ABSTRACT

Previous research in basic and clinical neurosciences has improved the understanding of genetic disturbances associated with human neurodegenerative disease, which has permitted the generation of an increasing number of transgenic mouse models of human neurodegenerative disease. While numerous studies have been performed on cell culture models, the detailed characterisation of cellular mechanisms in intact tissue remains an important challenge.

The automated confocal laser scanning system presented here permits structural and functional imaging of molecular / cellular mechanisms in acutely isolated CNS slice preparations. It therefore represents a valuable tool to analyse cellular pathologies in transgenic animal models of neurodegenerative disease with efficient analysis rates.

METHODS

For optical analysis, slice preparations were acutely isolated from CNS tissue and geometrically stabilized on the glass surface of 96 well microwell plates by light platinum grids. Figures illustrate data from wt and mSOD1 animal models of human motoneuron disease (ALS; Jaiswal & Keller, 2008).

TECHNOLOGICAL DEVELOPMENT

Optical design of confocal laser system:

Optimized slice handling and dye loading procedures permit microfluorometric measurements of cytosolic [Ca2+] in individual neurons of adult mSOD1 mice (140d) with clear symptoms of motoneuron degeneration (Lu2-2 AM, Hypoglossal motoneurons, rapid CCD Imaging).

CONCLUSIONS

• An automated confocal laser scanning system is presented for the analysis of acutely isolated slice preparations from CNS tissue

• The system is based on an automated confocal laser platform with excitation wavelengths of 405, 490 and 780 nm respectively

• A specialized software platform performs an automated recognition of organelles like cell nuclei and mitochondria, which can be monitored up to a distance 80 µm below the slice surface.

• The automated laser scanning system promises to serve as a valuable tool to characterize pathophysiological mechanisms in wild type and transgenic animal models of neurodegenerative disease

LITERATURE
