voxels, including but not limited to the bilateral precuneus, posterior cingulate gyrus, and lingual gyrus during HDBR. The reduction did not change throughout 60 days of HDBR until the first measurement during the recovery period when data were collected in supine position again. These findings were observed irrespective of the study site (*RSL* in Cologne vs. *Cocktail* in Toulouse), laterality, intervention groups (exercise, antioxidative supplementation, bed rest only), and frequency bands. Given the immediate attenuation of the EEG signal with the onset of HDBR, it is likely that these effects are related to the physiological manifestations associated with the –6° HDT posture. These effects may include a redistribution of cerebrospinal fluid (CSF), an upward brain shift in response to HDBR, changes in brain oxygenation, and alterations of the cortical neurocircuitry associated with baroreflex function and cardiovascular control. A change in posture from sitting to supine increases intracranial CSF, and CSF layer thickness has been shown to be inversely correlated with EEG spectral power (Alperin et al., 2005; Rice et al., 2013 as cited in Brauns et al., 2021). This is in line with the observation that the decreases in electrocortical activity were slightly but significantly smaller in anterior relative to the posterior brain regions. Head-down tilt posture has also been shown to increase cerebral blood flow (Marshall-Goebel et al., 2016), which in turn may decrease EEG spectral power (Lachert et al., 2017 as cited in Brauns et al., 2021). Likewise, it is also possible that these effects were moderated or mediated by the cortical circuitry associated with baroreflex cardiovascular control. The cardiovascular effects of bed rest are well-established (e.g., Maggioni et al., 2018). Various lines of research have speculated about the potential role of supramedullary sites in the alterations to baroreflex cardiovascular control associated with physical deconditioning. These studies implicate the ventral medial prefrontal and subgenual anterior cingulate gyrus regions in heart rate control, with a consistent inverse relationship between these regions and heart rate and heart rate variability (Critchley et al., 2004; Gianaros et al., 2004, 2012; Thayer

et al., 2012; Wong et al., 2007). Irrespective of these mechanisms accounting for the effects of altered EEG spectral power, these data lend support to the assumption that the cognitive effects observed in the first study are to some extent driven by different postural effects related to the data collections in sitting position before and after bed rest, and in –6° HDT position during bed rest. However, it should be noted that one of the ten tasks of the Cognition battery, the emotion recognition task, used in the first study showed a deterioration with increasing duration of HDBR as characterized by a gradual decline in response speed (Basner, Dinges, et al., 2021).

To elucidate the neurobehavioral of emotional processing in response to prolonged HDBR, another study was performed that investigated the neural bases of emotional processing employing a widely used set of stimuli of pictures that have been standardized relative to their valence, arousal, and dominance and shown to elicit positive and negative affect (Wilson et al., 2020). Briefly, participants were shown a random set of negative, positive, and neutral pictures. To exclude any confounding due to habituation or sensitization (Larson et al., 2000), the task was administered only once after 30 days of HDBR and compared to a control group. Further, to avoid changes in emotional processing related to different body postures, all testing was performed at –6° HDT in both groups, and sufficient time to account for the cephalic fluid shifts (Shirreffs & Maughan, 1994). Whereas no differences were observed for self-reported valence and arousal ratings between the bed rest and control group, neural indices of attention, memory encoding, and storage (late positive potential and P300 recorded in response to the onset of each picture) (Hajcak et al., 2010; Polich, 2007) were blunted for unpleasant and pleasant pictures. Source localization analyses identified the right insula, bilateral precuneus, and bilateral posterior cingulate gyrus as the neural generators of these effects, which is well in line with data with considerable evidence that these brain regions also critically contribute to emotion (Caria et al., 2010; Maddock et al., 2003; Ochsner et al., 2004). Further, the insula, posterior cingulate cortex, and precuneus also support various higher cognitive functions such as inhibitory and attentional control (Arrington et al., 2019; Brázdil et al., 2007; Crottaz-Herbette & Menon, 2006; Gur et al., 2007).

In the next study, it was therefore investigated how HDBR affects selective attention and response inhibition and their neural bases and whether an antioxidant supplement affects these outcomes because antioxidants have been shown to improve cognitive performance across a range of tasks (Ammar et al., 2020). A three-stimulus oddball task was used to elicit two event-related potentials, i.e., P3a and P3b, that indicate attentional orientating and the revision of

mental representations, respectively (Polich, 2007). The task was administered once before HDBR, on the 60th day of HDBR, and after one week of recovery. All testing was performed in –6° HDT posture to facilitate the comparison between sessions. Response efficiency significantly decreased in response to bed rest. Two large clusters of electrodes at the fronto-central and tempoparietal sites revealed significant reductions in P3a and P3b amplitudes. The antioxidant supplementation did not alter these effects, which persisted one after one week of recovery, suggesting that HDBR has considerable adverse effects on selective attention and that these effects do not recover quickly.

