Modularity of Swallow Networks via Optical Recordings in Medulla of Germline- GCaMP6F Neonate Mouse

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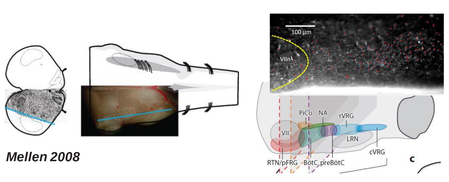
Introduction:
Swallow must be coordinated with breathing to prevent aspiration, but the mechanisms of this coordination are poorly understood. In the sagittally sectioned rodent hindbrain (SSRH) preparation, control networks for swallow are exposed at the dorsomedial edge of the preparation. In vitro swallow is defined as a burst at the hypoglossal nerve (XIIn) unaccompanied by phrenic (C4) motor output. Here we used transgenic mice in which the genetically encoded Ca^{2+} indicator GCaMP6F was expressed in the germline, enabling optical recording from all cells exposed at or near the sagittal surface of the SSRH. At the end of each experiment a 400 µm section from the sagittal face was processed immunohistochemically for ChAT, phox2b, and SST. We identified a novel population of neurons rostral to the pre-Bötzinger complex (PBC) that is immediately activated following stimuli. Seconds after stimulus offset, a discrete population of neurons in nucleus ambiguus become active, and their activity is accompanied by a burst on XIIn unaccompanied by C4 activity. This delayed activity occurred immediately before or after inspiration, consistent with gating of swallow with breath. Networks activated during swallow are spatially compact with sharp, well-defined borders. This functional anatomical organization is also found in IHC: networks activated during swallow strongly co-express phox2b and ChAT, consistent with modularity.

Purpose:
To identify central networks that control swallow, and profile them immunohistochemically.

Methods:
Electrophysiology: Sagittally-sectioned rodent hindbrain (SSRH) preparations isolated from neonate (P1-P6) B6.Cg-Tg(Camk2a-cre)T29-1Stl/J and B6.Cg-Edil3$^{Tg(Sox2\text{-}cre)1Amc}$/J mice (005359, 008454 Jackson Laboratories), crossed with B6J.Cg-Gt(ROSA)26Sor$^{tm95.1(CAG\text{-}GCaMP6f)Hze}$/MwarJ mice (028865Jackson Laboratories), so as to obtain germline GCaMP6F expression (i.e., expression in *all* cells). The SSRH was then transferred to an upright microscope (Axioskop 2, Zeiss). Hypoglossal and phrenic rootlets were recorded via suction electrodes to detect fictive swallow and/or inspiratory drive. A bipolar platinum/iridium electrode stimulated (20 Hz, 6V) regions described by others as command neurons for swallow. Optical recordings were made using a large-format sCMOS camera (30 Hz) (Prime 95B, Photometrics). Camera-control and machine- vision software were developed in-house (LabView, National Instruments).
Immunohistochemistry: Primary antibodies: somatostatin (rabbit 1:500, Invitrogen PA5-85759), choline acetyltransferase (goat 1:200, Millipore AB144P), Phox2b (mouse 1:100, Santa Cruz sc-376997). All secondary antibodies: Alexa fluor Plus, Invitrogen. Primary antibody exposure: 12 h at RT.



