TRIGEMINAL NEUROPATHY causes hypomyelination in the anterior cingulate cortex, disrupts the synchrony of neural circuitry and impairs decision-making in MALE rats

Li, Ying; Murugappan, Suresh K.; Hasan, Mahadi; Lei, Zhuogui; Iqbal, Zafar; Ramkrishnan, Aruna Surendran; Wong, Heung Yan

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Editor 1: Bradley Kerr
Editor 2: David McArthur
Editor 3: Junie Warrington
Reviewer 1: Min Zhuo
Reviewer 2: Tie-Jun Shi

1st Editorial Decision

**Decision letter**

Dear Professor Li:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We have now received the reviewer feedback and have appended those reviews below. As you will see, the reviewers find the question addressed to be of potential interest. Yet, they do not find the manuscript suitable for publication in its current form.

If you feel that you can adequately address the concerns of the reviewers, you may revise and resubmit your paper within 90 days. It will require further review. Please explain in your cover letter how you have changed the present version and submit a point by point response to the editors’ and reviewers’ comments. If you require longer than 90 days to make the revisions, please contact Dr Junie Warrington (jpwarrington@umc.edu). To submit your revised manuscript: Log in by clicking on the link below

https://rex-prod.resxchange.com/submissionBoard/1/804e76df-e1b5-4f11-9313-5419beb42dfd/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question “Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript’s Manuscript ID number in the space below” and including your original submission’s Manuscript ID number (jnr-2020-Sep-9072) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Junie Warrington
Editor-in-Chief, Journal of Neuroscience Research
Editorial Comments to Author:

1. The National Institutes of Health now mandates the inclusion of sex as a biological variable. To conform with this mandate, the Journal of Neuroscience Research has established new policy (please see our editorial: http://onlinelibrary.wiley.com/doi/10.1002/jnr.23979/full) requiring all authors to ensure proper consideration of sex as a biological variable. Please ensure that: 1) Any paper utilizing subjects of one sex state the sex of the sample in the title and abstract; 2) The number of samples/subjects of each sex used in the research must be clearly stated in the methods section; 3) The inability for any reason to study sex differences where they may exist should be discussed as a study limitation. We are also encouraging authors to report exploratory analyses of potential sex differences in studies not explicitly designed to address them.

2. Housing and husbandry must be clearly described. Please include light cycle, room temperature and humidity, how often cages are cleaned, whether animals are handled, the number of animals per cage and whether physical enrichment is provided.

3. Articles containing statistical analyses should state the name of the statistical test, the n value for each statistical analysis, the comparisons of interest, and justification for the use of the test. It should be clear what statistical test was used to generate every P value. Moreover, the authors must include the values from the appropriate statistical test (e.g., F(x,x) = xx; n = x; P = x.xxx). If the tests violate any assumptions, the authors must provide this information.

4. JNR does not allow the use of bar and line graphs for continuous data, as many different data distributions can lead to the same bar or line graph, and representation of the full data may suggest different conclusions (Weissgerber et al., 2017). The following are the new guidelines that authors should take into consideration when graphically illustrating data. For continuous data, JNR requires authors to use univariate scatterplots, violin plots or boxplots. If boxplots are used, their component parts need to be clearly identified in the caption. Use dotplot overlays if sample size is small.

5. Gels and blots should be presented with molecular weights noted. Cropping of images for clarity and conciseness purposes is acceptable, but at least several bandwidths should be kept above and below the cropped band and important bands must be shown. Authors must supplement these data with images of the entire blot. Merging images from different experiments is not acceptable. If splicing of data from a single experiment is necessary to reorder the samples, this should be clearly indicated on the figure and in the figure legend. The method of normalization of results must be explicitly stated in the figure legend.

