Direct and indirect mechanisms for alcohol damage to the brain

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Fetal Alcohol Spectrum Disorder (FASD) – Across the Lifespan

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IHE INSTITUTE OF HEALTH ECONOMICS ALBERTA CANADA Government of Alberta

Animal Models

- First developed to address initial skepticism that maternal alcohol consumption could cause FAS.
  - Biological and neurobehavioral effects of prenatal alcohol exposure in animals remarkably consistent with clinical effects seen in humans.
  - Effects exist on a continuum.
  - Data demonstrated that alcohol is a teratogen.
- Valuable for examining specific outcomes and investigating mechanisms of alcohol's actions on the developing fetus.
  - Despite hundreds of reports in human, animal and in vitro studies, mechanisms of teratogenesis are not fully known
- Outcomes in humans direct animals research; conversely, animal models can predict and inform deficits that might occur in humans.

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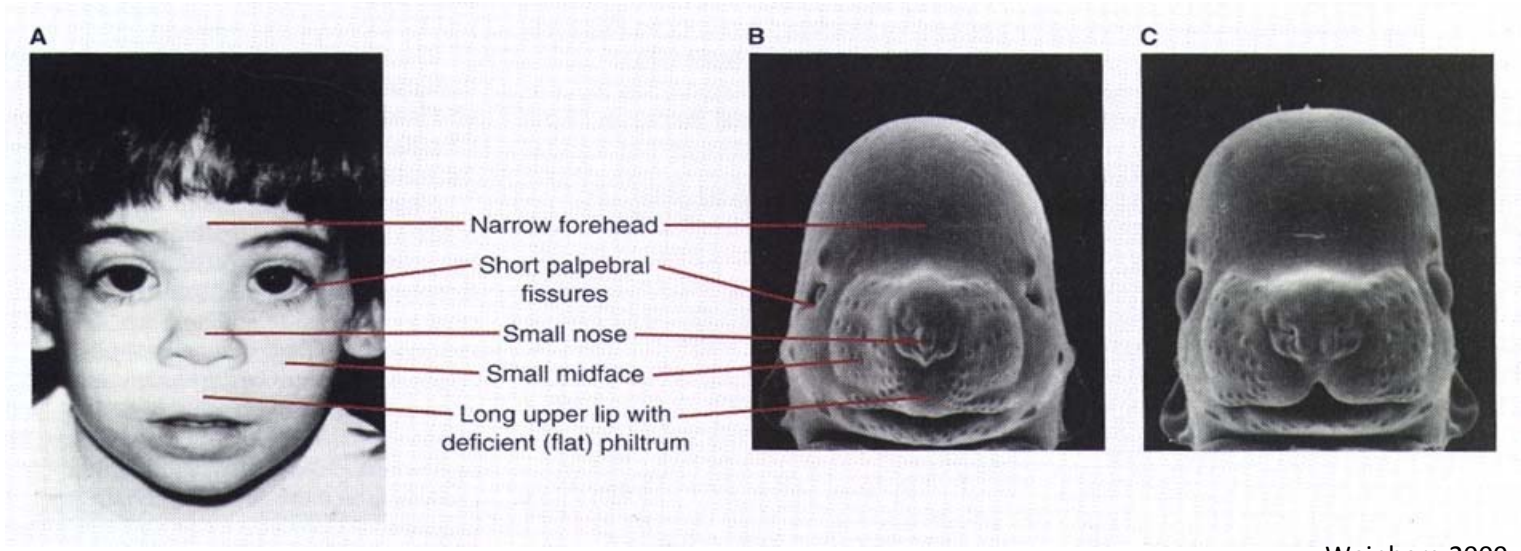
Rodent Models of Prenatal Alcohol Exposure Mirror the Effects Seen in Humans

(Sulik et al., Science 214:936-938, 1981)



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Why Use Animal Models?

- Control of environmental variables
  - Dose, timing of exposure, other drugs, maternal nutrition and health, prenatal/postnatal environment
- Control of genetic variables
  - Genetic differences in vulnerability or sensitivity to the same dose of alcohol
  - Genetic differences in absorption, distribution, metabolism, elimination of alcohol
  - Separate genetic from environmental effects
- Insight into mechanisms of action can suggest strategies for intervention (pregnant females) and treatment (exposed offspring)
  - Must consider both **direct** and **indirect** effects

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Direct effects

- Neuronal cell damage/cell death
  - Apoptosis
  - Brain particularly sensitive during brain growth spurt
- Direct inhibition of protein and DNA synthesis
  - Evidence for inhibition of protein synthesis in placenta and in fetal liver and brain → fewer cells, decreased growth and differentiation
  - Disruption of specific enzymes that play a role in metabolism in neural tissue
  - **Hippocampus, amygdala and cerebellum** particularly sensitive

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Secondary/Indirect effects

- Nutritional deprivation/malnutrition
- Abnormalities in calcium handling mechanisms
  - Increased calcium influx into neurons → inhibition of neuronal growth and migration
- Prostaglandins
  - Prostacyclin has vasodilatory effects, thromboxane has vasoconstrictive effects – balance important in regulation of umbilical and placental blood flow.
  - In a mouse model, low dose aspirin
    - Selectively reduced thromboxane levels without effects on prostacyclin → ↑ blood flow
    - Reduced alcohol-induced prenatal mortality and incidence of birth defects

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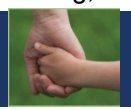

Secondary/Indirect effects (cont'd)

- Placental dysmorphology – structure and function of placenta altered
  - Direct effects on transport of amino acids and other nutrients (zinc, vitamin A) across placenta
- Ethanol-induced circulatory changes
  - Vascular constriction, vasospasms in placenta, fetus → decreased fetal blood flow, hypoxia
  - Umbilical arteries and veins particularly sensitive
  - Hypoxia-induced neuronal damage -

    Highest sensitivity in brain areas where excitatory neurotransmitters are particularly dense – **hippocampus, cerebellum, basal ganglia**

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Secondary/Indirect effects(cont'd)

- Disrupted cell-cell interactions (cell adhesion)
  - Interference with cell adhesion mechanisms (L1 CAM)
- Interference with growth factors and other cell-signalling mechanisms
- Oxidative stress and free radical damage
- Disruption of midline serotonergic neuronal development
- Distruption of endocrine balance

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Endocrine balance as a factor in the etiology of FASD

(Anderson, 1981)

- Among the physiological abnormalities induced by maternal ethanol intake are marked alterations in both maternal and offspring endocrine function.
- The endocrine system influences functions as diverse as reproduction, growth, metabolism, stress responsiveness, and behavior, and is critical in maintaining homeostasis.
- Can endocrine imbalance contribute to the etiology of FAS?
  - Changes in maternal endocrine function can:

