Supplementary Data

Supplements

Antioxidant measurements

Brains isolated from irradiated mice were subjected to comprehensive biochemical analyses conducted at the University of Iowa Radiation and Free Radical Research Core lab. Briefly, 2 and 4 weeks after gamma irradiation, the activity of brain antioxidants were determined using a suite of biochemical assays. For glutathione (GSH)/GSSH determination, one half brain was placed in a 5% aqueous solution of 5-sulfosalicylic acid dihydrate (SSA; Sigma), homogenized, and frozen at −20°C. For all other assays, the remaining half brain was frozen at −20°C. Frozen samples were then shipped to Iowa for analyses.

GSH and glutathione disulfide assay. Immediately after harvest whole brain sections were washed in cold PBS and homogenized in 5% 5-sulfosalicylic acid (Sigma) in water and stored at −80°C. Total GSH content was determined as described previously (8). Glutathione disulfide (GSSG) was determined by adding 2-vinylpyridine for at least 1 h prior to assaying as described previously (5). The rates of the reaction were compared to similarly prepared GSH and GSSG standard curves. GSH determinations were normalized to the protein content of the insoluble pellet from the SSA extracts dissolved in 2.5% SDS in 0.1 N bicarbonate using the BCA Protein Assay Kit (Thermo Scientific).

Catalase activity assay. Catalase activity was determined on whole brain homogenates in 50 m*M* potassium phosphate buffer (pH 7.8, with 1.34 m*M* diethylenetriaminepentaacetic acid) by measuring the disappearance of 10 m*M* hydrogen peroxide monitored at 240 nm and the units were expressed as m*k* units/mg of protein as described (2).

MnSOD activity assay. MnSOD activity of whole homogenates in 50 m*M* potassium phosphate buffer (pH 7.8, with 1.34 m*M* diethylenetriaminepentaacetic acid) was determined using an indirect competitive inhibition assay as described previously (2) using 5 m*M* sodium cyanide to distinguish between CuZnSOD and MnSOD activity.

GSH peroxidase activity assay. GSH peroxidase activity in whole homogenates was measured using H_2O_2 as the substrate as previously described by monitoring NADPH oxidation in the presence of reduced GSH and GSH reductase with 1 m*M* azide added to inhibit catalase activity (11).

GSH-S-transferase activity assay. Whole tissue homogenates prepared in 50 m*M*-phosphate buffer, pH 7.8, with 1.34 m*M* diethylenetriaminepenta- acetic acid (DETAPAC) were frozen at −20°C until being assayed for GSH-S-transferase activity using l-chloro-2,4-dinitrobenzene (CDNB) as the substrate as previously described (13).

Cognitive testing—novel object recognition

The novel object recognition (NOR) was performed as described (12). On day 1, the mice were habituated to an open field (16×16 inches; Kinder Scientific) three times for 10 min each. On day 2, the mice were placed in the open field containing two identical objects and were allowed to explore freely to become familiar with the objects. Mice were returned to the open field after a delay of 5 min or 24 h, but after replacing one familiar object with a novel object. NOR is sensitive to both hippocampal (4, 6, 7, 9) and cortical lesions in and around prefrontal areas surrounding the rhinal fissure (1, 3, 14). The mice were then allowed to explore for 5 min. Movement and time spent exploring each object was recorded and analyzed using Ethovision XT video tracking system (Noldus Information Technology). The discrimination index (DI) was used as a quantitative measure of NOR performance and was calculated as follows: the DI = ([novel object exploration time/total exploration time] − [familiar object exploration time/total exploration time])×100. Data shown for ^{56}Fe particle exposures were derived and re-plotted from a larger cognitive manuscript (10).

Cognitive testing—Morris Water Maze

Hippocampus-dependent spatial learning and memory was assessed in the water maze. A circular pool (diameter 140 cm) was filled with water made opaque with nontoxic chalk (24°C) and mice were trained to locate a submerged platform. To determine whether irradiation affected the ability to swim or learn the water maze task, mice were first trained to locate a clearly marked platform (visible platform, Days 1 and 2). The mice were subsequently trained to locate the platform when it was hidden beneath the surface of opaque water (Days 3–5). Training during the hidden platform sessions (acquisition) required the mice to learn the location of the hidden platform based on extra-maze cues. For both visible and hidden sessions, there were two daily sessions, morning and afternoon, which were 2-h apart. Each session consisted of three trials (with 5-min inter-trial intervals). A trial ended when the mice located the platform. Mice that failed to locate the platform within 60 s were led to the platform by placing a finger in front of their swim path. The mice were taken out of the pool after they were physically on the platform for a minimum of 3 s. During visible platform training, the platform was moved to a different quadrant of the pool for each session. For the hidden platform training, the platform location was kept constant. The mice were placed into the water facing the edge of the pool in one of nine randomized locations. The start location was changed for each trial. The swimming patterns of the mice were recorded with Noldus Ethovision video tracking software (Ethovision XT; Noldus Information Technology) set at six samples/s. The time to locate the platform (latency) was used as a measure of performance for the visible and hidden sessions. Because swim speed can influence the time it takes to reach the platform, they were also analyzed to assess whether there were genotype or treatment differences in this measure.

To measure spatial memory retention, probe trials (platform removed) were conducted 1 h after the last hidden trial of each mouse on each day of hidden platform training (*i.e.*, a total of three probe trials). The time spent in the target

quadrant, the quadrant where the platform was previously located during hidden platform training, was compared to the time spent in the three nontarget quadrants. For the probe trials, mice were placed into the water in the quadrant opposite of the target quadrant.

Statistical analysis

For the water maze, learning curves were analyzed using repeated measures analysis of variances (ANOVAs). For the analysis of spatial memory retention in the probe trials, one-way ANOVAs were used to assess the effect of quadrant in each group, followed up by *post hoc* tests when appropriate.

Supplementary References

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