COST Action CA 16122: Biomaterials and advanced physical techniques for regenerative cardiology and neurology
BIONECA

Advanced imaging of cellular markers and stem cell research for theranostics of ALS

Center for Laser Microscopy
Faculty of Biology
University of Belgrade, Serbia

Prof. Pavle Andjus group

Presented by Prof. Lidija Radenovic
MC substitute for Serbia
lidijar@bio.bg.ac.rs

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Soft and hard X-ray synchrotron radiation microscopy of intact hSOD1 G93A rat astrocytes

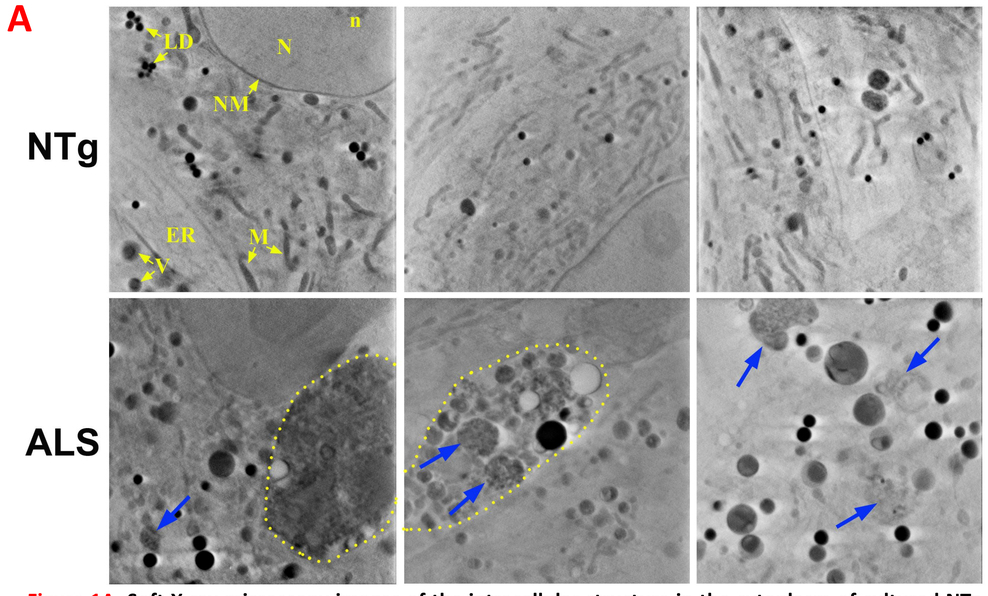
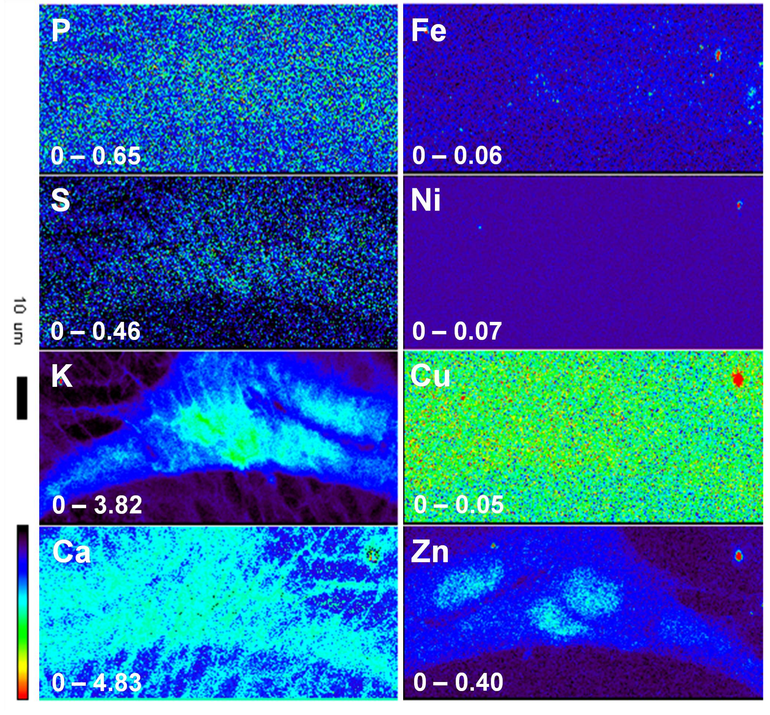


Figure 1A. Soft X-ray microscopy images of the intracellular structure in the cytoplasm of cultured NTg and ALS astrocytes. Image size 10 x 10 µm. Blue arrows indicate specific accumulations of intracellular material in ALS astrocytes which imply formation of SOD1 aggregates. Yellow dotted lines encircle complex multivesicular structures observed in the immediate vicinity of the nucleus in ALS astrocytes implying the formation of autophagosomes in these cells. N – nucleus, n – nucleolus, NM – nuclear membrane, M – mitochondria, V - vesicles, LD – lipid droplets, ER – endoplasmatic reticulum.



