CEREBRAD

Cognitive and Cerebrovascular Effects Induced by Low Dose Ionising Radiation

GA: 295552

www.cerebrad-fp7.eu

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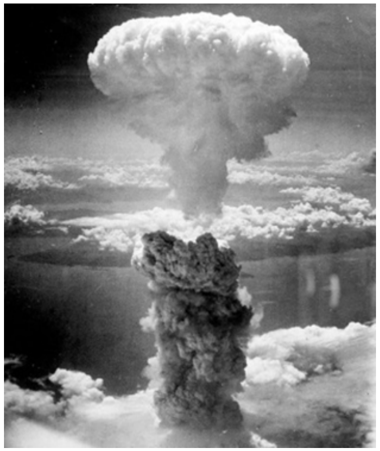
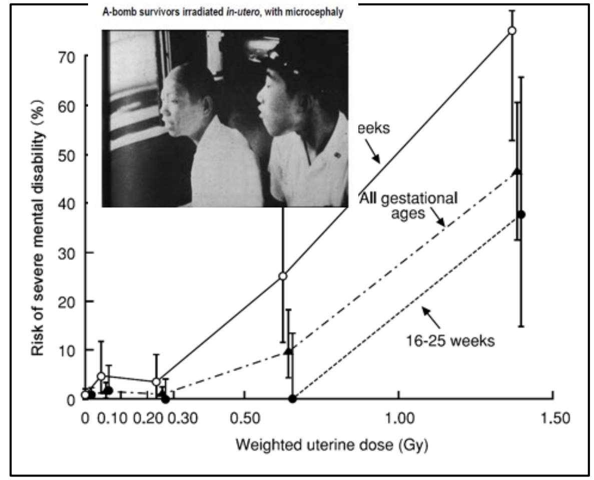
SCK•CEN

(Belgium)

Background





Verreet, Verslegers et al., Neural Plasticity, 2016

- Mental retardation
- Microcephaly
- Low IQ values
- …

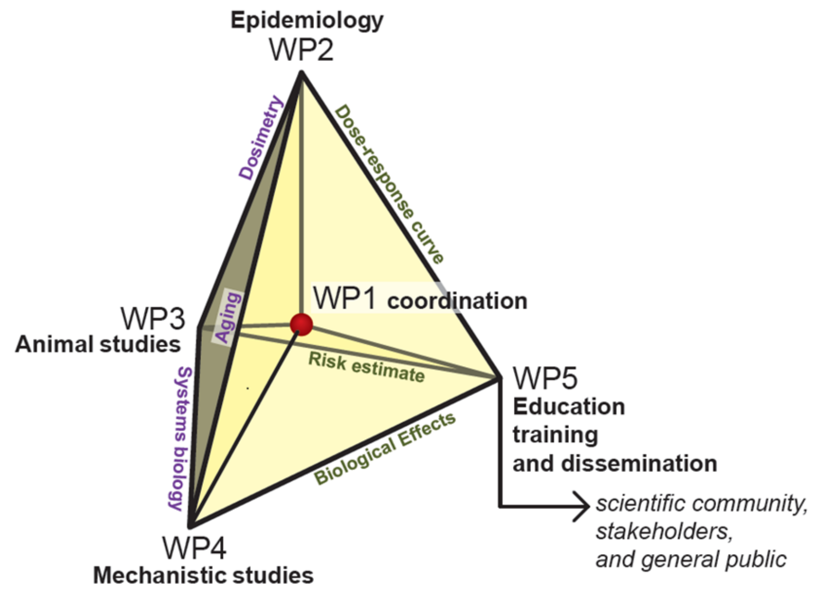
- Mental retardation
- Lower intellectual performances
- Stroke

- More sensitive than adults
- Higher metabolic activity
- Longer life expectancy

CEREBRAD objectives

- To increase the statistical power of epidemiological data about cognitive and cerebrovascular diseases following low-dose exposures.
- To employ appropriate dosimetry calculations for the human and animal studies that will allow the correct evaluation of the doses to the brain structures.
- To implement animal studies at low doses to provide experimental evidence on the shape of the dose-response curve for cognitive and cerebrovascular effects.
- To identify the molecular pathways and regulatory networks underlying the effects of low-dose irradiation, using an integrated systems biology approach, to combine data from different levels.

Partners: 11 (10 EU + Ukraine)
Total budget: M€ 4,72
EC contribution: M€ 3,00
Coordination: Dr. M. A. Benotmane, SCK•CEN (Belgium)
EC officer: André Jouve
Grant agreement n°: 295552



Acronym	Organisation name	Country
SCK-CEN	Belgian Nuclear Research Center	Belgium
EMC	Erasmus University Medical Center Rotterdam	The Netherlands
ENEA	Italian National Agency for New Technologies, Energy and the Environment	Italy
HMGU	Helmoltz Center for Environmental Health	Germany
NRIRR	Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene	Hungary
URV	University Rovira i Virgili	Spain
UU	Uppsala University	Sweden
RCRM	Research Centre for Radiation Medicine of the National Academy of Medical Sciences of Ukraine	Ukraine
AUTH	Aristotle University of Thessaloniki	Greece
UBHAM	University of Birmingham	UK
IGR	Institute Gustave-Roussy	France

CEREBRAD

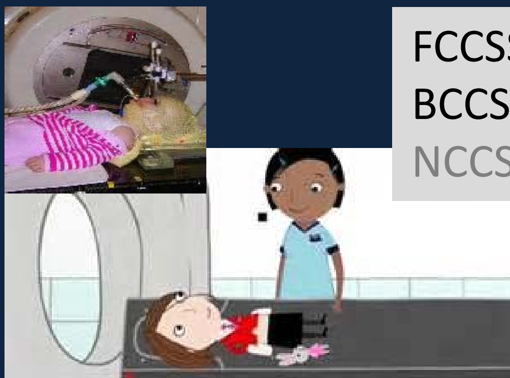
WP2: Human data of cerebrovascular and cognitive late efects

- To validate and localize patients who were treated with radiotherapy for childhood cancer and suffered from cerebrovascular diseases later in life.

- To assess cognitive dysfunctions in individuals exposed during and after the Chernobyl accident as well as subject from haemangioma cohort exposed during childhood < 1 year.

Human data of cerebrovascular and cognitive late efects

Cerebrovascular



FCCSS
BCCSS
NCCSS

233 cases and 233 matched controls

Cancer + RT
At age < 7 years

Stroke in average at
34 years old

Average 27 y

Congnitive

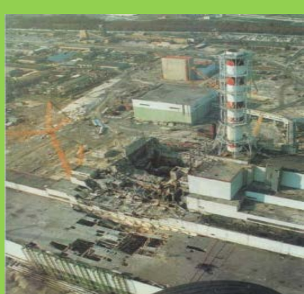
ANGIO cohort

115 cases were subjected to neurocognitive tests

treated with radiotherapy for skin hemangioma at IGR before the age of one year

167 individuals identified whose radiation received to the brain was less than 1 Gy

40-65 y



Chernobyl

210 in utero subject + 326 cleanup workers

In utero exposed & cleanup workers

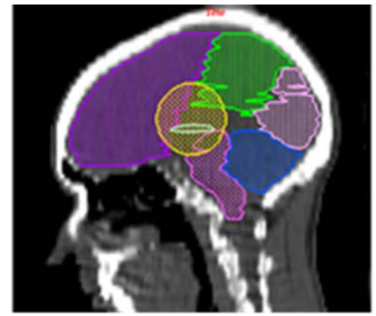
Neurocognitive test + TLS

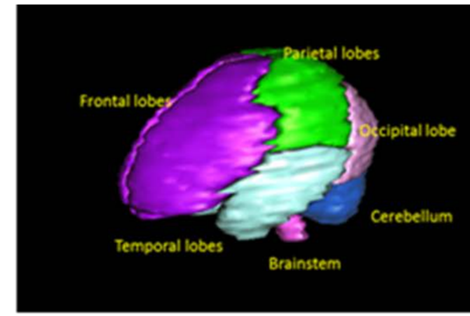
25-27 y

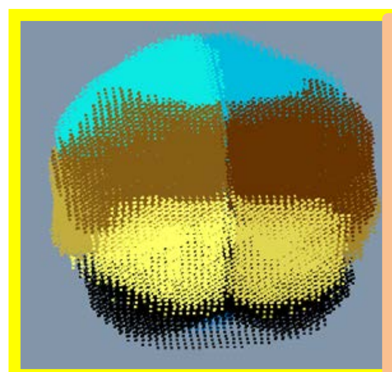
Dosimetry: brain model in the phantom

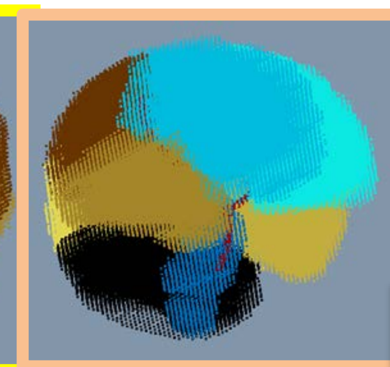
twenty-two structures segmented in the brain (about 60,000 voxels)

ANTERIOR COMMUNICATING ARTERY
INTERNAL CAROTID ARTERY R
INTERNAL CAROTID ARTERY L
CEREBELLUM
BRAINSTEM
FRONTAL LOBE R
FRONTAL LOBE L
PARIETAL LOBE R
PARIETAL LOBE L
TEMPORAL LOBE R
TEMPORAL LOBE L
OCCIPITAL LOBE R
OCCIPITAL LOBE L
BASILAR ARTERY
ANTERIOR CEREBRAL ARTERY R
ANTERIOR CEREBRAL ARTERY L
MIDDLE CEREBRAL ARTERY R
MIDDLE CEREBRAL ARTERY L
POSTERIOR CEREBRAL ARTERY R
POSTERIOR CEREBRAL ARTERY L
POSTERIOR COMUNICATING ARTERY R
POSTERIOR COMUNICATING ARTERY L





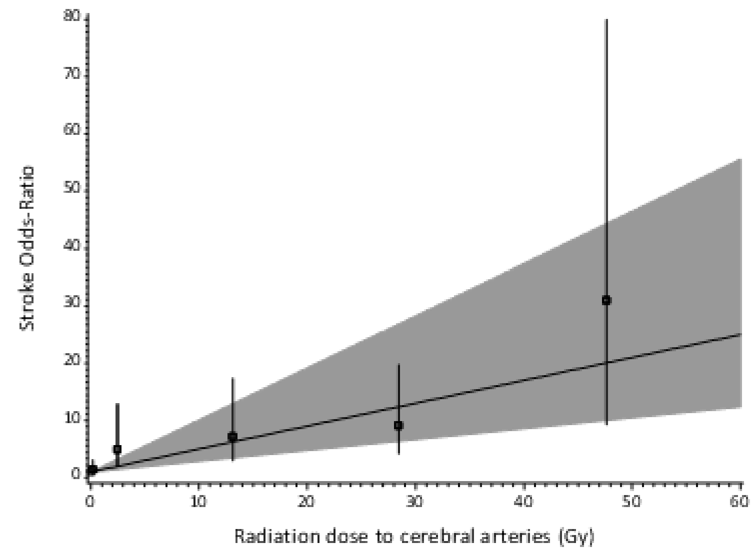


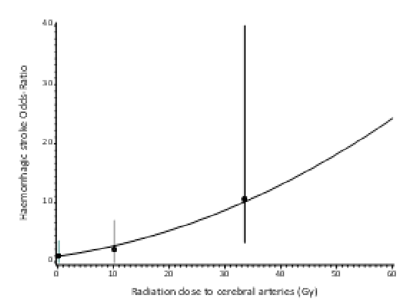


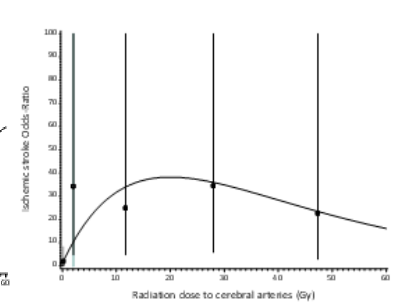
Circle of Willis

Cerebrovascular risk

- An average radiation dose to the cerebral arteries lower than 1 Gy (mean= 0.2 Gy) was associated to an OR=1.34 (95%CI:0.65 to 2.96).
- the only significant dose-response modifier was the delay between radiation therapy and the occurrence of cerebrovascular disease. The EOR/Gy was:
  - 0.25 (95%CI: 0.05% to 1.16) 5 to 14 years after radiation therapy,
  - 0.38 (95%CI: 0.09 to 2.59) 15 to 24 years
  - 0.93 (95%CI: 0.31 to 3.1) 25 years or more after.
- In a linear model, the Excess of Odds Risk at 1Gy being estimated to EOR/Gy = 0.49 (95%CI: 0.22 to 1.17).





