SEVENTH FRAMEWORK PROGRAMME

CEREBRAD

www.cerebrad-fp7.eu

Cognitive and Cerebrovascular Effects Induced by Low Dose Ionising Radiation

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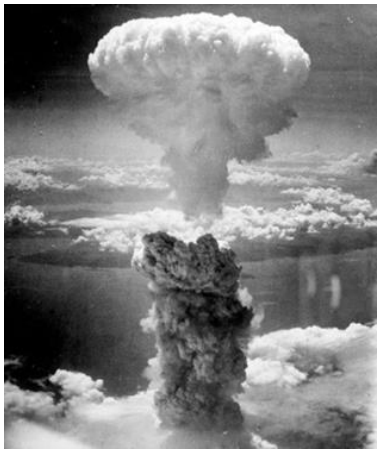
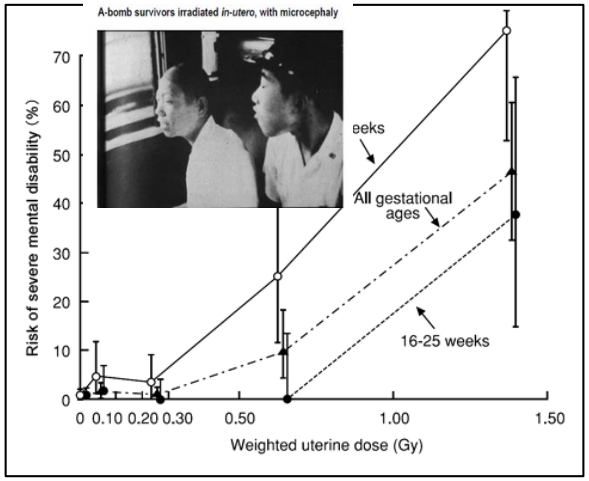
Background





- Mental retardation
- Microcephaly
- Low IQ values
- …

- Mental retardation
- Lower intellectual performances
- Stroke

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

CEREBRAD

GA n°:	295552
Duration:	36 months (+ 6M extension)
Partners:	11 (10 EU + Ukraine)
Total budget:	M€ 4,72
EC contribution:	M€ 3,00
Coordinator:	M. A. Benotmane, (SCK•CEN.be)
EC officer:	André Jouve (EU)
SAB:	Francis Cucinotta (USA)
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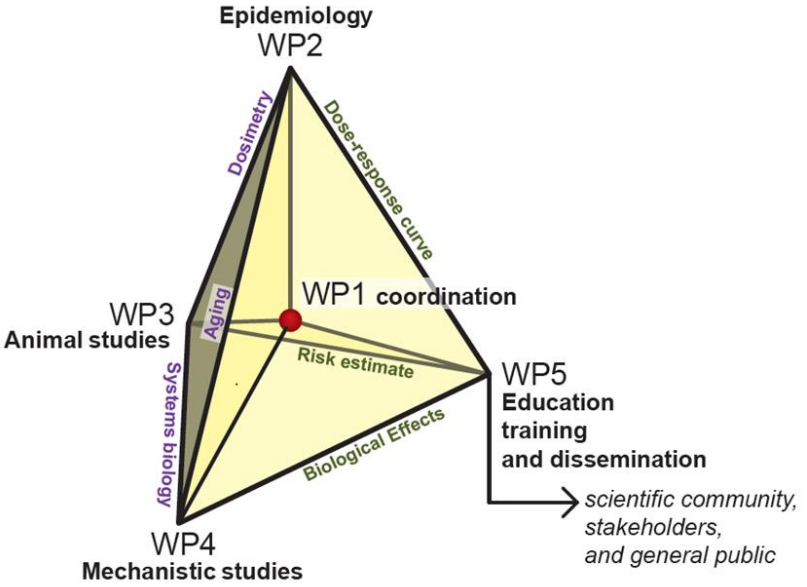


Acronym	Organisation name	Country
SCK-CEN	Belgian Nuclear Research Center	Belgium
EMC	Erasmus University Medical Center Rotterdam	The Netherlands
ENEA	Italian National Agency for New Technologies, Energy and the Environment	Italy
HMGU	Helmoltz Center for Environmental Health	Germany
NRIRR	Frédéric Joliot-Curie 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 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.

CEREBRAD concept and structure

Human epidemiology WP2

- cognitive dysfunctions in individuals exposed in utero or during childhood
- cerebrovascular diseases detected later in life from childhood cancer survivors

Animal studies WP3

- cognitive abilities and effects during in utero and neonatal brain development
- interplay between the vascular and central nervous system (BBB)

Mechanistic studies WP4

- determine the initial direct events induced by low-dose ionizing radiation
- To identify cellular and molecular fingerprints of late disease occurrence

Cognitive late effects
"Human data"

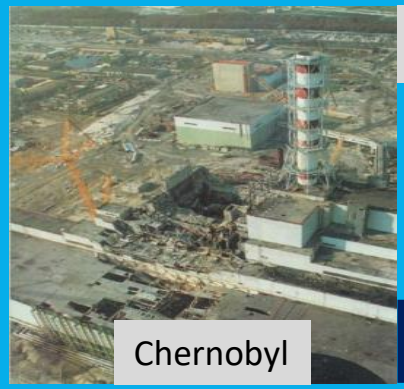
ANGIO cohort

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

Neurocognitive assessment

- The neurocognitive assessment are performed by neuropsychologists both at RCRM and IGR. Interviews are performed in the native language and independent of the dose of a subject. Additional information on health risks other than ionizing radiation like smoking, alcohol consumption, work with hazardous chemicals was collected.

- Exclusion criteria included infectious, metabolic, tumors', major head trauma, degenerative, major depression or psychosis, as well as severe visual or auditory disorders, inability to comply with protocol or procedures and or to understand the language used.

- The psychological test used:
  - The **Mini–Mental State Examination** (**MMSE**) or **Folstein test** is a 30-point questionnaire that is used to measure cognitive impairment. It is commonly used to screen for dementia.
  - The **Rey Auditory Verbal Learning Test (RAVLT)** is a neuropsychological assessment designed to evaluate verbal memory in patients. The RAVLT can be used to evaluate the nature and severity of memory dysfunction and to track changes in memory function over time.
  - **Functional Assessment of Cancer Therapy–Cognitive Function (FACT-Cog)** is a self-report questionnaire that assesses cognitive changes after Radiotherapy/chemotherapy
  - **Zung Depression Scale (SDS),** to determine the mood of the patient.
  - **Irritability, Depression and Anxiety Scale (IDA),** to rate irritability, depression and anxiety.
  - **Hospital Anxiety and Depression (HAD)** scale. Used for screening anxious and depressive patients.

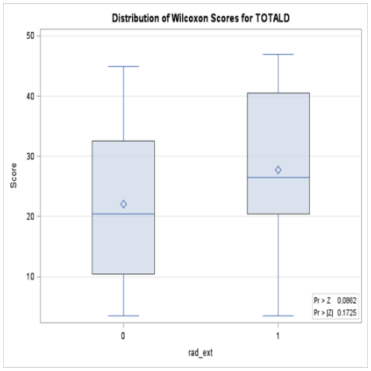
Cognitive effects (ANGIO cohort)

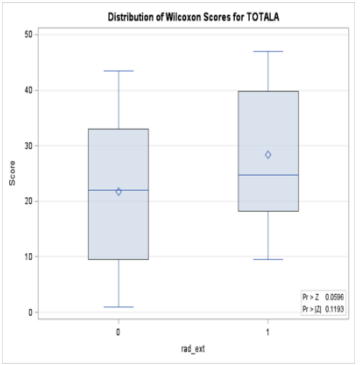
- Neuropsychological assessments are designed to measure cognitive functions in both healthy and test (IR) populations and remain important tools for research studies, clinical diagnoses, patient outcomes, and intervention monitoring.

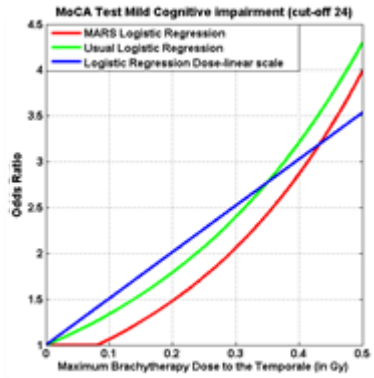
- A significant relation was established between depression and other stress-related states in proportion to radiation dose, which seems to be rather psychological radiation risk perception effect in case of Chernobyl workers.

