Microglia and Astrocyte Activation in Select Ventilatory Control Regions Following Exposure to Chronic Sustained Hypoxia

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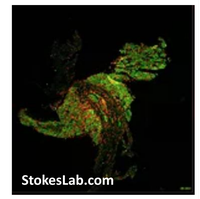


Abstract

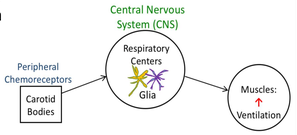
Understanding the role of glial cells in respiratory control regions is pertinent to understanding ventilatory control during chronic sustained hypoxia (CSH). The respiratory regions of interest in our lab are the Pre-Bötzinger Complex (PBC) and the nucleus tractus solitarius (NTS). The first part of this study optimized the Sholl analysis method to detect morphological changes in microglia. The second part of this study assessed the activation of microglia and astrocytes in the NTS and PBC following CSH. Based on previous research, we hypothesized that both astrocytes and microglia would be activated following CSH. Rats were exposed to normoxia, 60-minutes of CSH, or 12-hours of CSH. In the NTS, microglia analysis revealed a trend towards activation, but only one Sholl shell, 21-30μM, was statistically significant ($p<0.05$). In the PBC, microglia analysis revealed a trend towards activation with statistically significant Sholl shells of 11-20μM and 21-30μM ($p<0.05$). Astrocyte activation was not statistically significant across the conditions using the pixel intensity quantification method. Additional section analysis will be performed, as well as analysis of microglia cell body size and endpoint number. However, these data provide a great starting point assessing the activation profiles of glial cells in select respiratory control regions.

Background

Model of Central Glial Cell Activation During Hypoxia

(Arbogast et al., 2016)

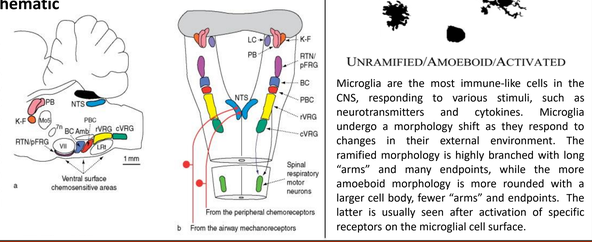
Microglia and astrocytes in the CNS respond to neurotransmitters and release their own gliotransmitters which influence synaptic transmission. Current research is investigating the role of glial cells in respiratory centers and ventilatory control.



Respiratory Control Schematic

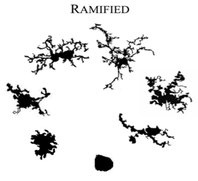
(Gourine and Spyer, 2009)

Ten respiratory control centers in the brainstem comprise a complex network involved in the control of ventilation. This study investigates the NTS and PBC regions. During CSH, the NTS is activated by stimuli from the peripheral chemoreceptors in the carotid body causing changes in ventilation to maintain homeostasis, while the PBC is thought to generate the normal breathing rhythm in mammals.



Model of morphological changes of microglia

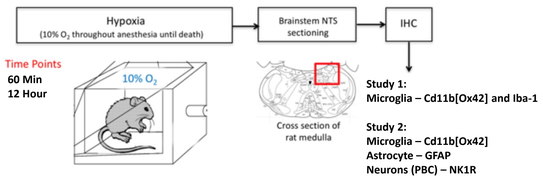
(Karperien et al., 2013)



Microglia are the most immune-like cells in the CNS, responding to various stimuli, such as neurotransmitters and cytokines. Microglia undergo a morphology shift as they respond to changes in their external environment. The ramified morphology is highly branched with long "arms" and many endpoints, while the more amoeboid morphology is more rounded with a larger cell body, fewer "arms" and endpoints. The latter is usually seen after activation of specific receptors on the microglial cell surface.

Methods

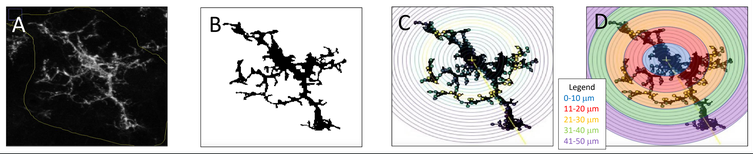
Animals: Rats were exposed to experimental conditions and tissue was perfused and fixed at the University of California San Diego lab of Dr. Frank Powell (Division of Physiology). All animal experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego (under the Guide for Care and Use of Laboratory Animals, National Institutes of Health publication 85-23, Bethesda, MD, USA).



