*In vivo* Synaptic Density Imaging with 18F-UCB-H Detects Effects of Space Irradiation in Rat Brain

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**Introduction:** Previous findings have shown that doses of charged particles as low as 5 cGy can result in substantial reductions of synapse number in the mouse that are persistent over many months ( Parihar *et al.* Sci. Adv. 2015;1:e1400256). To non-invasively assess which brain regions are most sensitive to chronic, low-level radiation exposure, we examined adult male Wistar rats irradiated with 252Cf neutrons with PET imaging using a radioligand (18F-UCB-H) which binds specifically to the synaptic vesicle protein SV2A and serves as a surrogate marker of synaptic density. For comparison, region-specific c-fos expression following a behavioral stimulus in a subset of animals was performed to determine the strength of behavior-neuron activation coupling.

**Methods:** Adult male, 6 month old Wistar rats (n=15) were irradiated chronically with 252Cf neutrons at 1 mGy/day for 6 months to a total dose of 180 mGy and compared to controls (n=16). PET images of the brain were acquired in twelve rats (6 irradiated and 6 control; 869±74 g) 65-67 days post-irradiation using the Rodent R4 microPET scanner (Concorde Systems). A 40 minute dynamic list mode scan was performed immediately after injection of 18F-UCB-H (76.6±5.5 MBq). Corrected images were reconstructed by OSEM 2D (4 iterations, 256x256, ramp filter, pixel size of 0.4x0.4x1.2 mm). Summed images (0–40 min) were used for rigid coregistration with MRI T2 weighted brain images (11.7T Bruker) and a standard rat brain template available in Vivoquant™ software (version 4.0; InVicro). Atlas based volumes of interest, representing the brain distribution of SV2A measured as Standard Uptake Value (SUV) were used for comparisons between unirradiated and irradiated animals. A separate cohort of animals (14 irradiated and 12 controls) were tested in four behavioral paradigms (1 x 1 meter square Open Field Task, Elevated Zero Maze test, Novel Object recognition task, and Social Dominance Tube test) two months after irradiation. At 3 months after irradiation animals were allowed to explore a 1 meter diameter open field for 30 min.to drive c-fos expression then euthanized at 90 min after completion of the task for brain extraction and fixation followed by antibody staining of the immediate early gene, c-fos, which is expressed in activated neurons.

**Results:** PET imaging analysis revealed three brain regions with statistically decreased mean SUV values in irradiated rats compared to controls; the entorhinal cortex (p=0.038), the ventral tegmental area (p=0.045), and the substantia nigra (p=.046). C-fos imaging after open field exploration showed significant decrements in c-fos positive cells in hippocampus CA1 (p = 0.0014, t-test) and a trend in primary motor cortex (p = 0.08, t-test) but not in prelimbic, cingulate and entorhinal cortex, dentate gyrus, amygdala or substantia nigra. Open field and elevated zero mazes showed reduced movement and exploration in neutron-irradiated animals (P < 0.03), while no statistically significant changes were observed for novel object recognition or social dominance.

**Conclusion:** 18F-UCB-H imaging revealed brain areas involved in motor, memory and behavior control with significantly decreased synaptic density following chronic neutron exposure at 0.2 Gy. Irradiated rats also exhibited altered exploratory behavior and altered behaviorally-induced c-fos expression. The brain regions associated with the various outcome measures were not well correlated.

**Acknowledgements:** Supported by NASA Grant NNX15AI22G (to G.N.) & Loma Linda Univ. School of Med. institutional support (to B.H.).

**Supporting Data:**

