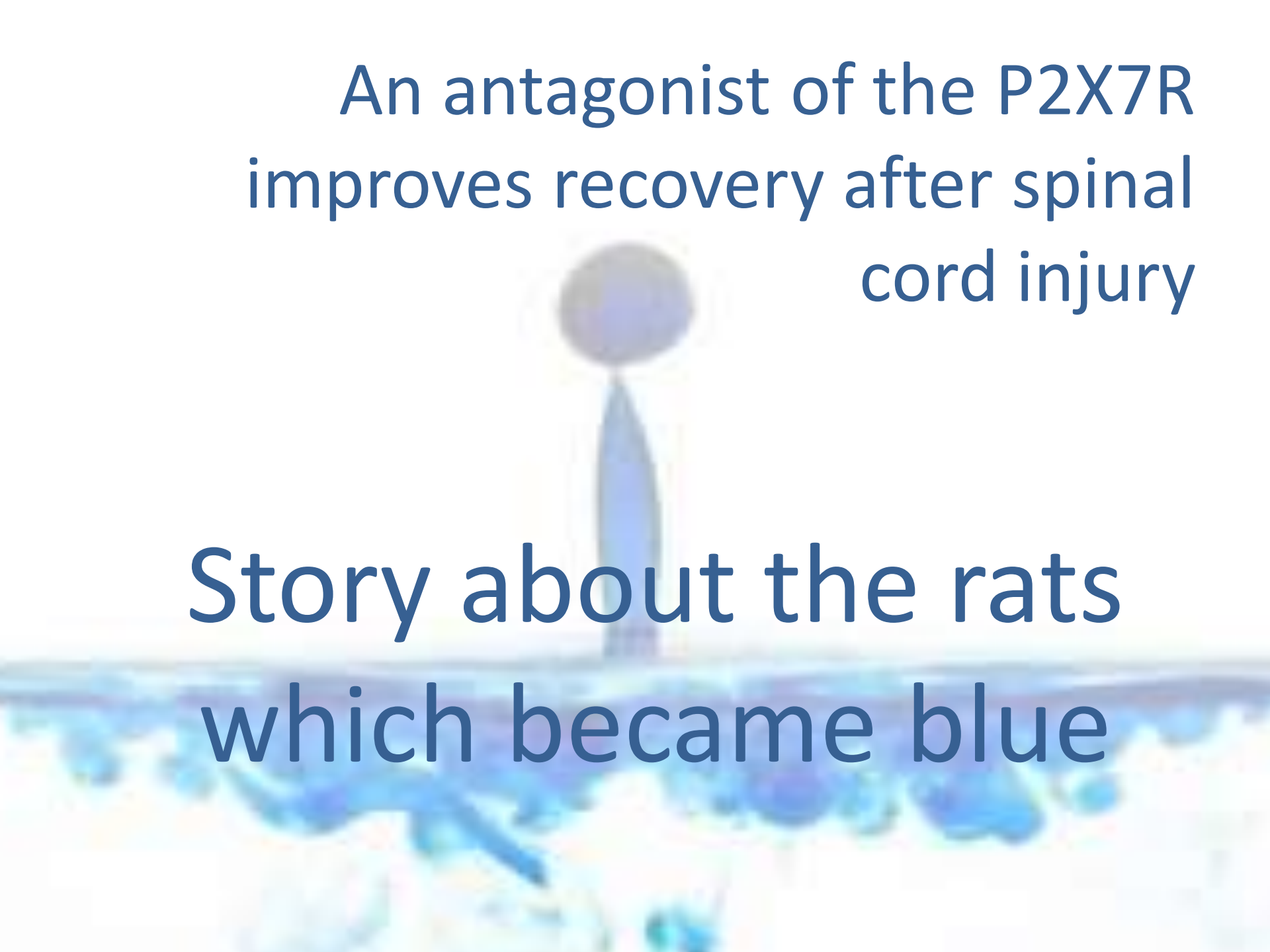
An antagonist of the P2X7R improves recovery after spinal cord injury



