Ophthalmic Changes in a Spaceflight Analog Are Associated with Brain Functional Reorganization

Heather R. McGregor, PhD [1], Jessica K. Lee, PhD [1,2], Edwin R. Mulder, PhD [2], Yiri E. De Dios, B.S.[3], Nichole E. Beltran, M.S.[3], Igor S. Kofman, M.S.[3], Jacob J. Bloomberg, PhD [4], Ajitkumar P. Mulavara, PhD [3] & Rachael D. Seidler, PhD [1*]

[1] Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL;
[2] Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany;
[3] KBR, Houston, TX;
[4] NASA Johnson Space Center, Houston, TX;

*Corresponding author:
Rachael D. Seidler, Ph.D.
University of Florida
1864 Stadium Rd.
Gainesville, FL 32611
Phone: (352) 294-1722
Email: rachaelseidler@ufl.edu

Author Contributions: HRM analyzed fMRI data. JKL collected data. JKL, YED, NEB, ISK set up the experiment. RDS, ERM, JJB, APM designed the experiment and secured funding. HRM drafted the manuscript. HRM, JKL, JJB, APM, RDS edited the manuscript.

KEY POINTS

Question Does optic disc edema development during head-down tilt bed rest with elevated carbon dioxide impact brain resting-state functional connectivity?

Findings A subset of participants developed optic disc edema during the head-down tilt bed rest intervention with elevated ambient CO_2. Participants who developed optic disc edema exhibited a distinct pattern of resting-state functional connectivity changes within visual and vestibular-related networks during the spaceflight analog compared to participants who did not. Participants who developed optic disc edema exhibited different resting-state brain activity prior to the spaceflight analog within a visual cortical network and within a large-scale network of brain areas involved in multisensory integration.

Meaning Development of optic disc edema was associated with distinct patterns of brain resting-state functional connectivity during and prior to the spaceflight analog.

Funding/Support: NASA grant #80NSSC17K0021. HRM was supported by a NSERC postdoctoral fellowship and a NASA HRP augmentation grant.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Declaration of Interests: None

Keywords: bed rest, spaceflight, CO_2, optic disc edema, SANS, resting-state fMRI, functional connectivity

ABSTRACT

Importance Following long-duration missions onboard the International Space Station, some astronauts develop ophthalmic abnormalities collectively referred to as Spaceflight Associated Neuro-ocular Syndrome (SANS). Optic disc edema is a common sign of SANS. SANS presents significant potential risk to astronaut health and performance; however, the origin and effects of SANS are not understood as signs of SANS have not manifested in previous spaceflight analog studies.

Objective To investigate whether development of optic disc edema during a spaceflight analog impacts resting-state functional connectivity.

Design, Setting and Participants Eleven healthy volunteers participated in this 58-day longitudinal study conducted at the :envihab facility at the German Aerospace Center.

Interventions or Exposures Baseline data were collected during a 14-day ambulatory phase in standard ambient air. All participants then underwent a spaceflight analog intervention: 30 days of strict head-down tilt bed rest in elevated ambient carbon dioxide (HDBR+CO_2). The elevated CO_2 level (0.5%) was matched to the hypercapnic environment of the International Space Station. The intervention was followed by a 14-day ambulatory recovery phase in standard ambient air. During the HDBR+CO_2 spaceflight analog, 5 participants developed optic disc edema (SANS subgroup) and 6 did not (NoSANS group).

Main Outcomes and Measures Using functional magnetic resonance imaging (fMRI), we acquired resting-state data at 6 time points throughout the study: before (2), during (2), and after (2) the HDBR+CO_2 intervention. We assessed the time course of resting-state functional connectivity changes from before, during, to after the HDBR+CO_2, and contrasted longitudinal changes between the SANS and NoSANS subgroups. We also assessed if the SANS and NoSANS subgroups exhibited differential patterns of resting-state functional connectivity prior to the HDBR+CO_2 intervention.

Results The SANS and NoSANS subgroups exhibited differential patterns of resting-state connectivity changes during the HDBR+CO_2 spaceflight analog within visual and vestibular-related brain networks. We further found that the SANS and NoSANS subgroups exhibited differential resting-state brain activity prior to the spaceflight analog within a visual cortical network and within a large-scale network of brain areas involved in multisensory integration.

Conclusions and Relevance Subgroup differences in resting-state functional connectivity changes may reflect differential patterns of visual and vestibular reweighting as optic disc edema develops during the HDBR+CO_2 spaceflight analog. This finding suggests that SANS impacts not only neuro-ocular structures, but also brain function. Future prospective investigations incorporating sensory assessments are required to determine the functional significance of the observed connectivity differences.

INTRODUCTION

As NASA prepares for the first human mission to Mars, it is critical to understand how spaceflight affects the brain and body. During long-duration missions onboard the International Space Station (ISS), some astronauts develop ophthalmic abnormalities including optic disc edema, choroidal folds, cotton wool spots, posterior globe flattening, distention of the optic nerve sheath [1–3]. These findings have been collectively termed Spaceflight Associated Neuro-ocular Syndrome (SANS). The origin and effects of SANS are not understood, and present significant potential risk to astronaut health and performance. Here we explored whether the development of signs of SANS during a spaceflight analog is associated with altered brain functional organization.

