JOURNAL OF Neuroscience Research

Is Cyclooxygenase-1 involved in Neuroinflammation?

Vries, Erik F.J. de; Ghazanfari, Nafiseh; Waarde, Aren van; Dierckx, Rudi A.J.O.; Doorduin, Janine

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Editor 1: Patricia Schuck
Editor 2: Cristina Ghiani
Reviewer 1: Ayse Er
Reviewer 2: Jean Jean Harry

1st Editorial Decision

Decision letter

Dear Dr Vries:

Thank you for submitting your manuscript to the Journal of Neuroscience Research. We've 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 and have indicated that considerable further work would be necessary to support your conclusions.

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. If you require longer than 90 days to make the revisions, please contact Dr Cristina Ghiani (cghiani@mednet.ucla.edu). To submit your revised manuscript: Log in by clicking on the link below https://wiley.atyponrex.com/submissionBoard/1/1f238cac-4478-4a51-bbcf-e40eb0b80a45/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-2021-Jan-9482) 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,

Professor Patricia Schuck
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Editors Comments:
The topic of the review is of high interest, but a series of problems have been identified and should be thoroughly addressed for it to be considered for publication in JNR. Importantly a thorough reorganization of the manuscript has been suggested along with revision of the language. Additionally, a more clear description of how the inclusion and exclusion criteria used by the authors, and a more clear critical review of the literature should be included.

JOURNAL OF Neuroscience Research

Reviewer: 1

Comments to the Author
The old references were written as new references are not used generally in this article. As a result, up-to-date information is not provided.

Although the search resulted in 32 individual articles in this article is declared, 29 different references are used in Tables 1, 2, 3 and 4.

There is a lot of typo and careless spelling (although references are cited in different ways in the article, reference years are not specified in some places).

It must be edited in English.

Reviewer: 2

Comments to the Author
The manuscript by Vries et al represents a review of the literature on Cox-1 and neuroinflammation. The topic is timely and well worth investigation. There are however a number of issues that should be addressed. Below please find comments on Introduction and the In vitro section. Note that these comments are similar across the different sections.

There are a number of statements in the Introduction on basic biology and relevance of arachidonic acid that do not have supporting citations. – Example line 14-37
"In contrast to most peripheral organs, there is basal expression of COX-2 in the brain".

The authors may want to define exactly what they are identifying as "activated" microglia as there is a distinction between reactive and activated and using this as a catch all term has raised concerns. In addition, there is not a requirement for cells to proliferate and in fact, much of the original literature demonstrates that macrophage like cells do not increase pro-inflammatory production when they are actively proliferating. This section of the Introduction requires some attention to more accurately reflect the biology of microglia. Or rather to simply remove this aspect of the "characterization" as it is really not relevant to the paper. "An early characteristic of neuroinflammation is the activation of microglia, which are the resident macrophages of the brain. Microglial activation is characterized by alterations in morphology and increased proliferation."

One interesting comment in the abstract is that it is proposed the Cox1 would act differently in the CNS than in the periphery. It would strengthen the manuscript in trying to identify a unique CNS role, if a comparison to the periphery was conducted.

The statement that Cox1 and Cox 2 function may be reversed in the CNS as compared to the periphery requires more specific information on the periphery. But also, one needs to include information on Cox 2 in the CNS to support the statement that Cox-1 plays the more dominant role in the brain.

The background information for cox-1 and cox-2 should more clearly outline what is general cell biology or what is in periphery or central. Thus, while the statement is made that the assumption was mainly based on peripheral organs there is no information from lines 44-55 as to what cell types these studies were conducted. It is suggested that this section be expanded with the inclusion of more details. And then that the neuro aspect from lines 58 be a separate paragraph with additional information on "some studies suggest an up-regulation of COX-2 . . .
It is not clear how therapeutic interventions of Cox-2 inhibitors in AD and PD failed to show efficacy in treating the disease symptoms says that COX-2 is not involved. This section laying out the rationale for considering COX-1 in neuroinflammation needs a bit more development.

