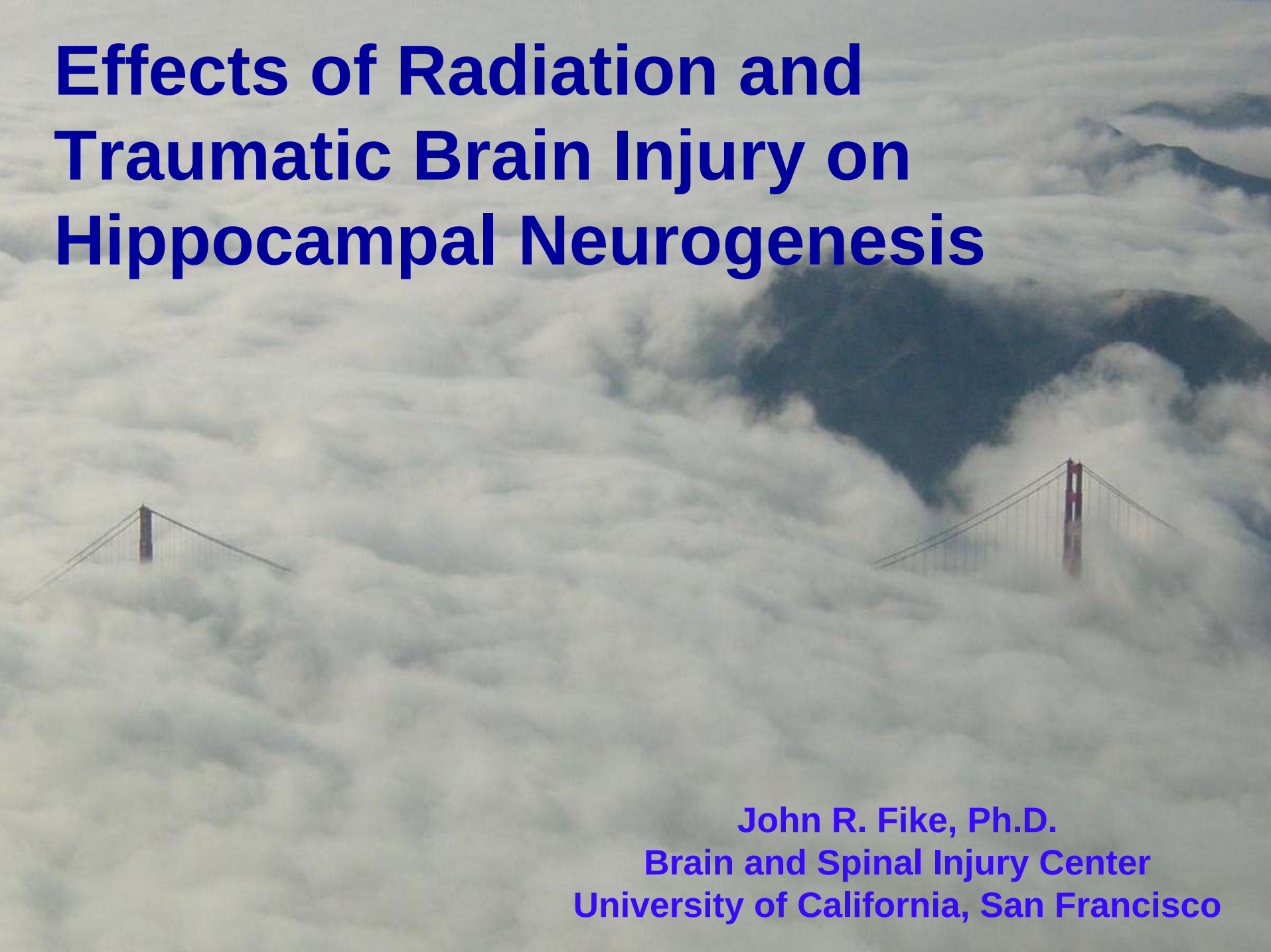
Effects of Radiation and Traumatic Brain Injury on Hippocampal Neurogenesis



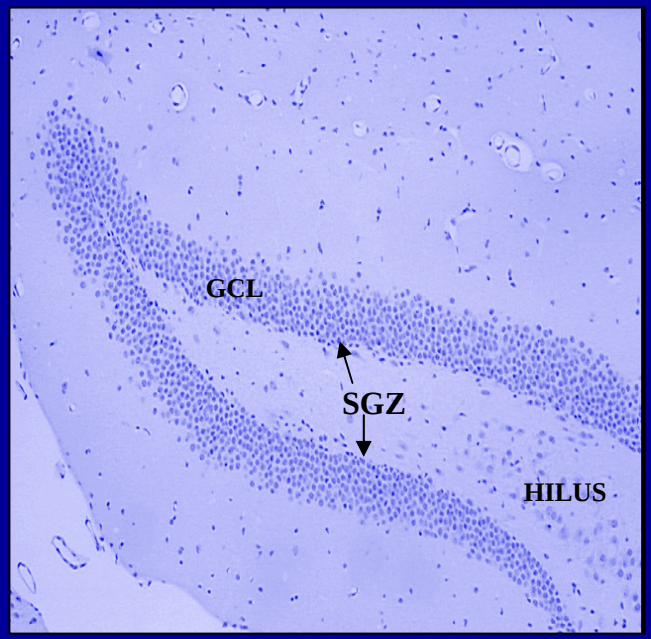
John R. Fike, Ph.D.
Brain and Spinal Injury Center
University of California, San Francisco

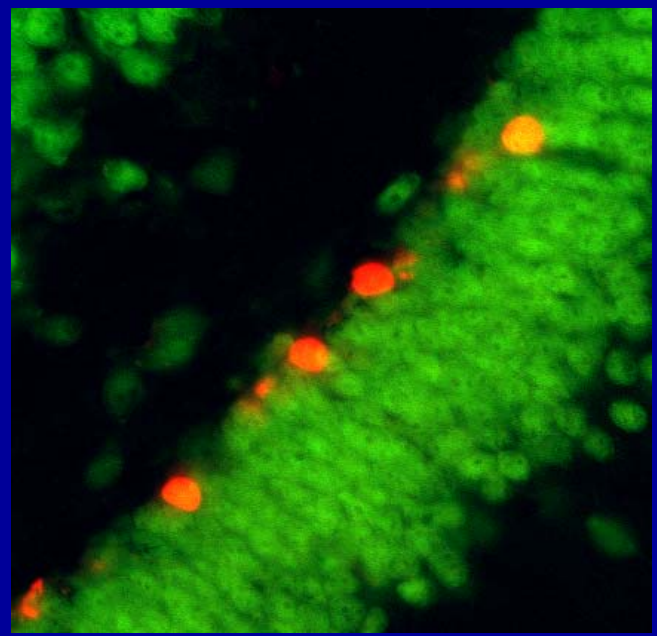
The Central Nervous System: Effects of Relatively Low Radiation Doses

- Neurocognitive effects occur after radiation doses that do not result in overt tissue destruction:
  - Progressive, currently untreatable and poorly understood;
  - Involves hippocampal functions of learning, memory and spatial information processing;
  - May involve neurogenesis.

Hippocampus

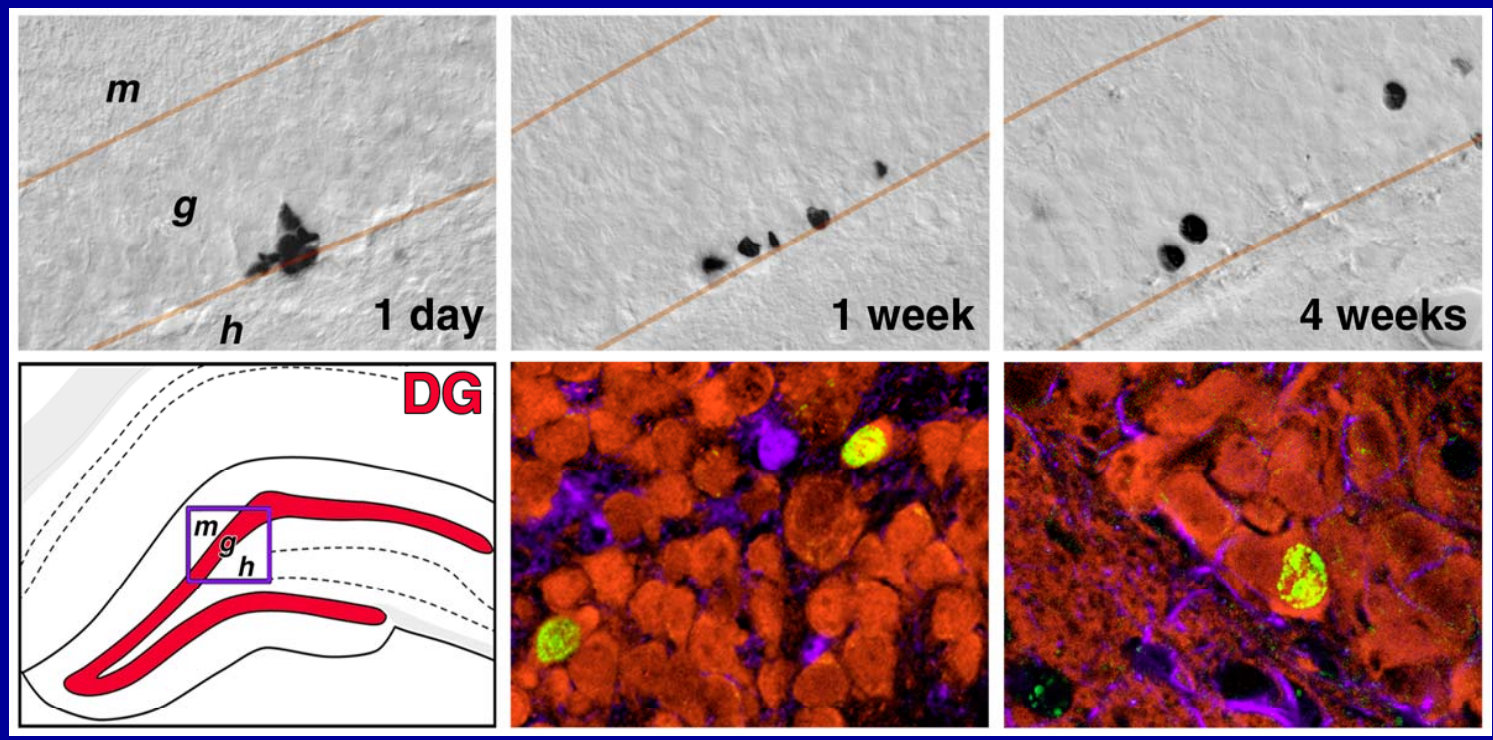
- Integral part of temporal lobe memory system.
- An active site of neurogenesis throughout life.





