CEREBRAD & BEYOND

Role of epigenetic events in late effects after prenatal and early postnatal exposure to IR

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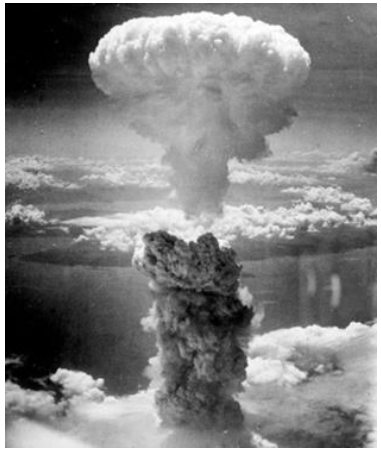
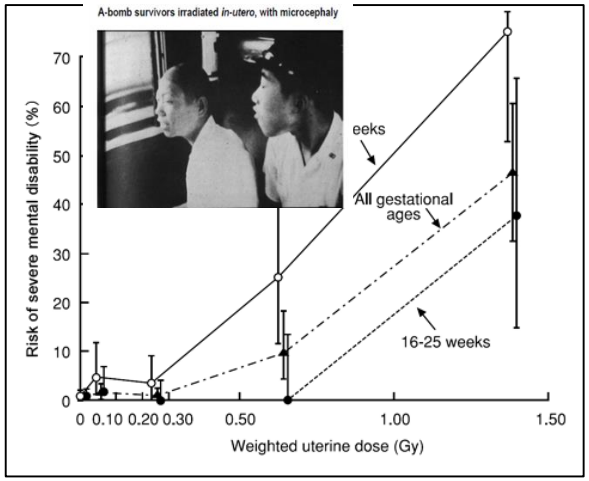
Radiation-induced Brain deffects





- Mental retardation
- Microcephaly
- Low IQ values
- …

- Mental retardation
- Lower intellectual performances
- Stroke

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

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

Cerebrovascular late effects “Human data”

Childhood Cancer survivor studies

Cancer + RT At age < 7 years

FCCSS
BCCSS
NCCSS

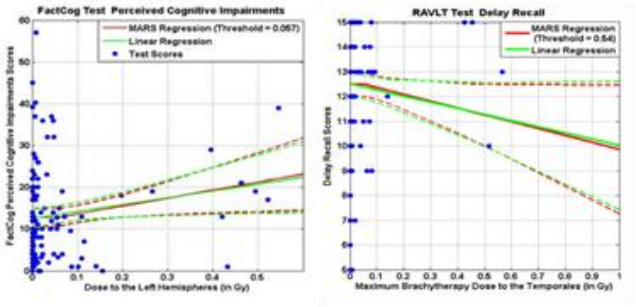
233 cases and 233 matched controls

Stroke in average at 34 years old

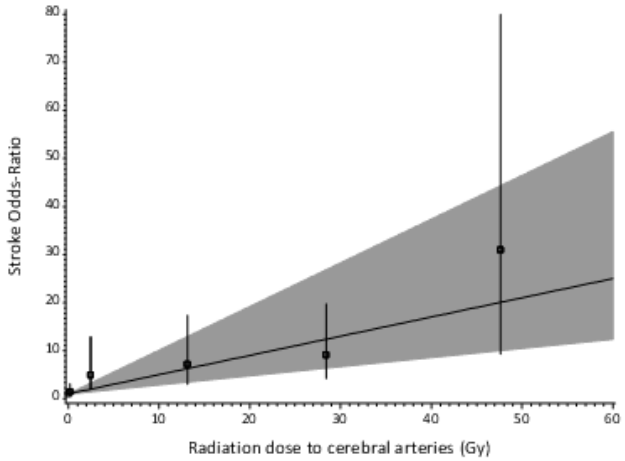
Average 27 y

Epidemiology results

- 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.