    - affect the ability to maintain a successful pregnancy

    - disrupt maternal-fetal hormonal interactions which in turn could have marked effects on many aspects of offspring development

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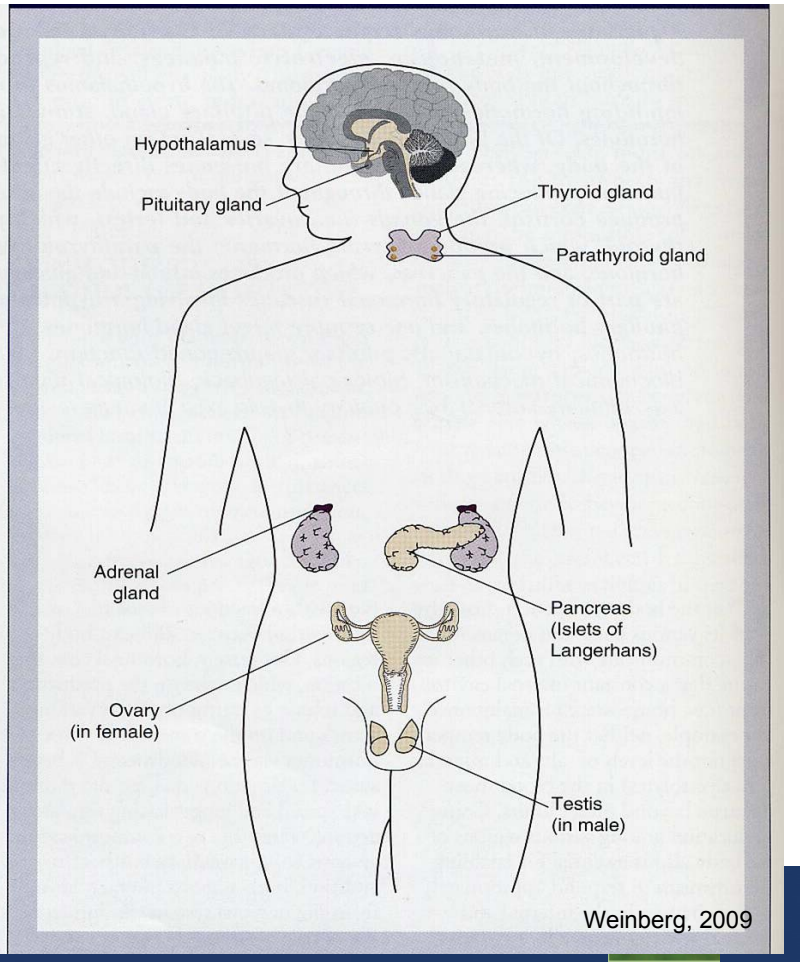
The Endocrine System
(Hiller-Sturmhofel & Bartke, 1998)

Location of the major endocrine (hormone-producing) glands in the body

We have been studying the stress system – hypothalamus, pituitary, adrenal (HPA) axis.



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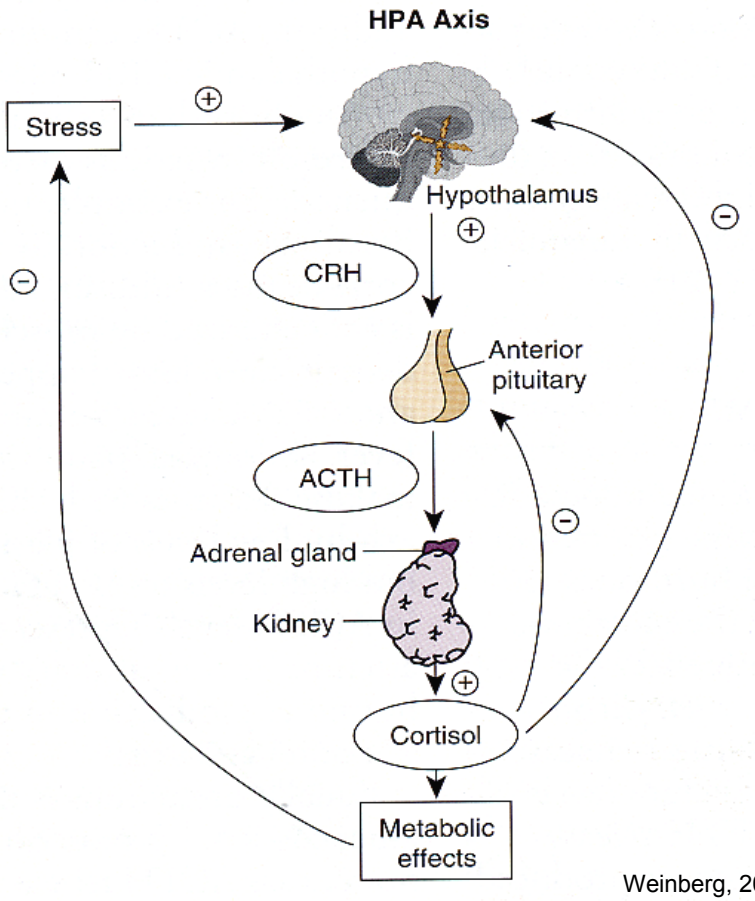
The HPA or Stress Axis

(Hiller-Sturmhofel & Bartke, 1998)

Stress, circadian changes
→ activate HPA axis
↓
Cascade of responses
↓
Increased hormone levels
(ACTH, glucocorticoids:
cortisol, corticosterone)
↓
Feedback to pituitary,
hypothalamus, hippocampus,
prefrontal cortex and other areas
→ Decreased stress hormone
levels



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Our studies

- Hypothesis 1: Fetal programming of HPA activity by alcohol permanently sensitizes neuroadaptive mechanisms that mediate the stress response, resulting in hyper-reactivity to subsequent life stressors, and increased vulnerability to illnesses, including depressive symptomatology
- Hypothesis 2: increased prevalence of drug addiction in individuals with FASD may be mediated by a pre-existing neurobiological vulnerability related to an altered responsiveness to both stress and the rewarding properties of drugs.

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Do the HPA changes induced by prenatal ethanol exposure underlie the increased risk for depression in children with FASD?

- Children with FASD problems in numerous life domains, including a high percentage of depression and anxiety disorders
  - These are commonly referred to as “secondary disabilities”, but are they really secondary?
- Children with FASD also show increased HPA responsiveness to stressors
- HPA hyperactivity and dysregulation are common findings in depression – reminiscent of changes with FASD.
- Strong relationship between depression in adulthood and adverse early life events.
- Brain areas implicated in depression overlap with areas that mediate responses to stress and emotional regulation – and addiction
  - HPA axis a key player in all of these

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Do the HPA changes induced by prenatal alcohol exposure underlie the increased risk for depression in children with FASD?