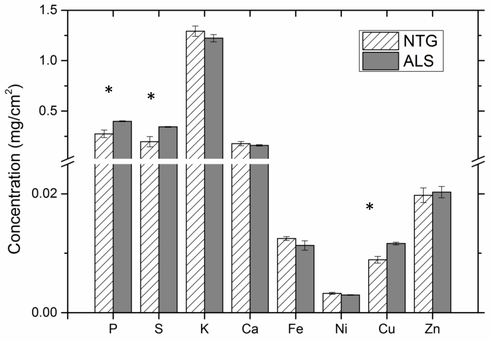


Figure 1B. Examples of elemental maps of P, S, K, Ca, Fe, Mn, Ni, Cu, and Zn of cryo-preserved ALS cells obtained by hard X-ray fluorescence imaging. Image size 84 x 40 µm, pixel size 300 nm and dwell time 200 ms. The numbers on the bottom of each map show the range of concentrations for the corresponding element in µg/cm^2. The graph presents elements concentration in NTg (stripped bars) and ALS (grey bars) cells after X-ray fluorescence imaging of P, S, K, Ca, Mn, Fe, Ni, Cu and Zn of cryo-preserved cells. n=6 cells for NTg and 10 cells for ALS. Values are given as mean ± standard error of the mean. Asterisks indicate significant differences at $p < 0.05$.

Tanja Dučić, Stefan Stamenković, Barry Lai, Pavle Andjus, Vladan Lučić, unpublished.

Differentiation of stem cells from apical papilla (SCAP) into neural lineage, application in the spinal cord rat tissue and MRI imaging