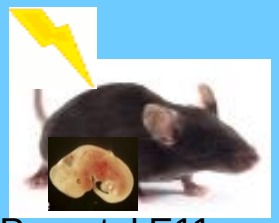
- Dose-dependent increase in cerebrovascular complications several years after exposure in FCCSS and BCCSS
  - The Excess of Odds Ratio (EOR) of stroke per Gy of average radiation dose to the cerebral arteries, was equal to EOR/Gy = 0.49 (95% CI: 0.22 to 1.17) in a linear model.
  - This data is in line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

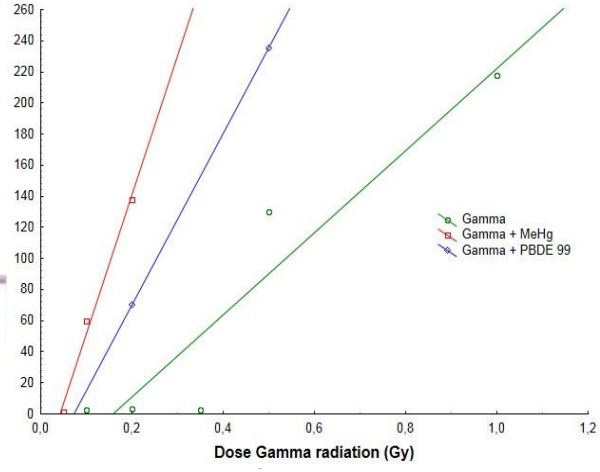
Late effects

1M 2M 4M 6M

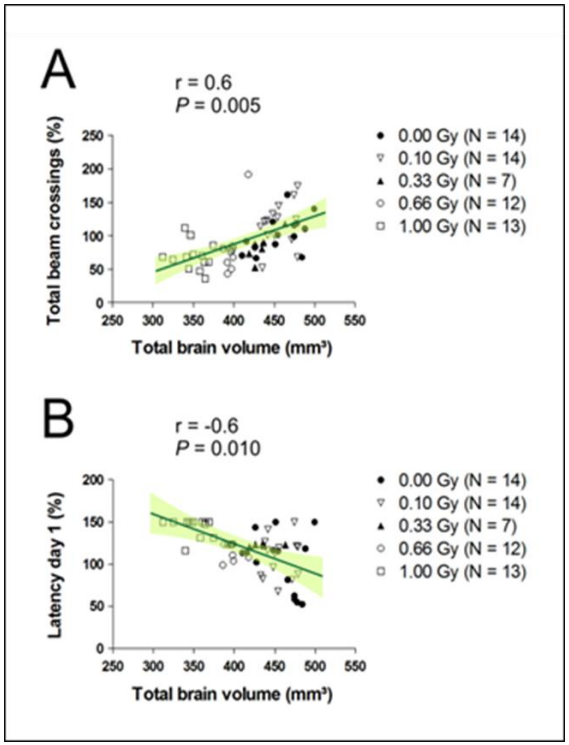
Behavior
Molecular Imaging
Adult neurogenesis
Mitochondrial Redox
Transcriptomic
Proteomic

Functional and structural changes

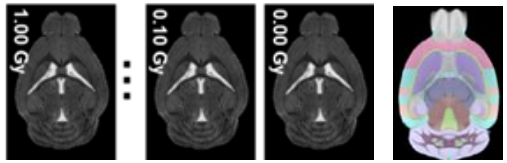
Behavioral test battery



Correlation analysis



MRI structural assessments



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.