The P3a characterizes focal attention, and its generation relies on the integrity of the frontal lobe, whereas the P3b relates to attentional and memory processes in temporal and parietal brain regions (Polich, 2007). Whereas the hippocampus may not directly affect P3a and P3b characteristics, the neural circuitry between the frontal and temporoparietal brain regions seems critical for their generation (Polich, 2007). It should be noted that the avoidance to respond to non-targets and distracters also relates to the ability to suppress prepotent responses. There is increasing evidence from animal and human studies that support the role of the hippocampus for behavioral inhibition and resolving response conflict. These abilities may relate to the role of the hippocampus in pattern separation (Oehrn et al., 2015). Using a fMRI pattern separation paradigm that reliably activates the hippocampus (Kirwan & Stark, 2007), it was therefore investigated whether prolonged HDBR also affects hippocampal activity associated with episodic memory encoding and retrieval.

Given that regular physical activity is a key driver for hippocampal plasticity (Domingos et al., 2021; Erickson et al., 2014; Firth et al., 2018; Ji et al., 2021), it was also hypothesized that a structured exercise program affects the neurobehavioral effects of HDBR. Testing was performed four days before the start of HDBR and on the 58th day of HDBR. Irrespective of the exercise intervention, the ability to identify similar pictures significantly improved at the second test session, suggesting a learning effect because of the repeated task exposure. No significant interactions between HDBR and group (exercise vs. no exercise) were detected for any behavioral outcomes. However, the group receiving the exercise numerically improved in the ability to recognize previously presented pictures. In contrast, the group that did not receive any countermeasure to mitigate the effects of HDBR showed a slight decrease in recognition performance.

Further, analyses of the fMRI data revealed a significant interaction between HDBR and groups, characterized by a decrease of the BOLD signal response in the hippocampus and parahippocampus during the encoding and retrieval phases in the HDBR group that received the exercise. Whereas the direction of the effects may seem counterintuitive, it is in line with research showing that aging and cognitive decline are associated with hyperactive hippocampal signaling during episodic memory formation (Friedl-Werner et al., 2020). Besides a compensatory response reducing the neuronal efficiency, it is also possible that HDBR induced dysfunctional mechanisms of the neural circuitry involved in memory encoding and retrieval, which were mitigated by the exercise program (Friedl-Werner et al., 2020).

Taken together, the research summarized here highlights that the health risks associated with prolonged HDBR go beyond cardiovascular deconditioning and muscle and bone loss. In line with the hypothesis, the studies confirm that HDBR can impair brain function and cognitive performance. The strongest effects were observed for tasks assessing emotion processing, selective attention, and memory formation. Some caution is warranted in situations where cognitive performance is examined in different body positions (i.e., sitting before and after bed rest, and in supine or –6° HDT during bed rest) because it was shown that some of the behavioral and neurophysiological data follow a characteristic pattern that suggests postural effects. It is possible that these effects are to some extent explained by altered reference frames associated with different body postures, as well as the role of cortical circuitry associated with the vestibular system and the autonomic control of cardiovascular reactivity. The complexity of potential mechanisms between HDBR and cognitive performance was also highlighted by recent studies investigating circadian disruptions associated with bed rest. Exercise was found to mitigate circadian delays (Mendt et al., 2021) and alter the neural responses during episodic memory encoding and retrieval (Friedl-Werner et al., 2020). In contrast, neither supplementation with antioxidants nor artificial gravity mediated the effects of HDBR (Basner, Dinges, et al., 2021; Brauns et al., 2021). However, it possible that potential effects were masked by the inability of the outcomes to detect these differences. This may be particularly true for the study investigating the use of AG as a countermeasure. Preliminary data analyses from the NASA project *Effects of Artificial Gravity on Brain Structural and Functional Plasticity During Head-Down Tilt Bed Rest* show that AG counteracts HDBR related reductions in hippocampal plasticity, and it could be potentially more potent than exercise for maintaining brain health during prolonged HDBR (Stahn, Roalf, et al., 2020). It can be

speculated that interventions combining AG with physical activity may reveal the full potential of AG.

