Umweltchemikalien und Hirnentwicklung

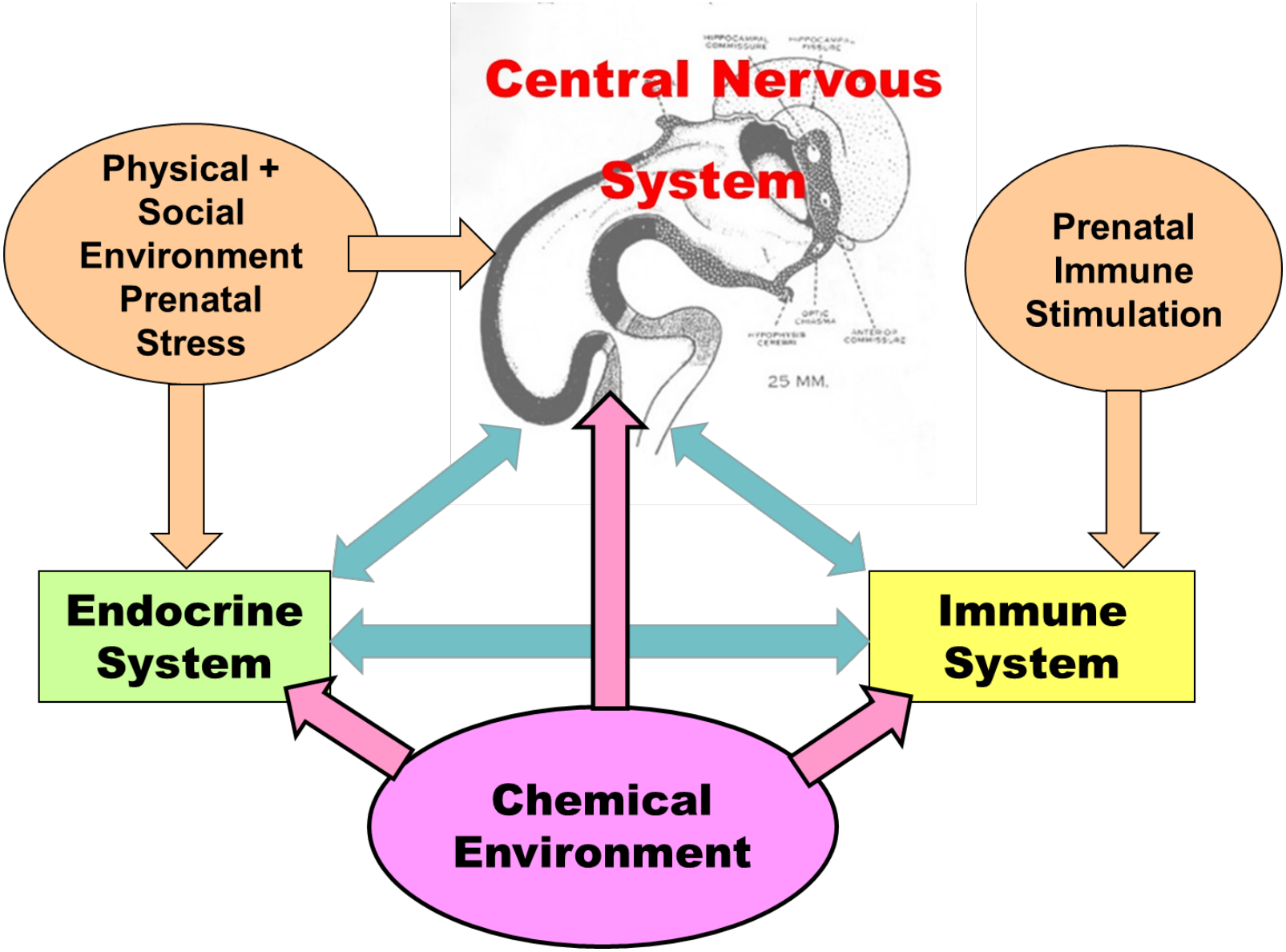
Walter Lichtensteiger

GREEN Tox

Zürich

AefU Tagung Solothurn, 19.05.2016

Brain Development is Shaped by Interactions of
Genetic Programs *with* ***Environmental Factors***



1. ***Welche Verhaltensstörungen beobachten wir bei Kindern und im Tiermodell ?***

2. Was wissen wir über molekulare Mechanismen?
   Neuroendokrine Systeme und Endokrine Disruptoren

3. Epigenetik: eine mögliche Basis für Langzeiteffekte
   Somatische Zellen: Individuum
   Keimzellen: transgenerationale Wirkungen

4. Stoffgemische: Wie weiter in der Risikobeurteilung ?

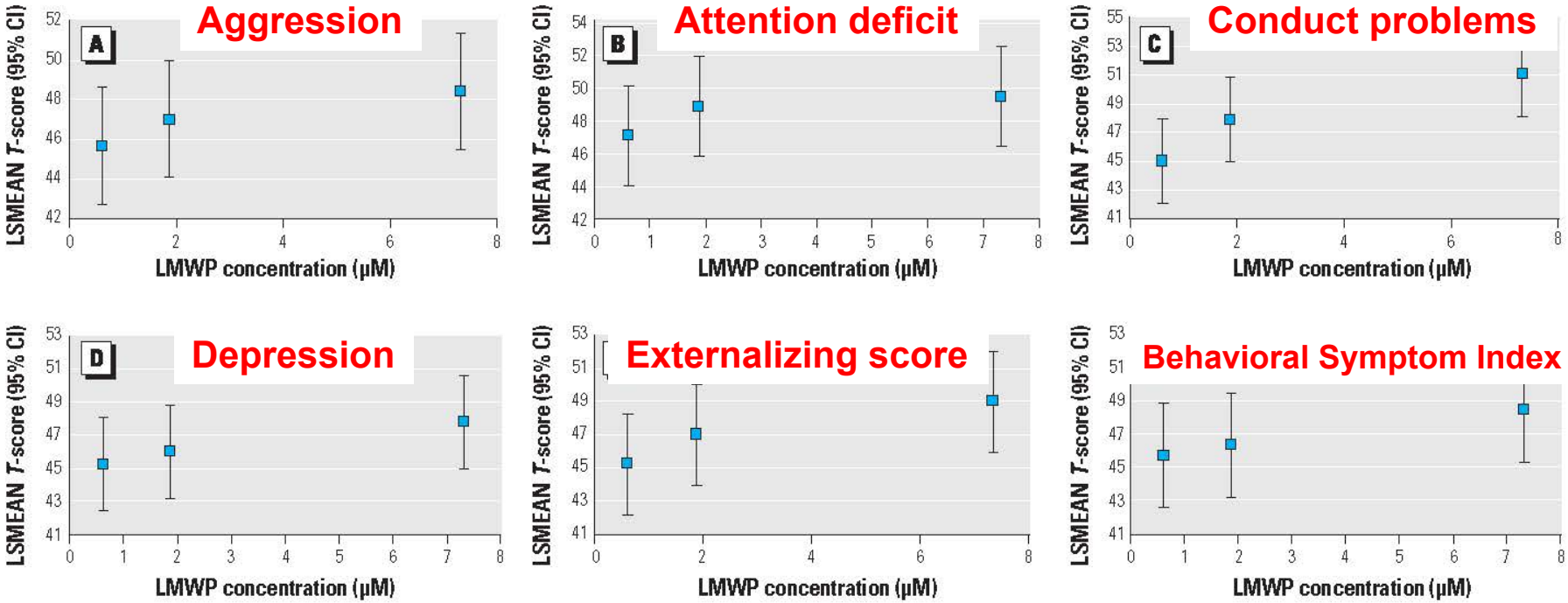
5. Take-Home Message

*Prenatal **PCB** Exposure: Neuropsychological Effects in Children*

Function in relation to PCB in maternal blood, cord blood, breast milk 9 birth cohort studies with **4459 children**	Performance	Confirmed / Studied
General cognitive abilities: IQ	decreased	4 / 6
Verbal ability (vocabulary, verbal comprehension)	decreased	3 / 4
Executive Functions Management of cognitive processes		
Planning	decreased	2 / 2
Response inhibition Suppression of actions interfering with goal-driven behavior	decreased	2 / 2
Task switching (Set shifting) Ability to shift attention between one task and another	decreased	1 / 1
Working memory	decreased	1 / 1
Information processing (speed)	decreased	2 / 3
Short-term memory	decreased	2 / 4
Visual recognition memory in young (6 Mt–4 Y) children	decreased	2 / 3

Meta-analysis of 9 birth cohort studies (Boucher et al., 2009):
Collaborative Perinatal Project (CPP) USA (Birth year 1959-65), North Carolina (1978-82), Michigan (1980-81), Faroe Islands (1986-87), Netherlands (1990-92), Oswego NY State (1991-94), Germany (1993-95), Nunavik 1st (1995-98), Hokkaido (2002-04).