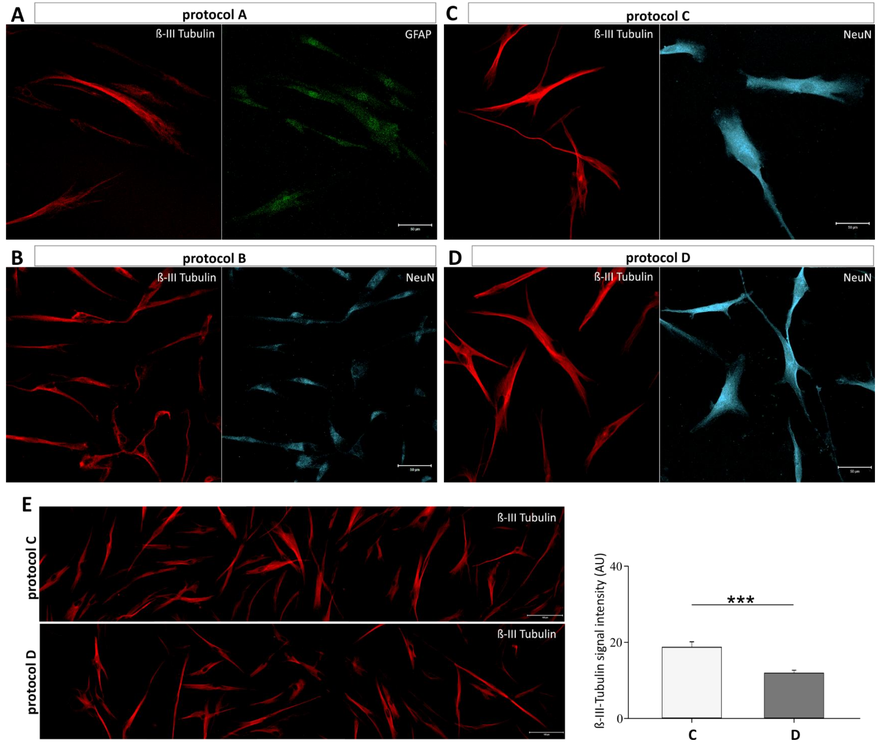


Fig 1. Investigation the role of various protocols on differentiation of SCAP into neuronal lineage cells. using graphene dispersion and carbon nanotubes.
Protocol A - neural induction medium without materials; **Protocol B** - neural induction medium and single walled carbon nanotubes (SWCNT) activated with poly-m-aminobenzene sulphonic acid (PABS), SWCNT-PABS; **Protocol C** – neural induction medium with graphen dispersion (GD); **Protocol D** - combination of SWCNT-PABS and GD. B-III-Tubulin and NeuN were used as markers of neuronal lineage., and GFAP as marker of glial cells.
Expression of B-III-tubulin using different protocols of differentiation showing different levels of immunoreactivity, depending on protocol. Protocol A shows low neuronal marker expression but presence of glial cell marker, GFAP, while the protocol B shows low expression of both neuronal markers. Protocols C and D show the highest immunoreactivity of B-III-tubulin. Medium with graphene dispersion, protocol C, shows significantly higher level of neuronal marker compared to protocol D, implying graphene dispersion has the most significant role in SCAP differentiation into neuronal cells (Simonovic et al. J Biomed Mater Res A. 2018)

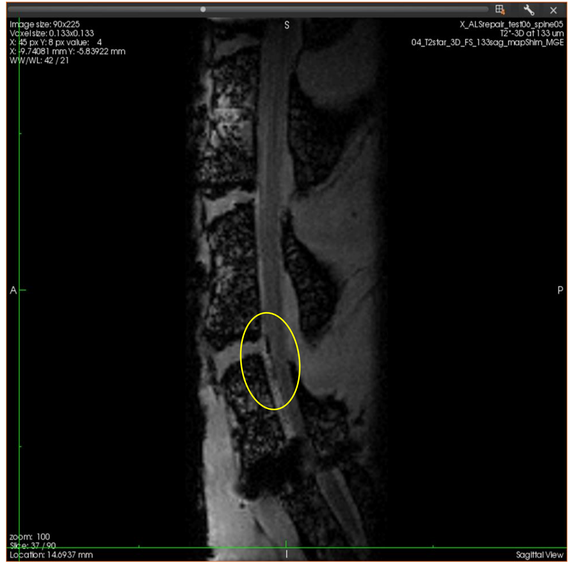


Fig 2. Aplication of labeled SCAP into rat spinal cord. MRI (Bruker 7T) - followup of lumbal injection of stem cells labeled with magnetic nanoparticles (Fe2O3) in the rat spinal cord (collaboration with Dinko Mitrečić, Zagreb)

Calcium imaging in brain cell cultures upon acute ALS IgG application

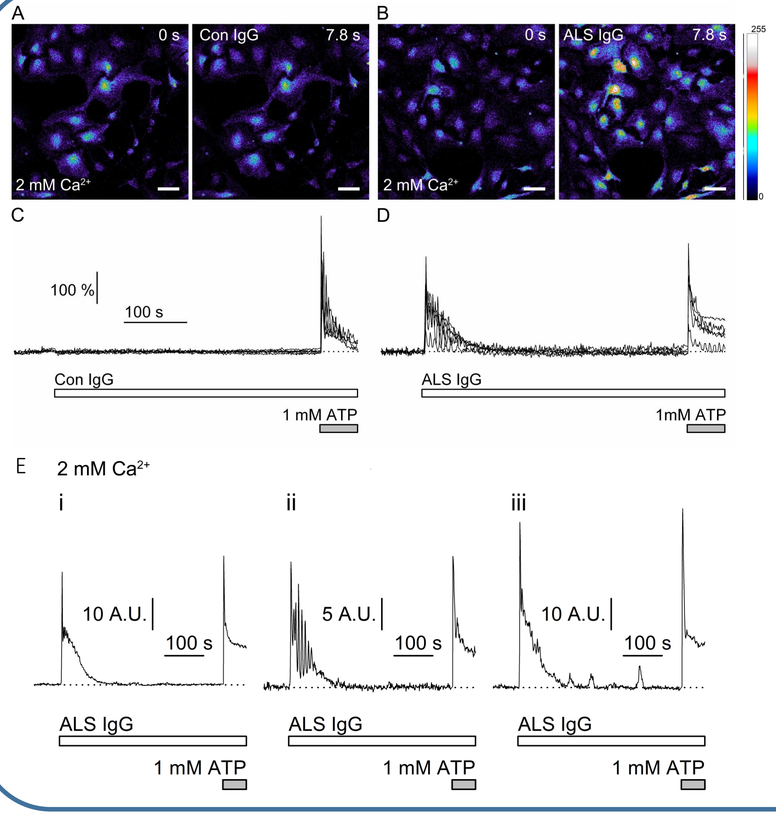
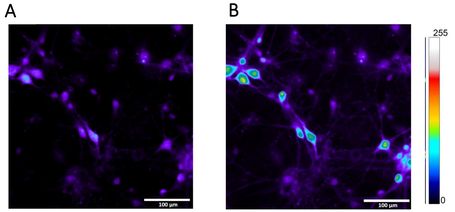