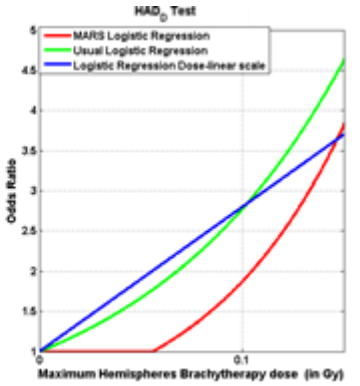
- A threshold (0.059 Gy) of the radiation dose to the left hemisphere lobe show a significant increase of the **Functional Assessment of Cancer Therapy–Cognitive Function (FACT-Cog)** Perceive cognitive impairments scores.

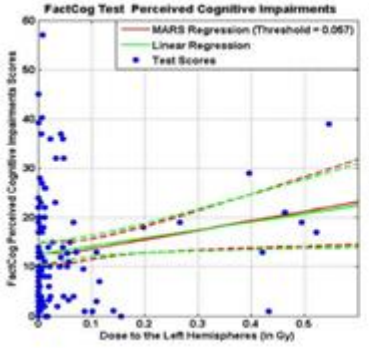
- A near from significant decrease of RTL was observed when increasing cognitive deficit (MMSE scale) and between age vs degree of cognitive deficit

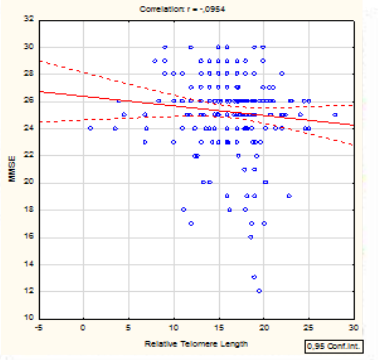












Cognitive tests in cleanup workers

- Low doses received in utero during Chernobyl accident: Overall, no significant difference was observed between the two irradiated groups and the control group for central nervous system diseases

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

Groups divided by dose (mSv)	Mean dose		Mean group values by test:							
			MMSE		GHQ-28		Zung		BPRS, scores 1-18	
	M	+SD	M	+SD			M	+SD	M	+SD
1 (0-20)	8,5	5,2	25,65	3,12	24,18	15,27	45,58	13,63	9,76	5,97
2 (20-100)	54,7	21,7	25,54	2,82	25,46	12,06	49,05	12,16	11,57	5,67
3 (100-250)	184,5	43,5	25,71	2,53	**32,72***	16,84	**53,19***	11,54	**12,55***	6,27
4 (250-500)	320,8	78,3	25,83	3,02	**30,45 ***	13,16	**50,58**	11,49	**12,07**	6,01
5 (>500)	1257,9	618,8	24,63	3,51	**36,00***	15,22	**55,96***	13,47	**14,57***	6,90

Cerebrovascular late effects
"Human data"

Childhood Cancer survivor studies

Cancer + RT At age < 7 years

FCCSS
BCCSS
NCCSS

233 cases and 233 matched controls

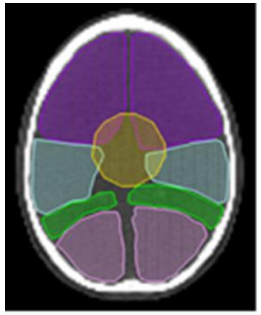
Stroke in average at 34 years old

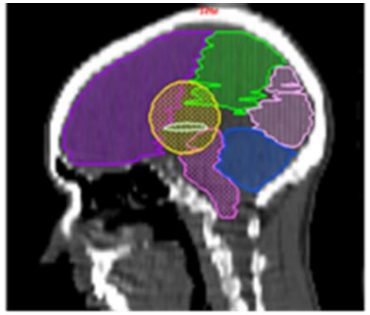
Average 27 y

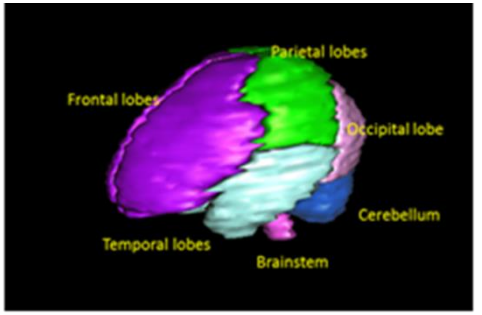
Dosimetry

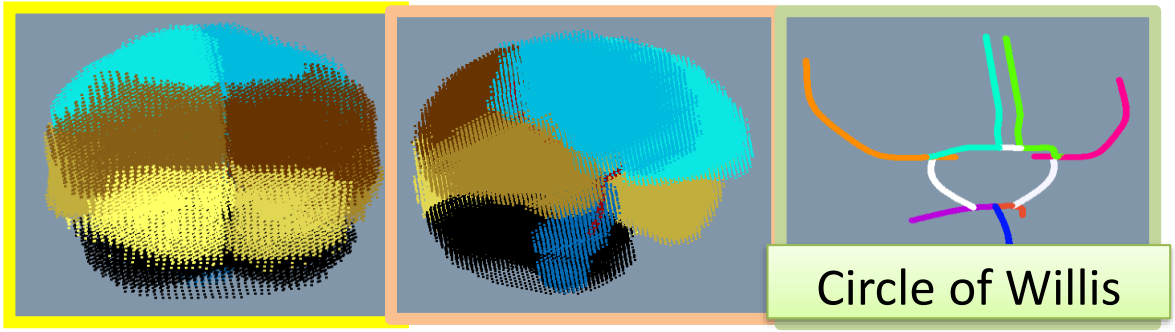
twenty-two structures segmented in the brain (about 60,000 voxels)

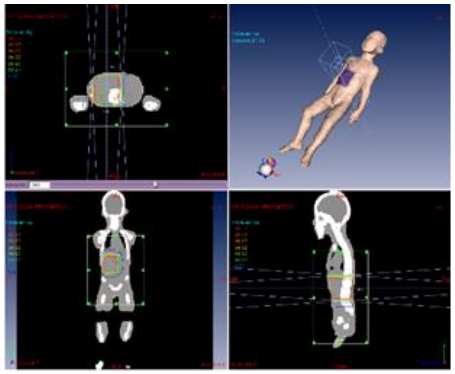
- ANTERIOR COMMUNICATING ARTERY
- INTERNAL CAROTID ARTERY R
- INTERNAL CAROTID ARTERY L
- CEREBELLUM
- BRAINSTEM
- FRONTAL LOBE R
- FRONTAL LOBE L
- PARIETAL LOBE R
- PARIETAL LOBE L
- TEMPORAL LOBE R
- TEMPORAL LOBE L
- OCCIPITAL LOBE R
- OCCIPITAL LOBE L
- BASILAR ARTERY
- ANTERIOR CEREBRAL ARTERY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

