Formation of the Blood-Brain Barrier and Susceptibilities to Toxicants

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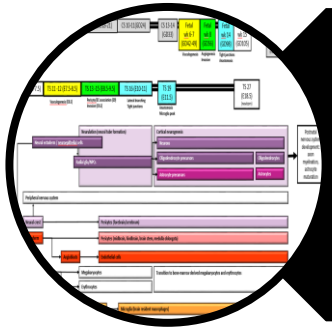
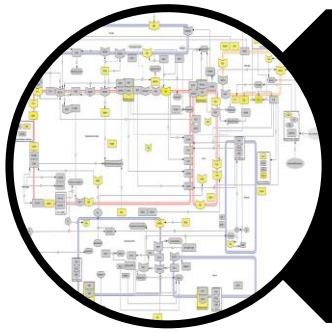
- The views expressed in this presentation do not reflect US EPA policy.

Roadmap

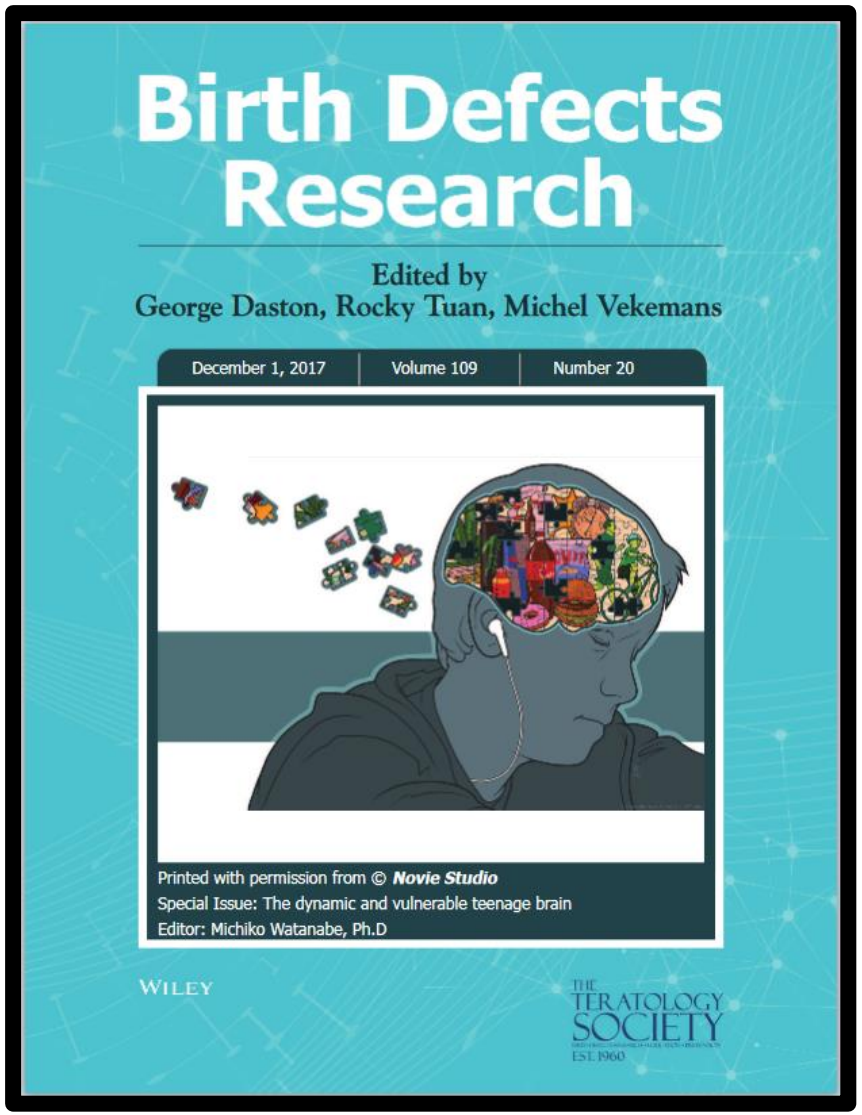
Background / hypothesis



Timeline of brain / BBB development



Modeling BBB developmental susceptibility



Background / hypothesis

The ‘black box’ underlying neurodevelopmental disorders

Genetic mutations
Infection
Chemical exposure
Nutrient deficiency
Metabolic disorder

Autism (ASD):

1 in 59 children aged 8 (1.7%)

300,000+ children

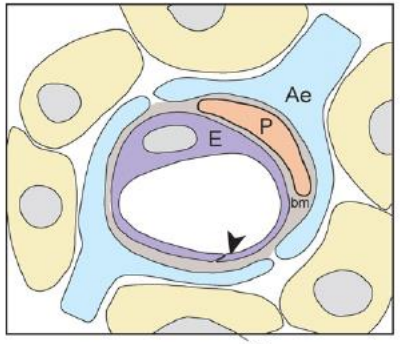
11 US survey sites (2014)

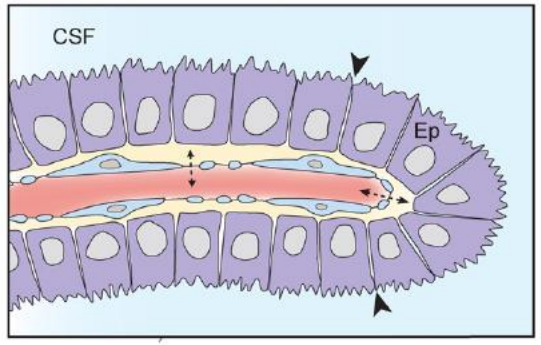
ADDM 2018 report

Autism and Developmental Disabilities Monitoring (ADDM) Network

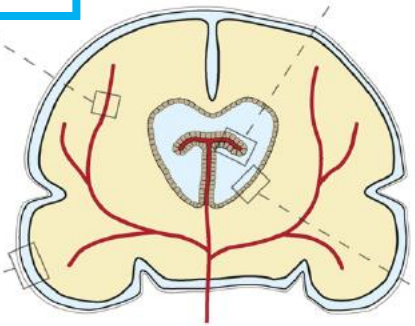
Four types of barriers in the brain

Blood-brain barrier
(cerebral cortex)

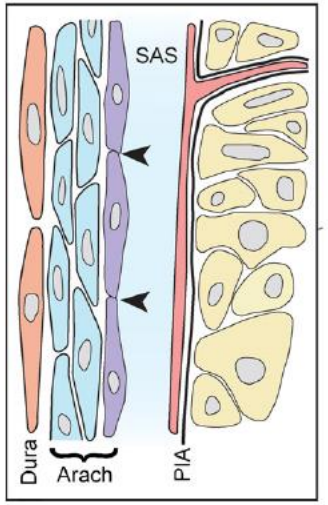


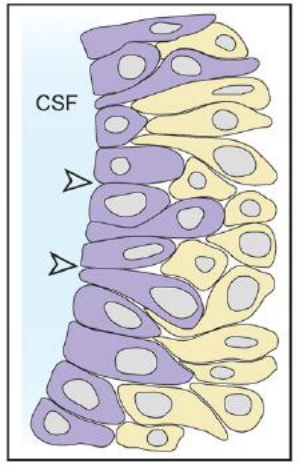


Blood-CSF barrier
(choroid plexus)



Meningeal barrier



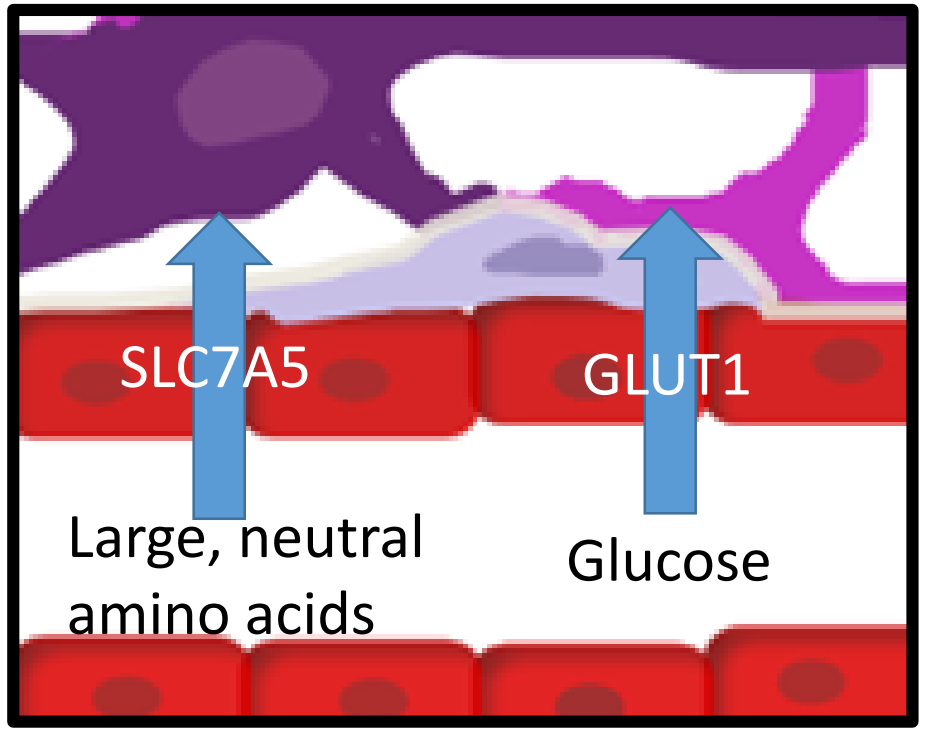


Fetal CSF-brain barrier
(neuroependyma)

