OPEN PEER REVIEW REPORT 2

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-19-00455
Title: GFAP expression in the optic nerve and H2S generation in the trout’s Oncorhynchus mykiss brain after unilateral eye injury
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Reviewer’s country: USA
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COMMENTS TO AUTHORS
Overall this is a very descriptive study of the response to unilateral eye injury in trout. The language and results are clearly stated and illustrated by figures, but reorganization of the manuscript would allow the reader to better follow the results of the study. Please see in particular my comments about the results and discussion sections below.

Some minor phrasing in the text that should be modified:
Pg 3 lines 35-39 is a run-on sentence
Pg 5 line 50 should read "…of glial fibrillary acidic protein…"
p 6 line 20 remove "…in situ…"
pg 9 line 59 & 61 should say "intensely" not "intensively", as well in other areas in the manuscript
pg 11 line 11 should read “were observed” instead of "were determined"
p 11 line 53 should read "…(data not shown)…”

Awkward phrasing in the following lines:
Pg 3 lines 31-32
Pg 4 line 37 "…are more significant sources…”

Introduction
Includes important background information but needs to be reorganized. For example, the brief description of the goals of this study that is in the middle of the introduction should go at the end. Make sure to start the introduction with the big picture and narrow down the information to support the goal of this study at the end of the introduction.

Methods
For morphometric analysis, were any landmarks in the tissue used to orient the five randomly chosen fields? How did the researchers ensure the fields were randomly chosen?

Pg 7-8 the description of Western blotting is readable, but should be edited to improve flow and separate to perhaps 3 paragraphs for readability. Pg 7 line 60 starting with “Concentration of GFAP…” should be moved to the last paragraph of the Western blot section.

Results
In general the figures present the data well, but the figures in conjunction with the text are challenging to read and interpret. For example, the panels in figure 1 should be labeled and organized in the order in which they are presented in the text. (The current panel 1D should be last, for example.) The organization of panels across figures is challenging to follow while reading the results. I recommend
extensively reorganizing the data to fit the order in which the authors discuss the results and also the goals of each experiment. For example, why are all of the data graphs in figure 2 when there are images in both figures 1 and 3 that illustrate those results? This may help streamline the text of the results as well to better summarize the interesting findings.

Because previous studies have found both a light and a heavy GFAP isoform depending on the age and location in the nervous system, and these researchers found only the light isoform in the intact optic nerve, figure 1A should include the full range of protein sizes to show the absence of the heavy isoform. Additionally, a positive control should be added to this figure to show that the antibody and protocol the researchers are using would detect both isoforms.

Pg 9 line 26 should read "qualitative analysis of GFAP content in the optic nerve…” because there is no quantitative data provided for this figure.

Table 1. GFAP is an intermediate filament protein. Do the researchers have an explanation for why there would be immunoreactivity in cell nuclei? Are there previous studies that also observed this?

Figure 4A is a quantitative analysis of CBS and 4B is a qualitative demonstration of CBS, and description of this figure should reflect this. The y-axis of 4A needs to be edited, as it says 'SBC'.

Throughout the results, the authors should provide the rationale for why they label as cell as "undifferentiated" or "microglia". For example, on pg 13 line 0, the authors state "...number of CBS-microglia cells migrating...". It should be clear how the determination of cell type was made. Perhaps providing more description of the morphometrics in the methods section would help the reader to interpret these findings. In this way, the results could be streamlined to summarize observations and leave the interpretation to the discussion.

Discussion
Page 18 lines 44-46 should be rephrased to highlight what previous studies showed and what was in this study.

Work to rewrite to remove repeating results reporting and to interpret the data. For example, paragraphs 2-4 of the GFAP expression section could be streamlined to focus on the interpretation of the data from the current study (mainly paragraphs 2 & 3). Page 17 lines 37-44 are redundant with the results section and should be removed from one of the two sections.

A second example of this is on page 19 starting at line 10. This paragraph begins as a restatement of results, ending with how this relates to studies in goldfish. The restatement of results could be removed to explicitly explain how the observation relates to nerve injury and repair. The discussion should be rewritten with this goal in mind.