6. Please incorporate Research Resource Identifiers (RRIDs) in your citation of all resources used in your manuscript (antibodies, software tools, databases, model organisms) where applicable in the text, exactly as you would a regular citation or Genbank Accession number. Please also be sure these RRIDs are included in your keywords list in addition to the required keywords. For any antibodies, we ask that you also include the RRIDs in your antibody table, in addition to citing them in the text. An example of how to list RRIDs in your antibody table can found in the example antibody table attached and for more information about how to obtain and cite RRIDs within your text, please visit the "Resource Identification Initiative" section of our author guidelines: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

7. Furthermore, in accord with JNR requirements, you will have to provide additional information on each of the antibodies that you use. This must include the source (not just the manufacturer and catalog or lot number, but the species it was raised in, and the exact structure of the immunizing antigen - including the amino acid sequence for peptide antigens); characterization (e.g., what does it recognize on immunoblots); and appropriate controls (i.e. the effect of blocking peptides for immunohistotoxic localization).

8. Please upload a graphical abstract, which we are asking of all authors submitting original research articles. This is intended to provide readers with a visual representation of the conclusions and an additional way to access the contents and appreciate the main message of the work. What we require is a.tif image file and a .doc text file containing an abbreviated abstract. For the image, labels, although useful, must be kept to a minimum and the image should be 400 x 300, 300 x 400, or 400 x 400 pixels square and at a resolution of 72 dpi. This can be one of the figures from your article, or something slightly different, as long as it represents your study. Instructions for this can be found in our author guidelines online at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html
9. Please state whether the behavioral tests and cell counting were done by investigators blinded to the groups/treatments.

Associate Editor: Kerr, Bradley
Comments to the Author:
(There are no comments.)

Statistics Editor: McArthur, David
Comments to the Author:
How the study's sample size was determined is not noted. There is no evidence of power analysis. On the mandated Questionnaire (not provided) item 3a asks that a sample size rationale be provided.

Results shown in Figure 1A: Clearly the study involves group, time, and a group x time interaction but only a single F value is reported. The same holds true for several additional figures. There are multiple other grouping figures involving two factors that are also presented in the narrative as having a single F test. Note as well that Figure 3B shows two factors but the results narrative gives a single F value on line but a different anova result four lines later.

Do the data shown in Figure 2C, 2D have no corresponding statistical tests? The phrase "clearly distinct from one another" is not a result.

Figure 7B's results are given by an F test with what appear to be incorrect degrees of freedom. In other places you use t-tests for this type of comparison.

The use of SEM is not provided with a rationale. SD's are preferred. SEM's calculated on separate data distributions that all have identical means and identical variances will be the same only if the number of cases in all groups is also the same. Thus SEMs cannot be interpreted as a stable reflection of variability for a given set of data distributions, and the visualizations using SEMs are thus also not stable. It is for these reasons that 21st century statistical thinking has pushed for SDs.

This journal does not accept "dynamite plots" (also called "plunger plots", "toiletbrush plots", or "bogbrush plots") as they are often severe distortions of the actual data distributions. When samples are small, kindly use dotplots, else use boxplots or violin plots with or without dotplot overlays.

This journal requests that test results and p-values be shown using a consistent decimal exactness: numerous values in this text are given to varying number of digits or they are expressed only as "P<0.05". Kindly choose a decimal, say 3, and stay with it throughout, with "p<0.001" reserved as appropriate.

Reviewer: 1
Comments to the Author
In this study, authors performed different sets of experiments to show changes in the ACC after the injury. Most of these findings are not surprising, except that different area of the body was investigated. The paper lacks of basic new mechanism to explain these changes. Additional work are needed to link these changes together with a new molecular mechanism.

Minor:
Some of recent key literatures on ACC and plastic changes have not been cited, authors are encouraged to read more publications related to ACC and plasticity.

Reviewer: 2
Comments to the Author
The authors have examined the effect of trigeminal nerve injury on the decision performance and the trigeminal and limbic systems. It is an interesting study, which will be of interest to a wide range of researchers who are interested in studying not only the sensory aspect of pain but also the emotional and interpretive or cognitive aspects of pain. Overall, the manuscript flows well and is generally easy to comprehend. However, there are a few concerns with the manuscript.
Title:
'Neuropathic pain causes hypomyelination...........’ Better to use ‘Trigeminal neuropathy causes...........’
Abstract:
1. Page 2, line 6, preferred to define abbreviation, particularly only when mentioned for the first time, for example ACC, and so on.
2. Page 3, line 4, ‘Chronic pain causes demyelination…….’ Better to use ‘Chronic neuropathy causes…..’