In general, the review is trying to take on the aspects of a systematic review process however, details that are normally related to such a process are not included. https://dal.ca.libguides.com/systematicreviews/writing
It is not clear if 325 articles represented those on COX-1 in inflammation or an earlier filtering. What was the criteria for the inclusion of the 32 studies and what was the exclusion criteria for the remaining studies? However, much of the criteria for systematic reviews often does not include an evaluation of the quality of the study but rather looks at things like experimenter blinding, statistical analysis, etc. What is very helpful to any review is a critical evaluation of each study with identification of limitations or alternative interpretations.

The evaluation of each study states significant increases but does not provide any details as to the level of change (%) for perspective, there is no information on things like the number of samples examined, how the cells were obtained, number of replications, nor any limitations identified with the individual studies.
"could be detected, but this finding is contradicted by several other subsequent in-vitro studies." Requires citations
In the section on in vitro studies there are statements suggesting that there is or is not an associated impact on neurodegenerative disease. One would suggest to remove such statements as they are not relevant to the review and in vitro models are difficult to relate back to a human disease process or therapeutic intervention.
Bate et al ( ) used primary neurons and microglia – how did they generate the co-culture and what was the age in culture. Was the cell viability lost only in co-cultures or were neurons alone vulnerable. What was the level of decreased cell viability and was IL-6 the only cytokine examined?
One assumes the Gu et al ( ) would be a separate paragraph and not sure why it mentions A53T mice in the first line then goes to microglia cells. Curious why they used brainstem astrocytes. What was the source of the microglia cells. What was the control for astrocyte conditioned medium of A53T mice, was it regular medium or conditioned medium from normal mice?
While the organization of the paragraphs appears to follow an alphabetical pattern, it might be of more interest to maybe put Calvello after Hoozemans as it showed contradictory findings. - if one mentions possible reasons for the differences rather than a general list, specifics would be better.

In general, the types of comments for the in vitro section apply to the rest of the manuscript. Of particular note
Iba-1 is a general marker for microglia not specific to activated microglia alone (page 10)
The disease based conclusions from the individual study authors for the in vitro experiments are interesting but not really appropriate. The point of the paper was to determine if there is a sufficient body of literature to identify COX-1 as an inflammatory factor unique to the nervous system.
Given that a lot of the references in the in vitro and the in vivo section rely on studies that propose to look at biological processes associated with AD it might be an easier transition if the human studies were placed first, followed by the in vivo animal models, and then the more mechanistic studies with cell cultures.
To assist the nomenclature of protein versus mRNA and species – following the appropriate format for each species for mRNA would be helpful. For the post-mortem studies, it is not clear if the studies are in contrast or if they demonstrate different responses of mRNA and protein.
The individual section summaries might be better presented if combined at the end of the manuscript as a summary to demonstrate a role for COX-1. Citations need to be consistent and follow the journal guidelines. There are inconsistent citation formats throughout the manuscript.
In general, attention should be given to maintaining a constant verb tense in the paragraphs. Most of the paper is in past tense however, there are some statements in present tense and not clear if it is a general statement or specific to the paper.
The section on in life imaging would benefit from a more detailed description for the average reader
The Discussion seems to really just present data that would be available in the "Results" section. The Discussion should go back to the original premise that COX1 might be more important the COX2 in the brain as compared to the periphery and do a brief discussion of how the overall review of the literature was found to support this assumption or to provide enough data for future studies in defining the differential roles.

How many articles looked at COX1 only, how many COX2 only, how many both?

Table 1 – the first col "Study" and the last col "Ref" are redundant

Author response

REBUTTAL LETTER

A point-to-point reply to the reviewer comments is given in the following letter. Comments of the reviewers are listed between string quotes ("). Our response is printed in italics.

REVIEWER 1

1. "The old references were written as new references are not used generally in this article. As a result, up-to-date information is not provided".

In response to this comment of the reviewer, the manuscript has been greatly expanded. In the revised version, the number of cited references has been increased from 65 to 113, and many recent references were added.

2. "Although the search resulted in 32 individual articles in this article is declared, 29 different

references are used in Tables 1, 2, 3 and 4".

This error has been corrected in the revised version of the manuscript. Some references were cited and described in the text, but not listed in the Tables. Please note that not all references listed in Table 4 concern imaging in neuroinflammation models, and that some references are listed in more than one Table. A list of the 32 papers is presented in the Appendix at the end of this rebuttal letter.

3. "There is a lot of typo and careless spelling (although references are cited in different ways in the article, reference years are not specified in some places)".