The findings and conclusions reached from the studies summarized here could help map the directions for a better understanding of the adverse neurobehavioral effects of HDBR. An interdisciplinary approach should characterize such prospective work, integrating brain neuroimaging, psychological and behavioral, neurovestibular, cardiovascular, biochemical, and circadian data. It could be guided by the National Institute of Mental Health's (NIMH) Research Domain Criteria (RDoC) (Sanislow et al., 2015), a heuristic framework to integrate various levels of methodologies and outcomes from genomics, molecules, cells, and brain imaging to behavior and self-reported data. A single experiment cannot leverage the complexity, expertise, effort, and costs. However, the way bed rest studies are conceptualized can already address such a framework because they typically integrate a series of physiological and behavioral research experiments, including a broad range of cardiovascular, musculoskeletal, vestibular, immunological, and neurobehavioral outcomes. It is intriguing that researchers and space agencies could leverage the integrative nature of such study protocols. Such an approach goes beyond data use agreements between experiments and starts with identifying deliverables using a hypothesis-driven rationale that promotes a holistic understanding of intellectual frameworks that together exceed individual disciplinary perspectives. The combination of various levels of methodologies and outcomes could provide the basis to better understand and characterize the type, extent, cause, and mechanisms of neurobehavioral changes in response to bed rest and their phenotypic signatures. The knowledge, technologies, and tools derived from such work could contribute to the space agencies' goal of providing knowledge, technologies, and tools to enable safe, reliable, and productive human space exploration.

Using bed rest for simulating inactivity associated with space travel also can benefit research and applications on Earth. Exploring the effects of bed rest on cognitive functioning and their mechanisms will promote research on the role of inactivity in health and disease. As highlighted by former Japanese astronaut Chiaki Mukai, space medicine can be considered the ultimate preventive space. It focuses on the health problems before they occur (Mukai, 2009). Along these lines, the use of bed rest as a spaceflight analog allows characterizing the entire process of pathogenesis and convalescence by examining healthy people before they are

exposed to inactivity, monitoring the early onset and time course of any adverse effects, and following subjects up through recovery.

Given that bed rest induces similar physiological processes observed in the aging population, bed rest can be considered a model of aging. Changes that typically evolve over years can be mimicked in time-lapse, providing unique opportunities to explore aging-related diseases and their prevention. In addition, the high standardization and control of bed rest studies (e.g., nutrition, sleep/wake cycles, and social activities) could promote the understanding of causal relationships between inactivity, brain function, and cognitive alterations. Collectively, these characteristics can translate the knowledge gained from bed rest studies to the prevention and treatment of various clinical conditions associated with cognitive impairments, and for which reduced physical activity levels are a critical risk factor such as chronic heart failure, Type II diabetes, obesity, myotonic dystrophy, fibromyalgia, various types of cancer, depression, anxiety, and dementia.

4 Summary

As space-faring nations across the globe are fueling a new race of human space exploration that goes well beyond the Moon, national agencies and private entities across the globe have accelerated the research and development that will promote the safety and success of such missions. Prolonged body unloading and reduced physical activity levels associated with space travel could adversely affect brain and behavior. Long-duration bed rest in –6° head-down tilt (>1 month) is an established spaceflight analog on Earth to simulate the physiological and psychological adaptations of prolonged inactivity and headward fluid shift during space travel. The present work investigated the effects of long-duration bed rest on brain function and cognitive performance. In a series of five studies combining behavioral, electrocortical and magnetic resonance imaging data it was shown that bed rest can induce significant functional brain changes and cognitive impairments including emotion processing, memory formation, and selective attention, and that these effects may not recover quickly. Structured physical activity programs superimposed to bed rest were found to mitigate cognitive impairments. The benefits of antioxidant supplementation and artificial gravity or their combination with exercise remain to be determined. Some caution is warranted when behavioral data are collected in different body positions, i.e., seated vs. head-down tilt, because the neurophysiological reactions associated with postural changes may mask the effects attributed to physical inactivity. Future work in this field should be characterized by an interdisciplinary approach, integrating multimodal brain imaging, psychological and behavioral, neurovestibular, cardiovascular, biochemical, and circadian data. Such an approach could promote a holistic understanding of intellectual frameworks that together exceed individual disciplinary perspectives. The knowledge from such approaches could go beyond their application to spaceflight. It can translate to the prevention and treatment of various clinical conditions associated with cognitive impairments, and for which reduced physical activity levels are a critical risk factor.

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Acknowledgment

First and foremost, I would like to acknowledge DLR and NASA for funding my research. It is my pleasure to thank Drs. Markus Braun, Peter Gräf, Christian Rogon and Katrin Stang from DLR management, and Drs. Thomas Williams and Alexandra Whitmire from NASA's Human Research Program for their trust in me and my work and their continuous support and advice.

