



This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.



EJP-CONCERT

European Joint Programme for the Integration of Radiation Protection Research

H2020 – 662287

D 9.49 – Behavioural analyses

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Reviewer(s): CONCERT coordination team

Work package / Task	WP 9	Task 9.2	SST 9.2.3.2
Deliverable nature:	(Draft) manuscript		
Dissemination level: (Confidentiality)	Public		
Contractual delivery date:	Month 30 (CONCERT M52)		
Actual delivery date:	Month 30 (CONCERT M52)		
Version:	1		
Total number of pages:	9		
Keywords:	Ionising Radiation; Lens; Cataract; Mouse Models; Behavioural Phenotypes		
Approved by the coordinator:	M52		
Submitted to EC by the coordinator:	M52		

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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 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 9.49 of the project, describes a further consequence of low dose ionising radiation, short- and long-term effects on locomotor, sensorimotor and cognitive behaviour. The impact of lens alterations on behavioural performance is assessed wherever possible.

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Progress summary

Introduction

In terms of wider radiation effects, for decades, the brain was considered an organ with very low mitotic activity, until it was discovered that in the adult mammalian brain, including humans, in the subventricular zone and in the subgranular zone of the dentate gyrus of the hippocampus neurogenesis is a lifelong process. We now know that the rate of this adult neurogenesis influences emotional development, learning and memory, supports repair processes and brain plasticity and that it is very sensitive to ionising radiation (Mizumatsu *et al.* 2003). It was shown that in humans and animals, brain irradiation led to apoptosis, neuroinflammation, loss of oligodendrocyte precursor cells and of myelin sheaths, and to damage of neuronal stem cells with long-term consequences on adult neurogenesis and on emotion and cognition (Marazziti *et al.*, 2012). Thus, via effects on the pool of neural stem cells and consequently their neural and glial daughter cells in the brain, ionising radiation can affect mood, learning, repair, plasticity, memory and olfaction, which in turn are biomarkers for early stages of neurodegenerative diseases.

Such disease-relevant radiation effects on behavior have reliably been demonstrated in the high dose range used in radiotherapy, but not in the low to moderate dose range. To test the hypothesis that via effects on adult neurogenesis and neuroinflammation also lower doses of radiation may cause long-term behavioural consequences, behavioural testing was performed over the course of a life-time in the INSTRA project. Significant reductions in mouse spontaneous locomotor behaviour at 12 months following 0.5 Gy irradiation, a dose effect over time, and late genotype-dose interactions were found in this project. Considering whether lens changes might be used as biomarkers of global radiation sensitivity and might also contribute to behavioural changes, LDLensRad built heavily on the INSTRA project and combined behavioural and lens assessments.

Methods

Mice of both sexes were irradiated at HMGU at the age of 10 weeks (0, 0.5, 1 and 2 Gy, dose rate 0.3 Gy/min). Irradiated and sham-irradiated control mice on a hybrid (C57BL/6 X C3HeB/FeJ) F1 genetic background as well as heterozygous *Ercc2*^{S737P+/-} mutants on the same hybrid genetic background. The *Ercc2* gene has DNA/RNA helicase activity and is involved in transcription and DNA repair. The heterozygous mutants are expected to be impaired in DNA repair, because lymphocytes of this mouse line showed a higher number of γH2AX foci 6 hours after *in vitro* exposure to 1 Gy compared to those of control animals (Kunze *et al.*, 2015). Thus, by including the *Ercc2*^{S737P+/-} mutants we want to test the hypothesis that a deficiency in DNA repair increases the risk for radiation-induced disease development.

Behavioural assessments were performed at 4, 12 and 18 months after irradiation. Locomotor and emotional behavior were assessed in the Open Field Test, learning and memory by spontaneous alternation in the Y-Maze and a Social Discrimination Test, and sensorimotor function by measurement of the Acoustic Startle Reflex and its Prepulse Inhibition. After finishing all tests of the 18 months after irradiation time point the animals were sacrificed and brains of a subset of mice were harvested and archived.

