INTRODUCTION: Compression of the spinal cord by fracture or tumor causes extensive damage to spinal cord neurons and axons. The concept of "secondary damage" postulates that a large amount of this damage is caused by the breakdown of the blood-brain-barrier which results in the infiltration of hematogenous macrophages and the activation of intrinsic spinal microglia. These activated cells are known to liberate neurotoxic substances such as oxygen free radicals and proinflammatory cytokines. It was our aim to establish an in vitro-model of spinal cord injury that enables the observation and analysis of a) the degree of macrophage activity and b) axonal and neuronal integrity in order to study the efficacy of various neuroprotective substances.

METHODS: This objective was achieved by the establishment of organotypic hippocampal slice cultures from p10 Wistar rats, containing either fluorescent dye prelabeled microglial cells or fluorescent dye prelabeled myelinated fiber tracts. Conventional and double fluorescence immunohistochemistry and electron microscopy confirmed that the myelinated fiber tract preserved synaptic connectivity with granule cell dendrites. Both prelabeled microglial cells or spleen derived macrophages as well as the integrity of a myelinated fiber tract were visualized inside slice cultures up to 21 days in vitro. Confocal laser microscopy enabled direct observation of fluorescent dye labeled microglia or fiber tracts inside living slice cultures. Subsequently, two different lesion models were established: Neuronal damage was induced by excitotoxic injury using N-methyl-D-aspartame (NMDA) and axonal damage was caused by fiber tract compression or transsection.

RESULTS: It was shown that excitotoxic damage caused a reproducible loss of neurons in NMDA-treated slice cultures, as visible by decreased numbers of neurons visualized by Nissl or Calbindin stains and increased numbers of neurons labeled by propidium iodide. Microglial cells were rapidly activated, visible by transformation from the ramified to the amoeboid morphological phenotype. Activated, prelabeled microglial cells showed preferential migration into areas of excitotoxic neuronal injury. Transsection of a prelabeled fiber tract caused anterograde degeneration of prelabeled myelinated axons. Axonal material was incorporated by activated microglia, as shown by both double fluorescence labeling and electron microscopy. The addition of transforming growth factor (TGF)-beta, a substance with well characterized neuroprotective properties and the capacity to deactivate microglial cells and macrophages, resulted in enhanced neuronal survival and an increase in the degree of microglial ramification, indicating deactivation of these cells.

DISCUSSION: In summary, an in vitro-model of spinal cord injury providing the potential for confocal microscopic observation of fluorescent dye prelabeled microglial cells or axons was established. Two lesion models based on either excitotoxic neuronal damage or axonal injury resulted in reproducible activation of microglial cells showing morphological transformation, chemotaxis and phagocytic activity. Future investigations will be focussed on the effect of different therapeutic strategies such as treatment with immunosuppressants or neuroprotective substances in order to suppress activated microglia and macrophages. Thus, the capacity of various treatments to reduce the amount of secondary damage in spinal cord injury can be systematically analyzed.