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Central Nervous System Oxidative Stress interplay with inflammation in a rat model of Type C Hepatic Encephalopathy – brothers in arms?

K. Pierzchala[1 2 3], D. Simicic[1 2], A. Sienkiewicz[4 5], D. Sessa[6], S. Mitrea[1 2], O. Braissan[7], V. McLin[6], C. Cudalbu[1 2]

[1]CIBM Center for Biomedical Imaging, Lausanne,[2]Animal Imaging and Technology, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, [3]Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, [4]Laboratory for Quantum Magnetism, Institute of Physics, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, [5]ADSresonances Sàrl, Préverenges, [6]Swiss Center for Liver Disease in Children, University Hospitals Geneva,[7]Neurometabolic Unit, Service of Clinical Chemistry, University Hospital of Lausanne, VD

BACKGROUND

- Although oxidative-stress (OS) and neuroinflammation play a role in type C hepatic encephalopathy (C HE), their involvement and synergistic action is not well understood[1].
- Under normal conditions the physiological levels of intracellular reactive oxygen species (ROS) are controlled by the counteracting antioxidant response to maintain redox homeostasis[1].
  - ROS are acknowledged for defense mechanisms - signaling messengers in immune system,
  - ROS are critical for hippocampal long-term potentiation (LTP) / long term dementia (LTD),
  - Excess of ROS exceeds the capacity of natural cellular antioxidant mechanisms, resulting in the pathological modification of proteins, lipids, and nucleic acids.

AIMS

Multidisciplinary approach implementation

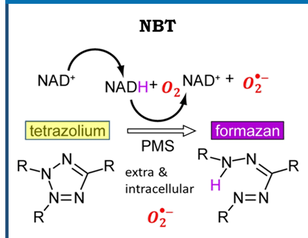
- **Longitudinal tracking of CNS OS** in a rat model of type C HE using *in-vivo*-^{1}H-MRS and *ex-vivo*-EPR spin-probing combined with UV-Vis spectroscopy and histological assessments (IHC).

Analyzing **synergistic participation of CNS OS and inflammation** in the progression of type C HE.

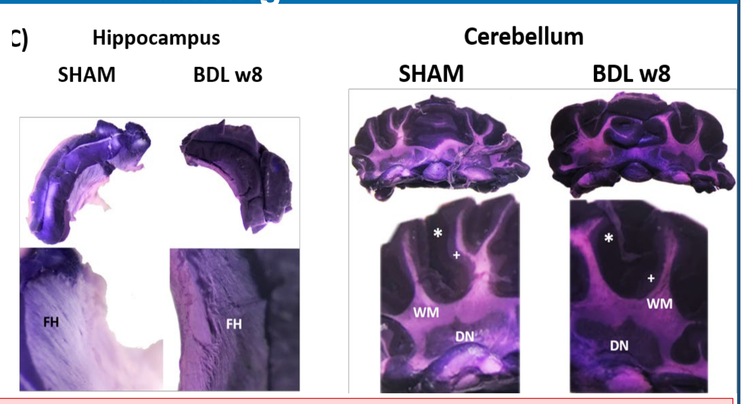
METHODS

- ***In-vivo*-^{1}H-MRS indirect OS detection – ascorbate and glutathione concentrations**: adult rats were scanned before and after BDL surgery (n=18) at 9.4T-MR (Varian/Magnex-Scientific) using SPECIAL-sequence[2] (TE=2.8ms).
- ***Ex-vivo* ESR direct and quantitative detection of OS ($O_2^{\cdot-}$) with CMH spin-probe:** X-band ESR, Model ESP300E with TE_{102} cavity (Bruker-BioSpin, Germany).
- **Histology:** BDL rats at 4 and 8-weeks post BDL (n=3 per group) and SHAM rats (n=3)
  - **NBT:** histo-enzymatic technique for ROS visualization
  - **SOD1/SOD2:** to differentiate between Cu/ZnSOD (SOD1) and MnSOD (SOD2) activity,
  - **GPX1:** glutathione peroxidase (intracellular antioxidant enzyme) activity,
  - **Oxo-8-dG**: to determine the presence of DNA/RNA oxidation,
  - **IL-6:** to determine the presence of neuroinflammation.
- **UV-Vis** spectroscopy: for quantitative evaluation of brain inflammation.