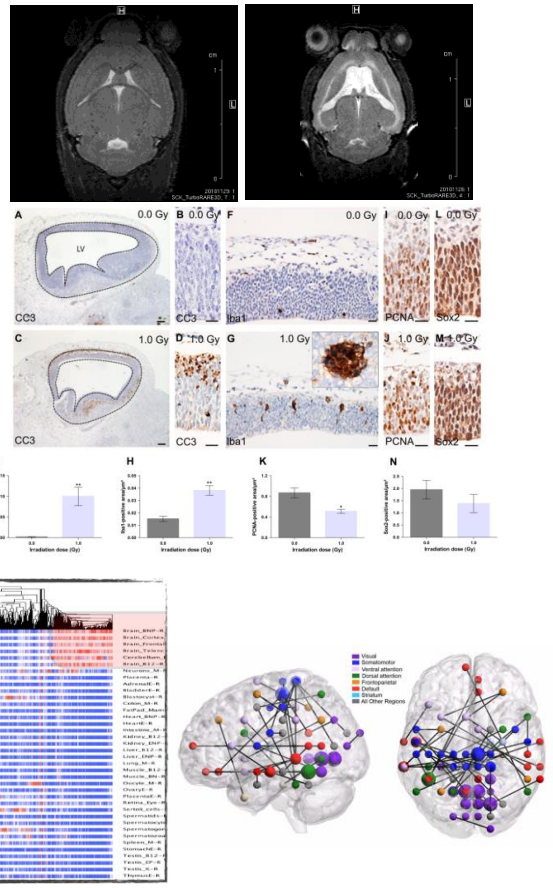
	Volume (mm³)					
	Whole brain	Ventricles	Posterior cerebral cortex	Frontal cortex	Striatum	Cerebellum
Cage activity Beam crossings (%)	0.6**	-0.3	0.15	0.028	0.3	0.24
Elevated plus maze Open/total (%)	-0.21	0.5**	-0.13	-0.28	-0.08	-0.09
Elevated plus maze Open/closed (%)	-0.20	0.5**	-0.12	-0.27	-0.09	-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

T. Verreet et al. Front Behav Neurosci. 2016

CEREBRAD

CEREBRAD biological outcome

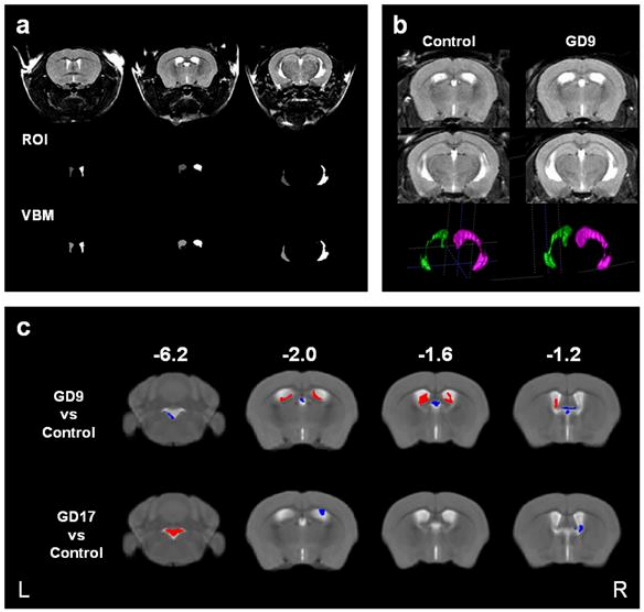
- Massive apoptosis in the brain and induction of p53 stress-related neurogenic targets are the major early markers identified after in utero exposure to radiation
- Persistent morphological changes at adult age (microcephaly-like phenotype, enlarged ventricles) leading to cognitive impairments
- Cognitive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

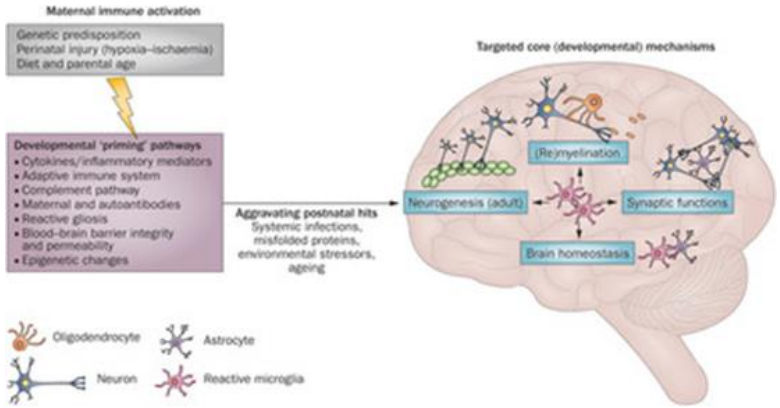


A dynamic interaction between multiple cell types (i.e. neurons, microglia and astrocytes) and the processing of the late response could in part be mastered through epigenetic events, requiring thus additional future investigations.