The effects of spaceflight are often studied using ground-based analogs, with head down-tilt bed rest (HDBR) being one of the most widely used. Head-down tilt posture simulates several effects of microgravity including headward fluid shift and axial body unloading. Headward fluid shift is hypothesized to be a contributing factor of SANS [4]. Spaceflight alters cerebrospinal fluid hydrodynamics [5], and results in ventricular volume increases [5–7] which have been associated with ocular globe deformation [6] and visual acuity decreases [7]. However, until recently, signs of SANS have not manifested in any spaceflight analog [8]. Another potential contributing factor to SANS development during spaceflight is the mild hypercapnic environment of the ISS [9] where CO_2 levels (0.5%) are approximately 10 times higher than on Earth [10]. In order to better replicate the ISS environment, NASA conducted the current study employing 30 days of strict HDBR in 0.5% ambient CO_2 (HDBR+CO_2). A subset of participants developed optic disc edema -- a sign of SANS -- by the end of the intervention [3,11,12]. These ophthalmic findings have been described in detail elsewhere [11,12].

The HDBR+CO_2 intervention used in the current study was thus the first spaceflight analog to induce signs of SANS. This occurrence afforded us the unique opportunity to explore functional brain differences between participants who developed optic disc edema (SANS subgroup) during the spaceflight analog and those who did not (NoSANS subgroup). We show that the SANS subgroup exhibited a distinct pattern of resting-state connectivity changes involving visual and vestibular brain regions during the HDBR+CO_2 intervention. Recent work has shown that genetic and B vitamin status are predictors of optic disc edema development during this HDBR+CO_2 intervention [12,13], and during spaceflight [14,15]. In light of this, we further investigated resting-state connectivity differences between the SANS and NoSANS subgroup prior to bed rest. Indeed, the SANS and NoSANS subgroups exhibited differential resting-state brain activity before bed rest within a visual cortical network and within a large-scale network of brain areas involved in multisensory integration. While preliminary, these findings suggest

that the development of optic disc edema during this spaceflight analog is associated with functional brain reorganization.

METHODS

Participants

Eleven participants completed the study. Five participants developed optic disc edema by the end of the HDBR+CO_2 intervention ("SANS subgroup"), and 6 did not ("NoSANS subgroup"). Participant demographics are presented in eTable 1. All participants provided written informed consent to study protocols approved by the local medical association (Ärztekammer Nordrhein) and institutional review boards at The University of Florida and NASA Johnson Space Center.

Study Design

The study design is shown in Figure 1A and has been detailed in the eMethods and elsewhere [16]. Baseline data collection (BDC) occurred during a 14-day ambulatory phase in standard (i.e., not hypercapnic) ambient air. Participants then underwent 30 days of strict 6° HDBR in 0.5% CO_2 (HDBR+CO_2). During the HDBR+CO_2 phase, participants remained in the head down tilt position at all times and were not permitted to use a standard pillow. The bed rest intervention was followed by a 14-day ambulatory recovery (R) phase in standard ambient air. See eMethods for further details.

Resting-state fMRI scans were acquired at six time points: 13 and 7 days before bed rest (BDC-13 and BDC-7, respectively), on days 7 and 29 of HDBR+CO_2 (HDT7 and HDT29, respectively), and 5 and 12 days post-bed rest (R5 and R12, respectively).

Using this same dataset, we have recently reported group-wise resting-state connectivity changes throughout the HDBR+CO_2 intervention as well as brain-behavior relationships [16].

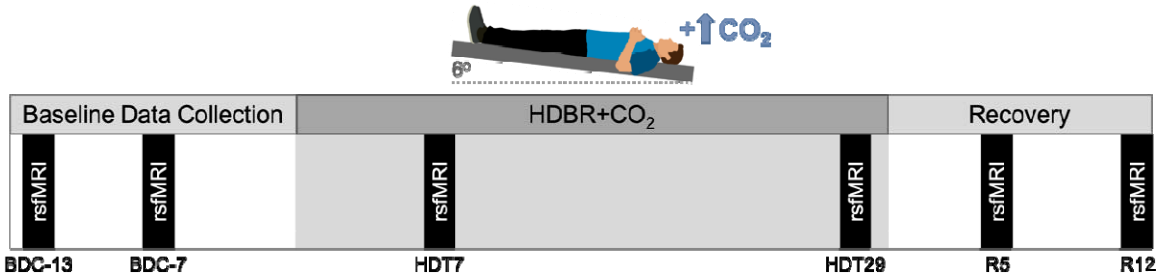

Figure 1. Baseline data collection (BDC) was carried out during a 14-day ambulatory phase in standard ambient air. Participants then underwent 30 days of strict 6° head-down tilt bed rest with 0.5% ambient carbon dioxide (HDBR+CO_2). The bed rest intervention was followed by a 14-day ambulatory recovery

(R) phase in standard ambient air. Resting-state fMRI scans were acquired on BDC-13, BDC-7, HDT7, HDT29, R5, R12.

Resting-state fMRI analyses

A detailed analysis description has been described in eMethods and elsewhere [16]. Briefly, image preprocessing in CONN followed a standard resting-state fMRI pipeline including slice timing correction, motion correction, and segmentation into grey matter, white matter, and CSF. Functional and anatomical images were then coregistered and normalized to MNI standard space. To improve normalization of the cerebellum, the cerebellum was extracted from each native space anatomical scan and preprocessed functional image using CERES [17], coregistered, normalized to the SUIT cerebellum template [18], and the functional image was smoothed (see eMethods for details).

Denoising in CONN was performed in CONN using the ARTifact detection tool to detect volumes where head movement was excessive, and using aCompCor to create nuisance regressors modelling non-neuronal physiological noise[19]. Functional data were then band-pass filtered between 0.008–0.09 Hz [20,21].

