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

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also called COVID-19, targets the respiratory system, leading to symptoms such as cough, fever, and difficulty breathing[1]. As the mechanisms behind SARS-CoV-2 infections continues to be studied, understanding of how this virus targets specific tissues becomes more apparent. Angiotensin-converting enzyme-2 (ACE2), the target of SARS-CoV infections and a functional receptor for SARS-CoV-2 invasions[1], is highly expressed in many tissues throughout the body, including the lungs, kidneys, and brain. Notably, our group previously reported that neurons are the main cells within the brain expressing ACE2. Accordingly, we hypothesized that neuronal infection of brainstem respiratory centers by SARS-CoV-2 could contribute to respiratory symptoms associated with COVID-19, specifically respiratory failure.

C57Bl6/J mice were mock infected or received SARS-CoV-2 ($7.5x10^3$ or $1.5x10^4$) intranasally in a BSL-3 facility (University of Texas in Galveston) and were euthanized by CO_2 hypoxia after 5 days post infection. The heads were harvested and immersed in formalin for 3 days before being transferred to LSU Health-NO for processing. Brains were carefully dissected out and preserved in optimal cutting temperature (OCT) before sectioning on a cryostat. Sections (30 µm) containing the retrotrapezoid nucleus (RTN) were selected as its neurons are part of the pontomedullary regions that generate the respiratory rhythm and pattern[2].

Immunohistochemistry for c-Fos, a marker of neuronal activity[3], was used to access neuronal activity in the RTN. Sections were incubated overnight with a primary monoclonal antibody (rabbit anti c-Fos, 1:1000) followed with a secondary goat antirabbit fluorescent IgG (GFP, 1:200, 1h) antibody. Sections were then mounted to slides in the dark to preserve the fluorescence and observed under the microscope. Images of neurons present in the RTN were captured and neurons were then hand counted and averaged.

Neuronal activity in the RTN was decreased with the amount of SARS-CoV-2 that has infected cells in the brain. The greater the infection of SARS-CoV-2, the least number of neurons tagged with c-Fos was indicated. Mock brains had the greatest average of neurons and brains infected with $1.5x10^4$ SARS-CoV-2 had the least average of neurons in the RTN. This was demonstrated by counting neurons at 3 different intervals and taking the average. We conclude that SARS-CoV-2 targets neurons in respiratory centers, possibly contributing to impaired respiratory function in infected patients.