Ek et al. 2012. *NeuroToxicology*

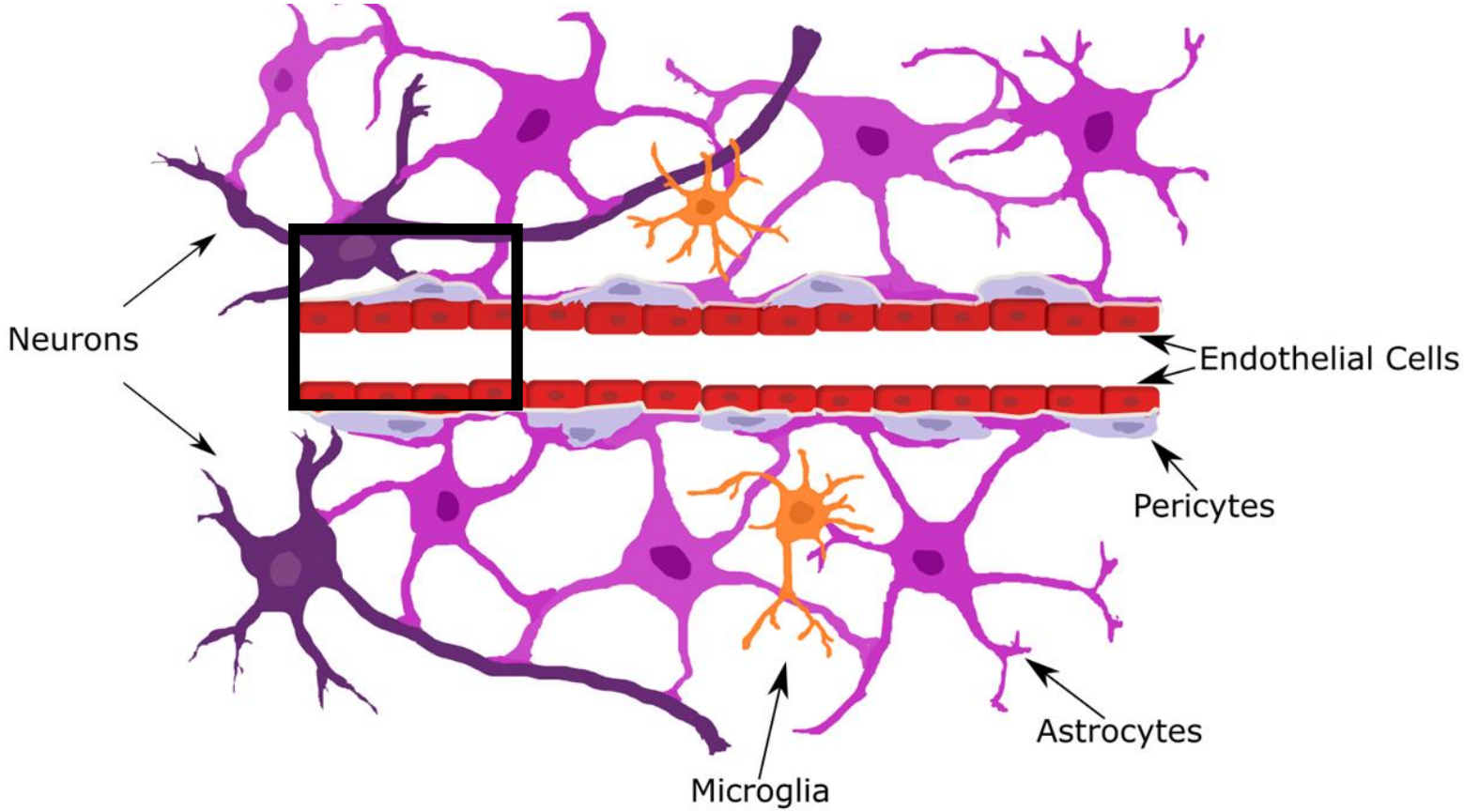
Blood-brain barrier transporters linked to neurodevelopmental disorders

- SLC7A5 (LAT1) linked to autism*
- GLUT1 deficiency syndrome
  - Epilepsy, learning disabilities



*Tarlungeanu et al. 2016. *Cell*

Neurovascular unit (NVU)

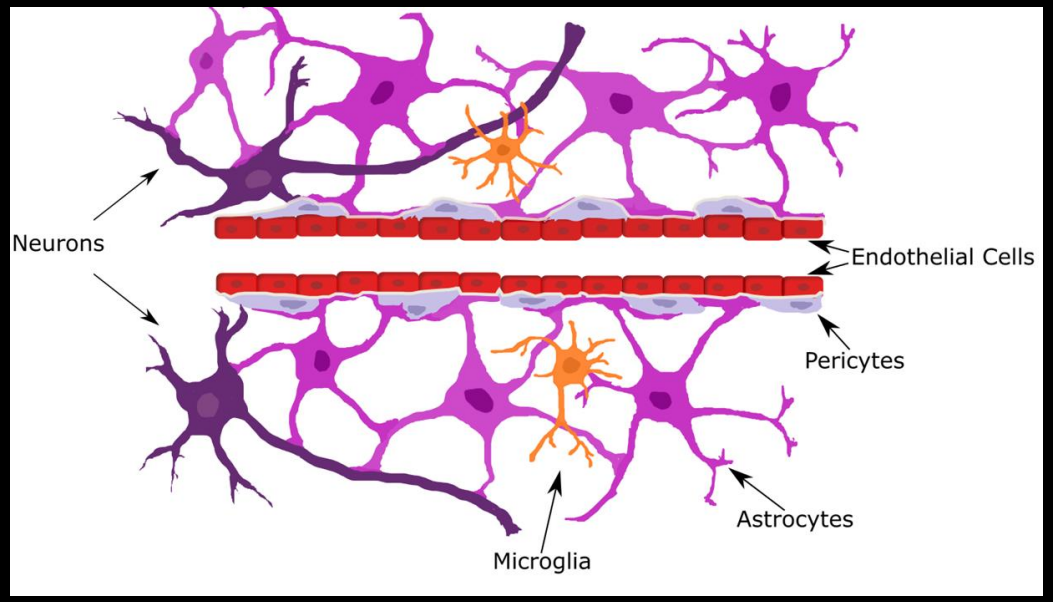


Saili et al. 2017. *Birth Defects Research*. *The Dynamic and Vulnerable Teenage Brain (special issue)*

Hypothesis

Putative BBB disrupting compounds

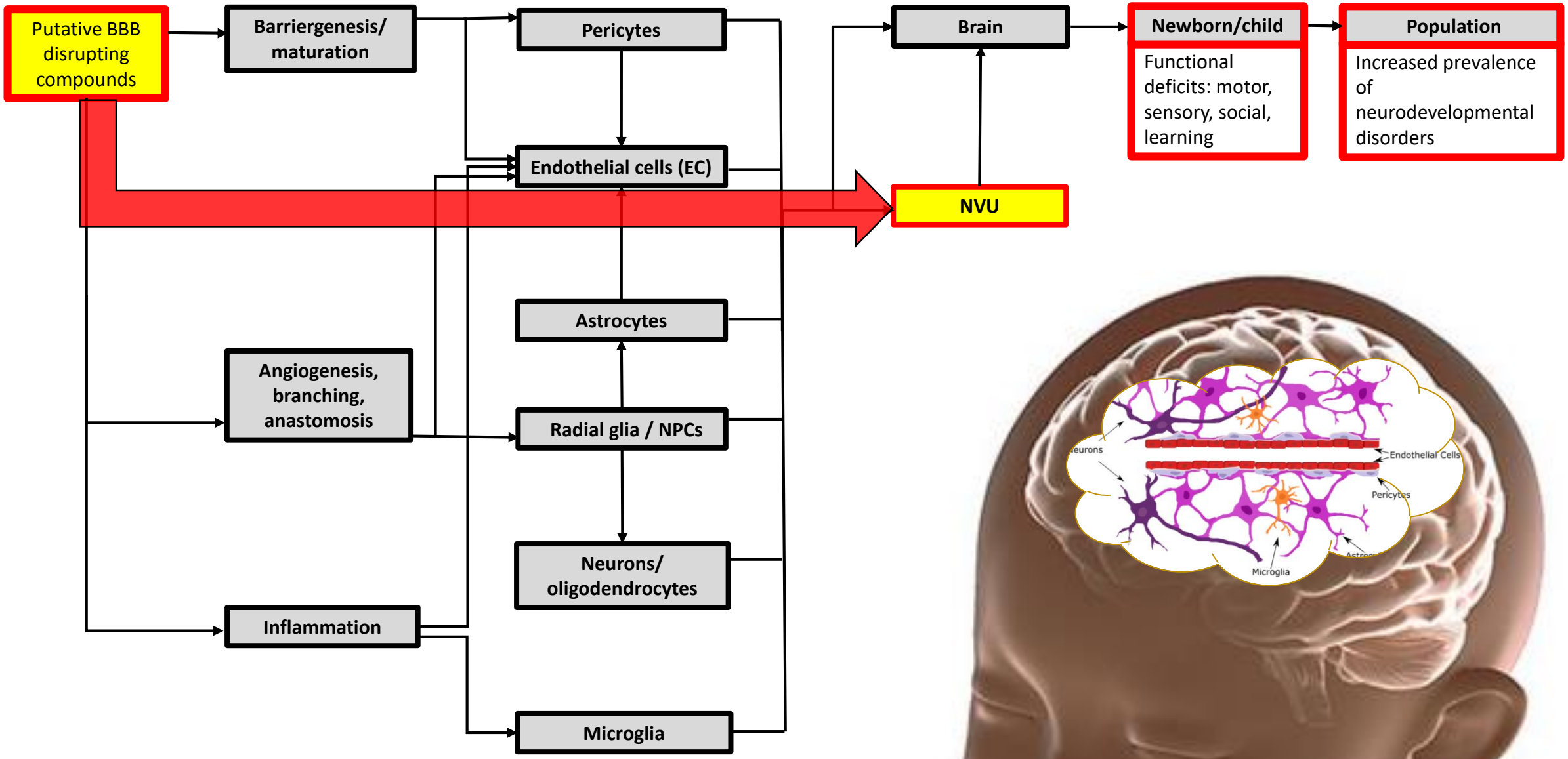
Chemical disruption of blood-brain barrier (BBB) formation leads to abnormal brain development and function