Story about the rats which became blue

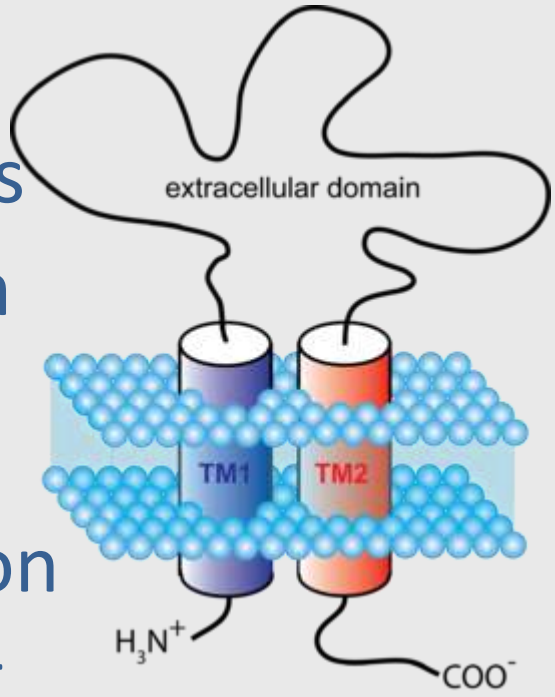
What is the spinal cord injury (SPI)?

- immediate, irreversible loss of tissue at the lesion site
- excessive release of ATP by the traumatized tissue, followed by activation of P2X7 receptors
- irreversible increases in cytosolic Ca^{2+} contribute to neuronal death
- local inflammatory responses
- a secondary expansion of tissue damage
- at last, immobility and paralysis!

No effective treatment options currently exist for patients with acute spinal cord injury

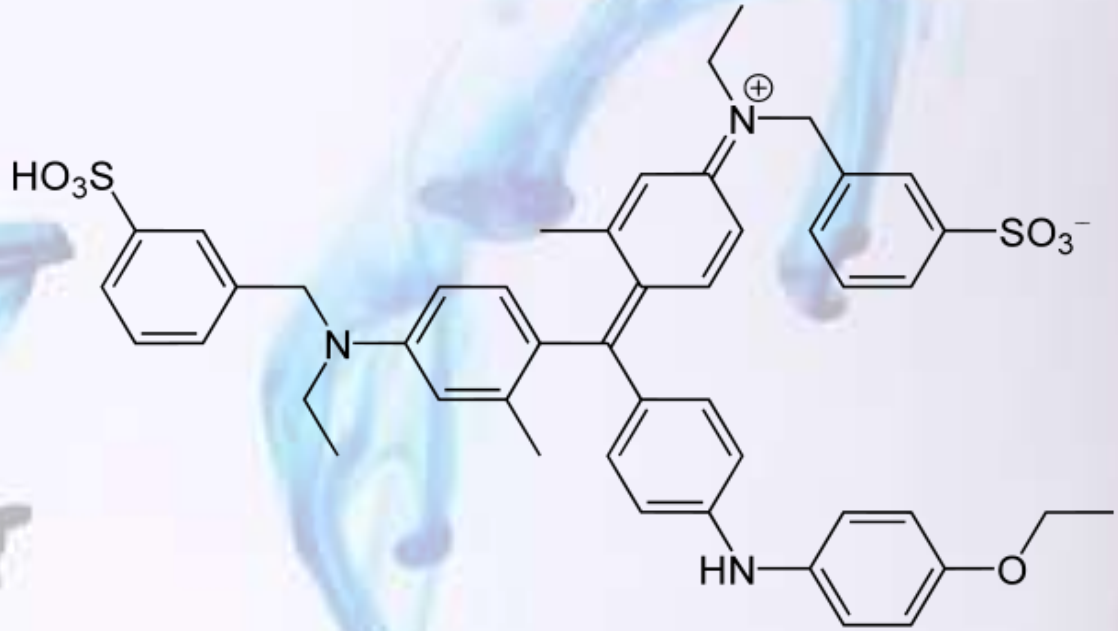
What about P2X7R ?

