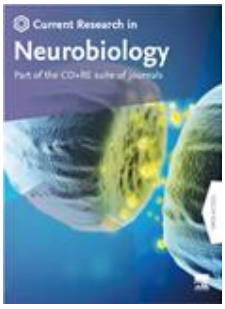
Peer Review Overview



Manuscript Title: The transcriptional repressor Rev-erbα regulates circadian expression of the astrocyte Fabp7 mRNA

Received	Dec 30, 2020
1st Decision	Jan 26, 2021
1st Revision Submitted	Feb 09, 2021
Accepted	Mar 05, 2021

1st Decision letter

Reference: CRNEUR-D-20-00024
Title: The transcriptional repressor Rev-erbα regulates circadian expression of the astrocyte Fabp7 mRNA
Journal: Current Research in Neurobiology

Dear Dr. Gerstner,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Feb 25, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

Current Research in Neurobiology values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Anna S Mitchell, Ph.D.
Editor in Chief
Current Research in Neurobiology

Comments from Editors and Reviewers:

Thank you for your short communication. Please revise the manuscript taking into account the comments of the two reviewers and re-submit within 30 days. Please also ensure all details about the mice are provided, e.g. sex and numbers used. We look forward to receiving your revised manuscript.

Reviewer #1:

The authors provide data which builds on a previous publication from this group implicating Rev-Erbalpha as a key regulator of Fabp7. This paper advances the story by providing ChIP-seq data which clearly demonstrates that Rev-Erba binds to the Fabp7 locus, with appropriate + and - controls. They also show that regulation by Rev-Erba is unique to Fabp7, and that this occurs across multiple brain regions. While the advance of this paper is perhaps a bit incremental, the data is convincing and well presented, and the conclusions are important and well supported by the data. The only additional thing that might fill out the paper a bit would be to show that there is no Rev-Erba binding peak at the Fabp3 or 5 loci, though this is not critical.

Reviewer #2:

In "The transcriptional repressor Rev-erba regulates circadian expression of the astrocyte Fabp7 mRNA", authors describe the transcriptional repression of Fabp7 by Rev-erba in the mammalian brain using ChIP-seq of the VTA. Using Rev-erba knockout mice, authors show dysregulation of Fabp7 in several areas of the brain, including the hippocampus, cortex, hypothalamus, striatum, cerebellum, and the VTA. Overall, the manuscript is well-written and the data clearly presented. I have only a few recommendations for improvement:

1. Though addressed briefly in the discussion, I think that the authors should spend more time in the introduction on the role of Fabp7 in astrocytes, particularly as it relates to the physiological function in astrocytes. (For example, what does loss or overexpression of Fabp7 do in terms of synaptic plasticity and region-specific function?) This would provide some context as to the importance of Rev-erba regulation of this gene in particular.
2. Since the authors are presenting the data for the ChIP-sequencing, I think that an additional figure with gene annotation or pathway classification would be of interest to readers and would greatly augment the paper in terms of understanding potential roles for Rev-erba in the brain outside of Fabp7 gene regulation.

Minor concerns:
1. Figure 4 graphs need y axis labels

1st Author Response Letter

Response to comments from Editors and Reviewers:

Comments from Reviewer 1

The authors provide data which builds on a previous publication from this group implicating Rev-Erbalpha as a key regulator of Fabp7. This paper advances the story by providing ChIP-seq data which clearly demonstrates that Rev-Erba binds to the Fabp7 locus, with appropriate + and - controls. They also show that regulation by Rev-Erba is unique to Fabp7, and that this occurs across multiple brain regions. While the advance of this paper is perhaps a bit incremental, the data is convincing and well presented, and the conclusions are important and well supported by the data. The only additional thing that might fill out the paper a bit would be to show that there is no Rev-Erba binding peak at the Fabp3 or 5 loci, though this is not critical.

Thank you for your careful review and comments – we have now included your suggested edit in a new Figure 1 showing lack of Rev-erba ChiP-seq peaks at Fabp3 and Fabp5 promoters in the VTA, with the edit in results "Here we identified positive Rev-erb interactions within the first kilobase upstream of the transcription start site of the *Fabp7* promoter, but not in the *Fabp3* or *Fabp5* promoters (Figure 1A-C)." We have also included the following statement in the discussion: "These reductions in *Fabp5* mRNA may represent compensatory mechanisms that are in response to the large increases in *Fabp7* mRNA expression in glial cells, however, to rule out a direct role of Rev-erba in transcriptional regulation of these other Fabp types throughout brain, binding assays for Rev-erb at their respective genetic loci across multiple brain regions would be required."

Figure 1.

ChIP-seq binding profile of Rev-erb around the *Fabp7* locus (A), but not in the *Fabp5* (B) or *Fabp3* (C) loci in the VTA of WT mice.

We have edited the current version so that Table 1 includes data from Figure 1B of the previous version:

Table 1.

The top 20 Rev-erbα binding site loci, peak score, distance to the translational start site (TSS), and gene names, as identified by Rev-erb ChIP-seq.