Newborn/child

Functional deficits: motor, sensory, social, learning

AOP framework of BBB developmental toxicity



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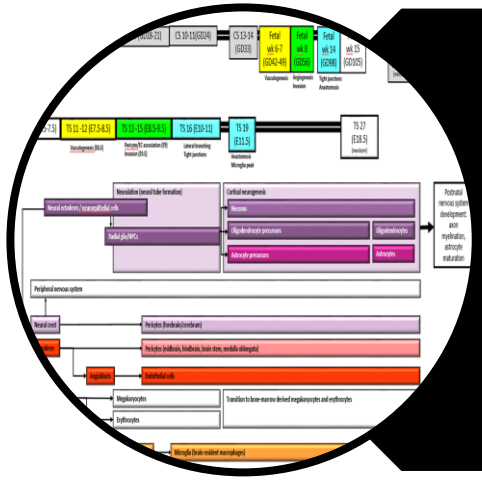
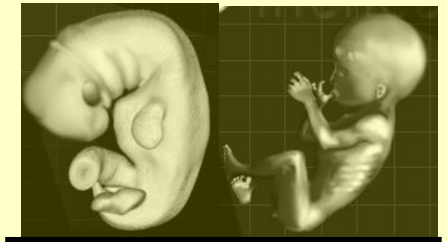


Timeline of brain / BBB development

Timeline of human brain development

Neurulation

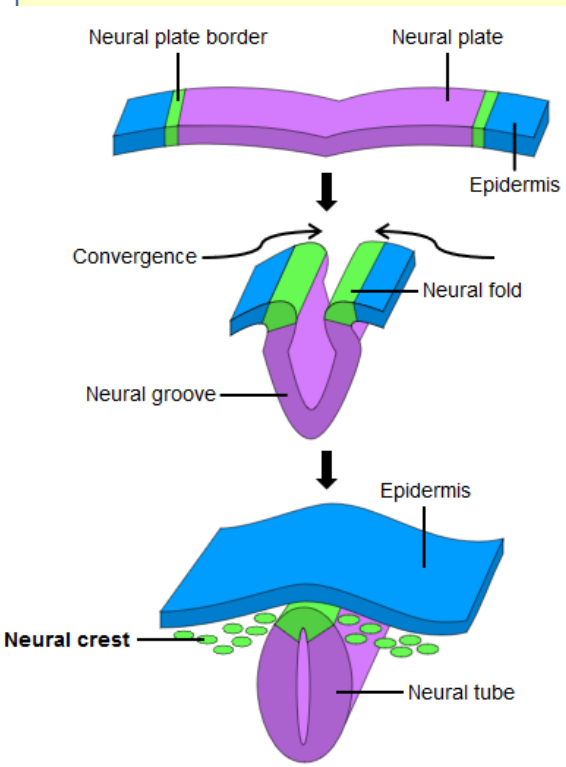
Neurogenesis/Synaptogenesis

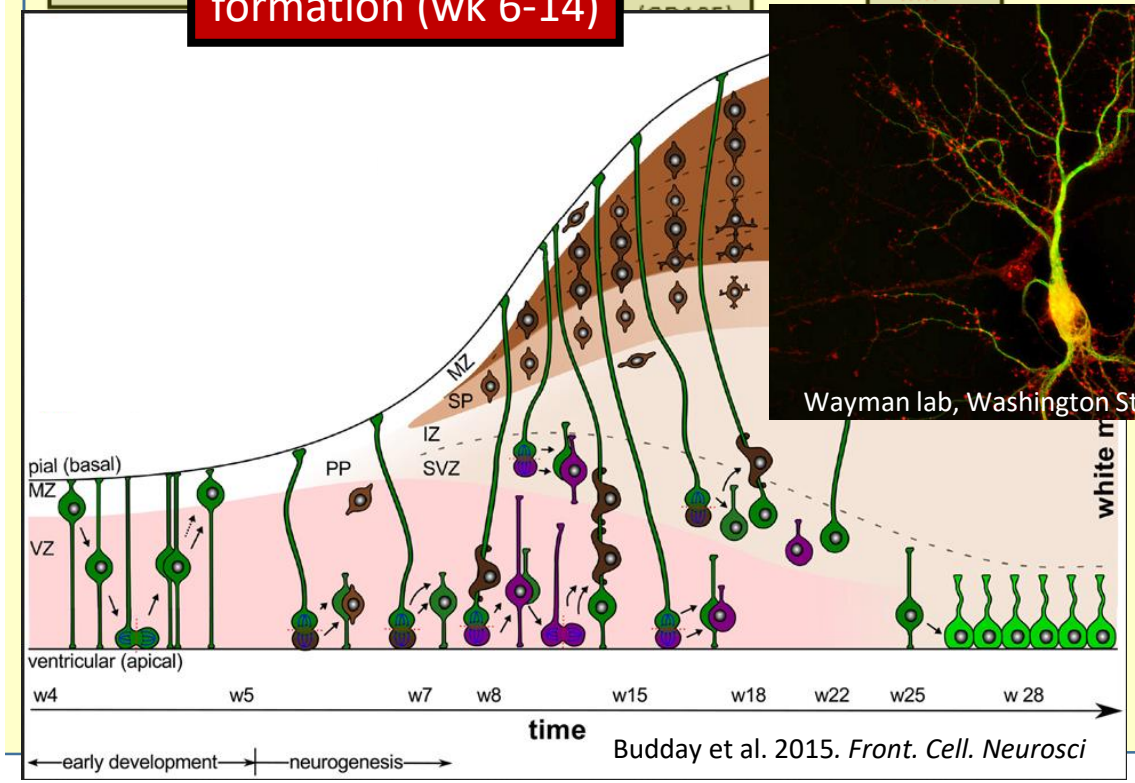


Human

CS 6-7 (GD14-16)	CS 7-8 (GD16-18)	CS 8-9 (GD18-21)	CS 10-11(GD24)	CS 13-14 (GD33)	Fetal wk 15	Fetal wk 38

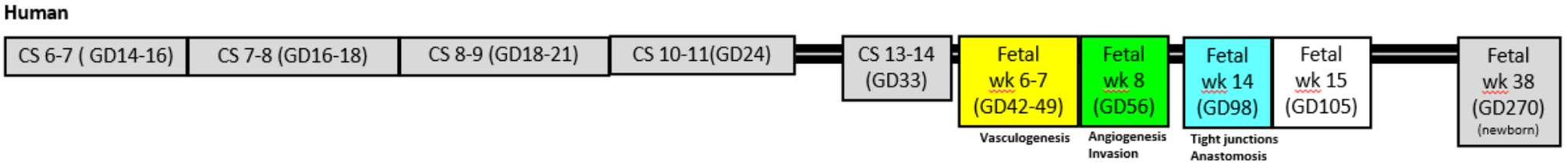
Human BBB formation (wk 6-14)