Results

Statistical analysis of behavioural data of all animals that were still alive and could be tested at the 18 months p.i. time point revealed a clear radiation dose effect on spontaneous locomotor activity, exploration and anxiety as shown in Fig. 1. There was also a significant interaction between radiation dose and time with respect to locomotor activity and anxiety. The strongest dose-dependent effects were seen at the first behavioural testing time point 4 months p.i. At this time point 0.5 Gy increased spontaneous locomotor activity (as measured by distance) and decreased anxiety (as measured by increased centre time) in the Open Field (Fig. 1), increased social interest as measured in the Social Discrimination Test (Fig. 2), and increased the number of entries made in the Y-Maze (Fig. 3), which corroborates the Open Field results indicating increased locomotor activity and decreased anxiety. At the same time point p.i. 2 Gy decreased locomotor activity (distance) and exploration (rearing) and increased anxiety (decreased centre time) (Fig. 1), which is in line with the decreasing effect of this dose on the number of entries made in the Y-Maze (Fig. 3).

Interestingly, both 1 Gy and 2 Gy reduced social interest 4 months p.i. (Fig. 2, top). At 12 months p.i. animals irradiated with 0.5 Gy still showed increased, and those irradiated with 1 Gy still decreased social interest (Fig. 2, bottom). Since this behaviour mainly relies on olfaction, these results most likely reflect long-term radiation effects on olfactory function, presumably mediated via effects on adult neurogenesis in the subventricular zone that is in mice a life-long source of new neurons travelling via the rostral migratory stream to the bulbus olfactorius.

Of note, 4 months p.i. 1 Gy significantly reduced working memory performance as measured by spontaneous alternations (SPA, Fig. 3 bottom) in the Y-Maze. However, this effect was not seen with the higher 2 Gy dose and there was also no radiation dose effect on working memory detectable 12 months p.i. (data not shown).

Further analysis of data of the 18 months p.i. time point as well as an integrative analysis approach taking into account genotype, sex and potential interactions is still ongoing.

