Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-19-00116
Title: The experimental evidence on the characteristic response of rat striatal astrocytes under dopamine-depletion
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Date sent for review: 2019-03-09

COMMENTS TO AUTHORS
Strength: The images obtained by immunohistochemistry are of good quality.

Weaknesses: The objective of the article is not very ambitious. The experimental design does not allow to achieve the proposed objectives. The results do not contribute any significant novelty to the knowledge of Parkinson's disease. The presence of astrogliosis and reactive astrocytes is a phenomenon already known in neurodegenerative diseases.

The present study aimed to examine and compare the characteristic changes of astrocytes and astrocyte-related protein levels under striatal dopamine (DA)-depletion. The results obtained show that the injection of 6OHDA in the medial forebrain bundle induces an increase in the presence of reactive astrocytes, an increase in the expression of S110B and the transcription factor STAT3 in the striatum tissue.

The introduction should be written more precisely. So, in lines 32-33, it is said that: "the role of striatal astrocytes in PD may be either to facilitate or prevent neuronal damage". However, in line 36 it is affirmed that "some neurodegenerative diseases may be caused by over-reactive astrocytes (10,11). Since the most common opinion is that astrogliosis and astrocyte reactive is a response not a cause to injury, the claim contained in line 36, because of its importance, should be better argued or eliminated.

The references cited to support that "the mechanisms of the "dying-back degeneration" are not well known" are from 6 years ago (lines 30-31). Have not there been advances in the last 6 years? It seems that if, because increasing evidence suggests that striatal astrocytes are involved in this degeneration (lines 31.32), but the references of such evidence are earlier. The most recent is for the year 2010. It is convenient to update the references.

In line 36, the possibility arises that "some neurodegenerative diseases can be caused by reactive astrocytes". Since the depletion of DA occur in the dopaminergic synapses from substantia nigra, pars compacta (SNC) to striatum, the changes in the astrocytes of the striatum seem to a consequence of the deficiency of dopaminergic synapses from the SNC and not the cause, although they may participate in the neurodegenerative mechanisms of the disease (line 40-42). It is the loss of dopaminergic signaling in the synapses of dopaminergic neurons from the SNC with postsynaptic neuron in striatum the pathologic hallmark of PD. To avoid confusion, it must be specified that in the design of this study,
which consists in evaluating changes in striated astrocytes, the possibility of knowing if astrocytes cause degeneration should be discarded.

In Materials and Methods some precisions seem necessary. Line 100, Apomorphine-induced rotation (APO, Tocris, Bristol, UK) was measured three weeks immediately after the 6OHDAA injection at the MFB. However, the results of these measurements do not appear in the manuscript. The results are obtained from "sections that contained striatal tissue were cut into 30 μm sections with a vibratome" (line 111-112). For the validation of the results, it should be indicated how the striatum samples are obtained and in particular how the globus pallidus has been eliminated. The striatum is not an anatomical concept. It includes the caudate and putamen nuclei that are anatomically separated. Also, especially for the results obtained from Western blott, it is important to describe how neurons have been removed from the sample. If the samples used for the Western are complete striated homogenates, since S100B and especially for STAT3 are not specific for astrocytes, the values obtained correspond to total values and not only to values in astrocytes.

Results

The results presented in fig. 1 show a significant increase in the presence of GFAP-positive cell (astrocytes) in the samples of animals of the 6OHDAA group compared with the control group. In the text, a reference is missing that indicates where the quantitative data are expressed. Fig. 4A, as presented in the results section, seems more appropriate to include it as part of fig. 2. In fact, it is the result of applying a program of image analysis to the images in fig. 2. The same comment serves for fig. 4B, which as it appears in the results text, it seems more appropriate to include it with fig. 3. Thus, fig.4 contains the Western blotting results, current fig. 4C and fig. 4D, it would be as fig. 4A and fig. 4B.

Discussion

In general, the discussion is very speculative with statements that are not supported by experimental results.

The content from lines 212 to 238 could be summarized and included in the Introduction. In lines 210-212, it is said that: Our study showed that the 6OHDAA-induced degeneration of dopaminergic striatal neurons mediated the up-regulation of S100B and STAT3 expression in astrocytes. This statement is not supported by the experimental data presented. There is no result in the manuscript that assesses the death of neurons.

In addition, the methods used - sections with stratal tissue - do not allow to state that the increase in S100B and STAT3 observed has been evaluated specifically in astrocytes. The method used does not adequate to differentiate which cells of the striatal tissue contribute to STAT3. According to the methodology used, the values of STAT3 correspond to the expression in neurons, astrocytes and microglia present in the tissue sample analyzed, not only that expressed by astrocytes.

In L 245-246, it is said that: S100B is released from reactive astrocytes and activates RAGE to promote neuroinflammation in the brain.[46] However, the authors do not seem to consider that S100B is also localized in many neural cell-types besides by astrocytes. These are important in order to avoid
misinterpretation in the identification of normal and pathological cell types in situ and in clinical studies since S100B is continuously used as an astrocytic marker in animal models and various human diseases (Steiner, J et al. (2007). Evidence for a wide extra-astrocytic distribution of S100B in human brain. BMC Neuroscience, 8. https://doi.org/10.1186/1471-2202-8-2).

Regarding the role of STAT3, the importance is not whether there is an increase in its expression. The importance is if there is an increase in phosphorylated STAT3 form, which has not been valued in the study. On the other hand, STAT3 is not an exclusive transcription factor of astrocytes. It also intervenes in important transcription pathways in neurons. As has been pointed out, the methodology raises doubts about the fact that the data presented correspond only to astrocytes.

Minor questions:
- Lines 69-70: Repeats what is indicated in lines 33-34.
- Line 103: where says ICH should say IHC.
- When describing the results, the term up-regulated is used to refer to an increase in a parameter.

However, the experimental design is not very suitable for regulatory studies. In this study I do not find information about any regulatory mechanism. Applying the term regulation, up-or down-, is not very appropriate. In this study I do not find information about any regulatory mechanism. For example, "Statistical data showed that the S100B positive cells were significantly upregulated in the 6OHDA group (2.00 ± 0.28) compared with the control group (1.10 ± 0.23, P = 0.0256), lines 179-181. It would be more rigorous to write "Statistical data showed that the S100B positive cells were significantly increased in the 6OHDA group (2.00 ± 0.28) compared with the control group (1.10 ± 0.23, P = 0.0256).

- In fig. 4C there are bands identified as GAPDH. In the manuscript and more specifically in the legend of Figure 4, there is no reference to GAPDH.