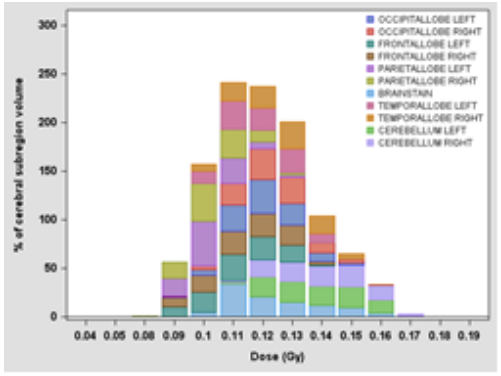










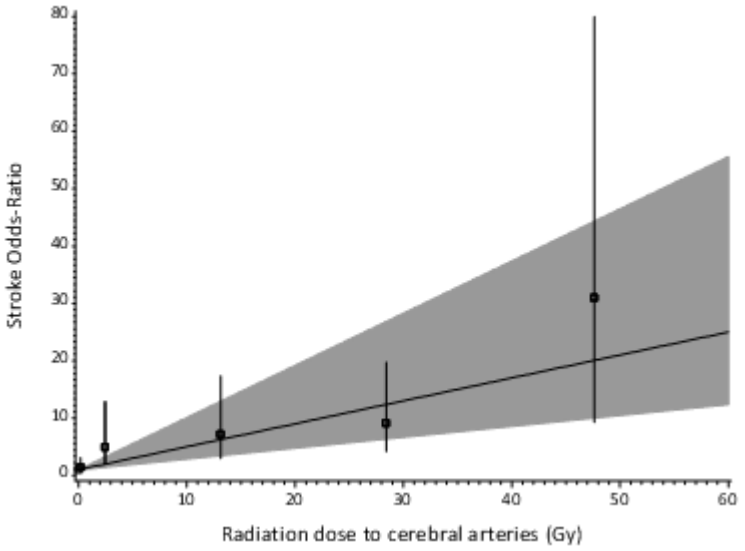


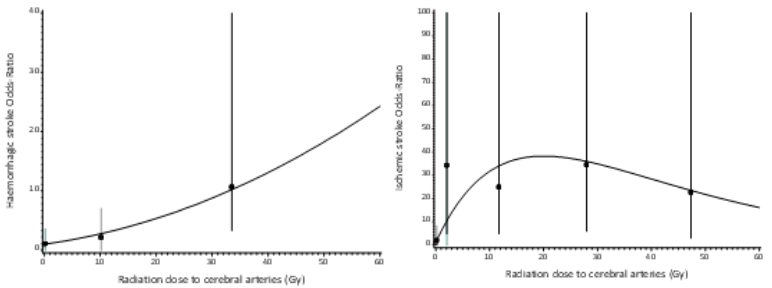
Cerebrovascular risk

- 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.

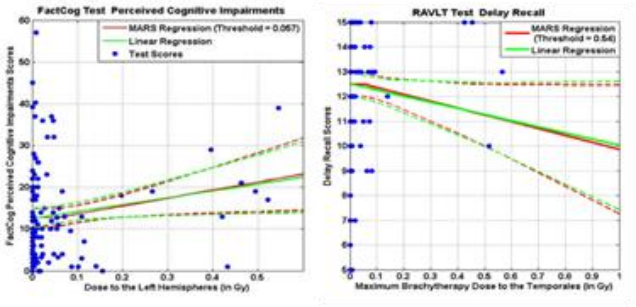
- In a linear model, the Excess of Odds Risk at 1Gy being estimated to EOR/Gy = 0.49 (95%CI: 0.22 to 1.17).



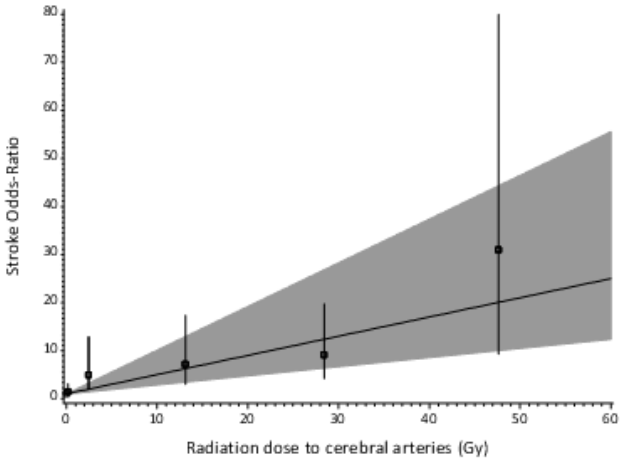


Epidemiology conclusions

- 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.



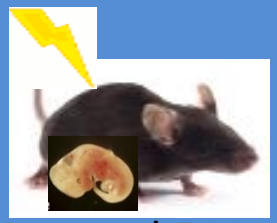
- Dose-dependent increase in cerebrovascular complications several years after exposure in FCCSS and BCCSS
  - The Excess of Odds Ratio (EOR) of stroke per Gy of average radiation dose to the cerebral arteries, was equal to EOR/Gy = 0.49 (95% CI: 0.22 to 1.17) in a linear model.
  - This data is in line with A-bomb and Mayak cohorts regarding cerebrovascular disease, increasing thus the statistical power.



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

CEREBRAD concept and structure

Human epidemiology
WP2

- cognitive dysfunctions in individuals exposed in utero or during childhood
- cerebrovascular diseases detected later in life from childhood cancer survivors

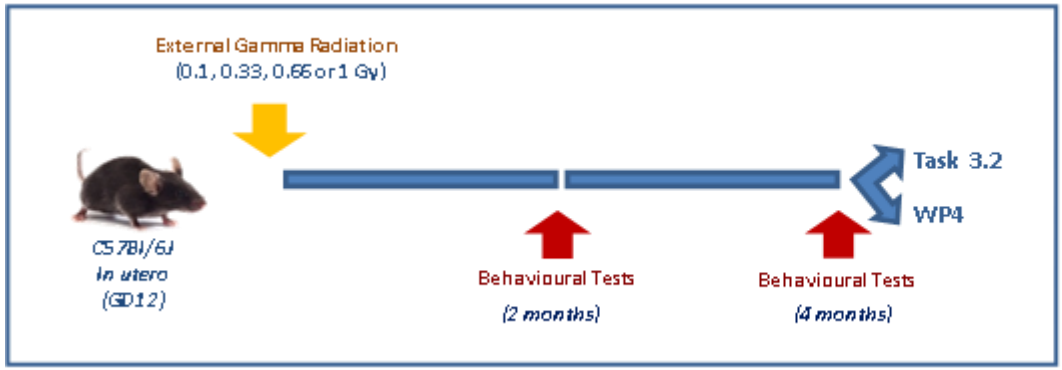
Animal studies
WP3

- cognitive abilities and effects during in utero and neonatal brain development
- interplay between the vascular and central nervous system (BBB)

Mechanistic studies
WP4

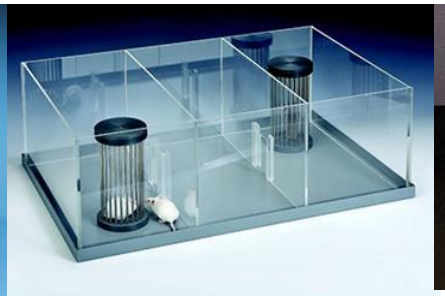
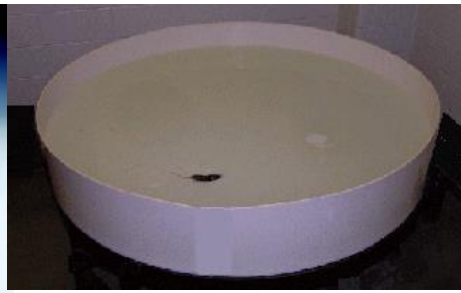
- determine the initial direct events induced by low-dose ionizing radiation
- To identify cellular and molecular fingerprints of late disease occurrence