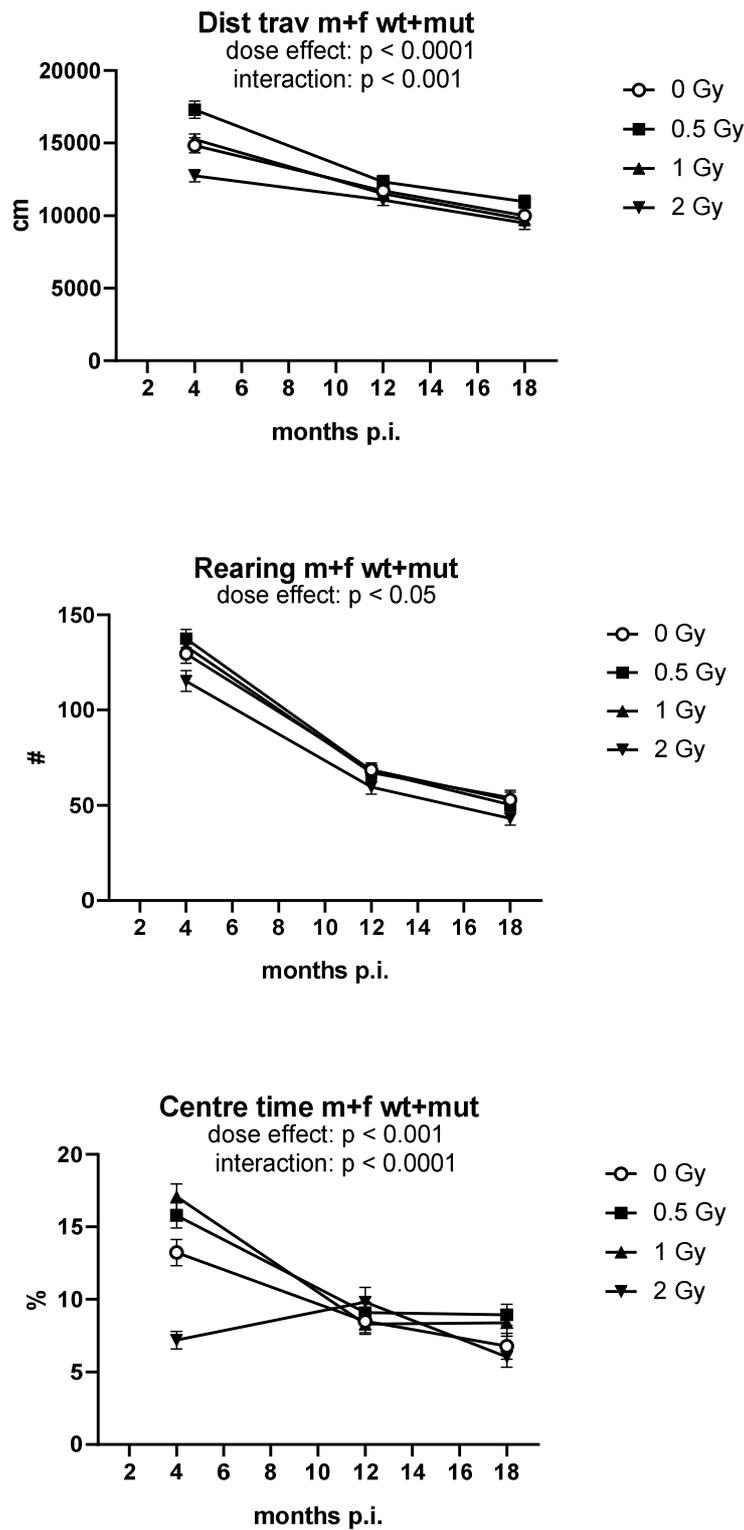


Fig. 1: Dose-dependent radiation effects on spontaneous locomotor activity (Distance), exploratory activity (Rearing) and anxiety (Centre time).

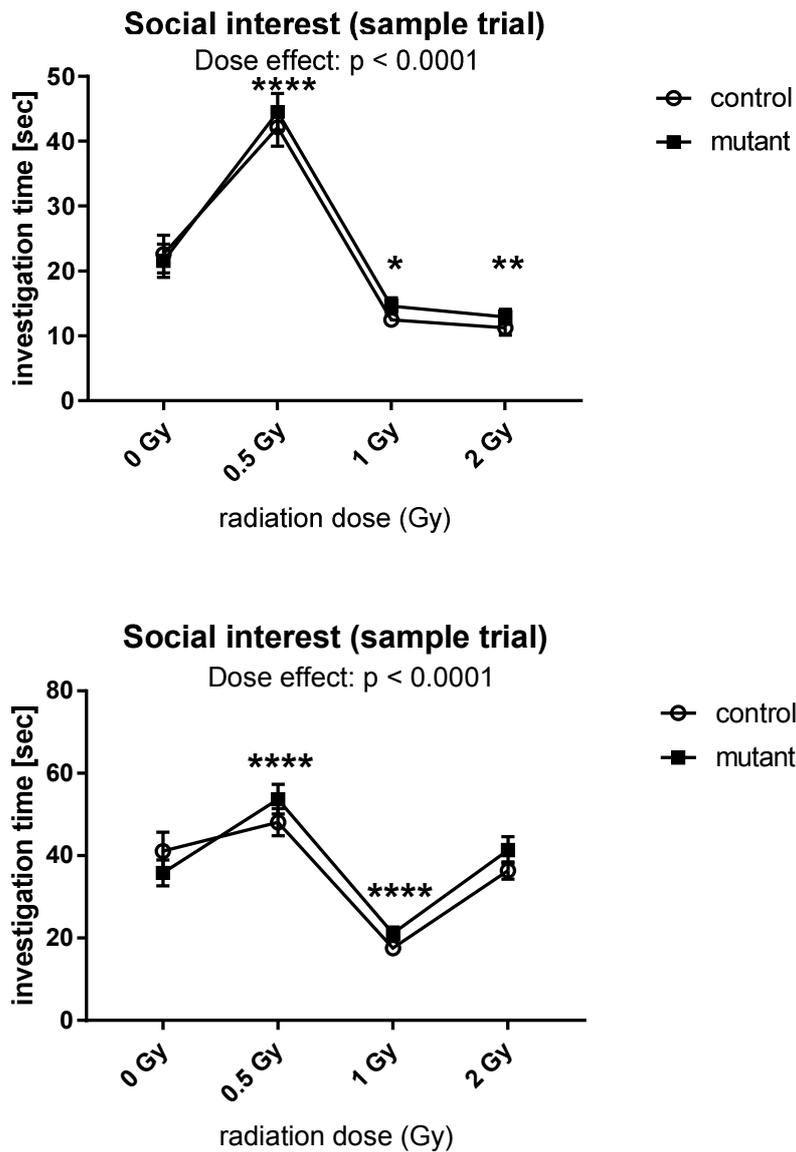


Fig. 2: Dose-dependent radiation effects on social interest as measured during the Social Discrimination Test 4 months p.i. (top) and 12 months p.i. (bottom). * indicates significant difference vs. 0 Gy in post-hoc comparisons.

