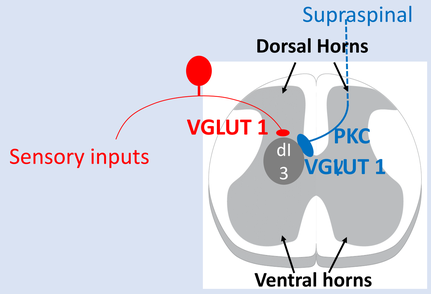
Characterization of spinal sensory circuits comprising dorsal interneuron 3 (dI3 IN) in mice.

Het Vaishnav, Nicolas Lalonde & Tuan Bui
Department of Biology, University of Ottawa, Ottawa ON

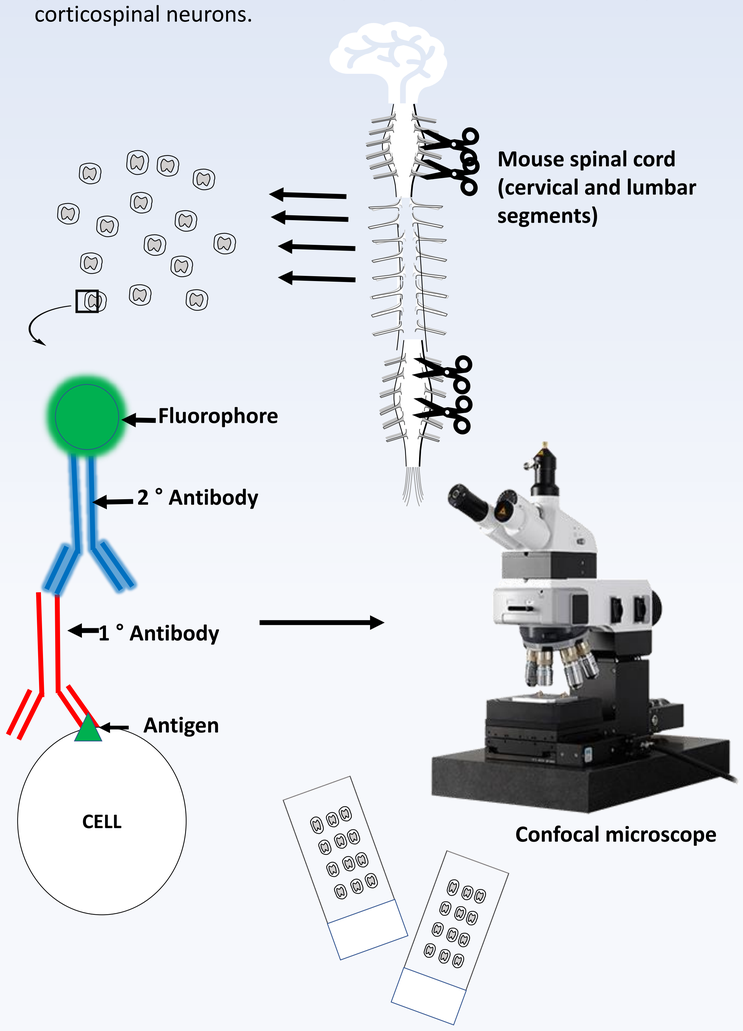
INTRODUCTION

- The palmar grasp reflex is a reflex observed in infants where the paws (in mice) and hands (in human) close when the skin is stroked. It emerges 11 weeks in utero and continues up until the age of 2-3 months in humans. It is believed to be essential in the development of voluntary hand grasp in adults.
- Corticospinal tract inputs (CST; labelled by PKCg and VGLUT1) convey volitional motor commands from the cortex. Sensory inputs from the skin and muscles (labelled by VGLUT1 but not PKCg). Studying the development of CST and sensory inputs to dI3 INs will provide unique insights into how the nervous system matures to control movements through the cortical control of reflexes. **My aim is to determine the number of sensory and CST inputs to dI3 INs.**



