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Imaging reticulospinal neurons in the lamprey brainstem using calcium indicator

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Imaging reticulospinal neurons in the lamprey brainstem using calcium indicator In the lamprey, a lower vertebrate, reticulospinal (RS) neurons in the brain are the output elements of the command system that activate spinal pattern generators and initiate swimming. In order to better understand the locomotor command system in the lamprey, it is necessary to determine the locations of neurons in the network, as well as their connectivity and patterns of activity. Calcium indicator dyes are an important technique for labeling and monitoring neuron activity. During impulse, calcium enters neurons and binds to the dye, increasing the fluorescence of the dye and creating an optical image that can be recorded and analyzed. In the present study, Calcium Green dextran amine was applied to the transected spinal cord at 20% body length (BL). After retrograde transport of the dye and labeling of RS neurons, the brain and spinal cord were removed and placed on a slide for viewing under a microscope equipped for fluorescence. Electrical stimulation of the spinal cord activated labeled RS neurons in the brain, resulting in a fluorescence increase that was recorded by an S-VHS video camera. The next step will be to image RS neuron activity during actual swimming movements. For this purpose, RS neurons will be labeled in a semi-intact preparation in which the brain and upper spinal cord are exposed and the lower half of the body is free to produce swimming movements. As a control experiment, the spinal cord was transected and Calcium Green applied at 60% BL. Semi-intact preparations were observed to produce swimming movements. Imaging of the isolated brain and rostral spinal cord showed RS neuron labeling and fluorescent changes similar to when tracer was applied at 20% BL. These results lay the groundwork for imaging brain neuron activity during actual swimming behavior.