Similar studies

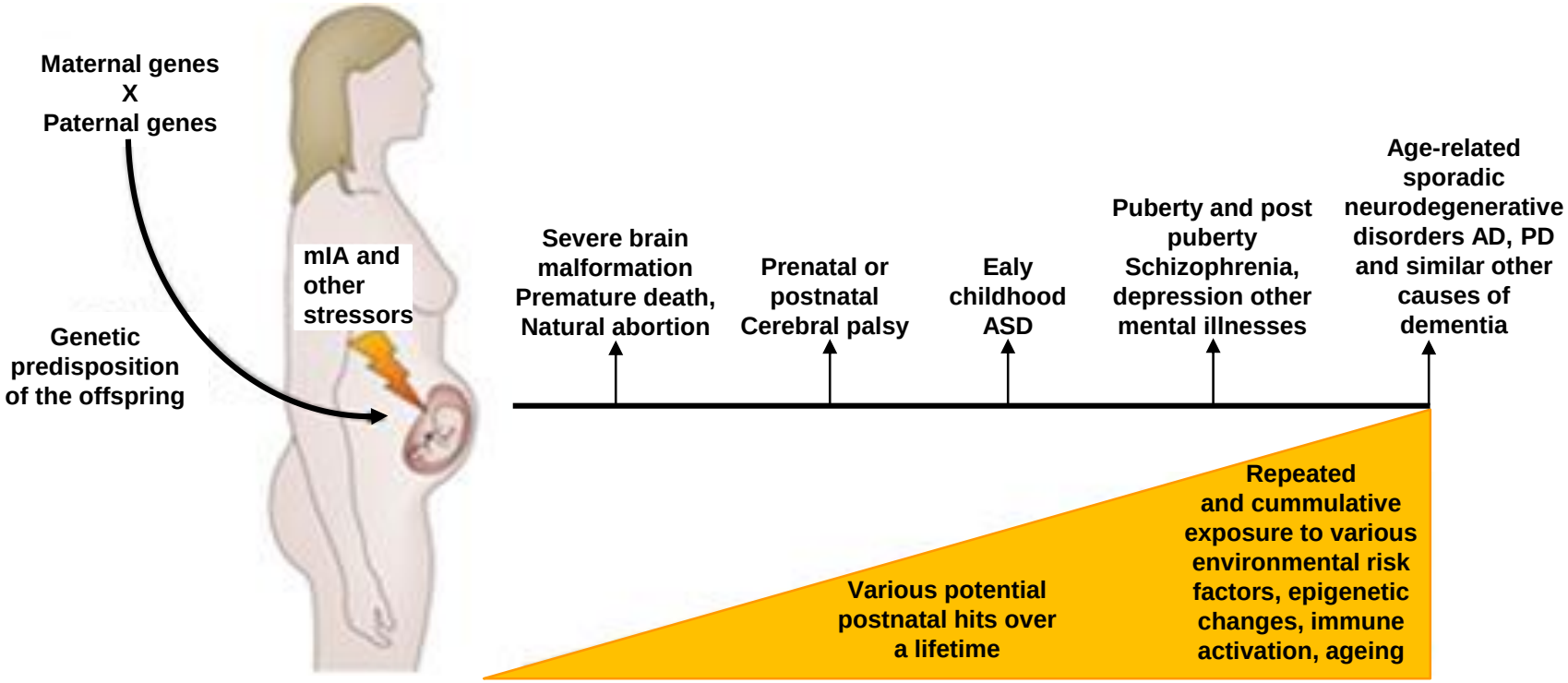
- Similar effects to IR have been observed for maternal alcohol intake on the neuropsychological development of the offspring known as Foetal Alcohol Spectrum Disorders (FASD)
- Infectious exposure during pregnancy is associated with schizophrenia, epilepsy or autism and cerebral palsy in the progeny
- 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





Maternal Immune Activation 'MIA'

Early life stress such as maternal immune activation and exposure to other stressors are environmental risk factor for brain and behavior change relevant to schizophrenia, and other neurological impairments



Maternal Immune Activation 'MIA'