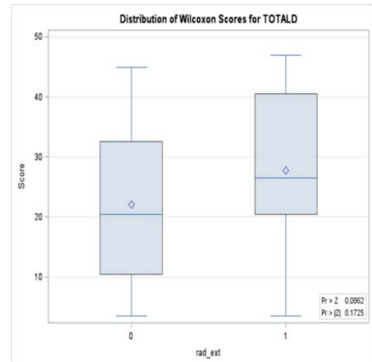


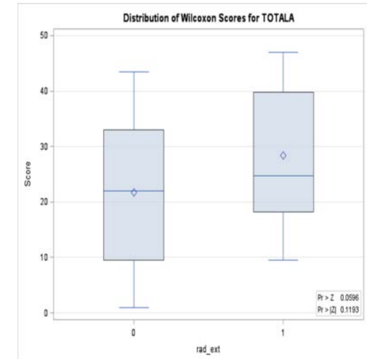
Cognitive effects (ANGIO cohort)

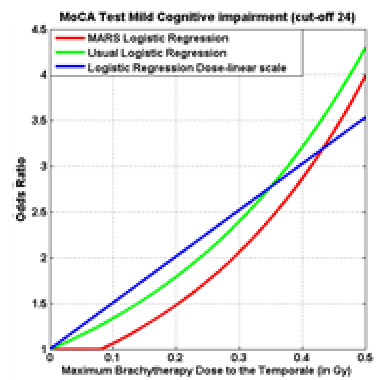
- For the HAD-D test there was a trend for increasing scores with increasing dose to the thyroid and with the maximum brachytherapy dose to the Hemispheres from thresholds equal to 0.12 Gy and 0.054 respectively.

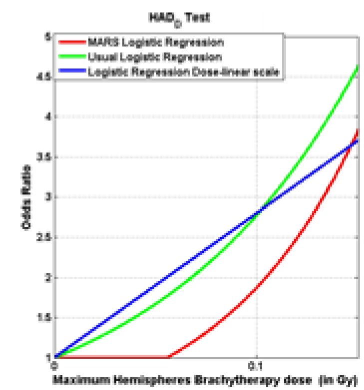
- Approximately the same threshold (0.059 Gy) of the radiation dose to the left hemisphere lob is obtained to show a significant increase of the FactCog Perceive cognitive impairments scores.

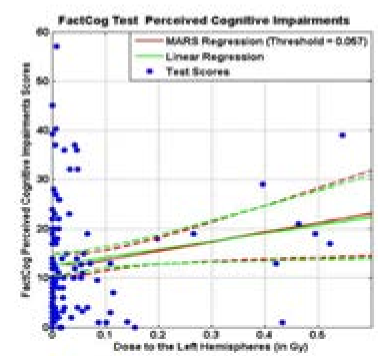
- RALVT delay recall scores according the years of schooling (threshold = 3 years). The maximum brachytherapy dose to the temporal lobes was also significantly associated to this test scores above 0.054 Gy.

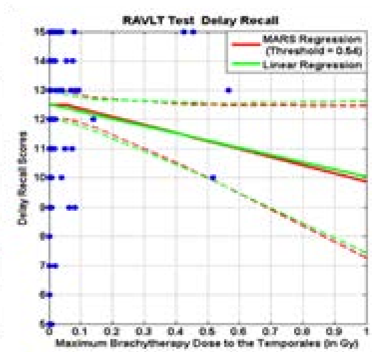




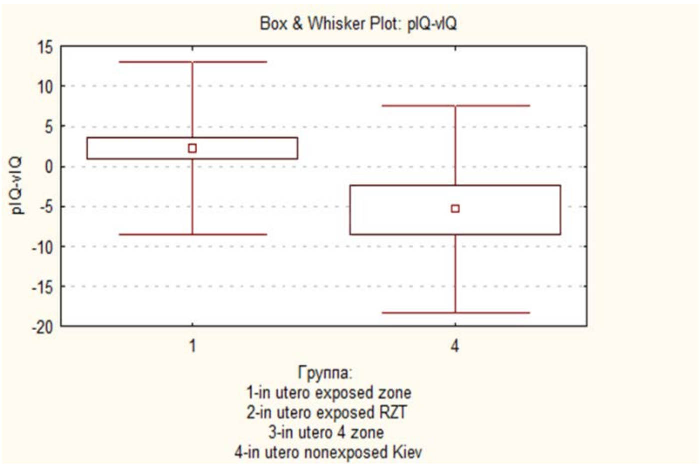


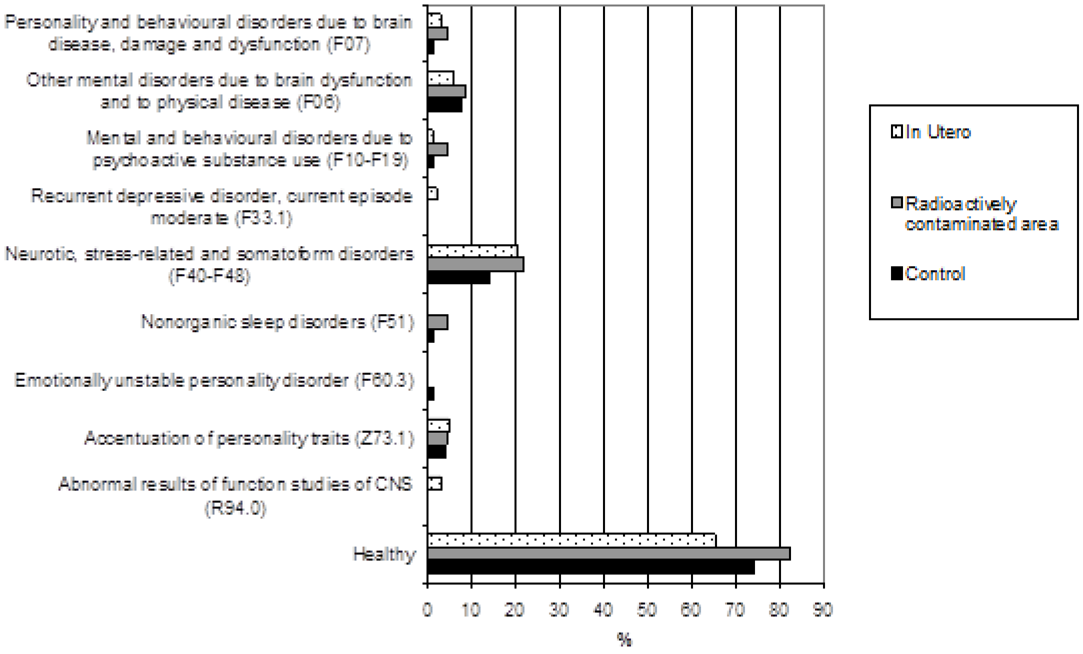






Chernobyl: in utero exposed





At age 27-28 years prenatally exposed people from Pripyat still have IQ discrepancies due to verbal IQ decrement ($p<0.01$)

Cognitive tests of cleanup workers who worked in the 30 km zone around the Chernobyl NPP between 1986 and 1990

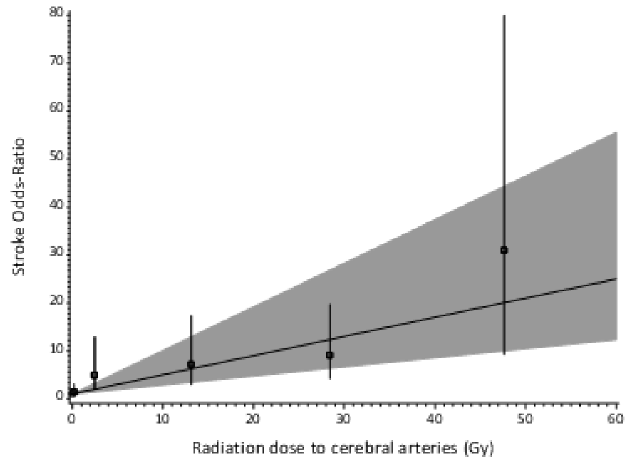
- Four groups of 100 Chernobyl cleanup workers are tested for cognitive dysfunctions, inclusion criteria:
  - Males, aged 18+ at cleanup exposure
  - 1986-1990 clean up
  - Dose records
- Dose groups: (0.0 to 20 mSv as internal control), (20 to 100 mSv), (100 to 250 mSv), (250-500 mSv) and (> 500 mSv)

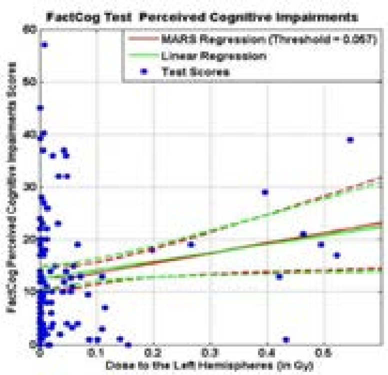
COGNITIVE FUNCTION GROUPS	Mini-Mental State Examination (MMSE)	Other criteria	N	%
Normal	28 or more	No cerebrovascular disease, confirmed by neurologist	77	25
Mild Cognitive Impairment (MCI)	24-27	Cerebrovascular disease, confirmed by neurologist	183	60
Dementia (VaD, mainly vascular)	23 or less	Cerebrovascular disease, confirmed by neurologist	46	15
Total			306	100

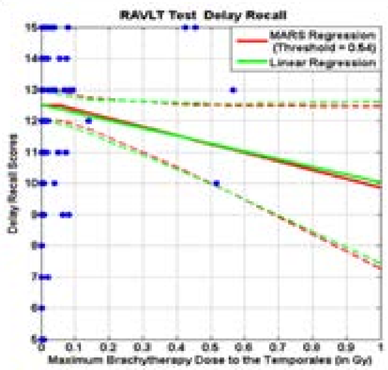
Main epidemiology conclusions

- Dose-dependent increase in cerebrovascular complications several years after exposure in FCCSS and BCCSS

- The Excess of Odds Ratio (EOR) of stroke per Gy of average radiation dose to the cerebral arteries, was equal to EOR/Gy = 0.49 (95% CI: 0.22 to 1.17) in a linear model.







Age-dependent change in cognition

- in in utero exposed cohort, effects are observed below 0.1 Gy,
- in the medical cohort (exposure at childhood below the age of one year), changes from thresholds of 0.12 Gy and 0.054 Gy, respectively to the thyroid and cerebral hemispheres.
- In cleanup workers demonstrated significant cognitive deficits when exposed to doses over 0, 25 Gy.

Biological assessments

Human data: prenatal and childhood exposure to radiation

Adult cognitive and cerebrovascular diseases

Prenatal E11

Postnatal P10

Early events

1d 7d

- Apoptosis
- Proliferation
- Differentiation
- Inflammation
- Transcriptomic
- Proteomic

Late effects

1M 2M 4M 6M

- Behavior
- Molecular Imaging
- Adult neurogenesis
- Mitochondrial Redox
- Transcriptomic
- Proteomic

WP3: Cerebrovascular effects in animal studies

- To study the cognitive abilities and neuro-toxicological effects during in utero and neonatal brain development.

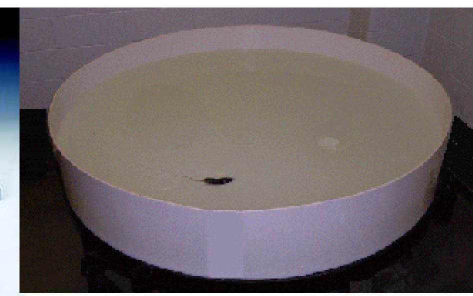
- To illustrate the interplay between the vascular and central nervous system in the processing of the response of brain damage induced by low doses of radiation.

