Distinct Astrocytic Changes during Synaptopathogenic Events Mediated by Organophosphate Toxicity in Hippocampal Explants

Caitlyn A. Young, Ben A. Bahr, and Karen L.G. Farizatto

[1]Biotechnology Research and Training Center, [2]Department of Biology, and [3]Department of Chemistry and Physics, University of North Carolina-Pembroke, Pembroke, North Carolina USA

Abstract

For my research, I am focusing on detecting neuronal and astrocytic alterations to study brain damage after an excitotoxic insult. An astrocyte is a star shaped glial cell of the central nervous system. Astrocytes are the most abundant glial cells in the brain responsible for maintaining neuronal functions, homeostasis, and undergoing numerous changes when they react to neurological injuries. Importantly, astrocytes also contribute to the neutralization and metabolism of toxins, such as organophosphates (OP). OP-induced neurological damage comprises complex pathophysiological signaling mechanisms involving neuron and glial cells, and astrocytes monitor and respond to synaptic alterations and modulate behavioral state in health and disease. Understanding how anticholinesterase toxins influence neural integrity is important since evidence indicates post-insult repair responses are mediated through synaptic signaling, and astrocytes appear to actively participate in this process by exchange information with multiple neurons. OP are anticholinesterase toxins that include dangerous nerve agents that produce cholinergic crises leading to seizures, memory deficits, neuronal loss, and eventually death, while survivors ordinarily experience long-term neurological problems. The view of astrocyte's role in pathogenic response has been altered in the recent years, and the astrocytic responses linked to OP-mediated neuropathology has not been well explained. Informative in vitro studies investigated the effects of OP and metabolites but do not address cell-type specific effects, such as in astrocytic cells. Pxn-treated tissue elicited a noticeable increase in the density of GFAP-positive cells while synaptic decline occurred in hippocampal dendritic zones. Note, the Pxn effect on synaptic markers was indeed found to be synapse-specific, noting no changes occurred in the density of neurons assessed by Nissl stain. In fact, the extent of GFAP increase correlated with the extent of reduced dendritic staining of synapsin II in double-labeled confocal images, however, contrasting the robust decline of the presynaptic marker presenting similar response across major dendritic fields, the GFAP response in the molecular layer of dentate gyrus (ml) appears to be heightened, comprising almost two-fold increase in the area fraction. To further examine Pxn-mediated astrocytic changes, our model showed that the signs of reactive astrocytes also comprises a greater number of branch points. Although, our data demonstrated that these events are not due to astrocyte or any other cell-type proliferation revealed by immunohistochemistry detection of GFAP-positive and DAPI counterstained cells. OP-mediated distinctive reactive astrocytes involved i) moderate to severe reactive astrocytosis, in which astrocytes abundantly exhibited GFAP-positive labeling along with no cellular proliferation, ii) increased branching profile, iii) morphological alterations, iiii) presence of complement component C3 protein co-localized with hypertrophic astrocytes. These findings suggest that astrocytes responded to acute Pxn exposure by presenting increased density of GFAP-positive cells, disruption of astrocytic phenotype, and adopted reactive profiling, characteristics of astrocytosis. This study showed that exposure to organophosphate Pxn can lead to synaptic loss along with distinct astrocytic alteration. Studying astrocyte profile during an anticholinesterase model is important to better understand different responses that are linked during synaptic perturbation, allowing to also study how to properly intervene in order to promote synaptic repair.

1. Potential of Astrocytes as Immune Modulators

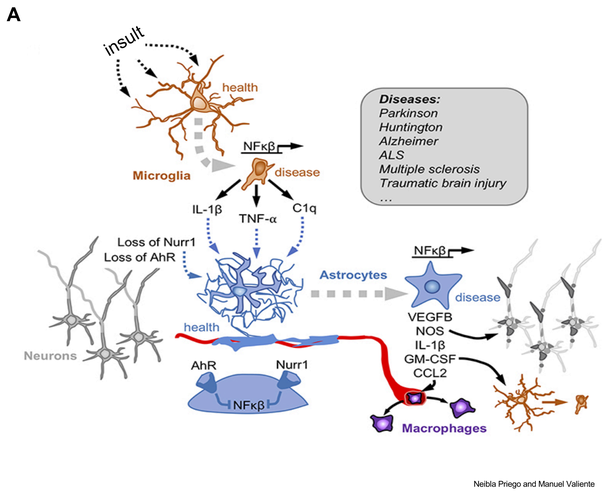

Figure 1. Positive and Negative aspects that can occur in Astrocytes. Astrocytes become reactive when responding to different insults in the brain. This response might engage the offset of the pathological cascade and can also be apart of the damage. Positive aspects can include reorganization of host priorities, tissue repair and neuroprotection. Negative aspects can include collateral damage, cognitive impairment and neuronal damage.