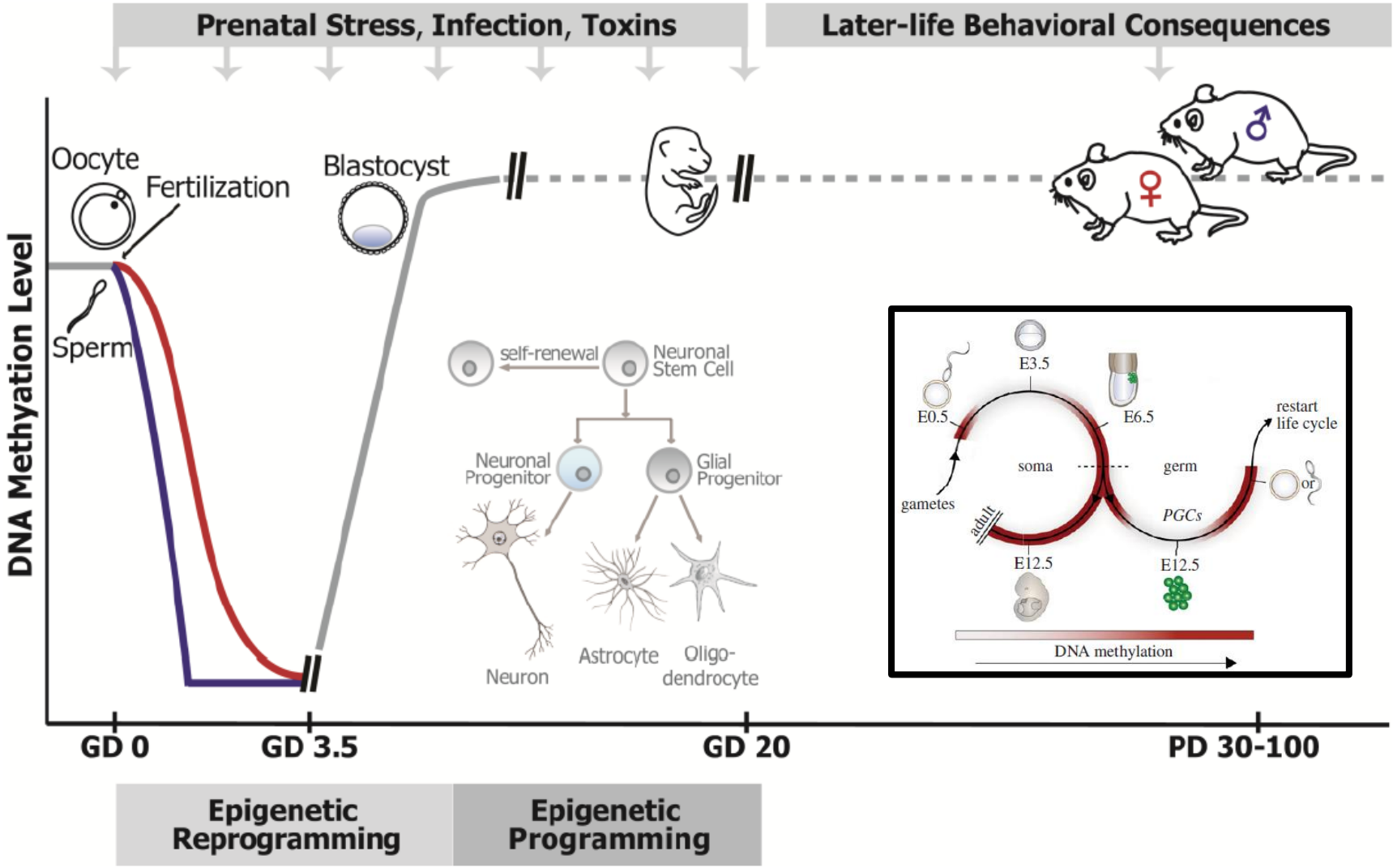
- Prenatal adverse environments, such as maternal stress, toxicological exposures, and viral infections, can disrupt normal brain development and contribute to neurodevelopmental disorders

- Alterations in environmental conditions during development produce long-lasting and often permanent changes in the structure and function of the brain that reflect altered expression of key genes involved in neuronal development and plasticity

- Increasing evidence shows that these short- and long-term effects of prenatal exposures on brain structure and function are mediated by epigenetic mechanisms.

These processes regulate gene activity, and determine when a gene is expressed throughout the course of a lifetime.