Behavioral testing



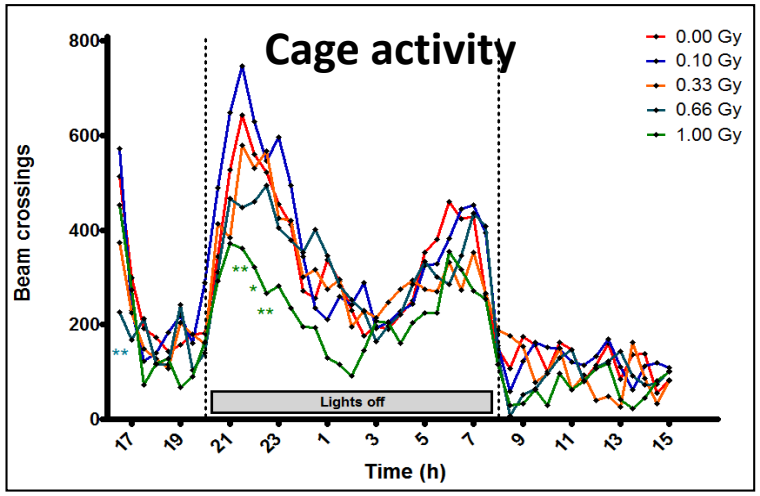
- **Behavioral test battery**

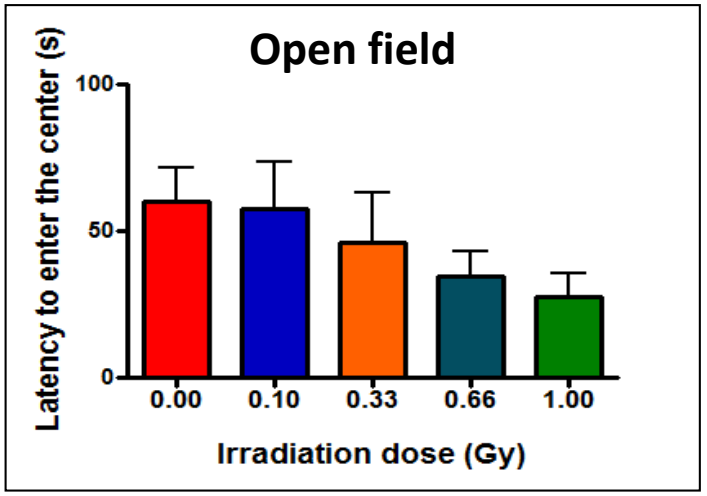


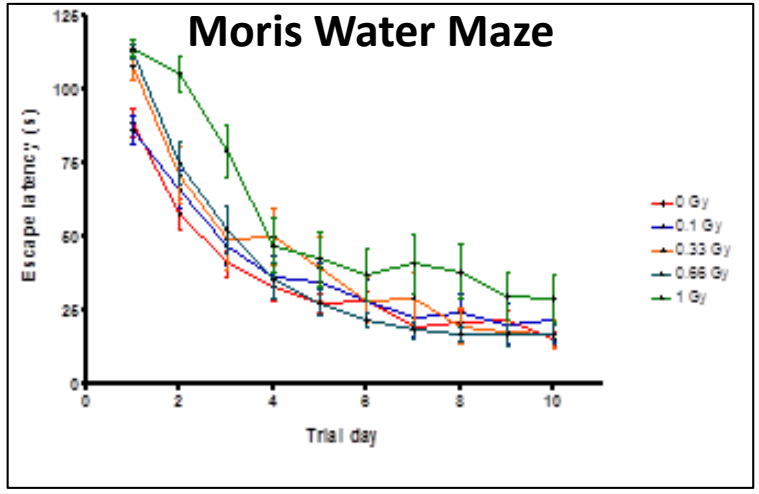




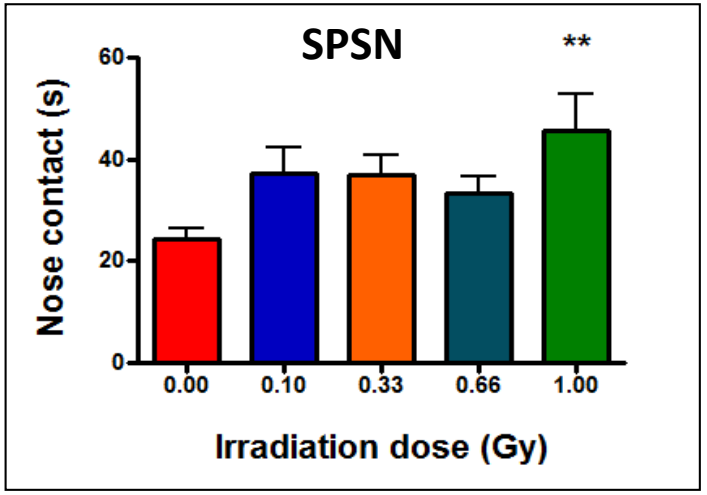
- Neuromotor tests: Cage activity, Rotarod, Gait analysis
- Exploratory tasks: Open field, Social exploration, Elevated plus maze, Sociability/preference for social novelty
- Learning & memory: Morris water maze, Contextual fear conditoning

Behavioral testing





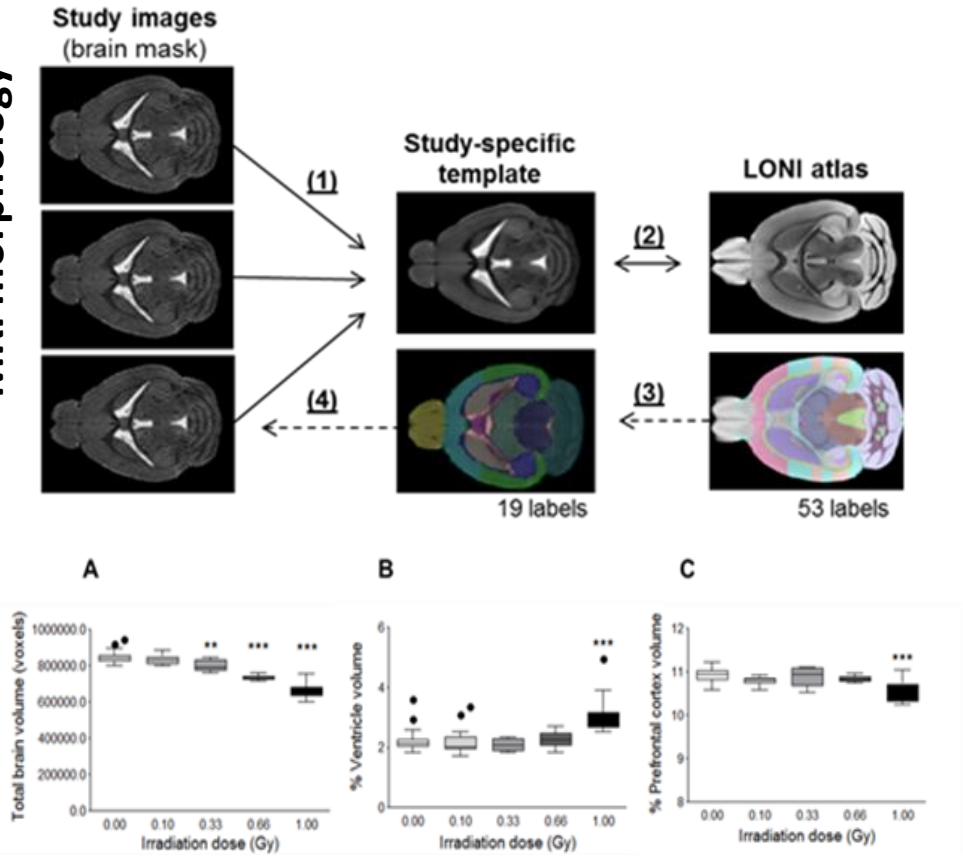




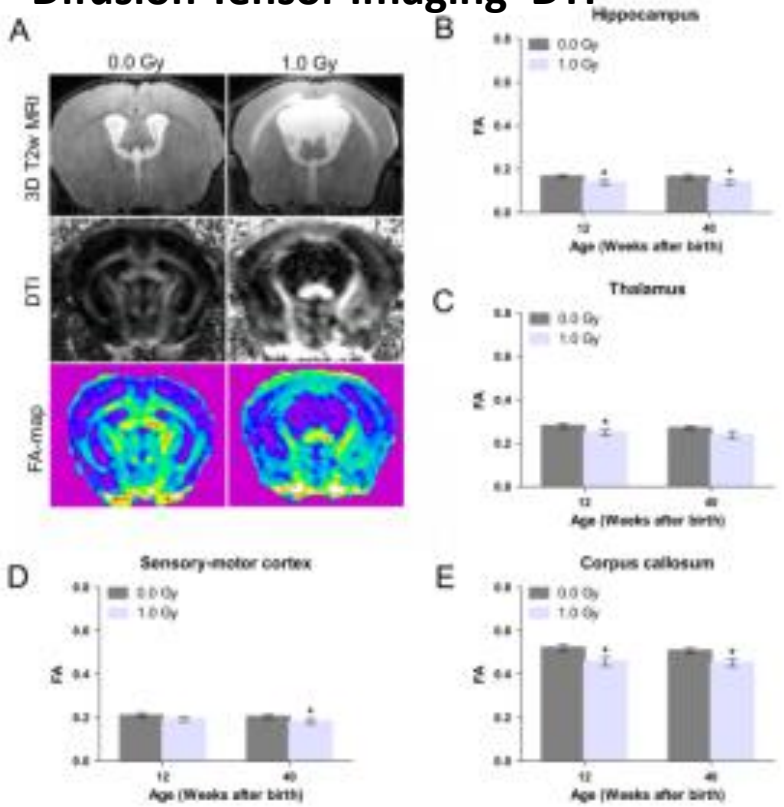
Prenatally irradiated animals exhibit reduced anxiety and increased sociability-related behavior

