## OPEN PEER REVIEW REPORT 2

**Name of journal:** Neural Regeneration Research
**Manuscript NO:** NRR-D-20-00920
**Title:** Subacute Metformin Treatment Leads to Better Functional Recovery after Spinal Cord Injury
**Reviewer's Name:** Harun Najib Noristani
**Reviewer's country:** USA

**COMMENTS TO AUTHORS**
In this study, the authors demonstrate the optimum dosage and therapeutic widow of metformin in mice following spinal cord contusion. The manuscript is well-written, and the data are informative to SCI field.
However, there are some clarifications necessary to further improve the quality of this manuscript:

Major:
Please comment on the difference in survival rate in control group between Figure S1 (around 10% survived by 14 days) and Figure 1 (around 50% survived by 14 days).

Figure S2: Does it mean that the n = 45 mice for this study includes mice that did not survive the SCI? If so, please add this information in Figure S2 i.e. BMS data for control after 14 days would have around 5 mice.
It may be beneficial to readers to see the survival rate throughout the 6 weeks post SCI. This can be added as a supplementary figure.

Figure 3B-D: Please change the bar graphs to scatter dot plot showing individual values for each mouse.

Page 12, line 50; If the p value is not close to 0.05, the statement "The test results showed that the motor function recovery in the 3 dpi group tended to be better than that in the 0 dpi and the Control group in terms of the rearing activity and average speed.." needs to be revised accordingly.

Page 13; What is measured and shown in Figure 4D is lesion area ($\mu m2$) NOT volume ($\mu m3$). The authors need to multiple the measured surface area ($\mu m2$) with tissue thickness ($\mu m2$) to convert the values into volume.

Page 14; Figure 4D: What does each dot represent in the graph. In the Materials and Methods section, it was suggested that 5 mice per groups were analyzed for histology at 2 weeks post-SCI. However, in the figure legend it states n = 10. Please clarify.

Figures 5M&N: As mentioned above for Figure 4D, please clarify n. In addition, why are the n different between NeuN and GFAP quantifications? It is important that both analyses examined in the same number of mice in each group.

The authors may wish to examine the effect of metformin on 5-HT axon regeneration after SCI. Given the important role of 5-HT in functional recovery after SCI, this data would provide additional mechanism on therapeutic effect of metformin.

Minor:
Contusion SCI in mice using 50 kDynes force is generally considered "moderate" contusion (PMID: 17439350). Please include "moderate" before stating contusion in the text.

Page 3, line 44 & 45 "…The formation of astroglia scar is beneficial in stabilizing the fragile tissues after injury, repairing the blood-brain barrier…" Please include "or blood-spinal barrier".

Page 4, line 23 "…Anti-inflammation strategy to facilitate functional recovery after SCI was proposed more than two decades ago.(Fehlings et al., 2017b)…" Please site the original paper that proposed the anti-inflammation strategy.

Page 6, line 6, "…of Penicillin as antibiotic drug and 0.05 mg/kg Buprenorphine as analgesic drug..." please delete the word "drug" after antibiotic and analgesic.

Page 6, line 9, "…Bladders of all injured rats…" Please replace "rats" with "mice".

Page 6, lines 39-45 "…The low-dose and high-dose groups received 100 mg/kg and 200 mg/kg metformin, respectively, immediately after surgery. The subacute high-dose group received 200 mg/kg metformin three days after surgery…". Please confirm if metformin was given for 7 consecutive days.

Page 9; Please include the catalog numbers for all the primary and secondary antibodies used.

Page 11, line 12; "...mortality of mice and inhibited the recovery from SCI..." Please delete "inhibited the recovery from SCI"

Page 12, line 29; "…and kept a trend better than the 0 dpi group (5.600 ± 0.927) albeit statistically insignificant..." Please include the p value.

Page 12, line 51; "…the rearing activity and average speed (Figure 3B-D), without much…" Please replace "much" with "Notable" or "marked".

Please describe in the Materials and Methods section how the lesion volume (or lesion area) was measure. i.e. how many sections were used per mice.

Discussion section:
Page 20, line 12; "…Our data showed that metformin administration at subacute phase  was more effective in inhibiting microglia/macrophages proliferation after injury…" Reduced Iba1 immunoreactivity could mean: Reduced microglia/macrophages activation, reduced monocyte derived macrophage infiltration into the injury site OR (as the authors suggest) reduced microglia/macrophage proliferation. Please revise this sentence accordingly.

Although, BMS is useful to indicate potential differences in functional recovery after SCI among groups, additional behavioral test such as Horizontal Ladder or CatWalk analyses are more reliable, particularly for fine motor functions. Though, the authors have not used these additional tests, they need to mention this as another potential limitation of their study.