Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

Convenience sample

Longitudinal data from a convenience sample of 15 individuals (hereafter referred to as "control" subjects) were collected two to four times over a period of 90 days (i.e., day 1, 7.5, 43.5, and 83.5). The age of the controls ranged from 26 to 60 years (Median = 40.5, MAD= 7.5) and four were female. The controls otherwise went about their daily lives for the duration of the study.

Image Acquisition

Controls: dMRIs were acquired on a 3T SIEMENS Verio scanner using the following protocol: diffusion-weighted 2D echo-planar imaging sequence TR = 11300 ms, TE = 95 ms, flip angle = 90°, FOV= 225 × 225 mm, matrix = 128 × 128, slice thickness = 2 mm, 80 contiguous slices (no gap), resulting in a voxel size of 1.95 × 1.95 × 2 mm. The b-value was 1,000 s/mm^2 in 30 non-collinear directions. Each direction was measured twice and two additional volumes without diffusion weighting (b-value = 0 s/mm^2) were acquired at the beginning and middle of the sequence.

In four time points of one control subject and one time point of another, the scan parameters were unintentionally altered. Seventy-five slices were collected with a TR of 10500ms without other parameter changes. Complete data from four time points was available for 10 of the 15 control subjects. Three time points were available for four control subjects, and data from two time points were available for another control subject. Reasons for missing and exclusion of data include time constraints (n = 1), incomplete data (n=2) and extreme scan artifacts (n = 3).

Image Analysis

All image analysis tools and steps including pre-processing, tensor fit, normalization, smoothing were kept constant between the astronauts and controls.

Statistical Analysis

Between group analyses: Comparisons between astronauts and the convenience control sample were carried out in order to gauge the degree of change brought about by spaceflight vs. time. To this end, the linear slope of FW and FA_T, AD_T, RD_T changes over time was first estimated.

Individual intercept and slope images were constructed utilizing all available time points, considering the time intervals between each assessment. Then the percentage signal change of these measures was calculated by expressing the slope as a percentage of the intercept, taking into account individual baselines. For FW measures and diffusion indices, tests of group differences 1) at baseline, 2) changes over time and 3) this change accounting for random variation in baseline values were conducted by entering the intercept, slope and percentage signal change images into two-sample t-tests. In order to take into account the significant difference in age between the two groups, age was entered as a covariate when conducting all group comparison analyses.

In order to aid the inference process, one sample t-tests were conducted to determine whether the controls as a reference group showed any significant changes in FW and FA_T, AD_T, RD_T over time. This test was carried out only within the control group, utilizing the slope images of each measurement. The results revealed that the control group slope images were not significantly different from zero for any of the brain metrics.

All one- and two sample t-tests were carried out carried out using a nonparametric permutation based test using a threshold-free cluster enhancement[1] approach with 15,000 random permutations implemented in FSL's randomize[2]. Variance smoothing of 2.5 mm full-width at half-maximum (FWHM) was applied and analyses were adjusted for multiple comparisons by applying a voxel level family wise error (FWE) correction (P<0.05). Anatomical labels of peak voxels showing significant white matter changes were defined using the JHU white-matter tractography atlas. If no labels were detected, the ICBM-DTI-81 white-matter labels atlas and the Harvard-Oxford Cortical Structural Atlas were consulted. The peak voxels within clusters showing FW changes were labeled using the Harvard-Oxford Cortical Structural Atlas.

Change in FW and white matter microstructure astronauts vs. controls:

Analyses of both slope and percentage change revealed that astronauts, in comparison to controls, showed significant increases in FW over time in areas throughout the base of the cerebrum (see Supplementary Figure 1 (B) and (C)).

Group analyses of the slope and percentage change images revealed that the FA_T change from pre-to postflight in astronauts was not significantly different from that in controls over time. Astronauts exhibited an increase in RD_T over time that was significantly greater than controls in white matter structure underlying the right supramarginal gyrus and in the right superior longitudinal fasciculus (Supplementary Figure 2 (B)). When the slope was adjusted for the baseline, astronauts showed significantly greater RD_T increases in the somatosensory association cortex white matter (supramarginal gyrus) in comparison to controls (Supplementary Figure 2 (C)). The AD_T change from pre-to postflight in astronauts was not significantly different from the AD_T change in the controls over time.