Magnatic Resonance Imaging 'MRI'

MRI morphology



Difusion Tensor Imaging 'DTI'



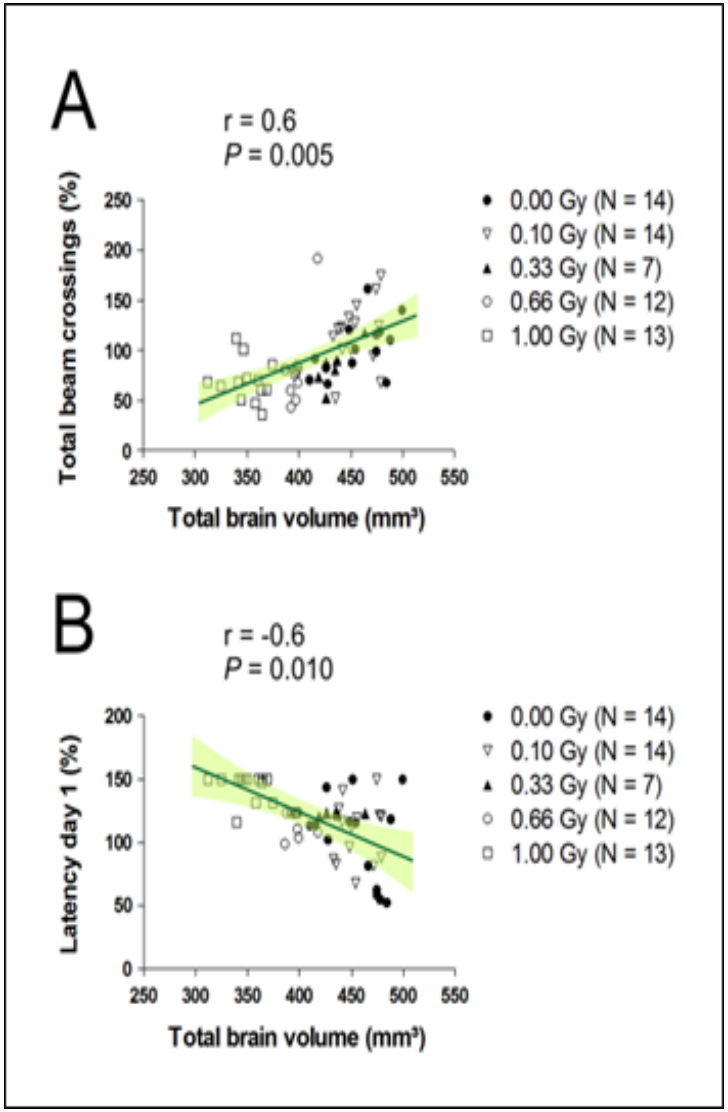
Reduction in cortical thickness and increase in ventricle size and reduced FA values (in ≠ regions)

Correlation studies

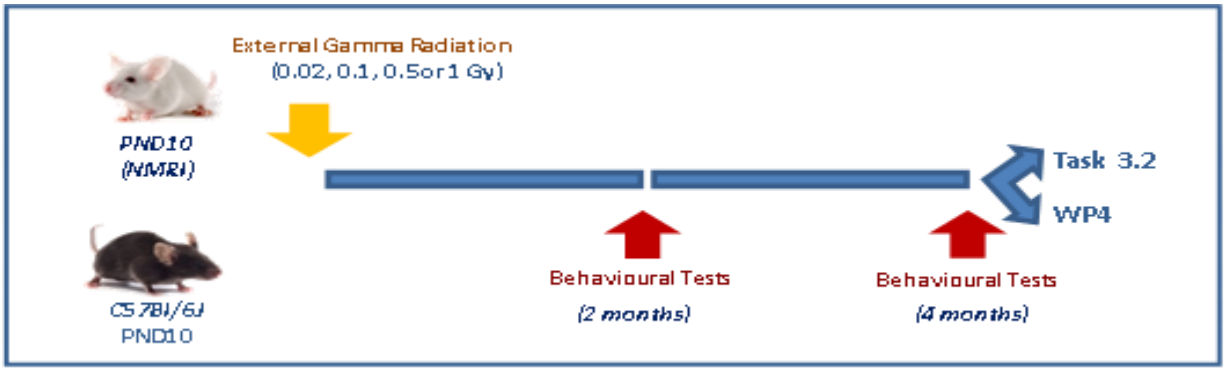
Correlations between behavioural variables and MRI-based volumetric data.

	Volume (mm³)					
	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

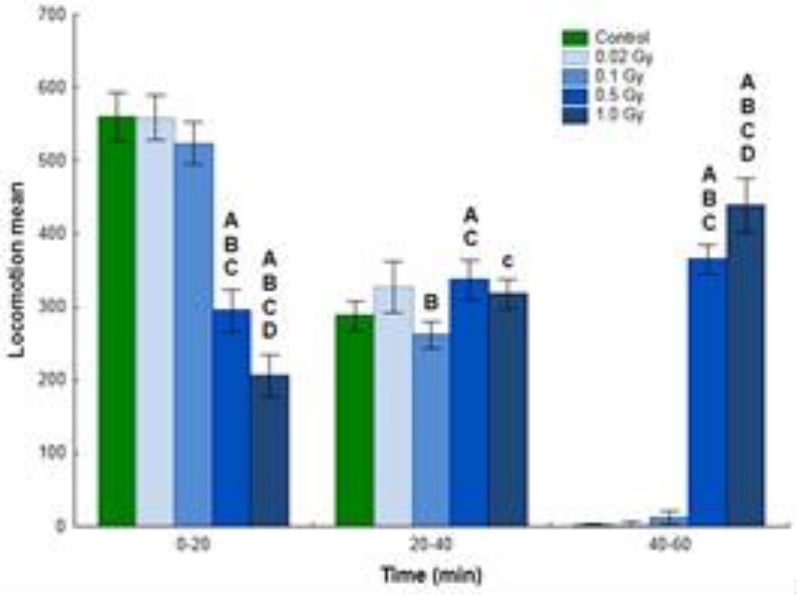
- Dose-dependent changes in activity, social behaviour, anxiety-related exploration and spatio-cognitive performance in mice exposed to 0.10 Gy
- Microcephaly from 0.33 Gy onwards, with deviations in regional brain volumes
- Whole-brain volume, as well as relative ventricle and prefrontal cortex volume,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