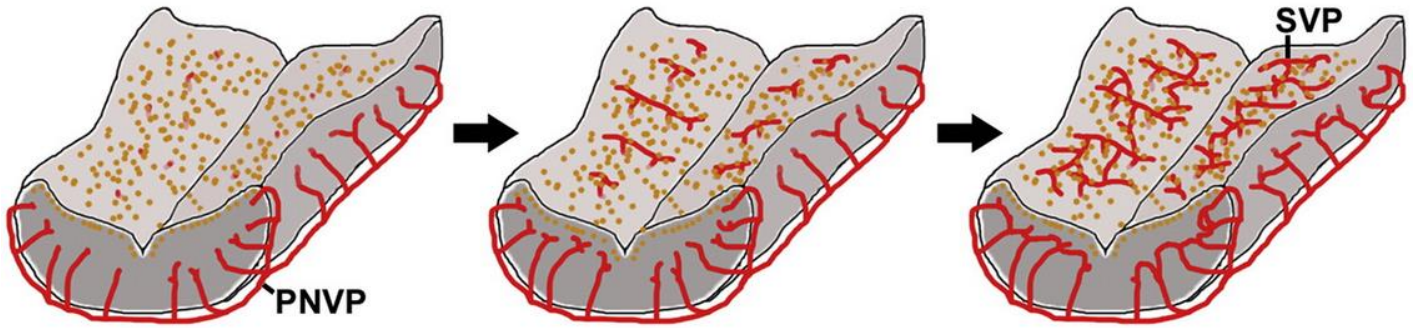




Budday et al. 2015. *Front. Cell. Neurosci*

Timeline of blood-brain barrier (BBB) development

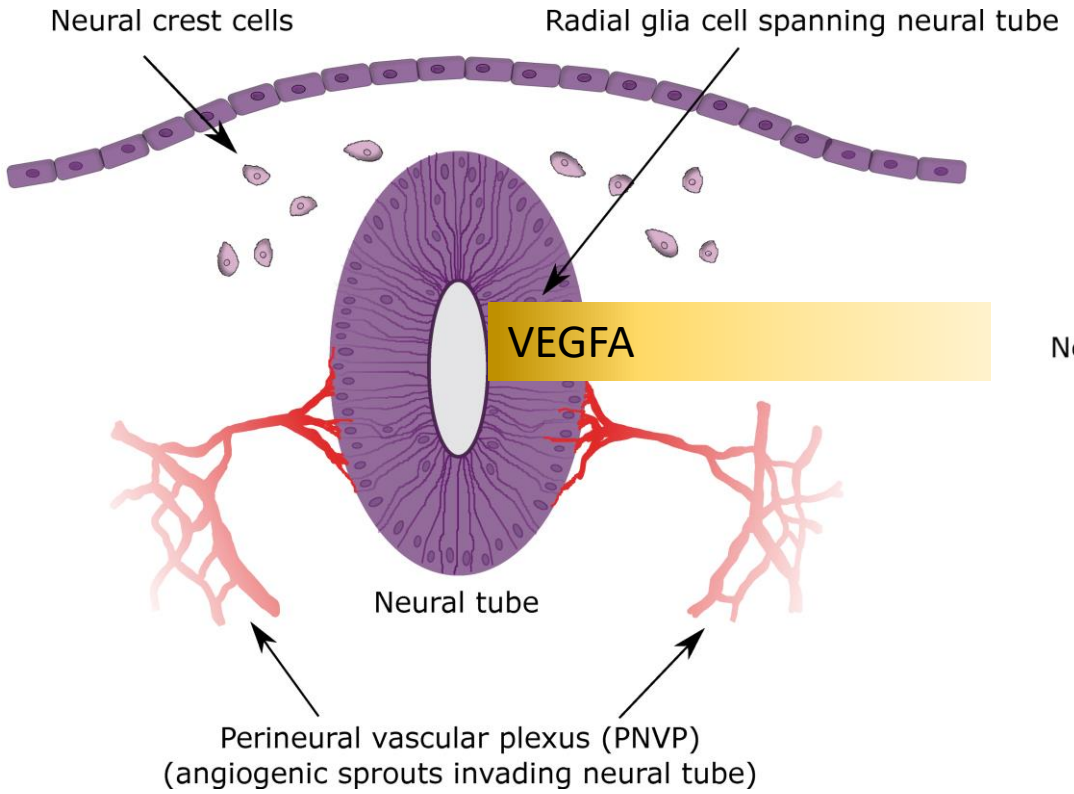


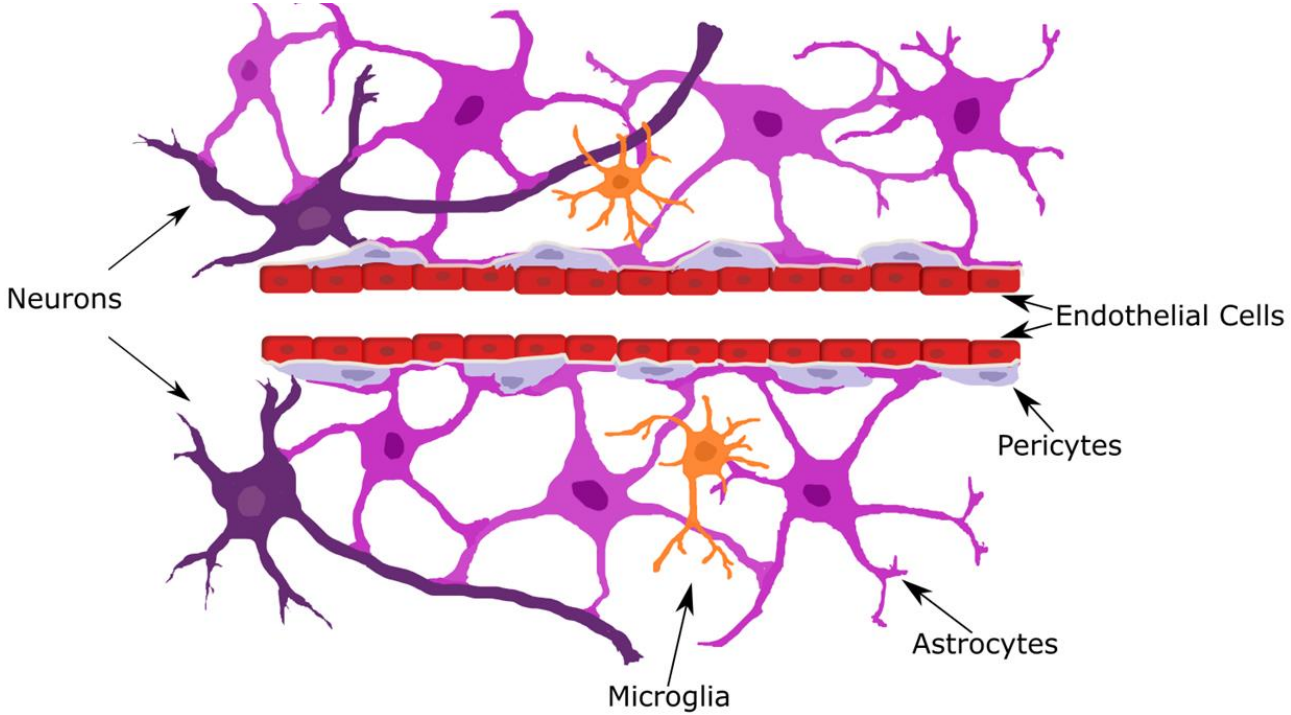


Tata et al. 2015. *Mechanisms of Development*

Sprouting angiogenesis leads BBB formation

- Hypoxic NPCs release VEGFA
- VEGFA attracts tip cells that express VEGFR2
- Tip cell recruits pericytes via PDGFB

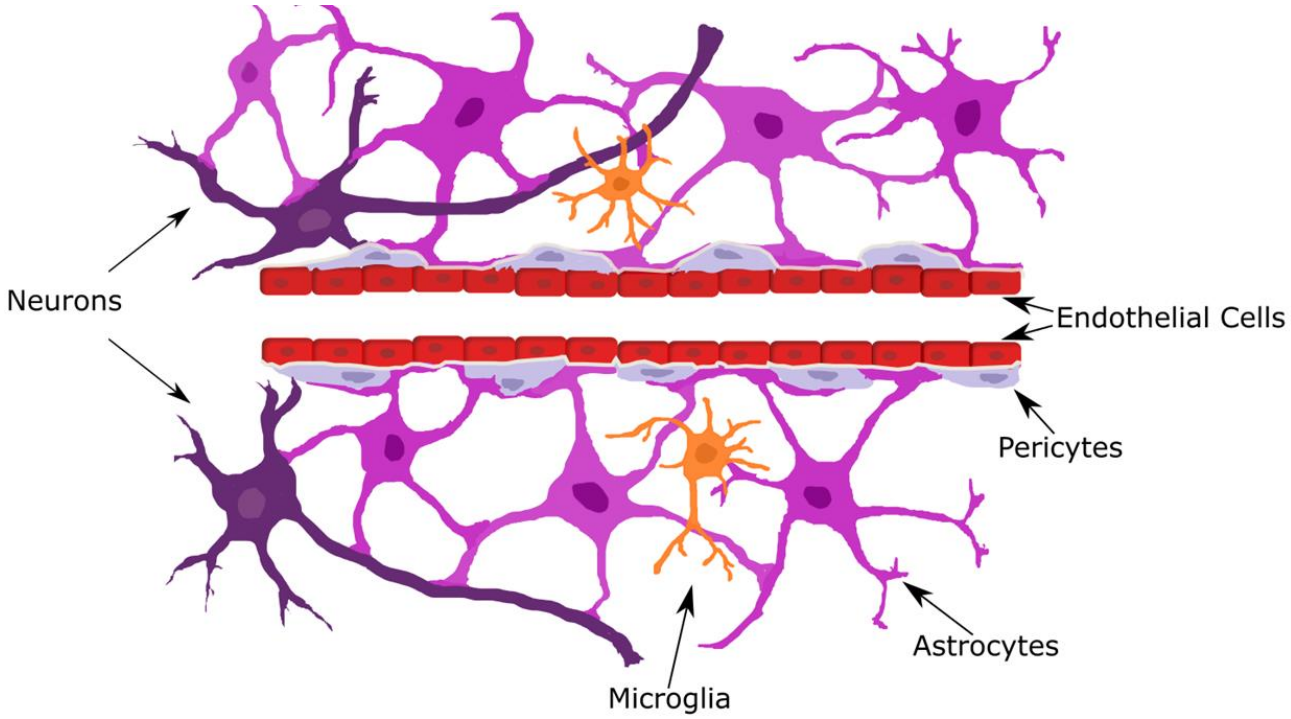




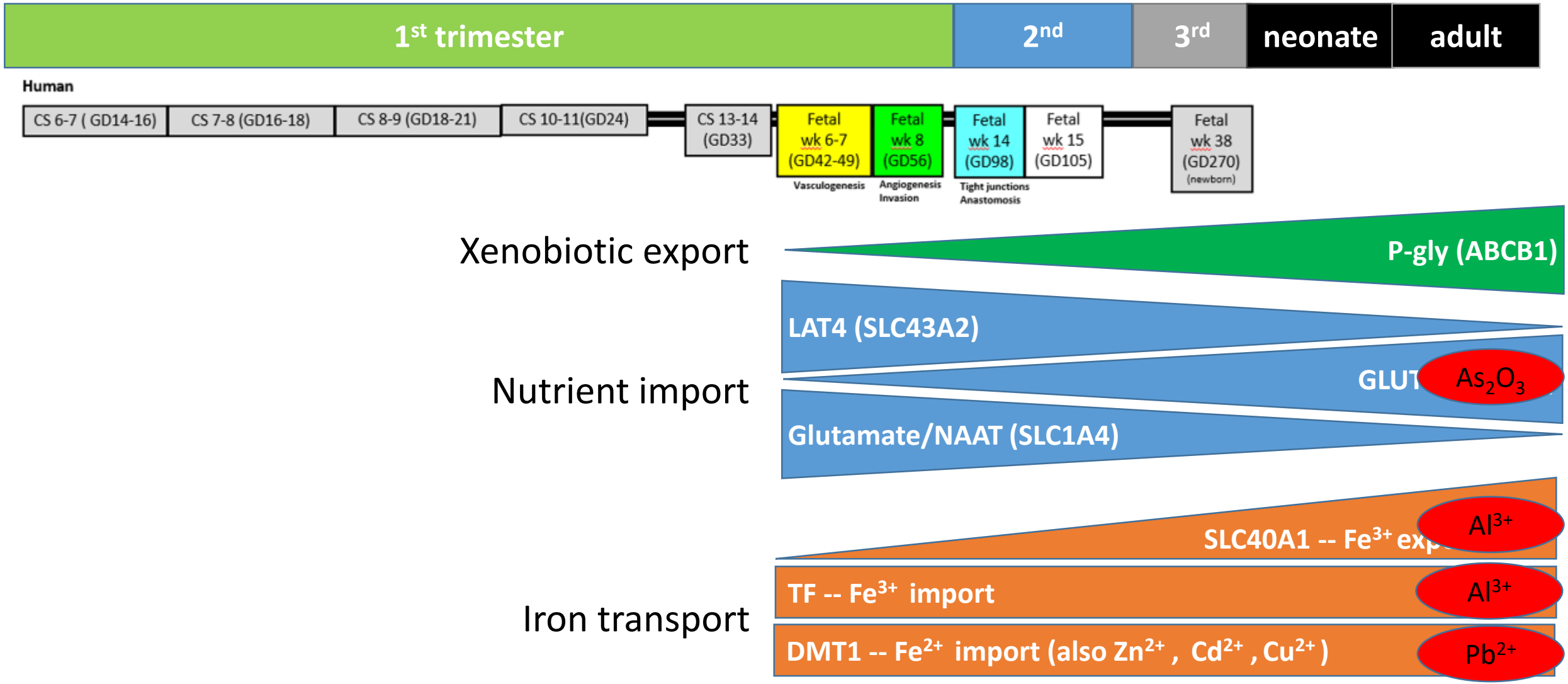
Maturation follows barrier differentiation