Introduction:
1. Page 4, paragraph 2 line 7, ‘...we examine......’ should be ‘...we examined......’.

Material and methods:
1. Page 13, paragraph 1 line 5, ‘The myelin MBP+ intensity is ......’ should be ‘The intensity of MBP+ myelin was.....’
2. Page 13, paragraph 1 line 9, ‘...1000um’ should be ‘...1000 µm......’
3. Page 13, paragraph 2, ‘1x1x2mm3.’ should be ‘1 × 1 × 2 mm3..’

Results:
1. Page 15, paragraph 2, line 3 ‘C-fos..’ should be ‘c-Fos..’
2. Page 15, paragraph 2, regarding the expression of c-Fos in ACC neurons, surprised to see so many c-Fos positive neurons in the sham animals (also see Fig 1C). Could this be because of mechanical stimuli, any investigation was made on the rats without stimuli?
3. Page 18, paragraph 4, subtitle ‘Chronic neuropathic pain......, better to use ‘Trigeminal neuropathy causes......’.
4. Page 18, paragraph 4, line 4, ‘...effect of chronic neuropathic pain (TNP) on----’ better to be ‘effect of chronic neuropathy on......’
5. Page 19, paragraph 1, line 8, ‘MBP+’ should be ‘MBP+’; line 10-11, ‘...electron microscopy (TEM) analysis..' should be ‘... TEM analysis.’
6. Page 19-20, paragraph 2, all the ‘+’ should be ‘+’; line 7, ‘Oligodendrocytes’ should be ‘oligodendrocytes’; line 14, ‘anti-adenomatous......’ should be ‘Anti-adenomatous......’
7. Page 20, line 4, ‘...lower that the.....’ better to be ‘lower than that......’
8. Page 20, line 8-9, it is hard to see ‘less MyRF staining in ION-CCI rats compared to sham ones (Fig 9G)’. Please provide better images/photos. Have authors verified the specificity of the antibodies used in this study, e.g. Olig2, CC1 and MyRF. Alternatively, additional methods should be used to confirm the immunohistochemical findings, such as western blot.

Discussion
1. Page 21, paragraph 1, line 2-3, use ‘TNP’ instead of ‘trigeminal neuropathic pain (TNP)’; line 23, ‘......were significantly greater when compared with’ should be ‘....were significantly greater in the TNP rats when compared with...’
2. Page 22, paragraph 2, line 6, better to be ‘ION-CCI induced chronic neuropathy causes plasticity in the ACC’.
3. Page 23, paragraph 1, line 8-10, ‘A plethora of evidence has........neural circuitry’ please add references here. Paragraph 1, line 29-30, ‘In TNP rat, very few oligodendrocytes express............ (Figure 10)’ please revise this sentence and it should be Figure 9.
4. Page 24, last paragraph, ‘Chronic pain leads....’ Should be ‘Chronic neuropathy leads---’

Figures
1. Page 38, in Figure 2 C chart title should be ‘ION-CCI’ instead of ‘Sham’ and Figure 2D chart title ‘Sham’ instead of ‘ION-CCI’.

Authors’ Response

Editorial Comments to Author:
1. The National Institutes of Health now mandates the inclusion of sex as a biological variable. To conform with this mandate, the Journal of Neuroscience Research has established new policy (please see our editorial: http://onlinelibrary.wiley.com/doi/10.1002/jnr.23979/full) requiring all authors to ensure proper consideration of sex as a biological variable. Please ensure that: 1) Any paper utilizing subjects of one sex state the sex of the sample in the title and abstract; 2) The number of samples/subjects of each sex used in the research must be clearly stated in the methods section; 3) The inability for any reason to study sex differences where they may exist should be discussed as a study limitation. We are also encouraging authors to report exploratory analyses of potential sex differences in studies not explicitly designed to address them.

