The Contribution of RTN to Ventilatory Acclimatization in Response to Hypoxia

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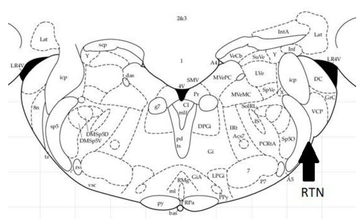
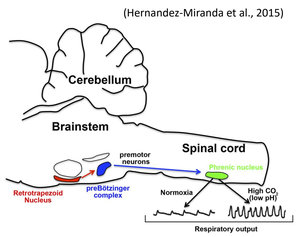
Abstract

Hypoxia is a condition where the tissues in your body are deficient in oxygen. If this condition is sustained in the body, it evokes a rapid increase in ventilation or breathing. This process is referred to as ventilatory acclimatization to hypoxia (VAH) and depending on the extent of the hypoxic event, this could last from minutes to months. This chronic sustained hypoxia arises from injury, disease, or your environment. The acclimatization to hypoxia is understood to take place in the nervous system, although the specific mechanisms are not yet understood. With this knowledge of the mechanisms behind your body's response to hypoxia and we can produce applicable strategies to combat many disease states like COPD and sleep apnea.
The RTN region which is ancillary to the process of VAH, is a thin sheet of cells that extend rostrally up to the caudal region of the trapezoid body in the pons. In this study the RTN was looked at and speculated to have a chemosensory response in the glial cells of RTN region following hypoxia based on the fact that glial cells have been shown to be active in other regions in response to the change in chemical composition of the blood. Response as determined by "activation" of the glial cells either by protein upregulation or a morphology change. The morphology of the Microglia will be determined by Scholl Analysis while the Astrocytes will be assessed by Area Analysis, from these analyses we will determine the "activation" of each glial cell quantified.

Methods



(http://labs.gaidi.ca/rat-brain-atlas/)



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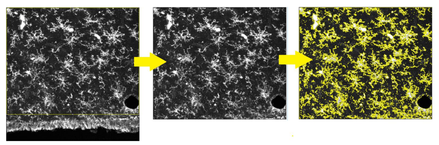
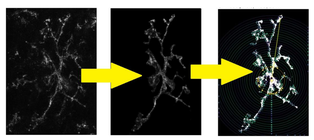
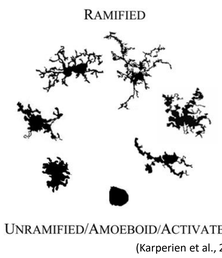
Area Analysis



Relative Ox42 and GFAP expression was assessed via fluorescent intensity analysis using FIJI/ImageJ.

Sholl Analysis





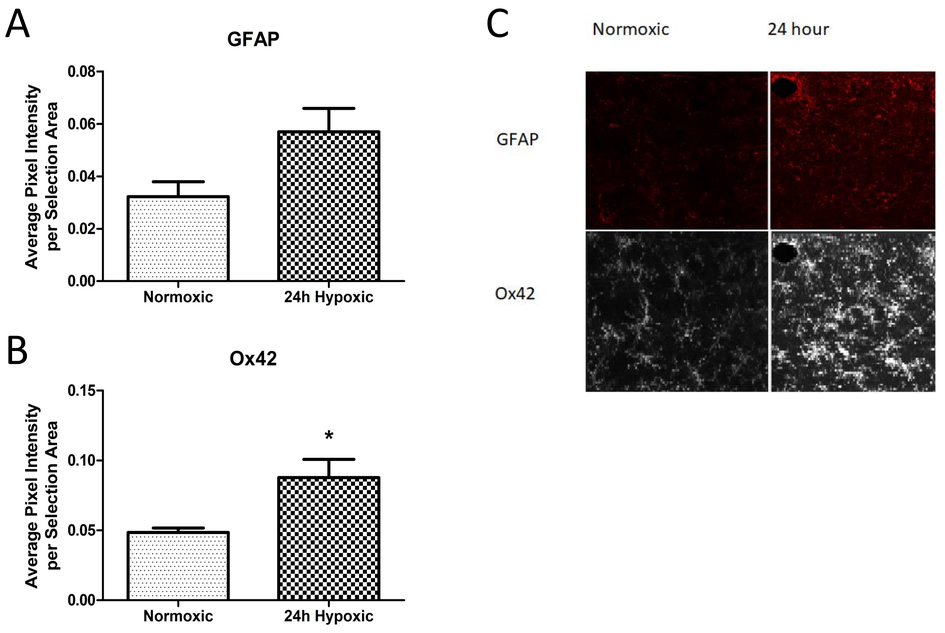
(Karperien et al., 2013)

The Sholl Analysis method is quantifying the activation of the microglia by measuring the branching of each microglia. The microglia is quantified based on the branch length and number of endpoints. In the more ramified state the microglia has a lot of branching and functionally is primarily sensing its environment (see figure above at right). While, the closer the microglia is toward an amoeboid state, which is little to no branching, and has a larger cell body, functionally the microglia is releasing cytokines in greater number and/or engulfing dead cell particles. The branch length, and the possible activity of each microglia was determined. To the side, is a picture that demonstrates the activation (ameoboid) in comparison to the resting (ramified) state.

Results

Figure 1: Microglia morphology shift quantification was performed using FIJI/ImageJ and the Sholl Analysis Plug-In order to assess the number of branches, branch length, and number of endpoints. 1A: Ox42 showed a significant change in the microglia morphology with the branch lengths becoming shorter, in contrast to the normoxic sholl analysis in which the opposite was observed. This difference however was not significant ($p>0.05$) except for the 11-20, which indicates that the microglia trended toward being stimulated by hypoxia at the 24-hour mark. 1B: The resulting microglia (on the left) is analyzed by sholl mask (on the right) and the branching the passes through each ring is counted.

Figure 2: *Activation of astrocytes and microglia by immuno fluorescent quantification*
2A: GFAP trended toward an increase in fluorescent intensity in the RTN region after 24-hours of hypoxia when compared to normoxic conditions although it was not significant ($p>0.05$). This was not significant, therefore no conclusive increase in GFAP after hypoxic exposure. 2B: Ox42 showed a significant increase in fluorescent intensity in the RTN region after 24-hours of hypoxia when compared to the normoxic conditions ($p < 0.05$). This suggests that there was an increase in Ox42 protein after hypoxic exposure. 2C: Representative images of those analyzed.



Conclusions and Future Directions

- Microglia were activated at the 24-hour hypoxic exposure, as measured by Ox42 expression and a slight morphology shift.
- GFAP trends toward activation, it was not significant. This may indicate the astrocytes were significantly active at an earlier time point or even at a later time point.
- For further research on microglia activation, we could quantify each cell's soma. Since the amoeboid cells have an enlargement of the soma of a cell we measure this in the normoxic and later time points to see if there is a change in the later time points of Microglia activation.
- We could also try to determine the time point in which the astrocytes become active, by choosing an earlier and/or later time points in the RTN.
- By knowing and understanding this mechanism behind the acclimatization toward hypoxia, we can fix resulting conditions caused by mutations in this area like apnea and even COPD.
- Therefore, there is a huge need for research on the contribution of RTN in ventilatory acclimatization to hypoxia.

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