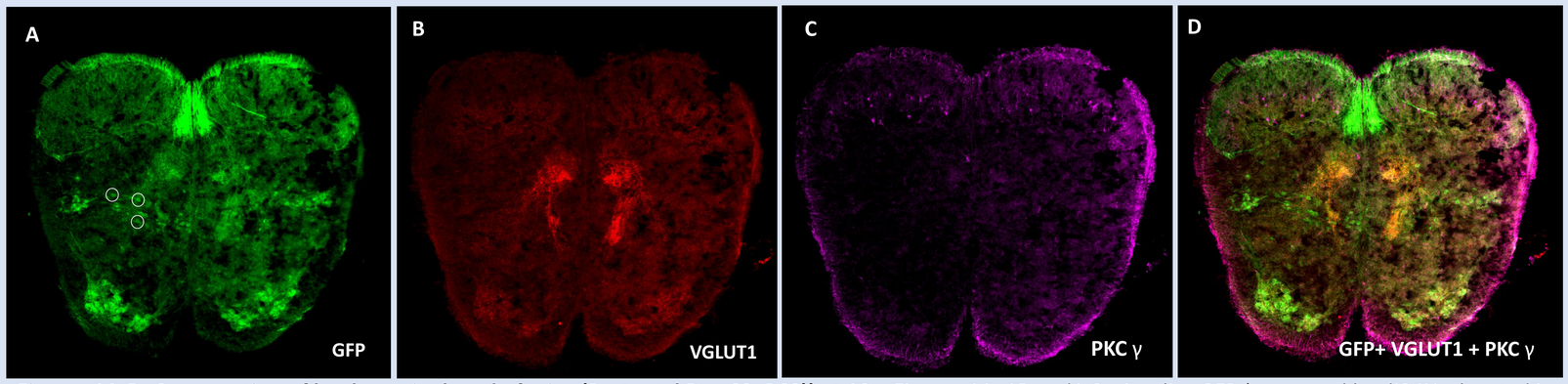
METHODOLOGY

The integration of sensory and motor commands by DI3 IN is studied through immunohistochemical labelling of sensory and descending inputs from the motor cortex using antibodies against proteins expressed selectively by synapses from sensory and corticospinal neurons.

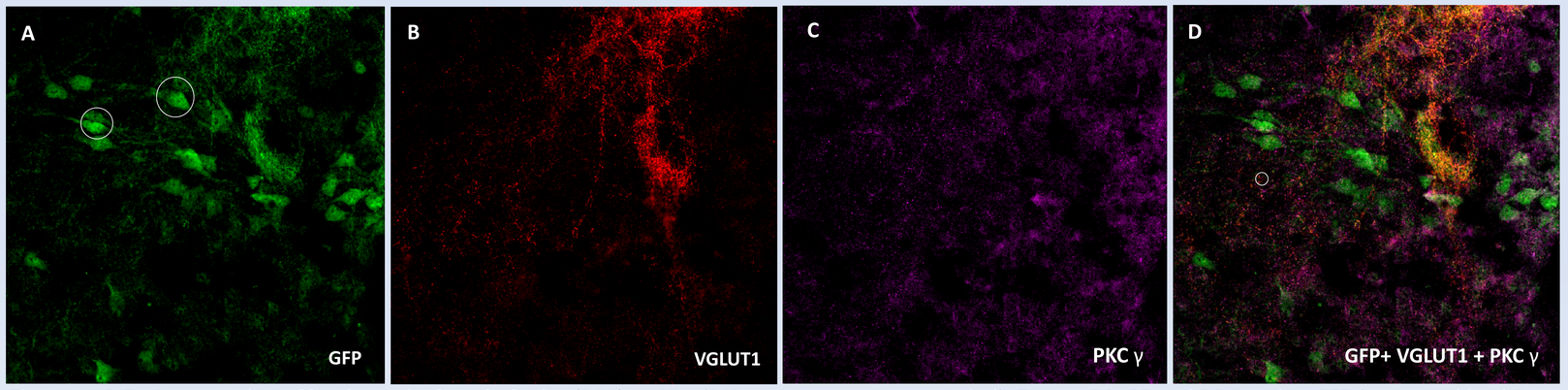


Dual antibody labelling is used to visualize the proteins because several 2 ° antibodies can bind to a single 1° antibody (polyclonal), forming large 1 °-2 ° antibody complexes, therefore amplifying the signal.