- purinergic receptors
- cation-permeable ligand gated ion channels that open in response to the binding of extracellular ATP
- abundantly expressed by spinal cord neurons and microglial cells
- were first discovered in macrophages
- P2X7R activation leads to production and release of interleukins (IL-1β), cycloxygenase-2 and tumor necrosis factor-α (TNF-α), to caspase activation
- they posses a low affinity for binding to ATP (≈ 140 μM)



Brilliant Blue G (BBG)

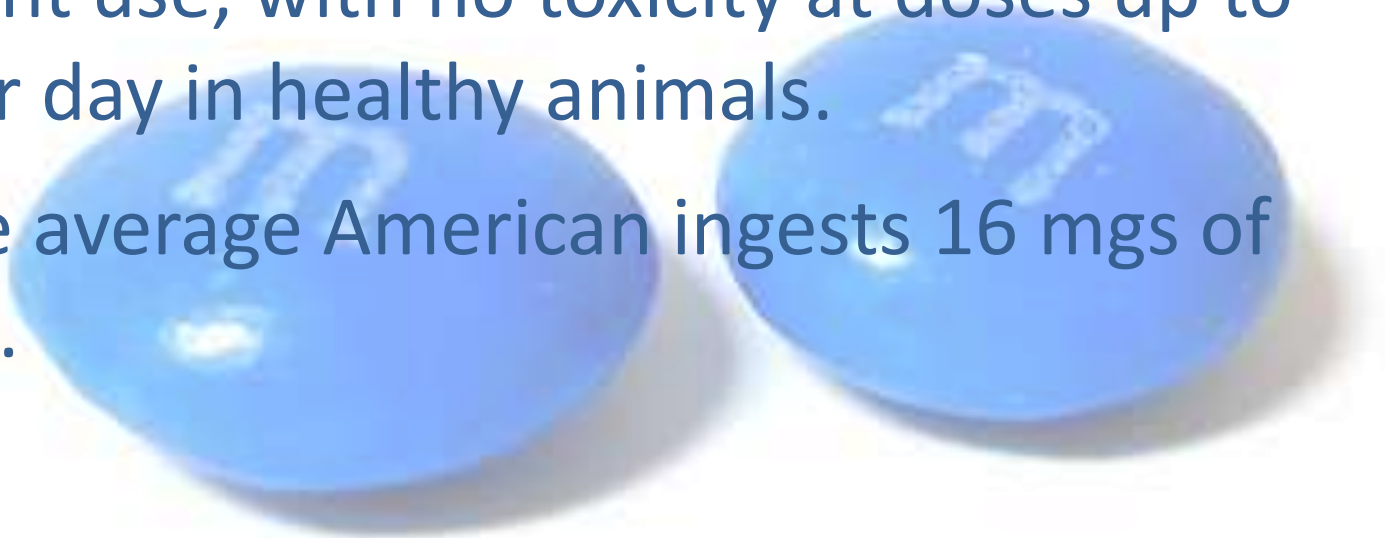
- is an analog of FD&C blue dye No. 1
- is a commonly used highly selective P2X7R antagonist



