Connectivity of dI3 Interneuron microcircuits in developing mice spinal cords

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Introduction

Our movements are strongly influenced by sensory information that is processed by sensorimotor microcircuits in the spinal cord. From the moment of birth, these circuits undergo developmental changes that allow us to master our movements.

A recently described spinal microcircuit centers on dI3 interneurons[1]. These neurons reside within the V-VII laminae of mice spinal cords, and they form disynaptic circuits which relay cutaneous information from sensory neurons to motoneurons that control hand grasp as well as locomotor activity.

Our interest in these interneurons is to visualize their connectivity to important neural pathways and to see how they mature during mouse development. Our findings will allow us to characterize sensorimotor microcircuits within the spinal cord during development to better understand motor control maturation.

Methods

Transgenic mice models (Isl1-Cre and Rosa26-YFP) were genotyped using PCR to identify the presence of dI3 INs by heterozygous expression of the Isl1-Cre transcription factor and YFP reporter gene. Mice only expressing the YFP gene will be used as the control group.

Mice spinal cords were fixed the through use of transcardial perfusion and sectioned with a thickness of 50 µm from the cervical, thoracic and lumbar regions.

Visualization of dI3 IN connectivity was done through the use of immunohistochemistry to label sensory inputs to dI3 INs (vGLUT1+ boutons), spinal excitatory inputs to dI3 INs (vGLUT2+ boutons), and excitatory motoneuron projections from dI3 INs (vGLUT2+/GFP+ boutons on ChAT+ or GFP+ motoneuron).

Results

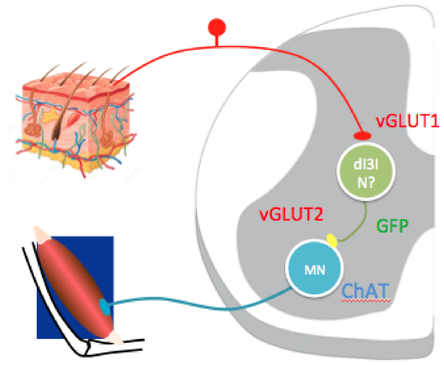

Figure 1. Diagram of spinal microcircuit connectivity. Cutaneous sensory input enters by neuronal cells through the dorsal horn and synapse on dI3 INs within the V-VII laminae forming excitatory boutons (vGLUT1). The dI3 INs (GFP) extend projections to motoneurons forming yellow boutons (vGLUT2/GFP). Motoneurons project out through the ventral horn to form neuromuscular junctions (ChAT) creating a sensorimotor microcircuit.

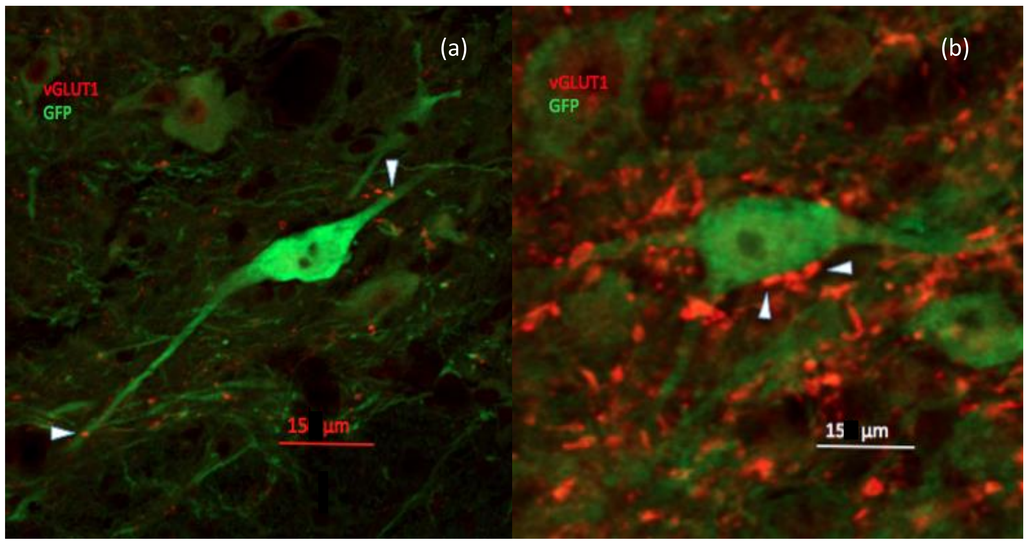

Figure 2. Immunohistochemistry of sensory neuronal circuitry. Labeling of sensory inputs (vGLUT1) with red Alexa Fluor 555 antibodies on dI3 Interneurons (GFP) labeled with green Alexa Fluor 488 antibodies. Arrows indicate location of excitatory (glutamatergic) sensory boutons on (a) axonal and (b) neuronal cell bodies.

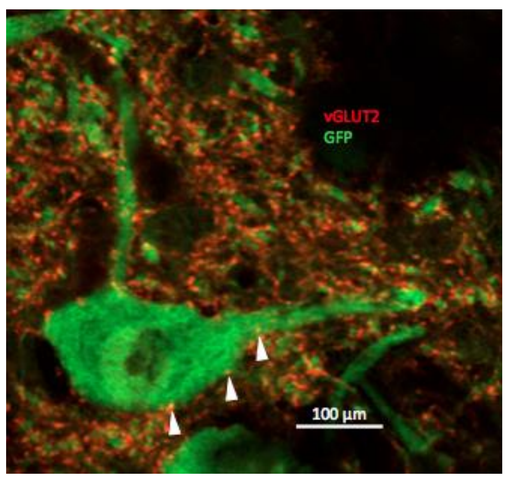

Figure 3. Immunohistochemistry of dI3 to motoneuron circuitry. Labeling of motoneurons (GFP) and excitatory outputs from dI3 INs (vGLUT2 and GFP) forming yellow boutons connecting on motoneurons indicated by the arrows.

Conclusions

- Successful visualization and labeling of dI3 INs and their synaptic boutons from sensory inputs and spinal neurons, and excitatory projections to motoneurons. The dI3 INs are seen to relay the cutaneous sensory inputs to motoneurons forming a microcircuit involved with motor control.

Future steps

- Continue to optimize immunohistochemistry labeling of ChAT, GAD65, and GAD67 neurotransmitters to better visualize dI3 IN connections to motoneurons as well as to analyze inhibitory neuronal inputs to dI3 INs.
- Compare the microcircuit connectivity in the cervical and lumbar regions of the spinal cord to compare and contrast the circuitry involved with hand grasp as compared to locomotion.

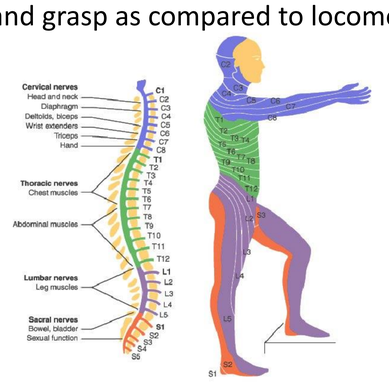

Figure 4. Diagram depicting somatotopic map of spinal cord control of the body (Singh, 2015).

References

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2. Singh A. 2015. Spinal cord injury levels [Intenet]. Bone and Spine. Available from http://boneandspine.com/spinal-cord-injury-levels/

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