NBT – qualitative $O_2^{\cdot-}$ detection by histochemical staining

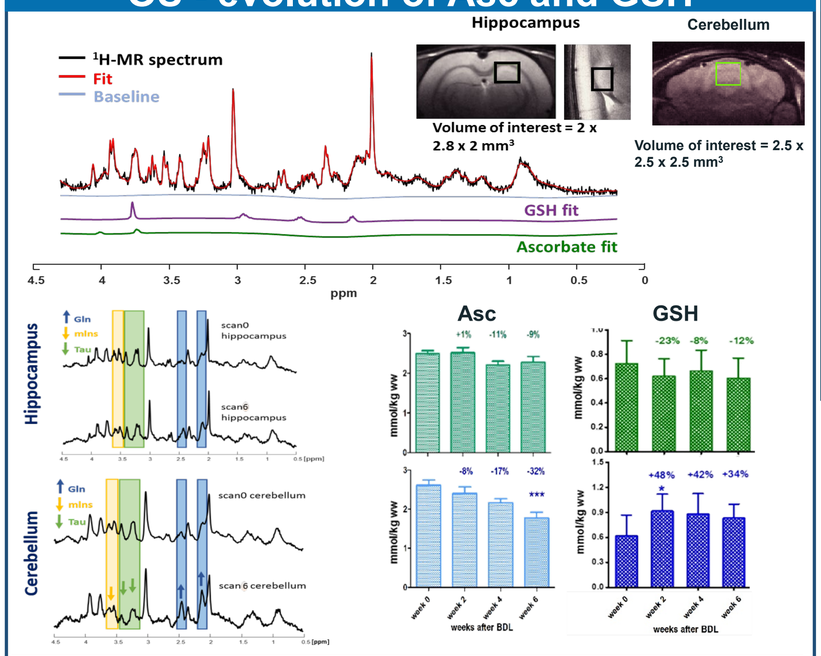


NBT specifically reacts with $O_2^{\cdot-}$ and forms a purple / blue formazan precipitate.



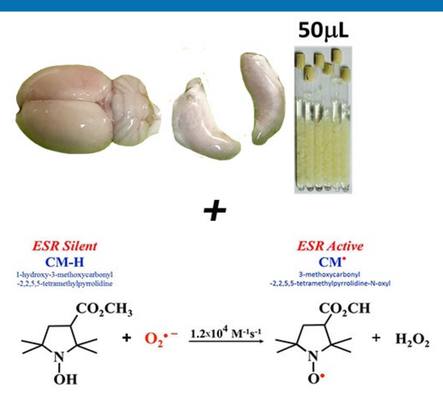
❖ **Increased $O_2^{\cdot-}$ production in cerebellar granular and molecular cell layer, DN and WM tracts, FH structures and cerebellum[1].**

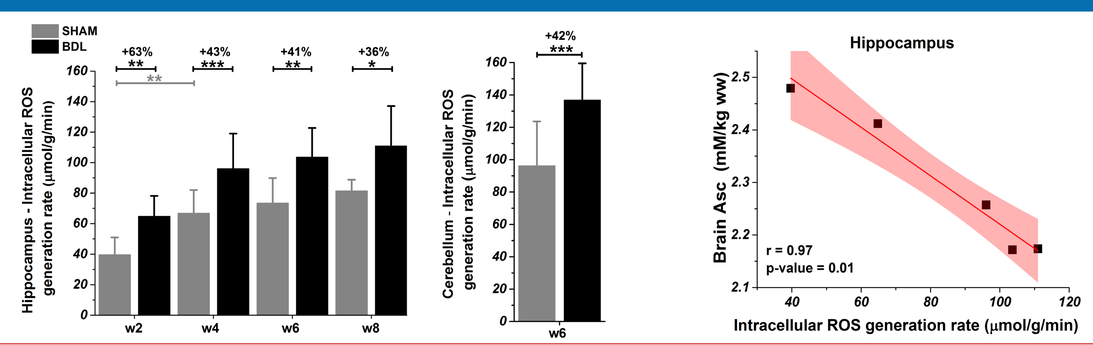
^{1}H-MRS: in-vivo indirect detection of OS - evolution of Asc and GSH



❖ **Asc and GSH alterations in BDL rats – indirect finding of OS[3].**

ESR: *ex-vivo* direct detection of the presence of OS - qualitative $O_2^{\cdot-}$