Response: We have mentioned the sex of the animal used in our study in the title and abstract, and mentioned the total number of animals used in our study in the ‘Materials and Methods’ section on page 7.
2. Housing and husbandry must be clearly described. Please include light cycle, room temperature and humidity, how often cages are cleaned, whether animals are handled, the number of animals per cage and whether physical enrichment is provided.

Response: We have included the details of housing and animal husbandry in the ‘Materials and Methods’ section on page 7.

3. Articles containing statistical analyses should state the name of the statistical test, the n value for each statistical analysis, the comparisons of interest, and justification for the use of the test. It should be clear what statistical test was used to generate every P value. Moreover, the authors must include the values from the appropriate statistical test (e.g., F(x,x) = xx; n = x; P = x.xxx). If the tests violate any assumptions, the authors must provide this information.

Response: According to the reviewer’s suggestions we have included the statistical analyses for all the datasets, mentioning the name of the test and the number of animals used for each experiment. We have presented all the results as Mean ± SD.

4. JNR does not allow the use of bar and line graphs for continuous data, as many different data distributions can lead to the same bar or line graph, and representation of the full data may suggest different conclusions (Weissgerber et al., 2017). The following are the new guidelines that authors should take into consideration when graphically illustrating data. For continuous data, JNR requires authors to use univariate scatterplots, violin plots or boxplots. If boxplots are used, their component parts need to be clearly identified in the caption. Use dotplot overlays if sample size is small.

Response: In our revised manuscript, we have provided all the results as Mean ± SD and presented as scatter plots for small datasets.

5. Gels and blots should be presented with molecular weights noted. Cropping of images for clarity and conciseness purposes is acceptable, but at least several bandwidths should be kept above and below the cropped band and important bands must be shown. Authors must supplement these data with images of the entire blot. Merging images from different experiments is not acceptable. If splicing of data from a single experiment is necessary to reorder the samples, this should be clearly indicated on the figure and in the figure legend. The method of normalization of results must be explicitly stated in the figure legend.

Response: We have provided the Western blot images from single experiment as mentioned.

6. Please incorporate Research Resource Identifiers (RRIDs) in your citation of all resources used in your manuscript (antibodies, software tools, databases, model organisms) where applicable in the text, exactly as you would a regular citation or Genbank Accession number. Please also be sure these RRIDs are included in your keywords list in addition to the required keywords. For any antibodies, we ask that you also include the RRIDs in your antibody table, in addition to citing them in the text. An example of how to list RRIDs in your antibody table can found in the example antibody table attached and for more information about how to obtain and cite RRIDs within your text, please visit the "Resource Identification Initiative" section of our author guidelines: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

Response: Research Resource Identifiers information for the immunohistochemistry and western blot provided in the text of ‘Materials and Method’, section “Immunohistochemistry” and ‘Western Blot analysis” on pages 12-14 and in TABLE 1 on pages 14-16. Research Resource Identifiers information for the reagents and softwares used for analysis are provided in TABLE 2 on page 16.

7. Furthermore, in accord with JNR requirements, you will have to provide additional information on each of the antibodies that you use. This must include the source (not just the manufacturer and catalog or lot number, but the species it was raised in, and the EXACT structure of the immunizing antigen - including the amino acid sequence for peptide antigens); characterization (e.g., what does it recognize on immunoblots); and appropriate controls (i.e. the effect of blocking peptides for
immunohistotochemical localization).

**Response:** We have provided the required information in TABLE 1 on pages 14-16.

8. Please upload a graphical abstract, which we are asking of all authors submitting original research articles. This is intended to provide readers with a visual representation of the conclusions and an additional way to access the contents and appreciate the main message of the work. What we require is a .tif image file and a .doc text file containing an abbreviated abstract. For the image, labels, although useful, must be kept to a minimum and the image should be 400 x 300, 300 x 400, or 400 x 400 pixels square and at a resolution of 72 dpi. This can be one of the figures from your article, or something slightly different, as long as it represents your study. Instructions for this can be found in our author guidelines online at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-4547/homepage/ForAuthors.html

**Response:** We have attached graphical abstract and the required text file containing an abbreviated abstract.

9. Please state whether the behavioral tests and cell counting were done by investigators blinded to the groups/treatments.