Subject-level analyses

We used a hypothesis-driven approach to examine resting-state functional connectivity (FC) between 14 regions of interest (ROIs) and the rest of the brain. Drawing upon previous spaceflight and bed rest studies, we hypothesized that the two subgroups would exhibit differential patterns of FC involving brain areas related to multisensory integration, visual and sensorimotor processing, and regions within the default mode network [22–28]. ROIs were 8mm spheres centered on coordinates presented in eTable 1. For each resting-state run and each ROI, FC was estimated by computing the correlation coefficient between the time series of the ROI and that of every other voxel in the brain. An uncorrected voxel threshold was set at $p < 0.001$. Analyses used a cluster-size threshold of $p < 0.05$ (two-tailed), and were corrected for multiple comparisons according to the FDR method. We also used a hypothesis-free voxel-to-voxel analysis (see eMethods).

Subgroup Differences in Longitudinal Changes in Functional Connectivity

We first examined if the SANS and NoSANS subgroups exhibited differential patterns of FC changes from before, during, to after the HDBR+CO_2 intervention. In our general linear model (GLM), subgroup was used as a between-subjects factor and session was used as a within-subjects factor (i.e. 6 time points). We created a within-subjects contrast model which was informed by the time course of optic disc edema onset and recovery [3]. Laurie and colleagues

measured total retinal thickness in these same participants using optical coherence tomography on days 1, 15, and 30 of HDBR and on the 6th and 13th day of the recovery phase. The SANS subgroup showed a gradual increase in retinal thickness during HDBR+CO_2 which was followed by a gradual post-bed rest decrease towards baseline values [3]. Our longitudinal analysis aimed to identify brain networks in which FC changes mirrored the time course of optic disc edema onset and recovery. We thus used a within-subject contrast model which identified brain areas in which FC was stable across the baseline phase (BDC-13, BDC-7) and then showed a linear FC change throughout HDBR+CO_2, followed by a gradual return towards baseline in the subsequent recovery phase (eFigure 1). Participant age and sex were included in the model as covariates.

Subgroup Differences in Functional Connectivity at Baseline

In light of recent work identifying genetic and biochemical predictors of optic edema development in these same participants, we also tested if there were FC differences between the SANS and NoSANS subgroups during the BDC phase. This analysis aimed to uncover functional brain networks in which resting-state activity was predictive of which participants would develop signs of SANS in the subsequent HDBR+CO_2 intervention. Our GLM for this analysis included subgroup as a between-subjects factor and baseline sessions (i.e., BDC-13, BDC-7) as a within-subjects factor. The within-subjects contrast modeled the average FC across the 2 BDC sessions. Confounding variables included participant age and sex.

For each identified network, we assessed the reliability of FC values from BDC-13 to BDC-7 by computing the intraclass correlation coefficient (ICC) for each unique ROI-cluster connection (see eMethods).

RESULTS

Subgroup Differences in Longitudinal Changes in Functional Connectivity

Our hypothesis-based seed-to-voxel analyses revealed that the ROI in right posterior parietal cortex (PPC) and clusters within bilateral insular cortices exhibited differential longitudinal changes between the SANS and NoSANS subgroups (Figure 2A). The NoSANS subgroup showed decreased FC between these regions during HDBR+CO_2 followed by a post-bed rest FC increase. In contrast, the SANS subgroup showed the opposite pattern, increasing FC during HDBR+CO_2 followed by a post-bed rest decrease.

Furthermore, the ROI in left PCC exhibited differential longitudinal changes with right V1 between the two subgroups (Figure 2B). The NoSANS subgroup showed a FC increase between these regions during HDBR+CO_2 which decreased after bed rest whereas the SANS

subgroup showed decreased FC between these regions during HDBR+CO_2 followed by a post-bed rest reversal.