Multiplexed networks along the VRC mediate swallow and BH

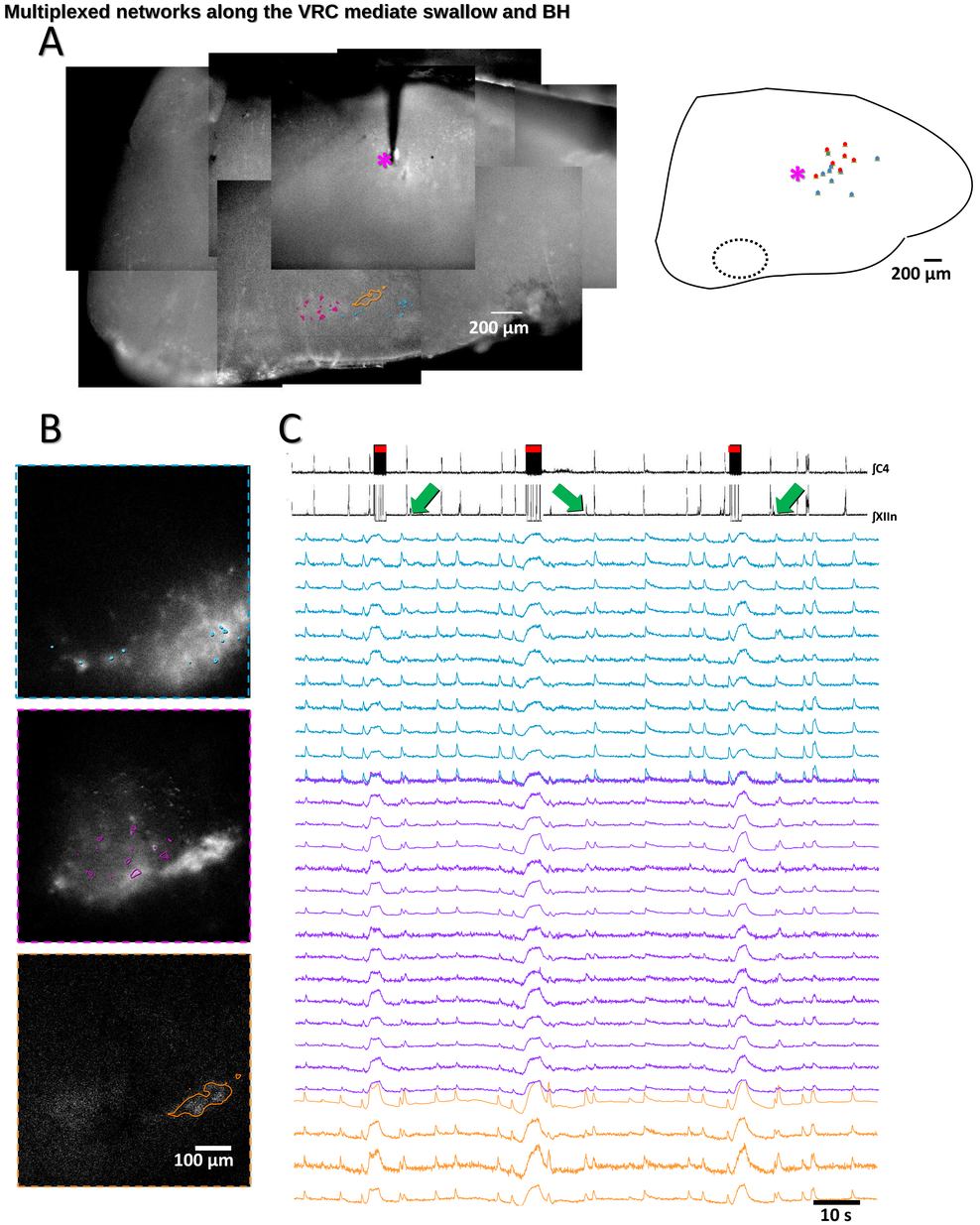


Figure 1: A. View of the preparation. Colored regions of interest (ROIs) are matched with traces in C below. VIIn outlined in white. Stimulus location for swallow is indicated by ✱. Inset shows locations where swallow was evoked **B.**. Averaged differenced view of respiratory activity (top), interneuron network (middle), and premotor network (bottom). **C.** Rectified integrated root recordings from XIIn and C4 (green arrows indicate delayed swallow, red bars stimuli), and time-varying Ca^{2+} signals obtained from color-coded regions of interest..

Cross-indexing immunohistochemistry with electrophysiology

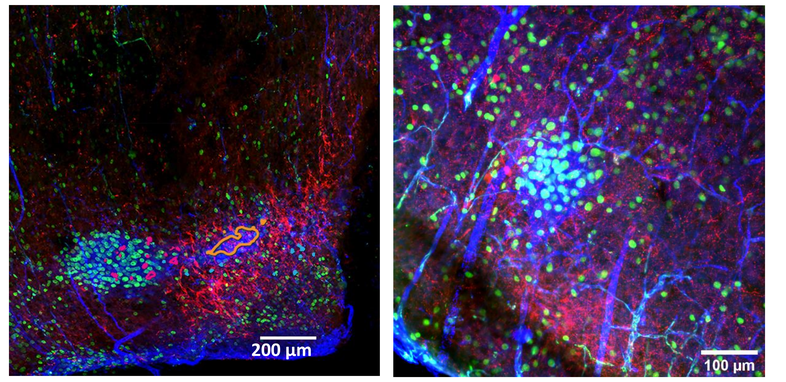


Figure 2: Merged maximum intensity images obtained from a confocal imaging stack obtained from the same preparation as shown in Fig. 1 in the sagittal plane. ChAT is shown in blue, SST in red, and phox2b in green. ROIs from figure 1 are superimposed **B.** The phox2b$^+$, ChAT$^+$ region is also well-defined in the transverse plane.

Conclusions:
- The ventrodorsal tilt of the sagittally sectioned mouse hindbrain preparation exposes populations of neurons in mediodorsal medulla that modulate the respiratory pattern and intercalate orofacial behaviors.
- Fictive swallow, identified as evoked activity on XIIn but not C2, was obtained by stimulating regions first described by Jean (1985).
- A spatially compact network of neurons that may be a constituent of the intermediate reticular nucleus was strongly activated during evoked swallow.
- A more caudal network, also ChAT$^+$ and phox2b$^+$ was active during spontaneous swallow that occurred seconds after stimulus offset before or after inspiration (green arrows in figure 1). This population appears to have no overlap with SST$^+$ respiratory rhythmogenic networks.
- This preliminary study lays the groundwork for future studies investigating interactions between respiratory rhythmogenic networks and central networks coordinating breath and swallow

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