

Supplementary Materials for

What happens to your brain on the way to Mars

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Supplementary Text

Fig. S1. Correlation of PSD-95 puncta and DI.

Supplementary Materials

Behavioral testing

Novel Object Recognition (NOR) test

The NOR test was administered after 3 days of habituation (10 min/day). On the testing day, mice were exposed to two duplicate objects within the arena for 5 min (familiarization). They were then allowed to rest for 5 min in their home cage within the behavioral room while the objects were cleaned (70% ethanol), and a novel object was added. Mice were then returned to the arena for 5 min, where they explored the familiar and novel object (testing). Trials were later hand scored by an individual blinded to the experimental groups and calculated by using the discrimination index: A positive score was counted when the nose of the mouse was within 1 cm and pointed in the direction of the object. Time was not scored for mice that were near but not facing the object (*1*).

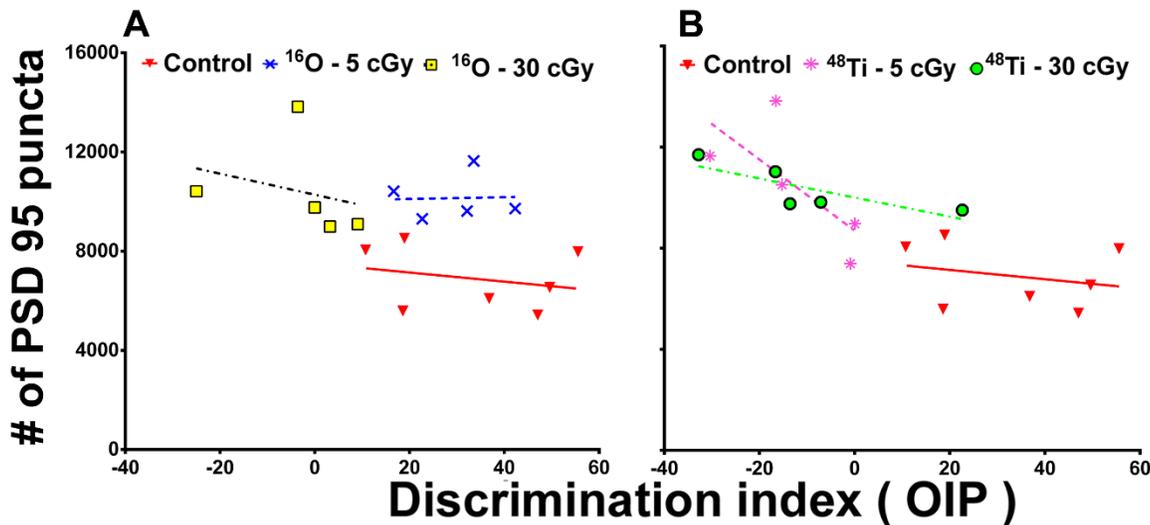
Object in Place (OiP) test

One week after completion of the NOR task, OiP testing was started. Mice were habituated to their arenas (10min/day) for 2 consecutive days. During the familiarization phase (day 3), mice were placed in the arena containing 4 different objects and allowed to explore for 5 min before being removed. After a 5 min delay, each mouse was replaced in the arena for exploration (test phase), in which 2 of the objects exchanged positions. The time spent investigating the objects in the switched position was compared with the time spent investigating the objects in the same positions. Intact object-in-place memory occurs when the subject spends more time investigating the 2 objects in different locations, compared with the 2 objects in the same locations. Trials were later hand scored by an individual blinded to the experimental groups and calculated by using the discrimination index as described earlier. Positive scores were calculated based on the same criteria detailed for the NOR (*13*).

Supplementary Materials

Correlation of altered cognition to synaptic puncta

To investigate the functional importance of quantifying synaptic puncta after various irradiation paradigms, behavioral performance of each individual mouse, expressed in terms of DI, was plotted against that animal's corresponding level of PSD-95 puncta (per $400\ \mu\text{m}^2$; **Supplementary Fig. 1**). While these correlations did not reach statistical significance, a clear trend indicates that behavioral decrements corresponded to elevations in the number of PSD-95 puncta. Thus, quantification of structural and synaptic parameters in the irradiated brain provides insights into the types of radiation-induced change most likely to underlie performance-based decrements that may prove problematic during long term deep space missions.



Supplementary Figure 1: Correlation of PSD-95 puncta and DI. PSD-95 puncta (per $400\ \mu\text{m}^2$) are plotted against the corresponding performance of each animal on the OiP task. Data clearly show that increased levels of PSD-95 puncta are associated with decreased behavioral performance 6 weeks following exposure to 5 or 30 cGy of ^{16}O (A) or ^{48}Ti (B) charged particles.