**Response:** The cell counting is performed by 2 people blinded to the type of experiments (stated in ‘Materials and Methods’, page 13). The data were then averaged and presented in the final results.

**Associate Editor:** Kerr, Bradley

Comments to the Author:
(There are no comments.)

**Statistics Editor:** McArthur, David

Comments to the Author:
How the study’s sample size was determined is not noted. There is no evidence of power analysis. On the mandated Questionnaire (not provided) item 3a asks that a sample size rationale be provided.

**Response:** Power analysis was not performed prior to start of study to determine the sample size but are based on our previous publications (Cao et al., 2016a; Cao, et al., 2016b; Cao et al., 2016c; Rivalan et al., 2009; Wang et al., 2017). We added the information in the Materials and Methods section on page 7.

Results shown in Figure 1A: Clearly the study involves group, time, and a group x time interaction but only a single F value is reported. The same holds true for several additional figures. There are multiple other grouping figures involving two factors that are also presented in the narrative as having a single F test. Note as well that Figure 3B shows two factors but the results narrative gives a single F value on line but a different anova result four lines later.

**Response:** We have included the F-statistic including surgery, time, and a surgery x time interaction for Figure 1A in the results section in page 18.

Do the data shown in Figure 2C, 2D have no corresponding statistical tests? The phrase "clearly distinct from one another" is not a result.

**Response:** We have reorganized the Figure 2 and included the One sample t test to show the choices significantly different from chance level (50%) in Figure 2D, 2E as shown in the results section in page 19. Figure 7B’s results are given by an F test with what appear to be incorrect degrees of freedom. In other places you use t-tests for this type of comparison.

**Response:** Thank you for pointing out the discrepancies in the statistics. We have amended and provided a 2-tailed unpaired t-test for Figure 7B in the Results section in Page 22.

The use of SEM is not provided with a rationale. SD’s are preferred. SEMS’s calculated on separate data distributions that all have identical means and identical variances will be the same only if the number of cases in all groups is also the same. Thus SEMs cannot be interpreted as a stable reflection of variability for a given set of data distributions, and the visualizations using SEMs are thus also not stable. It is for
these reasons that 21st century statistical thinking has pushed for SDs.

Response: Thank you for the valuable suggestion about the rationale for using SD’s instead of SEM’s. We understand SEM’s are unstable and have redone the statistical analysis for all the data and provided the results as Mean ± SD instead of Mean ± SEM. We have also changed the figures to show the data as Mean ± SD.

This journal does not accept "dynamite plots" (also called "plunger plots", "toiletbrush plots", or "bogbrush plots") as they are often severe distortions of the actual data distributions. When samples are small, kindly use dotplots, else use boxplots or violin plots with or without dotplot overlays.

Response: We have replaced the dynamite plots used for data presentation with scatterplots for small datasets to display the individual data.

This journal requests that test results and p-values be shown using a consistent decimal exactness: numerous values in this text are given to varying number of digits or they are expressed only as "P<0.05". Kindly choose a decimal, say 3, and stay with it throughout, with "p<0.001" reserved as appropriate.

Response: We have provided the P-Value for all datasets with 3 decimal places.

Reviewer 1

Comments to the Author

In this study, authors performed different sets of experiments to show changes in the ACC after the injury. Most of these findings are not surprising, except that different area of the body was investigated. The paper lacks of basic new mechanism to explain these changes. Additional work are needed to link these changes together with a new molecular mechanism.

Response: We have related the cognition-like behavior deficits in the chronic pain state in neuropathy rats with the plastic changes taking place in the ACC. Further, we associated the cognitive impairment in neuropathy with the demyelination in the ACC region and the possible mechanism of neuroinflammation leading to demyelination in the neuropathy rats. According to the reviewer’s suggestions, we performed additional experiments to show that chronic neuropathy is associated with microglia plasticity and inflammatory cytokines expression in the ACC region. A new Figure 10 has been provided in our revision. Details of additional results have been included in pages 23-24. A paragraph of discussion of microglial plasticity contributing to hypomyelination in chronic pain has been added in Discussion section on pages 27-28.

