

Using the IR-DIC microscope to identify locomotor activity-labelled spinal cord neurons in green fluorescent protein transgenic Mice

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Abstract

To study in depth and understand the generation of locomotion in spinal cord neurons, we used a transgenic mice expressing enhanced green fluorescent protein (EGFP) which was generated by a purified c-fos promoter-EGFP plasmid. The spinal cord slices were transferred to a chamber mounted in the stage of an upright Leica DM LFSM microscope equipped with a camera sensitive to wavelengths from blue to infrared. EGFP-positive neurons were identified using epifluorescence with a narrow band GFP cube and analogue integration of the video output. Infrared-differential interference contrast (IR-DIC) microscopy was used to visualize EGFP-labeled cells in the living whole mount slices. The results showed the position of the EGFP-expression cells that are concentrated in the intermediate gray matter (lamina VII) or near the central canal (lamina X). To address the sensitivity and specificity of EGFP expression, double-labelling for cfos and EGFP expression was undertaken. The immunohistochemical analysis demonstrated that most EGFP+ cells were also positive for cfos expression. Conversely, there were many cfos+ cells that were not EGFP positive. EGFP+ cells were seen in the intermediate and ventral thoracic spinal cord; many more were seen in the lumbar cord. The cfos labeling was present dorsally and ventrally in lower thoracic and upper to mid lumbar spinal cord. Double labeling for cfos and EGFP was observed in cells within ventral lamina VII, VIII, and lamina X. The distribution of EGFP neurons in the mouse spinal cord seems likely to cfos-labeled cells. But the cfos expressions are more than EGFP expressions. Must EGFP neurons coexpress cfos. A few EGFP cells that are cfos negative. This may relate to differences in timecourse of expression of both cfos and EGFP. To examined the neurons responsible for the locomotion, we performed EGFP/ c-fos double-labeled immunocytochemistry to locate and identify EGFP expression-neurons using fluorescence microscopy. The results indicate that EGFP can be used as a marker of activity to define the spinal neurons relative locomotion. This study demonstrates the feasibility of using transgenic animals that express EGFP to study electrophysiological properties of identified spinal cord neurons.