We have checked the spelling throughout the manuscript. And references are now consistently cited, using the journal style.

4. "It must be edited in English".

The entire manuscript has been read by several persons who have rephrased many sentences and have improved the English language.

REVIEWER 2

1. "The manuscript by Vries et al represents a review of the literature on Cox-1 and neuroinflammation. The topic is timely and well worth investigation. There are however a number of issues that should be addressed. Below please find comments on Introduction and the In vitro section. Note that these comments are similar across the different sections. There are a number of statements in the Introduction on basic biology and relevance of arachidonic acid that do not have supporting citations. – Example line 14-37 'In contrast to most peripheral organs, there is basal expression of COX-2 in the brain'

This reviewer comment is correct. We have added supporting citations to all our statements, not only in the Introduction but also in later sections of the revised manuscript.

2. "The authors may want to define exactly what they are identifying as "activated" microglia as there is a distinction between reactive and activated and using this as a catch all term has raised concerns. In addition, there is not a requirement for cells to proliferate and in fact, much of the original literature demonstrates that macrophage like cells do not increase pro-inflammatory production when they are actively proliferating. This section of the Introduction requires some attention to more accurately reflect the biology of microglia. Or rather to simply remove this aspect of the "characterization" as it is really not relevant to the paper. "An early characteristic of neuroinflammation is the activation of microglia, which are the resident macrophages of the brain. Microglial activation is characterized by alterations in morphology and increased proliferation."

This reviewer comment is correct. "Apparently quiescent" microglia is not inactive, but is in fact actively monitoring the internal conditions of the brain. Thus, the term "activated microglia" is misleading. We have replaced the term "activated microglia" by "reactive microglia" throughout the manuscript. And we have deleted the statement about increased proliferation from the Introduction. The incorrect statement has been modified to: "An early characteristic of neuroinflammation is the change of microglia to a reactive phenotype, which is characterized by altered cellular morphology".

3. "One interesting comment in the abstract is that it is proposed the Cox1 would act differently in the CNS than in the periphery. It would strengthen the manuscript in trying to identify a unique CNS role, if a comparison to the periphery was conducted. The statement that Cox1 and Cox 2 function may be reversed in the CNS as compared to the periphery requires more specific information on the periphery. But also, one needs to include information on Cox 2 in the CNS to support the statement that Cox-1 plays the more dominant role in the brain".

Our statement about a reversal of COX1 and COX2 functions in the brain as compared to the periphery was probably too strong. We have changed the sentence in the "Purpose" section of the

Abstract to: "However, recent evidence suggests that COX-1 can also be upregulated and may play a prominent role in the brain during neuroinflammation". This sentence is better supported by the cited literature.

4. "The background information for cox-1 and cox-2 should more clearly outline what is general cell biology or what is in periphery or central. Thus, while the statement is made that the assumption was mainly based on peripheral organs there is no information from lines 44-55 as to what cell types these studies were conducted. It is suggested that this section be expanded with the inclusion of more details. And then that the neuro aspect from lines 58 be a separate paragraph with additional information on "some studies suggest an up-regulation of COX-2 . . .
It is not clear how therapeutic interventions of Cox-2 inhibitors in AD and PD failed to show efficacy in treating the disease symptoms says that COX-2 is not involved. This section laying out the rationale for considering COX-1 in neuroinflammation needs a bit more development".

We have completely rewritten the last three sections of the Introduction. Many incorrect references were replaced by more appropriate citations. We have indicated whether findings concerned peripheral organs or the brain. Additional details were provided. The neuro aspect has now been discussed in a separate paragraph, as the reviewer requested and additional information on the upregulation of COX-2 has been included. The sentence about therapeutic interventions with COX-2 inhibitors in AD and PD has been deleted from the revised Introduction. It is true that failure of such interventions does not prove that COX-2 is not involved in the pathophysiology of neurodegerative diseases. The section about a potential role of COX-1 in neuroinflammation has been rewritten and expanded.

5. "In general, the review is trying to take on the aspects of a systematic review process however, details that are normally related to such a process are not included. It is not clear if 325 articles represented those on COX-1 in inflammation or an earlier filtering. What was the criteria for the inclusion of the 32 studies and what was the exclusion criteria for the remaining studies? However, much of the criteria for systematic reviews often does not include an evaluation of the quality of the study but rather looks at things like experimenter blinding, statistical analysis, etc. What is very helpful to any review is a critical evaluation of each study with identification of limitations or alternative interpretations".