2. Paraoxon leading to synaptic compromise

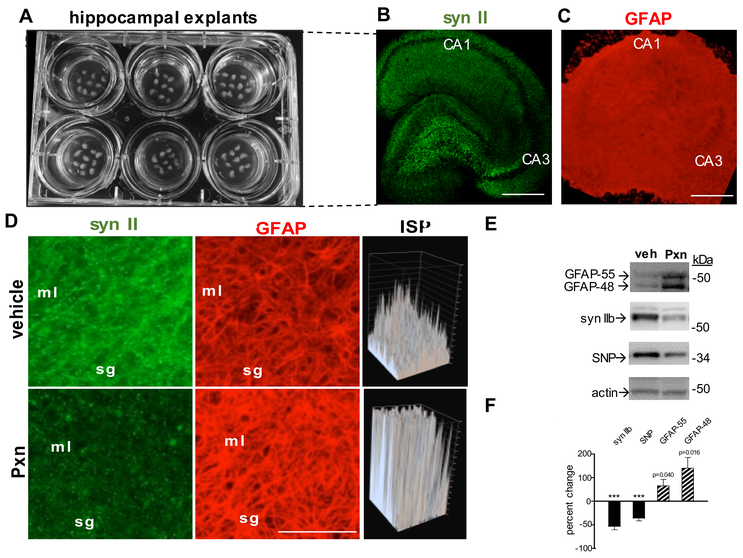

Figure 2. Paraoxon mediates a cascade synaptic alterations. Hippocampal slice cultures treated with vehicle or 200 µM Pxn for 24 h and assessed by immunoblot for Synapsin II (syn II), synaptophysin (SNP), and load control actin (A). Synapsin II was assessed in CA1 and CA3 astrocyte regions. (B). Additionaly, tissue samples from Pxn-treated hippocampal slice cultures were assessed for GFAP, an indicator of reactive astrocytes (C). Similarly, hippocampal tissue were fixed and stained for syn II, GFAP, and ISP and images are shown with compressed maximal intensity projection alongside Nissl staining from the same view-field in the molecular layer (D). Hippocampal slice cultures treated with vehicle or 200 µM Pxn for 24 h and assessed by immunoblot for GFAP, Synapsin IIb (syn IIb), synaptophysin (SNP), and load control actin (E). Percent change profiles for synapsin IIb, synaptophysin, and GFAP from the molecular layer are shown to illustrate immunostaining (F)

3. Organophosphate-mediated synaptopathology induce Astrocytes to become reactive

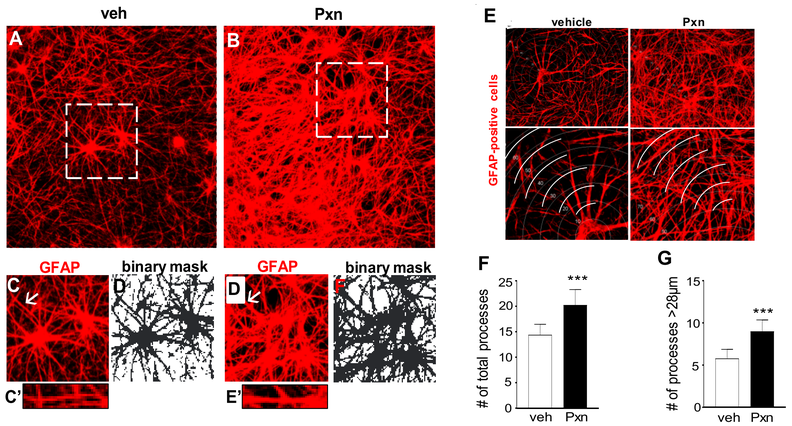

Figure 3. Astrocytes become reactive when responding to synaptic vulnerability after Pxn exposure. Hippocampal slice cultures treated with vehicle or 200 µM Pxn for 24 h were fixed and stained for GFAP, and images are shown with compressed maximal intensity projection (A-E). Area fraction analyses of acquired z-stacks (percent of control; mean ± SEM) show Pxn-induced increase in GFAP-positive immunostaining area (D). Similarly, slices were assessed for GFAP and lipocalin-2 by immunoblot (E). In addition, astrocytic morphological analysis were performed and the number of astrocyte branching as well as the process length were assessed (Mean±SEM, F-G).). ***P< 0.001.