Cognitive effects after Prenatal exposure to IR

mouse pups

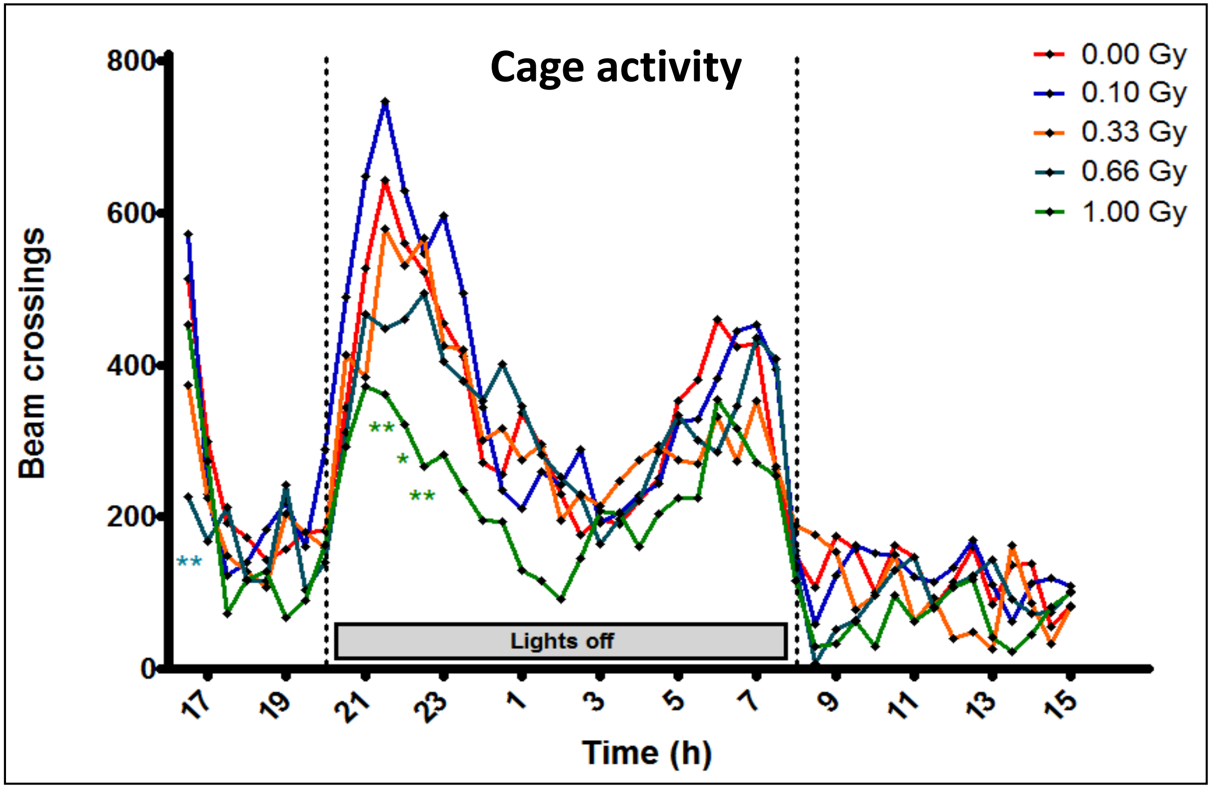
adult mouse

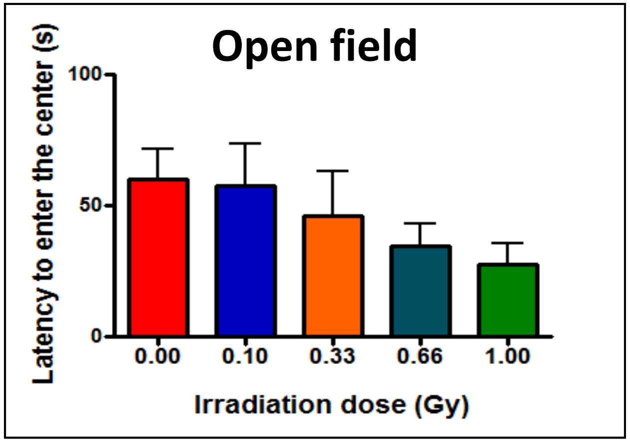
- **Behavioral test battery**

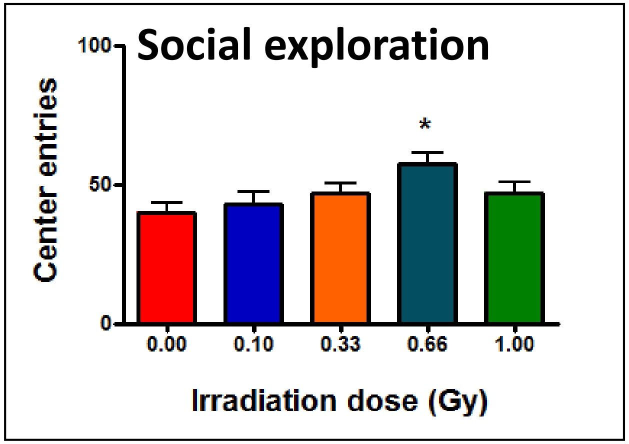


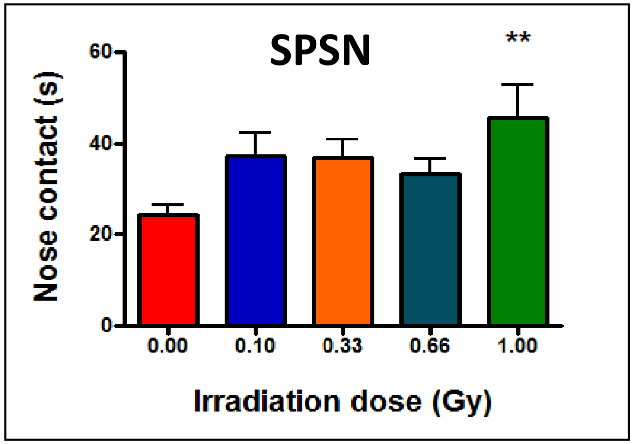
- **<u>Neuromotor tests</u>:** **Cage activity, Rotarod, Gait analysis**
- **<u>Exploratory tasks</u>:** **Open field, Social exploration, Elevated plus maze, Sociability/preference for social novelty**
- **<u>Learning & memory</u>:** **Morris water maze, Contextual fear conditoning**

Prenatal exposure to IR





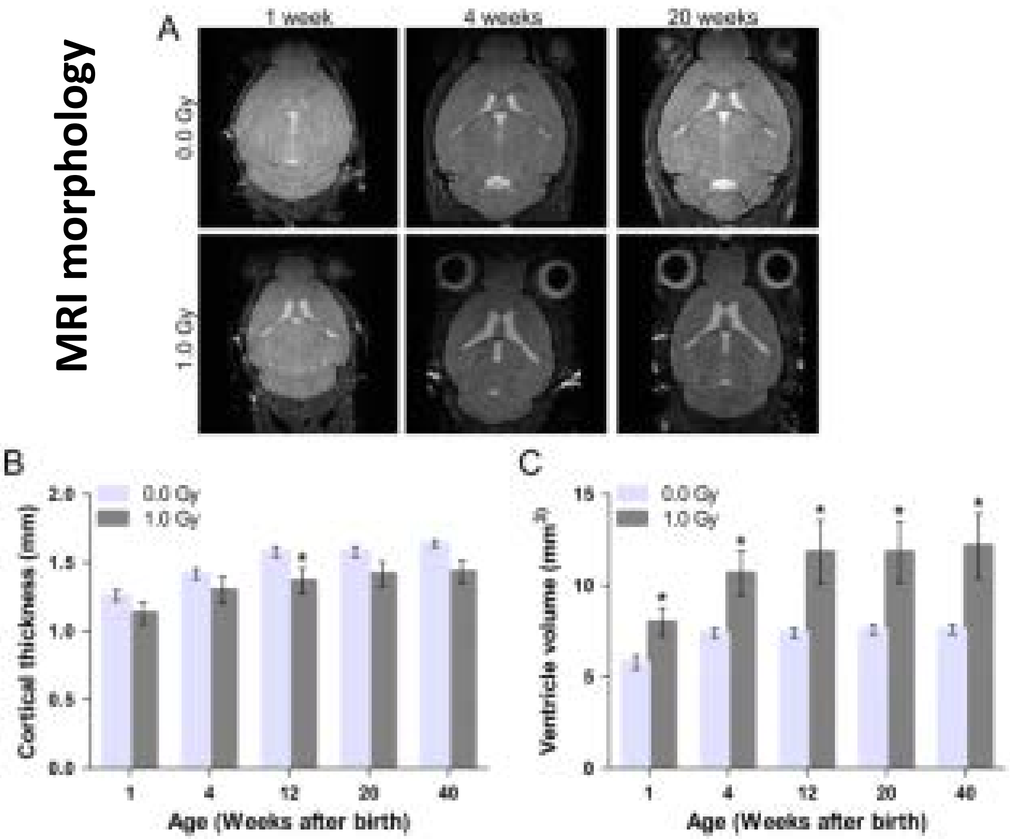




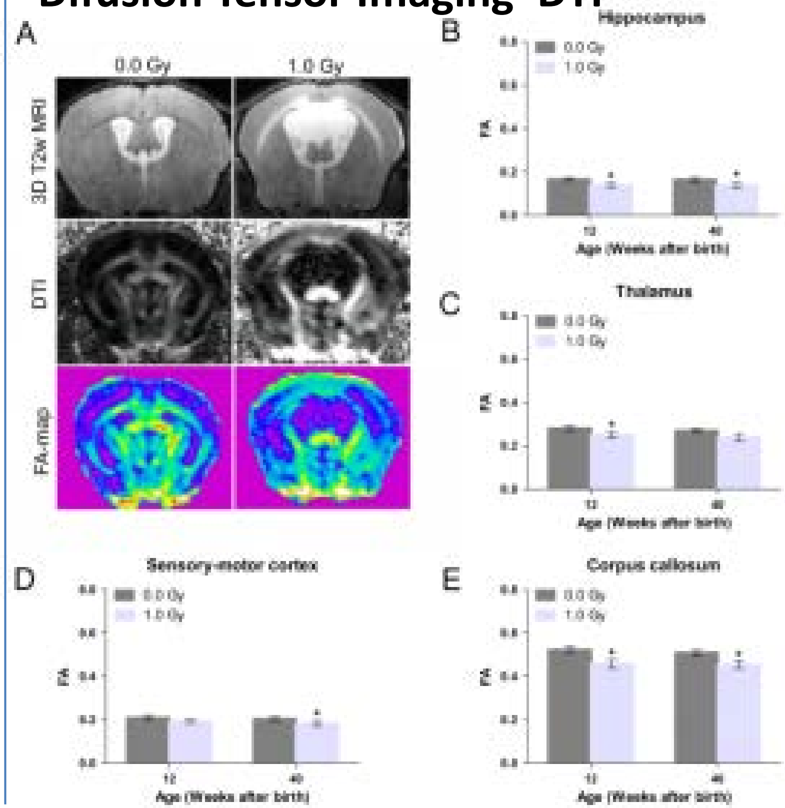
Prenatally irradiated animals exhibit reduced anxiety and increased sociability-related behavior.