Most information that reviewer 2 requested was provided in Figure 2 and Appendix 1. His/her comments seem to be based on reading just the review text. The text of our Methods section was indeed rather incomplete. We have expanded it in the revised version of our manuscript, in order to clarify our selection procedure.

6. "The evaluation of each study states significant increases but does not provide any details as to the level of change (%) for perspective, there is no information on things like the number of samples examined, how the cells were obtained, number of replications, nor any limitations identified with the individual studies. 'Could be detected, but this finding is contradicted by several other subsequent in-vitro studies.' Requires citations. "

We have included details concerning the magnitude of changes (in %), the number of samples or replications, etc to the text of the revised version (or the text of the Tables), as the reviewer requested. Citations to support our statements have also been added.

7. "In the section on in vitro studies there are statements suggesting that there is or is not an associated impact on neurodegenerative disease. One would suggest to remove such statements as they are not relevant to the review and in vitro models are difficult to relate back to a human disease process or therapeutic intervention".

This reviewer criticism is correct. We have therefore removed all extrapolations and unfounded conclusions regarding human disease, based on data from in vitro or animal studies.

8. “Bate et al ( ) used primary neurons and microglia – how did they generate the co-culture and what was the age in culture. Was the cell viability lost only in co-cultures or were neurons alone vulnerable. What was the level of decreased cell viability and was IL-6 the only cytokine examined? One assumes the Gu et al ( ) would be a separate paragraph and not sure why it mentions A53T mice in the first line then goes to microglia cells. Curious why they used brainstem astrocytes. What was the source of the microglia cells. What was the control for astrocyte conditioned medium of A53T mice, was it regular medium or conditioned medium from normal mice?”

The information requested by the reviewer has been added to the revised version of our manuscript.

9. “While the organization of the paragraphs appears to follow an alphabetical pattern, it might be of more interest to maybe put Calvello after Hoozemans as it showed contradictory findings. - if one mentions possible reasons for the differences rather than a general list, specifics would be better. “

The comment of the reviewer that articles are cited in alphabetic order is incorrect. We have cited articles in chronologic order, which seems a logical procedure. This has now been clearly pointed out at the beginning of the in vitro study section and in other places, such as the Table headers. The reasons for discrepant findings in different articles have now been more extensively discussed.

10. “In general, the types of comments for the in vitro section apply to the rest of the manuscript. Of particular note: Iba-1 is a general marker for microglia not specific to activated microglia alone (page 10).

Any erroneous statement concerning Iba1 has been corrected. And improvements proposed by reviewer 2 concerning the in vitro section have also been made to the following sections of the review.

11. “The disease based conclusions from the individual study authors for the in vitro experiments are interesting but not really appropriate. The point of the paper was to determine if there is a sufficient body of literature to identify COX-1 as an inflammatory factor unique to the nervous system”.

This reviewer criticism is correct. As we wrote earlier, we have removed all extrapolations and unfounded conclusions regarding human disease from the In Vitro and Animal Study sections of our revised text. These conclusions were indeed speculative and not really appropriate.

12. “Given that a lot of the references in the in vitro and the in vivo section rely on studies that propose to look at biological processes associated with AD it might be an easier transition if the human studies were placed first, followed by the in vivo animal models, and then the more mechanistic studies with cell cultures”.

We decided to not change the order of the sections, since the order of the sections is also largely a chronologic order. Reversing the order of the sections would have the confusing result that very recent papers were discussed first and old papers were discussed much later.

13. “To assist the nomenclature of protein versus mRNA and species – following the appropriate format for each species for mRNA would be helpful. For the post-mortem studies, it is not clear if the studies are in contrast or if they demonstrate different responses of mRNA and protein. “

We have added information to the text indicating whether observed changes concerned mRNA or protein levels. In cases where both protein and mRNA levels were measured, the magnitude of change of both parameters was different, but the direction of their change was identical.

14. “The individual section summaries might be better presented if combined at the end of the manuscript as a summary to demonstrate a role for COX-1”.

We considered this a valuable suggestion and we have thus combined the individual section summaries in a general “Summary of literature findings”, after the Results section and before the Discussion.