- **Stress-diathesis model of depression:**
  - Adverse early life experiences sensitize or prime the stress system.
  - A sensitized stress axis will be hyperactive in response to subsequent, even mild, stressful life events.
  - Repeated stress → maladaptive cascade of events and increased vulnerability to depression and anxiety disorders.
- Our hypothesis: fetal programming of HPA activity by alcohol permanently sensitizes neuroadaptive mechanisms that mediate the stress response, resulting in hyper-reactivity to subsequent stressors, and increased vulnerability to illnesses, including depressive symptomatology

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Diagnostic Criteria for Depression

(adapted from American Psychiatric Association, 1994)

- Depressed or irritable mood*
- Decreased interest in pleasurable activities and ability to experience pleasure = Anhedonia*
- Significant weight gain or loss (>5% change in a month)
- Insomnia or hypersomnia
- Psychomotor agitation or retardation
- Fatigue or loss of energy
- Feelings of worthlessness or excessive guilt
- Diminished ability to think or concentrate
- Recurrent thoughts of death or suicide

✓ For diagnosis, a patient must display at least five of these symptoms for at least 2 weeks. One of these five symptoms must be from the core symptoms (*).

How do you assess depression in an animal model?

Clinical Symptom	Endpoint in animal studies
• Sex differences	• Sex differences
• Significant weight gain/loss	• Weight gain or loss
• Decreased ability to think	• Performance in learning/memory tasks
• Elevated basal HPA tone	• Basal/stress CORT levels
• Increased HPA responses to stress	• Increased CORT response and prolonged stress CORT levels
• Anxiety	• Behavior in elevated plus maze
• Psychomotor agitation/ retardation	• Locomotor activity in home cage or novel arena
• Anhedonia	• Sucrose contrast test

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Our Model of Prenatal Ethanol Exposure
(ARND – Neurobehavioral abnormalities)

- Pregnant Sprague-Dawley females maintained on 1 of 3 diets throughout gestation (d 1-21):
- **Ethanol (E):** liquid diet (36% ethanol-derived calories) throughout pregnancy.

  (BALs ~ 150-200 mg/dl)
- **Pair-fed (PF):** liquid diet with maltose-dextrin isocalorically substituted for ethanol (g/kg body wt/day of gestation).
- **Control (C):** ad-lib access to liquid control diet or standard lab chow and water.

- Offspring of these females are tested at various ages

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Prenatal alcohol exposure increases later life vulnerability to depression-/anxiety-like disorders

(Hellemans et al., 2008; 2009)

- Prenatal alcohol exposure = early life adversity
- Animals subjected in adulthood to 10 days chronic mild stress (CMS) [2 stressors/day, unpredictable, mostly psychological]
- Tested on a multidimensional behavioral test battery to assess depressive- and anxiety-like behaviors:
  - E males show increased anxiety, impaired hedonic responsivity, locomotor hyperactivity, and alterations in social behavior compared to controls.
  - E females show greater anxiety, altered social interactions, and 'behavioral despair"

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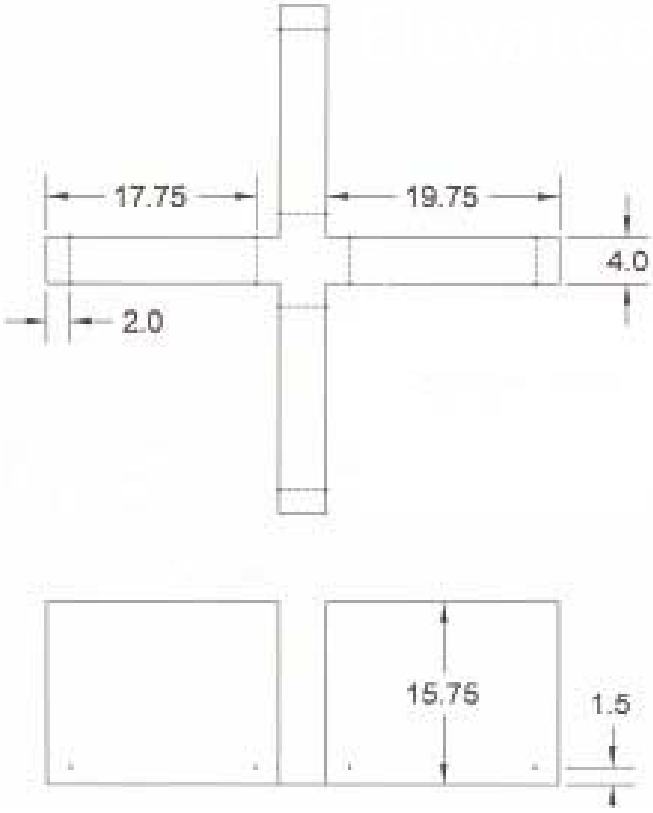
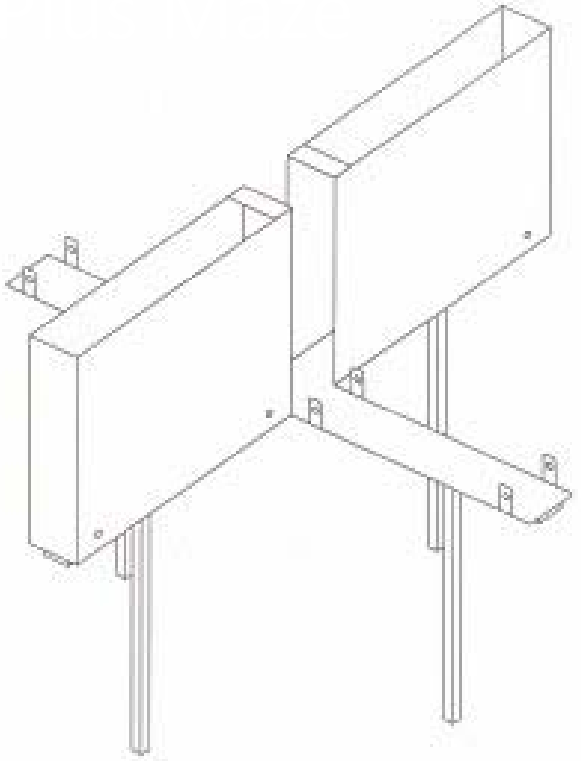
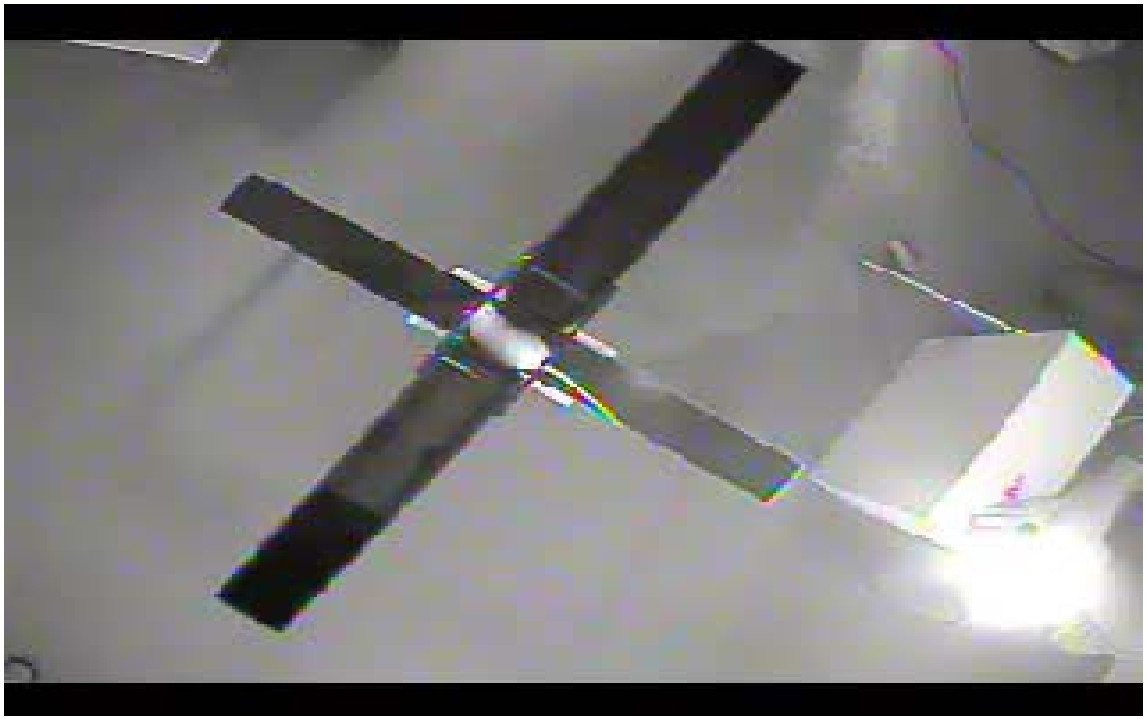