Postnatal exposure

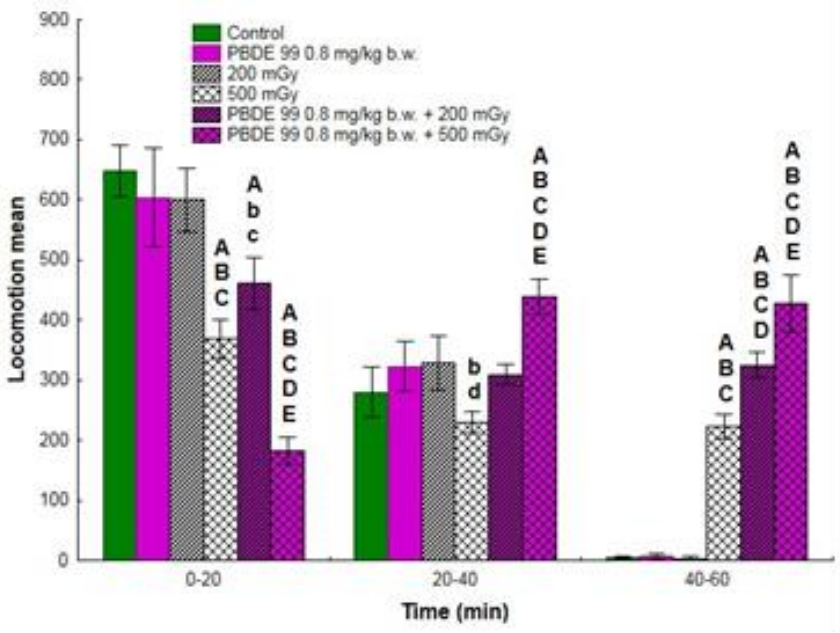


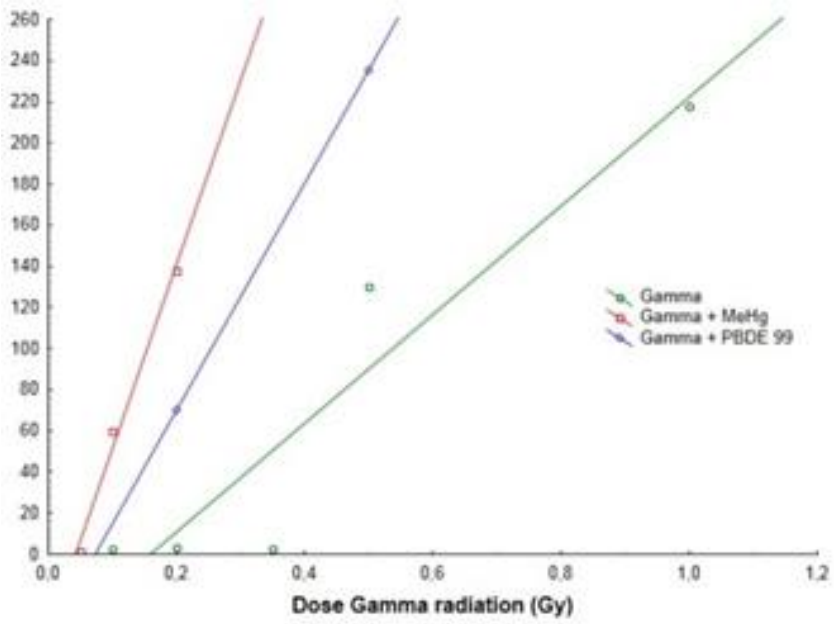
- Significant behavioural deviation was seen in male mice exposed to 0.5 and 1.0 Gy
- Persistent changes observed both at 2 and 4 Month
- Similar effects were seen in female mice as observed for males
- This study indicates also a similar reaction to gamma radiation in the C57 black strain as in NMRI strain



Co-exposure study

- Human being are indirectly or directly exposed to IR as well as environmental and social toxicants.
- Epidemiological studies indicate that exposure to IR and environmental toxicants during early human development can have deleterious effects on cognitive development
- External and internal exposure to IR, together with persistent environmental toxicants (pentabromodiphenyl ether (PBDE 99) and bisphenol–A (BPA)), metals (methylmercury (MeHg)), pesticides (paraquat) and social drugs (nicotine)



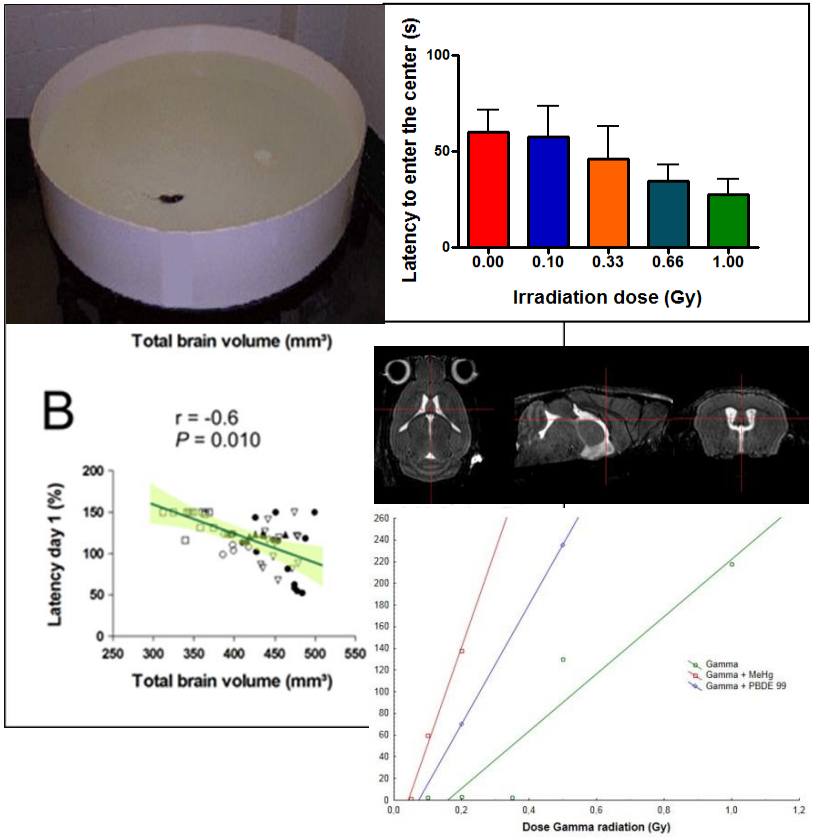


IR-induced blood–brain barrier ‘BBB’ damage

- (BBB) breakdown has been reported in several studies to explain the acute radiation syndrome and the delayed brain radiation injury following radiotherapy clearly occurring at high doses (>10 or 20 Gy)
- In CEREBRAD Local brain irradiations at young (P10) and adult (W10) have shown acute endothelial cell activation in the cortex, hippocampus and cerebellum only in W10 irradiated mice
- A higher sensitivity of older mice to radiation induced acute inflammatory reactions compared to young animals (age-related effect)
- Unfortunately the large variation and the small number of animals used for these experiments, prevent to draw final conclusions. Requiring thus further validation

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.
- MRI data showed morphological changes that might be linked with the functional behavioral impairments (as shown for other mental disorders e;g; schizophrenia)
- Postnatal co-exposure with environmental toxicants (such as MeHg, nicotine and PBDE) showed defects at a dose below 0.1 Gy.



In all, CEREBRAD data confirms the linearity of the dose-response curve and indicate there might be no threshold below which no effects are observed.

CEREBRAD concept and structure

Human epidemiology
WP2

- cognitive dysfunctions in individuals exposed in utero or during childhood
- cerebrovascular diseases detected later in life from childhood cancer survivors