15. “Citations need to be consistent and follow the journal guidelines. There are inconsistent citation

formats throughout the manuscript."

We have adjusted the citations, they are now consistent throughout the manuscript and Tables. From a recent review published in JNR, we learned that references should be cited als "Author et al., year" in the text and be sorted as an alphabetic list at the end of the manuscript.

16. "In general, attention should be given to maintaining a constant verb tense in the paragraphs. Most of the paper is in past tense however, there are some statements in present tense and not clear if it is a general statement or specific to the paper."

We have adjusted the verb tense and have tried to use the past tense as much as possible.

17. "The section on in life imaging would benefit from a more detailed description for the average reader."

We have expanded the imaging section (by including additional references) and have also added several details to our descriptions in order to assist the average reader.

18. The Discussion seems to really just present data that would be available in the "Results" section. The Discussion should go back to the original premise that COX1 might be more important the COX2 in the brain as compared to the periphery and do a brief discussion of how the overall review of the literature was found to support this assumption or to provide enough data for future studies in defining the differential roles."

We have reorganized and modified the Discussion. Duplicate information was removed as much as possible. However, text concerning the interpretation and linking of published data, and the identification of potential mechanisms underlying changes of COX-1 expression in the brain has been retained in the revised Discussion.

19. "How many articles looked at COX1 only, how many COX2 only, how many both?"

A statement concerning these numbers has been added to the Methods section. See also the Appendix of this rebuttal letter.

20. "Table 1 – the first col "Study" and the last col "Ref" are redundant".

This reviewer criticism is correct. However, it concerns not only Table 1 but also Tables 2, 3 and 4. We have deleted the first column (Study) from each Table as this column was indeed redundant.

APPENDIX: Articles resulting from the search procedure

Article no.	**Author, year**	**Providing information on involvement in neuroinflammation of:**
1	Hoozemans 2002	Both COX-1 and COX-2
2	Qin 2003	Both COX-1 and COX-2
3	Bate 2006	Both COX-1 and COX-2
4	Gu 2010	Both COX-1 and COX-2
5	Calvello 2012	Both COX-1 and COX-2
6	Calvello 2017	Both COX-1 and COX-2
7	Choi 2008	COX-1
8	Choi 2009	COX-1
9	Garcia-Bueno 2009	Both COX-1 and COX-2
10	Aid 2010	Both COX-1 and COX-2
11	Choi 2010	Both COX-1 and COX-2
12	Matousek 2010	Both COX-1 and COX-2
13	Teeling 2010	Both COX-1 and COX-2
14	Dargahi 2011	Both COX-1 and COX-2
15	Russo 2011	COX-1
16	Griffin 2013	Both COX-1 and COX-2

17 Choi 2013 COX-1
18 Nazmi 2019 COX-1
19 Nie 2019 Both COX-1 and COX-2
20 Griffin 1994 COX-1
21 Yasojima 1999 Both COX-1 and COX-2
22 Kitamura 1999 Both COX-1 and COX-2
23 Yermakova 1999 COX-1
24 Hoozemans 2001 Both COX-1 and COX-2
25 Schwab 2002 COX-1
26 Deininger 2003 Both COX-1 and COX-2
27 Maida 2006 Both COX-1 and COX-2
28 Kim 2011 Both COX-1 and COX-2
29 Takashima-Hirano 2010 COX-1
30 Shukuri 2011 COX-1
31 Ohnishi 2016 COX-1
32 Shukuri 2016 COX-1

Thus, 20 articles concerned the involvement of both COX-1 and COX-2 in neuroinflammation, and 12 articles concerned the involvement of COX-1.

2nd Editorial Decision

Decision Letter

Dear Dr Vries:

Thank you for submitting your manuscript "Is Cyclooxygenase-1 involved in Neuroinflammation?" by Ghazanfari, Nafiseh; Waarde, Aren van; Dierckx, Rudi A.J.O.; Doorduin, Janine; Vries, Erik F.J. de.

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,

Professor Patricia Schuck
Associate Editor, Journal of Neuroscience Research

Dr Cristina Ghiani
Editor-in-Chief, Journal of Neuroscience Research

Authors' Response

3rd Editorial Decision

JOURNAL OF Neuroscience Research

Decision Letter

Authors' Response

4th editorial decision

Decision Letter

Author response