Immunohistochemistry: Glial cells were visualized using immunofluorescent techniques. Primary antibodies used are listed above. Images were taken at LSUHSC-Shreveport and analyzed using FIJI/ImageJ.

Astrocyte Quantification: Activation was assessed by measuring the mean GFAP intensity within the isolated region, as astrocytes upregulate GFAP upon activation using FIJI by ImageJ software version 2.0.0. The Analyze-Measure tool in FIJI was used to analyze the mean positive area GFAP intensity within the region of interest. (See Figure 3 A-B)

Microglia Morphology Quantification: Microglia activation was quantified using ImageJ (FIJI) software version 2.0.0. The extent of activation was measured by analyzing the change in branch proliferation and length using the Sholl analysis plugin in FIJI to assess morphological changes. The number of crossings by each branch was assessed using a starting radius of 2 mm with a step size of 2 mm. The extent of branching at different time points was compared by analyzing the number of crossings at different step intervals (shell/bracket).



Results

Figure 1: Comparison of microglia morphology as visualized with Iba-1 and Cd11b[Ox42] antibodies. *Rats were exposed to normoxic conditions (2C), 60-minute hypoxia (2F), and 12-hour hypoxia (2I) and microglia morphology in the NTS regions was assessed. There was no significant difference in the branching patterns between the two antibodies as defined by Sholl analysis and assessed by a 2-way ANOVA. Figures 2A and 2B are the same normoxic condition microglia, 2D and 2E are the same 60 minute hypoxic condition microglia, and 2G and 2H are the same 12 hour hypoxic condition microglia, but visualized with different antibodies: Iba-1 and CD11b [Ox42] as labeled on the image. This suggests that Iba-1 and Ox42 are staining the same cell and can be used for the same morphological analysis. All data presented as mean ± SEM. (n=2-3 animals/group; 2-way ANOVA).*

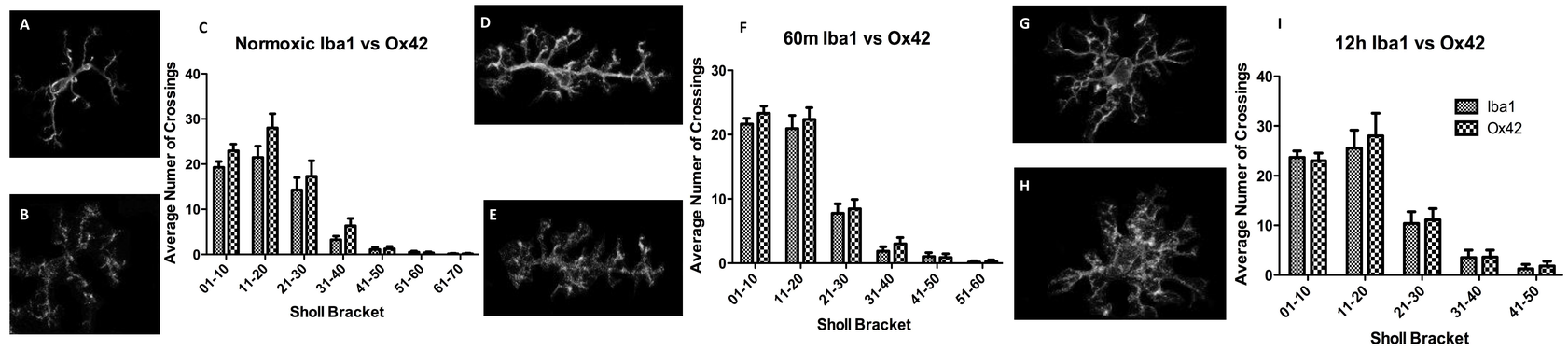


Figure 2: Sholl analysis of microglia stained with CD11b[Ox42] in the PBC. *Average number of crossings counted for each treatment group across five different Sholl shells/brackets. Intervals 0-10 μm and 41-50 μm do not suggest a significant microglia morphology shift ($p> 0.05$). However, intervals 11-20 μm , 21-30 μm , and 31-40 μm show a significant decrease in branch crossings for the 60 minute hypoxia group compared to the normoxic (N) group ($p < 0.05$). All data presented as mean ± SEM. (n=2-3 animals/group; 1-way ANOVA with Dunnett's post-hoc test; *$p < 0.05$)*

