

## **Effects of chemical vagotomy on the neonatal mouse paraventricular nucleus of the hypothalamus**

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Birth is marked by a dramatic transition from the sterile womb to a world full of microorganisms that colonize every body surface. We previously found that this colonization exerts rapid effects on brain development, as in mice born in the absence of microbes had increased cell death in the paraventricular nucleus of the hypothalamus (PVN), an effect not seen 12 h prior to expected delivery. The gut microbiota influences the development of many body systems, and communicates bi-directionally with the brain. We hypothesize that the microbiota exerts effects on neuronal cell death via the vagus nerve, which connects the gut to the PVN via two synapses. To test this, we plan to use capsaicin to perform chemical vagotomies, but we must first validate the approach. In preliminary studies, we confirmed that capsaicin injections to newborn mice caused marked increases in death of vagal afferents. To further validate our technique, in this experiment we evaluated whether capsaicin also induces cell death in the PVN (which could present a confound to our study) and whether it influences neural activity in this nucleus. Mice were injected with capsaicin or vehicle subcutaneously on postnatal day 0 and their brains were collected 0.5, 2, 4, 6, or 24 hours later, and processed for immunohistochemical detection of markers of cell death (activated caspase-3, AC3) or neural activation (Fos). We found that capsaicin did not alter cell death in the PVN at any time point. It did, however, cause increased Fos expression 2 and 4 hours after treatment, which may be due to the activation of the vagal-PVN pathway. The absence of capsaicin-induced cell death in the PVN suggests that chemical vagotomies may be a useful approach to test whether the microbiota affects cell death in the PVN via the vagus nerve.