The neurovascular unit is a ‘target organ’ of toxicity

1. Chemical disrupts NVU interactions to perturb BBB formation
2. Chemical accesses brain cells



BBB transporter expression changes during development

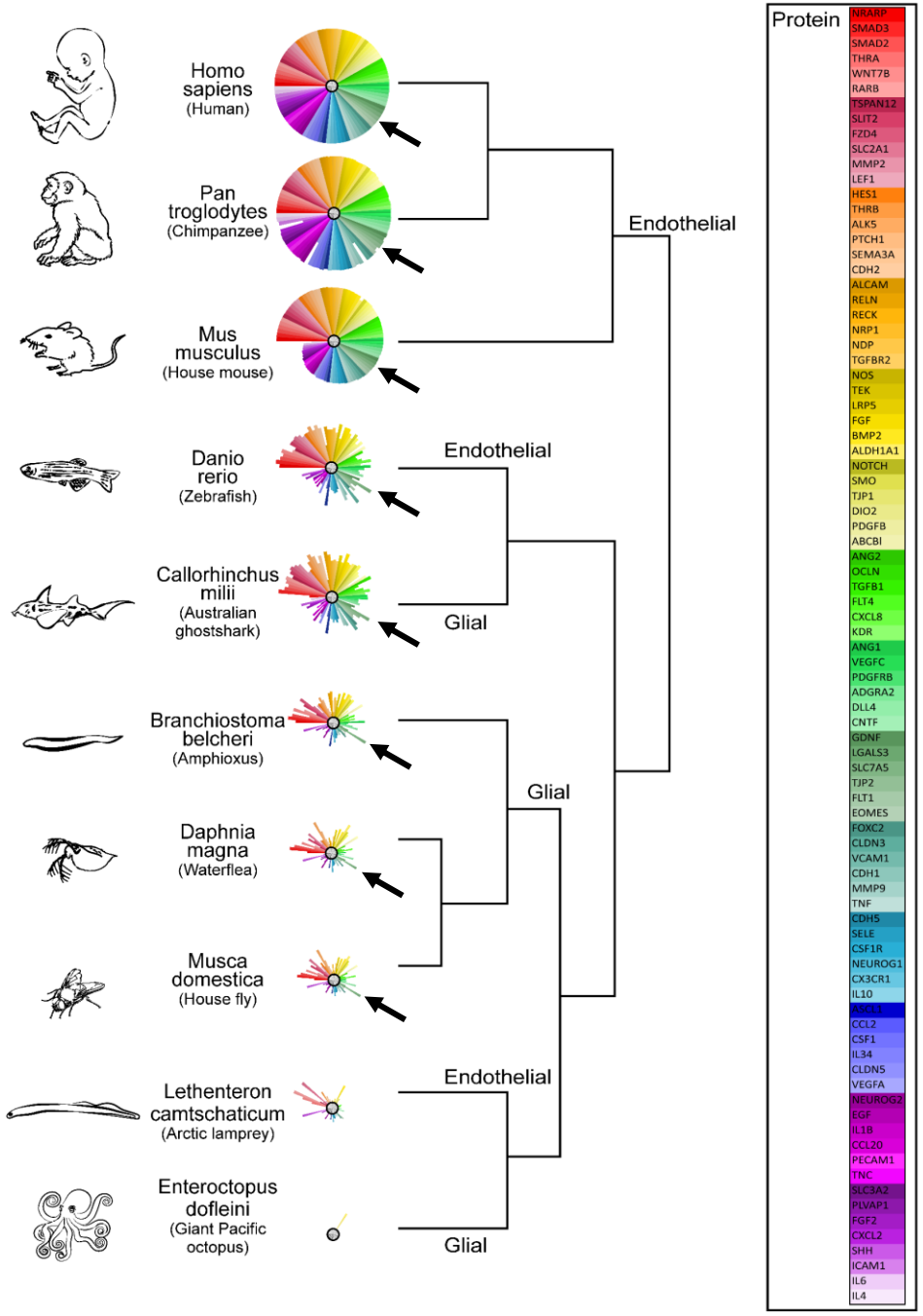


Daneman et al. 2010. *PLoS ONE*

Evolution of the neurovascular unit (NVU)

Key BBB transporters are evolutionarily conserved

Species	GLUT1	P-gly	SLC7A5
Human	100	100	100
Chimpanzee	99.7	99.5	99.6
House mouse	97.3	87.1	81
Zebrafish	81.3	64.8	77.7
Australian ghostshark	82.7	65.7	72.7
Amphioxus	38	54.5	61.6
Waterflea	46.1	48.5	45.4
House fly	50	41.5	48.5
Arctic lamprey	64.4	---	---
Giant Pacific octopus	---	---	---



Saili et al. 2017. *Birth Defects Research*. *The Dynamic and Vulnerable Teenage Brain (special issue)*

Produced using ToxPi (v2.0 beta) and SeqAPASs (v2.0)

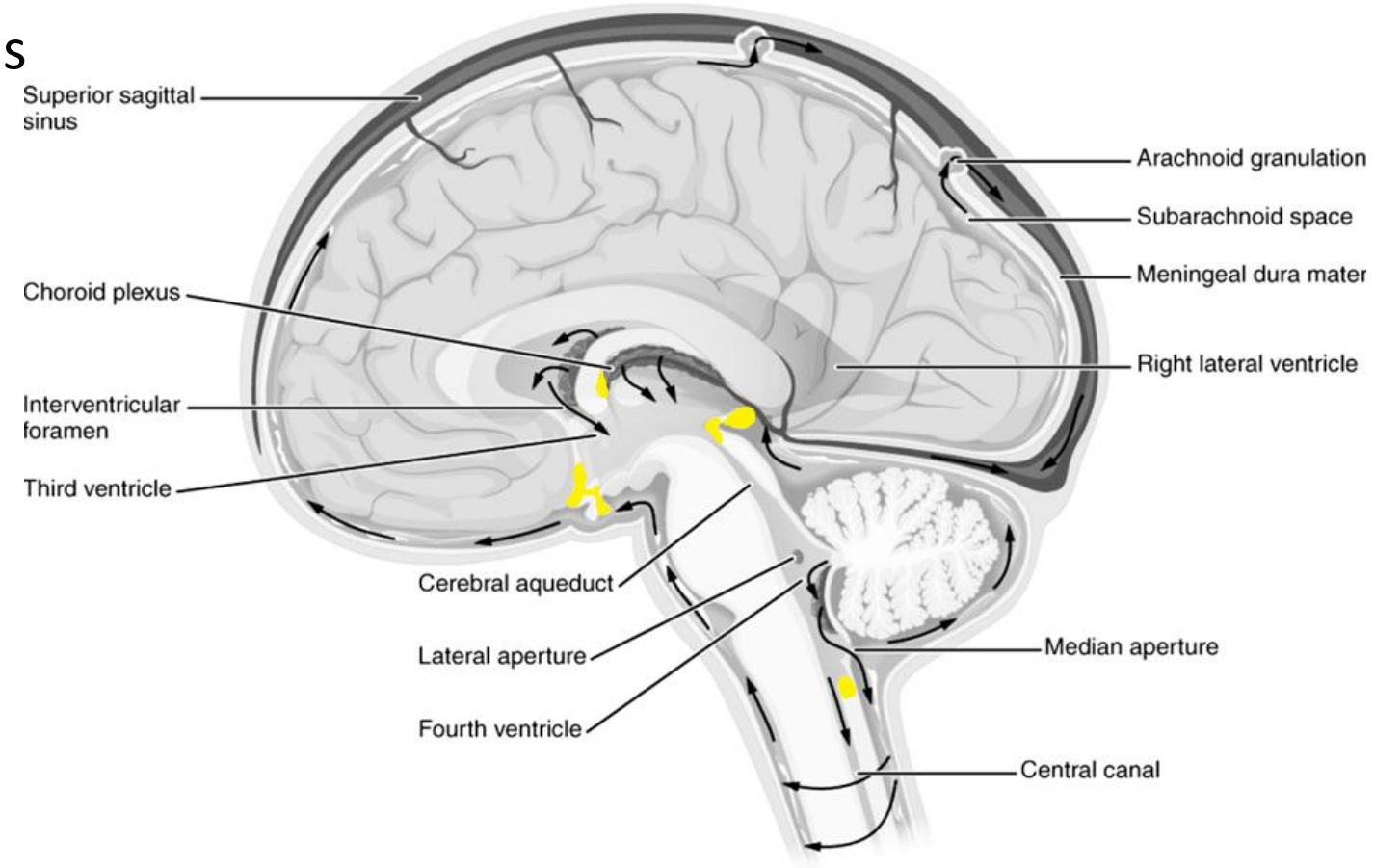
Barriers of the choroid plexus and circumventricular organs

Choroid plexus

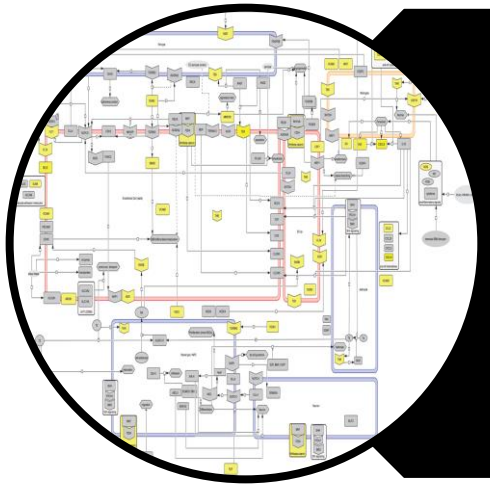
- Secretes CSF
- Glial, ependymal cells line the ventricles
- Barrier is more permissive than BBB