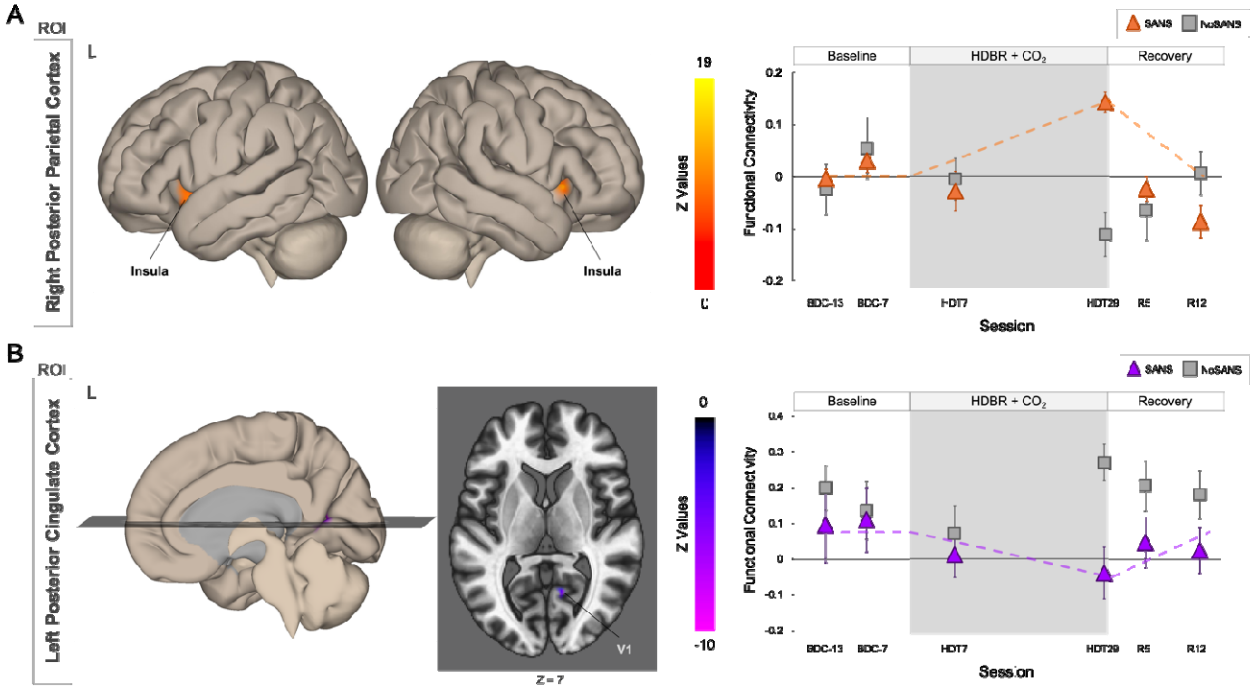

Figure 2. Differential longitudinal FC changes between the SANS and NoSANS subgroups. A) The NoSANS subgroup (gray squares) showed a FC decrease between the ROI in right posterior parietal cortex (PPC) and clusters in bilateral insular cortices during HDBR+CO_2 followed by a post-bed rest reversal. The SANS subgroup (triangles) exhibited FC increases between these regions during HDBR+CO_2 followed by a post-bed rest decrease. **B)** The NoSANS subgroup (gray squares) showed a FC increase between the ROI in left posterior cingulate cortex (PCC) and right primary visual cortex (V1) during HDBR+CO_2 followed by a post-bed rest reversal. The SANS subgroup (triangles) exhibited a FC decrease between these regions during HDBR+CO_2 followed by a post-bed rest increase. The dashed line on each graph represents the hypothesized longitudinal model. Error bars represent standard error. L, left; R, right; ROI, region of interest; FC, functional connectivity; SANS, spaceflight associated neuro-ocular syndrome; HDBR+CO_2, head down tilt bed rest with elevated CO_2.

Subgroup Differences in Functional Connectivity Before HDBR+CO_2

We also identified two brain networks in which average FC across the baseline scan sessions significantly differed between the SANS and NoSANS subgroups. The first network, shown in Figure 3A, consisted of right primary visual cortex (V1) and higher order visual areas V3 and V4 within the left hemisphere. The NoSANS subgroup exhibited positive connectivity between these visual brains areas at baseline. In contrast, the SANS subgroup exhibited lower FC or an

anti-correlation (i.e., negative FC) between right V1 and left V3 and V4 relative to the NoSANS subgroup (see eTable 4). The second network, identified using left insular cortex as a seed region, encompassed left primary motor cortex (M1), lateral occipital cortex (LOC), inferior and middle temporal gyri (ITG/MTG), parahippocampal gyrus (PHG), and right cerebellar lobule 7b. As shown in Figure 3B, the NoSANS subgroup exhibited FC within this network during the baseline phase. The SANS subgroup exhibited lower FC or an anti-correlation (i.e., negative FC) within this network during the baseline phase (see eTable 5).

