Prenatal Stress: impact on Neurodevelopmental diseases
Introduction

- Adverse *in utero* events can alter the development and function of numerous physiological systems, giving rise to lasting neurodevelopmental deficits.

- In humans, untreated infection, exposure addictive drugs (EtOH, THC,…), environmental chemicals (bisphenol A,…) during pregnancy are risk factors for the occurrence of neurodevelopmental diseases in children often appearing at puberty.

- These diseases include a wide array of conditions as Autism spectrum disorder, Attention Deficit Hyperactivity disorder, Bipolar disorder…

- Fetal alcohol spectrum disorder (FASD) refers to the broad spectrum of structural, neurocognitive, physiological and behavioral abnormalities or deficits that can occur following prenatal alcohol exposure. The most severe condition is fetal alcohol syndrome (FAS), which can occur with chronic exposure to high doses of alcohol. It is associate with anatomical alterations and mental retardation.

- Prenatal stresses induce reprogramming of the normal brain developmental leading to diseases at later stages.

- Reprograming often includes epigenetic modifications which can be transmitted to the next generation, i.e. transgenerational transmission of stress.

- Neurodevelopmental alterations are now widely recognised as prominent factors for the occurrence of brain diseases.
Experimental models of prenatal stress

- There are several experimental rodent models which recapitulate the traits of the diseases observed in humans. There are:
  - Maternal immune activation (MIA):
    - LPS → bacterial infection
    - polyI:C → viral infection
  - Valproic acid
  - Methylazoxymethanol (MAM)
  - Addictive drugs, ethanol
  - Environmental toxins: pesticides, herbicides.

- Although these exposures are all detrimental to brain development, their outcomes may be different.
- In addition, the outcomes may differ according to the time of the insult.
- Some models exhibit gender specificity, others do not.
- It is sometimes hard to reconcile the data obtained with these models.
Rodent models of prenatal stress

LPS, polyI:C
EtOH, CB1R activation
Valproic acid

Read-outs:
Cognition
Behaviour
Synaptic plasticity
Biochemical and genomic modifications
Behavioural tests

- ‘Psychotic condition’: Pre-pulse inhibition (PPI) or sensorimotor gating/Social interactions
- Learning/memory: Morris’ watermaze, Y maze, Light/Dark box…
- Anxiety: elevated-plus-maze, open field
- Depression: anhedonia, forced swim
PPI (or startle reflex)

Strong aversive sound

Low intensity sound

Strong aversive sound

Decreased startle reflex when pairing a low (neutral) intensity sound with an aversive sound. This reflex is lost in ‘psychotic conditions’ including in humans.
Diminished or lost PPI reflects dysfunctional cortico-thalamic loop in the prefrontal cortex: the decreased activity of GABA interneurons leads to excessive excitation in this loop. Dopamine and 5HT are both regulating this loop.
Diminished or lost PPI in prenatally exposed animals to LPS at the adult stages. Gender specificity?
Reversal by haloperidol, an antipsychotic drug (D2 receptor antagonist).
Social behaviour

Reduced social interactions after prenatal polyI:C
Learning/memory (1)

Learning impairment (Morris’ watermaze) after LPS in utero and strong gender specificity.
Learning/memory (2)

Reduced exploration in the Y-maze after prenatal polyI:C
Loss of synaptic plasticity in the hippocampus after in utero LPS

Long-term potentiation, which is the neurophysiological mechanism of learning, including in the hippocampus is lost after prenatal in males.
Similarly, long-term depression is lost quicker with development in stressed animals than in control ones.
The loss of synaptic plasticity in the hippocampus after in utero LPS is due to specific alterations of the Glutamate- and GABA-mediated synaptic transmission. Prenatal stress (LPS) leads to decreased function of synaptic NMDA receptors which activation is required for synaptic plasticity.
Altered Glutamate and GABA-mediated synaptic transmission after prenatal stress (2)

Prenatal stress is associated with a decrease of GABA transmission (evoked and spontaneous) associated with reduced occurrence of interneurons (see next slide).
Reduced occurrence of GAD67 expressing cells in the hippocampus (CA3 area) after prenatal stress with LPS
Epigenetic modulation of GAD expression by prenatal polyI:C

Reduced expression of GAD65 and GAD67 after increased CpG methylation of their promoters *GAD1* and *GAD2*. This epigenetic regulation along to the reduced occurrence of interneurons support the deficit in GABA synaptic transmission after prenatal stress.
How can maternal immune activation (LPS) impact on the offspring future life?