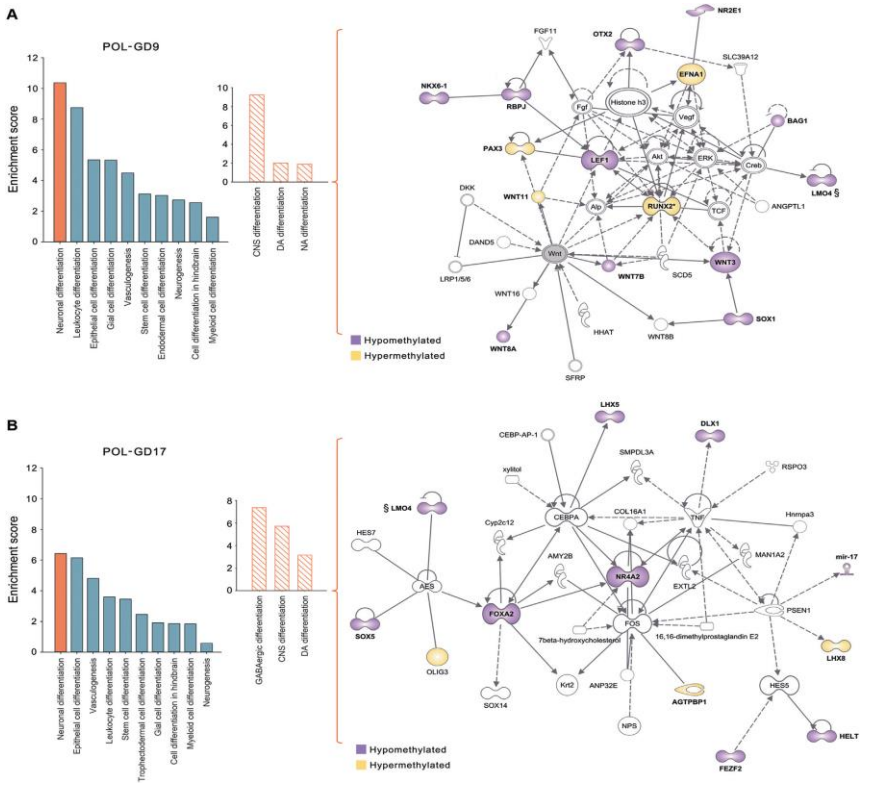
Epigenetic reprograming



Maternal Immune Activation 'MIA'

The study investigated GD9 and GD18 and provides a possible link between prenatal immune activation and widespread DNA methylation changes found in neurodevelopmental disorders including schizophrenia.

- Prenatal immune activation was induced by maternal treatment at midle (GD9) or late (GD18) gestation
- Adult offspring of immune-challenged mothers displayed behavioural and cognitive impairments
- Hyper- and hypomethylated CpGs were observed at numerous loci and corresponding alterations were associated with expression of genes relevant for gamma-aminobutyric acidergic 'GABAergic' differentiation and signaling (e.g., Dlx1, Lhx5, Lhx8), Wnt signaling (Wnt3, Wnt8a, Wnt7b), and neural development (e.g., Efnb3, Mid1, Nlgn1, Nrxn2).

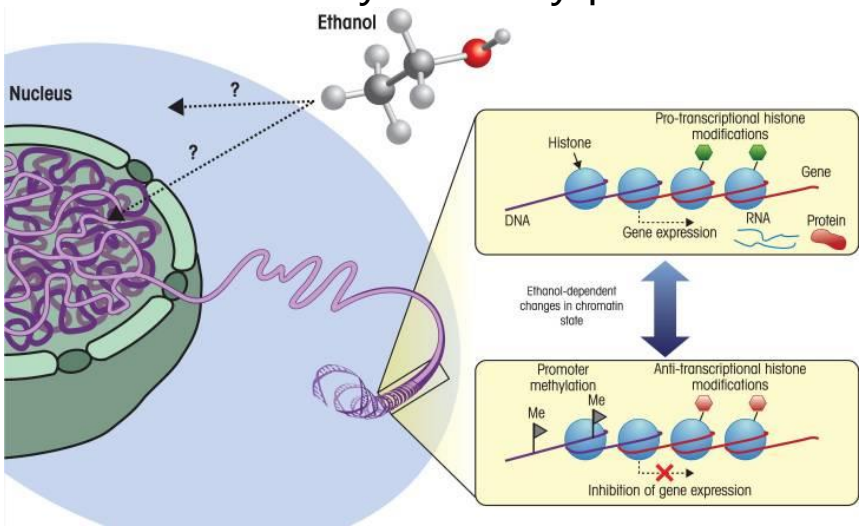


prenatal immune activation seems to interfere with epigenetic programs that are crucial for normal brain maturation at different stages of brain development