Minor:
Some of recent key literatures on ACC and plastic changes have not been cited, authors are encouraged to read more publications related to ACC and plasticity.

Response: We have included more recent evidences for ACC synaptic plasticity in acute and chronic pain state in the Introduction (pages 4 and 6) and Discussion (pages 26-27) sections. Several new references have been added in revised manuscript.

Reviewer 2

Comments to the Author

The authors have examined the effect of trigeminal nerve injury on the decision performance and the trigeminal and limbic systems. It is an interesting study, which will be of interest to a wide range of researchers who are interested in studying not only the sensory aspect of pain but also the emotional and interpretive or cognitive aspects of pain. Overall, the manuscript flows well and is generally easy to comprehend. However, there are a few concerns with the manuscript.

Title:
‘Neuropathic pain causes hypomyelination..........’ Better to use ‘Trigeminal neuropathy causes..........’

Response: We have rewritten the title as mentioned and included the sex of the animal. The new title is “Trigeminal neuropathy causes hypomyelination in the anterior cingulate cortex, disrupts the synchrony
of neural circuitry and impairs decision-making in male rats.

**Abstract:**
1. Page 2, line 6, preferred to define abbreviation, particularly only when mentioned for the first time, for example ACC, and so on.
   **Response:** We have included the abbreviation of ACC “anterior cingulate cortex” in page 2, line 6.
2. Page 3, line 4, ‘Chronic pain causes demyelination…….’ Better to use ‘Chronic neuropathy causes…….’
   **Response:** We have replaced the term and mentioned as ‘Chronic neuropathy’ in the text on page 3 line 4.

**Introduction:**
1. Page 4, paragraph 2 line 7, ‘…we examine……’ should be ‘…we examined……’.
   **Response:** We edited the sentence to “we examined” in page 4, paragraph 2, line 7.

**Material and methods:**
1. Page 13, paragraph 1 line 5, ‘The myelin MBP+ intensity is ……..’ should be ‘The intensity of MBP+ myelin was….’.
   **Response:** We have amended the sentence as suggested as “The intensity of MBP+ myelin was measured up to 1000 μm from the plial surface (midline) covering layers I-VI in the ACC region. The intensity of myelin MBP+ was calculated using Image J. The MBP+ myelin intensity ….” (page 13, paragraph 1, line 10-12)
2.1. Page 13, paragraph 1 line 9, ‘…1000um’ should be ‘…1000 μm….’
   **Response:** We corrected the unit “μm” in the text on page 13, paragraph 1, line 15.
2. Page 13, paragraph 2, ‘1x1x2mm3.’ should be ‘1 × 1 × 2 mm3.’
   **Response:** We corrected the dimensions as ‘1 × 1 × 2 mms’ on page 13, paragraph 2, line 3.