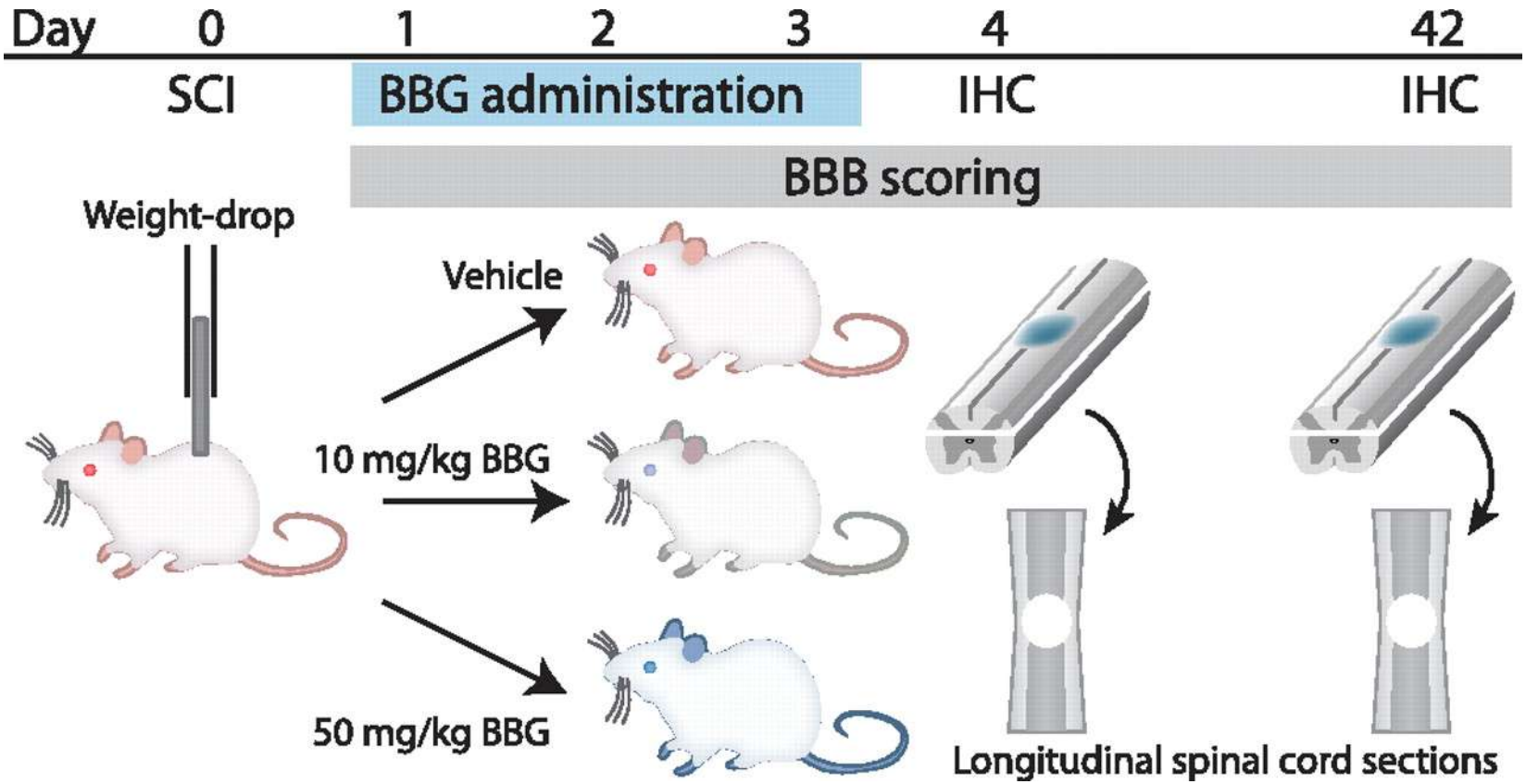
The low toxicity and high selectivity of BBG make this compound an ideal candidate for blocking the potential adverse effect of P2X7R activation in peritraumatic regions after SCI.

Some interesting…

- FD&C blue dye No. 1. is a synthetic dye approved as a food additive.
- blue food dye used to color the chocolate candies “M&M’s” and drink “Gatorade”.
- FD&C blue dye No.1 is regarded as one of the safest dyes in current use, with no toxicity at doses up to 12 mg/kg per day in healthy animals.
- each day, the average American ingests 16 mgs of this blue dye.

