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A novel FIJI workflow demonstrates dynamic changes in postnatal respiratory nuclei innervation by *Nkx2.2*- and *Olig3*-derived neurons.

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The autonomic nervous system undergoes rapid and significant change in the postnatal period of mammalian development. How the contributions of specific neural lineages to autonomic circuits change and develop during this phase remains to be determined. In this study, we utilized transgenic mouse modeling and immunofluorescent imaging to determine how *Nkx2.2*- and *Olig3*-derived neural lineages develop postnatally and, more specifically, how their innervations into three hindbrain respiratory nuclei are altered during this epoch. We generated a novel FIJI workflow, which allowed the systematic and rapid analysis of *Nkx2.2*- and *Olig3*- derived synapses in regions of interest in each of the three hindbrain respiratory nuclei. This workflow provided more precise quantifications in only 1/25 of the time when compared to human quantifications. We demonstrated that *Nkx2.2*-derived innervations into the PreBötzinger Complex significantly decreased in the first three weeks of postnatal life while *Olig3*-derived afferents showed no such change during the same period. These data demonstrate utility in the application of this novel FIJI workflow to the investigation of neural circuit contributions to the postnatal development of the autonomic nervous system. Further, they indicate that the role of *Nkx2.2*-derived neurons in autonomic function and respiration changes during the first three weeks of life.

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