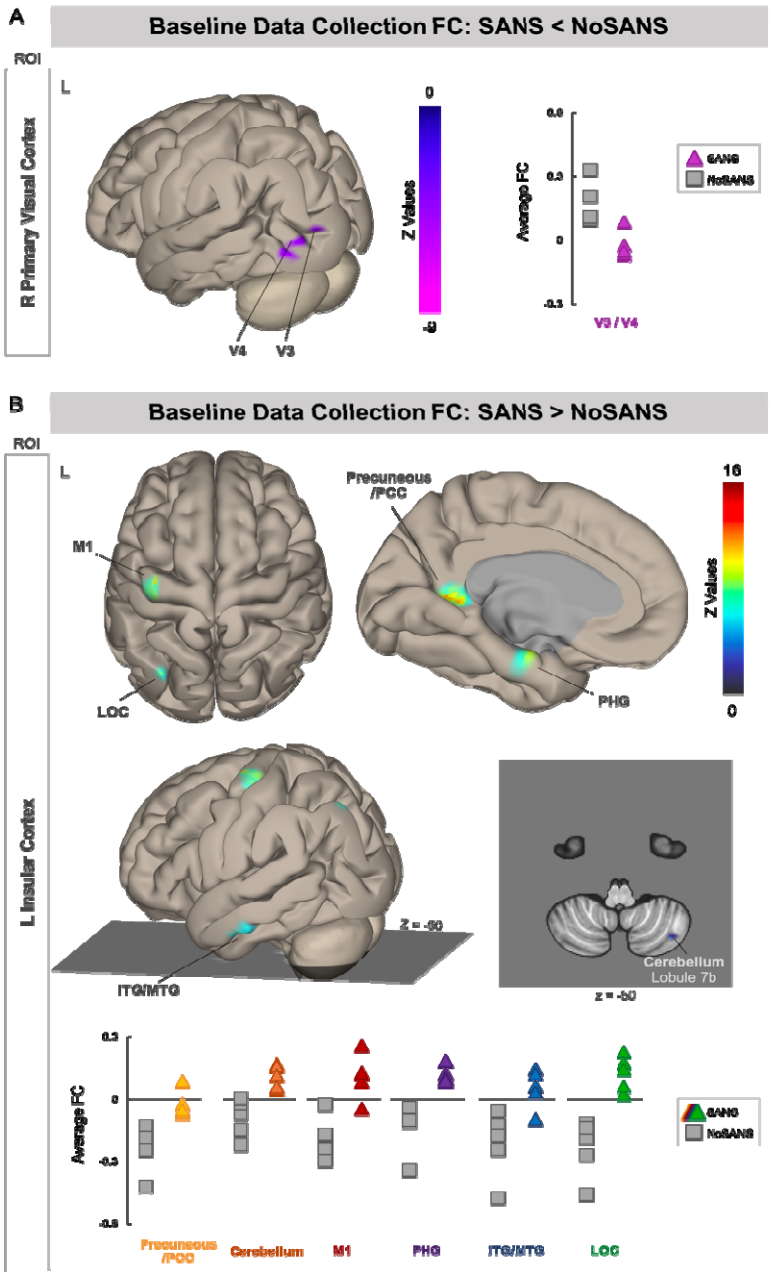

Figure 3. Pre-bed rest FC differences between the SANS and NoSANS subgroups. A) During the baseline phase, the NoSANS subgroup (gray squares) exhibited FC between the ROI in right primary visual cortex (V1) and visual areas V3 and V4 within the left hemisphere. The SANS subgroup exhibited an anti-correlation or lower FC between visual brain areas prior to bed rest (colored triangles) prior to the HDBR+CO_2 intervention. **B)** During the baseline phase, the NoSANS subgroup (gray squares) exhibited anti-correlations between the ROI in left insular cortex and left primary motor cortex (M1), lateral occipital cortex (LOC), inferior and middle temporal gyri (ITG/MTG), parahippocampal gyrus (PHG), precuneous/posterior cingulate cortex (PCC), and right cerebellar lobule 7b. The SANS subgroup (colored triangles) exhibited greater FC than the NoSANS subgroup during the baseline phase. FC, functional connectivity; L, left; R, right; ROI, region of interest; SANS, spaceflight associated neuro-ocular syndrome.

For the networks presented in Figure 3, we computed ICCs to assess the test-retest reliability of FC between BDC-13 and BDC-7 sessions (see eMethods). eTable 6 shows ICCs for each unique ROI-cluster connection. For the network shown in Figure 3A, the ICC analysis yielded substantial test-retest reliability. For the network shown in Figure 3B, ICC analyses yielded fair (0.2-0.4) to almost perfect (>0.8) test-retest reliability across baseline sessions although confidence intervals were large.

DISCUSSION

Subgroup Differences in Longitudinal Changes in Functional Connectivity

Here we show that the development of signs of SANS during a spaceflight analog is associated with a distinct pattern of resting-state FC changes involving visual and vestibular brain regions.

During the HDBR+CO_2 intervention, the SANS subgroup exhibited an increase in FC between PPC and the insular cortices which reversed post-bed rest. The NoSANS subgroup exhibited the opposite pattern, showing FC decreases during HDBR+CO_2 which reversed following bed rest. Posterior parietal cortex (PPC) is a region in which multisensory integration occurs, combining inputs from the visual, somatosensory and auditory systems [29]. The insula is a region within the vestibular system [30], which uses multisensory integration for self-motion perception, spatial orientation, gaze stabilization, and postural control [31]. During spaceflight and HDBR, the vestibular system receives altered multimodal sensory cues and it is thought that the brain adaptively reweights sensory inputs [32–34]. The brain increases the contribution of reliable sensory inputs to performance while decreasing contributions of altered or unreliable inputs [35]. The current results suggest differential patterns of vestibular reweighting during the HDBR+CO_2 between the SANS and NoSANS subgroups. The SANS subgroup's coupling between PPC and the insula may reflect progressively increased weighting of vestibular inputs during the spaceflight analog as signs of SANS develop. However, we did not find subgroup differences in vestibular processing in response to pneumatic skull taps from pre- to post-HDBR+CO_2 [36]. Future studies incorporating behavioral metrics of vestibular input reliance are required to investigate the functional significance of this finding.

In addition, the SANS subgroup exhibited reduced FC between PCC and V1 during HDBR+CO_2 and then recovered post-bed rest whereas the NoSANS subgroup exhibited FC increases between these regions during HDBR+CO_2. The posterior cingulate cortex (PCC) is a key region within the default mode network. Although its function remains a topic of debate, recent theories suggest that the PCC is involved in task-independent introspection and self-referential processes [37]. Primary visual cortex (V1) is the first cortical stage of the visual system wherein

low-level visual feature processing occurs [38]. We have recently provided evidence that the SANS subgroup became increasingly visually dependent during HDBR+CO_2 while the NoSANS subgroup became less so [39]. Performance on rod and frame tests during HDBR+CO_2 showed that the SANS subgroup's perception of verticality became increasingly affected by the tilt of the visual frame. Moreover, the SANS subgroup exhibited better post-bed rest balance control than the NoSANS subgroup during trials with the eyes open. These findings suggest that participants who developed signs of SANS increased their reliance on visual input during the HDBR+CO_2 intervention [39]. While counterintuitive, the SANS subgroup's increased visual dependence and the identified FC decreases during the spaceflight analog may reflect compensation in response to optic disc edema development.

Notably, recent research has demonstrated spaceflight-related functional connectivity changes involving a subset of those regions identified by our longitudinal analyses, namely the insula and posterior cingulate cortex. A case study of a single cosmonaut showed decreases in intrinsic connectivity within the right insula and within PCC from pre- to post-flight [40]. Moreover, a task-based connectivity study showed post-flight connectivity increases between the left and right insular cortices during plantar stimulation[41]. While it is unknown whether the cosmonauts who participated in these studies developed SANS during spaceflight, these studies have also provided evidence for vestibular cortex reorganization and multisensory reweighting following spaceflight.

Subgroup Differences in Functional Connectivity Before HDBR+CO_2

We also identified two functional networks in which FC during the baseline phase was predictive of which participants would subsequently develop signs of SANS during HDBR+CO_2.