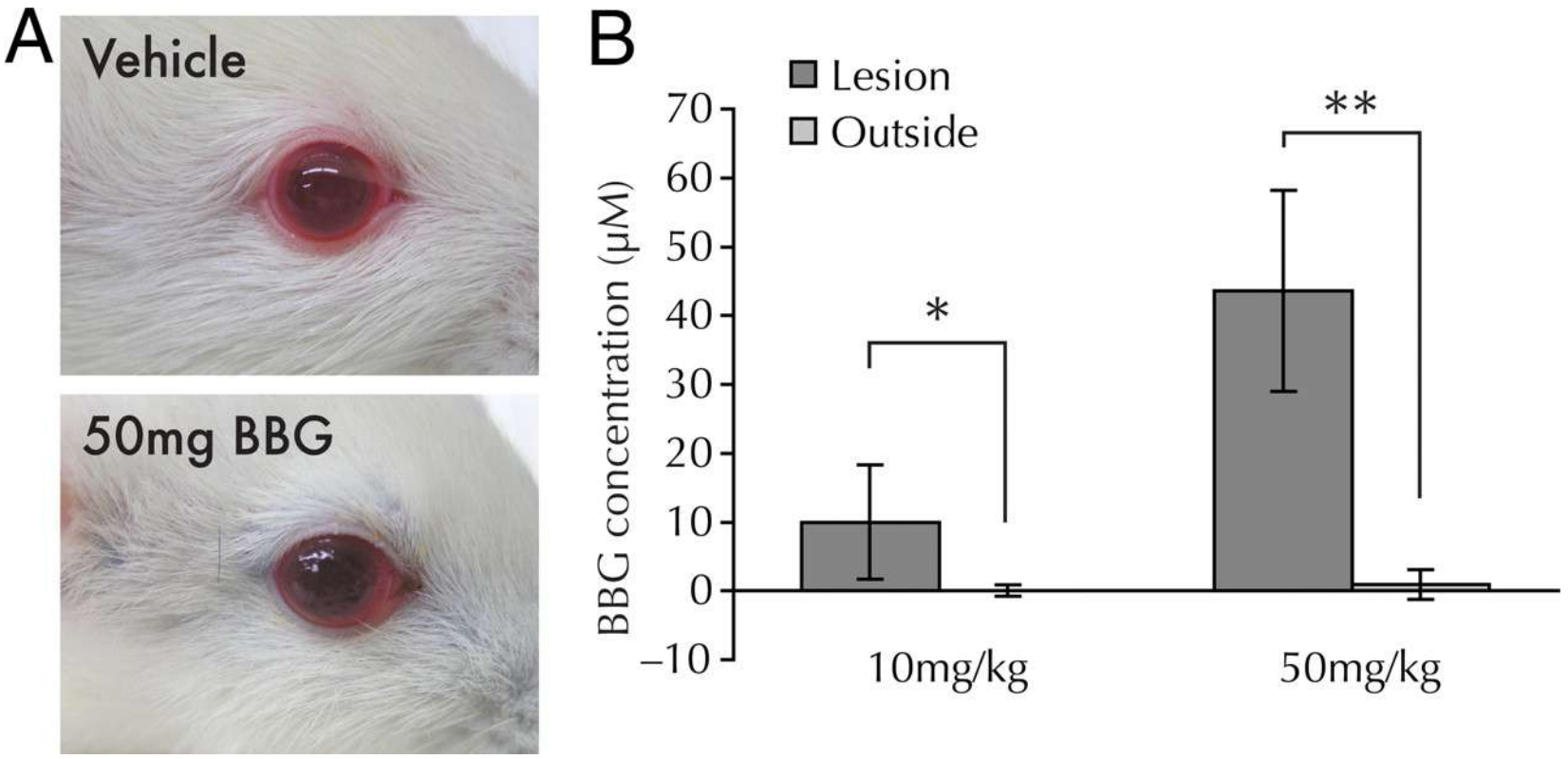


Schematic diagram of the experimental design.