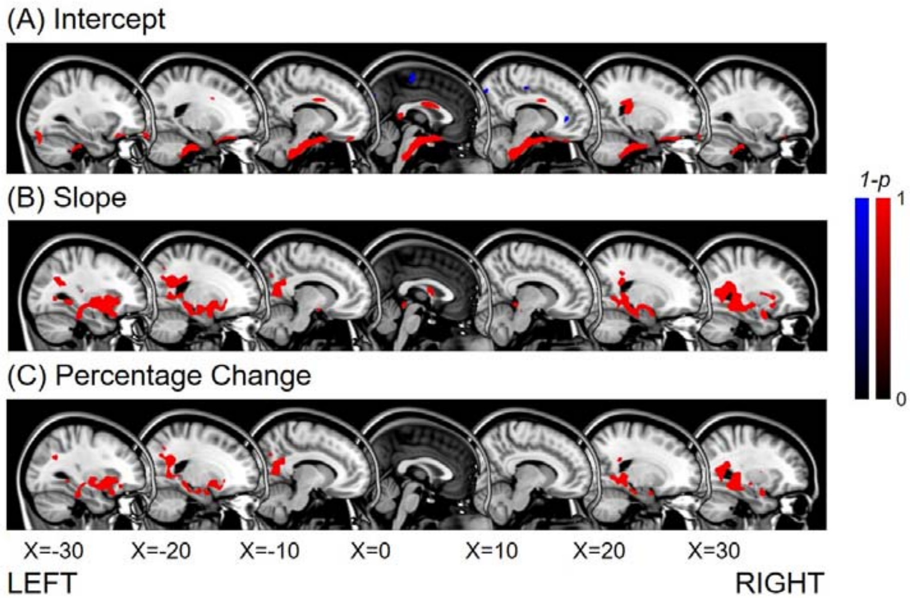
Difference in FW and white matter microstructure at baseline astronauts vs. controls:

With the exception of one, all of the other astronauts had varying numbers and lengths spent in space prior to the current preflight assessment. Consequently, the astronauts, as a group, may have different baseline FW and white matter microstructural measure in comparison to the controls.

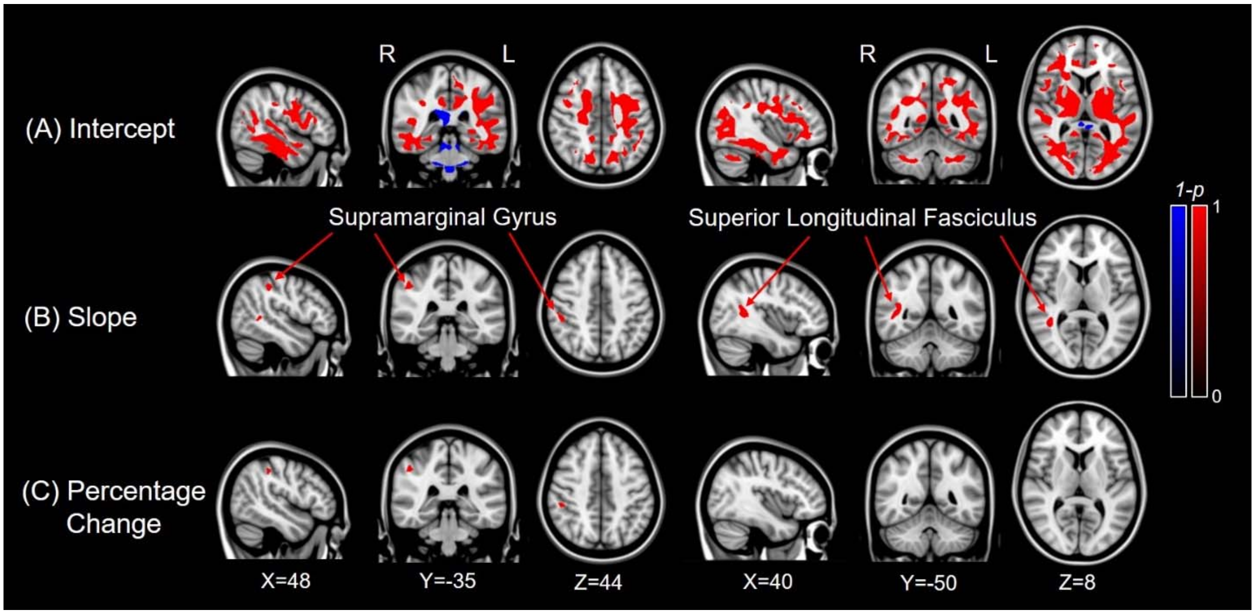
The astronauts, compared to controls, showed significantly less FW in an area located on the midsagittal plane of the brain at baseline. Conversely, significantly greater baseline FW was detected in the astronauts, adjacent to the interpeduncular cistern and pontine cistern and in areas located superior to the lateral ventricles (Supplementary Figure 1 (A)).