**Mother**
- LPS
- TLR-4
- ↑ TNF-α
- ↑ IL-1
- ↑ IL-6
- ↓ Zn
- Chemokines, Complement, Prostaglandins, Leukotrienes
- IL-1
- PGE₂
- POMC, NPY
- CRF, ACTH
- Fever
- Anorexia
- ↑ GCs

**Fetus**
- ↑ TNF-α, IL-1, IL-6
- Oxidative stress
- Altered cytokines
- Effects on brain development

**Placenta**
- ↑ IL-1
- ↓ Zn
- Oxidative stress
- Altered cytokines
- Effects on brain development

**Amniotic Fluid**
- ↑ IL-1

**Postnatal Offspring**
- Microglial activation
  - (Oxidative stress
  - ↑ cytokines, ↑ GFAP
  - with higher dose prenatal LPS)
- White matter injury.
  - ↓ myelin, ↓ oligodendrocytes
- Hippocampal changes
  - (e.g. ↑ AMPAR/NMDAR currents, ↑ neuronal excitability, ↓ neurogenesis, ↓ dendritic arborization)
- Altered DA and 5-HT markers
- Behavioral deficits
  - (e.g. PPI, learning and memory)
Cytokines are important mediators of prenatal stress. Delayed effect?
A prominent role of IL17a? (Nature 28<sup>th</sup> of September 2017 issue)

- IL17a is secreted by Th17 (‘helper’ lymphocytes) in the mother’s blood.
- IL17a by itself can induce detrimental effects compared to MIA.
- Th17a seem to require microbiote-secreted interleukins IL1β, IL23 and IL6 to differentiate into IL17a-secreting cells.
- Microbiote activating Th17 in the pregnant rat may increase the risk of neurodevelopmental diseases in the offspring.
Increased oxidative stress in the fetal brain after prenatal stress (LPS)
Strategies to reverse / prevent prenatal stress outcomes?

- ‘Handling’
- Antioxydants
- Vitamine D
- GABA
N-acetyl-cysteine (before stress) limits oxidative stress.
NAC prevents plasticity and behavioural defects observed in males subject to prenatal LPS.
Vitamin D

Vitamin D metabolism and associated transduction pathway

Vitamin D exhibits a lot beneficial and protective effects.

Its deficiency during pregnancy is also a risk factor for neurodevelopmental disorders occurrence in children.

→ effect on prenatal stress models?
Vitamin D (given with the stressor polyI:C) treatment reverses social deficits and anxiety (marble burying test, see below) observed in stressed animals.

This treatment also improves learning.
Action on GABA transmission?

Could the reinstatement of synaptic GABA improve the stressed phenotype? Effect of a GABA uptake blocker, tiagabine.

Tiagabine rescues LTD in the hippocampus of rats subjected to prenatal LPS.
Epigenetic mechanisms

• Increasing evidences for their occurrence in prenatal stress detrimental effects
• Epigenetic mechanisms: DNA methylation, Histone modifications (acetylation), microRNA
• They are involved in transgenerational transmission of stress, often via sperm.
Fetal Alcohol Spectrum Disorders and epigenetics

- **DNA Methylation**
  - Differential methylation of genes involved in learning (NMDA receptor subunit), development, imprinted genes, metabolism, and stress (Pomc gene)
  - Regional changes in total methylation and hydroxymethylation in hippocampus
  - Regional alterations in Mecp2 in cortex, striatum, hippocampus, and Pomc cells

- **Histone Modifications**
  - Histone modifications on genes regulating neurogenesis and neural precursor identity
  - Reduced CREB binding protein and reduced H3 and H4 acetylation in cerebellum
  - Decreases in histone activation marks and increases in repressive marks in hypothalamic Pomc neurons

- **Noncoding RNA**
  - Altered expression of miRNAs involved in neuronal maturation (miR-21, miR-335, and miR-153), pluripotent state (miR290), embryonic development (miR-10a, miR-10b), and hippocampal dendritic spine size (miR138-2)

- **Prenatal Alcohol Exposed Animal**
  - Blunted growth, fetal deformities, neural dysfunction, hippocampal defects, learning and memory deficits, motor deficits, increased anxiety, hyperactive stress axis
Recent studies indicate that prenatal ethanol is associated with modifications of histone methylations and acetylations which will impact on gene expression. Ethanol metabolism seems to be largely involved in these modifications.
The increase of response to stress can be transmitted from one generation to the other via sperm epigenetic modifications. Indeed, the exposure to alcohol of pregnant rats leads to epigenetic mechanisms activation in the offspring of both gender. This result in an enhanced response to stress (hyperactive HPA axis) via POMC gene decreased expression. These modifications occur in the sperm of the male from the first generation and are then transmitted to the next one. The offspring of F2 and F3 generation exhibit enhanced response to stress.
A similar transgenerational transmission has recently been evidenced for the maternal immune activation (polyI:C). The second generation exhibits reduced social interactions but also depressive behaviour. The PPI deficit is only observed in the first generation. Transmission occurs by male from the first generation.
Transgenerational transmission of early life stress

Early life stress consisting Maternal Separation and Unpredictable Stress (MSUS) is also transmitted from one generation to the other. The transmission seems to involve miRNA specific expression in sperm.