*Prenatal **Phthalate** Exposure and Children's Behavior*



Low mol. weight phthalate metabolites (MMP, MEP, MBP, MiBP), **Maternal Urine** (**3rd Trimester**)

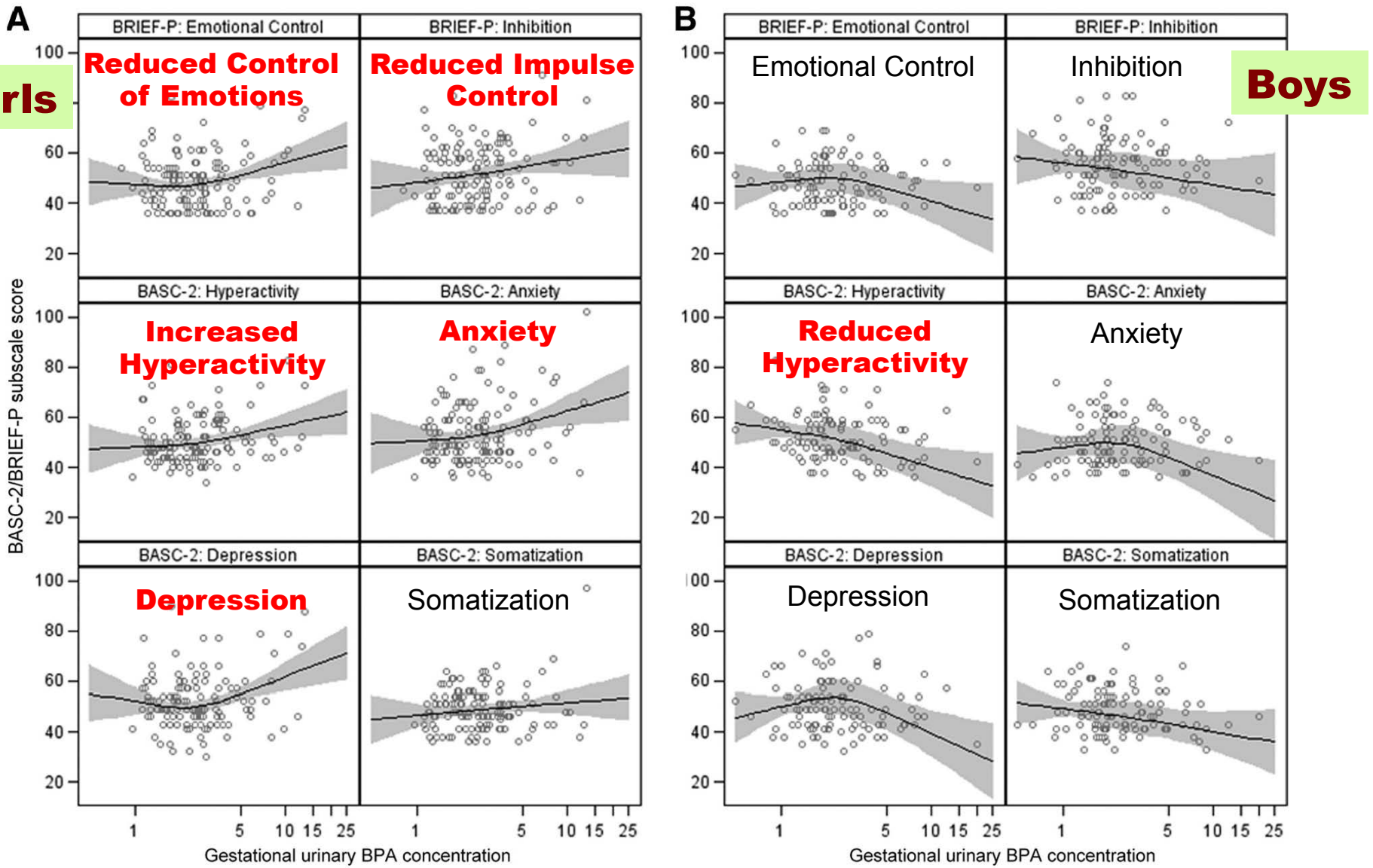
Combined data from **4.5-5.5 years** (102 children), **6-6.5 years** (113 children), **7-9 years** (146 children); 54% boys (Engel et al., 2010, Miodovnik et al., 2011).

BASC-PRS Test: **Problematic behavior** of children at home and in society.

Conduct problems: aggressive and destructive activities causing disruptions in the child's environments, and repetitive/persistent behaviors violating societal norms.

Externalizing score: hyperactivity, impulsiveness, aggression, and conduct problems combined.

*Prenatal **Bisphenol A** Exposure and Children's Behavior*



Bisphenol A in **maternal urine** (Week 16 - 26), Tests at **3 years of age**, 239 children (BRIEF, BASC-2).

Braun et al., 2009, 2011

Animal Models	Children
Learning and Memory **PCB**: ↓ M and F **Bisphenol A** ↓M **Phthalates** (DINP, DBP) ↓ M ↑ **Vinclozolin** ↓M (extinction of cond. response)	**Cognitive Performance, Memory** **PCB**: cognitive performance ↓ (executive functions, short-term memory, verbal ability)
Attentio **PCB**: **Atter**	**on** ↓M+F
Anxiety **Bisphenol A**: **Anxiety** ↑ M **Vinclozolin**: **Anxiety** ↓ M ↑ F	**Bisphenol A**: **Anxiety** ↑ F, **Depression** ↑ F **Phthalates**: ↑ **Anxiety** + ↑ **Depression** M+F combined
Aggression **Social interactions** **Bisphenol A** **Aggression** ↑ M **Social interaction** ↑ between Females **Self-grooming** (non-social behavior) ↓ F	**Aggression** **Social interactions** **Bisphenol A** **Externalizing behavior** ↑ F (Aggression) **Phthalates** (DMP, DEP, DBP), M+F combined **Social cognition** ↓ **Social communication** ↓ **Conduct problems** ↑

Analogien zwischen Kindern und im Tiermodell.

Nicht nur **kognitive Funktionen** betroffen.
Auch Wirkungen auf **Emotionen** und **Sozialverhalten!**

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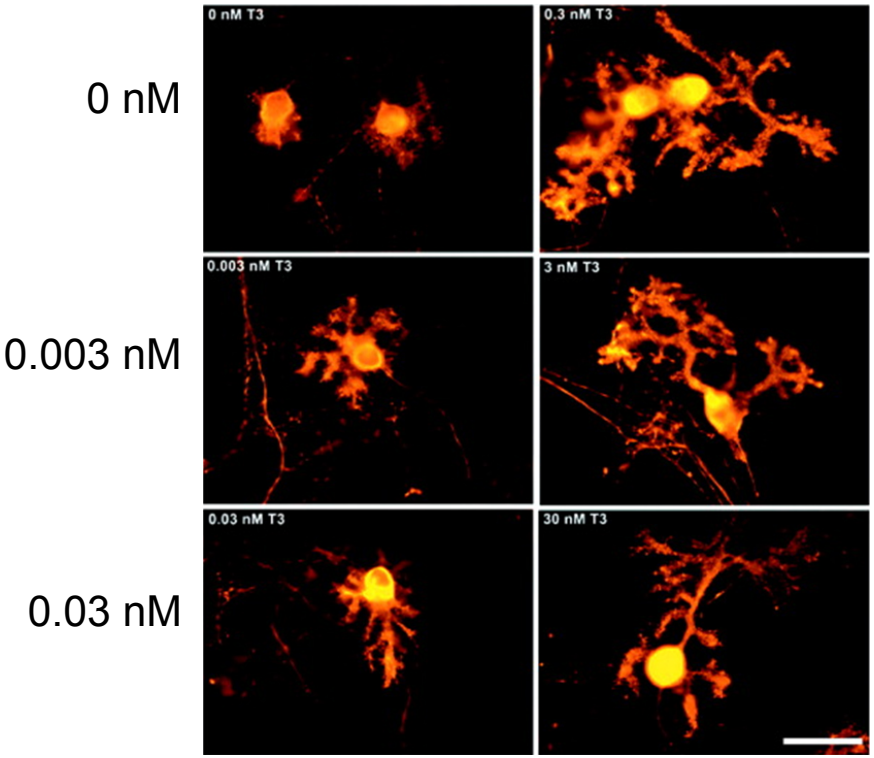
Interaktion mit Hormonen: Endokrine Disruptoren

Organochlor Pesticides (DDT, HCH, Lindane), **Linuron, Prochloraz, Vinclozolin**	Pesticide (Insecticide, Fungicide)
Polychlorinated Biphenyls (PCBs)	Isolation materials, Pesticides
Polybrominated Diphenylethers (PBDE)	Flame retardants
Alkylphenols (e.g., Nonylphenol)	Emulgators
Bisphenol A: Plastic Monomer	Plastics (Polycarbonates, Epoxy resins, others)
Phthalates (Diethylhexyl-, Dibutyl-, others)	Softeners, Colors, Personal Care-Products
Chemical UV-Filters	Sunscreans, other Cosmetics, Product Protection
Parabens (Methyl-, Propyl-, Butyl-paraben)	Preservatives
Perfluorooctanoic acid (PFOA), Perfluorooctane sulfonic acid (PFOS)	Oil- and water-repellants, Fire-fighting foams
Dioxins (TCDD)	Incineration products
Ethinylestradiol, other Estrogens	Contraception, Estrogens in Menopause
Phytoestrogens	Plant Estrogens
Paracetamol	Analgesic

Thyroid Hormones and Brain Development

Effects of Thyroid Hormones (TH)
Dependent on **Maternal TH** **prior to formation of fetal thyroid gland**
Proliferation of neuronal progenitor cells Hippocampus, cerebellum, cerebral cortex (?)
Commitment to cell type Differentiation program
Migration Neural precursors in cortex, hippocampus, cerebellum
Differentiation of Neurons Purkinje cells in cerebellum
Differentiation of Glial Cells **Myelinization**
Synaptogenesis



Triiodothyronin (T3) induces dendrite formation by cerebellar Purkinje cells
14 days in vitro
(Heuer et al., 2003)

*Interaction of **PCBs** with **Thyroid Hormones***

Multiple Interactions of PCBs with Thyroid Hormones

Maternal T3 levels (some also T4) decreased within normal range (humans)
Congener-dependent, variable

Reduced stimulation of T4 synthesis by TSH,
damage at thyroid gland level

PCBs bind to **Transthyretin** (TTR) and **Thyroid Hormone-Binding Globulin** (TBG)

Enhanced metabolism:
T4 glucuronidation (not T3):
UDP-glucuronyltransferase induced.

