Developmental, Physiological, and Transcriptomic Analyses of Neurons Involved in the Generation of Mammalian Breathing

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Abstract

Breathing is a rhythmic motor behavior with obvious physiological importance: breathing movements are essential for respiration, which sustains homeostasis and life itself in a wide array of animals including humans and all mammals. The breathing rhythm is produced by interneurons of the brainstem preBötzinger complex (preBötC) whose progenitors express the transcription factor Dbx1. However, the cellular and synaptic neural mechanisms underlying respiratory rhythmogenesis remain unclear. The first chapter of this dissertation examines a Dbx1 transgenic mouse line often exploited to study the neural control of breathing. It emphasizes the cellular fate of progenitors that express Dbx1 at different times during development. I couple tamoxifen-inducible Dbx1 Cre-driver mice with Cre-dependent reporters, then show that Dbx1-expressing progenitors give rise to preBötC neurons and glia. Further, I quantify the temporal assemblage of Dbx1 neurons and glia in the preBötC and provide practical guidance on breeding and tamoxifen administration strategies to bias reporter protein expression toward neurons (or glia), which can aid researchers in targeting studies to unravel their functions in respiratory neurobiology. The second chapter of this dissertation exploits the mouse model characterized in the first chapter and then focuses on mechanisms of respiratory rhythmogenesis. The breathing cycle consists of inspiratory and expiratory phases. Inspiratory burst-initiation and burst-sustaining mechanisms have been investigated by many groups. Here, I specifically investigate the role of short-term synaptic depression in burst termination and the inspiratory-expiratory phase transition using rhythmically active medullary slice preparations from Dbx1 Cre-driver mice coupled with channelrhodopsin reporters. I demonstrate the existence of a post-inspiratory refractory period that precludes light-evoked bursts in channelrhodopsin-expressing Dbx1-derived preBötC neurons. I show that postsynaptic factors cannot account for the refractory period, and that presynaptic vesicle depletion most likely underlies the refractory period. The third chapter of this dissertation focuses on transcriptomic analysis of Dbx1 preBötC neurons, and differences in gene expression between Dbx1-derived and non-Dbx1-derived preBötC neurons. I analyze and quantify the expression of over 20,000 genes, and make the raw data publicly available for further analysis. I argue that this full transcriptome approach will enable our research group (and others) to devise physiological studies that target specific subunits of ion channels and integral membrane proteins to examine the role(s) of Dxb1-derived neurons and glia at the molecular level of breathing behavior.