I would like to extend my gratitude to the DLR Institut für Luft- und Raumfahrtmedizin in Cologne, Germany, and the Institut de Médecine et de Physiologie Spatiales (MEDES) in Toulouse, France, for organizing and carrying out the experiments on behalf of ESA and NASA. They have been instrumental in defining and assisting the implementation of my research and shaping the project outcomes.

Special thanks also go to former Director of the Institute of Physiology (IfP) at Charité – Universitätsmedizin Berlin, Professor Axel Pries (now Dean of Charité – Universitätsmedizin Berlin), and his successor Professor Wolfgang Kübler, and the Director of the Unit of Experimental Psychiatry (UEP) at the Perelman School of Medicine at the University of Pennsylvania, Professor David F. Dinges, for the opportunity to undertake my studies, and providing assistance at every stage of my research.

I'm deeply indebted to Professor Hanns-Christian Gunga, speaker of the Center for Space Medicine and Extreme Environments Berlin, for his unlimited support, guidance, and exceptional opportunities for fostering my career in space life sciences. He taught me the fundamentals of gravitational physiology, introduced me to DLR, ESA, and NASA, provided me opportunities to implement research on ISS, and get first-hand experiences of working with astronauts. He took me to new heights in the Atacama Desert, valued my initiatives to engage in collaborative efforts with the South African Department of Environmental Affairs and Stellenbosch University, and laid the basis for my research on brain and behavior in Antarctica. He has always endorsed new and innovative ideas in and outside his realm of research. His generosity, open-mindedness, genuine interest, and tolerance were fundamental in building an independent line of research of neurobehavioral science in extreme environments. I consider myself exceptionally lucky and privileged for having his support and experiencing an atmosphere of unlimited individual scientific growth and development. It was also him who introduced me to Professor Karl Kirsch. The numerous discussions with him and his

encouragement for believing in my data helped me develop a fundamental understanding of integrative physiology and gain invaluable confidence in refining my own line of research.

I would like to extend my sincerest gratitude to Professor David F. Dinges for his profound belief in me and my line of research. Recruiting me to the Perelman School of Medicine at the University of Pennsylvania was an invaluable honor and milestone in my academic trajectory and personal life. Following Professor Martin T. Orne's legacy, he taught me the significance of critical thinking, stating controversial positions, and objective scientific data. His priceless professional and personal care and advice have encouraged me in my academic research and daily life. His commitment to excellent science, fearless questioning of concepts, diligence, expertise in neurobehavioral research, experience with all major US funding agencies, and network within and outside the University of Pennsylvania have been a constant source of inspiration. They provided limitless invaluable opportunities to shape my skills and knowledge and push my scientific career farther than I thought I could go. Most importantly, he has given my family and me a new home. We could not have had a warmer welcome when we moved to Philadelphia and experience unprecedented support as a family. The way he has affected my family and me is a priceless gift that will shape our lives forever.

My appreciation extends to all the staff and faculty whose assistance was pivotal in completing my work. I would like to acknowledge Drs. Martina Maggioni, Mathias Steinach, and Oliver Opatz, Sylvia Plog and Adrian Ecker. I have been supported by three outstanding and highly talented students, Katharina Brauns, Anika Friedl-Werner, and Stefan Mendt. They played a decisive role in each stage of the project, from the conceptualization and procedures to the experiments' final analysis. Their dedication, loyalty, motivation, immense commitment, management, and team skills are unparalleled. It has been a true privilege to see them grow personally and academically during the last five years. Special thanks also go to Professors Simone Kühn from the Max-Planck Institute for Human Development Berlin and Mathias Basner from the Division of Sleep and Chronobiology at the University of Pennsylvania for their collaborative efforts, continuous discussions, sharing their highly unique expertise, and providing treasured advice and contributions for maximizing the scientific output and deliverables.

My sincere and deepest gratitude goes to my family and in-laws for their love and unwavering support, patience, and understanding. I would like to thank them for encouraging me to pursue

my dreams throughout my life, appreciate and respect these goals, and always support and be there for my family and me.

Above all, I wish to wholeheartedly thank my wife, Daria, the most important person and greatest supporter in my life. I am deeply indebted to her unconditional support and genuine interest in my work. She has been the most skillful and relentless listener, raising vital questions, discussing my concerns, and constantly inspiring my research. She has stepped back from her own professional and personal commitments to support my aspirations. She took care of our family when I was away during various business trips, including stays in Antarctica, worked overtime to meet project and grant application deadlines, missed several family occasions, had my fits, was short-tempered, and felt broken. Yet, she has always stood by me through challenging times with a smile, lifted me up and kept me moving forward no matter how hard the hits were. I dedicate this work to her and our wonderful sons Carlo, Luca, Antonio, and Matteo. You have taught me what love and life are all about.

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

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Datum Unterschrift