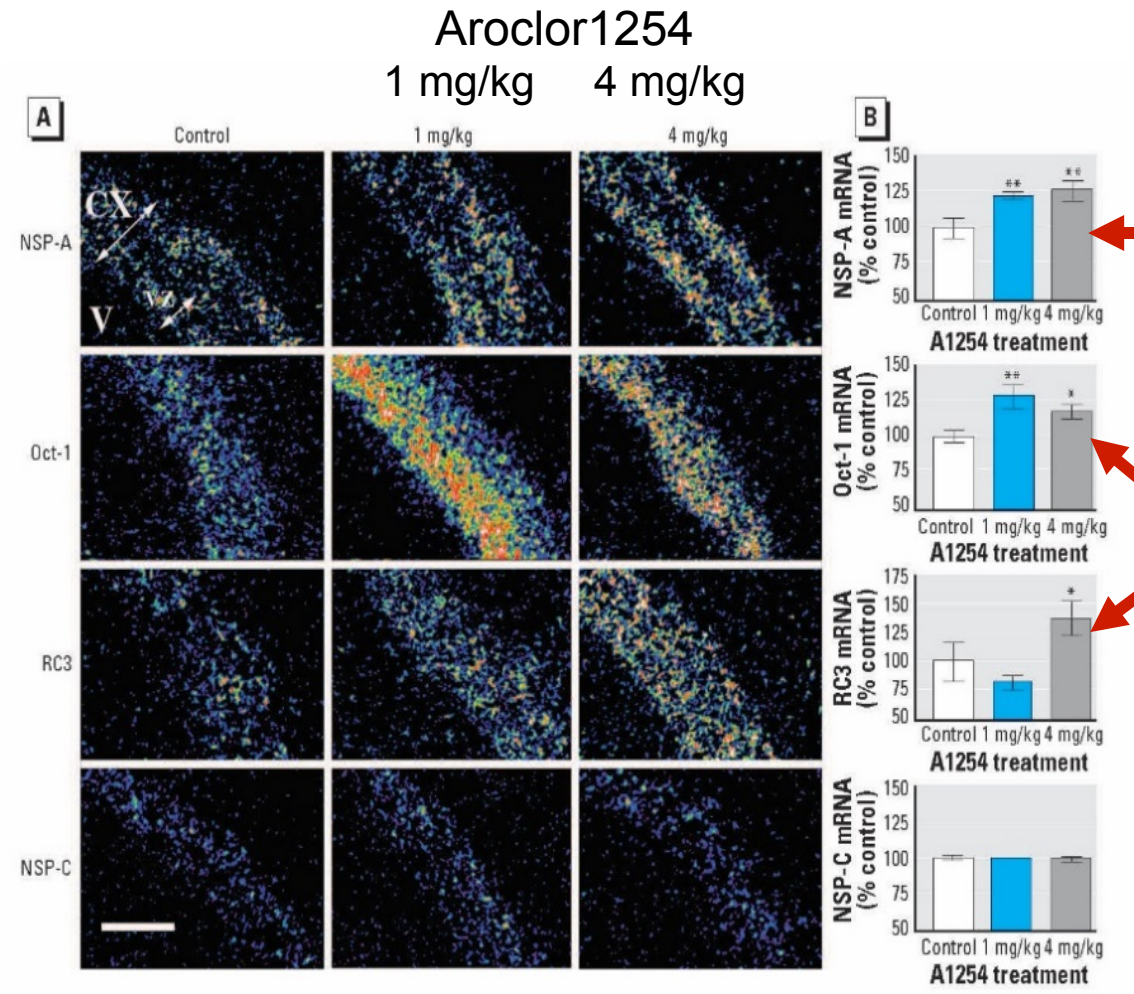
Thyroid Hormone Receptors
activated by specific PCB congeners

Table 4. Hormone levels and PCBs concentrations during pregnancy (Takser et al., 2005)

	TSH (mIU/L)		fT_4 (pmol/L)		TT_3 (nmol/L)	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
∑ PCB (µg/L)						
Estimate	0.65	0.45	−0.08	0.49	−0.37	−0.47
df	151	148	151	148	151	148
Type 3 *F*-value	0.50	0.21	0.05	1.6	6.4*	9.6***
∑ *mono-ortho* coplanar PCBs[b] (µg/L)						
Estimate	3.0	0.46	−2.6	2.7	−1.3	−2.1
df	151	148	151	148	151	148
Type 3 *F*-value	0.13	0.0	0.60	0.62	0.98	2.27
PCB-138 (µg/L)						
Estimate	0.90	−0.55	−0.48	3.1	−2.1	−2.8
df	151	148	151	148	151	148
Type 3 *F*-value	0.03	0.01	0.05	2.1	7.2**	11.2***
PCB-153 (µg/L)						
Estimate	−0.18	−0.93	0.57	2.5	−1.2	−1.5
df	151	148	151	148	151	148
Type 3 *F*-value	0.0	0.08	0.19	3.6	5.9*	8.6**
PCB-180 (µg/L)						
Estimate	7.8	7.5	−1.4	−0.27	−1.2	−1.4
df	151	148	151	148	151	148

Inverse correlation between **maternal total T3** levels and **non-coplanar PCBs 138, 153, 180**

PCBs in Developing Brain:
Hypothyroidism plus Activation of Thyroid Hormone Receptors



Cerebral Cortex, Rat Fetuses

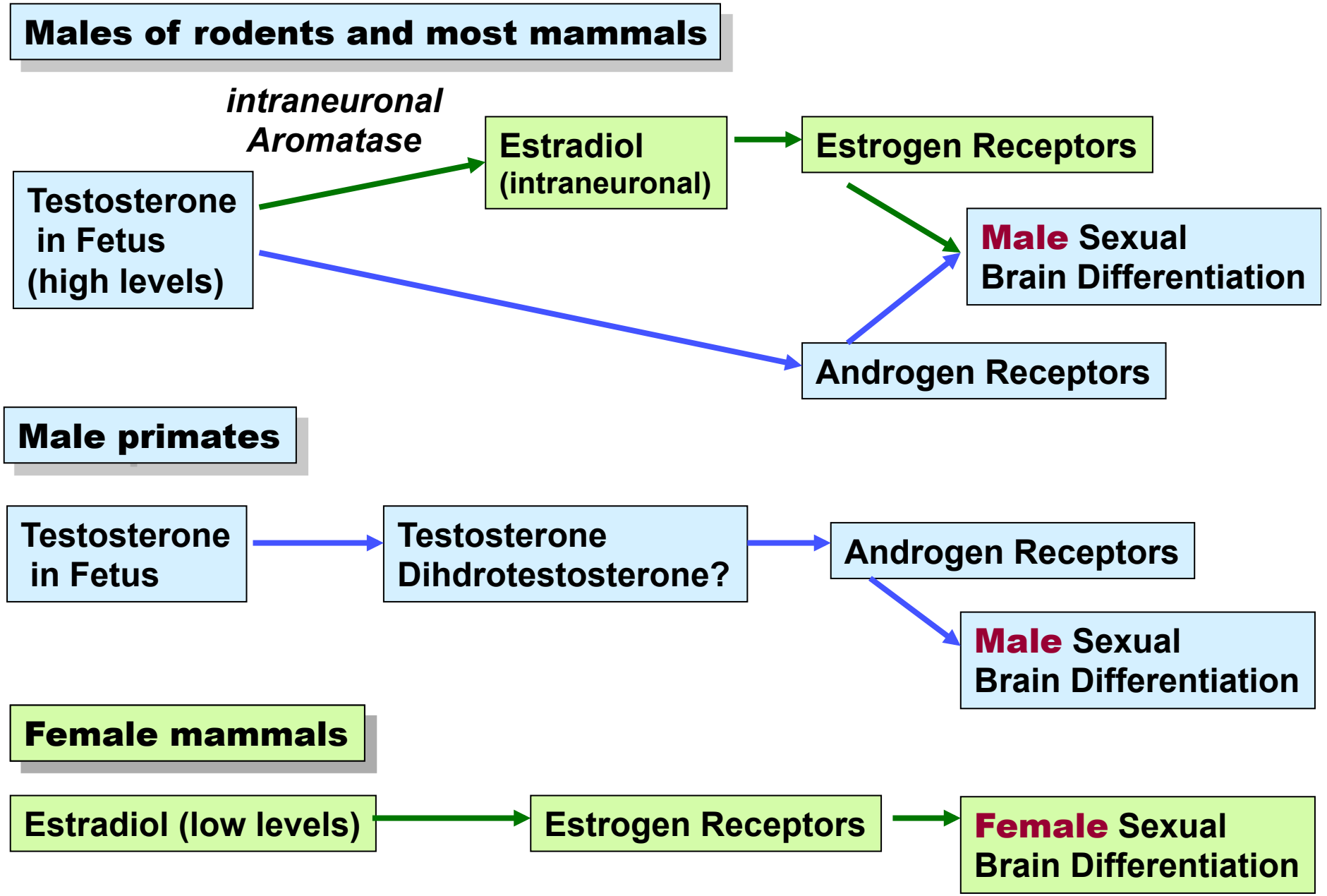
NSP-A up-regulated **indirectly** by **low maternal T4**

Thyroid Hormone-responsive **Oct1** and **RC3/Neurogranin** up-regulated by **activation of Thyroid Hormone Receptors**