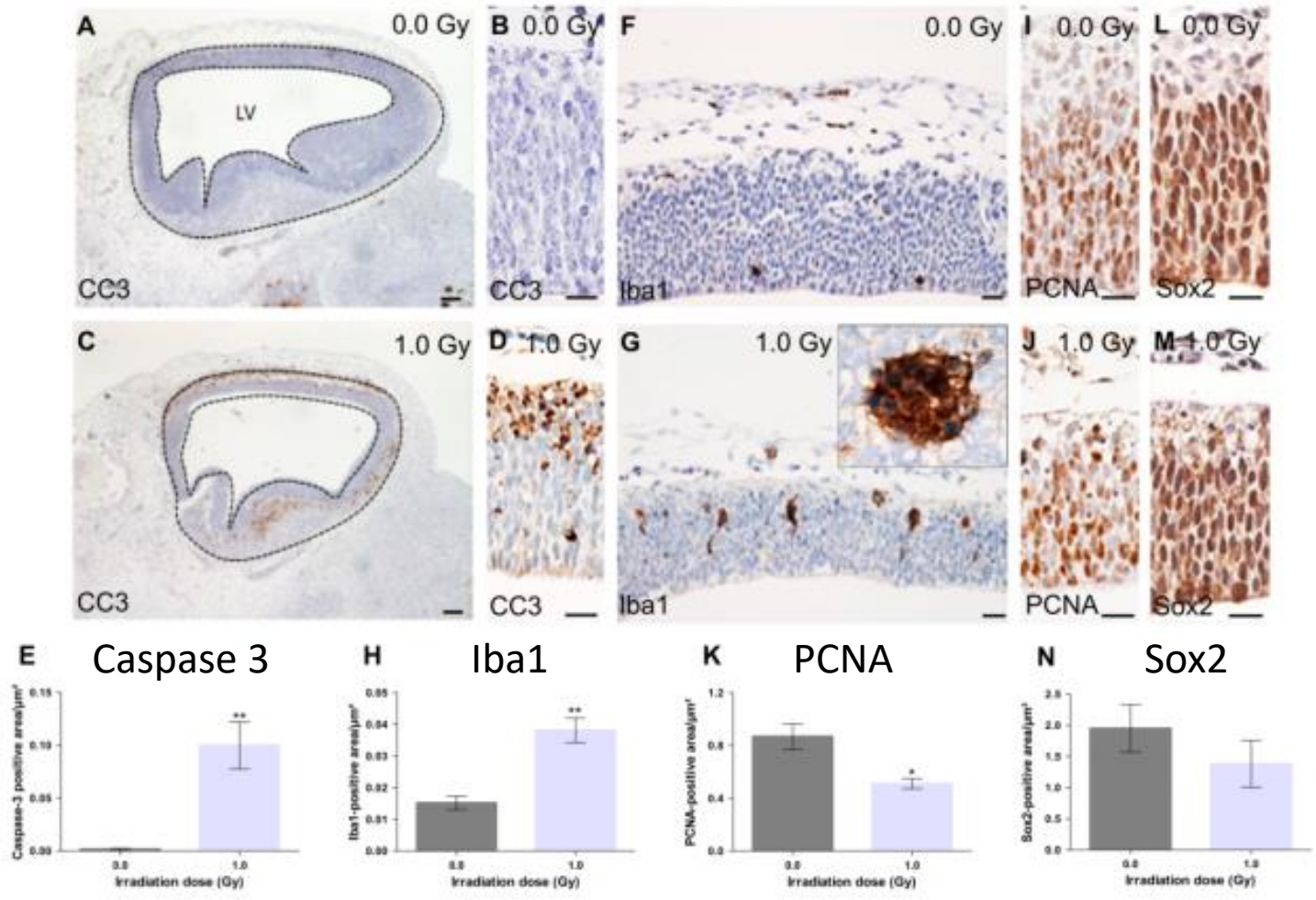
Animal studies
WP3

- cognitive abilities and effects during in utero and neonatal brain development
- interplay between the vascular and central nervous system (BBB)

Mechanistic studies
WP4

- determine the initial direct events induced by low-dose ionizing radiation
- To identify cellular and molecular fingerprints of late disease occurrence

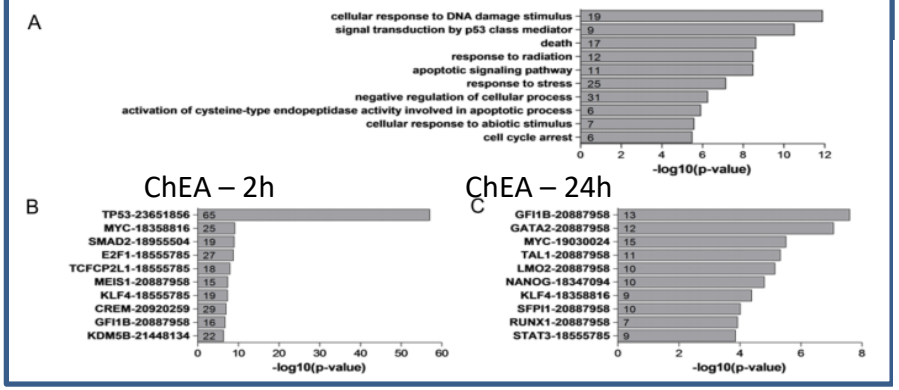
Early events



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

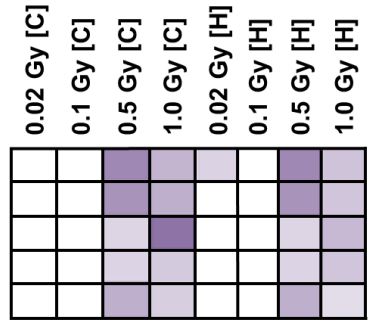
Omics analysis of brain exposure to IR

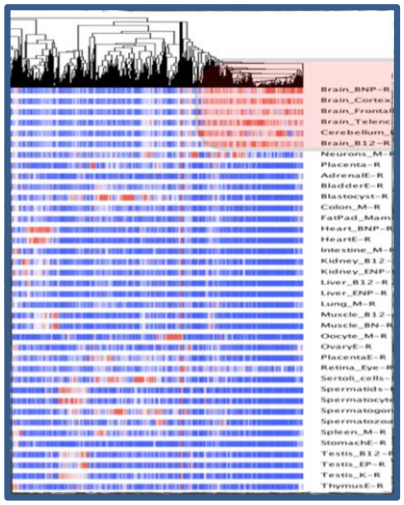
Functional enrichment analysis

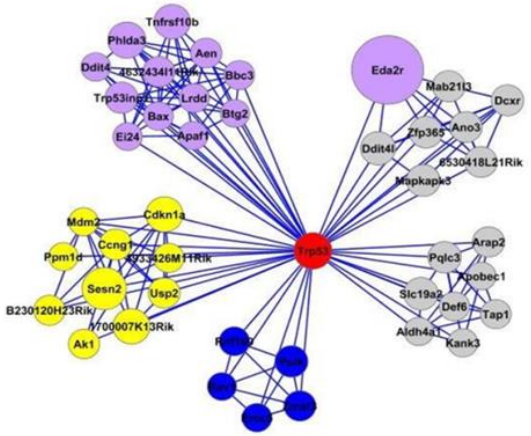


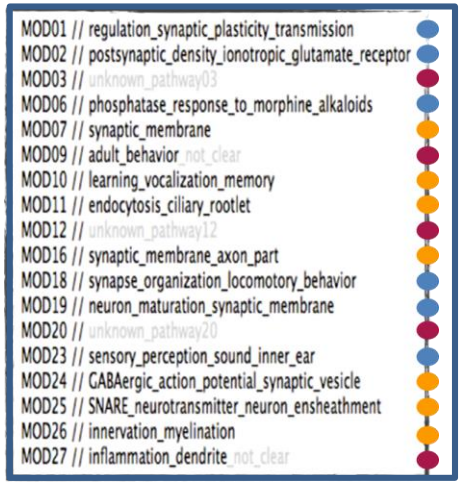
Mass-spectrometry based proteomics of cortex [C] and hippocampus [H]