At baseline, astronauts showed significantly lower FA_T values than controls throughout the white matter (Supplementary figure 3(A)). Complementing the baseline FA_T findings, the astronauts showed significantly increased RD_T values at baseline in comparison to controls in widespread regions. Significantly lower AD_T values in astronauts in areas such as the body of the corpus callosum were seen in comparison to controls at baseline.

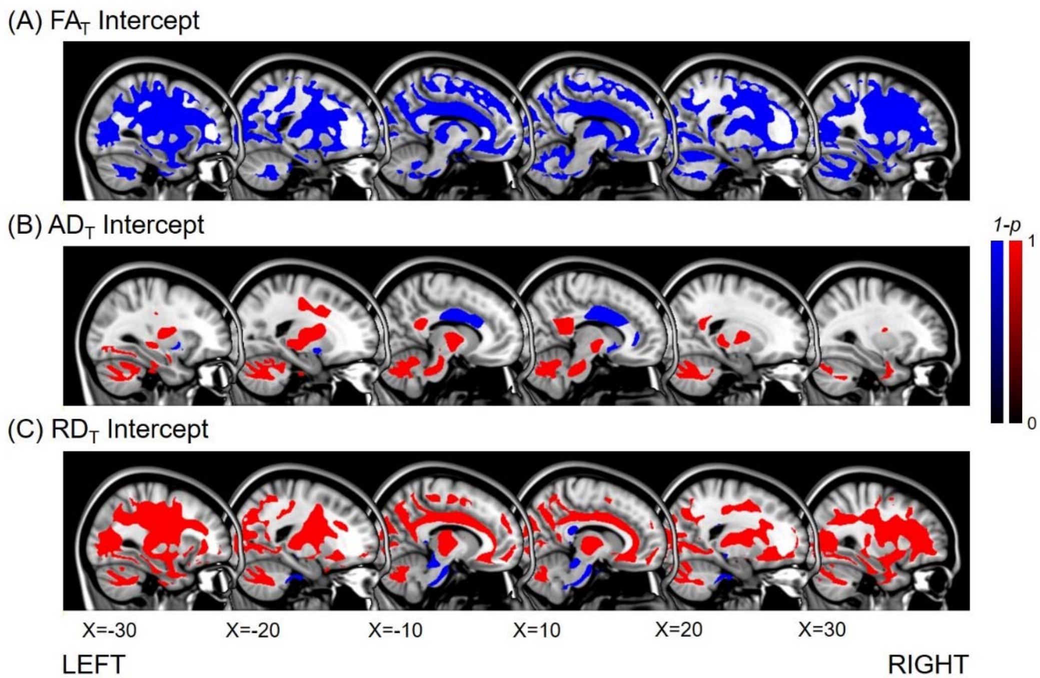
Interestingly, when compared to controls, the astronauts showed significantly lower baseline RD_T values in small areas of the splenium of the corpus callosum and the corticospinal tract. Higher AD_T values in the anterior thalamic radiation, splenium of the corpus callosum, corticospinal tract and cerebellar peduncles (For baseline group comparison AD_T and RD_T results see Supplementary figure 3 (B) and (C), respectively) were also observed in astronauts in comparison to controls at baseline.



eFigure 1. Comparison of control and astronauts' free water (FW) measure in (A) intercept (i.e. baseline), (B) slope (i.e. rate of change over time), and (C) percentage change (i.e. degree of change relative to baseline). The red and blue colors show regions in which crewmembers had significantly increased and decreased FW values in comparison to controls, respectively (P<0.05, FWE corrected). The results are overlaid on the MNI152 brain template.



eFigure 2. Comparison of control and astronauts' FW-corrected radial diffusivity (RD_T) measure in (A) intercept (i.e. baseline), (B) slope (i.e. rate of change over time) and (C) percentage change (i.e. degree of change relative to baseline). The red and blue colors show regions in which astronauts had significantly increased and decreased RD_T values in comparison to controls, respectively (P<0.05, FWE corrected). The results are overlaid on the MNI152 brain template.



eFigure 3. Intercept (i.e. baseline) differences between crewmembers and controls in FW-adjusted (A) fractional anisotropy (FA_T), (B) axial diffusivity (AD_T) and (C) radial diffusivity (RD_T) ($P<0.05$, FWE corrected). Clusters in red colors indicated areas where crewmembers had higher values, and blue colors indicate areas where controls had higher values.

eReferences

1. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage.* 2009;44(1):83-98. doi: 10.1016/j.neuroimage.2008.03.061.
2. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage.* 2014;92:381-397.