4. Detecting the pro-cytokine IL-6 as a marker of astrogliosis

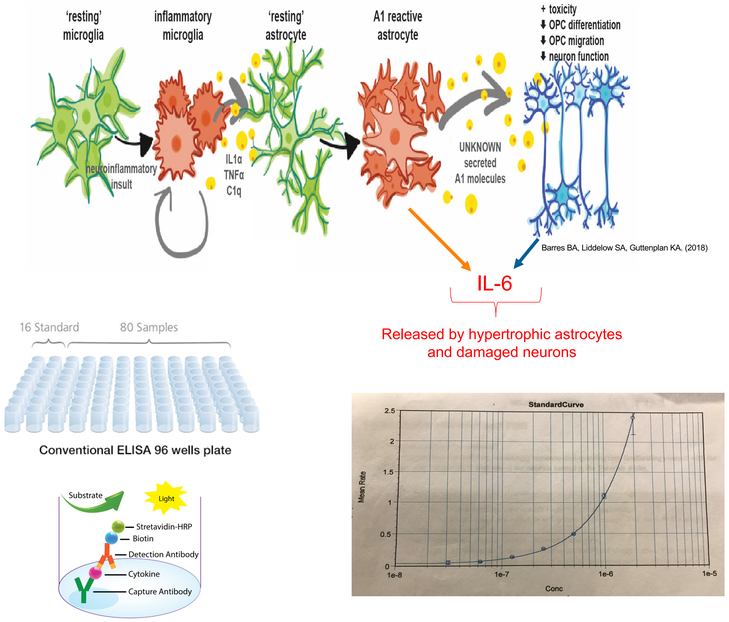

Figure 4. Secretion of interleukine-6 appears to be a important mediator of astrocytosis. Microglia are exposed to a neuroinflammatory insult which directly influences astrocytes. The astrocytes become reactive and molecules such as interleukine-6 (IL-6) is secreted as a response to the insult. This insult can cause elongation and increased number of branches in astrocytes. Measures of IL-6 released in the media samples from Pxn-treated slice cultures were performed. Upon completion of the ELISA assay the concentrations are shown compared to the different standards.

Conclusion

- Pxn exposure resulted in a presynaptic vulnerability;
- Reactive astrocytes in correspondence with synaptic decline was found in hippocampal tissue exposed to Pxn;
- Astrocytes became reactive when responding to Pxn exposure and morphological changes were shown such as elongation of processes and hypertrophy of the astrocyte body.
- The pro-inflammatory cytokine IL-6 is an indicator that astrocytes are responding to an insult; however media samples were not effective in this ELISA assay. Further research will be accomplished for IL-6 using brain tissue samples

References

Farizatto KLG, McEwan SA, Naidoo V, Nikas SP, Shukla VG, Almeida MF, Byrd A, Romine H, Karanian DA, Makriyannis A, Bahr BA (2017) Inhibitor of endocannabinoid deactivation protects against in vitro and in vivo neurotoxic effects of paraoxon. J Mol Neurosci. 63:115-122.

Barres BA, Liddelow SA, Guttenplan KA. (2018) Neuroprotective treatment for neurodegenerative disease, neuroinflammation and neuronal injury by targeting reactive astrocytes. Stanford University, https://stanford.resoluteinnovation.com/technologies/S16-144_neuroprotective-treatment-for.

Priego, et al. "The Potential of Astrocytes as Immune Modulators in Brain Tumors." *Frontiers*, Frontiers, 23 May 2019, www.frontiersin.org/articles/10.3389/fimmu.2019.01314/full.

Acknowledgments

I would like to thank the following people and institutions:

- Dr. Ben Bahr, Dr. Karen Farizatto and Michael Almeida for their mentorship.
- PURCC, for providing this opportunity.
- Fellow student researchers who assisted in the process.
- This work was supported by PURCC under a supply scholarship.