# OPEN PEER REVIEW REPORT 2

**Name of journal:** Neural Regeneration Research
**Manuscript NO:** NRR-D-21-00269
**Title:** Lithium promotes spinal cord injury recovery in rats by inhibiting inflammatory mediators, oxidative stress, neuronal pyroptosis via Nrf2/HO-1 signaling pathway
**Reviewer's Name:** Vedad Delic
**Reviewer's country:** USA

## COMMENTS TO AUTHORS

Pyroptosis is a recently described hyper inflammatory cell death signaling pathway. In contrast to apoptosis which occurs in relative immunological silence, mediated by caspase 3 and 7 effectors, pyroptosis is a lytic cell death process that is highly immunostimulatory. Pyroptosis is stimulated by caspases 1,4,5, and 11 and caspase 3 which activate pore forming gasdermin proteins resulting in rapid release of cellular content and high activation of the inflammatory response, which can serve to contain fast spread bacterial of viral infections. Nrf2 is a transcriptional activator of genes containing the antioxidant response element in their promoter region. Nrf2 is normally bound to cytoplasmic retention factor Keap1, which in responses to damage an elevated reactive oxygen species is degraded, and Nrf2 is translocated to the nucleus where it will promote transcription of anti-oxidant responsive genes. Nrf2 has been a therapeutic target of interest for many years due to its potential to help cells survive in a variety of diseases.

Millions of people live with spinal cord injury (SCI) and 180,000 new cases are reported each year. Limited treatment options are available for neurodegenerative diseases and also neurodegeneration that occurs as a result of spinal cord injuries. Lithium treatment was previously shown to improve locomotor recovery in injured rats, and it is thought that lithuium promotes survival and repair by inhibition activation of nod-like receptor protein 3 (NLRP3) inflammasomes thereby limiting cell death resulting from pryoptosis initiated excessive inflammation. The authors aimed to elucidate the mechanisms driven by lithium that provide neuroprotection and concluded that lithium achieves neuroprotection by dampening ROS production through NLRP3 inhibition and activation of Nrf2 signaling pathway. They use NYU in vivo rat model of SCI and also hypoxic injury in neuron-like PC12 cells. While the manuscript is generally well written, and the topic is of key interest to the field, it is the opinion of this reviewer that there are several critical and some minor issues remaining that should be addressed to improve the manuscript. Below, they are listed in the order as they appear in the manuscript.

Graphical abstract
Aspect ratio on the abstract should be adjusted to not appear stretched and fonts should be clear and readable.

Introduction
The authors present lithium treatment for spinal cord injury, and neurodegenerative diseases as a foregone conclusion. Lithium treatment has not been shown convincingly to prevent neurodegeneration. It has been shown to marginally improve mild cognitive impairment in AD patients with dementia. In a 2012 double blind, randomized, placebo-controlled study by Yang et al published in spinal cord, authors found no significant improvement in patients with spinal cord injuries. Therefore, the language and manner in which lithium as a treatment for neurodegeneration, is presented needs to rebalanced and presented in the context of neurodegeneration, inflammation. Considering that lithium treatment

was found to have no significant effect in humans with SCI, stronger justification must be provided for its potential application as an effective treatment.

Methods

It is unclear why Wistar rats, and not other strains were used for SCI.

Why were the sections cut at 6 microns considering that the thickness of a soma is in the ranges 12-25 microns?

Quantification methods for histology for multiple figures are not adequately explained. How were the cells counted?

Figure 3. It is unclear how exactly the ROS was quantified. The images are shown, alongside graphs that are not of the images? Were they obtained using a plate reader assay, and if so how exactly was that accomplished? It is unclear from the methods, and figure caption.

Figure 6. Gating strategy for flow cytometry is insufficiently explained in methods.

Results

Consider including the individual values within the bar graphs should be presented as a scatterplot inside the bar graph, showing individual values. This is easily achieved in the graphpad statistical and graphing software the authors used in this study.

Discussion

While the authors demonstrate that there was a marginal improvement in motor function, following treatment with LiCl, the results were not curative. This language should be tempered throughout text.

Figures

Fig. 2A-C- For these and other graphs throughout, the means by which the measurements were obtained should be indicated in the legend and/or the results section (not just the Methods). For these first 3 graphs, it appears to be ELISA, but this is not mentioned in the results or legend.

Fig. 2D and other micrographs: Arrows should be added pointing to example cells of interest with explanations of those arrows in the legend.

Also, all micrographs require a legible scale bar in at least one frame in each figure that includes micrographs.

For each figure, especially the micrograph sets, the spinal cord region must be indicated because there could be significant differences between ventral and dorsal horns, for example.

Figures 3G, 5C, and 7G- It is impossible to see anything. Better images are needed (along with the arrows mentioned above). This might require different colors for the channels and/or the background.