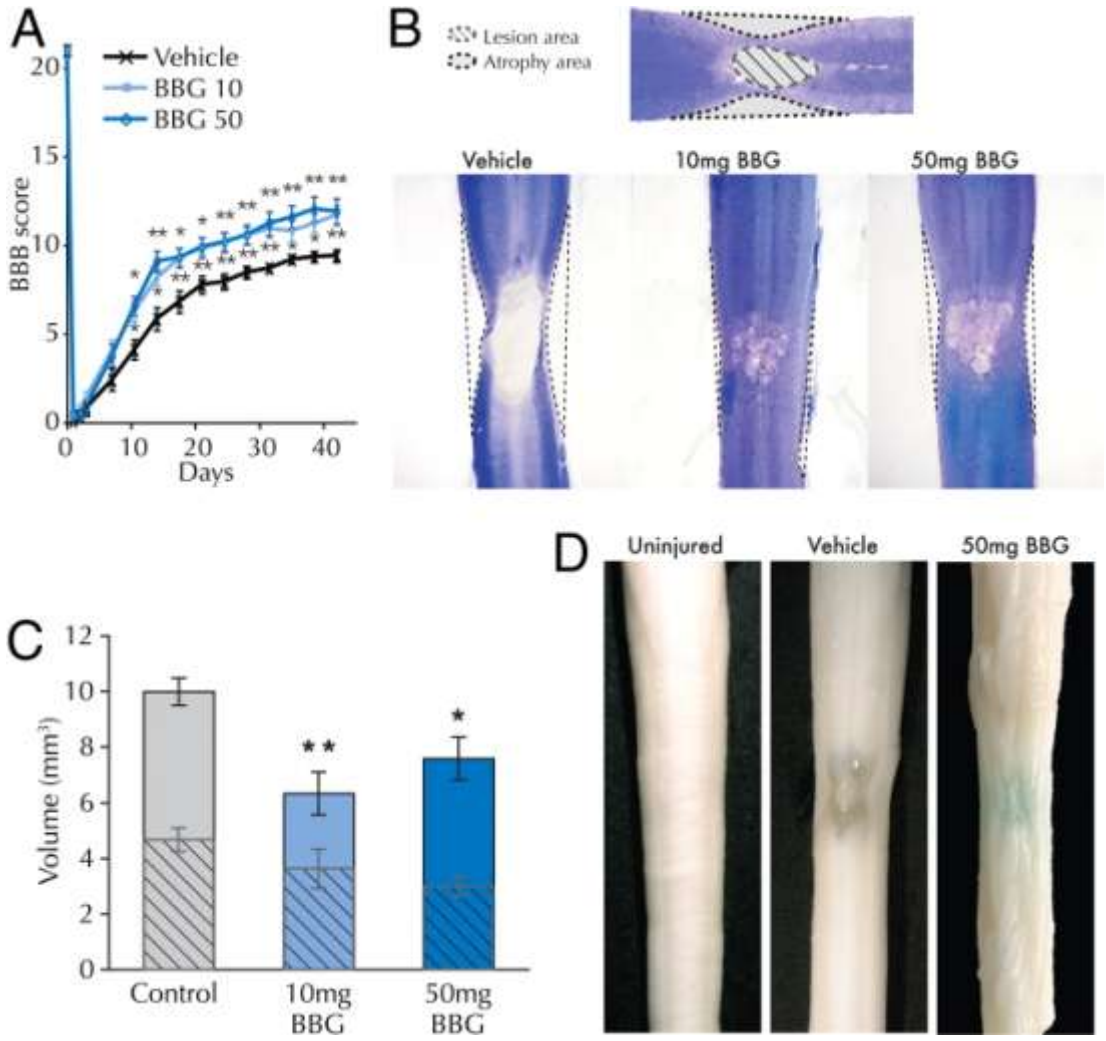


In a strange side effect…

BBG accumulates in the lesion area following spinal cord injury in rats.