17.75
19.75
4.0
2.0
15.75
1.5



Elevated Plus Maze



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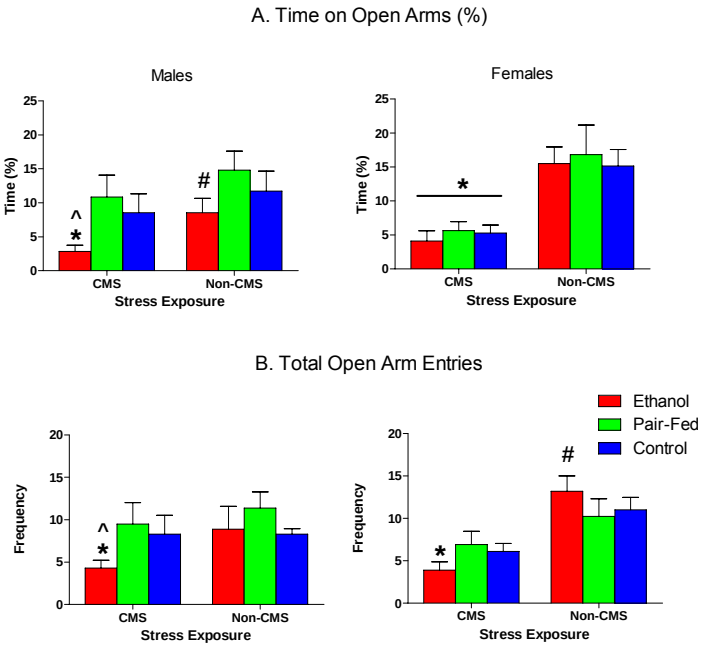
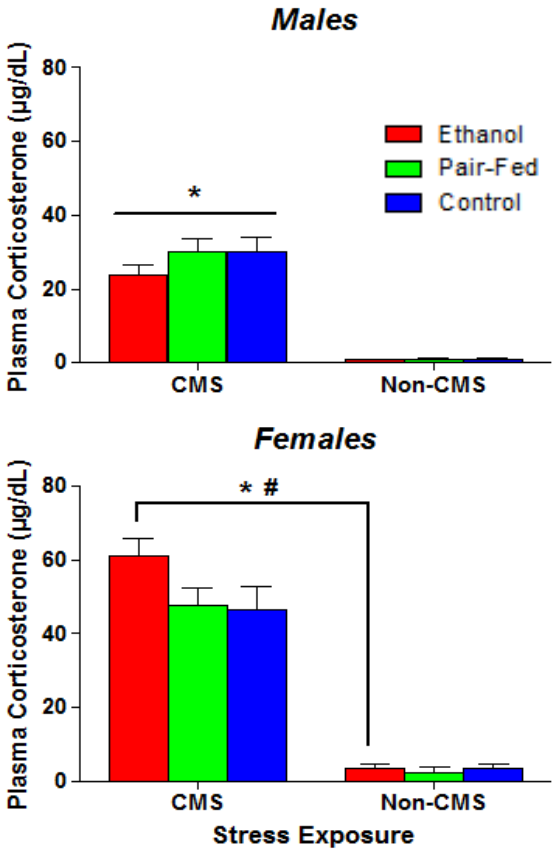
Anxiety (Elevated Plus Maze)



- Exposure to CMS caused increased anxiety for both E males and females:
- E males spent less time on OA and made fewer OA entries than PF and C males
- All females showed decreased time on OA
- CMS decreased total OA entries for E females; E< PF and C



EPM: CORT

Greater anxiety in E females, but not males, reflected in greater CORT levels (E>PF=C).

Epigenetic Mechanisms?

- Early life experiences such as prenatal exposure to alcohol may exert some of their long-lasting effects through epigenetic mechanisms that alter gene expression.
  - Epigenetic mechanisms refer to changes in hereditary information or heritable traits that do not occur through changes in the underlying DNA sequence.
  - Due to their dynamic nature, epigenetic mechanisms may function as mediators connecting the genome to environmental signals and exposures, and thus play a role in gene x environment interaction.
- A well studied epigenetic mechanism involves chemical modification of the DNA itself by methylation, the addition of methyl groups to cytosine (one of the four bases that make up DNA).
- The chemical modification of the histone proteins by acetylation, methylation, phosphorylation and other processes is another type of epigenetic alteration affecting gene expression

Summary and Conclusions

- Fetal/neonatal programming represents a mechanism for non-genetic inheritance of a predisposition for increased risk for disease
- HPA axis particularly susceptible to early life (prenatal, early neonatal) programming
- Increased fetal/neonatal exposure to glucocorticoids due to prenatal alcohol exposure may underlie, at least in part, the connection between the prenatal environment and adult stress-related and behavioral disorders.