Juliet Richetto, Renaud Massart, Ulrike Weber-Stadlbauer, Moshe Szyf, Marco A. Riva, and Urs Meyer. Genome-wide DNA Methylation Changes in a Mouse Model of Infection-Mediated Neurodevelopmental Disorders. Biological Psychiatry February 1, 2017; 81:265–276

Foetal Alcohol Spectrum Disorders (FASD)

- Alcohol exposure during development can cause variable neurofacial deficit and growth retardation known as fetal alcohol spectrum disorders (FASD).
- Individuals prenatally exposed to alcohol often have impaired spatial working memory (SWM).
- Cortical thickness, neuroepithelial proliferation, and neuronal migration and maturity were found to be deterred in the developing brain from alcohol exposure during gestation
- localized alterations in neural activity, aberrant fronto-parietal network synchrony, and poor coordination of neural responses with regions outside of this network may help explain SWM deficits in individuals with a history of heavy prenatal alcohol exposure.
- alcohol, is known to affect methyl donor metabolism, may induce aberrant epigenetic changes contributing to FASD



Kaminen-Ahola, N.; Ahola, A.; Maga, M.; Mallitt, K.A.; Fahey, P.; Cox, T.C.; Whitelaw, E.; Chong, S. Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model. PLoS Genet. 2010, 6, e1000811.

Bisphenol A 'BPA' (endocrine disruptor)

- Bisphenol A (BPA) is an estrogenic endocrine disruptor widely used in the production of plastics.
- BPA exposure induces persistent, largely sex-specific effects on social and anxiety-like behavior.
- in utero bisphenol A (BPA) exposure is a model of environmental exposure shown to disrupt neurodevelopment and exert long-term effects on behavior in animals and humans.
- prenatal BPA induces lasting DNA methylation changes in the transcriptionally relevant region of the Bdnf gene in the hippocampus and blood of BALB/c mice and that these changes are consistent with BDNF changes in the cord blood of humans exposed to high maternal BPA levels in utero
- BDNF expression and DNA methylation are altered in several psychiatric disorders that are associated with early-life adversity, including depression, schizophrenia, bipolar disorder, and autism
- BDNF DNA methylation in the blood may be used as a predictor of brain BDNF DNA methylation and gene expression as well as behavioral vulnerability induced by early-life environmental exposure (adversity)

Epigenetic biomarkers are good candidates because early life exposures can leave lasting marks on the epigenome of various tissues and these marks could be detected before the disease is fully developed.

Kundakovic, M.; Gudsnuk, K.; Herbstman, J.B.; Tang, D.; Perera, F.P.; Champagne, F.A. DNA methylation of BDNFas a biomarker of early-life adversity. Proc. Natl. Acad. Sci. USA 2015, 112, 6807–6813.

Kundakovic, M.; Gudsnuk, K.; Franks, B.; Madrid, J.; Miller, R.L.; Perera, F.P.; Champagne, F.A. Sex-specific epigenetic disruption and behavioral

Methyl mercury ‘meMHg’

- perinatal exposure to methylmercury (MeHg) causes persistent changes in learning and motivational behavior in human and mice.
- Behavioral alterations are associated with a decrease in brain-derived neurotrophic factor (BDNF) mRNA in the hippocampal dentate gyrus and fluoxetine treatment restores BDNF mRNA expression
- MeHg-exposure induces long-lasting repressive state of the chromatin structure at the BDNF promoter region, in particular DNA hypermethylation,
- antidepressant treatment with Fluoxetine significantly up-regulated H3 acetylation at the BDNF promoter IV in MeHg-exposed mice, but not in control animals, overcoming the repressive chromatin state and contributing to the restoration of BDNF mRNA levels and recovery of behavior in the FST

58. Onishchenko, N.; Karpova, N.; Sabri, F.; Castren, E.; Ceccatelli, S. Long-lasting depression-like behavior and epigenetic changes of BDNF gene expression induced by perinatal exposure to methylmercury. J. Neurochem. 2008, 106, 1378–1387.