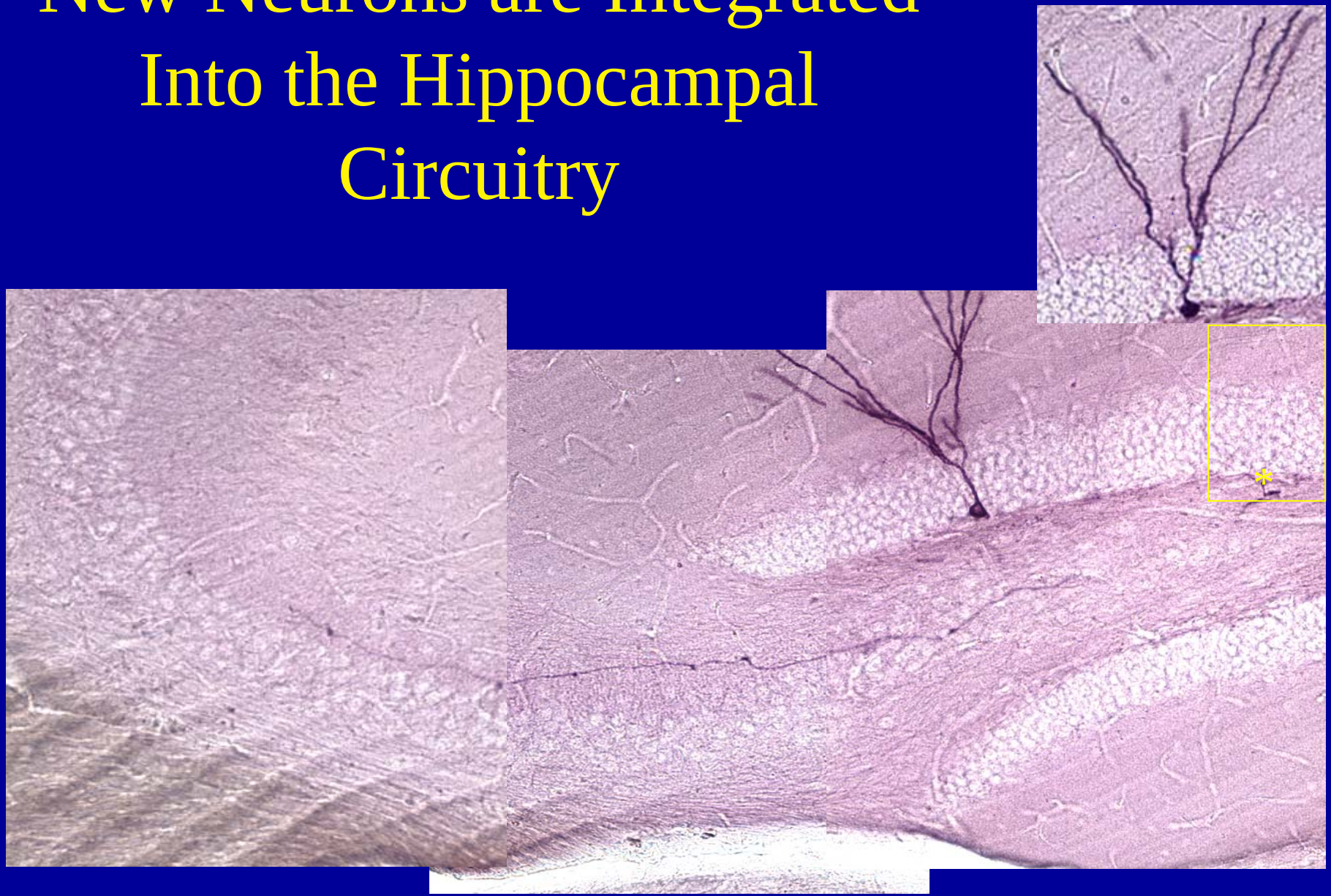
Neurogenesis in the Dentate Gyrus

Proliferation **Migration**



Differentiation

New Neurons are Integrated Into the Hippocampal Circuitry



ARTICLES

nature neuroscience

Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus

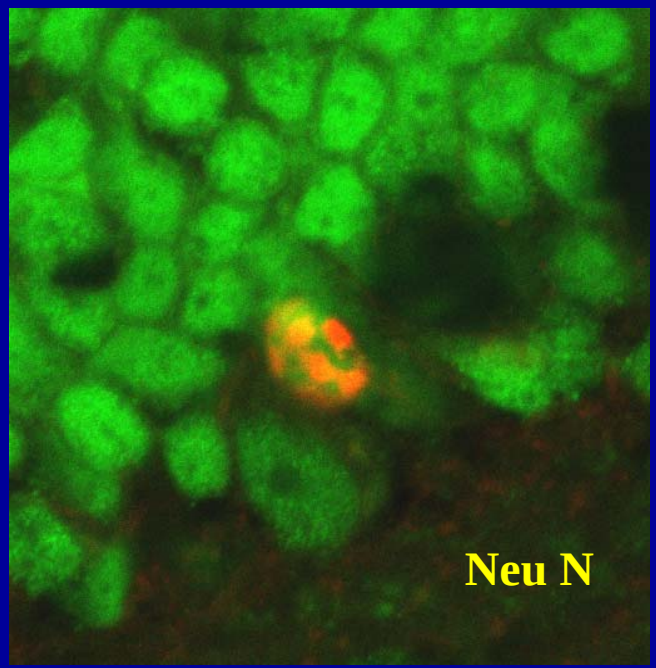
Nohjin Kee[1–3,5], Cátia M Teixeira[1–5], Afra H Wang[1,3] & Paul W Frankland[1–3]

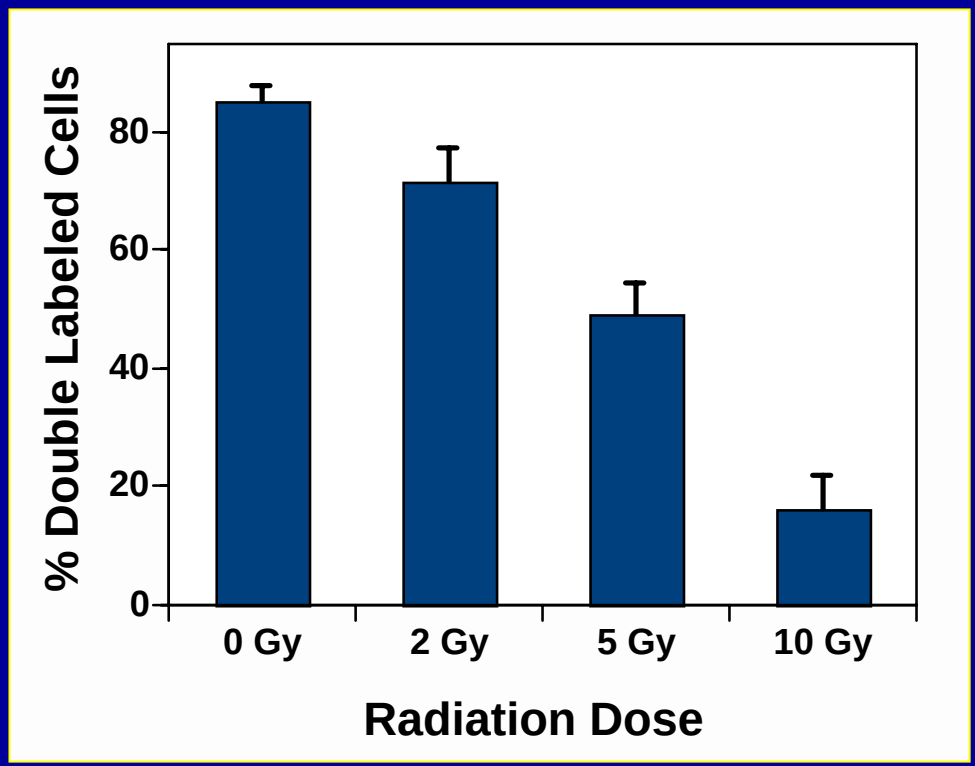
Throughout adulthood, new neurons are continuously added to the dentate gyrus, a hippocampal subregion that is important in spatial learning. Whether these adult-generated granule cells become functionally integrated into memory networks is not known. We used immunohistochemical approaches to visualize the recruitment of new neurons into circuits supporting water maze memory in intact mice. We show that as new granule cells mature, they are increasingly likely to be incorporated into circuits supporting spatial memory. By the time the cells are 4 or more weeks of age, they are more likely than existing granule cells to be recruited into circuits supporting spatial memory. This preferential recruitment supports the idea that new neurons make a unique contribution to memory processing in the dentate gyrus.

Neurogenesis Protocol

- 1-3 month post irradiation: BrdU 1x/day for 7 days
- 3 wks after BrdU perfuse with 4% PFA
- 50 μm floating sections
- Immunohistochemistry and confocal microscopy
  - Neurons: NeuN
  - Astrocytes: GFAP
  - Immature oligodendrocytes: NG2
  - Activated microglia: CD68

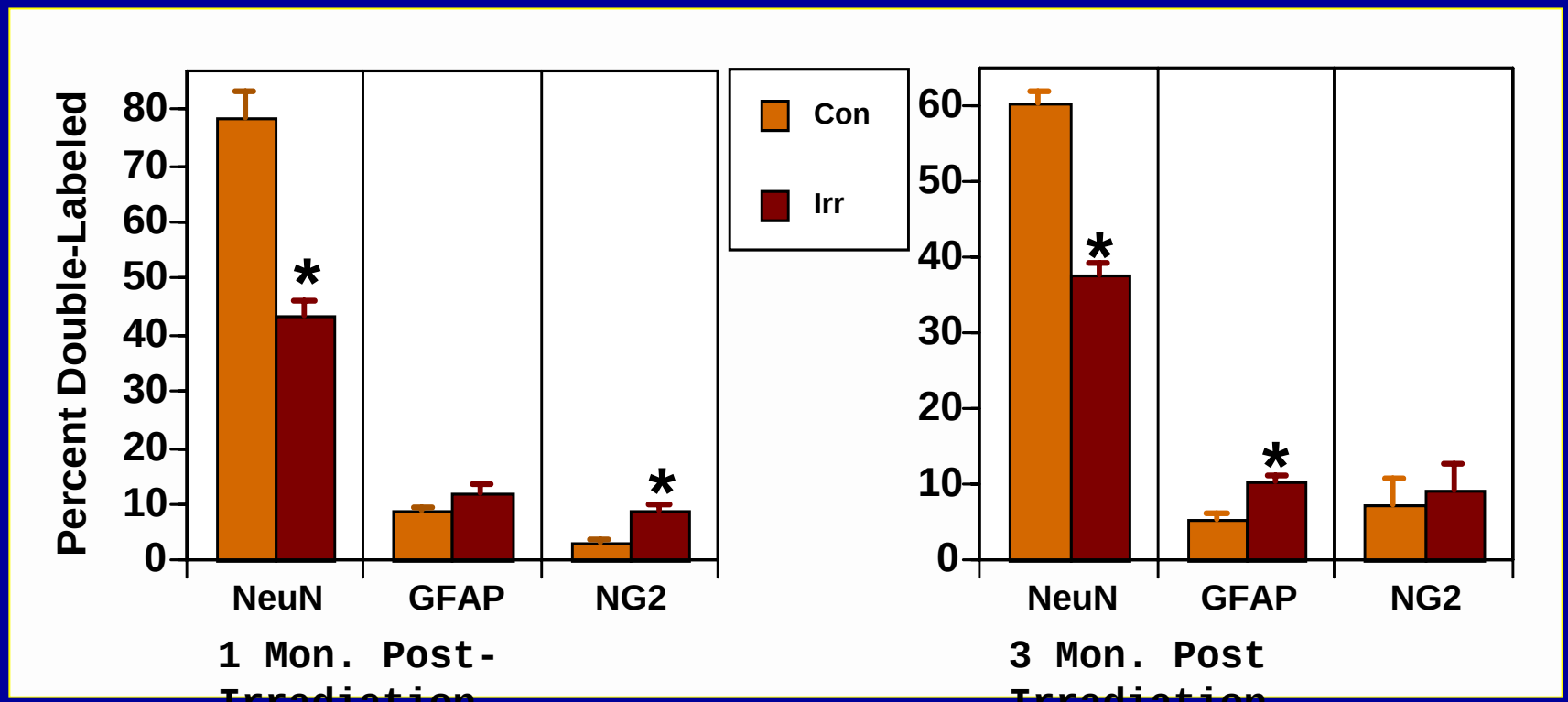
X-Irradiation and Newly Born Neurons in the Dentate SGZ





Mizumatsu et al, Can. Res. 2003

Changes in Neurogenesis are Persistent After Low Dose X-Irradiation (5 Gy)