Prenatal exposure to IR

MRI morphology



Difusion Tensor Imaging 'DTI'



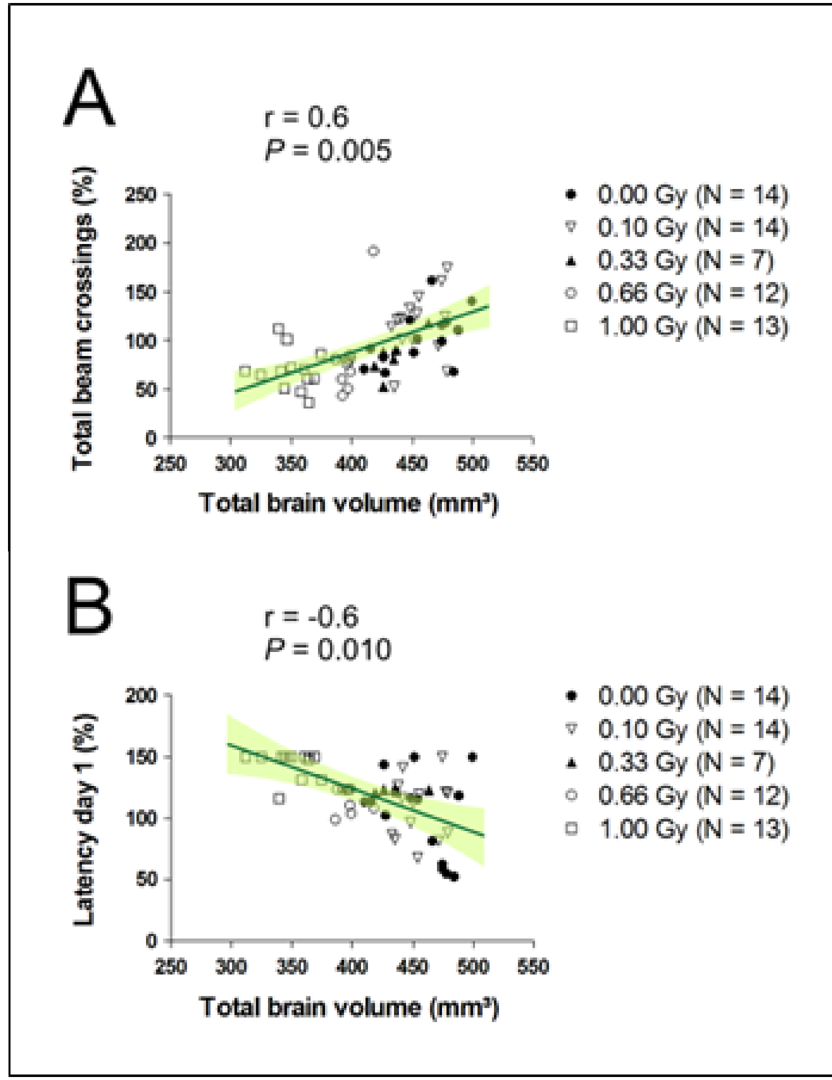
Reduction in cortical thickness and increase in ventricle size and reduced FA values (in ≠ regions)

Correlation studies

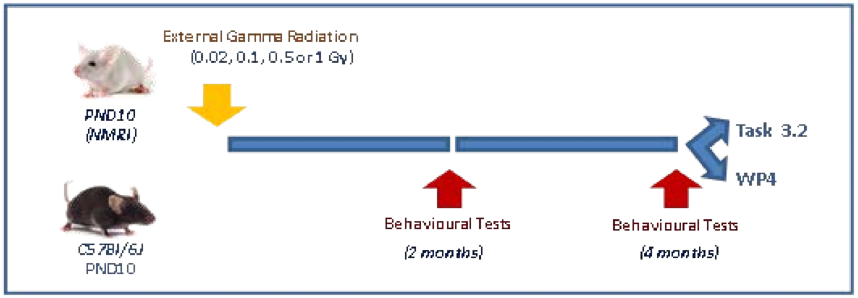
- Dose-dependent changes in activity, social behaviour, anxiety-related exploration and spatio-cognitive performancein mice exposed to 0.10 Gy
- Microcephaly from 0.33 Gy onwards, accompanied by deviations in regional brain volumes
- Whole-brain volume, as well as relative ventricle and prefrontal cortex volume, strongly correlated to aberrant behavioural parameters

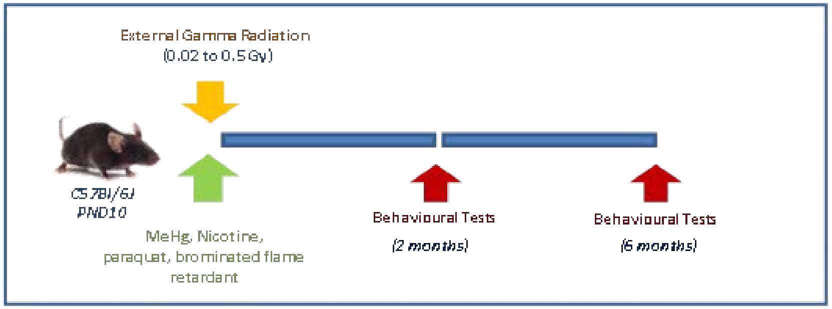
Table 5.3. Correlations between behavioural variables and MRI-based volumetric data.

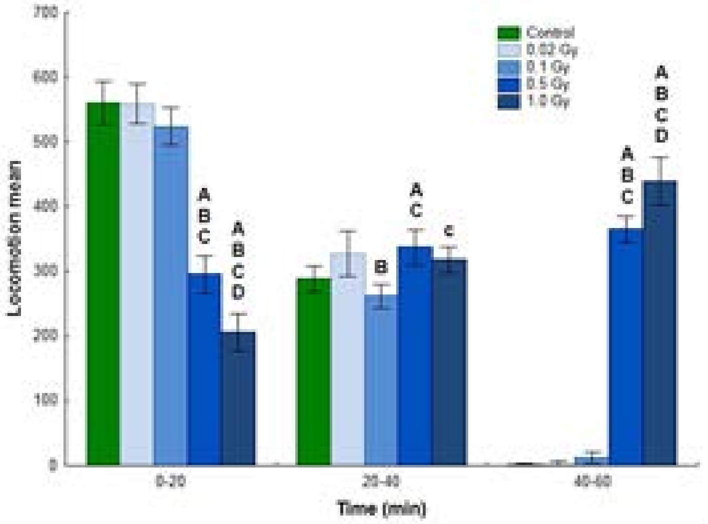
	Volume (mm^3)					
	Whole brain	Ventricles	Posterior cerebral cortex	Frontal cortex	Striatum	Cerebellum
Cage activity Beam crossings (%)	0.6••	-0.3	0.15	0.028	0.3	0.24
Elevated plus maze Open/total (%)	-0.21	0.5••	-0.13	-0.28	-0.08	-0.09
Elevated plus maze Open/closed (%)	-0.20	0.5••	-0.12	-0.27	-0.09	-0.07
SPSN Sociability (%)	-0.4•	0.4•	-0.22	-0.009	-0.11	-0.14
SPSN Social memory (%)	0.20	-0.3	0.09	0.24	0.21	0.05
MWM Latency day 1 (%)	-0.6••	0.4•	-0.3	-0.4•	-0.22	-0.4

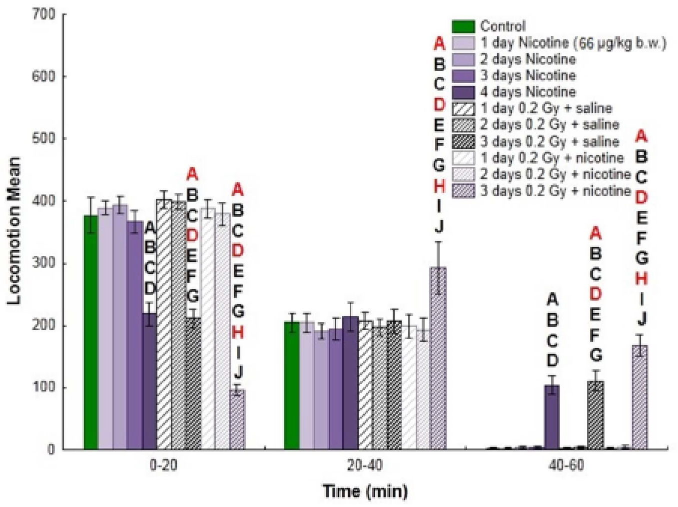


Postnatal exposure to IR



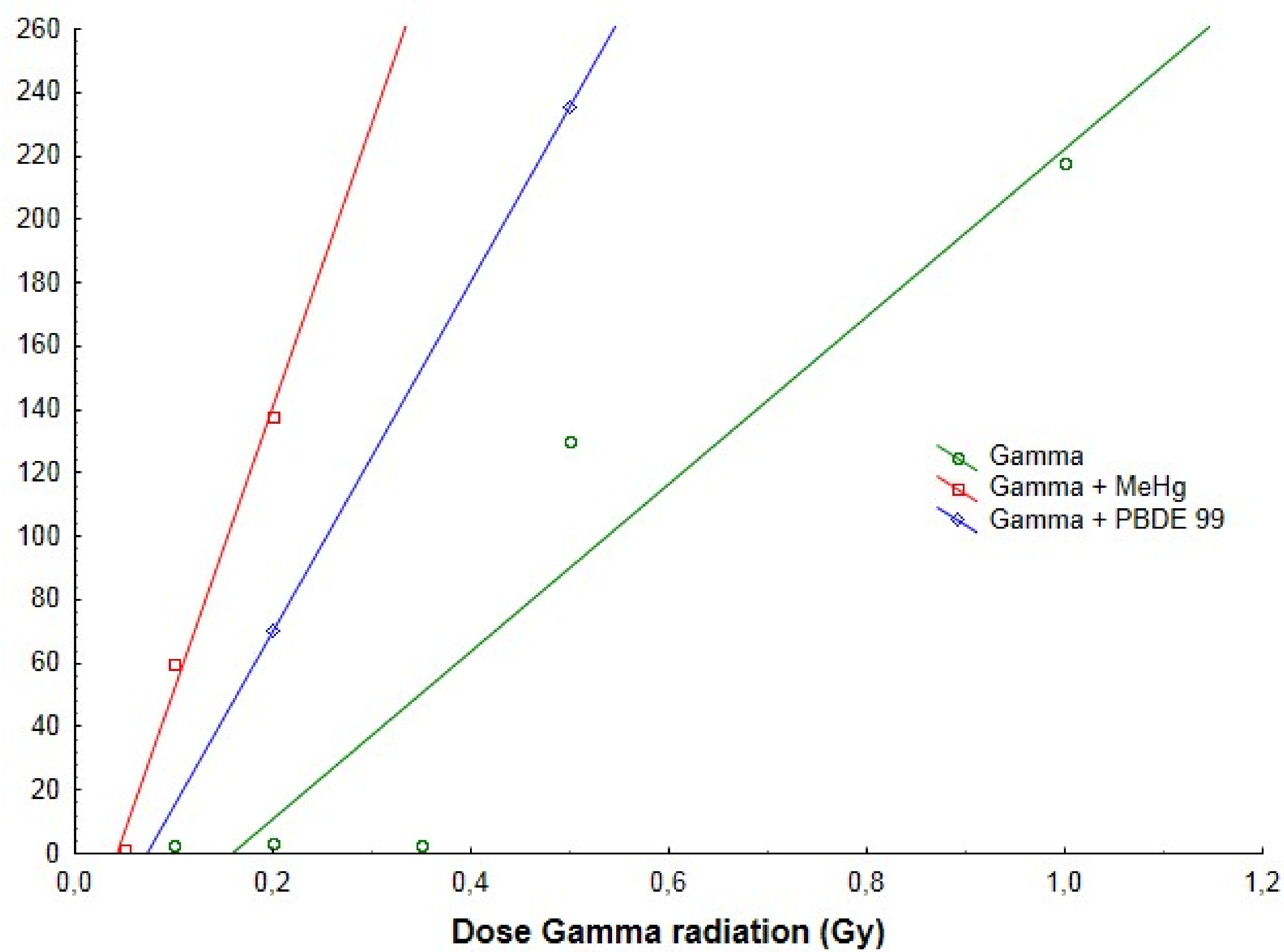






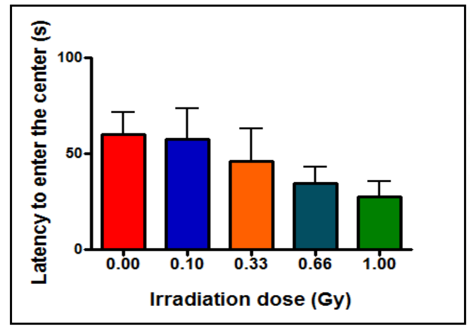
Buratovic S[1], Stenerlöw B[2], Fredriksson A[3], Sundell-Bergman S[4], Viberg H[3], Eriksson P[3]. **Neonatal exposure to a moderate dose of ionizing radiation causes behavioural defects and altered levels of tau protein in mice.** Neurotoxicology. 2014 Dec;45:48-55. doi: 10.1016/j.neuro.2014.09.002. Epub 2014 Sep 26.