Participants who exhibited higher FC between the ROI in right primary visual cortex (V1) and left visual areas V3 and V4 during the baseline phase were less likely to develop signs of SANS during HDBR+CO_2. Participants who lacked this coupling between primary and higher order visual areas during the baseline phase were more likely to develop signs of SANS during HDBR+CO_2. V1 processes low-level visual stimuli [38] while higher level visual processing subserving visual recognition and visual attention are believed to occur within visual areas V3 and V4 [42].

We further identified a functional network consisting of left insula, primary motor cortex, lateral occipital cortex, inferior and middle temporal gyri, parahippocampal gyrus, precuneous/posterior cingulate cortex, and right cerebellar lobule 7b. Participants who exhibited greater decoupling between the insula and the other regions within the network during the baseline phase were less likely to develop signs of SANS during HDBR+CO_2. In

contrast, participants who exhibited coupling among these regions during the baseline phase were more likely to develop signs of SANS during HDBR+CO_2. Many of the brain areas within this network support cognitive functions including visual perception, visuospatial processing, memory, and navigation [43–45].

A genetic predisposition to optic disc edema development has been identified in the SANS subgroup of this study [12,13] as well as in astronauts who develop SANS [46]. Low levels of B-vitamins (B_6, B_{12}, and folate) and 1-carbon pathway risk alleles (linked to homocysteine levels) predict optic disc edema during HDBR+CO_2 [12,13] and spaceflight [14,15]. In healthy older adults, lower levels of vitamin B_{12} and high levels of homocysteine are associated with greater whole brain [47] and cerebral white matter atrophy [48], respectively. It is thus feasible that subgroup differences in B vitamin levels and risk alleles in the current study [12,13] contribute to the structural connectivity differences at baseline. As there exists a close correspondence between brain structural connectivity and functional connectivity [49], it is possible that the observed pre-bed rest FC differences may arise from subgroup differences in structural connectivity within the identified networks. However, if this were the case, one would expect the SANS subgroup to exhibit lower FC compared to the NoSANS group for both networks. Thus, future research is required to further investigate the causes(s) of SANS, and whether or how these pre-bed rest FC differences relate to the cause(s).

Limitations

The small sample size is a significant limitation of our work. This study was the first long-duration bed rest study to implement strict HDBR and use a mild hypercapnic environment. As such, we are unable to determine whether the development of optic disc edema was due to elevated CO_2 levels, strict HDBR, or a combination thereof. Moreover, optic disc edema in the SANS subgroup was more pronounced than typically seen in astronauts post-flight [3]. In addition, cases of SANS in astronauts typically include other ophthalmic changes including globe flattening, choroidal folds, cotton wool spots, etc [1]. Thus it remains unclear how closely the signs of SANS we observed here mimic those seen in astronauts.

Conclusions

Visual deterioration during long-duration spaceflight represents a significant potential risk to human health and performance on current and future missions. Therefore, despite the limitations noted above, these data have provided important insights into the underlying cause of SANS[14,15] and understanding the breadth of its effects. This is particularly the case given that prior work has only related SANS to central nervous system structures, and not to connectivity or activity.

Here we have shown that the SANS and NoSANS subgroups exhibited differential resting-state brain activity prior to the spaceflight analog within a visual cortical network and within a large-scale network of brain areas involved in multisensory integration. We also showed that the development of signs of SANS during the spaceflight analog is associated with differential patterns of resting-state connectivity changes within visual and vestibular-related brain networks. This finding suggests that SANS impacts not only neuro-ocular structures, but also brain function.

REFERENCES

1. Mader TH, Gibson CR, Pass AF, et al. Optic disc edema, globe flattening, choroidal folds, and hyperopic shifts observed in astronauts after long-duration space flight. *Ophthalmology*. 2011;118(10):2058-2069.

2. Lee AG, Mader TH, Gibson CR, et al. Spaceflight associated neuro-ocular syndrome (SANS) and the neuro-ophthalmologic effects of microgravity: a review and an update. *NPJ Microgravity*. 2020;6:7.

3. Laurie SS, Lee SMC, Macias BR, et al. Optic Disc Edema and Choroidal Engorgement in Astronauts During Spaceflight and Individuals Exposed to Bed Rest. *JAMA Ophthalmol*. Published online December 26, 2019. doi:10.1001/jamaophthalmol.2019.5261

4. Stenger MB, Tarver WJ, Brunstetter T, Gibson C R, Laurie SS, Lee SMC, Macias BR, Mader TH, Otto CA, Smith SM, Zwart SR. *NASA Human Research Program Evidence Report: Risk of Spaceflight Associated Neuro-ocular Syndrome (SANS) (No. 2.0)*. NASA Johnson Space Center; 2017.

5. Kramer LA, Hasan KM, Stenger MB, et al. Intracranial Effects of Microgravity: A Prospective Longitudinal MRI Study. *Radiology*. 2020;295(3):640-648.

6. Alperin N, Bagci AM. Spaceflight-Induced Visual Impairment and Globe Deformations in Astronauts Are Linked to Orbital Cerebrospinal Fluid Volume Increase. *Acta Neurochir Suppl*. 2018;126:215-219.

7. Van Ombergen A, Jillings S, Jeurissen B, et al. Brain ventricular volume changes induced by long-duration spaceflight. *Proc Natl Acad Sci U S A*. 2019;116(21):10531-10536.

8. Taibbi G, Cromwell RL, Zanello SB, et al. Ocular Outcomes Comparison Between 14- and 70-Day Head-Down-Tilt Bed Rest. *Invest Ophthalmol Vis Sci*. 2016;57(2):495-501.