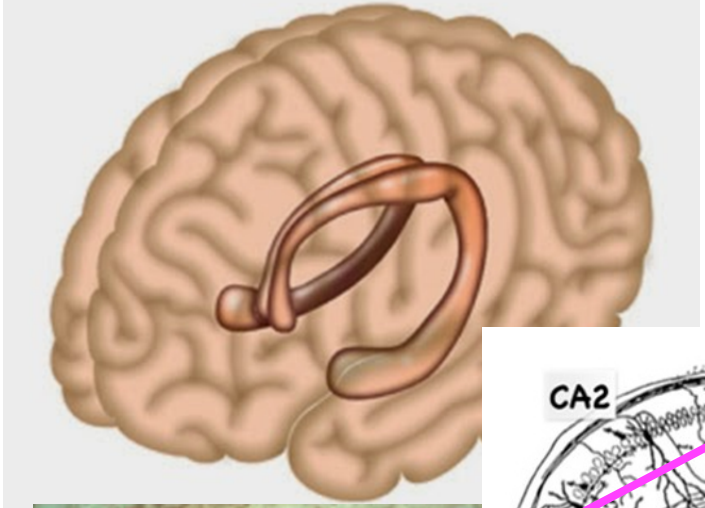
NSP-C: Non TH-responsive

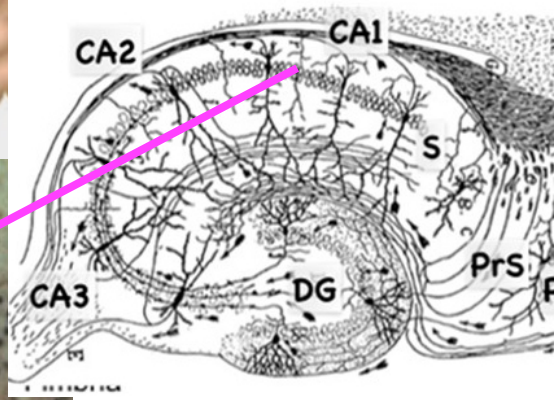
Gauger et al., 2004

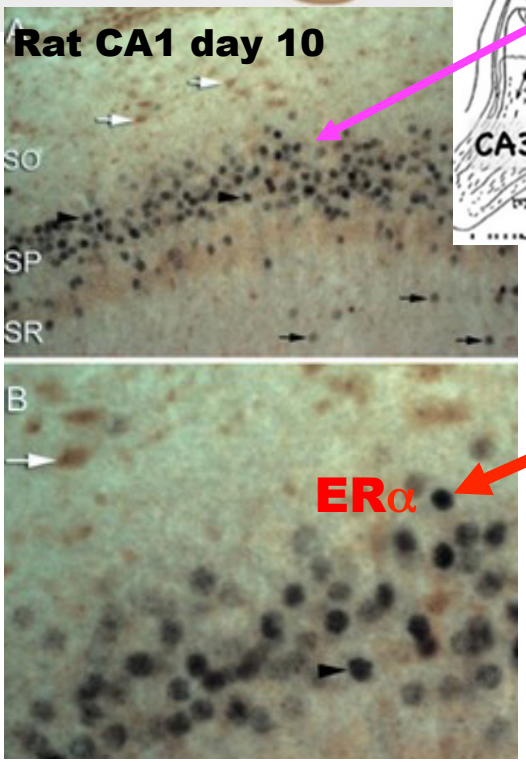
Sex Hormones and Brain Development: Sexual Phenotype



Non-Reproductive Brain Functions: Hippocampus – an Estrogen Target





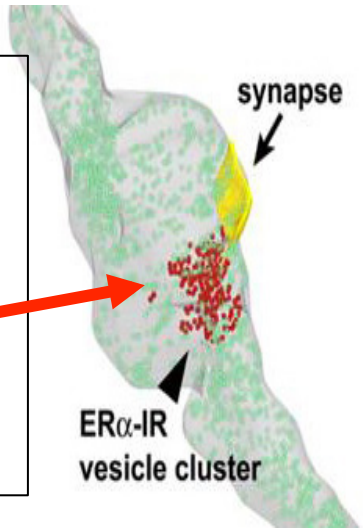


- The **Hippocampus** expresses **Estrogen Receptors (ERα, ERβ)** and Androgen Receptors, at high levels during **development**.
  O'Keefe and Handa, 1990; Ivanova and Beyer, 2000).

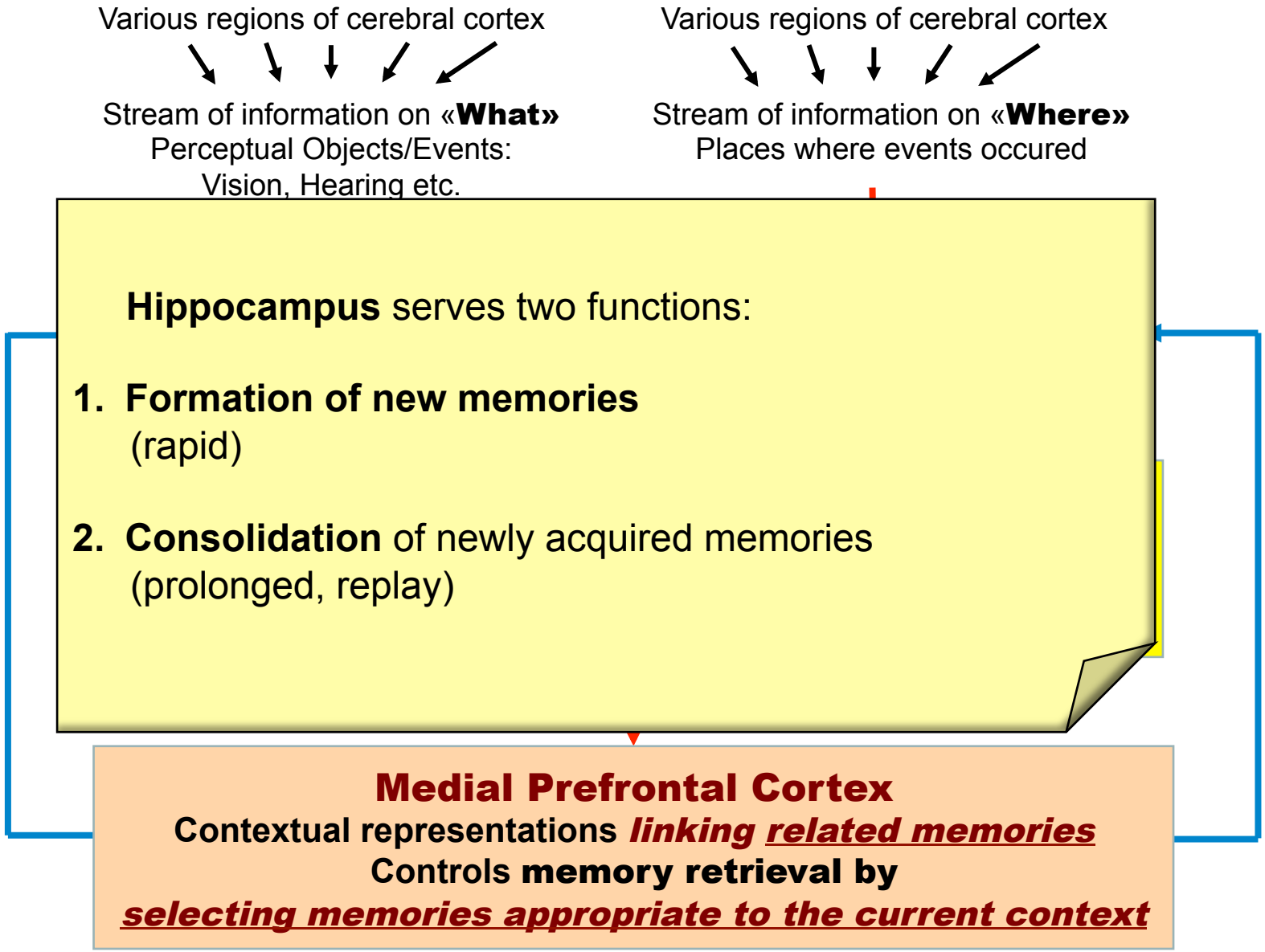
- The Hippocampus is capable of ***de novo*** **synthesis: Testosterone, Estradiol.**
  (Hojo et al., 2004; Prange-Kiel et al., 2009.

Estrogen Receptors:
Nuclear Estrogen Receptors,
in **pyramidal cells** (Solum & Handa, 2001)

Non-nuclear Estrogen Receptors
in **inhibitory nerve terminals**,
associated with synaptic vesicles
(Hart et al., 2007)

