Time-lapse imaging of pyramidal tracts in vivo as a tool to study axonal degeneration in HSP

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The advances in transgenic mouse technology expressing celltype-specifically fluorescent proteins as well as in laser-scanning microscopy allow identifying individual cells *in vivo*. Using two-photon microscopy we are capable to study axonal structures and glia-axon interaction in the spinal cord. For this purpose, adult transgenic mice with microglial-specific EGFP expression and neuronal/axonal EYFP expression are anaesthetized using pentobarbital i. p. and methohexital-Na i. v.. Vital functions are closely monitored and can be maintained for a period of at least 12 h. During anaesthesia, a laminectomy is performed at the main input region of the hind leg, at the spinal cord segment L4. Using this approach we can monitor *in vivo* responses of axons and microglial cells under physiological conditions, to an acute injury. Repetitive imaging is also possible over 1-2 weeks to study chronic neurodegenerative or neuroinflammatory processes. Adaptation of the surgical approach and positioning of the animal recently allowed us to image pyramidal tracts located in the ventrolateral tracts of the spinal cord. In the intact animal e. g., upon application of a local laser lesion we can observe immediate extensions of microglial branches towards an acutely applied single axonal dissection. Subsequently, over a period of several hours, microglial cells (including their somata) move towards the lesion site and accumulated there indicating early signs of microgliosis. Furthermore, activated microglial cells ensheath and phagocytose axonal structures promoting axonal degeneration. This approach enables us to study cell-cell interaction and axonal degeneration in pyramidal tracts of the spinal cord in the intact animal and may serve as tool to better understand the pathophysiology of axonal degeneration using HSP models