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Summary and Conclusions (cont'd)

- Can postnatal and later environmental events modulate effects of prenatal/early life programming?
- Interventions based on mechanisms of teratogenesis
  - May not fully reverse damage but can improve function
- Our data suggest that interventions targeted to the HPA axis may provide a novel approach to intervention
  - Normalize HPA activity with CRH antagonists, glucocorticoid receptor antagonists, antidepressants, behavioral interventions.
- Implications for development of policies that recognize basic science findings in structuring interventions and care of pregnant women and their children.

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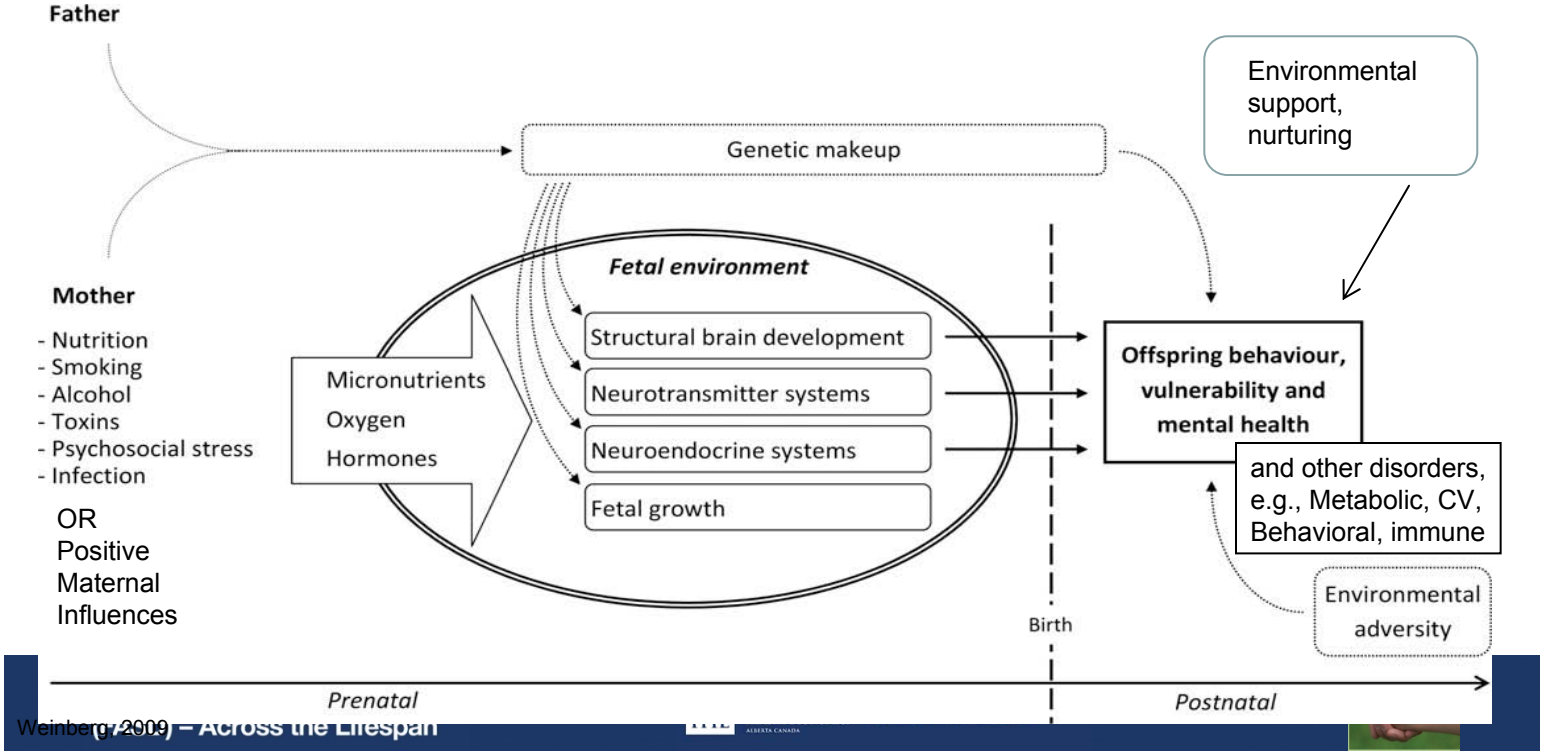
Links between fetal adversity/prenatal insult, vulnerability and health outcomes



Weinberg (2009)

Collaborators: Victor Viau, Sheila Innis, Angela Devlin, Michael Kobor, Gary Meadows