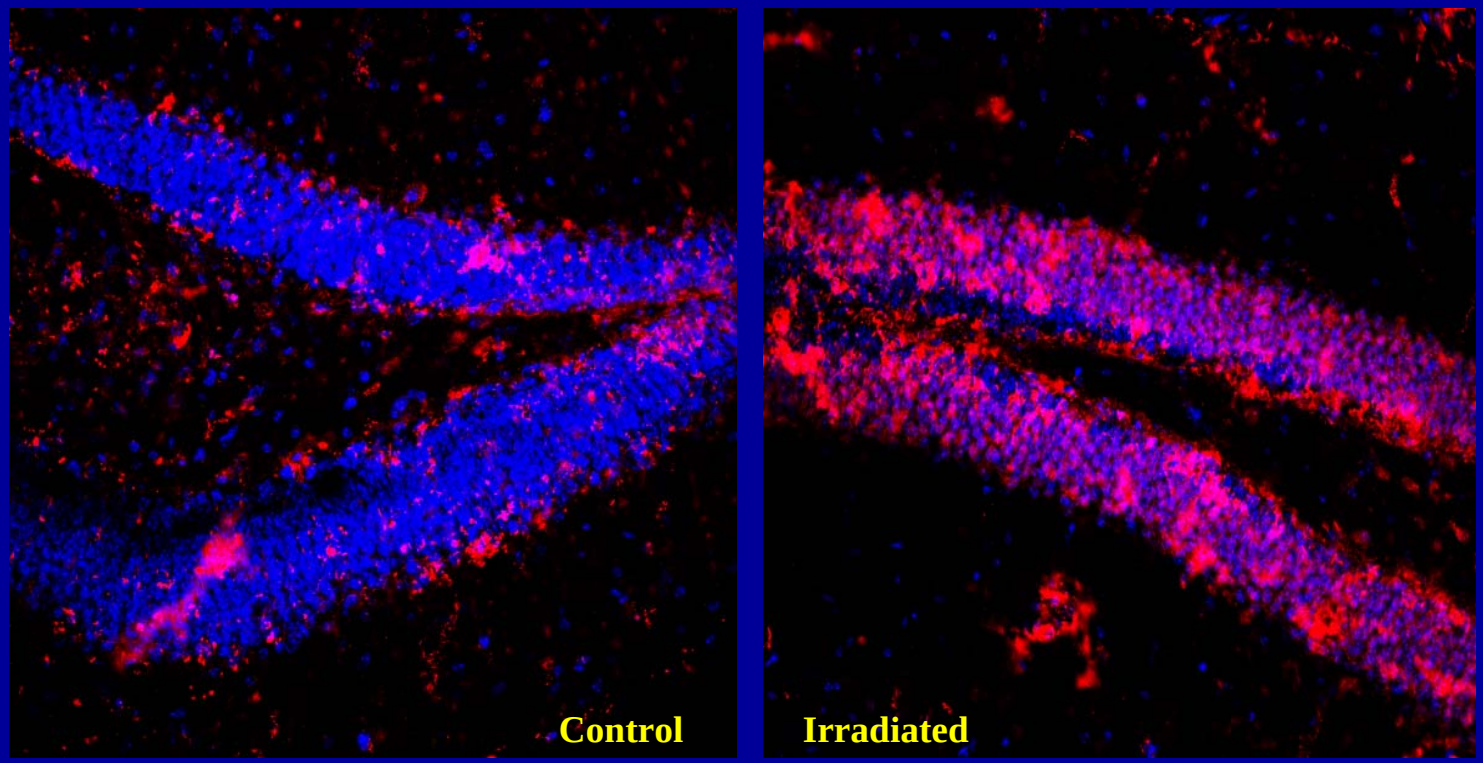
Rola et al, Exp. Neurol. 2004

Altered Neurogenesis is Associated with Radiation-Induced Cognitive Impairments

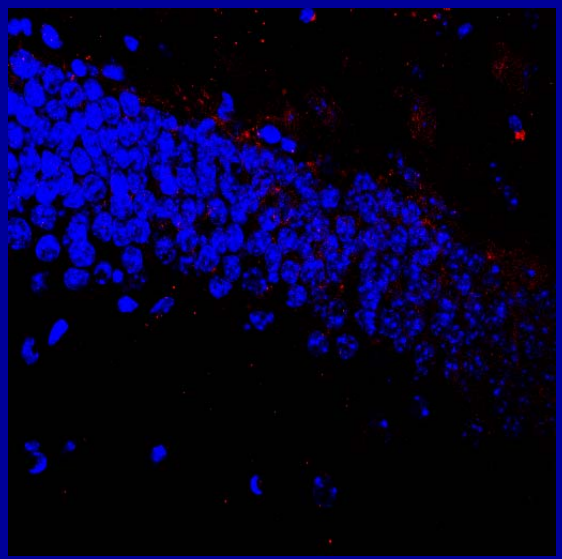
- Raber et al, Rad. Res. 162, 2004.
- Rola et al, Exp. Neurol. 188, 2004.
- Raber et al, Ann. Neurol. 55, 2004.
- Fan et al, Eur. J. Neurosci. 25, 2007.
- Madsen et al, Neurosci. 119, 2003.
- Snyder et al, Neurosci. 130, 2005.
- Shi et al, Rad. Res. 166, 2006

Are changes in neurogenesis associated with microenvironmental factors?

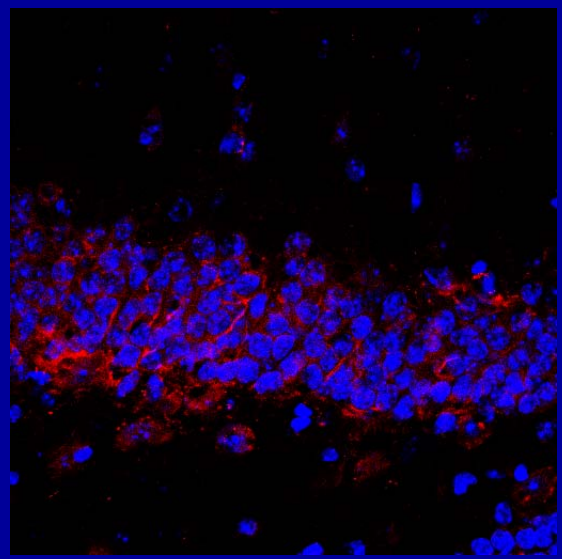
The Receptor (CCR2) for Monocyte Chemoattractant Protein 1 is Increased in the Dentate Gyrus After X-Irradiation



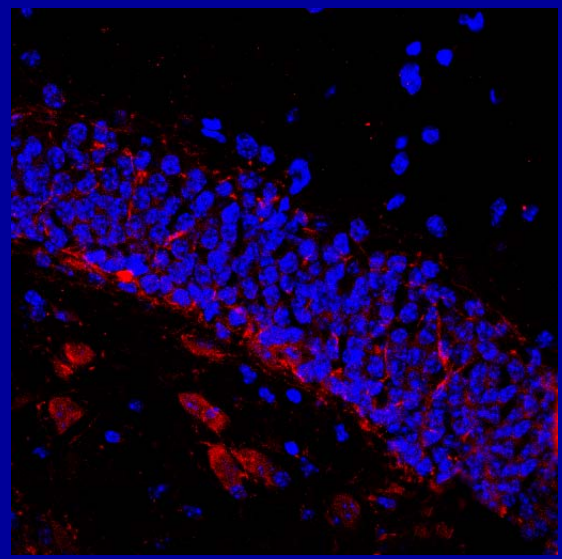
CCR2 Expression is Still Increased in the Dentate Gyrus 9 Months After High LET Irradiation



Control

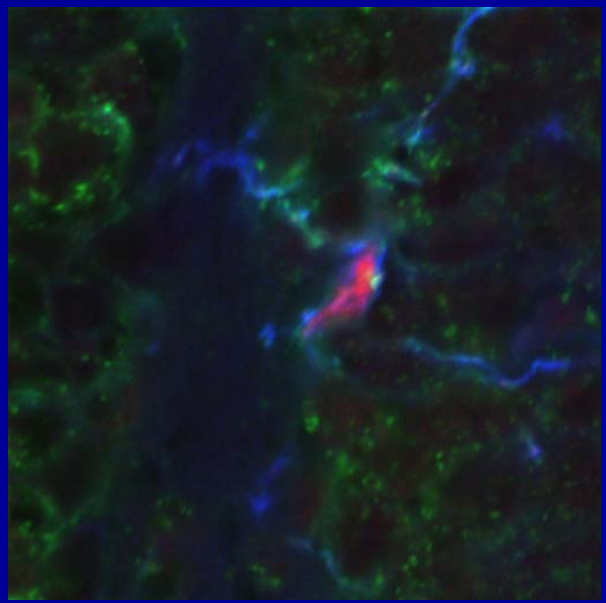


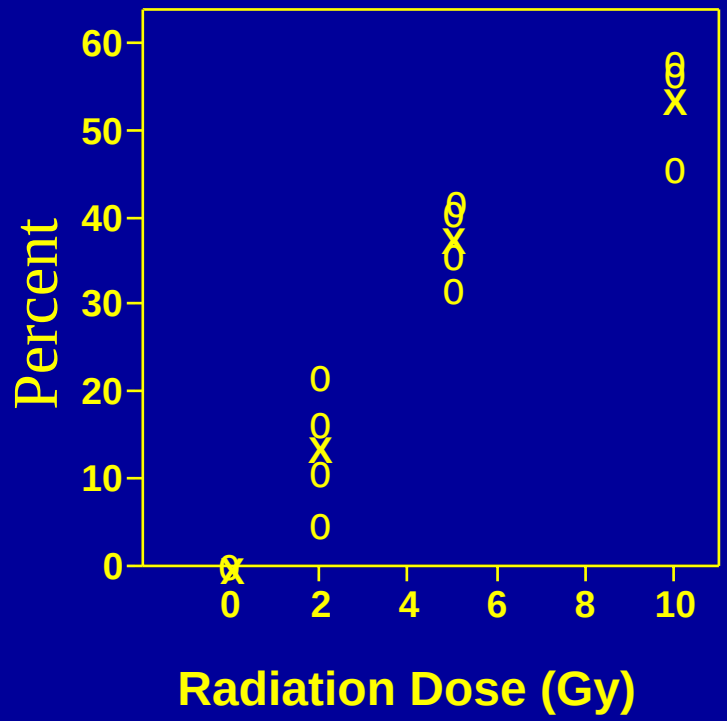
2 Gy ^{56}Fe



2 Gy ^{12}C

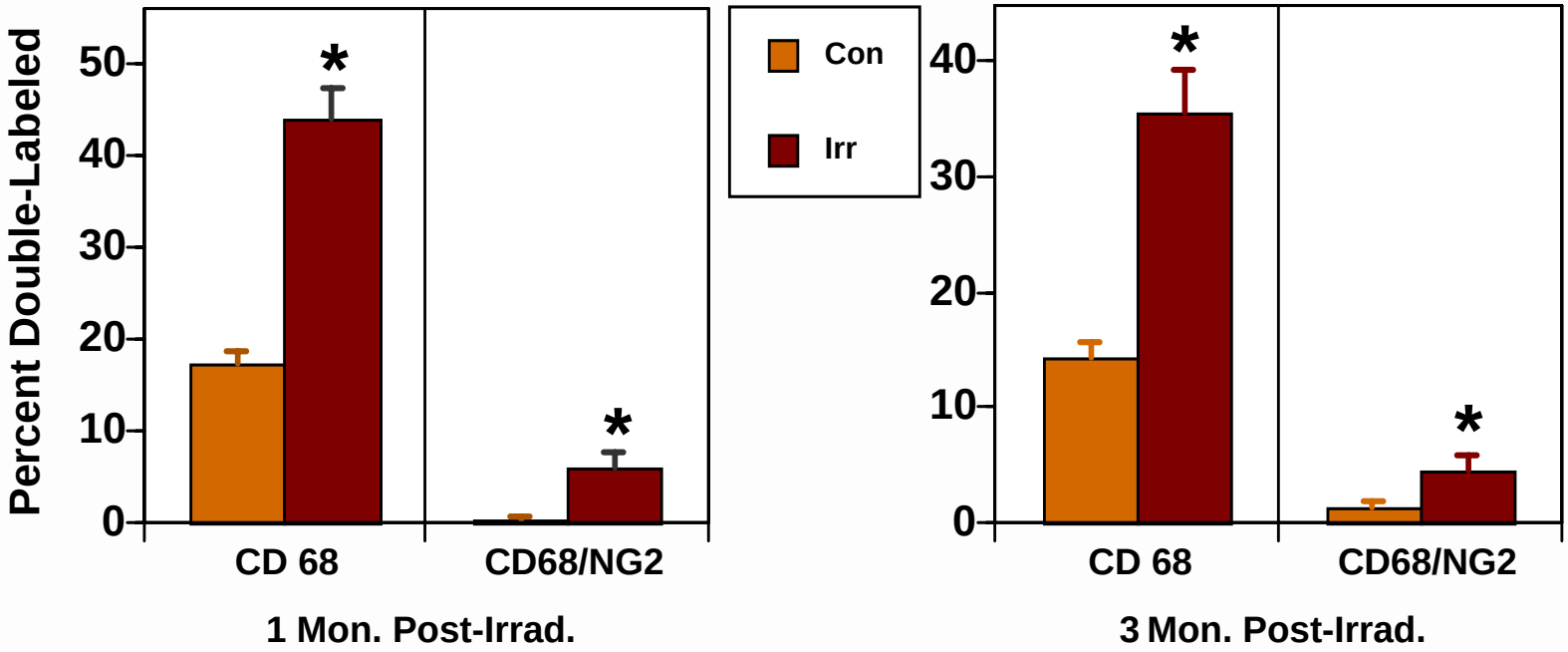
Increasing Numbers of Newly Born Activated Microglia as a Function of Radiation Dose