Comments from Reviewer 2

In "The transcriptional repressor Rev-erba regulates circadian expression of the astrocyte Fabp7 mRNA", authors describe the transcriptional repression of Fabp7 by Rev-erba in the mammalian brain using ChIP-seq of the VTA. Using Rev-erba knockout mice, authors show dysregulation of Fabp7 in several areas of the brain, including the hippocampus, cortex, hypothalamus, striatum, cerebellum, and the VTA. Overall, the manuscript is well-written and the data clearly presented. I have only a few recommendations for improvement:

Thank you for your review – we have addressed your comments and concerns as follows, in line:

1. Though addressed briefly in the discussion, I think that the authors should spend more time in the introduction on the role of Fabp7 in astrocytes, particularly as it relates to the physiological function in astrocytes. (For example, what does loss or overexpression of Fabp7 do in terms of synaptic plasticity and region-specific function?) This would provide some context as to the importance of Rev-erba regulation of this gene in particular.

We have added the following statement and 5 additional references in the introduction to address these concerns:

"Fabp7 has been shown to regulate dendritic morphology and excitatory cortical neuron synaptic function (8), as well as locomotor responses to NMDA-receptor activity (9), and other behavioral conditions including fear memory and anxiety (10). Therefore, Fabp7 may play an important role in regulating time-of-day dependent changes in astrocyte-derived and evolutionarily conserved plasticity-related processes (11-13)."

8.M. Ebrahimi et al., Astrocyte-expressed FABP7 regulates dendritic morphology and excitatory synaptic function of cortical neurons. Glia 64, 48-62 (2016).

9.A. Watanabe et al., Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype. PLoS Biol 5, e297 (2007).

10.Y. Owada et al., Altered emotional behavioral responses in mice lacking brain-type fatty acid-binding protein gene. Eur J Neurosci 24, 175-187 (2006).

11.M. Lavialle et al., Structural plasticity of perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors. Proc Natl Acad Sci U S A 108, 12915-12919 (2011).

12.J. Nagai et al., Behaviorally consequential astrocytic regulation of neural circuits. Neuron, (2020).

13.J. R. Gerstner, On the evolution of memory: a time for clocks. Front Mol Neurosci 5, 23 (2012).

2. Since the authors are presenting the data for the ChIP-sequencing, I think that an additional figure with gene annotation or pathway classification would be of interest to readers and would greatly augment the paper in terms of understanding potential roles for Rev-erba in the brain outside of Fabp7 gene regulation.

Thank you for this suggestion; we agree with the reviewer that these additional figures would be of interest to readers and have added GO molecular, biological, and cellular component tables of the ChIP-seq data, as well as a list for the top 20 KEGG pathways, in a new figure 2 with figure legend, edited results section, and appropriate methods/references as follows:

"Gene Ontology (GO) analysis revealed significant enrichment of several biological processes, molecular functions, and cellular components (Table 2) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis shows the top 20 pathways in Rev-erb ChIP-seq genes (Figure 2)."

Table 2.

Analysis of Gene Ontology in Rev-erb ChIP-seq genes. Highest fold enriched Gene Ontology classes for Biological Process, Molecular Function and Cellular Component are listed with most highly enriched on top.

Figure 2.

Analysis of the top 20 KEGG pathways enriched in Rev-erba ChIP-seq genes plotted with number of hits per pathway.

GO and KEGG Analysis

Gene ontology analysis was performed on the ranked list of Rev-erb ChIP-seq genes with peak score >2 [SUPPLEMENTAL dataset 1], using Panther GO-Slim against the mouse gene list (http://geneontology.org release 2021-01-01: 44,091; (53, 54). Top non-redundant categories are presented.

KEGG pathway analysis was performed on the same gene list using KEGG Mapper https://www.genome.jp/kegg/tool/map_pathway1.html (55) against mouse pathways.

53.M. Ashburner et al., Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25, 25-29 (2000).

54.The Gene Ontology resource: enriching a GOld mine. Nucleic Acids Res 49, D325-d334 (2021).

55.M. Kanehisa, Y. Sato, KEGG Mapper for inferring cellular functions from protein sequences. Protein Sci 29, 28-35 (2020).

Minor concerns:

1. Figure 4 graphs need y axis labels This has been added.

Accept Letter

Dear Dr. Gerstner,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I am delighted to inform you that your manuscript has been accepted for publication.

My comments, and any reviewer comments, are below.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and include details of the GEO number as outlined below. You will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Current Research in Neurobiology and hope you will consider us again for future submissions.

As you may be aware, we are new, gold open access neuroscience journal and have just published our Editorial highlighting our innovations; there is also a survey to complete. Please check out the details following the link here https://doi.org/10.1016/j.crneur.2021.100005

Thank you once again for submitting your manuscript to CRNEUR and Congratulations on its forthcoming publication.

Kind regards,

Anna S Mitchell, Ph.D.
Editor in Chief
Current Research in Neurobiology

Editor and Reviewer comments:

As per Reviewer 2, please can you ensure that you include the GEO number for the high throughput data so that your deposit of the data can be found and made publicly available.

Reviewer 1: The authors have addressed my critique completely. The addition of the pathways analysis, requested by the other reviewer, is also a nice addition. I have no further concerns.

Reviewer 2: The authors have thoroughly addressed all of my initial comments. Perhaps I missed it, but I did not see a GEO number for the high throughput data.

-------- End of Review Comments --------