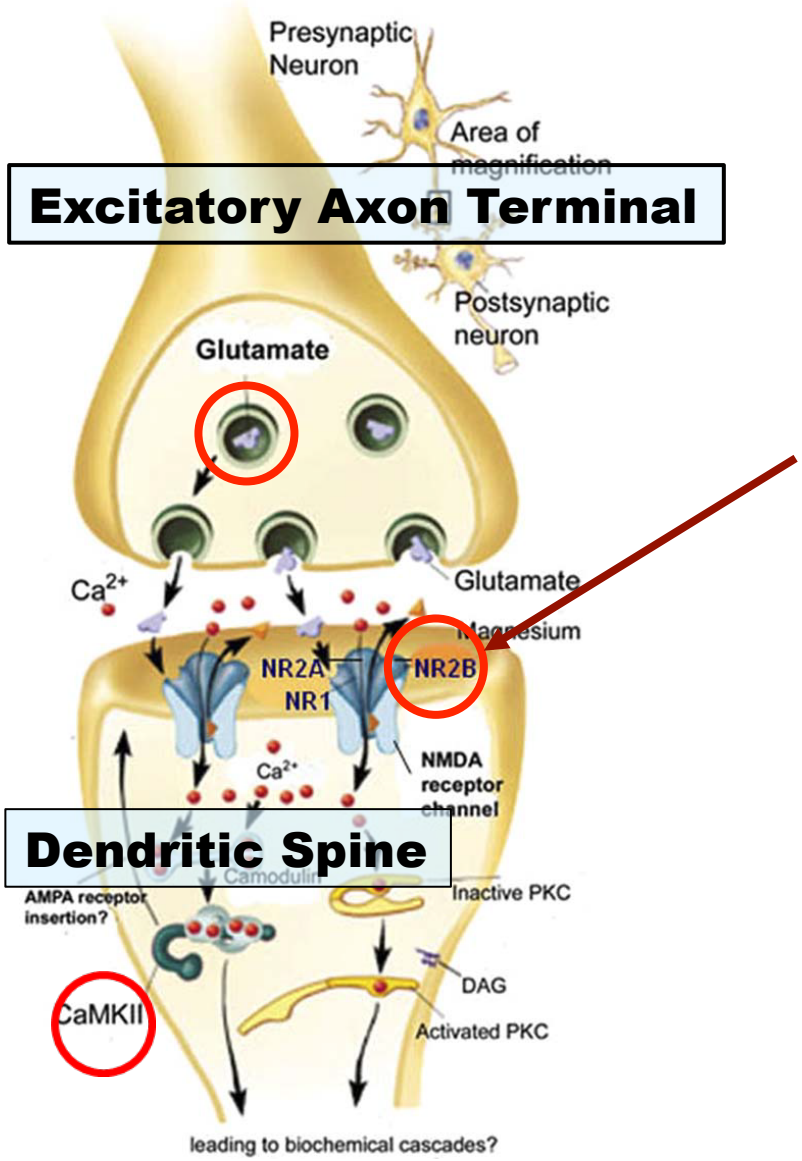


Central Role of Hippocampus in Memory Formation



Preston and Eichenbaum, 2013

Hippocampus: Excitatory Glutamatergic Synapses and Memory



H. Wang et al., 2006

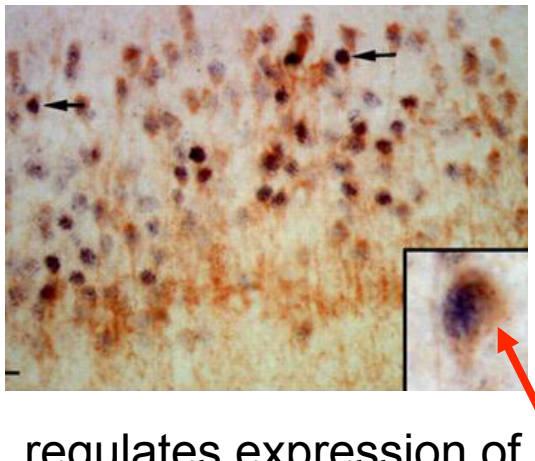
Memory Consolidation

- **Excitatory Glutamatergic Synapses** *mediate consolidaton of short-term memory into long-term memory.*
- **NMDA receptor** *at postsynaptic site functions as* ***«Coincidence Detector»:*** *Opening delayed* ***until the postsynaptic neuron is sufficiently depolarized (activated)****.*
- *Calcium ion influx* ➔ *Binding of protein kinase CaMKII at NMDA receptor subunit* ***NR2B*** ➔ *Activation of CaMKII and subsequent postsynaptic processes.*
- *Sequence is* ***many times repeated during memory consolidation*** *(synaptic reentry reinforcement).*

Developing Hippocampus, Estrogens, and Endocrine Disrupters

Estradiol

controls **growth processes**



regulates expression of ***Brain-Derived Neurotrophic Factor (BDNF, brown color)***

(Solum and Handa, 2002;
Luine and Frankfurt, 2013)

regulates expression of ***Glutamate receptor subunits*** *and of* ***synaptic proteins***

(Watanabe 1999, Morissette 2008, Ikeda 2010; Waters 2009)

Bisphenol A

administered **during gestation and lactation**.

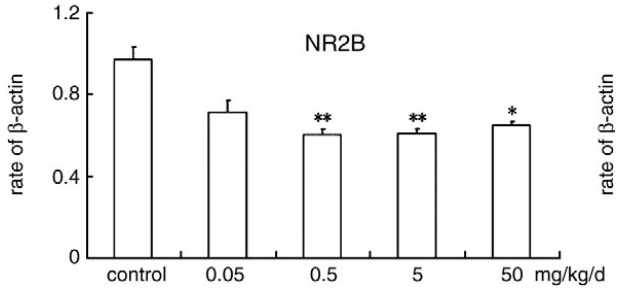
Adult male mice offspring (56 days) show:

1. Impaired Learning Behavior
(Morris Water Maze, passive avoidance test)

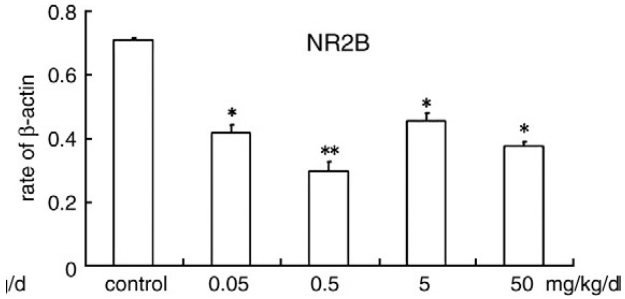
2. Impaired Synaptogenesis (Xu et al., 2013)

3. Reduced NMDA receptor subunit expression *in hippocampus* (NR1, NR2A, NR2B) at 50 μg/kg x day (X-H Xu et al., 2010)

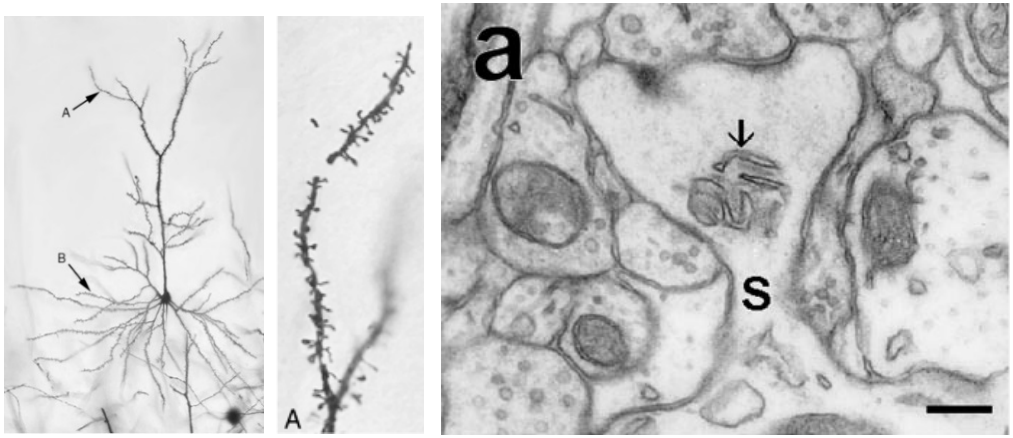
NR2B
Postnatal
Day 21



NR2B
Postnatal
Day 56



Dendritic Spines (Excitatory Synapses), Estradiol and Bisphenol A

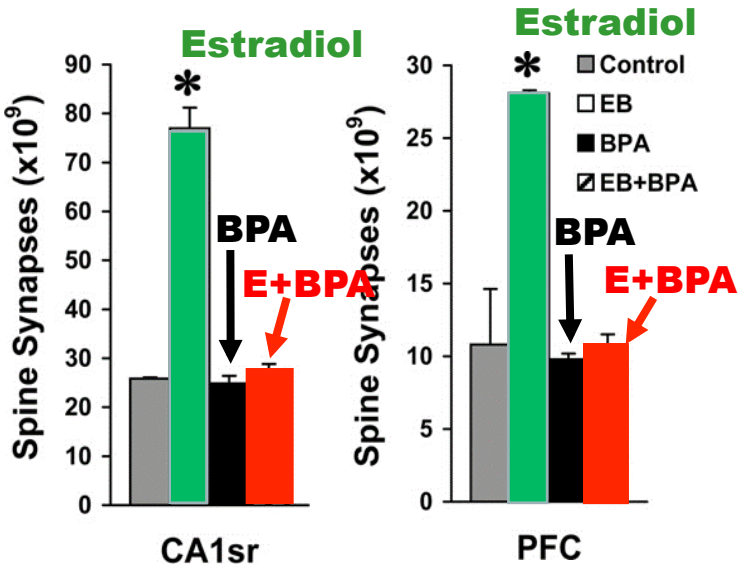


Estradiol stimulates formation of ***Dendritic Spines,*** site of **excitatory glutamatergic synapses,** indirectly via effects on neuronal networks, leading to increased activity of pyramidal cells (CA1)
(Cooke and Woolley, 2005)

Injection of **estradiol** ➔ increased of number of ***dendritic spines*** + ***improved spatial working memory*** in adult rats
(Sandstrom and Williams, 2001).

Estradiol*-induced formation of* ***dendritic spines*** *prevented by* ***Bisphenol A*** (50μg/kg x day) Hippocampus and prefrontal cortex of ovariectomized adult monkeys.
(Hajszan and Leranth, 2010).

BPA acts here as **antagonist**.



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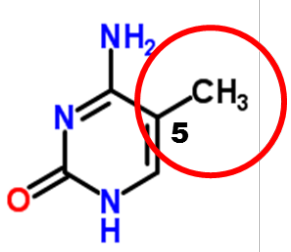
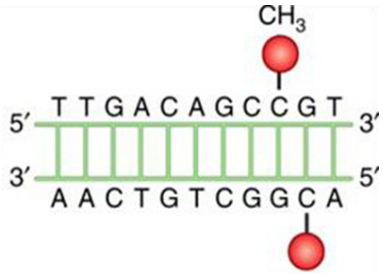
DNA Methylation – one Mechanism to Regulate Gene Expression

Methylation of the base **Cytosine blocks expression of a gene** in certain positions **(Silencing)**.