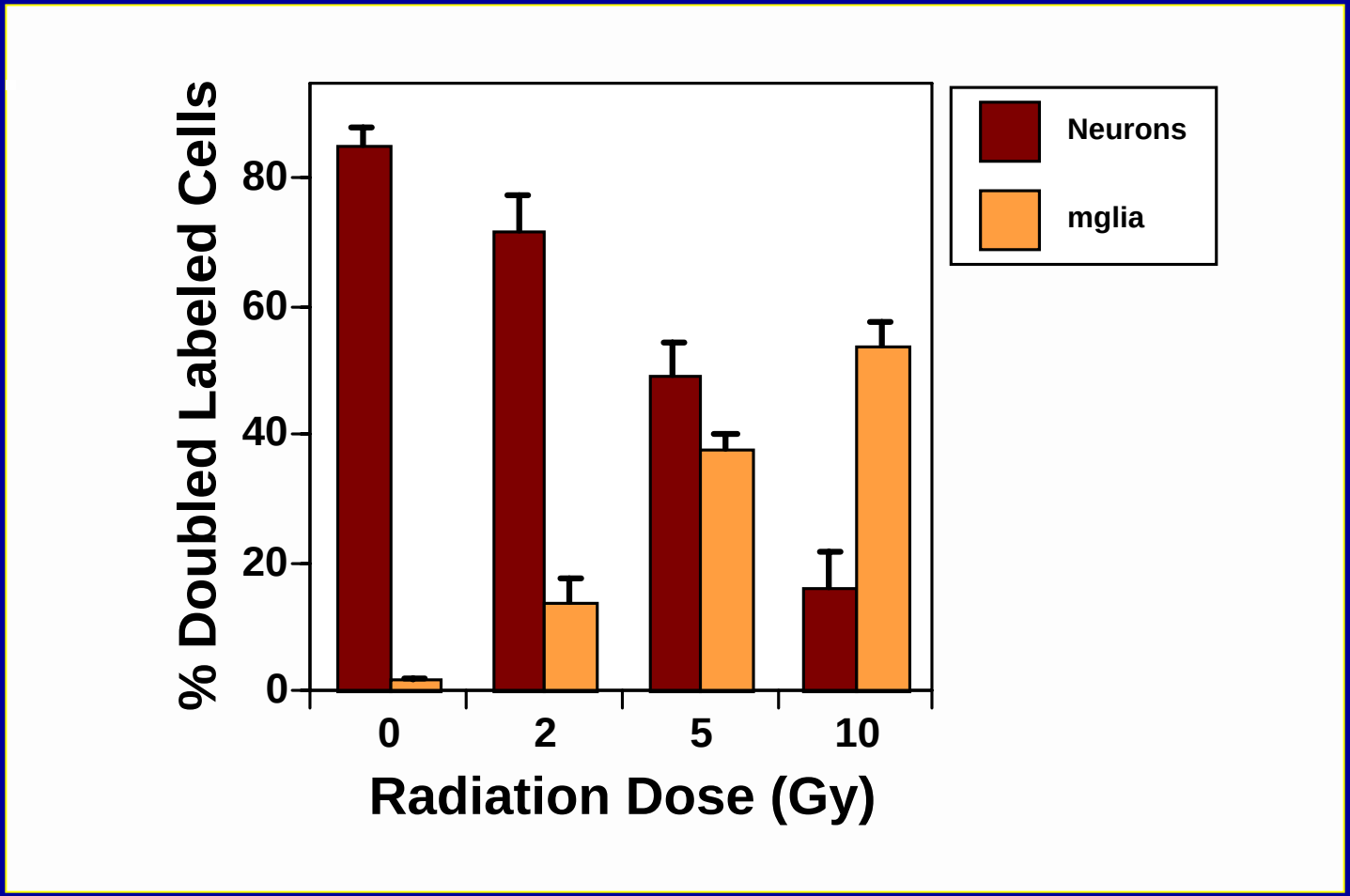
Mizumatsu et al, Can. Res. 2003

Radiation-Induced Inflammatory Changes in the SGZ are Persistent

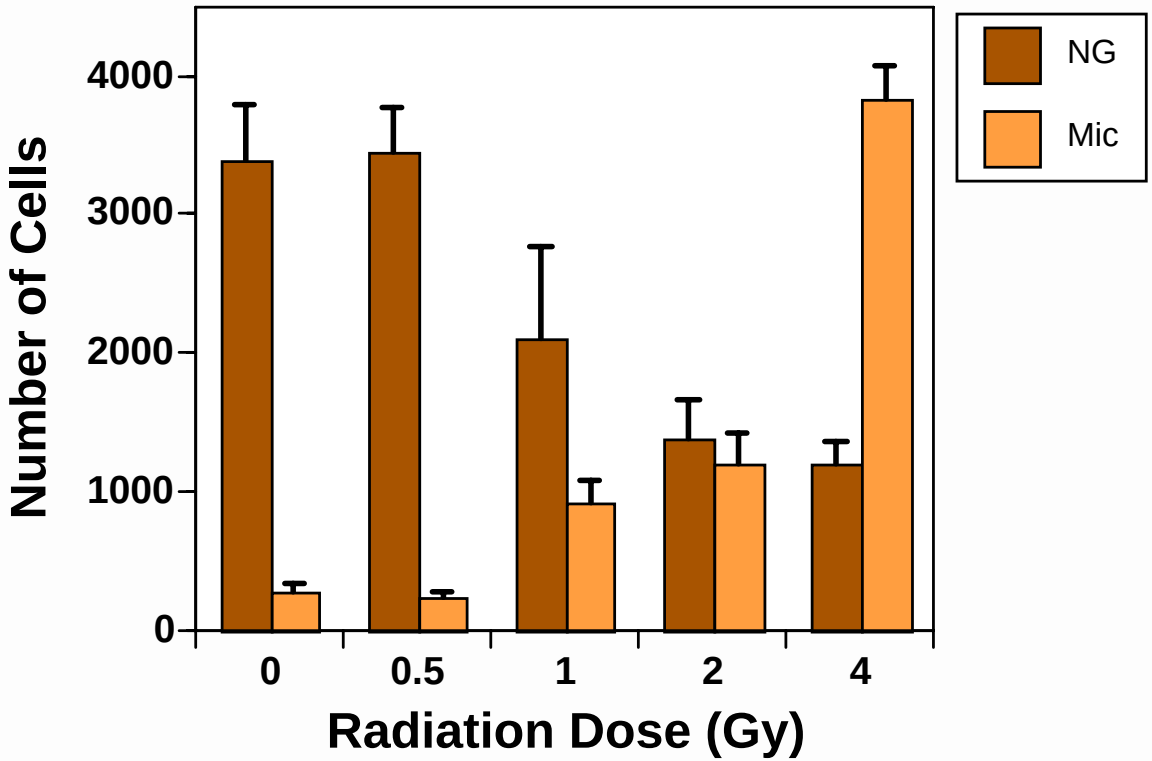


Rola et al, Exp. Neurol. 2004

Association between New Neuron Production and Inflammation After X-Irradiation

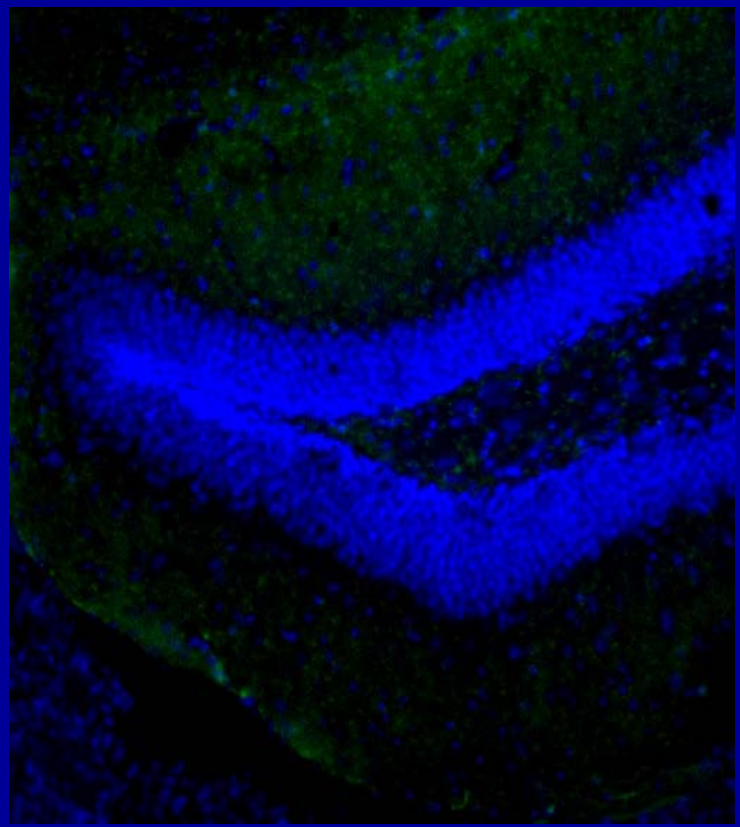


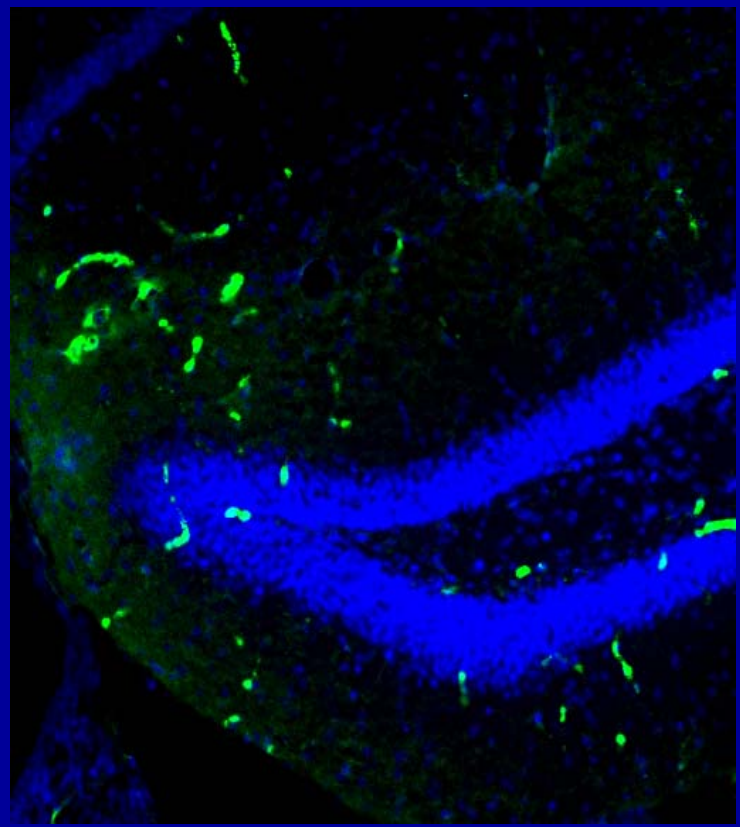
Association between New Neuron Production and Inflammation After ^{56}Fe Irradiation



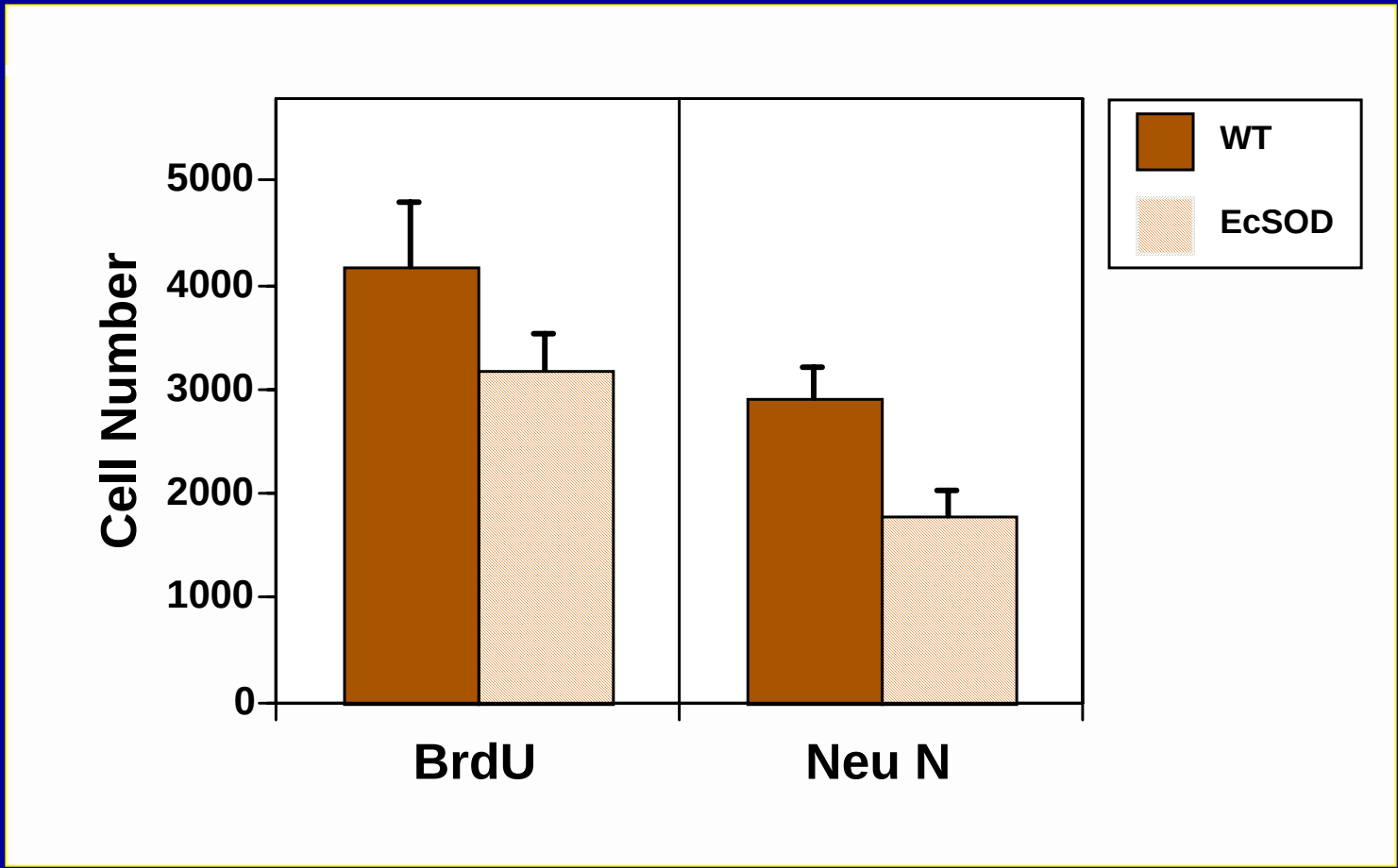
Oxidative Stress

Oxidative Stress (Lipid peroxidation:4-Hydroxynonenal) is Increased in Mouse Brain 9 Months After 2 Gy ^{56}Fe Irradiation





