Neuroanatomical and morphological properties of neurons that generate inspiratory related breathing rhythm and influence respiratory motor pattern in mice

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Abstract

The relationship between neuron morphology and function is a perennial issue in neuroscience. Information about synaptic integration, network connectivity, and the specific roles of neuronal subpopulations can be obtained through morphological analysis of key neurons within any given microcircuit. Breathing is essential behavior for humans and all mammals, yet the neural microcircuit that governs respiration is not completely understood. The respiratory neural microcircuit resides within the ventral respiratory column located in the medulla. Within the respiratory column, the site of respiratory rhythm generation is the bilaterally distributed preBötzinger complex (preBötC). Rhythm-generating neurons in the preBötC are derived from a single genetic line, i.e., precursor cells expressing the transcription factor Developing brain homeobox-1 (Dbx1). An analysis of over 40 dendritic morphological features of rhythmogenic Dbx1 preBötC neurons and putatively premotor Dbx1 neurons in the intermediate reticular formation, revealed these two populations are similar except reticular neurons have a larger dendritic diameter, which may contribute to a greater passive transmembrane conductance. Both populations showed commissural axon projections and reticular formation neurons show premotor-like projections to the XII motor nucleus. These morphological data provide additional evidence supporting bilateral synchronization the preBötC through Dbx1 neurons, and demonstrate that Dbx1 preBötC neuron connectivity includes recurrent interconnections. On the molecular level, the ion channels that mediate rhythm-generating whole-cell ion currents have not been not identified, and were investigated using principally an anatomical approach. The nonspecific cation current, I_{CAN}, underlies robust inspiratory drive potentials in the preBötC and the persistent sodium current, I_{NaP} may play a role in the production of robust bursts when respiration is challenged in such cases as anoxia or hypoxia. The leading candidate for ion channels that contribute to I_{CAN} belong to the transient membrane receptor (Trp) ion channel superfamily and the leading ion channel candidate for I_{NaP} is $Na_v1.6$. I determined the presence of Trpc3 ion channels and $Na_v1.6$ ion channels on Dbx1 preBötC neurons (as well as their expression in neighboring non-Dbx1 preBötC neurons). Finally, breathing behavior involves periodic sighs, which are slower than normal eupneic breathing but critical for lung function. I examined receptor expression for bomebsin-like peptides neuromedin B (NMB) and gastrin releasing peptide (GRP), which are important for sigh behavior. I show that NMB and GRP receptors are expressed in Dbx1 preBötC neurons and are not expressed by glia in the preBötC, as posited by some because of the low frequency of sigh breaths. These advances in morphological and anatomical knowledge can be used to design targeted *in vitro* and *in vivo* experiments to further explore their role in respiratory rhythm and pattern generation.