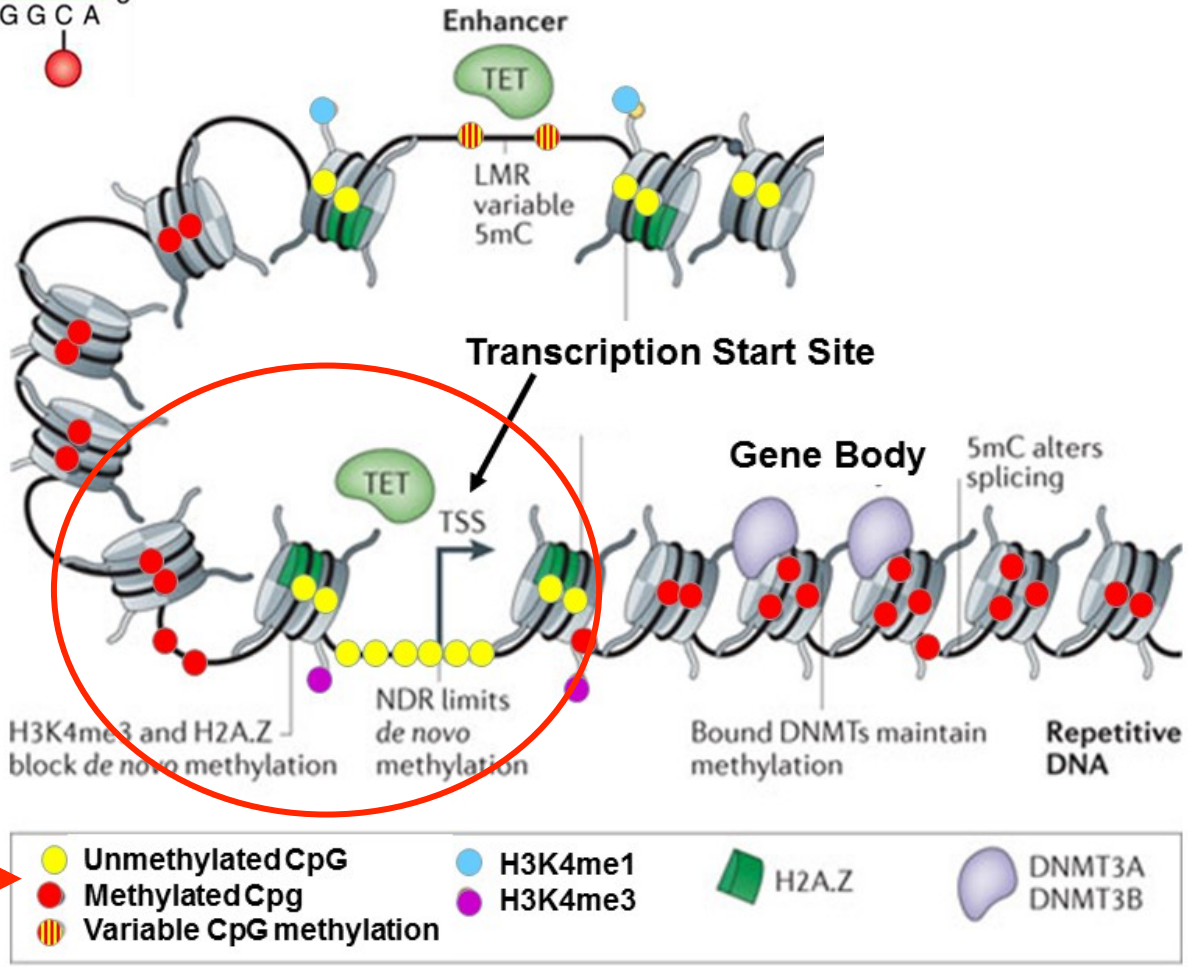
5-Methylcytosine



Histone H3 Methylation of lysines

- **H3K4me3 blocks** *de novo* DNA methylation
- **H3K9me1,2,3** and **H3K27me3** important roles in **early development**

DNA Methylation of CpG sites



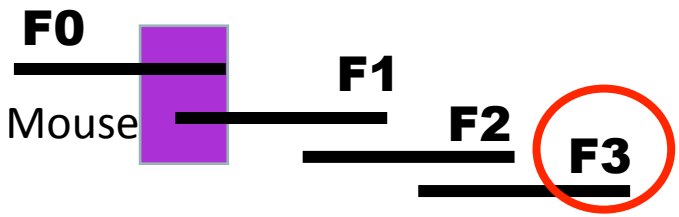
Jones, 2012,
Moore et al., 2013,

Jones 2012)13

Nature Reviews | Genetics

Transgenerational Effects of Endocrine Disrupters

Exposure to chemical:
Parents + F1 Embryo
Non-persisting chemicals



Emotionality
Vinclozolin (

F3 female mic
(shorter stay in ope

(n = 8-9) (Skinner et

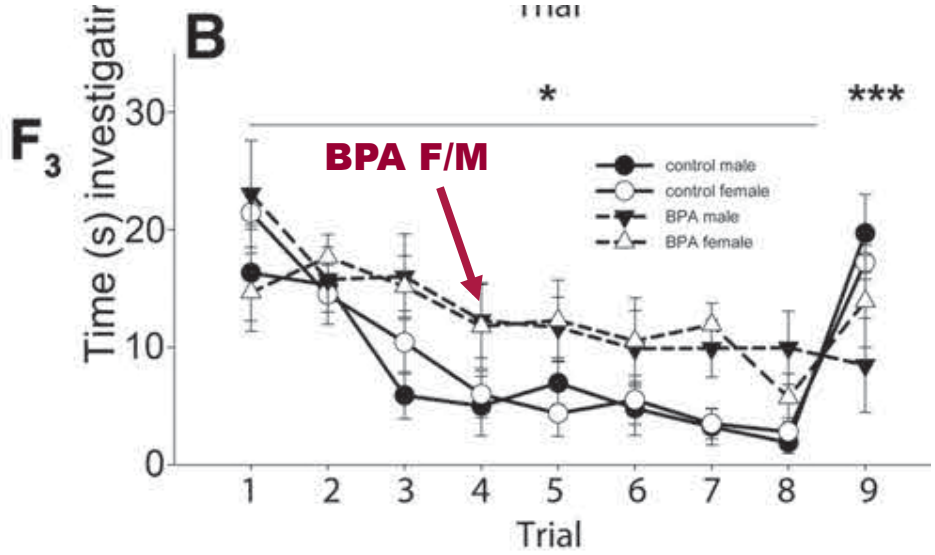
The alterations in F3 behavior are accompanied by changes in DNA methylation in germ cells.

Social Recognition
Bisphenol A (0.8 mg/kg to pregnant mice)

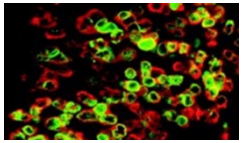
F3 mice ***don't stop investigating a previously known mouse***

Habituation decreased.
Altered interpretation of stimuli
(visual, auditory, other, independent of odor).

(F1 n = 12-14, F3 n = 7-8) (Wolstenholme et al., 2013)



DNA-Methylation and Development: Germ Cells and Somatic Cells



Primordial Germ Cells in the genital ridge of the embryo:
DNA de-methylated and later ***re-methylated***. **Environmental influences**?

Germ Line	**Somatic Cells**
F1 (exposed) ***Germ cells: altered DNA methylation***	
F2 (not exposed) ***Germ cells: altered DNA methylation***	**F2** ***Somatic cells*** with ***altered gene transcription***
F3 (not exposed) ***Germ cells***: ***altered DNA methylation***	**F3** ***Somatic cells*** with ***altered gene transcription***

Note:
Methylation processes (DNA, Histones) ***differ between developmental stages!***

Specific DNA methylation in **germ cells** (e.g. sperm cells)
is translated into Specific DNA methylation in **embryonic cells**
is translated into Specific DNA methylation in **differentiated cells**.

Mechanisms?

Embryonic Stem Cell | **Neural Precursor Cell** (**Neuro-genic** | **Glio-genic**) | **Neuron** | **Astrocyte**