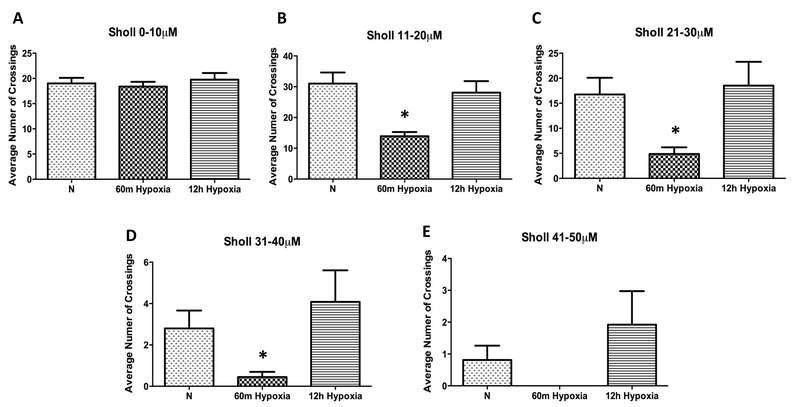
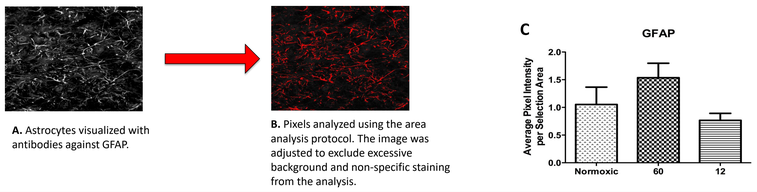


Figure 3: Pixel intensity quantification of astrocytes in the PBC. *Quantification of GFAP expression using area analysis and pixel intensity. While the data is not significant ($p = 0.2495$), there is a trend towards and increase in GFAP expression at 60 minutes of hypoxia. All data presented as mean ± SEM (1-way ANOVA, n=2-3).*



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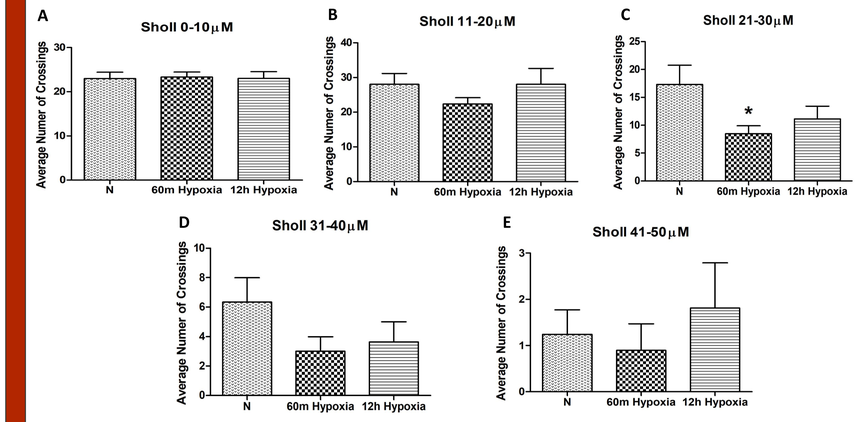
Acknowledgements: UC-San Diego Powell Lab, and LSU Health Science Center Shreveport Core Facilities

Figure 4: Sholl analysis of microglia stained with CD11b[Ox42] in the NTS. *Average number of crossings counted for each treatment group across five different Sholl shells/brackets. Intervals 0-10 μm , 11-20 μm, 31-40 μm, and 41-50 μm do not suggest a significant microglia morphology shift ($p> 0.05$). However, interval 21-30 μm shows a significant decrease in branch crossings for the 60 minute hypoxia group compared to the normoxic (N) group ($p < 0.05$). All data presented as mean ± SEM. (n=2-3 animals/group; 1-way ANOVA with Dunnett's post-hoc test; *$p < 0.05$)*



Conclusions

- The first study *(Figure 1)* indicates that both Iba-1 and CD11b[Ox42] antibodies can be used to visualize microglia and assess branching patterns.
- The second study *(Figures 2 and 4)* indicate a morphology shift of microglia during hypoxic exposure in two different central respiratory control regions during an early hypoxic time point (60 minutes), as well as possible astrocyte activation at the same time point *(Figure 3)*.
- A morphology shift of microglia to a more amoeboid state could indicate their localized response to an increase in synaptic transmission during the hypoxic exposure, as well as their own activation and release of gliotransmitters.
- Future studies include additional section analysis which will increase the n for each of the conditions, as well as analysis of microglia cell body size and endpoint number.
- Additionally, quantification of specific cytokine receptors hypothesized to play a role in CSH and ventilatory acclimatization to hypoxia will be quantified on neurons, microglia, and astrocytes.