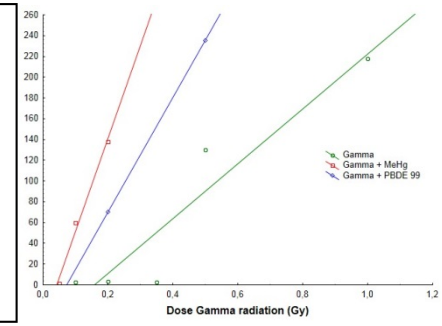
Dose-response curve and threshold value

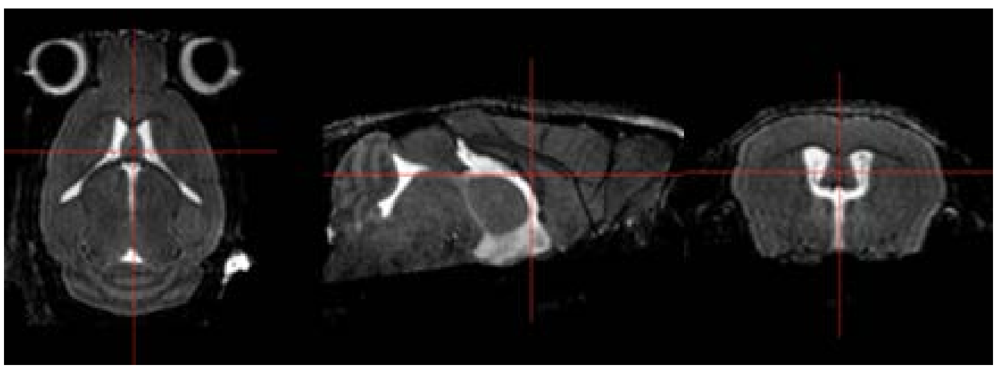


Animal studies main conclusions

- The shape of the dose-response curve for cognitive impairments in animal models shows a linear dose-response curve with age-dependent sensitivity.
- Postnatal co-exposure with environmental toxicants (such as MeHg, nicotine and PBDE) showed defects at a dose below 0.1 Gy.







- MRI data showed morphological changes that might be linked with the functional behavioral impairments

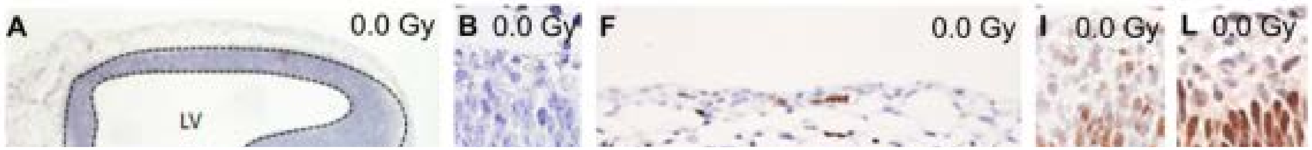
In all, CEREBRAD data indicate there might be no threshold below which no effects are observed, warranting thus further investigations.

WP4: Biological consequences of low-dose ionizing radiation exposure of the central nervous system

- To determine the initial direct events specifically induced by low-dose ionising radiation, which contribute to the etiology of diseases of the CNS.

- To identify a transcriptional and translational fingerprint characteristic for low-dose ionising radiation using advanced bioinformatics for data integration.

Prenatal exposure to IR



Verreet *et al. Journal of Neurodevelopmental Disorders* 2015, **7**:3
http://www.jneurodevdisorders.com/content/7/1/3

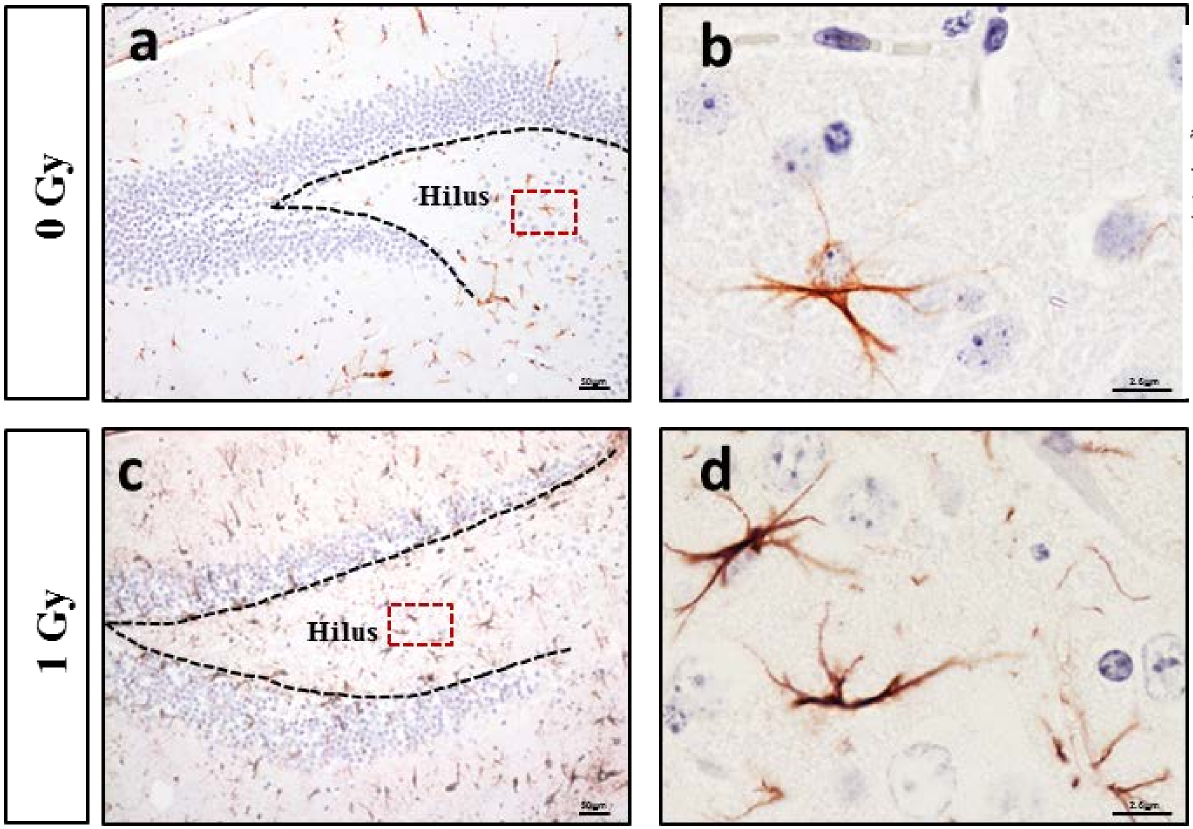
RESEARCH Open Access

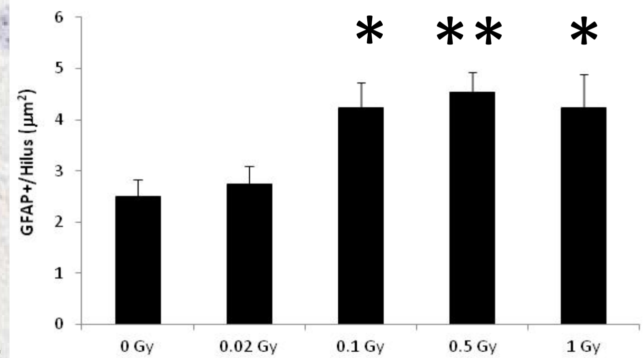
A multidisciplinary approach unravels early and persistent effects of X-ray exposure at the onset of prenatal neurogenesis

Tine Verreet[1,2], Roel Quintens[1], Debby Van Dam[3], Mieke Verslegers[1], Mirella Tanori[4], Arianna Casciati[4], Mieke Neefs[1], Liselotte Leysen[1], Arlette Michaux[1], Ann Janssen[1], Emiliano D'Agostino[5], Greetje Vande Velde[6,7], Sarah Baatout[1], Lieve Moons[2], Simonetta Pazzaglia[4], Anna Saran[4], Uwe Himmelreich[6,7], Peter Paul De Deyn[3,8] and Mohammed Abderrafi Benotmane[1*]

Huge increase in apoptosis 'Caspase3' and microglial activation 'Iba1', decreased proliferation 'PCNA', 24 h after exposure

A critical player in hippocampal neurogenesis



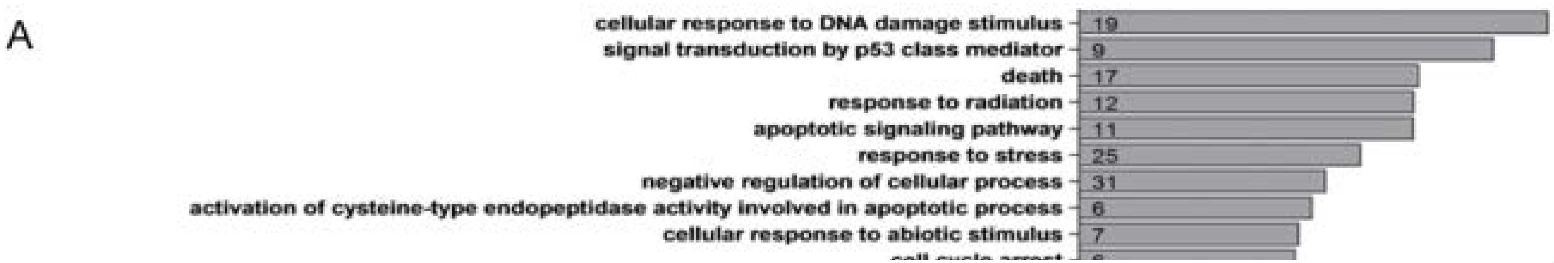


A significant increase of 70-82% in the number of GFAP+ astrocytes was detected in the hilus after irradiation with doses ≥ 100 mGy

Chronic inflammation may alter hippocampal neurogenesis causing defects in cognitive function

Transcriptomic analysis of prenatal exposure to IR

Functional enrichment analysis



 | Biology Open (2015) 000, 1–14 doi:10.1242/bio.20149969

RESEARCH ARTICLE

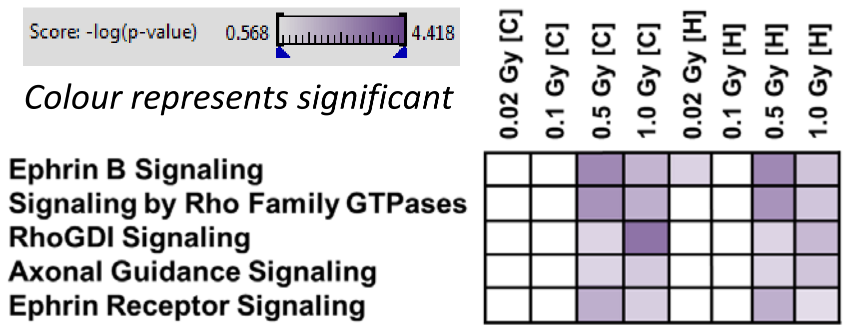
Identification of novel radiation-induced p53-dependent transcripts extensively regulated during mouse brain development

Roel Quintens[1,*], Tine Verreet[1,2], Ann Janssen[1], Mieke Neefs[1], Liselotte Leysen[1], Arlette Michaux[1], Mieke Verslegers[1], Nada Samari[1], Giuseppe Pani[1,‡], Joris Verheyde[1], Sarah Baatout[1,3] and Mohammed A. Benotmane[1]

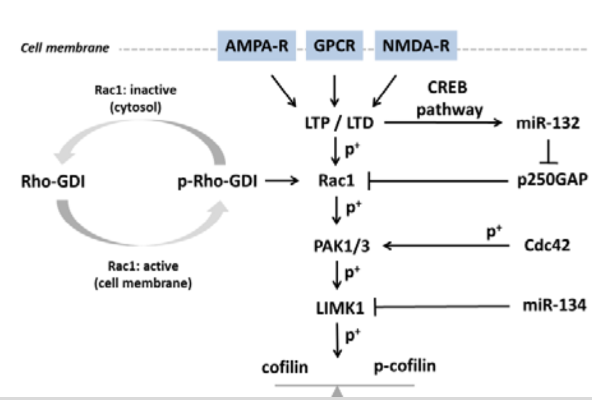
Activation of p53-responsive genes and processes at 2h after exposure to radiation

Proteomic analysis of postnatal exposure to IR

Mass-spectrometry based proteomics of cortex [C] and hippocampus [H]



The Rho family GTPase Rac1-Cofilin pathway involved in spine morphology – Reduced Rac1 levels 7 month post-irradiation at 0.5 Gy and 1.0 Gy

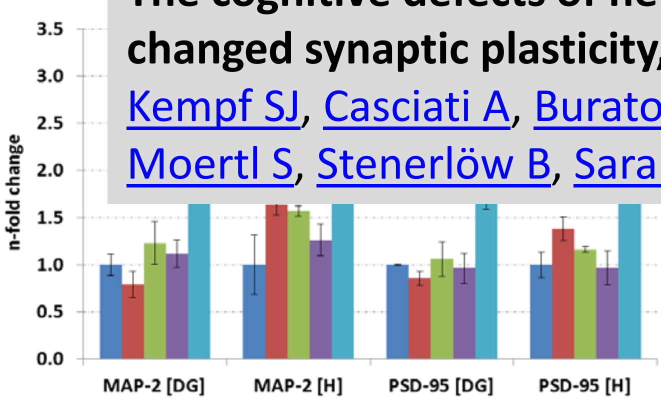


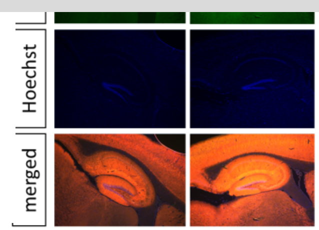
Sequen
and MA

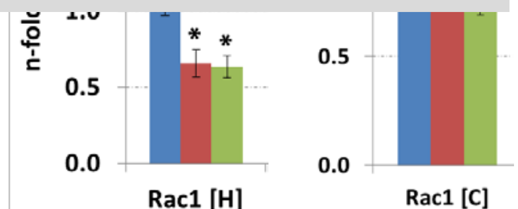
Mol Neurodegener. 2014 Dec 16;9:57. doi: 10.1186/1750-1326-9-57.