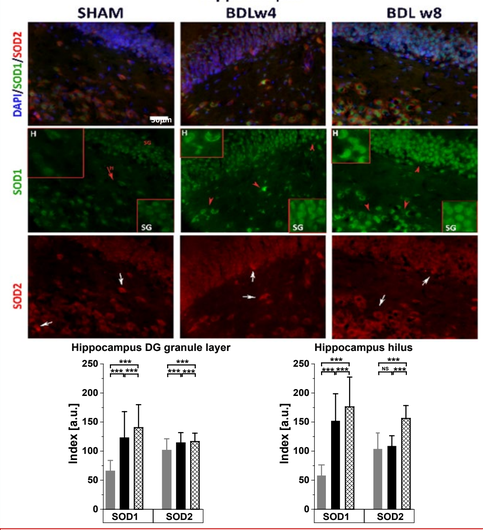


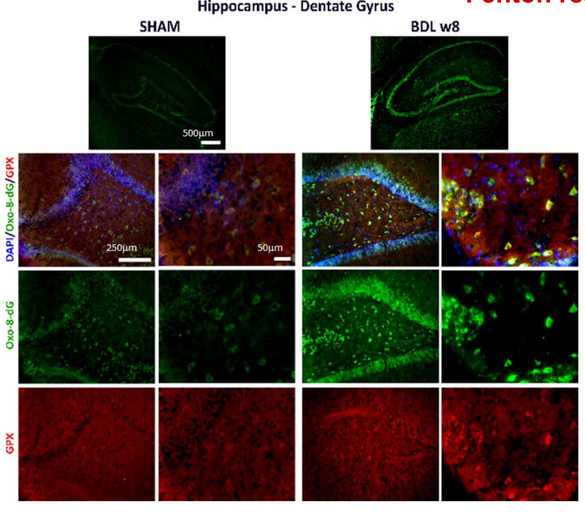


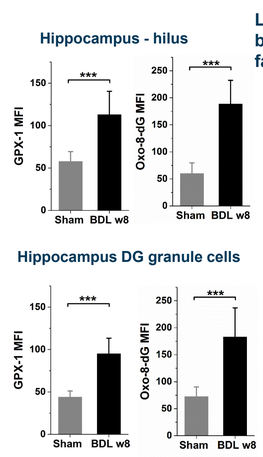
❖ **Significantly $O_2^{\cdot-}$ increase in the hippocampus, cerebellum of BDL rats [1].**

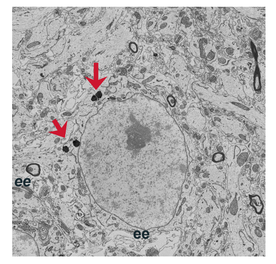
IHC OS detection – antioxidants and DNA/RNA oxidation

$O_2^{\cdot-}$ → SOD1/SOD2↑ → H_2O_2 + GPX↑ → H_2O → $HO^{\cdot}$ → Oxo-8-dG↑ (Fenton reaction: Fe^{2+} / Fe^{3+})



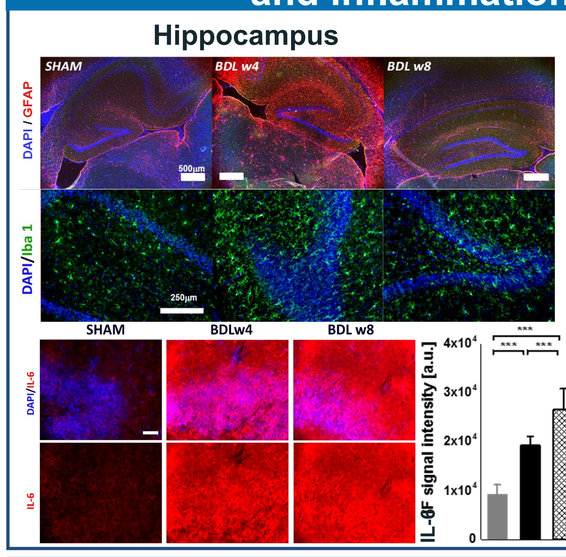






❖ **SODs, GPX-1 , and Oxo-8-dG ↑ in several brain regions of BDL rats[1].**
❖ **SOD1 relocation into the nucleus - genomic DNA protection in the presence intracellular $O_2^{\cdot-}$ elevated levels [1].**
❖ **Lipofuscin aggregates encourage the Fenton reaction and $HO^{\cdot}$ production, one of the most potent ROS, which can lead to lipids, proteins, and RNA/DNA oxidation [4].**

Synergistic participation of CNS OS and inflammation



❖ **Astrocytes and microglial activation → ↑ of cytokines and OS[1].**
❖ **Brain IL-6 ↑ → impaired cognition, learning loss[1].**

CONCLUSION

- **For the first time, longitudinal presence of CNS OS together with inflammation in a rat model of type C HE.**
- **OS increase is not due the declined antioxidants activity but rather a response to ROS increase.**
- **OS is one of the major pathways driving neurodegeneration. Therefore, CNS OS, together with inflammation, may strongly contribute to HE pathogenesis.**

References

[1]Pierzchala K. , et al., Free Radic Biol Med, 2022; [2]Mlynárik et al. ,Magn Reson Med 2006; [3]Braissant, O., et al., J Hepatol, 2019; [4]Simicic D, Cudalbu C, Pierzchala K., Anal Biochem. 2022.

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