"Maresin1 protection of dopaminergic neurons involves activation of astrocytes in vivo"

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Introduction

Parkinson's disease (PD) is a neurodegenerative disorder that affects the dopaminergic neurons in the Substantia Nigra (SN). Astrocytes, the largest and most abundant type of supporting cells in the central nervous system, participate in the detection and communication of stress signals from neurons to microglial cells (1). For this purpose, astrocytes change their phenotype, in some cases transforming themselves into reactive types with the subsequent release of cytokines and chemokines with the activation of NFkB/p65 transcription factor. These phenotypes direct the defensive neuroprotective efforts to modulate neuroinflammation (2).

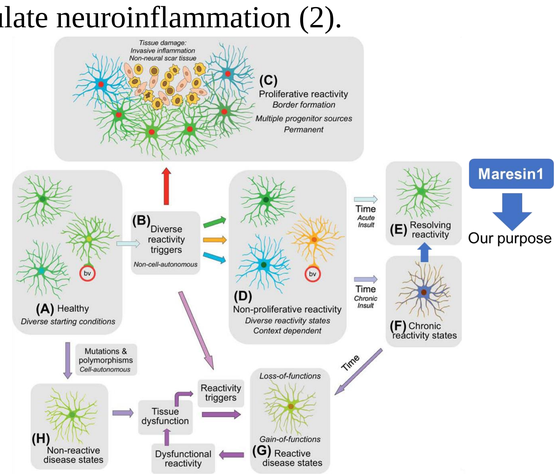


Figure 1. Model depicting how Maresin1 can be used to transform chronic reactivity states to resolving reactivity.

Our **overall hypothesis** is that Maresin1 induces the anti-inflammatory conversion of astrocytes when 6-hydroxydopamine (6HODA) exerts toxicity on dopaminergic neurons to help them survive. This hypothesis was tested in a 6HODA toxicity transgenic rat model that expresses GFP driven by the tyrosine hydroxylase (TH) promoter (3).

Materials and Methods

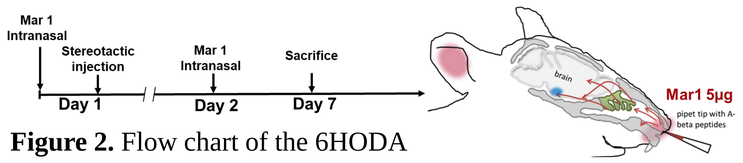
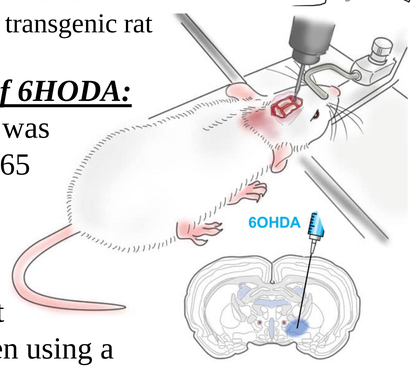


Figure 2. Flow chart of the 6HODA toxicity model in TH-GFP transgenic rat (3).

Stereotactic injection of 6HODA:



Immunohistochemistry was used to detect nuclear p65 in glial fibrillary acidic protein positive cells, a marker of astrocytes in different areas of the rat brain. Images were taken using a FluoView 3000 confocal microscope and an Olympus BX61VS fluorescence microscope. The confocal captured z-stacks were processed using IMARIS 10 and the data was statistically analyzed using Microsoft Excel.

Results

Figure 3

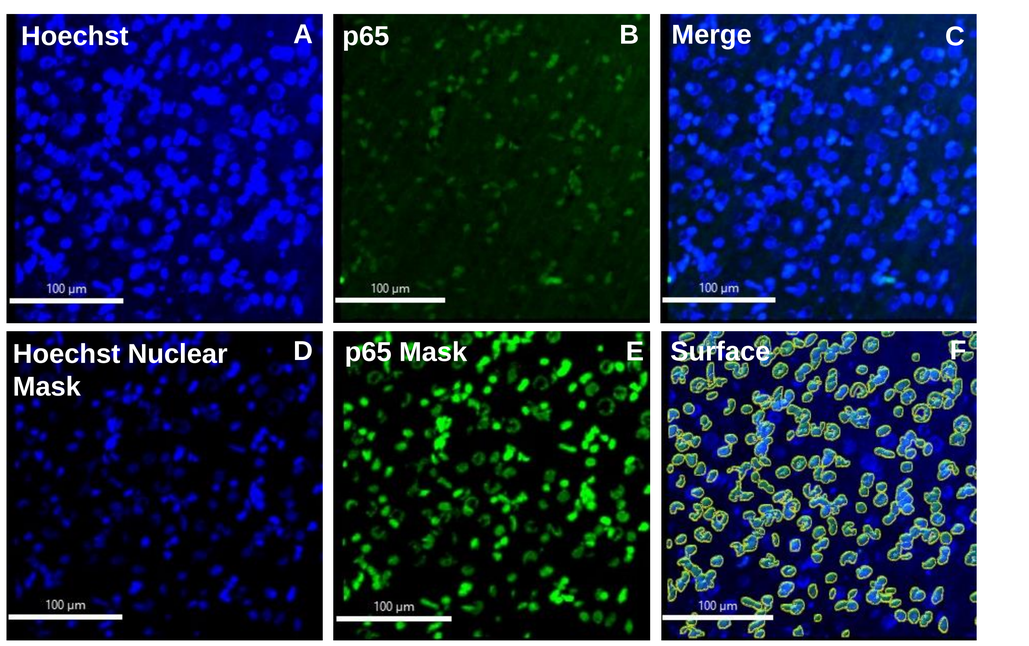


Figure 3. Activation of astrocytes is evidenced by increase in nuclear p65. A) Nuclei (blue); B) Neurons with p65 (green); C) Merged A and B; D) Mask of neurons to reduce background (blue); E) Mask of p65 to reduce background (green); F) p65 surface (green)