**Results:**
1. Page 15, paragraph 2, line 3 ‘C-fos..’ should be ‘c-Fos..’.
   **Response:** We amended the word C-fos to ‘c-Fos’ on page 18.
2. Page 15, paragraph 2, regarding the expression of c-Fos in ACC neurons, surprised to see so many c-Fos positive neurons in the sham animals (also see Fig 1C). Could this be because of mechanical stimuli, any investigation was made on the rats without stimuli?
   **Response:** We applied mechanical stimuli in the orofacial region in ION-CCI and sham rats for c-Fos study and increased the sample size to n=6 for each group. In addition, we have included the results for c-Fos cells in the sham and ION-CCI rats without mechanical stimulation (n=5 for each group) for comparison.
3. Page 18, paragraph 4, subtitle ‘Chronic neuropathic pain……., better to use ‘Trigeminal neuropathy causes…….’.
   **Response:** We have replaced the subheading ‘chronic neuropathic pain’ with ‘Trigeminal neuropathy causes…….’ on page 22.
4. Page 18, paragraph 4, line 4, ‘…effect of chronic neuropathic pain (TNP) on----‘better to be ‘effect of chronic neuropathy on…….’
   **Response:** The sentence has been edited in the text on Page 22.
5. Page 19, paragraph 1, line 8, ‘MBP+’ should be ‘MBP+’; line 10-11, ‘….electron microscopy (TEM) analysis..’ should be ‘... TEM analysis..’
   **Response:** We have made the suggested changes in the text on page 22.
6. Page 19-20, paragraph 2, all the ‘+’ should be ‘+’; line 7, ‘Oligodendrocytes’ should be ‘oligodendrocytes’; line 14, ‘anti-adenomatous…..’ should be ‘Anti-adenomatous…..’.
   **Response:** We have replaced the term as suggested to ‘Anti-adenomatous’ (page 23).
7. Page 20, line 4, ‘…lower that the…..’ better to be ‘lower than that…….’
   **Response:** We have replaced the phrase as suggested to ‘lower than that…….’ on page 23.
7. Page 20, line 8-9, it is hard to see ‘less MyRF staining in ION-CCI rats compared to sham ones (Fig 9G)’. Please provide better images/photos. Have authors verified the specificity of the antibodies used in this study, e.g. Olig2, CC1 and MyRF. Alternatively, additional methods should be used to confirm the immunohistochemical findings, such as western blot.

Response: We have replaced the confocal images of Olig2, CC1 stained Oligodendrocytes and NG2, Olig2 stained oligodendrocyte progenitor cells in the ACC region with better quality images. Further, we could not confirm the specificity of MyRF antibodies and hence removed Figure 9G and H.

Discussion
1. Page 21, paragraph 1, line 2-3, use ‘TNP’ instead of ‘trigeminal neuropathic pain (TNP)’.; line 23, ‘…….were significantly greater when compared with’ should be ‘….were significantly greater in the TNP rats when compared with…’.

Response: We have rewritten the sentence to ‘were significantly greater in the TNP rats when compared with’ on page 25.

2. Page 22, paragraph 2, line 6, better to be ‘ION-CCI induced chronic neuropathy causes plasticity in the ACC’.

Response: We have edited the sentence to ION-CCI induced chronic neuropathy causes plasticity in the ACC’ on page 26.

3. Page 23, paragraph 1, line 8-10, ‘A plethora of evidence has.........neural circuitry’ please add references here. Paragraph 1, line 29-30, ‘In TNP rat, very few oligodendrocytes express......... (Figure 10)’ please revise this sentence and it should be Figure 9.

Response: We have cited the references for the above-mentioned sentence. We have removed the figure on MyrF staining as we could not confirm the specificity of the antibody and have reorganized Figure 9 in this submission, edited the sentence about MyRF in the Results section, Discussion section and the related references.

4. Page 24, last paragraph, ‘Chronic pain leads….’ Should be ‘Chronic neuropathy leads---’

Response: We have replaced the term ‘Chronic pain’ to ‘Chronic neuropathy’ on page 29.

Figures
1. Page 38, in Figure 2 C chart title should be ‘ION-CCI’ instead of ‘Sham’ and Figure 2D chart title ‘Sham’ instead of ‘ION-CCI’.

Response: We have reorganized the sub-figures in Figure 2 and corrected the title for Sham and ION-CCI rats.

2nd Editorial Decision

Decision Letter

Dear Professor Li:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We have now received the reviewer feedback and have appended those reviews below. I am glad to say that the reviewers are overall very enthusiastic and supportive of the study. Before your manuscript can be accepted for publication, all bar graphs must be converted to violin or dot plots (please refer to statistics editor's comment). Additionally, images of full Western blots must be provided as supplementary materials.

We ask that you return your manuscript within 15 days. Please explain in your cover letter how you have changed the present version and submit a point-by-point response to the editors’ and reviewers’ comments. The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please see https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/data-sharing-citation/data-sharing-policy.html#standardtemplates for examples of an appropriate DAS. Please include the DAS in the manuscript as well.

