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# EJP-CONCERT

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# D9.41 – Mouse stress, communication studies

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## Abstract

The lens of the eye is known to be more radiosensitive than previously thought but, despite a substantial reduction in occupational dose limits based on recent epidemiological information and reanalyses, the mechanisms of low dose radiation cataract induction are still unclear. This is an important current public health issue, for instance for medical radiation workers, many of whom will need to amend their working practices despite a clear understanding of the underlying process and ultimate effects of chronic, low dose, ionising radiation exposure.

The LDLensRad project aims to bring together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.41, 1.0.8 of the project, describes the motivation and plans for stress and communications studies using the mouse models investigated under LDLensRad. Once collected, the data will be submitted for publication in peer-reviewed scientific journals.

## Progress summary

**As work on this has not yet started, the report is in the form of a *short overview of the plans and the methods that will be used.***

Oxidative stress is a well-known factor following radiation exposure, and data from the literature suggest that ROS and other intracellular communication factors may play a role in cataractogenesis (Berthoud and Eric, 2009; Brennan et al., 2012). To investigate these factors the eyes from whole body irradiated mice (0, 0.5, 1 and 2 Gy) were collected at different time points after exposure (4 and 24 hrs, 4, 12 and 18 mth). After fixation in PFA the eyes were embedded in paraffin and stored at 4°C. All samples up to 12 months after exposure were collected and embedded. The remaining mice from the 18 mth long-term groups were sacrificed in June 2019 and eyes were collected.

The paraffin-embedded eyes will be sectioned and used for immunohistochemistry. At early time points (4 and 24 hrs) ROS-mediated DNA damage will be investigated by immunofluorescence staining with anti-8OHG and DNA double strand breaks will be measured with the γ-H2AX-assay. Apoptosis will be determined with the TUNEL assay.

At later time points (4, 12 and 18 mth) we have planned immunostainings with different antibodies (against TGFβ, connexin 43, connexin 46 and Aquaporin 0), in order to investigate different components of the signal transduction pathway of the lens. As part of the ROS protection system, we will also stain for SOD1, SOD2, Catalase, GPX1 and αA-Crystallin. Additionally, distribution of the ion pump $Na^+/K^+$-ATPase will be investigated.

Qualitative changes of these antibodies will be quantitatively counterchecked with western blots.

Berthoud VM and Eric CB, Oxidative stress, lens gap junctions, and cataracts. Antioxid Redox Signal. 2009 Feb;11(2):339-53. doi: 10.1089/ars.2008.2119.

Brennan LA1, McGreal RS, Kantorow M, Oxidative stress defense and repair systems of the ocular lens. Front Biosci (Elite Ed). 2012 Jan 1;4:141-55.