Colour represents significant



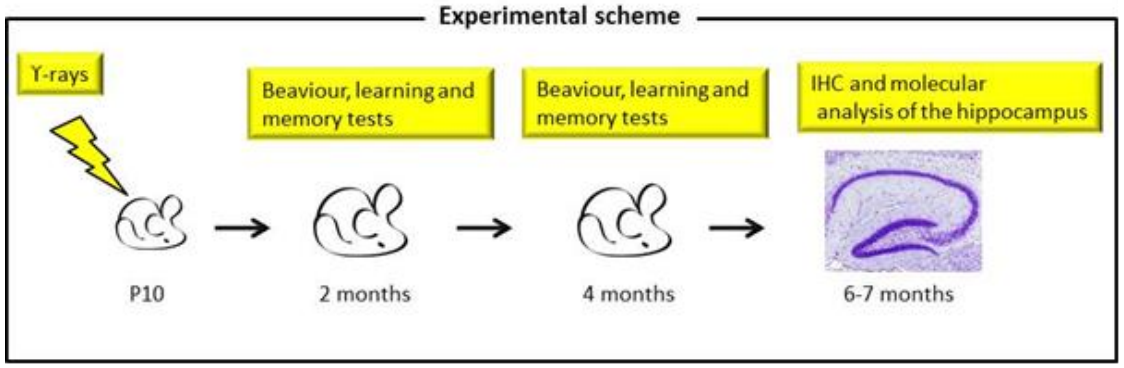


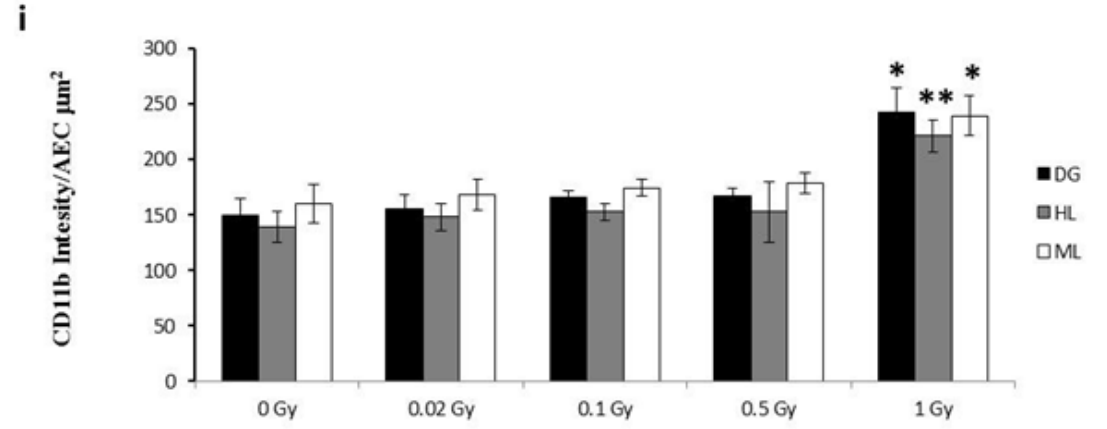


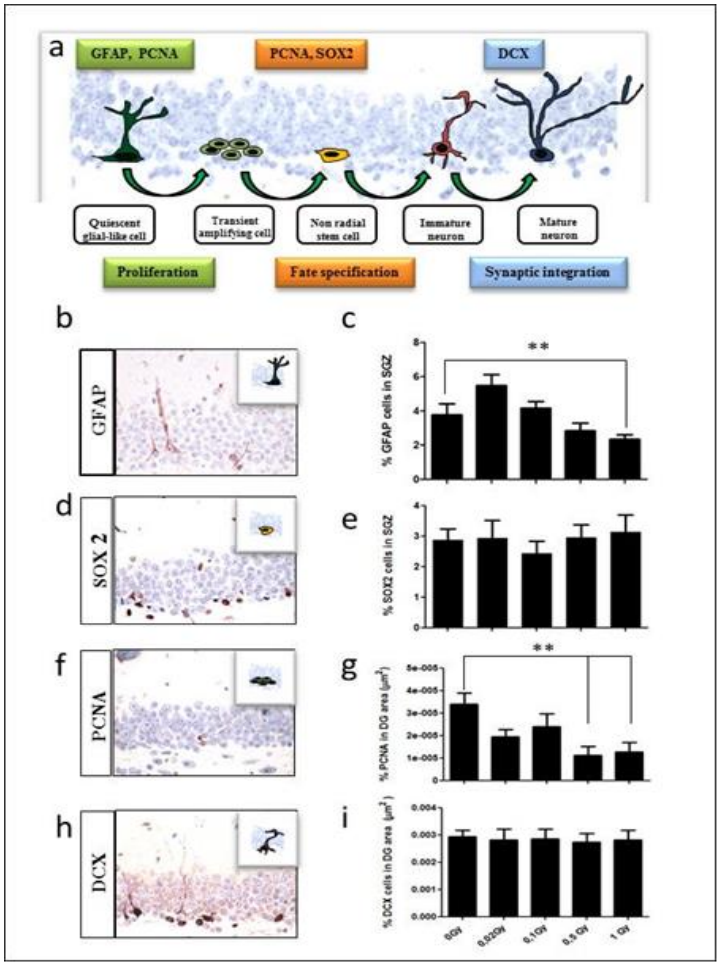


Early activation of p53-responsive genes and late effect on synaptic plasticity and neurogenesis

Late effect on hippocampal neurogenesis



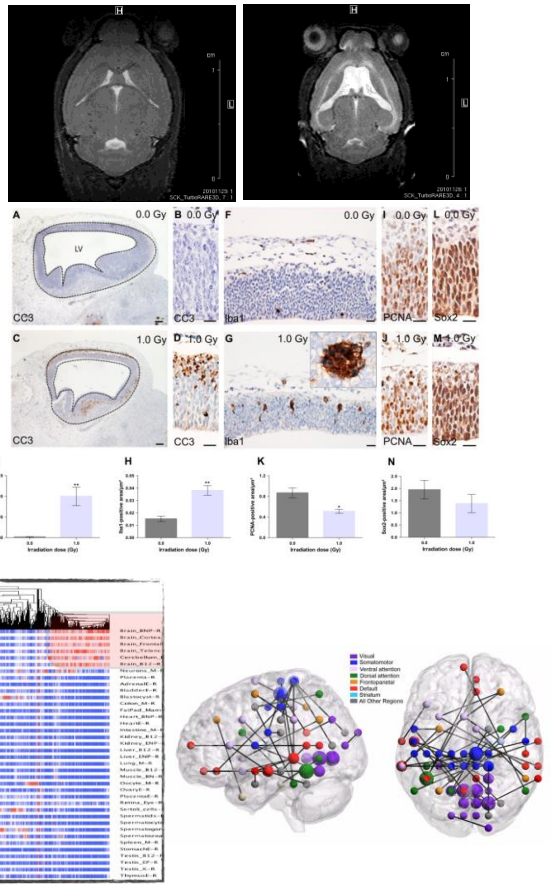




Significant reduction in proliferation (GFAP, PCNA) at 7 month after exposure

Conclusion

- Massive apoptosis in the brain and induction of p53 stress-related neurogenic targets are the major early events identified after in utero exposure to radiation
- Persistent morphological changes at adult age (microcephaly-like phenotype, enlarged ventricles) leading to cognitive impairments
- Cognitive dysfunction appeared to be 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

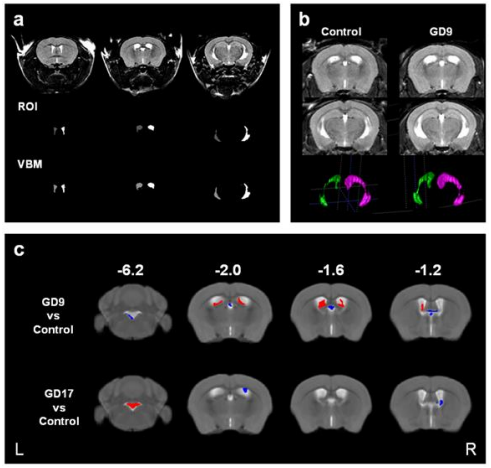
Conclusion

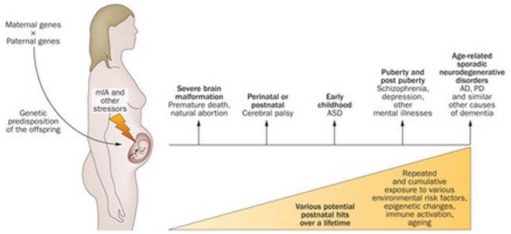
- Similar effects to IR have been observed for maternal alcohol intake on the neuropsychological development of the offspring known as Foetal Alcohol Spectrum Disorders (FASD) or Alcohol-Related Neuro-developmental Disorder (ARND)

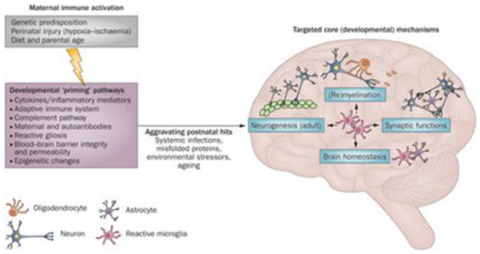
- Maternal immune activation is an environmental risk factor for brain and behavior change relevant to schizophrenia, causing marked enlargement of lateral ventricles in adulthood

- Infectious exposure during pregnancy is associated with schizophrenia, epilepsy or autism and cerebral palsy in the progeny

- Transcriptomic changes in prenatal radiation exposed brain is highly similar to ZIKV infection, including induction of p53 gene and its target genes involved in premature neuron differentiation







E&T and Dissemination

- Three students day organized during the annual meetings bringing together PhD students, post-docs and more experienced scientists
- A special mentoring program has been started and this tool has been used to improve career development of all junior scientists within the consortium
- The students have been given the possibility to spend time in other laboratories within the consortium to learn new methods. Some of them participated to the EU master course organized by DoReMi
- Three annual meetings jointly with Procardio
- Active website regularly updated with project results
- More than 30 peer reviewed international articles
- Several presentations at international events (IRPA14, Melodi, RPW)
- Several posters at various events (EU projects, Melodi, national and international societies)
- A satellite meeting "CEREBRAD and beyond" was organized at RPW 2016 in Oxford UK
- …..

SEVENTH FRAMEWORK PROGRAMME

CEREBRAD

Cognitive and Cerebrovascular Effects Induced by Low Dose Ionising Radiation

www.cerebrad-fp7.eu

Thank you for your attention

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STUDIECENTRUM VOOR KERNENERGIE
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RCRM

Institut de cancérologie GUSTAVE ROUSSY

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