The cognitive defects of neonatally irradiated mice are accompanied by changed synaptic plasticity, adult neurogenesis and neuroinflammation.

Kempf SJ, Casciati A, Buratovic S, Janik D, von Toerne C, Ueffing M, Neff F, Moertl S, Stenerlöw B, Saran A, Atkinson MJ, Eriksson P, Pazzaglia S, Tapio S[1].







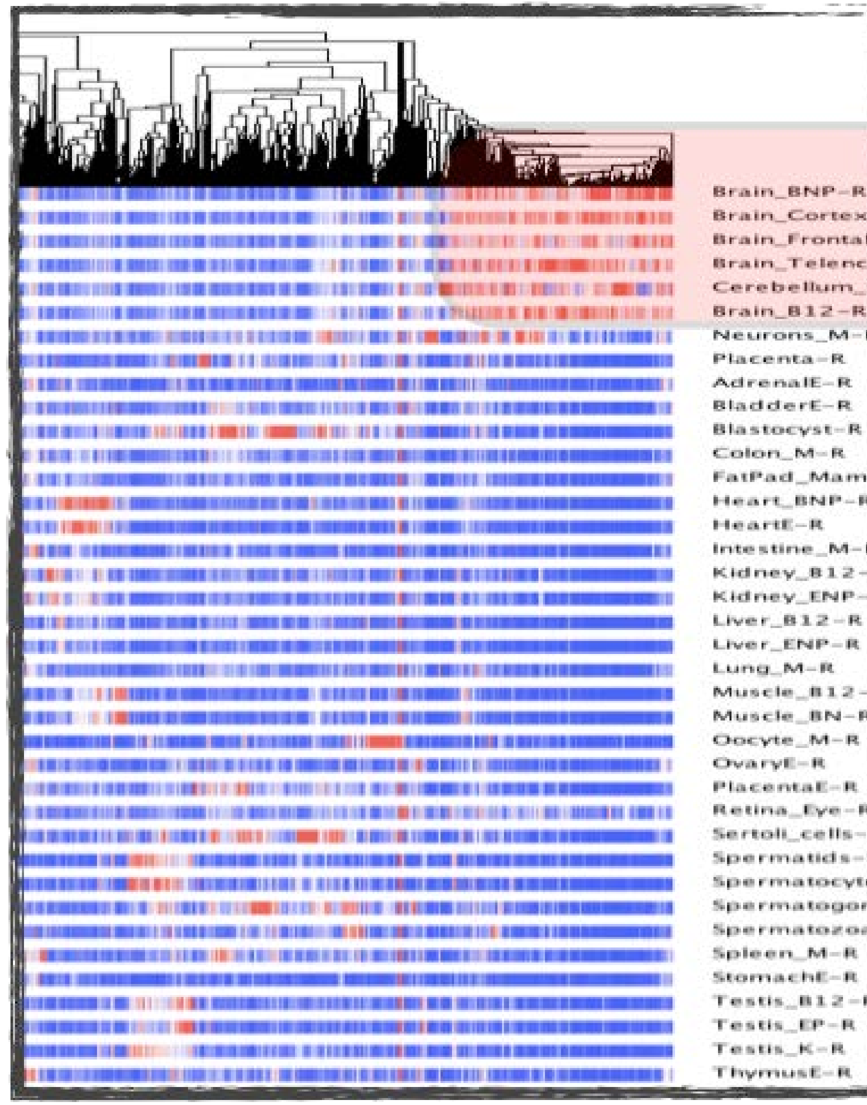
Defects in synaptic plasticity 7 months post-irradiation at 0.5 Gy and 1.0 Gy

molecular biomarkers

- 1381 genes filtered for tissue-specificity with independent RNAseq data (998 genes retained)

- clustered with Pearson index-based hierarchical bi-clustering, retained high-specificity class with 290 putative highly specific biomarkers

- biomarkers detected both by proteomics & RNASeq expression



cellular pathways

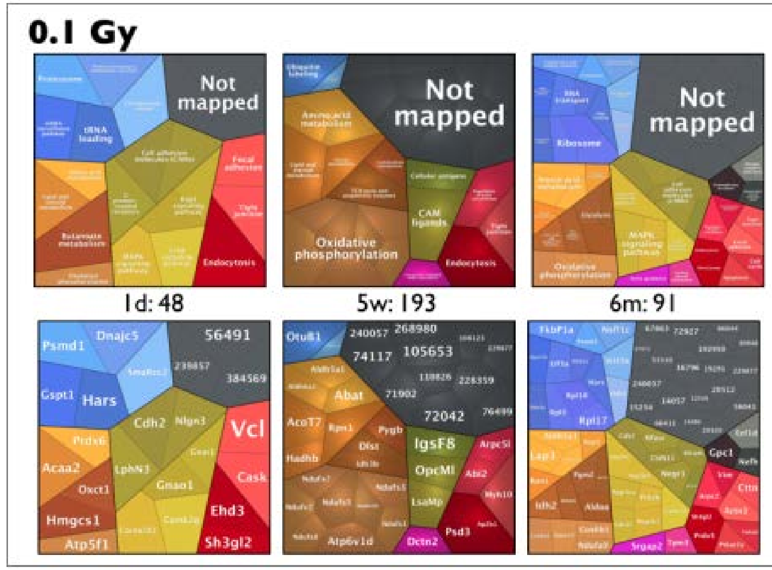
- 290 genes further clustered, resulting in 270 genes across 18 modules with >5 genes – shown »
- broadly annotated with composite queries *vs.* public-domain omics data
- we discover 5 unclear cases (•), 7 promising but non-significant (•), and 6 highly significant (•) – detected across dose & time points (*in prep.*)

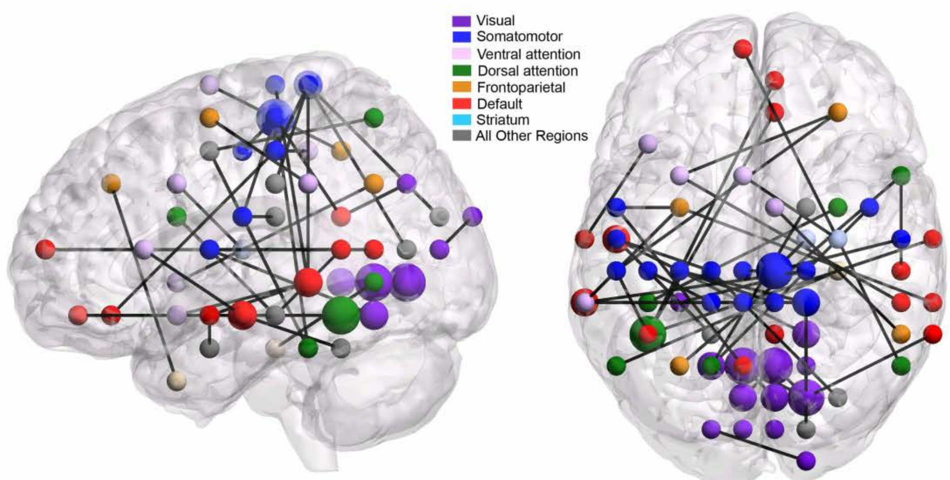
MOD01 // regulation_synaptic_plasticity_transmission
MOD02 // postsynaptic_density_ionotropic_glutamate_receptor
MOD03 // unknown_pathway03
MOD06 // phosphatase_response_to_morphine_alkaloids
MOD07 // synaptic_membrane
MOD09 // adult_behavior not_clear
MOD10 // learning_vocalization_memory
MOD11 // endocytosis_ciliary_rootlet
MOD12 // unknown_pathway12
MOD16 // synaptic_membrane_axon_part
MOD18 // synapse_organization_locomotory_behavior
MOD19 // neuron_maturation_synaptic_membrane
MOD20 // unknown_pathway20
MOD23 // sensory_perception_sound_inner_ear
MOD24 // GABAergic_action_potential_synaptic_vesicle
MOD25 // SNARE_neurotransmitter_neuron_ensheathment
MOD26 // innervation_myelination
MOD27 // inflammation_dendrite not_clear

Systems biology approach for data integration

Dose	Time						
	4h	24h	48h	4w	5w	24w	6m
0.1Gy	-	48	-	32	193	202	91
0.2Gy	-	-	-	33	-	-	-
0.5Gy	11	155	-	104	159	384	102
1Gy	31	223	56	-	-	-	117
2Gy	-	34	-	45	259	559	-
4Gy	50	91	-	-	-	-	-
Total		**305**		**77**	**452**	**761**	**208**

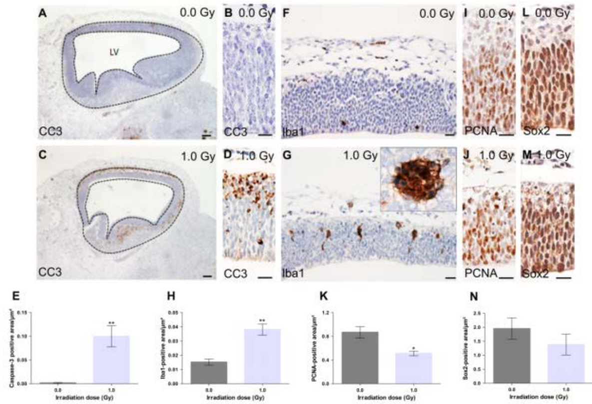
Grand total 1,803 non-unique proteins

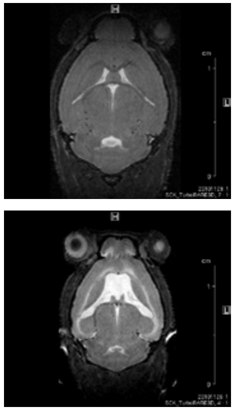


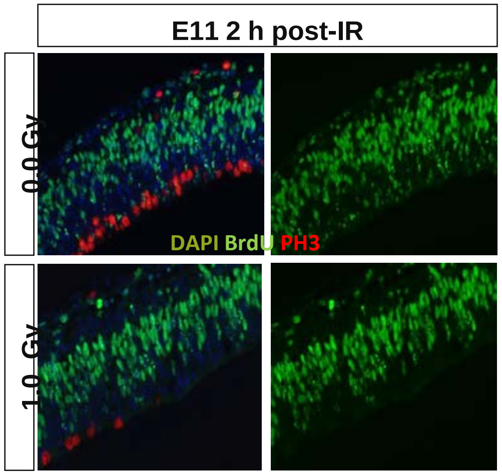


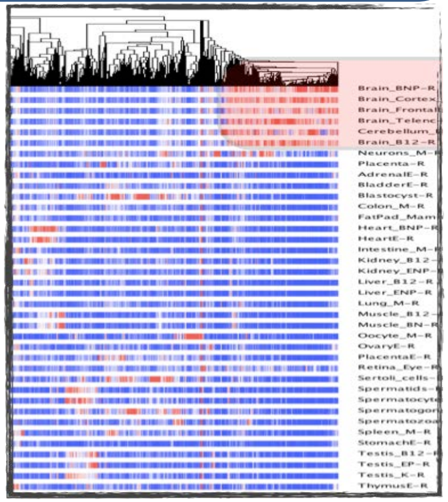
Biology main conclusions

- Massive apoptosis in the brain and induction of p53 stress-related and other neurogenic targets were observed early after in utero exposure to radiation
- Induction of microcephaly-like phenotype after in utero exposure to radiation
- Persistent morphological changes at adult age leading to cognitive impairments









- Cognitive dysfunction is linked with impaired neurogenesis and neuroinflammation in the hippocampus after early postnatal irradiation at doses (500-1000 mGy).
- Persistent effects (DNA damage, inflammation) are observed at low doses 20-100 mGy especially several months after exposure in mice (years in human).