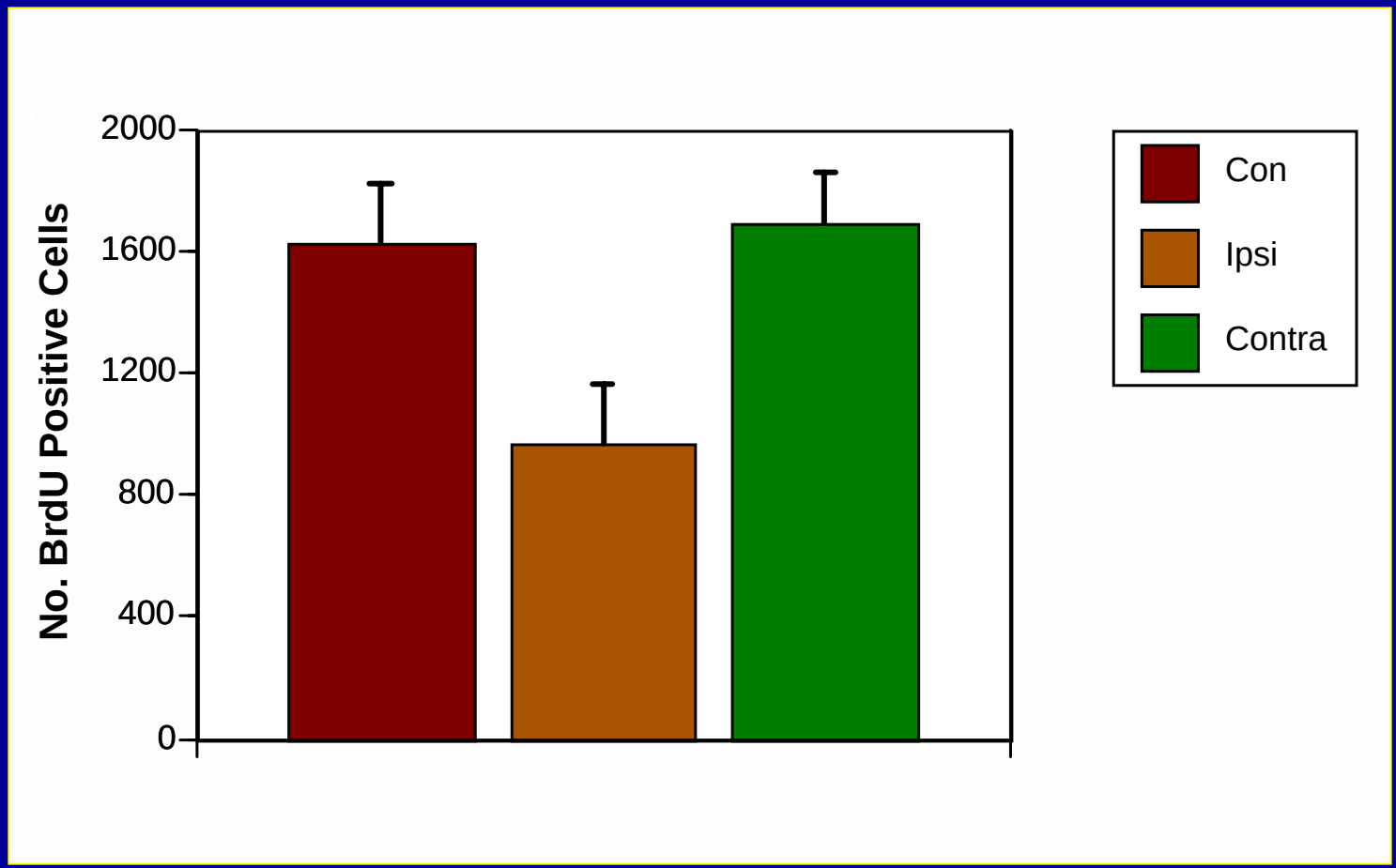
Persistent Oxidative Stress Affects Neurogenesis in the Dentate SGZ



Traumatic Brain Injury (TBI)

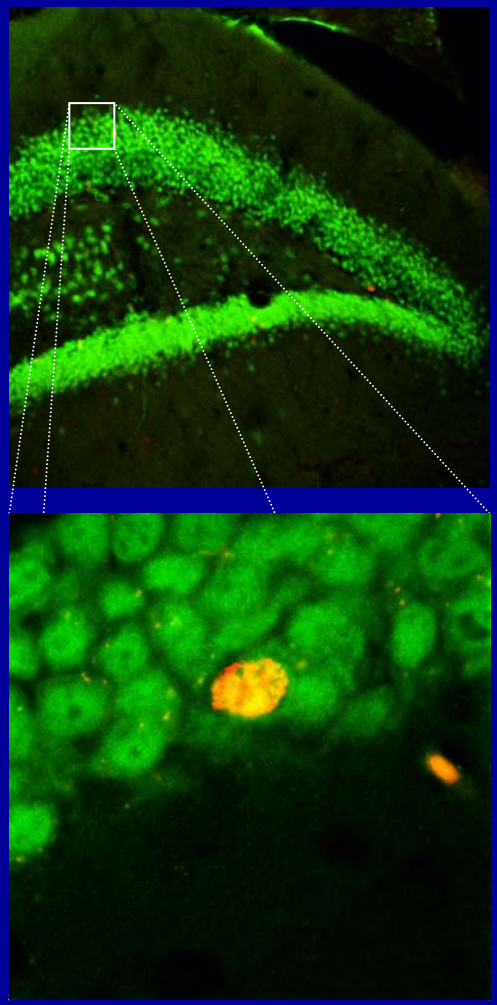
- Unilateral controlled cortical impact
- 7 days post TBI: daily injections BrdU x 7
- 1 month post BrdU tissues collected for immunohistochemistry
- Quantification of neurogenesis

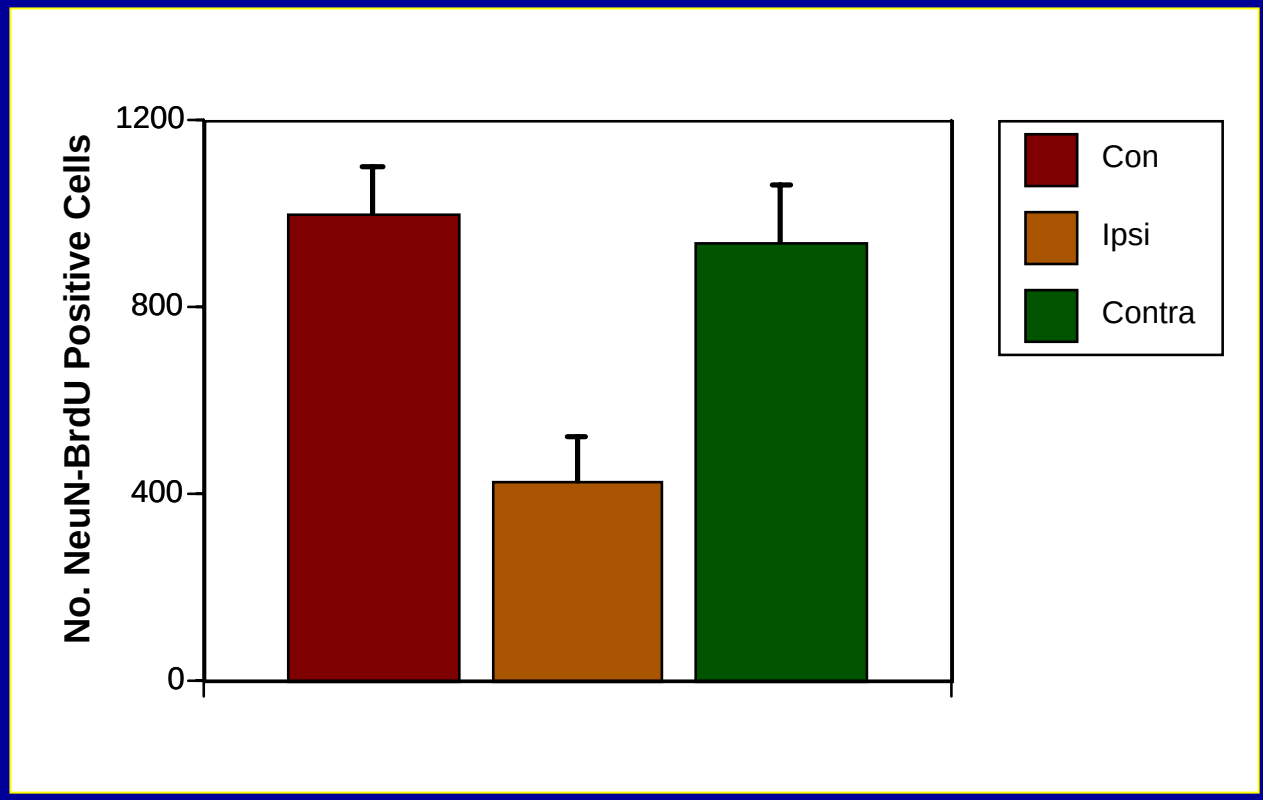
Newly Born SGZ Cells are Decreased After TBI