Figure 1. IgG isolated from patients with ALS increase cytosolic Ca activity in cultured rat astrocytes.

A and B Confocal images of live rat astrocytes preloaded with fluorescent Ca2+ indicator Fluo-3-AM. The left and right images display cells before (0 s) and after (7.8 s) the application of either control IgG isolated from a patient with Parkinson disease (Con IgG, **A**) or IgG isolated from a patient with ALS (ALS IgG, **B**). ALS, but not control IgG, triggered prominent increases in intracellular Ca activity as indicated by the color-coded intensity scale (right, 0–256 intensity levels). **C and D** Superimposed time-dependent fluorescence intensity plots obtained in 5 cells treated with 0.1 mg/ml control IgG and thereafter with 1 mM ATP **(C)**, and with 0.1 mg/ml ALS IgG and thereafter with 1 mM ATP **(D)** at the time periods indicated. The application of ALS IgG evoked transient and complex increases in intracellular Ca activity. (**E**) Examples of characteristic types of Ca transients observed in astrocytes treated with ALS IgG: (i) single, (ii) bursting, and (iii) repetitive. **C and E** The thin dotted lines indicate the zero fluorescence level (F0).

(Milošević et al. 2013, *Cell Calcium* 54:17)



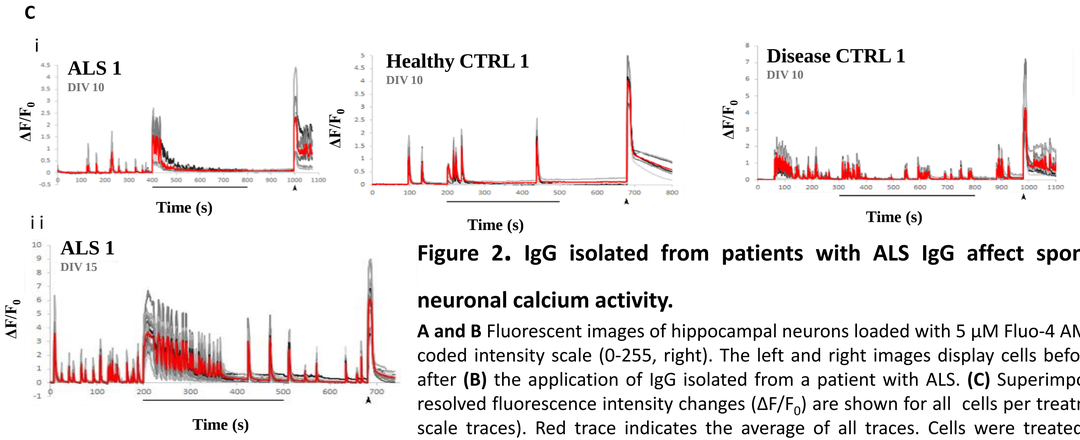


Figure 2. IgG isolated from patients with ALS IgG affect spontaneous neuronal calcium activity.

A and B Fluorescent images of hippocampal neurons loaded with 5 µM Fluo-4 AM, in color-coded intensity scale (0-255, right). The left and right images display cells before **(A)** and after **(B)** the application of IgG isolated from a patient with ALS. **(C)** Superimposed time-resolved fluorescence intensity changes ($\Delta F/F_0$) are shown for all cells per treatment (gray scale traces). Red trace indicates the average of all traces. Cells were treated with ALS patients and control IgGs (D CTRL –patients with neurological disease; H CTRL – helathy donors) as indicated with black timeline bar and subsequently depolarized for 5s with 50 mM K^+ in extracellular solution as indicated with black arrowhead. **i)** Comparison of ALS and CTRL IgG effect in developing neurons. **ii)** More prominent effect of ALS IgG in neurons with higher spontaneous activity. Dunja Bijelić, Milena Milošević, Irena Živković, Zorica Stević, Pavle Andjus, unpublished.