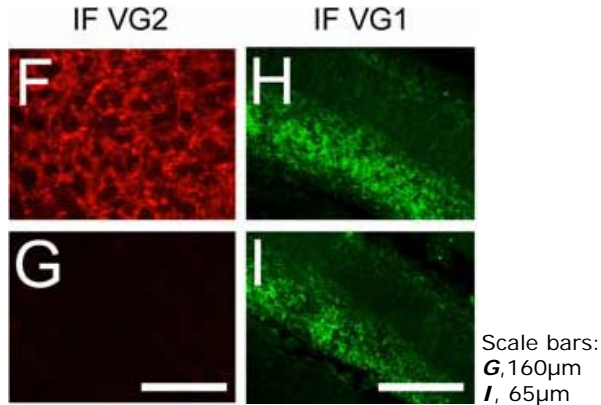


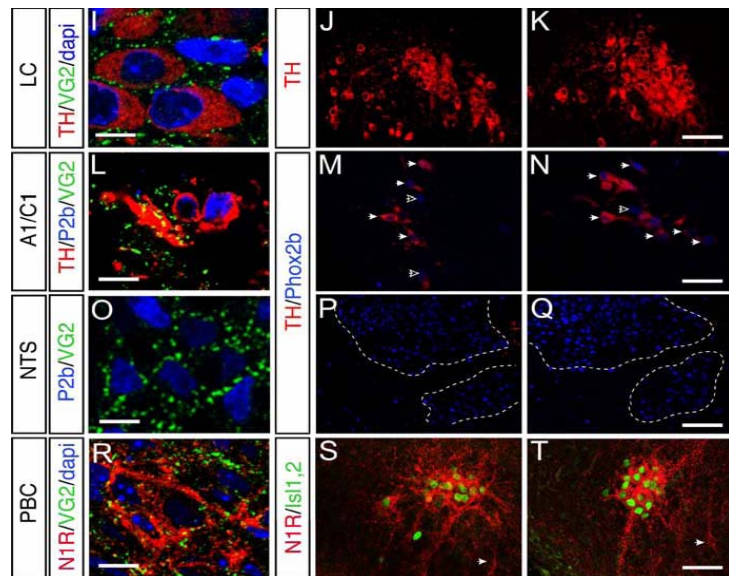


Role of the VGLUT2 transporter in the respiratory rhythm generation



Dr Kullander and colleagues are interested in the function of neuronal circuits in the central nervous system. Their goal is to increase our knowledge of how neuronal networks develop into functional units, and better understand the mechanisms behind neuroexcitotoxicity. In this article, the researchers study the glutamatergic excitatory neurotransmission which relies on glutamate release from vesicles that are carried by transporters called VGLUTs. Mice lacking VGLUT2 die from absence of respiratory behaviour. The aim is therefore to show that VGLUT2 signaling plays a key role in the respiratory rhythm generator.

In this experiment, the researchers firstly generated VGLUT2 null mice. In order to test the mutants, Dr Kullander examined their brains using the **Volocity Grid Confocal** imaging system which combines **Volocity** software and the **OptiGrid** structured light device to achieve confocal quality imaging with a standard fluorescence microscope without using laser scanning technology. **Volocity Acquisition** captured the first set of images which shows the expression of VGLUT2 and VGLUT1 proteins in mutant (G,I) and control mice (F,H). Image G clearly shows that VGLUT2 is not detectable in mutant mice. **Volocity Visualization** allowed the researchers to instantly render the objects, and work on each channel separately in order to obtain the required image.



Scale bars: **J**, 13µm; **K**, 75µm; **L**, 9µm; **N**, 35µm; **O**, 21µm; **R**, 13µm; **Q**, 150µm; **T**, 50µm.

Further results showed that VGLUT2 mutants had lung alveoli that were reduced in size, which lead researchers to focus on the ventilation and respiratory generating circuits in the brainstem. These appeared to show a complete absence of rhythmic activity in the PBC brain area in mutant mice. In order to understand this result, the team characterized the structural and cellular organization of nuclei located in the brainstem that are known to be part of the central respiratory network. The second set of images shows the expression of specific markers of four areas of the brain (LC, A1C1, NTS, PBC) in control mice (first two columns) and in mutants (third column). The data clearly indicates that the components of the respiratory network in mutant embryos are present and have normal locations and size, suggesting little impact of the VGLUT2 loss on brainstem structures and expression of genetic markers.

However, further experiments showed that the lack of VGLUT2 affects neuronal activity in the PBC area by abolishing the fast excitatory synaptic events and that the synaptic vesicles are redistributed and malformed in asymmetric synapses. The study therefore confirms that VGLUT2 is an obligatory component of the respiratory development.