Early life stress (ELS)

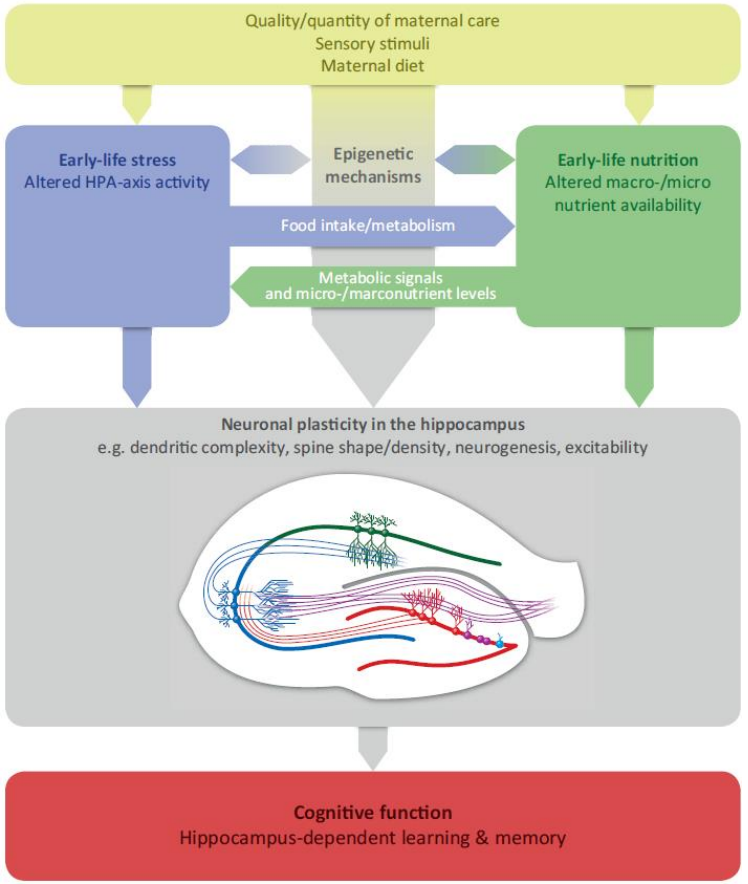
Early-life stress lastingly affects adult cognition and increases vulnerability to psychopathology, but the underlying mechanisms remain elusive

Hypothesis:

- Early nutritional input together with stress hormones and sensory stimuli from the mother during the perinatal period act synergistically to program the adult brain, possibly via epigenetic mechanisms.

- Stress during gestation or lactation affects the intake of macro- and micronutrients, including dietary methyl donors and/or impairs the dam's metabolism, thereby altering nutrient composition and intake by the offspring

- the hippocampus is particularly sensitive to the EL environment because it mostly develops postnatally, is highly plastic, and is rich in stress-hormone receptors.



Paul J. Lucassen, Eva F.G. Naninck, Johannes B. van Goudoever, Carlos Fitzsimons, Marian Joels, and Aniko Korosi. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. Trends in Neurosciences November 2013, Vol. 36, No. 11

Early life stress (ELS)

- Role of epigenetic mechanisms in maintaining the lasting changes in brain structure and function after adverse early-life experiences?
- How maternal **lifestyle**, genotoxic stress and intrauterine environment affect the critical developmental period and influence molecular modifications through epigenetic alterations in the offspring,
- Do the lasting alterations caused by early-life malnutrition (children) involve the same mechanisms as the early-life-stress-induced cognitive impairments and alterations in neuronal plasticity?
- Can the lasting deleterious effects of adverse early-life experiences be prevented or treated by nutrition-based intervention?

In general terms, epigenetic mechanisms would play a major role in radiation protection and contribute greatly in understanding the underlying molecular mechanisms of both cancer and non-cancer effects