A dynamic interaction between multiple cell types (i.e. neurons, microglia and astrocytes) and synaptic plasticity are most probably involved in radiation-induced cognitive injury. These persistent effects could in part be mastered through epigenetic events, requiring additional investigations.

SEVENTH FRAMEWORK PROGRAMME

CEREBRAD

Cognitive and Cerebrovascular Effects Induced by Low Dose Ionising Radiation

www.cerebrad-fp7.eu

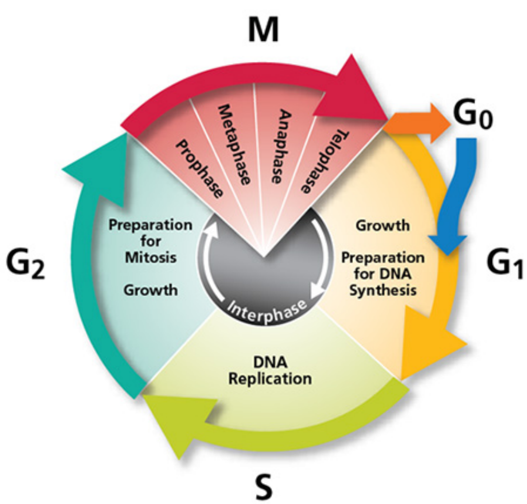
Many thanks for your attention

This work was supported by the EU Euratom FP7 (GA295552)

Prenatal radiaton-induced defects

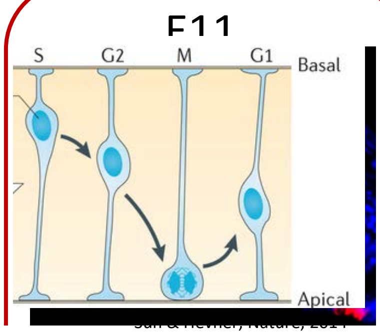
PROLIFERATION

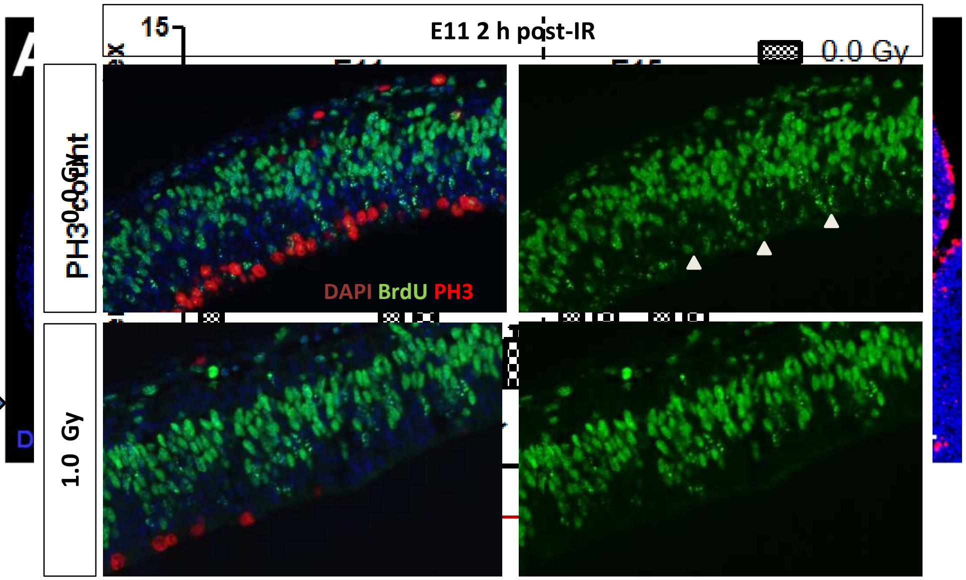
Phosphohistone H3



Ki67

BrdU pulse experiments

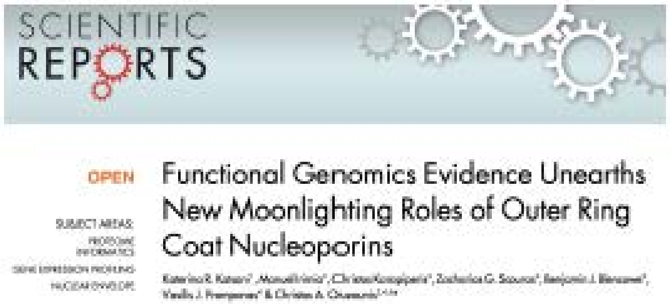




→ Stalling of VZ progenitors in the S/G2 phase?

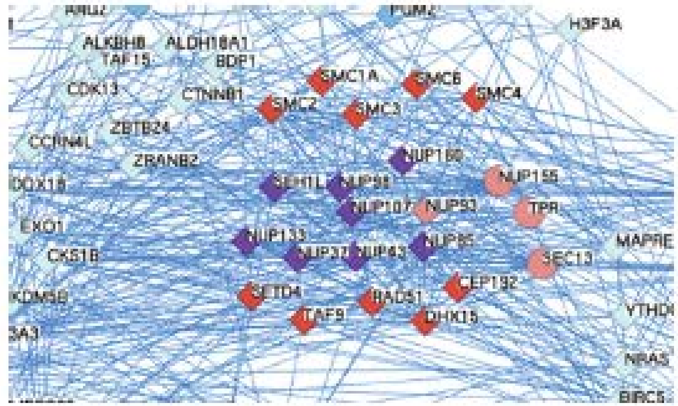
→ Transient effect immediately after X-radiatio

assessing tissue specificity

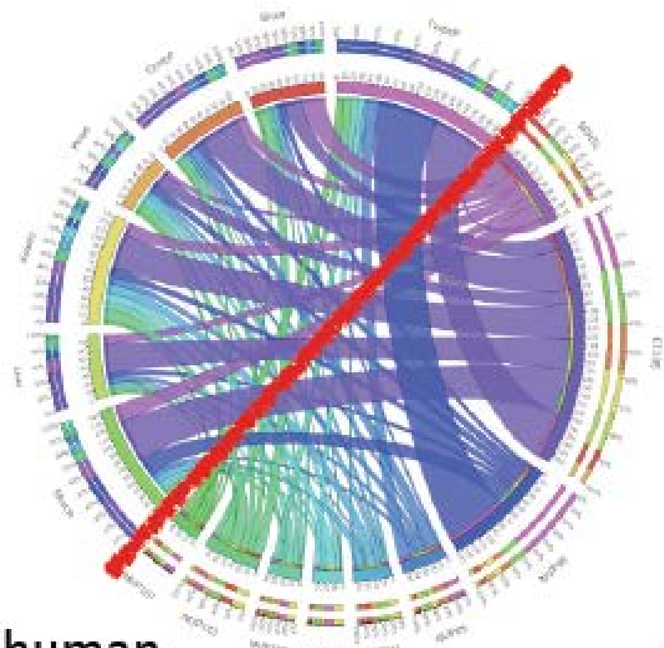

SCIENTIFIC REPORTS

OPEN

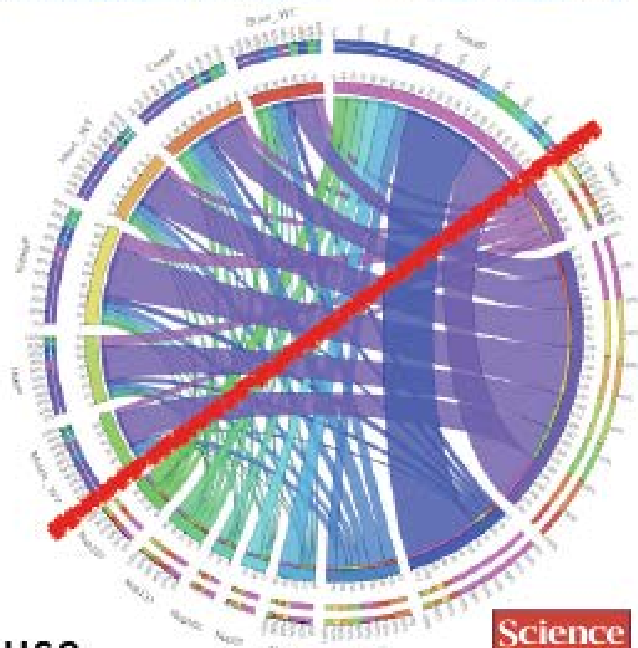
Functional Genomics Evidence Unearths New Moonlighting Roles of Outer Ring Coat Nucleoporins



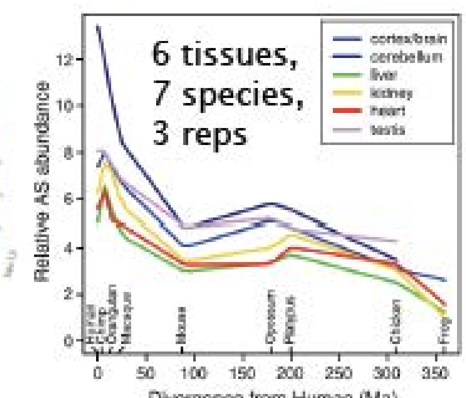
integration with other data types, module detection & inference (task 4.4.3)



human



mouse



Science AAAS

The Evolutionary Landscape of Alternative Splicing in Vertebrate Species
Nuno L. Barbosa-Morais et al.
Science 338, 1587 (2012);
DOI: 10.1126/science.1230612