9. Stenger MB, Tarver WJ, Brunstetter T, Gibson CR, Laurie SS and Lee, Stuart and Macias, Brandon R and Mader, Thomas H and Otto, Christian and Smith, Scott M. Evidence report: risk of Spaceflight Associated Neuro-ocular Syndrome (SANS). Published online 2017.

10. Law J, Van Baalen M, Foy M, et al. Relationship between carbon dioxide levels and reported headaches on the international space station. *J Occup Environ Med*. 2014;56(5):477-483.

11. Laurie SS, Macias BR, Dunn JT, et al. Optic Disc Edema after 30 Days of Strict Head-down Tilt Bed Rest. *Ophthalmology*. 2019;126(3):467-468.

12. Zwart SR, Laurie SS, Chen JJ, et al. Association of Genetics and B Vitamin Status With the Magnitude of Optic Disc Edema During 30-Day Strict Head-Down Tilt Bed Rest. *JAMA*

Ophthalmology. 2019;137(10):1195. doi:10.1001/jamaophthalmol.2019.3124

13. Smith S, Laurie S, Young M, Zwart S. MTRR 66 and SHMT1 1420 Variants Are Associated with Optic Disc Edema During 30-d Strict Head-down Tilt Bed Rest and CO2 Exposure (P24-036-19). *Current Developments in Nutrition*. 2019;3(Supplement_1). doi:10.1093/cdn/nzz044.p24-036-19

14. Zwart SR, Gibson CR, Gregory JF, et al. Astronaut ophthalmic syndrome. *The FASEB Journal*. 2017;31(9):3746-3756. doi:10.1096/fj.201700294

15. Smith SM, Zwart SR. Spaceflight-related ocular changes: the potential role of genetics, and the potential of B vitamins as a countermeasure. *Curr Opin Clin Nutr Metab Care*. 2018;21(6):481-488.

16. McGregor HR, Lee JK Mulder, EM, Beltran NE, Kofman IS, De Dios YE, Bloomberg JJ, Mulavara AP, Seidler RD (Under Revision at NeuroImage). Brain connectivity and behavioral changes in a spaceflight analog with elevated CO_2.

17. Romero JE, Coupé P, Giraud R, et al. CERES: A new cerebellum lobule segmentation method. *NeuroImage*. 2017;147:916-924. doi:10.1016/j.neuroimage.2016.11.003

18. Diedrichsen J. A spatially unbiased atlas template of the human cerebellum. *Neuroimage*. 2006;33(1):127-138.

19. Behzadi Y, Restom K, Liau J, Liu TT. A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. *Neuroimage*. 2007;37(1):90-101.

20. Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995;34(4):537-541.

21. Damoiseaux JS, S A R, Barkhof F, et al. Consistent resting-state networks across healthy subjects. *Proceedings of the National Academy of Sciences*. 2006;103(37):13848-13853.

22. Cassady K, Koppelmans V, Reuter-Lorenz P, et al. Effects of a spaceflight analog environment on brain connectivity and behavior. *Neuroimage*. 2016;141:18-30.

23. Zeng L-L, Liao Y, Shen H, Liu X, Hu D. Decoding Brain States with Simulated Microgravity from Baseline Using Functional Connectivity of Default Network. *Advances in Cognitive Neurodynamics (V)*. Published online 2016:325-330. doi:10.1007/978-981-10-0207-6_45

24. Zeng L-L, Liao Y, Zhou Z, et al. Default network connectivity decodes brain states with simulated microgravity. *Cognitive Neurodynamics*. 2016;10(2):113-120. doi:10.1007/s11571-015-9359-8

25. Liao Y, Lei M, Huang H, et al. The time course of altered brain activity during 7-day simulated microgravity. *Front Behav Neurosci*. 2015;9:124.

26. Liao Y, Miao D, Huan Y, Yin H, Xi Y, Liu X. Altered regional homogeneity with short-term simulated microgravity and its relationship with changed performance in mental transformation. *PLoS One*. 2014;8(6):e64931.

27. Demertzi A, Van Ombergen A, Tomilovskaya E, et al. Cortical reorganization in an astronaut's brain after long-duration spaceflight. *Brain Struct Funct*. 2016;221(5):2873-2876.

28. Zhou Y, Wang Y, Rao L-L, et al. Disrupted resting-state functional architecture of the brain after 45-day simulated microgravity. *Frontiers in Behavioral Neuroscience*. 2014;8. doi:10.3389/fnbeh.2014.00200

29. Lewis JW, Van Essen DC. Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey. *J Comp Neurol*. 2000;428(1):112-137.

30. zu Eulenburg P, Caspers S, Roski C, Eickhoff SB. Meta-analytical definition and functional connectivity of the human vestibular cortex. *Neuroimage*. 2012;60(1):162-169.

31. Balaban CD. Neurotransmitters in the vestibular system. *Handbook of Clinical Neurology*. Published online 2016:41-55. doi:10.1016/b978-0-444-63437-5.00003-0

32. Young L, Oman C, Watt D, Money K, Lichtenberg B. Spatial orientation in weightlessness and readaptation to earth's gravity. *Science*. 1984;225(4658):205-208. doi:10.1126/science.6610215

33. Young LR, Oman CM, Watt DG, et al. M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 1. Sensory adaptation to weightlessness and readaptation to one-g: an overview. *Exp Brain Res*. 1986;64(2):291-298.

34. Mulavara AP, Peters BT, Miller CA, et al. Physiological and Functional Alterations after Spaceflight and Bed Rest. *Med Sci Sports Exerc*. 2018;50(9):1961-1980.