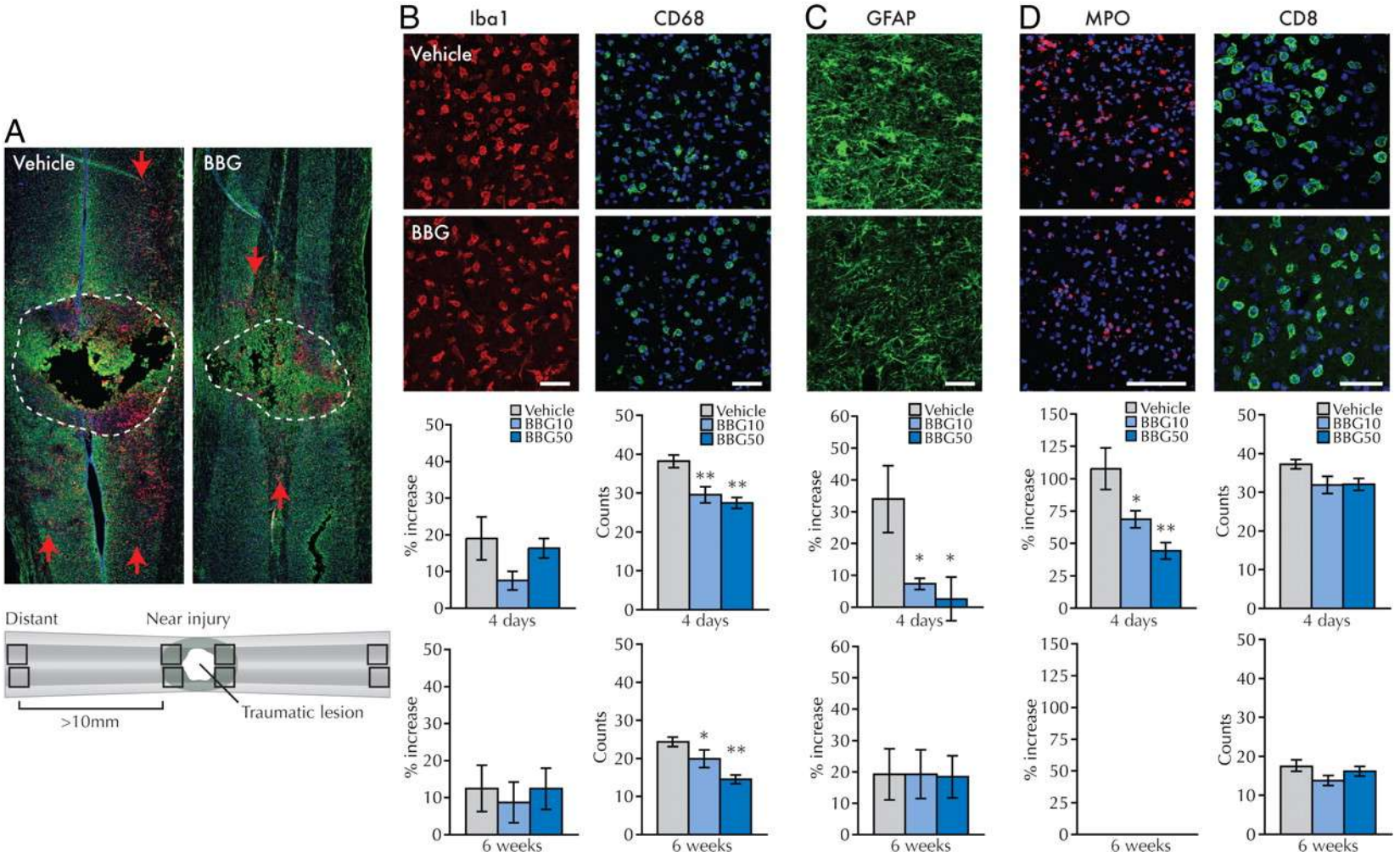
BBG (10 or 50 mg/kg) significantly improves motor function and reduces tissue loss following traumatic spinal cord damage.



Results

- BBG administered intravenously accumulates in the area of the spinal cord lesion.
- BBG improves behavioral recovery and reduces the size of the lesion.
- The blue color fades slowly and is not noticeable after 1 week.
- Neither regime of BBG administration had effects on behavior, weight, survival, or other physiological parameters, including body temperature, blood pH, blood gases, orblood pressure.

BBG reduces microglia cell activation, suppresses reactive gliosis, and decreases the number of infiltrating neutrophils in the spinal cord at 4 days after injury.



The plotted data of the immunoreactivity near the injury site compared with distant area.

BBG reduces microglial activation and reactive gliosis in the injured spinal cord

- Iba1 immunolabeling revealed that microglia in the BBG-treated group exhibited longer processes than in the vehicle group.
- Immunodetection of CD68 microglia showed clear reduction in the number of CD68 cells in the peritrau matic area after BBG treatment.
- Analysis of GFAP immunoreactivity showed that peritraumatic astrocytes from animals treated with 10 mg/mL BBG were morphologically indistinguishable from astrocytes located distant to the injury site.

BBG treatment diminishes neutrophil infiltration after SCI

- Both neutrophils and lymphocytes express high levels of P2X7R and infiltrate lesioned tissue as part of the systemic immune response to SCI.
- The increase in the intensity of MPO immunosignal in areas near the injury was reduced by treatment with 10 mg/kg BBG (compared with vehicle-treated control animals).
- Although CD8 T cells was observed in the BBG-treated groups, it did not reach statistical significance.

Conclusion

- I.v. administration of the P2X7R inhibitor BBG significantly reduced the severity of spinal cord damage without any evident toxicity. Its suppressed activation of *astroglial* and *microglial* cells, suppressed *neutrophil* infiltration, reduced the local *inflammatory response* and leukocyte infiltration from the periphery.
- Its only notable side effect was the transient acquisition of a blue tint to the skin.
- BBG injections might need to be given in the first 10 to 15 minutes after injury.

References: Peng W, et al. Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after SCI. PNAS. 2009 July 27. University of Rochester Medical Center.