National Institutes of Health

NIAAA National Institute on Alcohol Abuse and Alcoholism

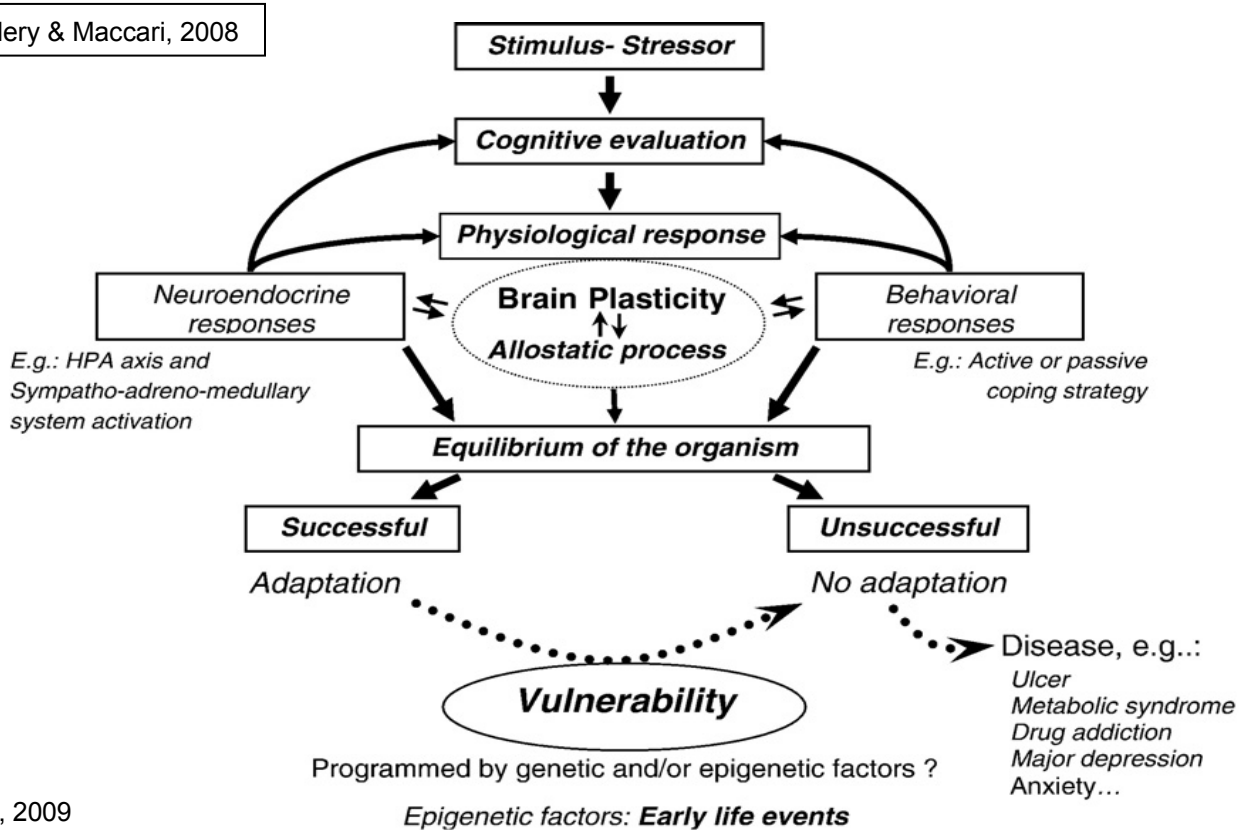
Human Early Learning Partnership HELP

Canadian Foundation on Fetal Alcohol Research

Impart Gender Women Addictions

coastcapital SAVINGS

Darnaudery & Maccari, 2008



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Sucrose Contrast

- ***Anhedonia:*** decreased interest in, and ability to experience, pleasure.

- **Positive Contrast:**

  2% Sucrose x 4 days; test @ 15 %.

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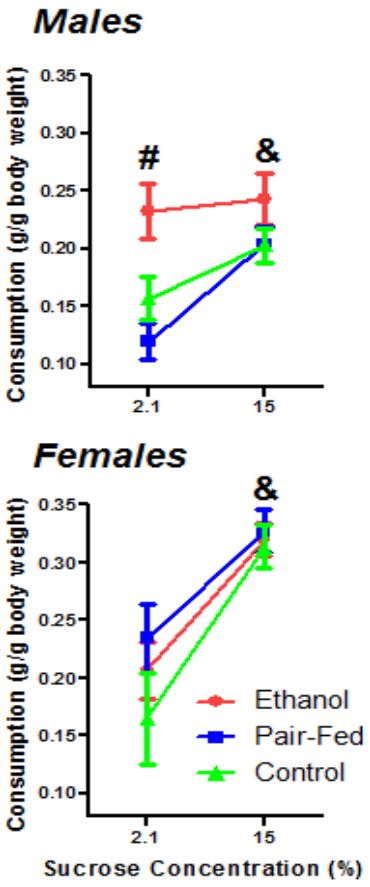

Anhedonia

Sucrose Contrast Test

- All females, and PF and C males showed increased intake with higher concentration
- E males had higher intake initially, and did not increase intake as concentration increased
  - Insensitivity to change in reward value of sucrose?
  - Greater intake of lower concentration may be compensatory response to modulate greater behavioral or HPA arousal

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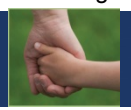
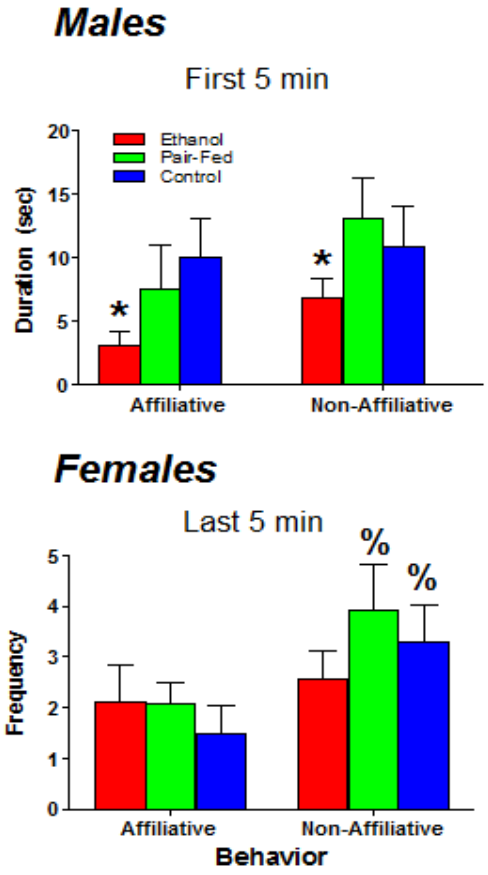