35. Assländer L, Peterka RJ. Sensory reweighting dynamics in human postural control. *J Neurophysiol*. 2014;111(9):1852-1864.

36. Hupfeld KE, Lee JK, Gadd NE, et al. Neural Correlates of Vestibular Processing During a Spaceflight Analog With Elevated Carbon Dioxide (CO2): A Pilot Study. *Frontiers in Systems Neuroscience*. 2020;13. doi:10.3389/fnsys.2019.00080

37. Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. *Brain*. 2014;137(1):12-32. doi:10.1093/brain/awt162

38. Tootell RBH, Hadjikhani NK, Vanduffel W, et al. Functional analysis of primary visual cortex (V1) in humans. *Proceedings of the National Academy of Sciences*. 1998;95(3):811-817. doi:10.1073/pnas.95.3.811

39. Lee JK, De Dios Y, Kofman I, Mulavara AP, Bloomberg JJ, Seidler RD. Head Down Tilt Bed Rest Plus Elevated CO2 as a Spaceflight Analog: Effects on Cognitive and Sensorimotor Performance. *Frontiers in Human Neuroscience*. 2019;13. doi:10.3389/fnhum.2019.00355

40. Demertzi A, Van Ombergen A, Tomilovskaya E, et al. Cortical reorganization in an astronaut's brain after long-duration spaceflight. *Brain Struct Funct*. 2016;221(5):2873-2876.

41. Pechenkova E, Nosikova I, Rumshiskaya A, et al. Alterations of Functional Brain Connectivity After Long-Duration Spaceflight as Revealed by fMRI. *Front Physiol*. 2019;10:761.

42. Roe AW, Chelazzi L, Connor CE, et al. Toward a Unified Theory of Visual Area V4. *Neuron*. 2012;74(1):12-29. doi:10.1016/j.neuron.2012.03.011

43. Aminoff EM, Kveraga K, Bar M. The role of the parahippocampal cortex in cognition. *Trends in Cognitive Sciences*. 2013;17(8):379-390. doi:10.1016/j.tics.2013.06.009

44. Stoodley CJ, Schmahmann JD. Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex*. 2010;46(7):831-844.

45. Aguirre GK, Singh R, D'Esposito M. Stimulus inversion and the responses of face and object-sensitive cortical areas. *Neuroreport*. 1999;10(1):189-194.

46. Zwart SR, Gregory JF, Zeisel SH, et al. Genotype, B-vitamin status, and androgens affect spaceflight-induced ophthalmic changes. *FASEB J*. 2016;30(1):141-148.

47. Vogiatzoglou A, Refsum H, Johnston C, et al. Vitamin B12 status and rate of brain volume loss in community-dwelling elderly. *Neurology*. 2008;71(11):826-832.

48. Feng L, Isaac V, Sim S, Ng T-P, Krishnan KRR, Chee MWL. Associations between elevated homocysteine, cognitive impairment, and reduced white matter volume in healthy old adults. *Am J Geriatr Psychiatry*. 2013;21(2):164-172.

49. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A*. 2005;102(27):9673-9678.

50. Laurie SS, Christian K, Kysar J, et al. Unchanged cerebrovascular CO reactivity and hypercapnic ventilatory response during strict head-down tilt bed rest in a mild hypercapnic environment. *J Physiol*. 2020;598(12):2491-2505.

51. Whitfield-Gabrieli S, Nieto-Castanon A. Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connect*. 2012;2(3):125-141.

52. Carass A, Cuzzocreo JL, Han S, et al. Comparing fully automated state-of-the-art

cerebellum parcellation from magnetic resonance images. *Neuroimage*. 2018;183:150-172.

53. Van Dijk KRA, Sabuncu MR, Buckner RL. The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage*. 2012;59(1):431-438. doi:10.1016/j.neuroimage.2011.07.044

54. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*. 2012;59(3):2142-2154.

55. Van Dijk KRA, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *J Neurophysiol*. 2010;103(1):297-321.

56. Cole DM, Smith SM, Beckmann CF. Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Front Syst Neurosci*. 2010;4:8.

57. Koppelmans V, Bloomberg JJ, De Dios YE, et al. Brain plasticity and sensorimotor deterioration as a function of 70 days head down tilt bed rest. *PLoS One*. 2017;12(8):e0182236.

58. Liao Y, Zhang J, Huang Z, et al. Altered Baseline Brain Activity with 72 h of Simulated Microgravity – Initial Evidence from Resting-State fMRI. *PLoS ONE*. 2012;7(12):e52558. doi:10.1371/journal.pone.0052558

59. Demertzi A, Van Ombergen A, Tomilovskaya E, et al. Cortical reorganization in an astronaut's brain after long-duration spaceflight. *Brain Struct Funct*. 2016;221(5):2873-2876.

60. Zhou Y, Wang Y, Rao L-L, et al. Disrutpted resting-state functional architecture of the brain after 45-day simulated microgravity. *Frontiers in Behavioral Neuroscience*. 2014;8. doi:10.3389/fnbeh.2014.00200

61. Martuzzi R, Ramani R, Qiu M, Shen X, Papademetris X, Todd Constable R. A whole-brain voxel based measure of intrinsic connectivity contrast reveals local changes in tissue connectivity with anesthetic without a priori assumptions on thresholds or regions of interest. *NeuroImage*. 2011;58(4):1044-1050. doi:10.1016/j.neuroimage.2011.06.075

62. Landis JR, Richard Landis J, Koch GG. The Measurement of Observer Agreement for Categorical Data. *Biometrics*. 1977;33(1):159. doi:10.2307/2529310