8 circumventricular organs

- Fenestrated capillaries

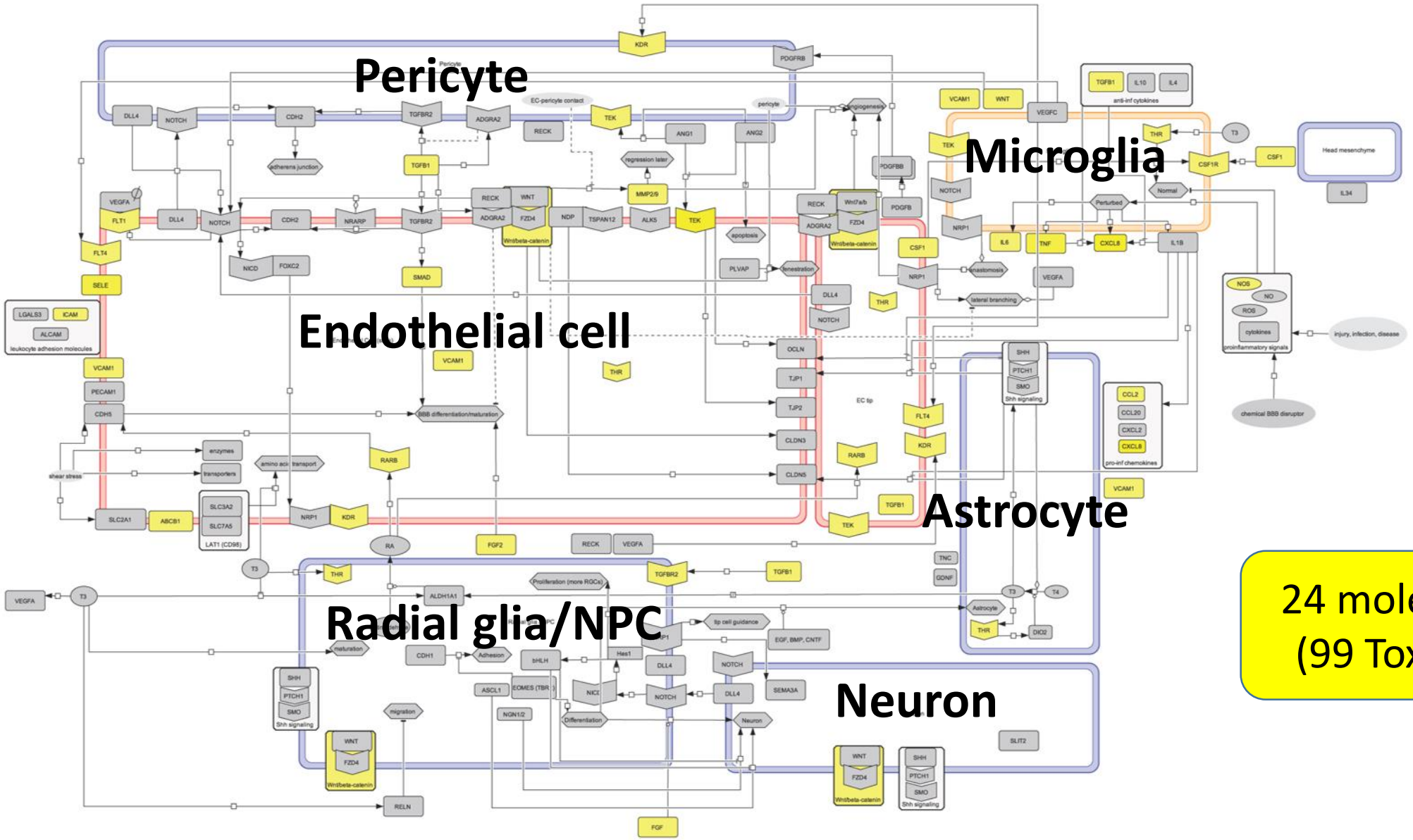


Modified from OpenStax Anatomy and Physiology Textbook (v8.25). 2016



Modeling BBB developmental susceptibility

Modeling the control circuit for BBB development



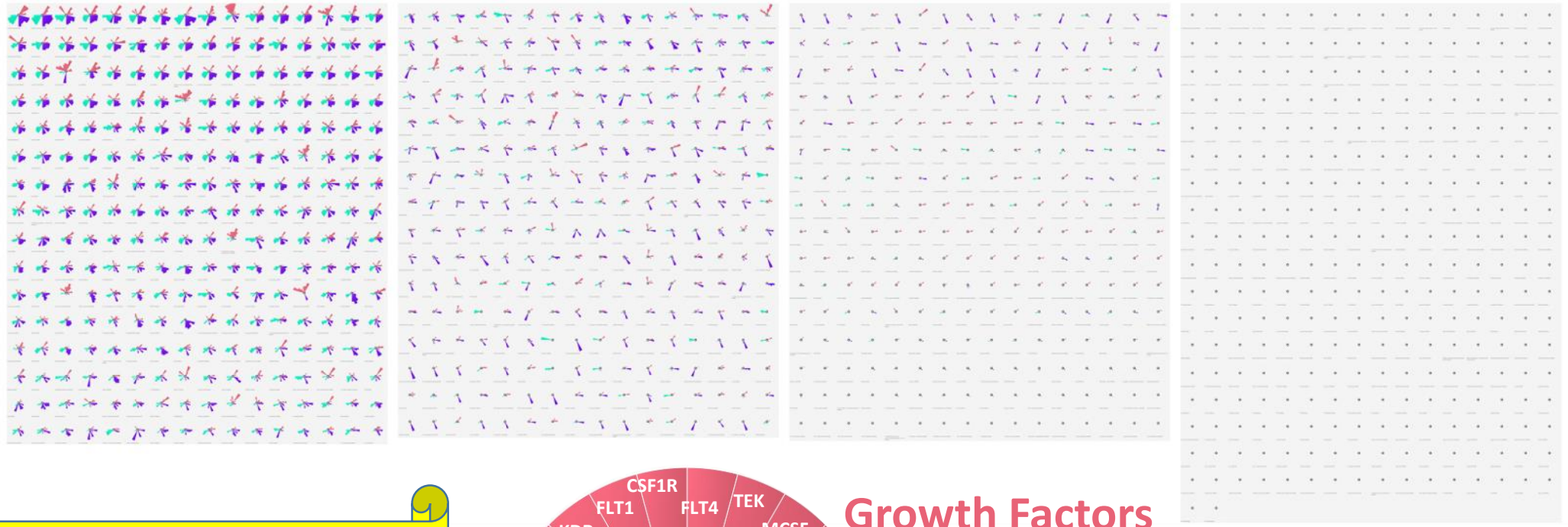
24 molecular targets
(99 ToxCast assays)

Produced in CellDesigner v4.4

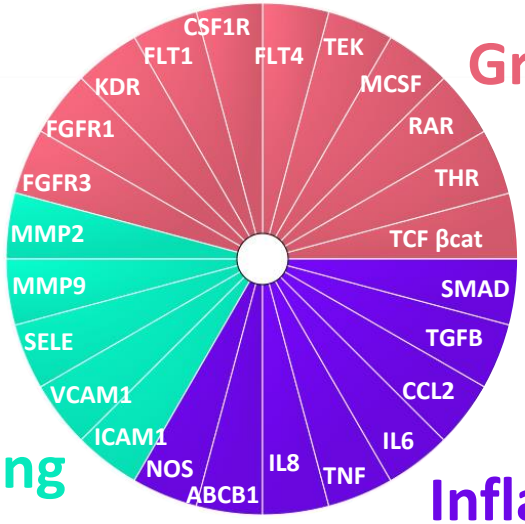
Modified from Saili et al. 2017. *Birth Defects Research*. *The Dynamic and Vulnerable Teenage Brain (special issue)*

Classifying putative developmental neurovascular toxicants by ToxPi score

1058 PhI/II chemicals ranked by ToxPi score



Ranking chemicals by predicted toxicity helps prioritize chemicals for follow-up *in vivo* testing and reduce animal use