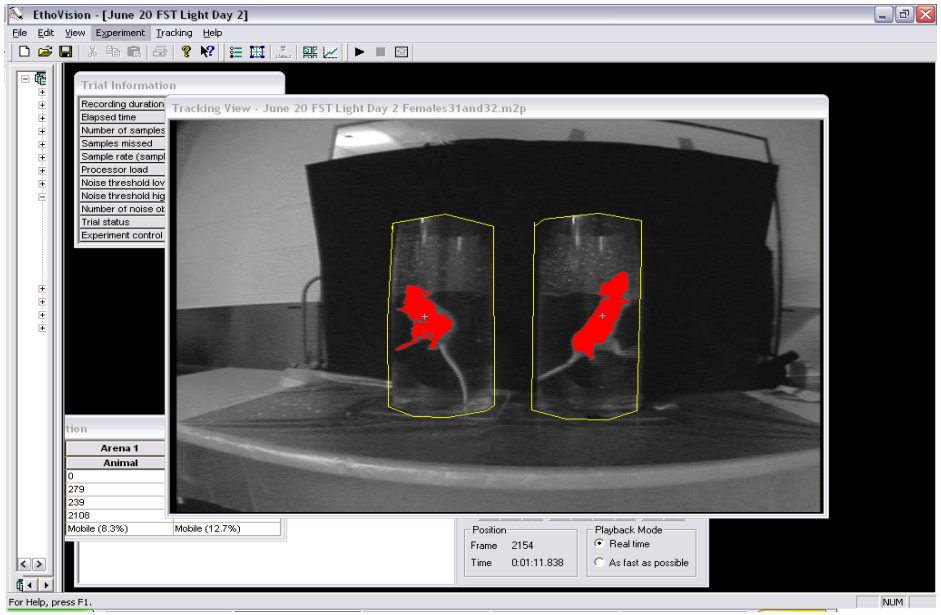
Social Interaction

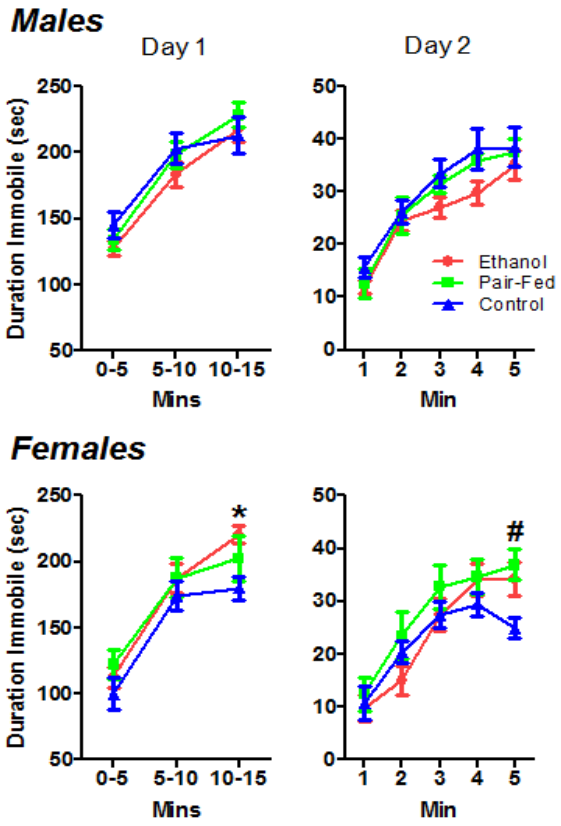
- Data suggest disturbed social interactions in both E males and females:
  - E males showed decreased Affiliative and Non-affiliative behaviors over first 5 min of testing
  - PF and C but not E females showed increased non-affiliative behaviors over last 5 min of testing

Porsolt Forced Swim Test

'Behavioral Despair"

- Duration & frequency of immobility
- Day 1: 15 min test
  Day 2: 5 min test



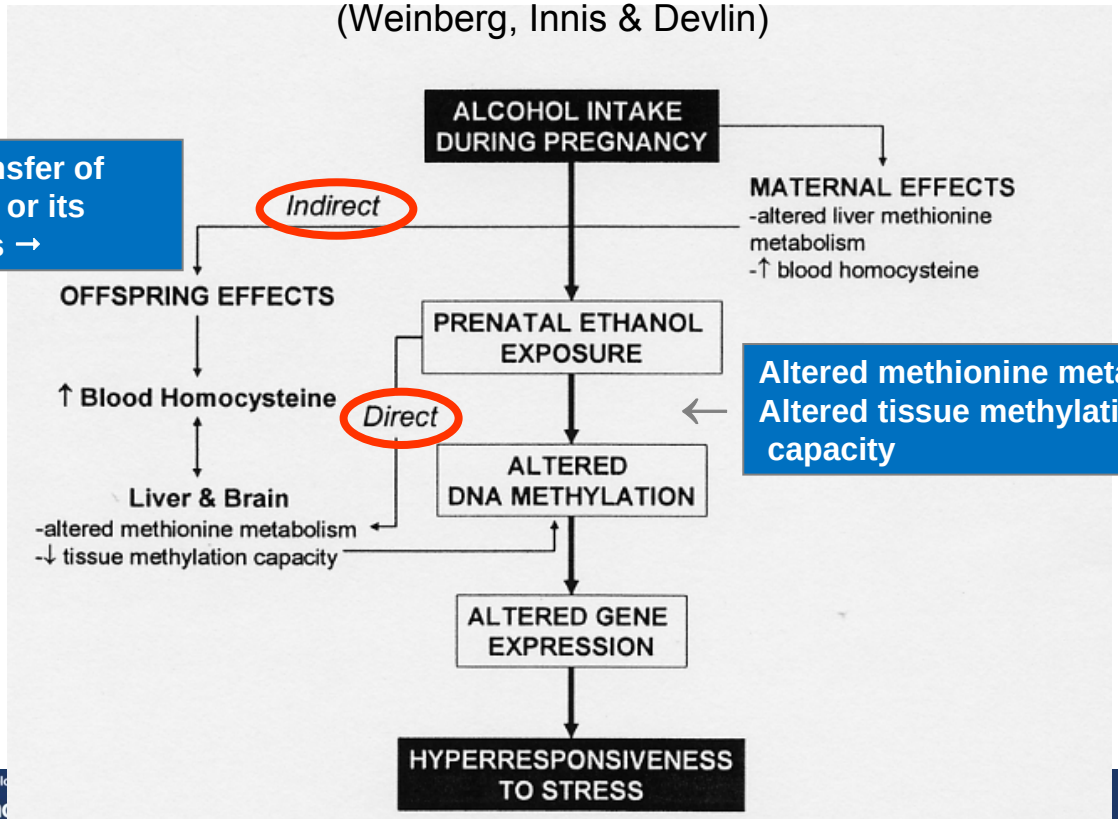


FST

- No differences among males
- E females show greater immobility than C on D 1
- Both E and PF show greater immobility than C on D 2

Proposed model for HPA programming

(Weinberg, Innis & Devlin)