Rola et al, Exp. Neurol. 2006

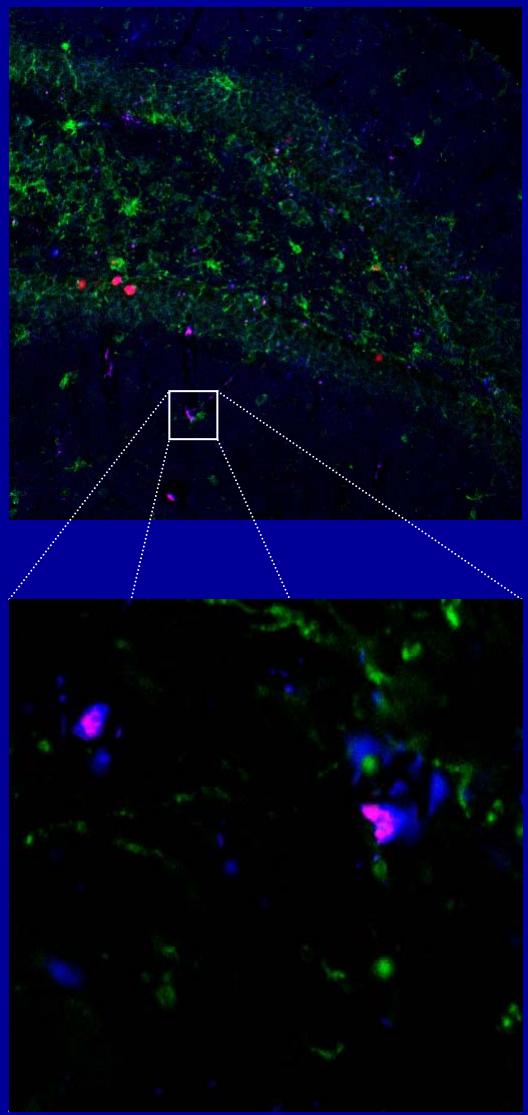
Effects of TBI on SGZ Neurogenesis

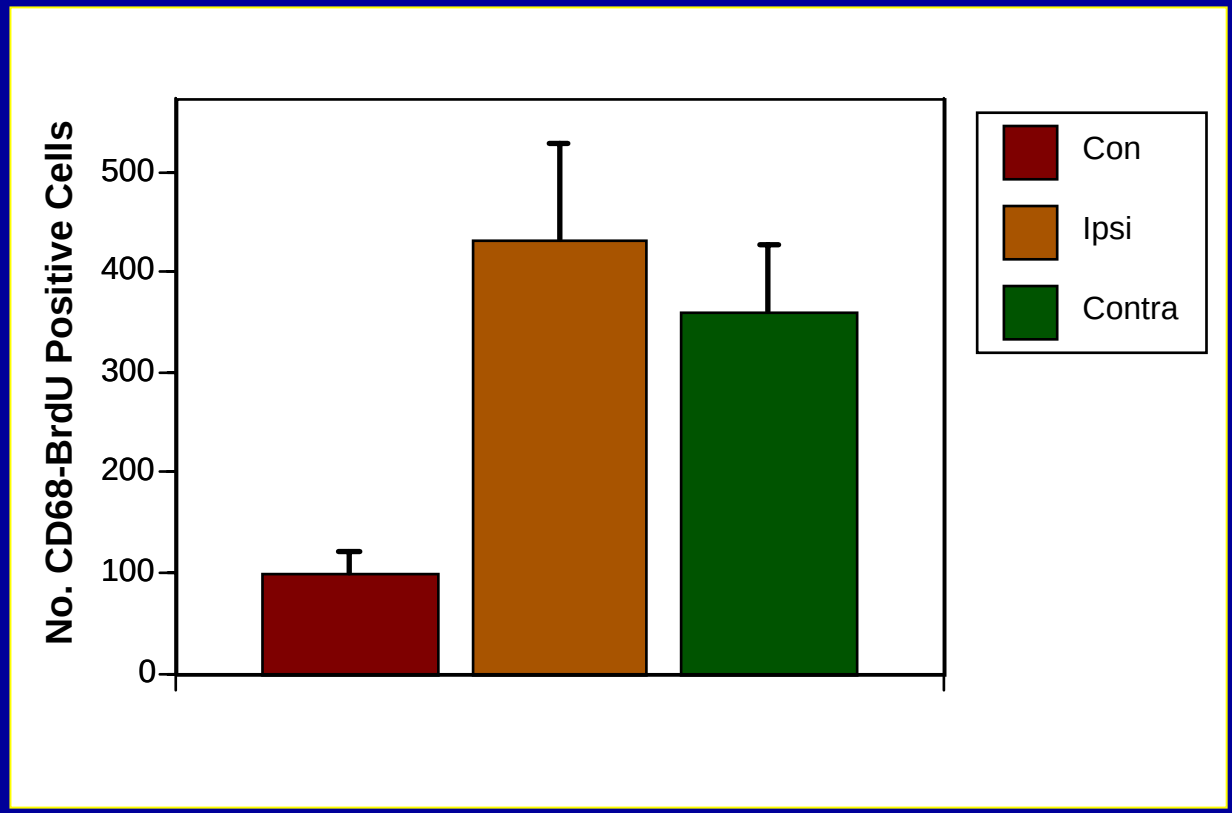




Rola et al, Exp. Neurol. 2006

TBI and Newly Born Microglia





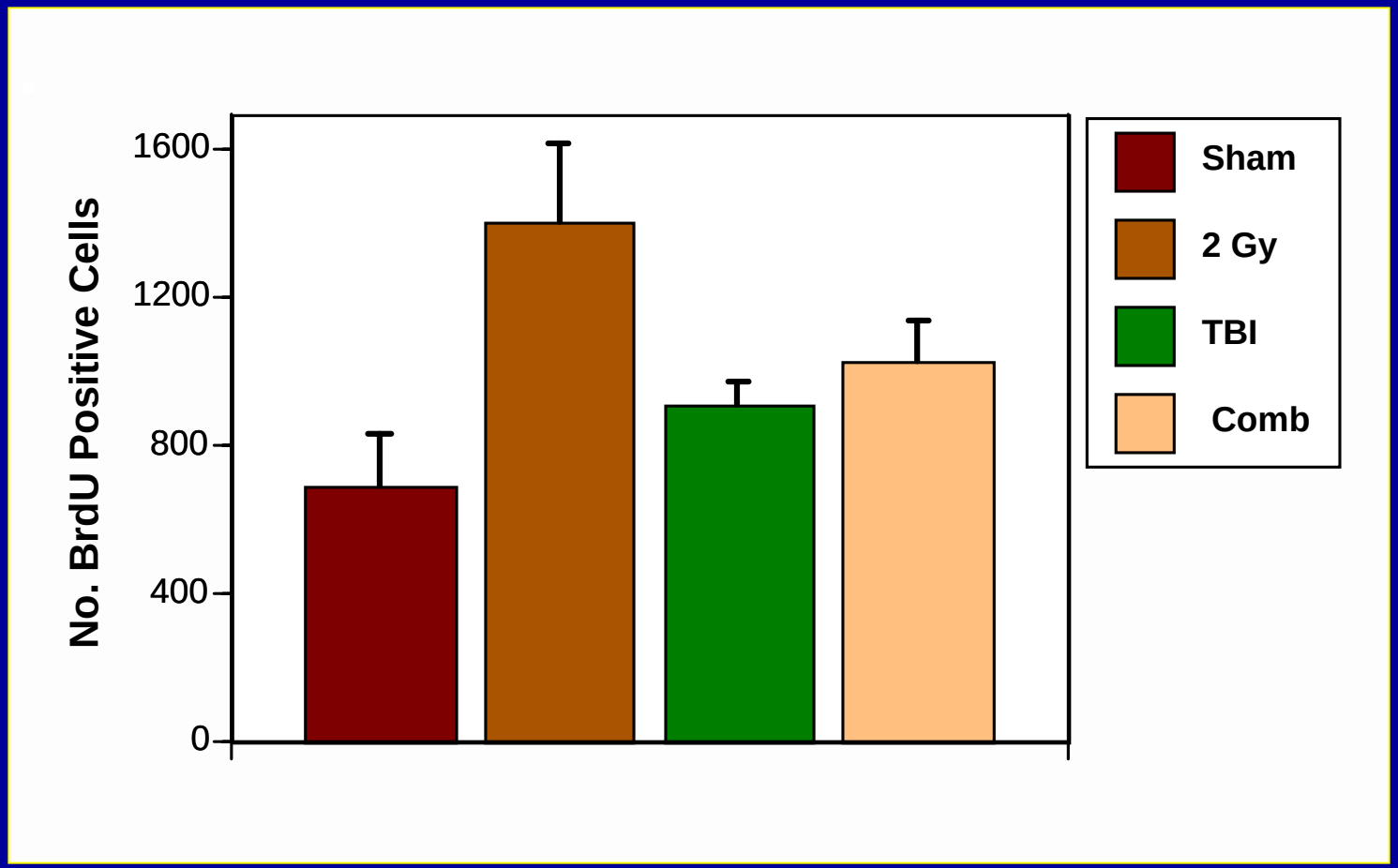
Rola et al, Exp. Neurol.

Does Irradiation Sensitize the Hippocampus to Subsequent TBI?

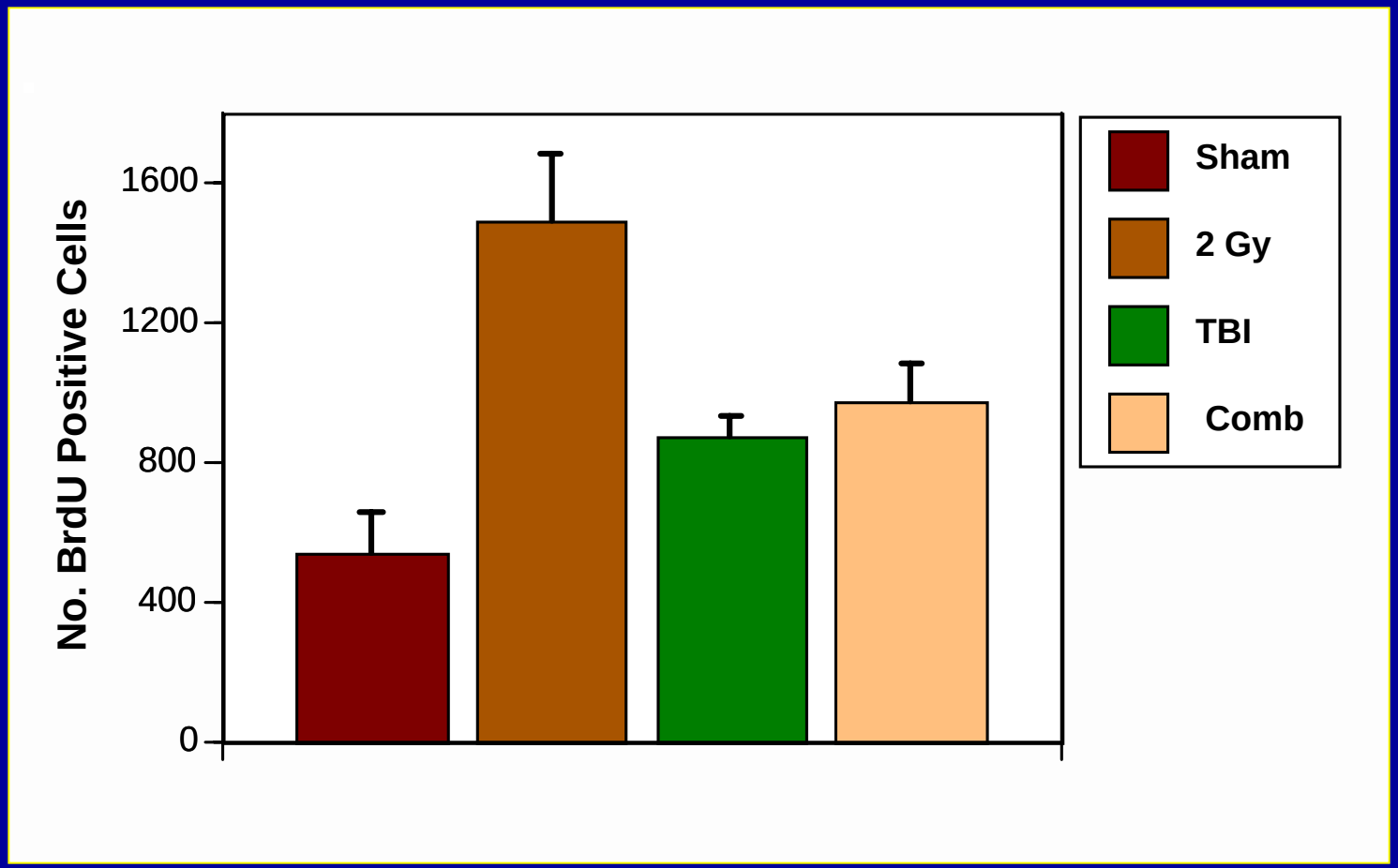
Protocol

- Single X-ray dose of 2 Gy (brain only)
- 1 month post irradiation: unilateral TBI
- 1 month post TBI: BrdU 1x/day for 7 days
- 3 wks after BrdU perfuse with 4% PFA
- Immunohistochemistry and confocal microscopy to quantify neurogenesis

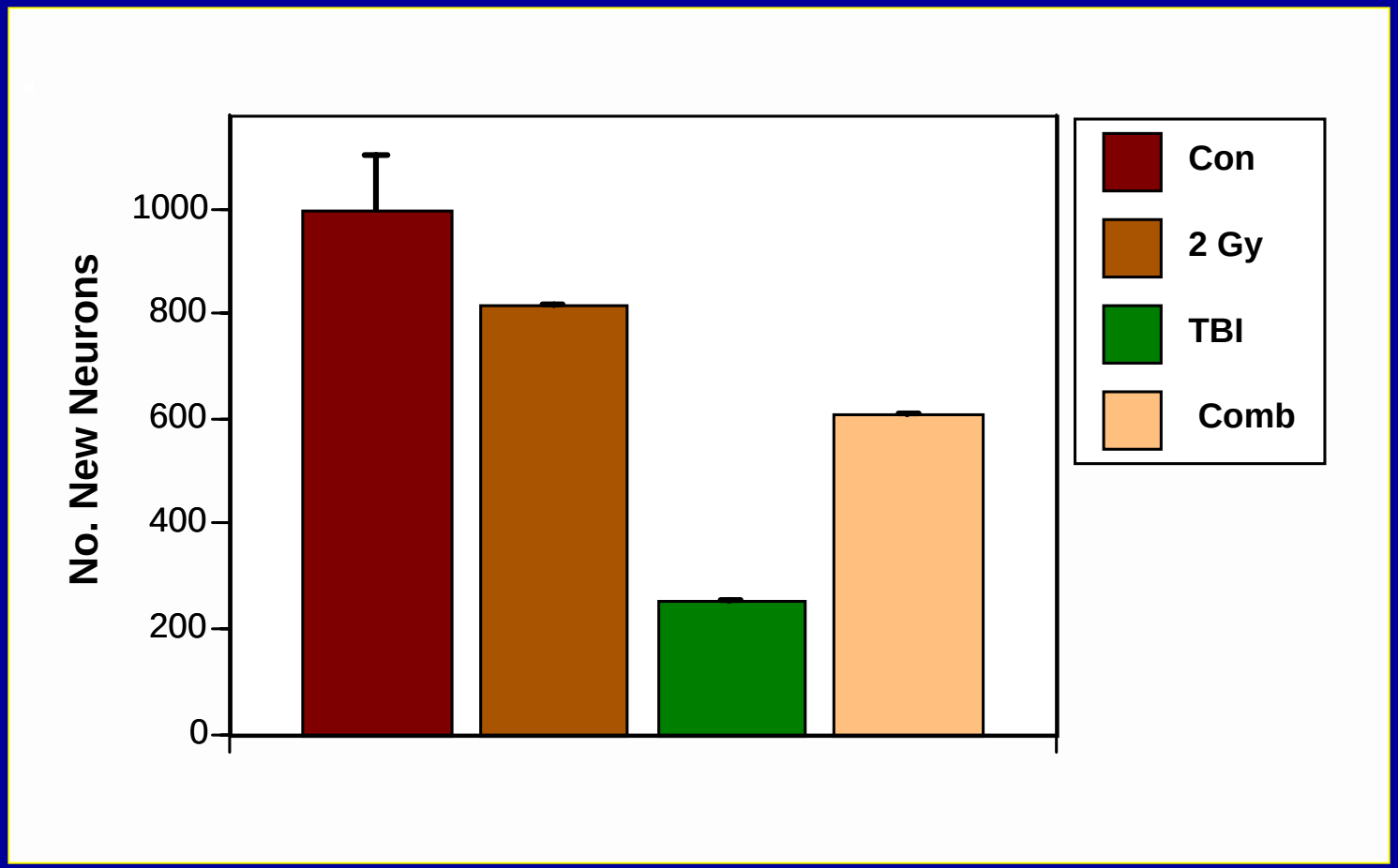
Effect of Combined Treatment on Total Newly Born Cells in the SGZ of the Ipsilateral Hemisphere