Growth Factors

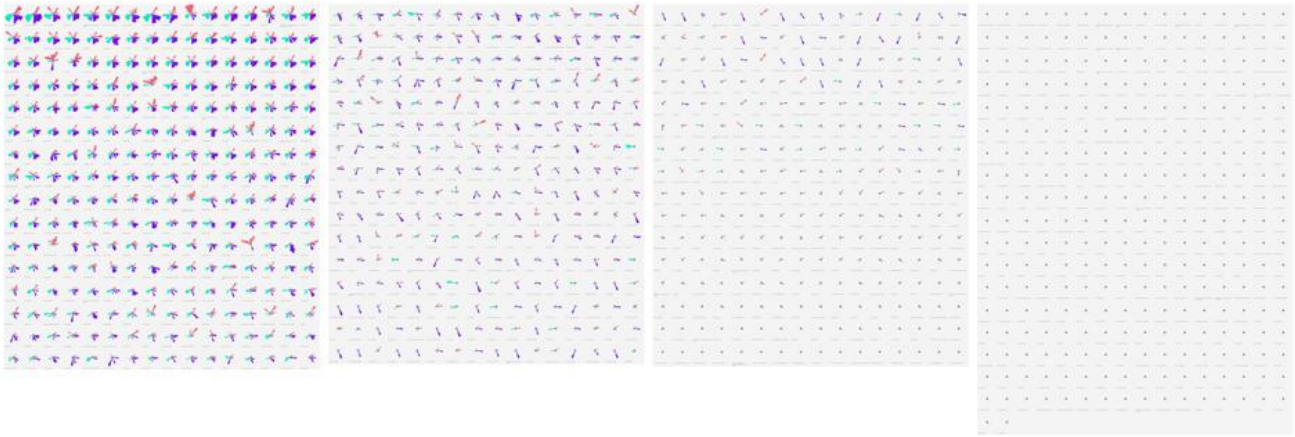
Tissue Remodeling

Inflammation

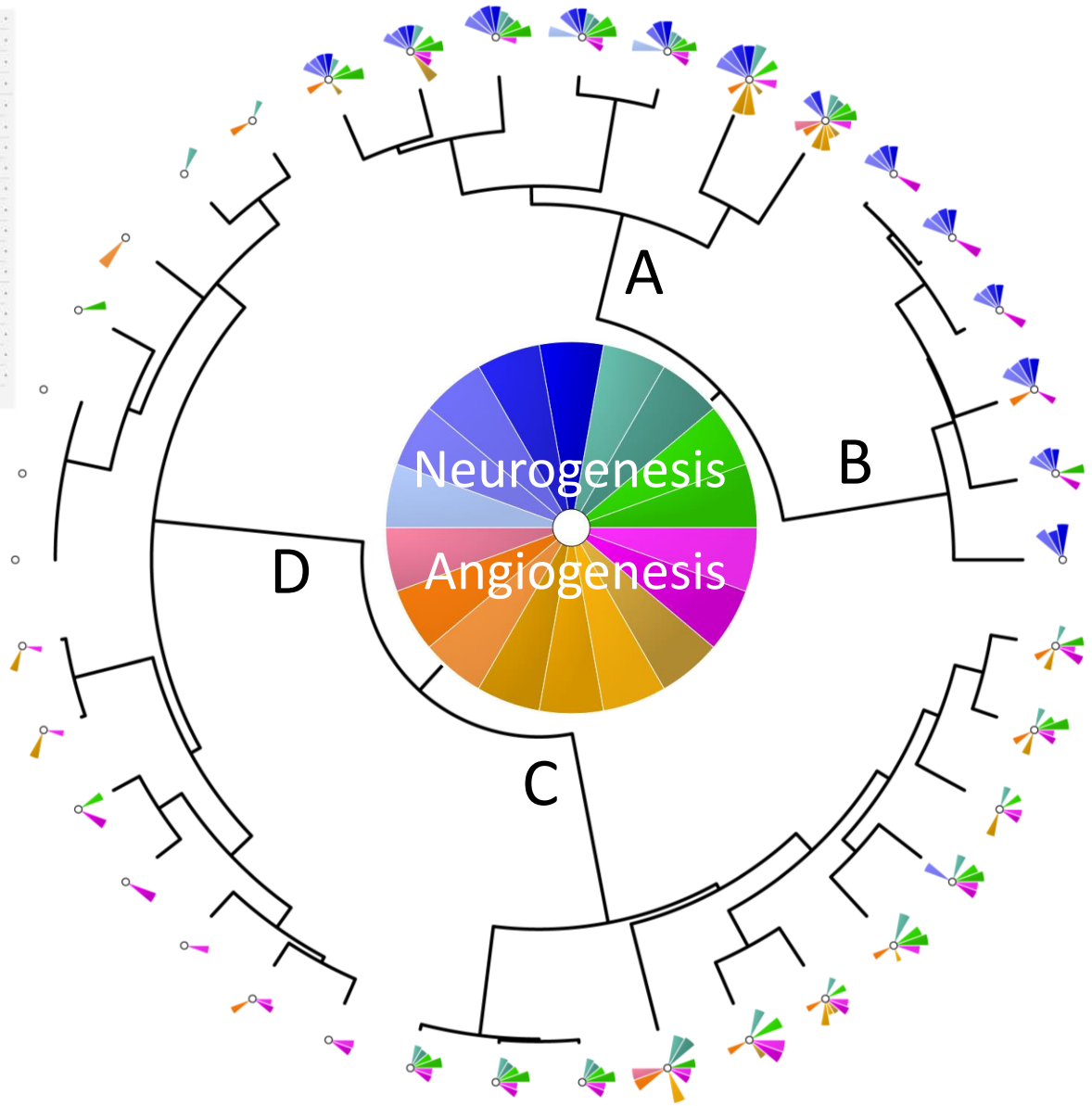
Saili et al. *In prep*

ToxPi 2.0 (beta, D. Reif, NCSU)

Evaluating predictions with *in vitro* angiogenesis/neurogenesis assays

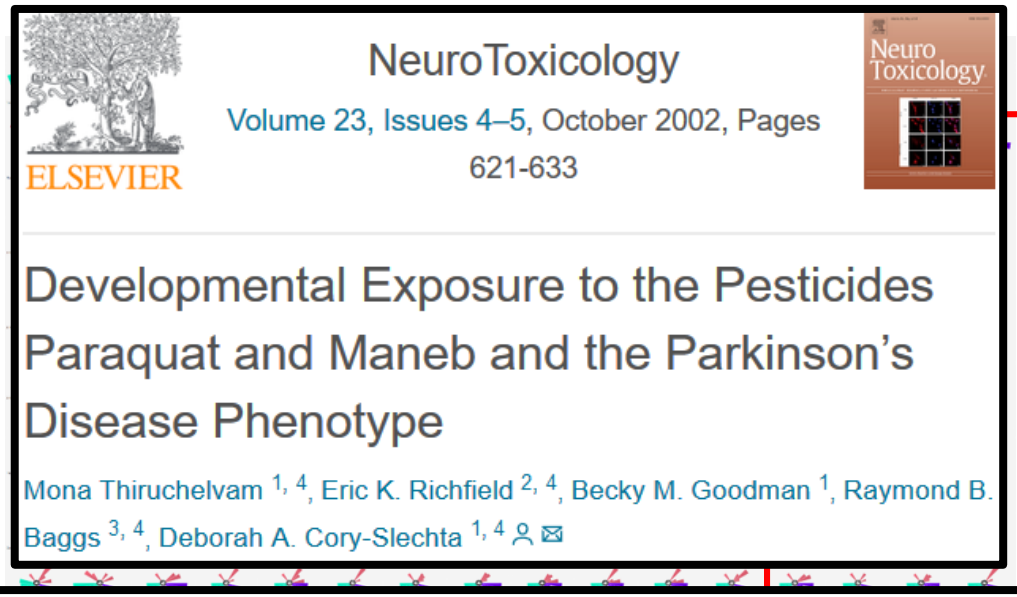


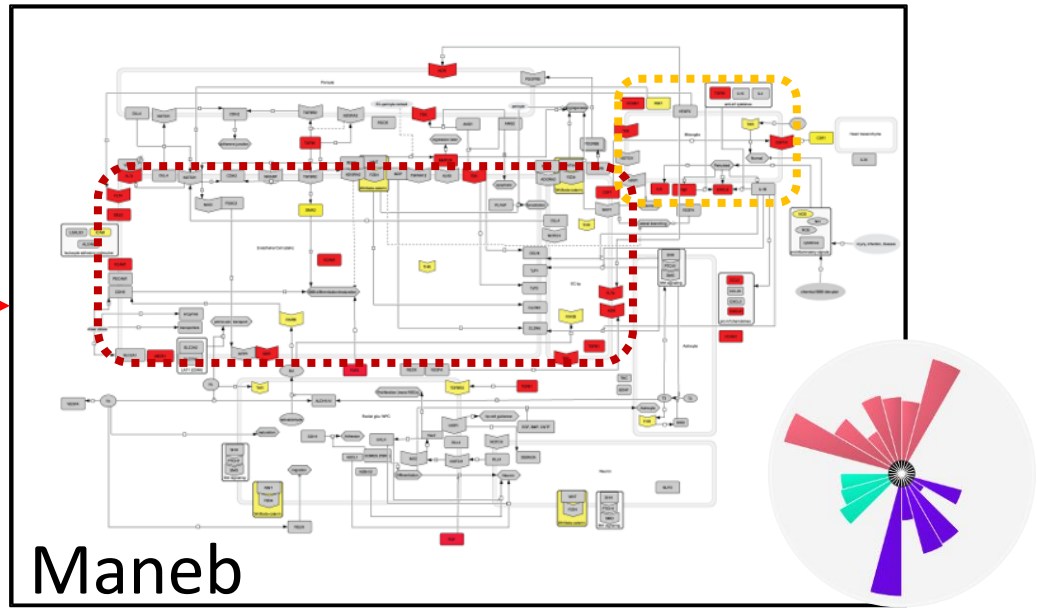
Wilcoxon Rank Sum Test
(ranks based on classification model)
C > D; p = 0.005
A > D; p = 0.018
B > D; NS