Figure 4

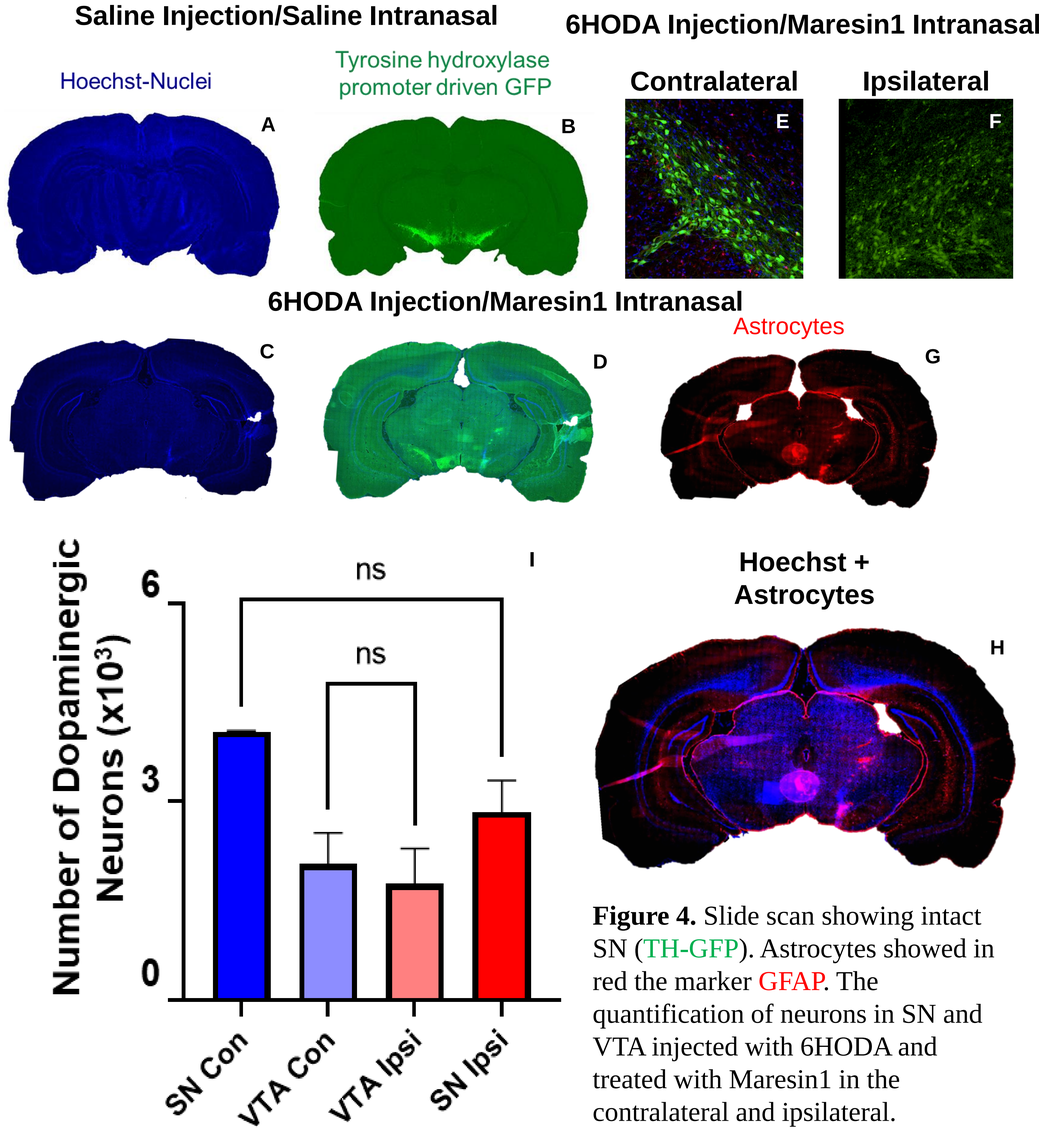


Figure 4. Slide scan showing intact SN (TH-GFP). Astrocytes showed in red the marker GFAP. The quantification of neurons in SN and VTA injected with 6HODA and treated with Maresin1 in the contralateral and ipsilateral.

Conclusion

- The rats that were administered saline showed activation of astrocytes in both hemispheres.
- In the contralateral hemisphere, p65 was present in lesser amount of astrocytes (Figure 3H) but its intensity in the nucleus was higher (Figure 3J).
- All the above suggests that the damage caused by 6HODA induced global inflammation that affected the contralateral side.
- In the Mareisn1-treated rats, intensity of p65 was higher in the side of injection than the contralateral, and the number of astrocytes containing p65 in the nucleus was equal suggesting that Maresin1 prevented the translocation of p65 into the nucleus.
- All the above implies that Maresin1 blocks the inflammation signal from being released out of the damaged region.
- Astrocytes in the 6HODA-injected hemisphere of Maresin1-treated animals showed double the intensity of nuclear p65 than the contralateral control.
- When dopaminergic neurons were counted, in the SN and VTA of ipsilateral and contralateral hemispheres in Maresin1-treated rats, no significant difference was found.
- All together, the data points to a neuroprotective role of Maresin1 that is possibly acting via transforming pro-inflammatory astrocytes into resolving reactive phenotype that helps neurons to overcome the toxicity of 6HODA.

References

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3. Iacovitti, L et al, 2014. *Plos One*. 9(12): e113151.

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