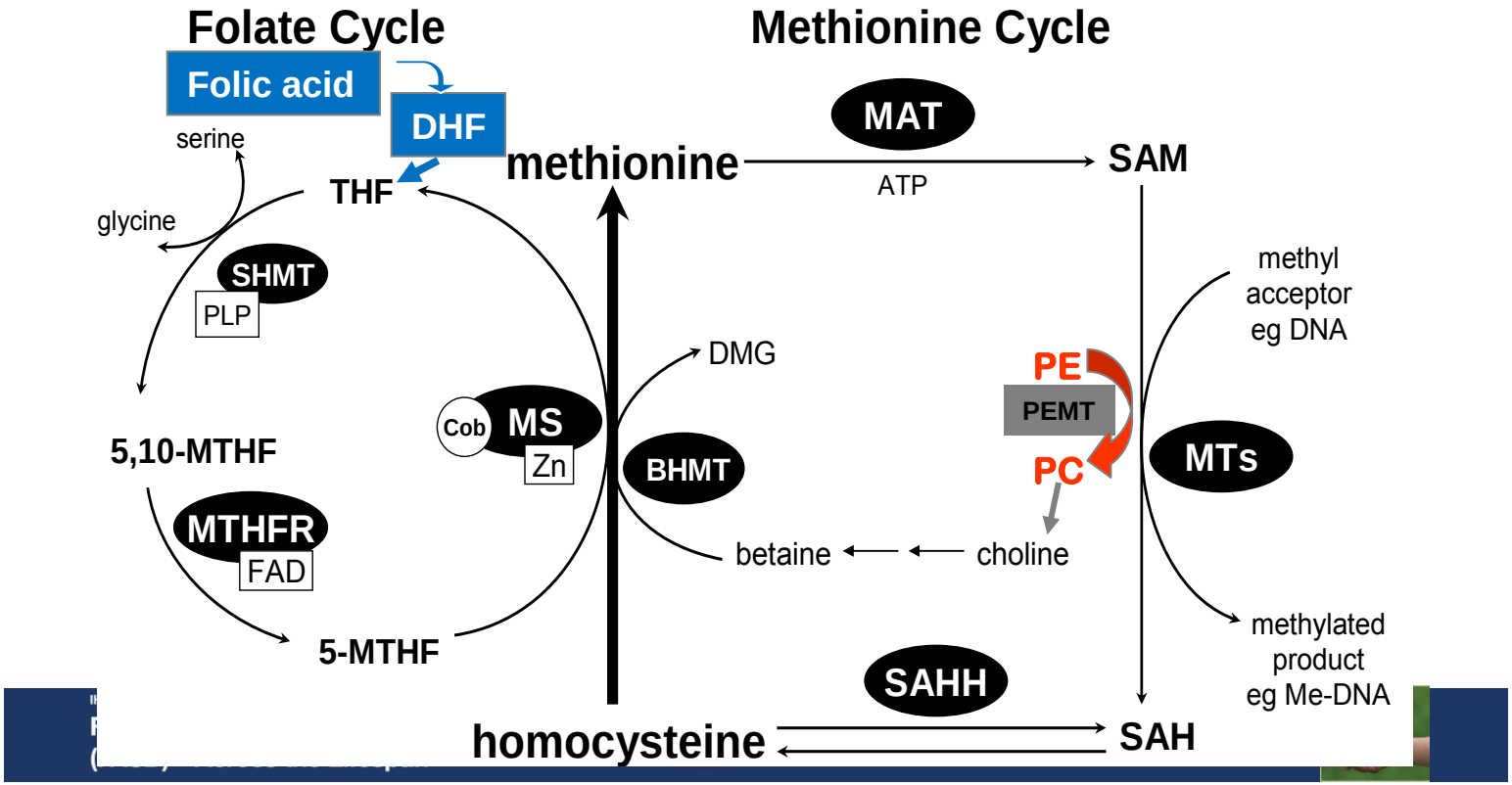


Altered transfer of methionine or its metabolites →

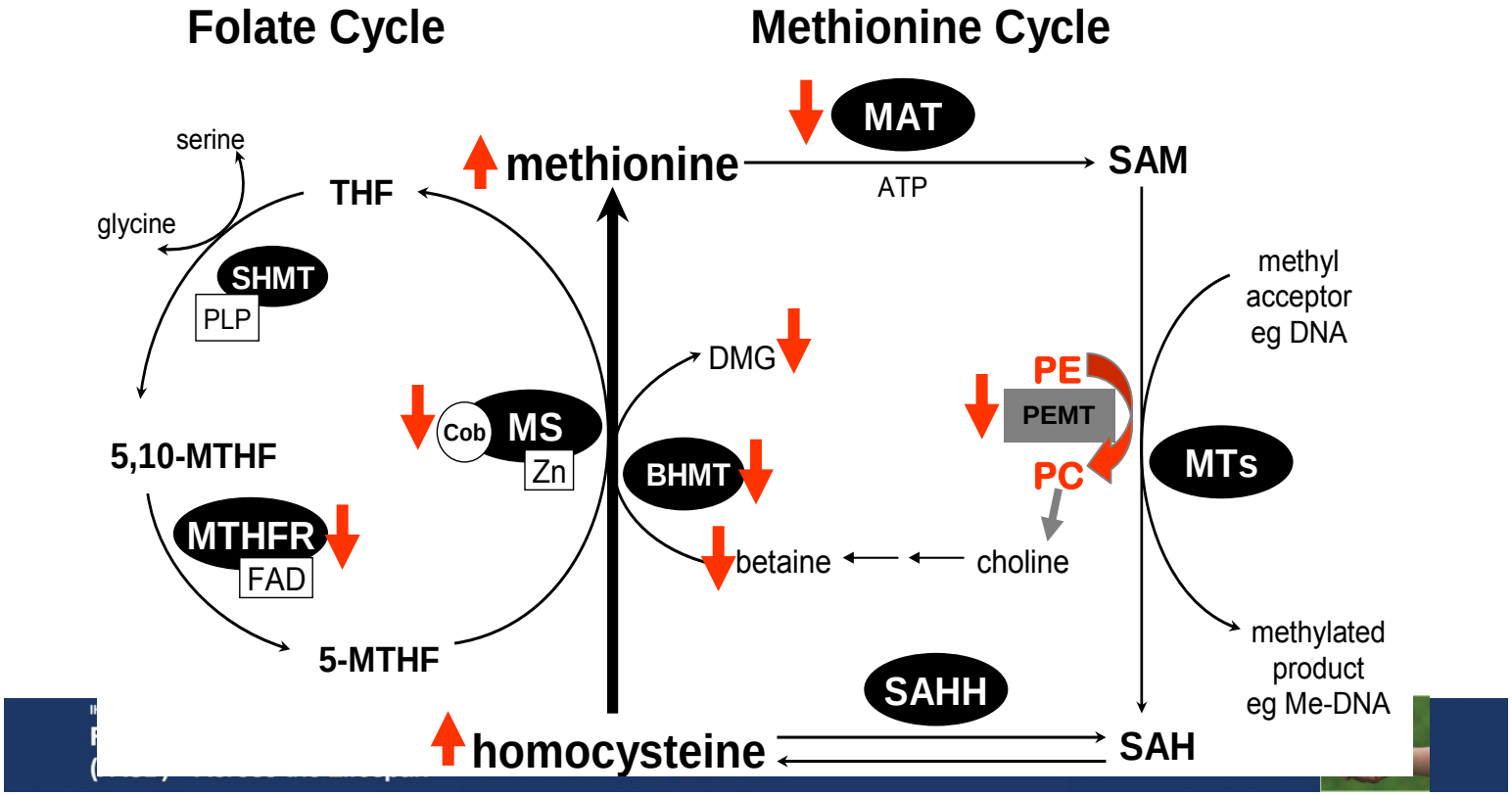
Altered methionine metab→ Altered tissue methylation capacity

Methionine cycle in liver

Methionine is an essential amino acid: role in cell function



Alterations in the maternal cycle



Alterations in the fetal cycle