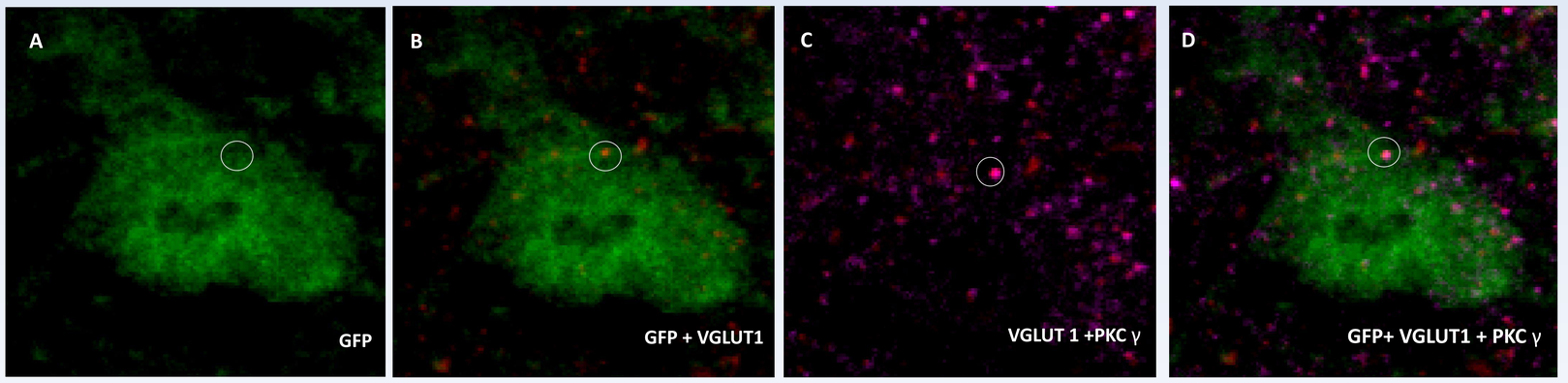
RESULTS



Figures 1A-D: Cross section of lumbar spinal cord of mice (Postnatal Day 60; P60)) at 10x. Figures 1A, 1B and1 C, visualize GFP (expressed by dI3 INs; located in the intermediate laminae; dI3 INs encircled), VGLUT1 and PKCγ, respectively. The secondary antibodies re-emit lights of wavelengths 488 nm, 555 nm and 647 nm, respectively. Figure A also visualizes motor neurons (bottom left corner) are in the ventral horn. Figure 1D shows an overlap of the thee signals.



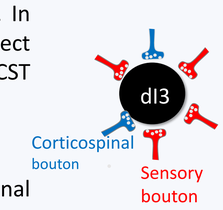
Figures 2 A-D: Cross section of lumbar spinal cord of mice (P60) at 40x. (DI3 interneurons are encircled). Figures 2A, 2B and 2C visualize GFP (expressed by dI3 INs), VGLUT1 and PKCγ, respectively. The secondary antibodies re-emit lights of wavelengths 488nm, 555nm, and 647 nm, respectively. Figure 2D shows the co-localization of the 3 signals (encircled in white).



Figures 3 A-D: Cross section of lumbar spinal cord of mice (P60) at 40x, zoomed in to show a single bouton with co-localization of GFP, VGLUT1 and PKCγ signals. Co-localization is encircled. Figures 3A, 3B, 3C and 3D visualize GFP (expressed by dI3 INs), GFP +VGLUT1, VGLUT1 +PKCγ, and GFP+ VGLUT1 +PKCγ, respectively. The secondary antibodies re-emit lights of wavelengths 488nm (GFP), 555 nm (VGLUT1), and 647 nm (PKCγ). Figure 3D shows a single bouton to dI3 with co-localization of all three signals (encircled). It can therefore be hypothesized that the encircled bouton corresponds to a supraspinal input. All images (figures 1 A-D, 2A-D and 3 A-D) are captured using a confocal microscope.

DISCUSSION

- To date, 20-30 samples from three different animals have been tested, however, not many co-localizations have been identified. In contrast, many VGLUT1+/PKC γ- boutons were observed. These results suggest that dI3 INs receive many sensory inputs whereas direct CST inputs to dI3 INs are rare. Control of dI3 INs by descending CST inputs must be through interneurons interposed between the CST terminals and dI3 INs.



Future directions

- Conducting more immunohistochemical labelling of (20-30) samples from at least 2-3 more animals in order to characterize the spinal sensory circuits comprising dI3 interneurons and determine the percentage of each type of input to dI3 INs.

ACKNOWLEDGEMENTS

I would like thank Tuan Bui (and the University of Ottawa) for providing me with this opportunity and all members of the Bui lab, especially Nicolas Lalonde, Benjamin Lindsey and Jonathan Tea.

Faculté des sciences
Faculty of Science