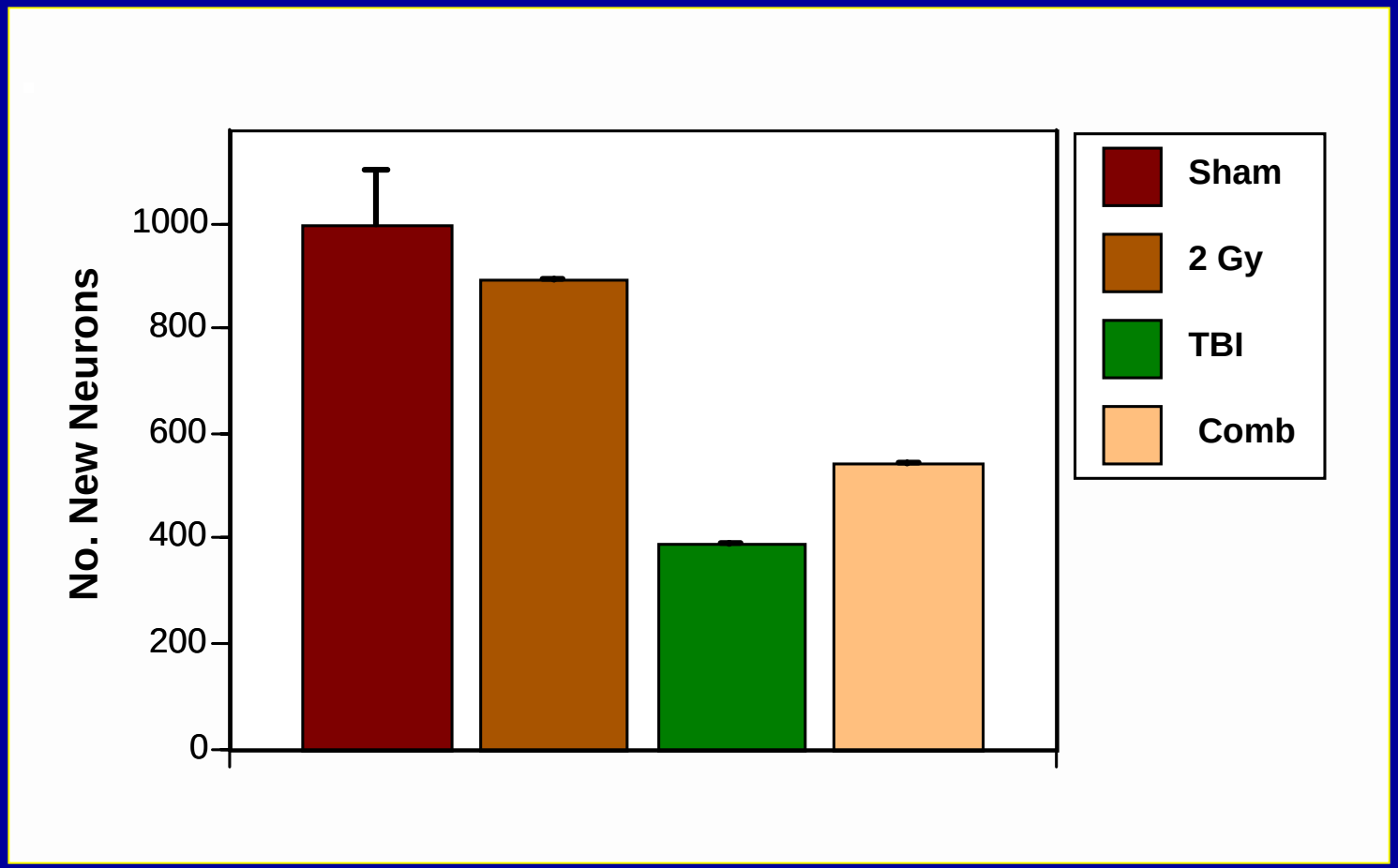
Effect of Combined Treatment on Total Newly Born Cells in the SGZ of the Contralateral Hemisphere



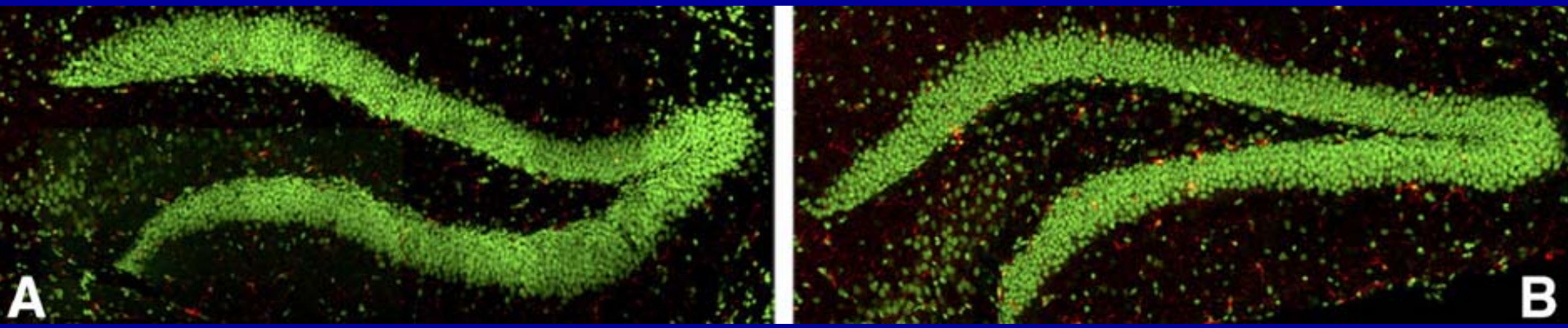
Effect of Combined Treatment on Newly Born Neurons in the SGZ of the Ipsilateral Hemisphere

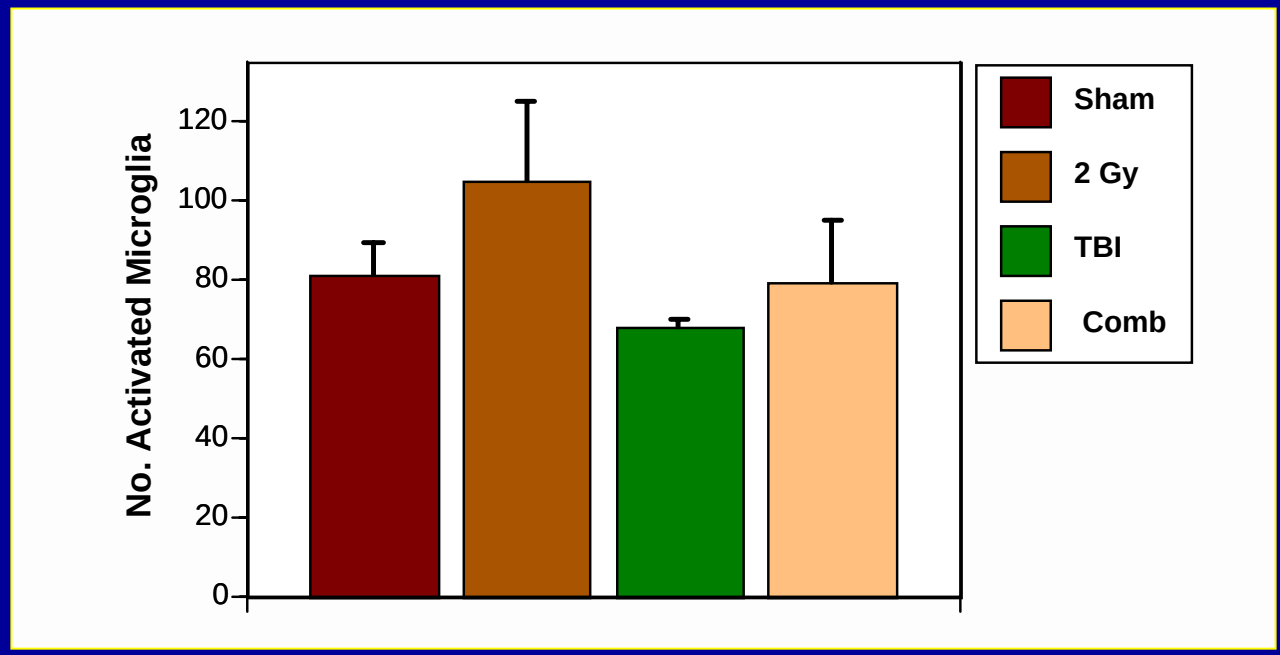


Effect of Combined Treatment on Newly Born Neurons in the SGZ of the Contralateral Hemisphere

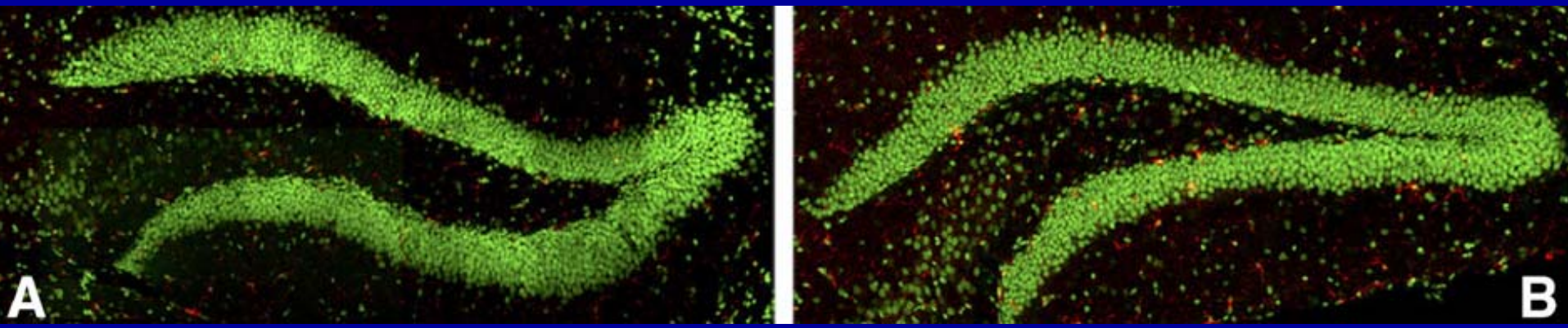


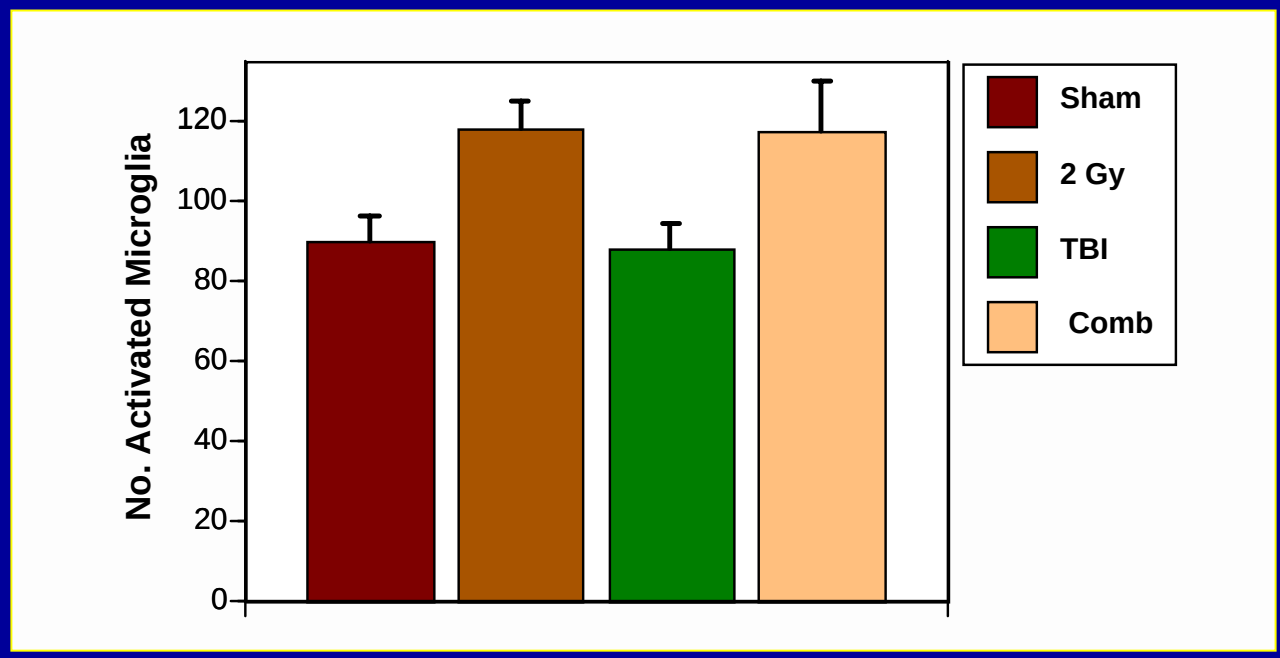
Effect of Combined Treatment on Total Activated Microglia in the Ipsilateral Hemisphere





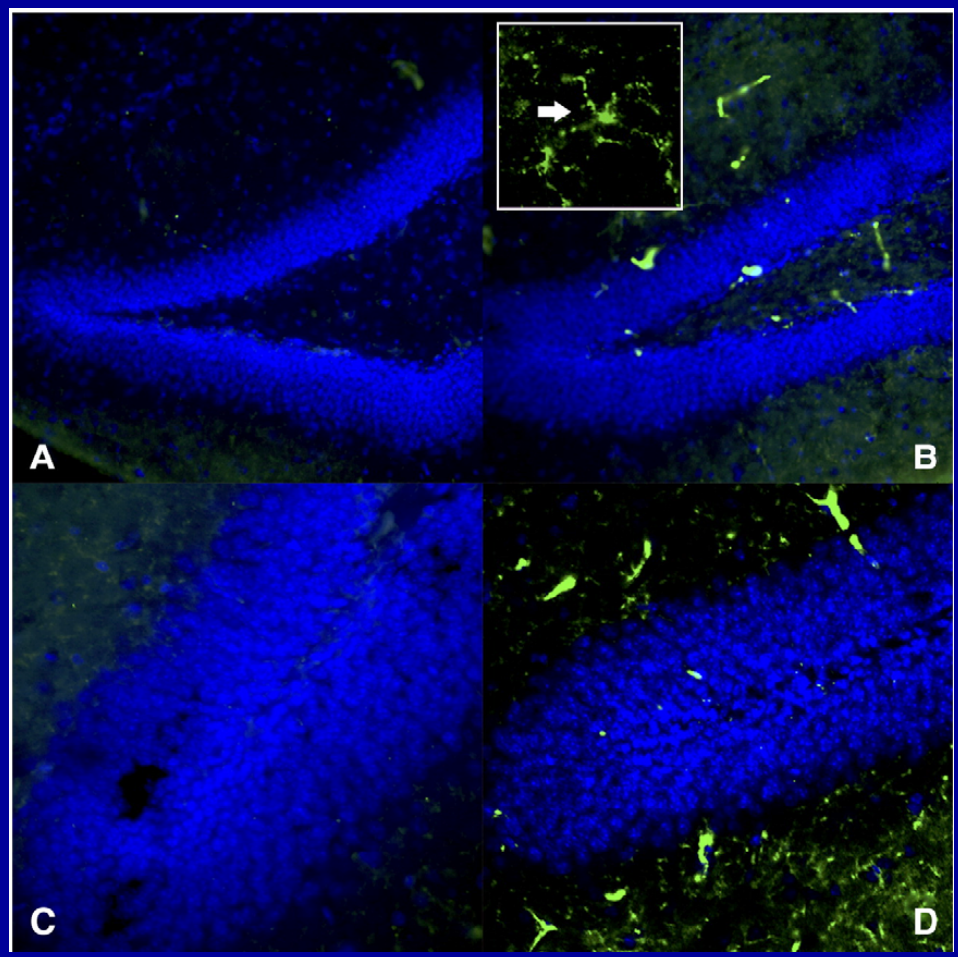
Effect of Combined Treatment on Total Activated Microglia in the Contralateral Hemisphere