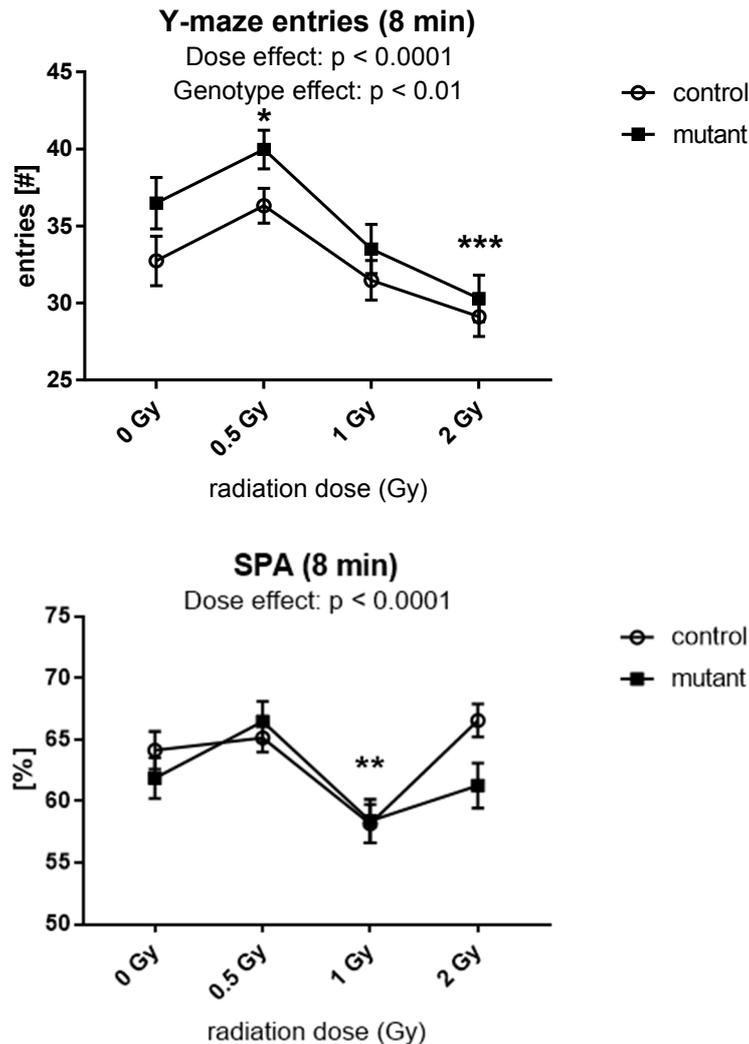


Fig. 3: Dose-dependent radiation effects 4 months p.i. on locomotor activity as measured by the number of entries during the Y-Maze Test (top) and on working memory as measured by spontaneous alternations (SPA) during the Y-Maze test (bottom). * indicates significant difference vs. 0 Gy in post-hoc comparisons.

Conclusions

While data analysis is still ongoing, the results acquired so far clearly demonstrate dose-dependent radiation effects on locomotion, anxiety, social behaviour and cognition in mice, with the lowest dose of 0.5 Gy used in this study having opposite effects to the highest dose of 2 Gy. These dose-dependent effects are largest at the first behavioural testing time point 4 months p.i., and decrease within the course of the natural age-related decline in activity and behavioural performance. The observed behavioural alterations 4 months p.i. are most likely independent of radiation effects on the eye lens, since no effects on the eye lens were detectable at this time point. They are more likely mediated via dose-dependent radiation effects on the neural stem cell pool in the brain, presumably resulting in different time courses of recovery and long-term effects on brain cell populations.

References

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