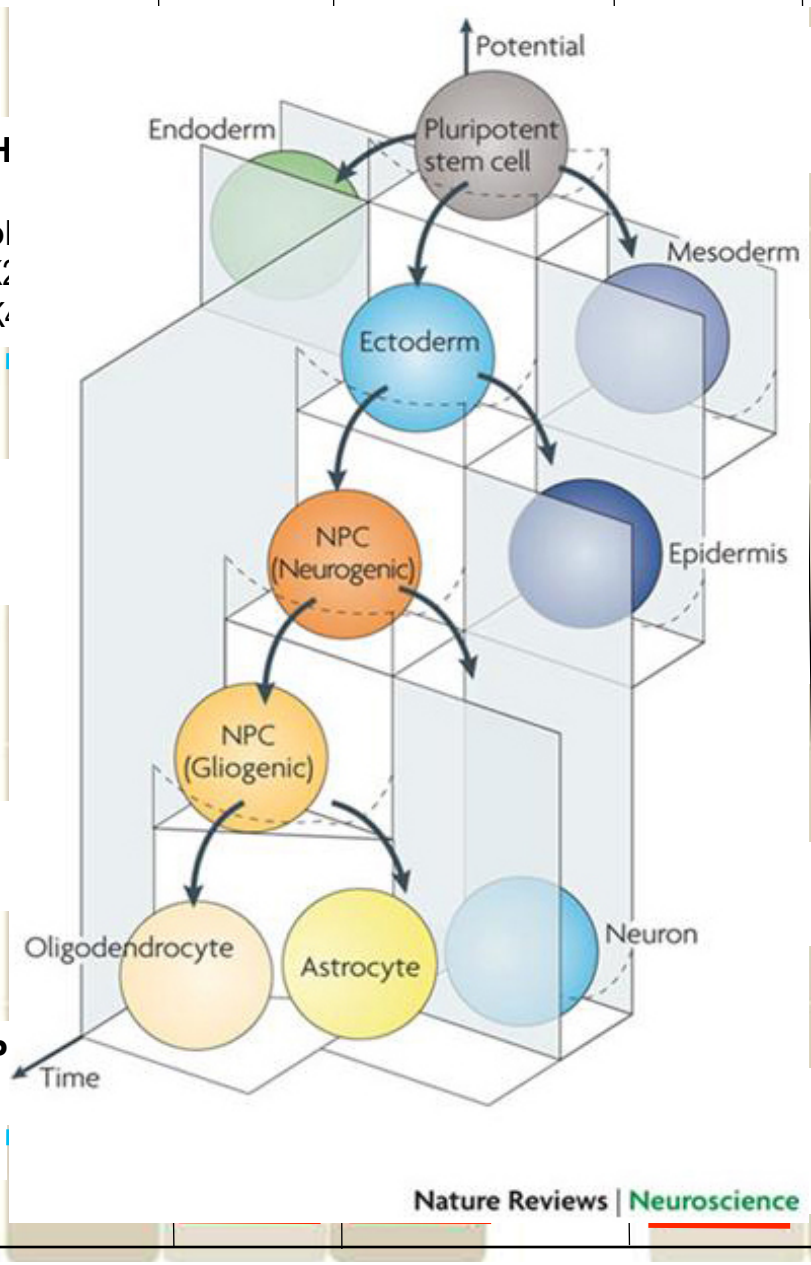
Pluripotent genes
Oct4, Gdf

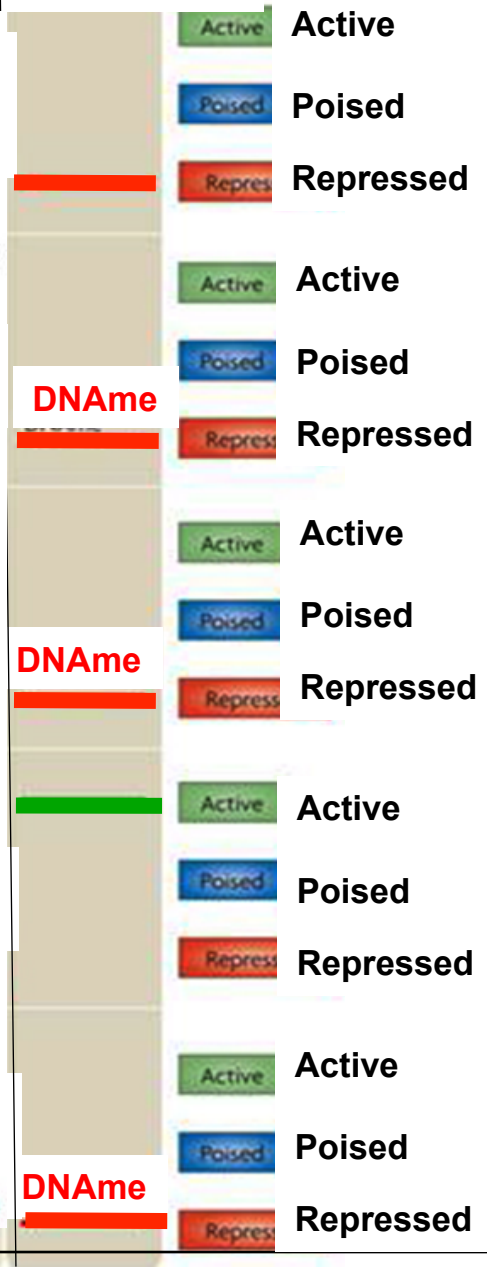
Neural genes
Ngn1, Pax6, Sox1

Neuronal genes
Syt1, Sema4f, Grid1

Astrocytic genes
Gfap, S100b, etc.

***Non-neuronal* genes**
Pparg, Sp7, etc.





Hirabayashi, Gotoh 2010

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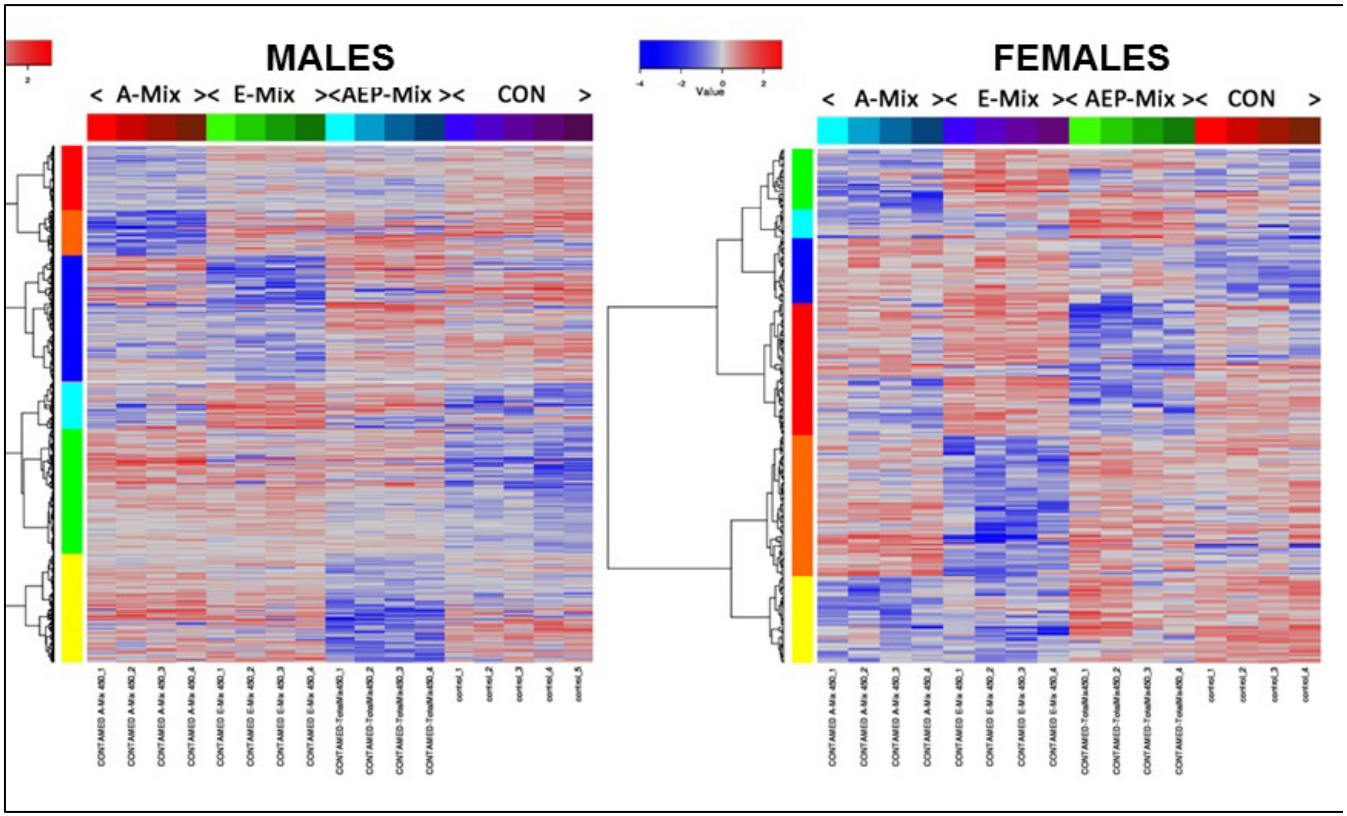
*Developmental Exposure to **Mixtures of Endocrine Disrupters**:*
Gene Expression in Medial Preoptic Area of Rat Offspring on Postnatal Day 6

A-Mix ***Anti-androgenic***	**E-Mix** ***Estrogenic***	**AEP-Mix** ***Anti-androg. + estrogenic***
DBP		DBP
DEHP		DEHP
Vinclozolin		Vinclozolin
Prochloraz		Prochloraz
Procymidone		Procymidone
Linuron		Linuron
Epoxy-conazole		Epoxy-conazole
pp'-DDE		pp'-DDE
		Paracetamol
	4-MBC	4-MBC
	EHMC/OMC	EHMC/OMC
	Bisphenol A	Bisphenol A
	Butyl-paraben	Butyl-paraben



Lichtensteiger et al., 2015

*Risk Genes for **Autism Spectrum Disorder** affected by EDC Mixtures*

Genes involved in

- ***Embryonic development***
  (NF1, Tsc2, Met)
- ***Brain Development, Cell Adhesion, Synaptogenesis***
  (Cadm, Cntn3, Cntn4, Dlg1 Mdga2, Nrxn1, Nlgn1, Nrcam, Reln)
- ***Glutamatergic Neurotransmission***
  Cacna1c, Fmr1, Grik2, Il1rapl1, Shank1, Shank2, Shank3, Syngap1, Tsnax
- ***GABAergic neurotransmission***
  Abat, Gabra4, Gabrb1
- *Other processes*
  Dpp6, Dpp10, Dpyd, Park2

