SARS-CoV-2 Decreases Neuronal Activity in Brainstem Respiratory Centers in C57Bl6/J Mice

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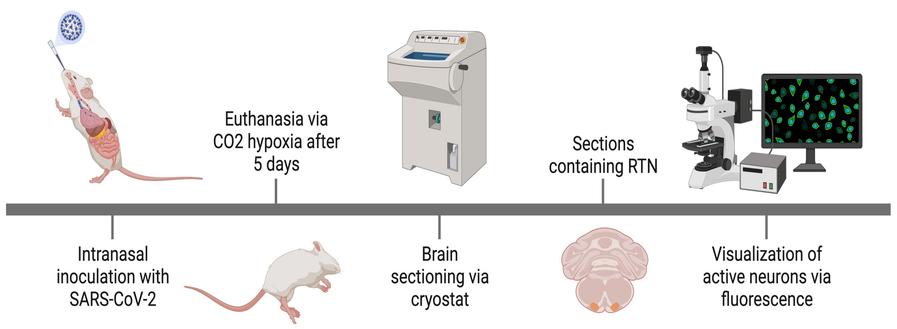
Introduction

- Respiratory symptoms such as cough, fever, and difficulty breathing are common in SARS-CoV-2, widely known as COVID-19, infections.
- Angiotensin-converting enzyme-2 (ACE2) is the functional receptor for SARS-CoV-2 and is highly expressed in many tissues throughout the body.
- Our group previously reported that neurons are the main cells within the brain expressing ACE2.
- This experiment demonstrated a decrease in neuronal activity the cells of the retrotrapezoid nuclei infected with SARS-CoV-2.

Hypothesis

Neuronal infections of brainstem respiratory centers by SARS-CoV-2 could contribute to respiratory symptoms associated with COVID-19, specifically respiratory failure.

Methods



C57BI6/J mice inoculated with different doses of SARS-CoV-2 virus ($7.5x10^3$ and $1.5x10^4$ units) or mock infected intranasally were euthanized 5 days post infection using CO_2 hypoxia. Harvested mice brains received from BSL-3 facility in Galveston were carefully dissected out and preserved in OTC before sectioning on a cryostat. Sections containing the retrotrapezoid nucleus (RTN) were selected. Immunohistochemistry for c-Fos, a marker for neuronal activity, was performed, and the sections were incubated overnight with a primary monoclonal antibody (rabbit anti c-fos, 1:1000) followed with a secondary goat anti-rabbit fluorescent IgG (GFP, 1:200, 1h) antibody. RTN and non-RTN (negative control region) images were taken using a confocal microscope. Activated neurons (c-Fos positive) were counted within a set area overlapping the RTN, by 2 investigators, averaged, and the data were analyzed using a two-way ANOVA followed by Bonferroni multiple comparisons test.

Results

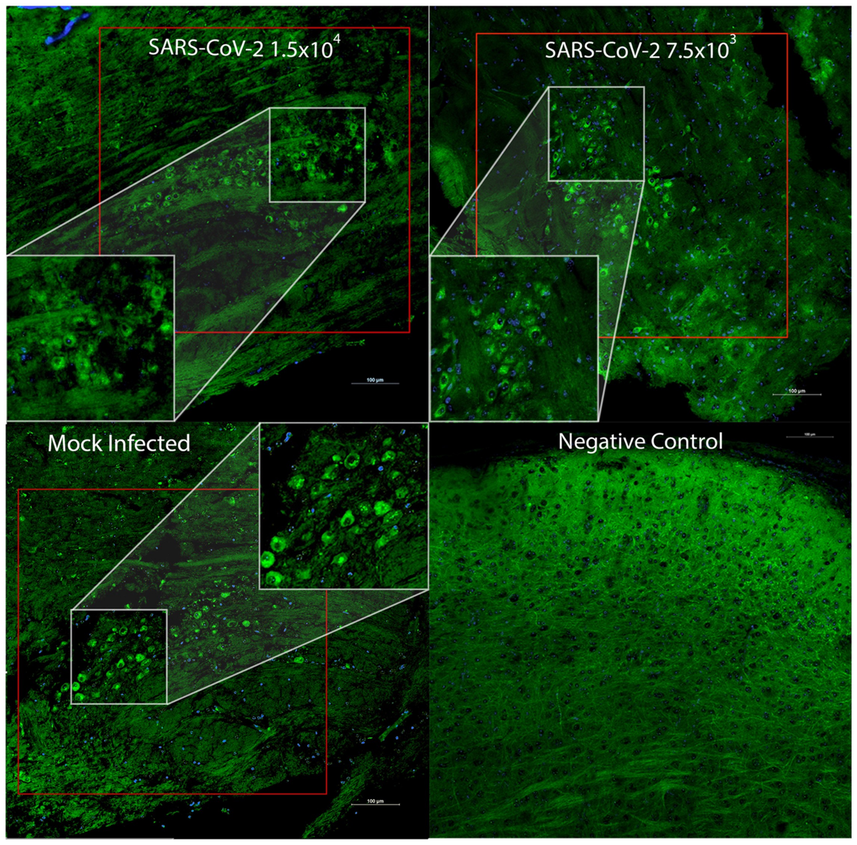


Figure 1: Neuronal activity of the RTN is reduced by SARS-CoV2. Hypoxia leads to activation of neurons in the RTN, as identified by c-fos immunoreactivity (bright green fluorescence). SARS-CoV-2 infection reduced the number of c-fos positive neurons within the RTN region (red box). No c-fos positive neurons were observed outside the RTN (negative control). DAPI: nucleus staining (blue).

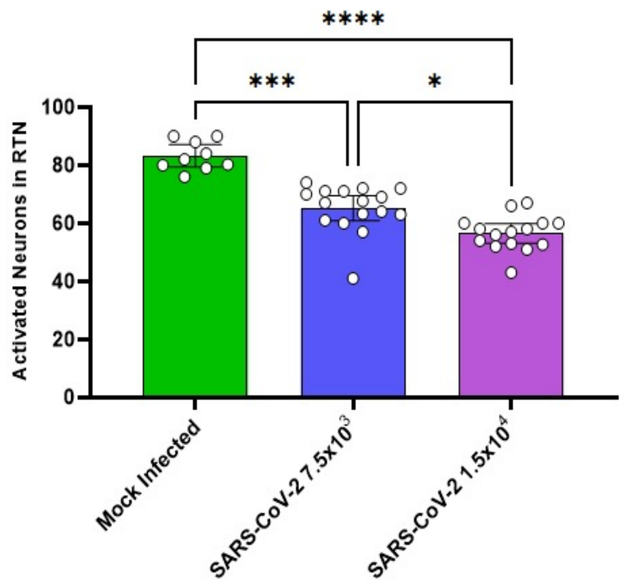


Figure 2: SARS-CoV2 dose-dependently reduces neuronal activity in the RTN. Mock infected mice exhibited the highest number of activated neurons in the RTN. Statistical significance: *p<0.05, ***p<0.001 and ****p<0.0001, two-way ANOVA.

Summary and Conclusion

- Our data show that euthanasia by hypoxia led to neuronal activation in RTN of mice, with mock-infected mice showing the highest level of activated neurons.
- SARS-CoV-2 exposure dose-dependently reduced neuronal activation most likely due to apoptosis.
- We conclude that SARS-CoV-2 targets neurons in respiratory centers, possibly contributing to impaired respiratory function in infected patients.

Future Directions

Further studies will investigate if SARS-CoV-2 tropism is restricted to neurons and if neuronal death is increased in infected brains.

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