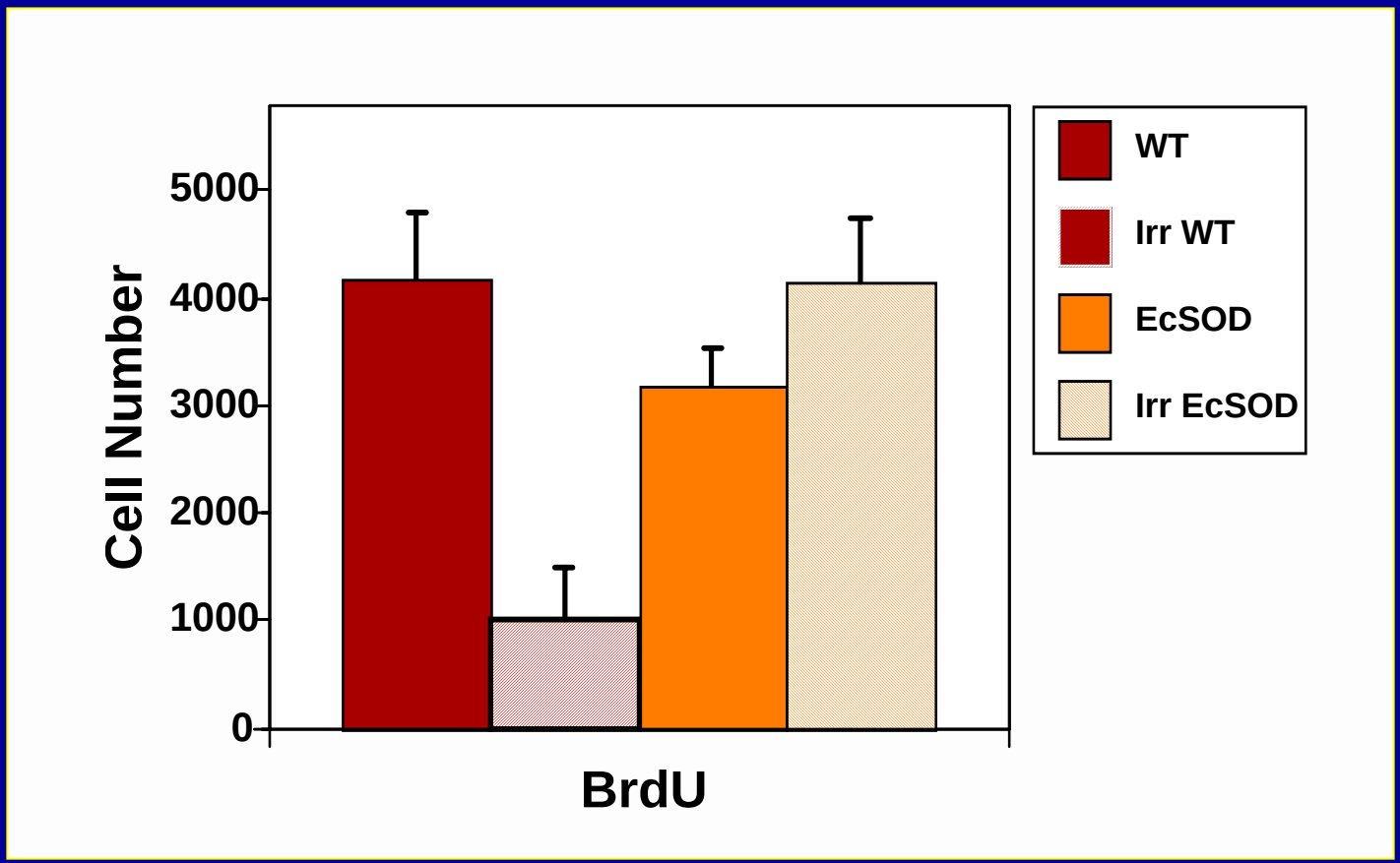
How might irradiation and TBI interact to affect the magnitude of combined injury?

Persistent Oxidative Stress is Observed in the Hippocampus of EC-SOD Knock-out Mice



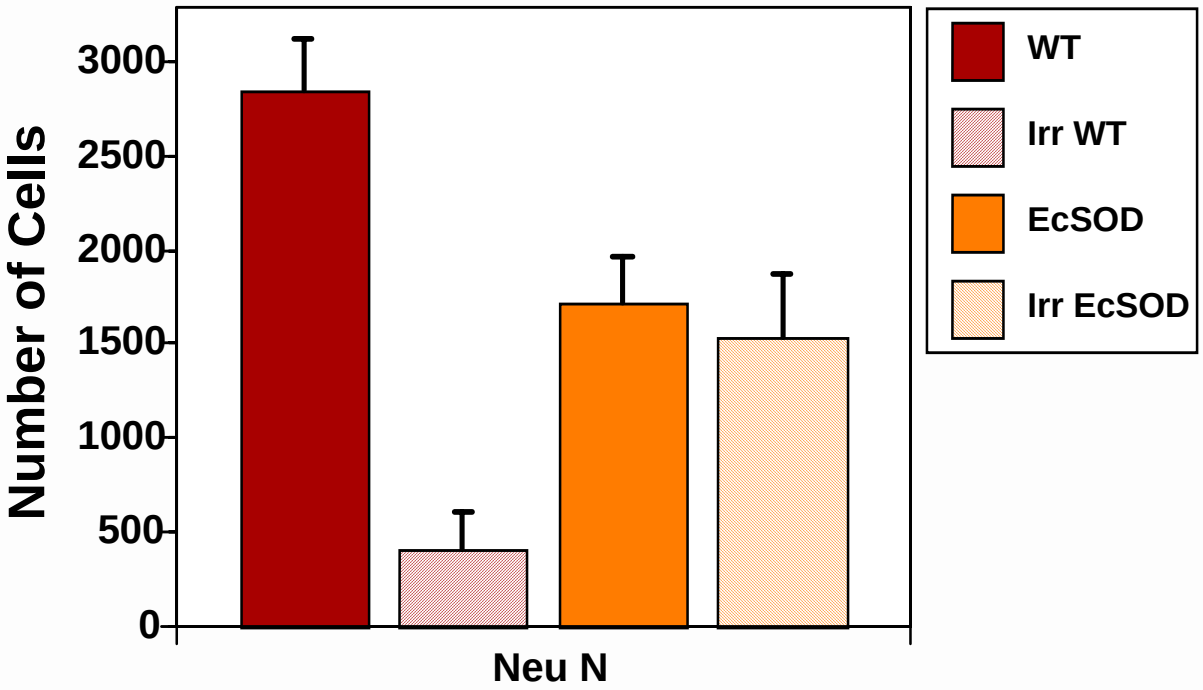
Rola et al, Free Rad. Biol Med., 2007

Lack of EC-SOD Impacts Radiation Response (5 Gy) of Newly Born Cells in the Dentate SGZ



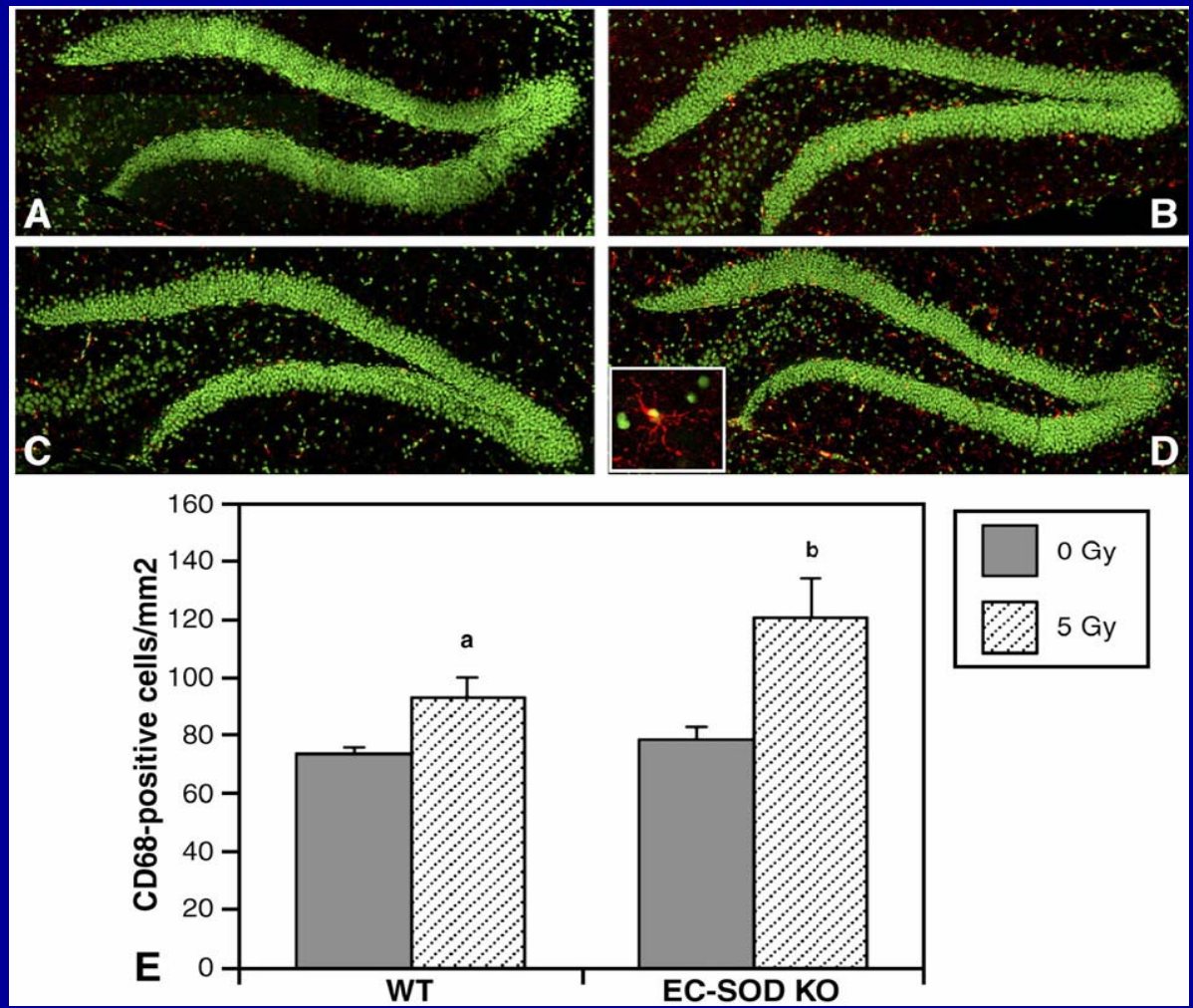
Rola et al, Free Rad. Biol Med., 2007

Lack of EC-SOD Affects Neurogenesis and Radiation Response in the Dentate SGZ



Rola et al, Free Rad. Biol Med., 2007

Irradiation Increases Numbers of Activated Microglia in WT and EC-SOD KO Mice



Rola et al, Free Rad. Biol Med., 2007

Summary

- Neurogenic cells in the dentate gyrus constitute a sensitive target for ionizing irradiation and TBI
- Microenvironmental factors are seen in conjunction with decreased neurogenesis;
- Combined injury leads to reduced effects relative to radiation alone;

Conclusions

- Altered neurogenesis is associated with significant hippocampal-dependent cognitive functions and may be contributory to cognitive impairments after combined injury.

- Microenvironmental factors may play an important role in the development and/or magnitude of combined effects.

Conclusions

- Understanding mechanisms associated with how independent insults interact to produce CNS injury may provide insight into development of effective countermeasures.

Acknowledgments

- **UC San Francisco**
  - Radoslaw Rola
  - Kelly Fishman
  - Jennifer Baure
  - Susanna Rosi
  - Linda Noble
- **NIH**
  - R01 NS46051
- **NASA**
  - NCC 9-118
  - NNJ04HC90G
  - NAG2-1632