➔ ***Possible interaction of chemicals with genetic background***

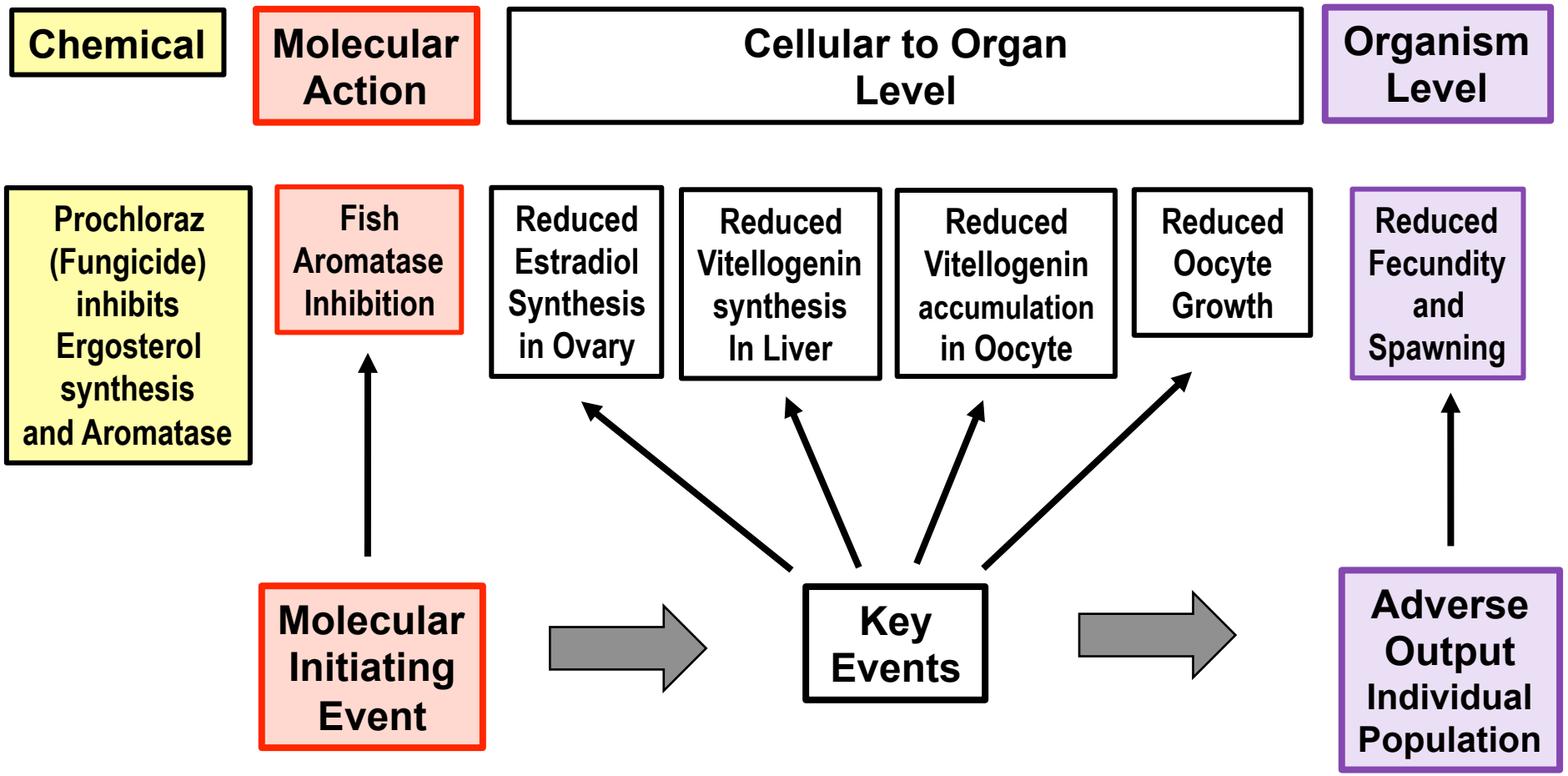
Lichtensteiger et al., 2015

oteins
Grip1,

acna1g,
b3,

nsteiger et al., 2015

How could we link in vitro with in vivo? ***Adverse Output Pathway (AOP)***



OECD, Testing of Chemicals: Adverse Outcome Pathways, Molecular Screening and Toxicogenomics
EAGMST (Extended Advisory Group on Molecular Screening and Toxicogenomics)

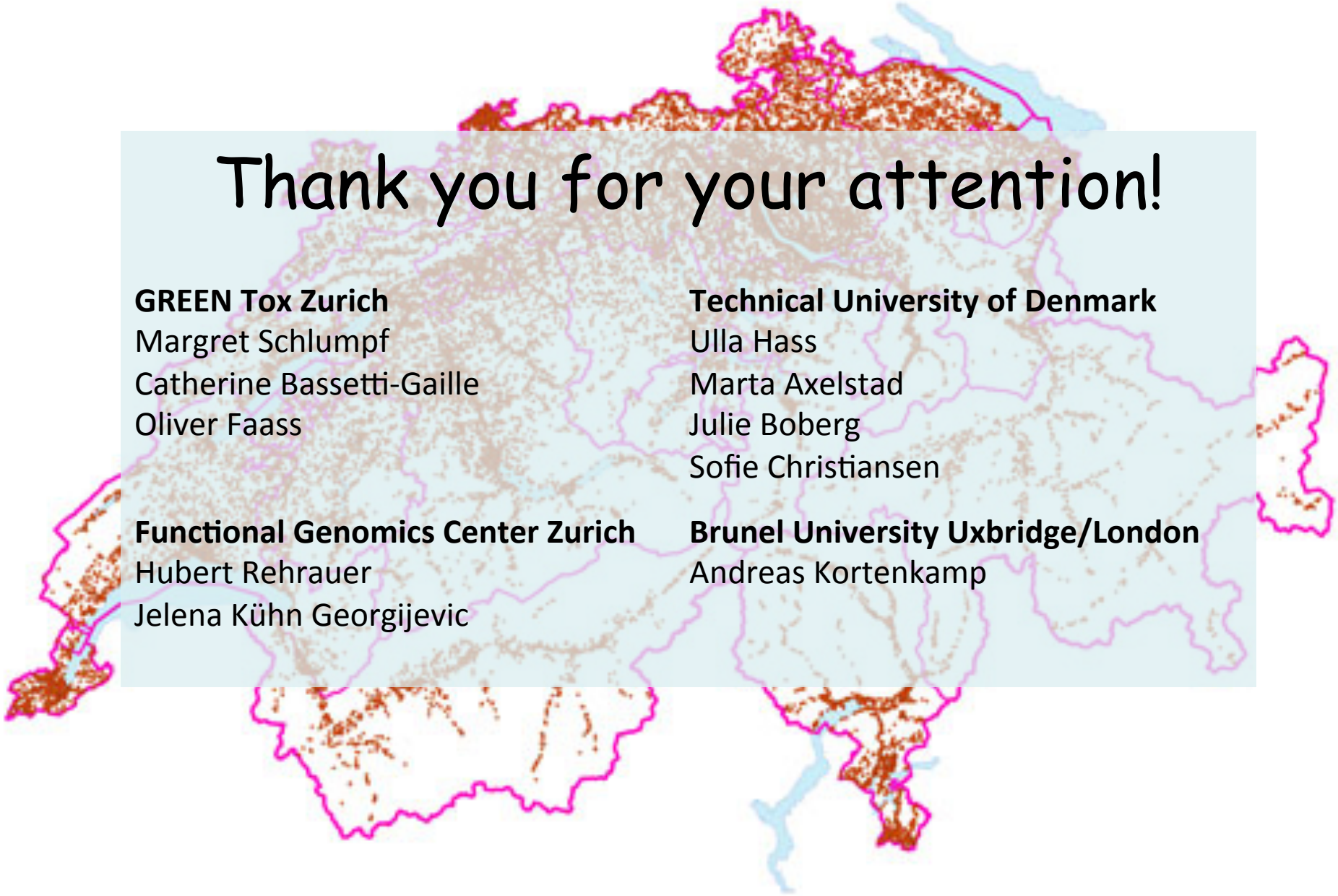
Web: Look for “OECD AOP” and “AOP-Wiki

AOP Example: Dan Villeneuve, (US EPA)

Take-Home Message

- ***Korrelationen** zwischen **Chemikalienexposition während der Entwicklung** und **Verhaltensstörungen** gut dokumentiert in epidemiologischen Studien an **Kindern** und im **Tiermodell**, z.B. für **Endokrine Disruptoren (EDCs).***
- Im Vordergrund **kognitive Leistungen, emotionales und soziales Verhalten**. Chemikalien stören die Entwicklung von Hirnregionen wie z.B. **Hippocampus**, die für diese Funktionen zentral sind, zum Teil über Interaktionen mit **Schilddrüsenhormonen** und **Sexualhormonen**.
- ***Epigenetische Mechanismen** wie **DNA Methylierung** ändern die Genexpression langfristig, in **somatischen Zellen** im Individuum, in **Keimzellen Generationen-übergreifend**. **Chemikalien** können die DNA-Methylierung ändern. Wie epigenetische Modifikationen in der Keimbahn zu Genexpressionsänderungen in somatischen Zellen führen, ist nicht bekannt.*
- **Gemische von EDCs** beeinflussen die Entwicklung von **Glutamat-Synapsen**, die u.a. in kognitive Prozesse und sexuelle Gehirndifferenzierung involviert sind, sowie die Expression von **Risikogenen für Autismus (ASD).**Über epigenetische und genetische Effekte können Umweltchemikalien mit dem **genetischen Hintergrund** interferieren.
- *Ein neuer Ansatz zur **Risikobeurteilung** ist der **Adverse Output Pathway (AOP),** der in vitro-Beobachtungen mit schädigenden Effekten im Organismus verknüpft. Noch ungelöst ist der Einbezug quantitativer Aspekte.*

The Environment of the Brain: Dumpsites in Switzerland (BAFU)



Thank you for your attention!

GREEN Tox Zurich
Margret Schlumpf
Catherine Bassetti-Gaille
Oliver Faass

Technical University of Denmark
Ulla Hass
Marta Axelstad
Julie Boberg
Sofie Christiansen

Functional Genomics Center Zurich
Hubert Rehrauer
Jelena Kühn Georgijevic

Brunel University Uxbridge/London
Andreas Kortenkamp