Impact

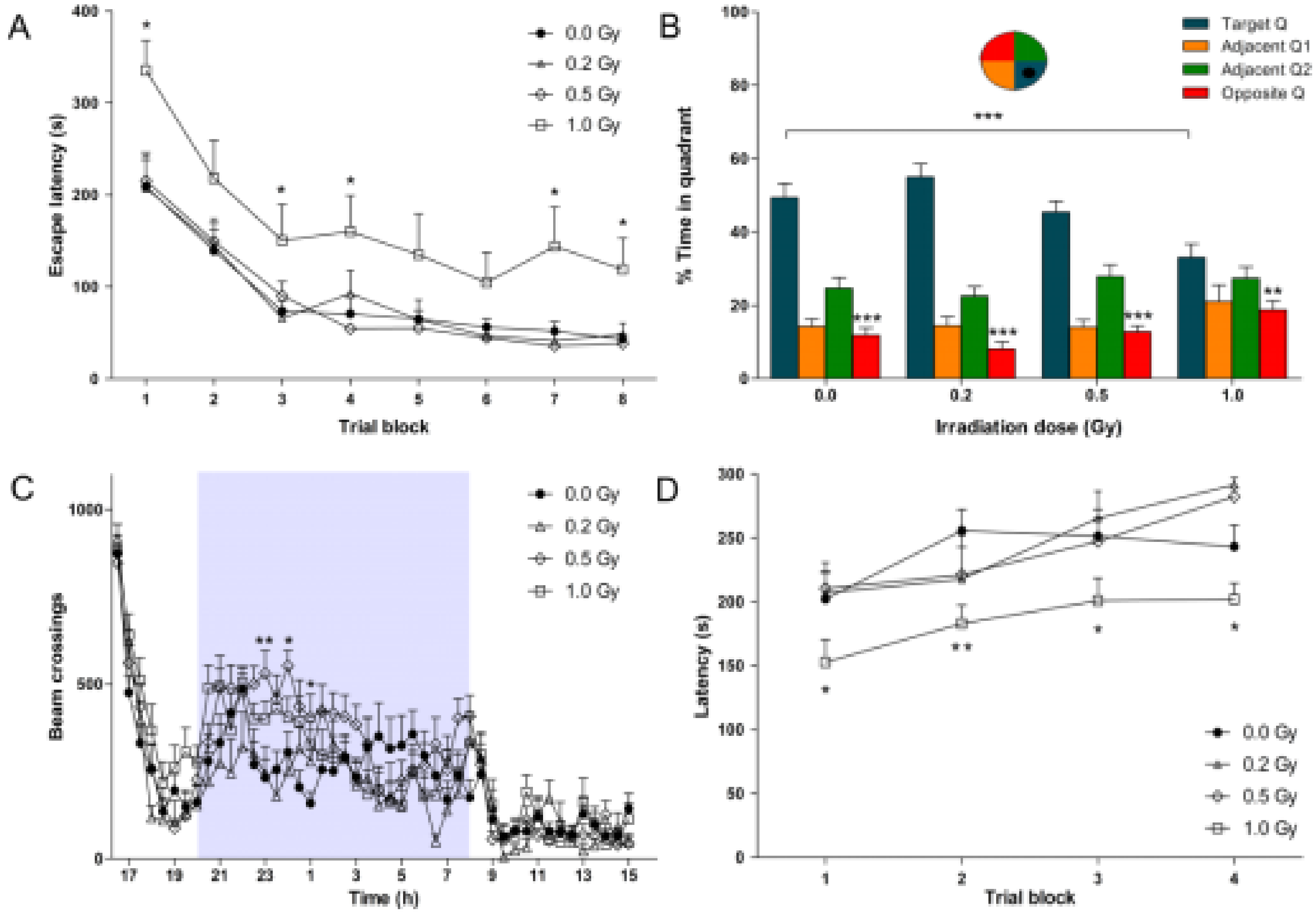
- The knowledge provided by CEREBRAD would improve the protection of children bellow the age of years towards exposure to radiation.

- The results of CEREBRAD increase our understanding of the effect of low vs. high doses of external and internal exposures similar in diagnostic, therapeutic and accidental situations.

- coexposures.

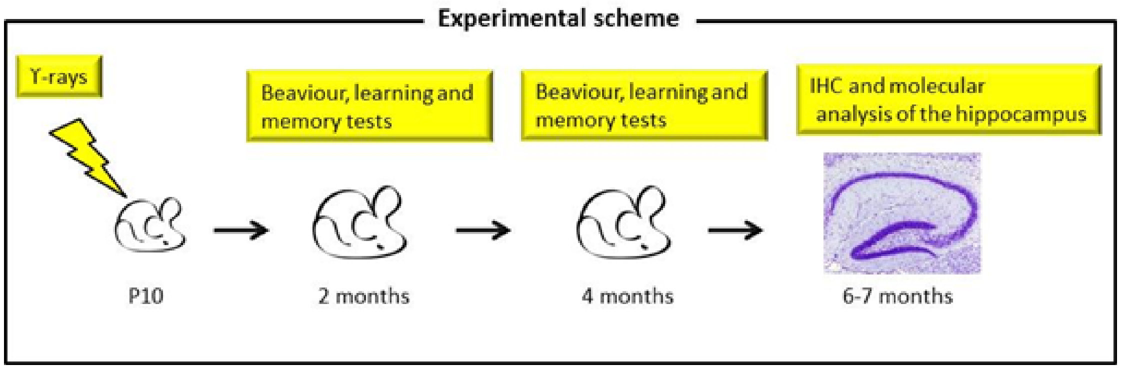
- Melodi

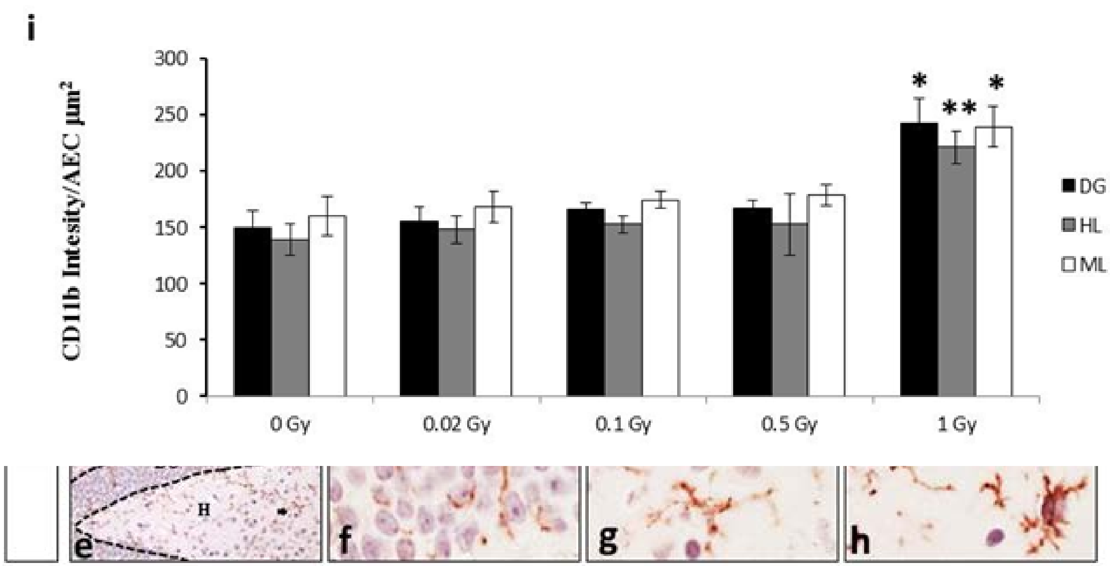
Prenatal exposure to IR

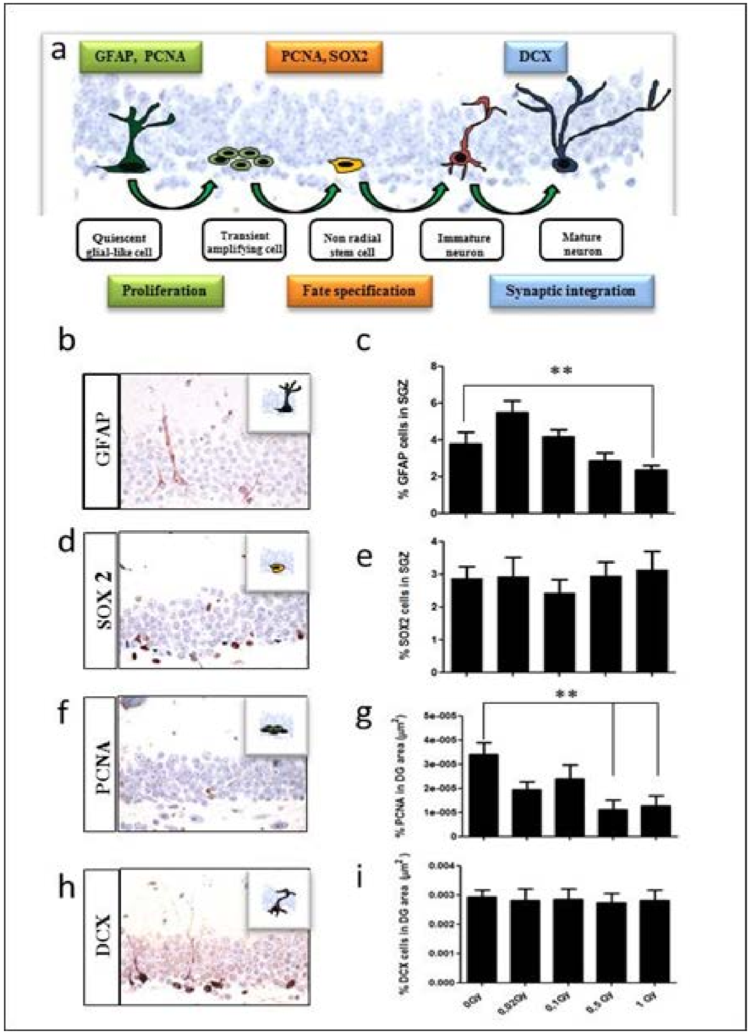


Deviant behavior in learning and memory, as well as cage activity and motor performances.

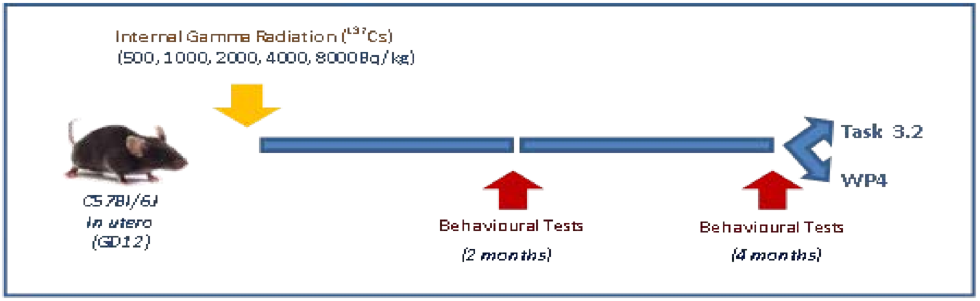
Postnatal exposure to IR

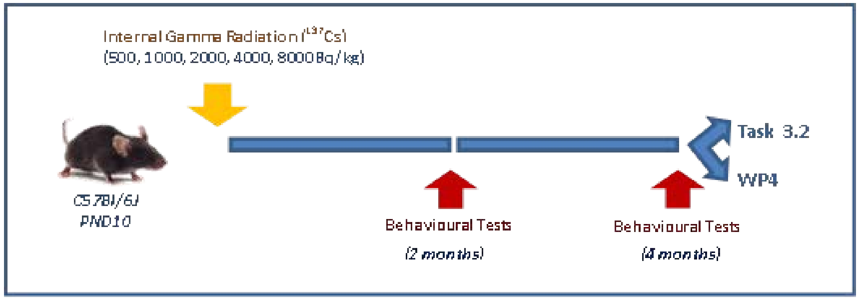


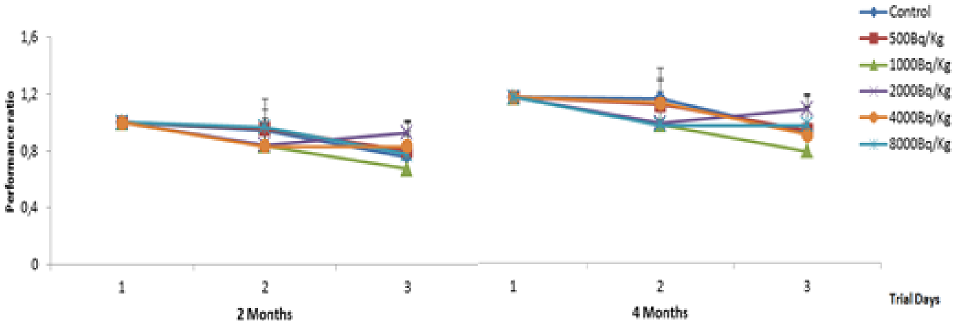


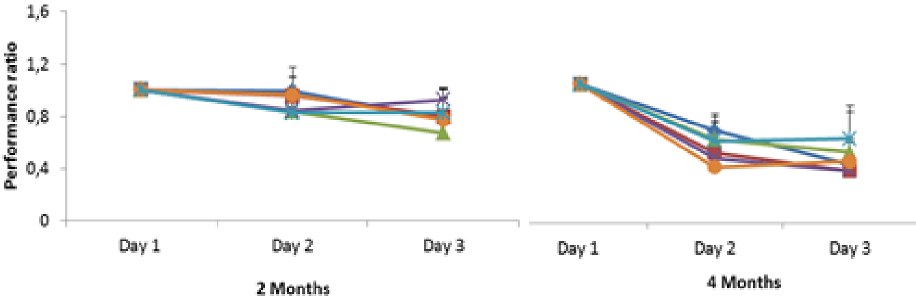


Significant reduction in proliferation (GFAP, PCNA) at 7 month after exposure









Performance ratios (number of errors each day/the number on the first day) at the age of two and four months, of mice exposed to 137Cs.