If you require longer than 15 days to make the revisions, please contact Dr Junie Warrington (jpwarrington@umc.edu). To submit your revised manuscript: Log in by clicking on the link below
https://wiley.atyponrex.com/submissionBoard/1/b4e4bc31-a942-4822-a79b-72b443ae2ea9/current

(If the above link space is blank, it is because you submitted your original manuscript through our old submission site. Therefore, to return your revision, please go to our new submission site here (submission.wiley.com/jnr) and submit your revision as a new manuscript; answer yes to the question "Are you returning a revision for a manuscript originally submitted to our former submission site (ScholarOne Manuscripts)? If you indicate yes, please enter your original manuscript's Manuscript ID number in the space below" and including your original submission's Manuscript ID number (jnr-2020-Sep-9072.R1) where indicated. This will help us to link your revision to your original submission.)

Thank you again for your submission to the Journal of Neuroscience Research; we look forward to reading your revised manuscript.

Best Wishes,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Junie Warrington
Editor-in-Chief, Journal of Neuroscience Research

Editorial Comments to the author:

- Please convert all bar graphs to violin or dot plots as recommended by the statistics editor.
- Additionally, images of full Western blots used to generate the cropped images in Figure 8B and 10 must be provided as supplementary materials.

Associate Editor: Kerr, Bradley
Comments to the Author:
(There are no comments.)

Statistics Editor: McAruthur, David
Comments to the Author:
Figure 3B and several panels of Figures 5, 6, and 7 remain as dynamite plots, which need redrafting as explained previously.

Reviewer: 2
Comments to the Author
For the revised version I have no further comments.

Authors’ Response

Editorial Comments to Author:

1. Please convert all bar graphs to violin or dot plots as recommended by the statistics editor: “Figure 3B and several panels of Figures 5, 6, and 7 remain as dynamite plots, which need redrafting as explained previously.”

Response: As suggested by the statistical editor, we have replaced the bar graphs/dynamite plots in Figure 3B, 5B, 5D, 5E, 5F, 6B, 6D, 7B, 7E as dotplots to indicate the individual values as suggested by the statistics editor. New Figures 3, 5, 6 and 7 are provided in .tif format.

2. Additionally, images of full Western blots used to generate the cropped images in Figure 8B and 10 must be provided as supplementary materials.

Response: Full unprocessed western blot images of MBP and β-actin used in Figure 8B and CD-68, IL-1 β and β-actin used in Figure 10A have been included as Figure S1 and Figure S2 respectively in a Supplementary Materials file. Figure S1 and Figure S2 have also been cited on page 22, paragraph 2 and page 24, paragraph 1 in the marked manuscript.

3. The journal has adopted the "Expects Data" data sharing policy, which states that all original articles and reviews must include a Data Availability Statement (DAS). Please include the DAS in the manuscript as well.

Response: The Data Availability Statement has been included on page 29 of the manuscript.
3rd Editorial Decision

Decision Letter

Dear Professor Li:

Thank you for submitting your manuscript "TRIGEMINAL NEUROPATHY causes hypomyelination in the anterior cingulate cortex, disrupts the synchrony of neural circuitry and impairs decision-making in MALE rats" by Murugappan, Suresh K.; Hasan, Mahadi; Lei, Zhuogui; Iqbal, Zafar; Ramkrishnan, Aruna Surendran; Wong, Heung Yan; Li, Ying.

You will be pleased to know that your manuscript has been accepted for publication. Thank you for submitting this excellent work to our journal.

In the coming weeks, the Production Department will contact you regarding a copyright transfer agreement and they will then send an electronic proof file of your article to you for your review and approval.

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Congratulations on your results, and thank you for choosing the Journal of Neuroscience Research for publishing your work. I hope you will consider us for the publication of your future manuscripts.

Sincerely,

Dr Bradley Kerr
Associate Editor, Journal of Neuroscience Research

Dr Junie Warrington
Editor-in-Chief, Journal of Neuroscience Research

Associate Editor: Kerr, Bradley
Comments to the Author:
(There are no comments.)

Authors’ Response

4th editorial decision

Decision Letter

Author response