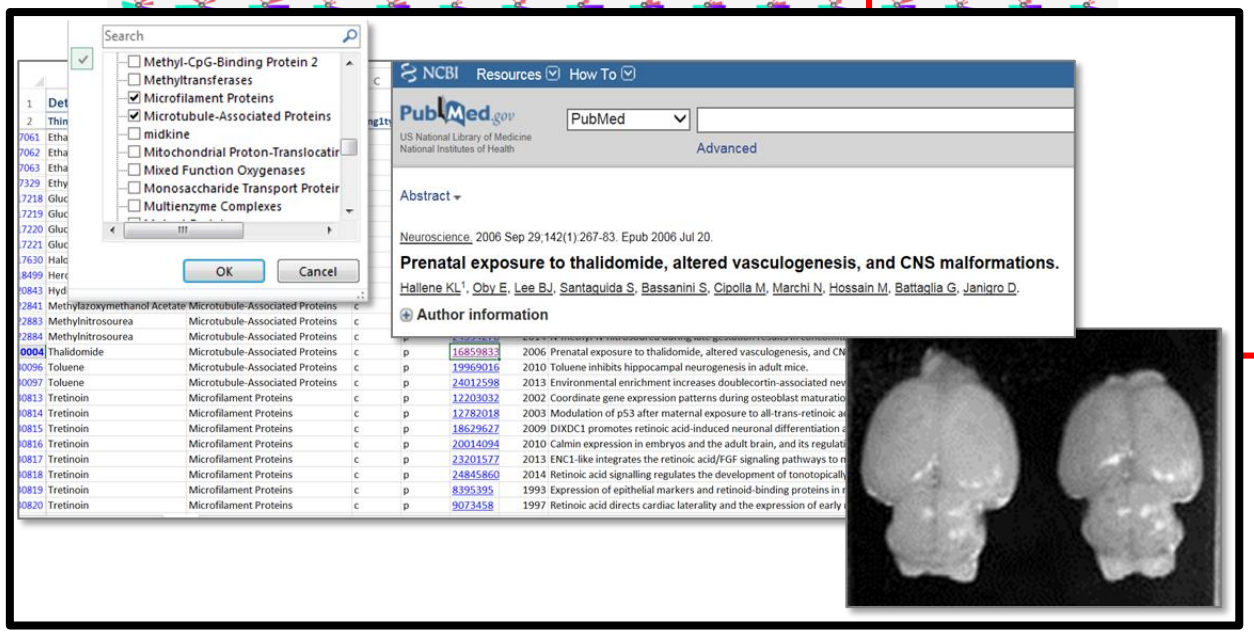


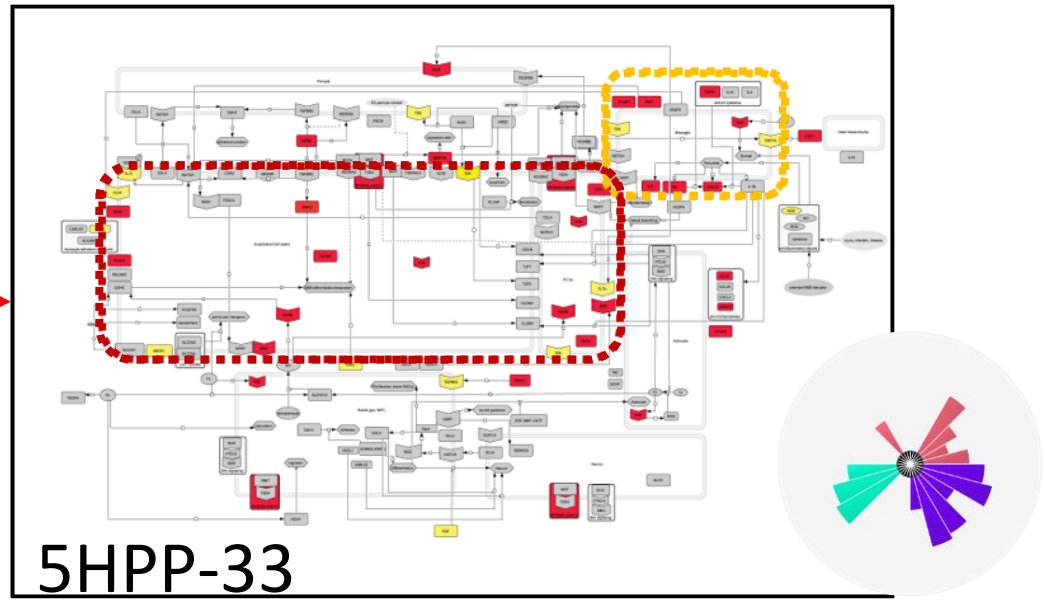
Zurlinden et al. *In prep*

Putative developmental neurovascular toxicant examples









AOP framework of BBB developmental toxicity

Putative BBB disrupting compounds

Barriergenesis/ maturation
- ↓TIE2 (TEK)
- ↓Wnt signaling
- ↓Norrin (NDP)
- ↓Shh signaling
- ↓OCLN, ↓TJP2
- ↓CLDN3, ↓CLDN5
- ↑PLVAP, ↓P-gly
- ↓GPR124 (ADGRA2)

Angiogenesis, branching, anastomosis
- ↓NRP1
- ↓NOTCH
- ↓VEGFR2 (KDR)
- ↓CSF1R

Inflammation
- ↑IL6
- ↑TNFa
- ↑TGFB

Pericytes
- ↓adherens junctions
- ↓basement memb.
- ↓EC contact

Endothelial cells (EC)
- ↓tight junctions
- ↓transporters
- ↑fenestrations

Astrocytes
- ↓endfoot contacts

Radial glia / NPCs
- ↓tip cell guidance
- ↓differentiation

Neurons/ oligodendrocytes
- Δ migration
- Δ proliferation
- Δ branching

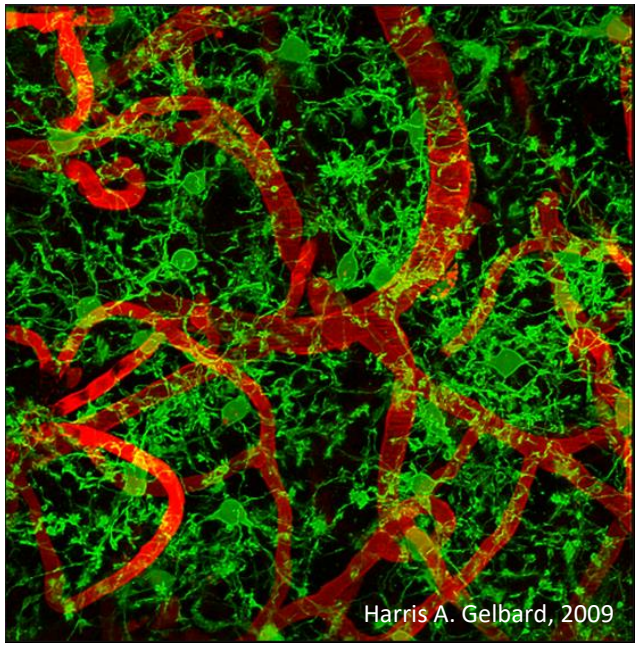
Microglia
- ↑inflammatory cytokine release
- ↑ameboid phenotype
- ↓recruitment to EC

Brain
- ↓ neuronal numbers
- ↓synapses
- ↑apoptosis

NVU
- ↓intercellular adhesions
- ↓barrier properties
- ↓microglia sensing

Newborn/child
Functional deficits: motor, sensory, social, learning

Population
Increased prevalence of neurodevelopmental disorders



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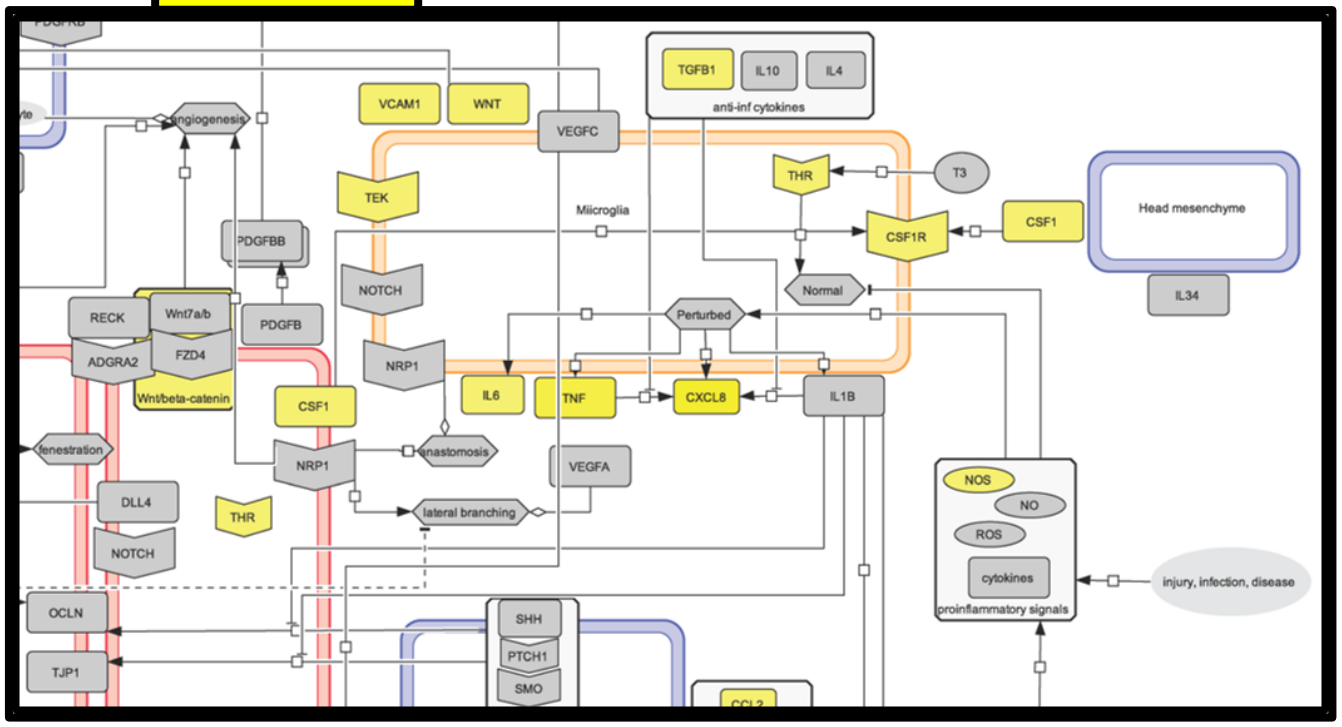
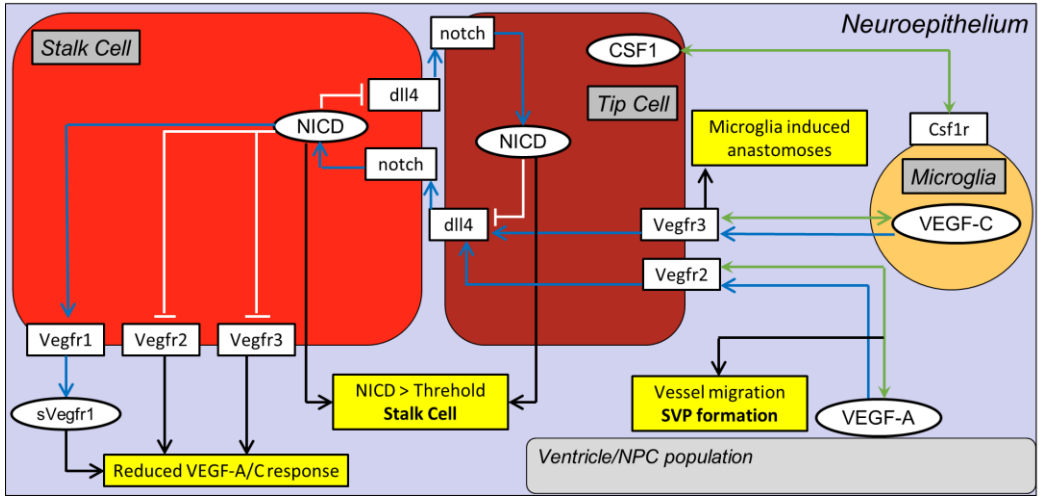
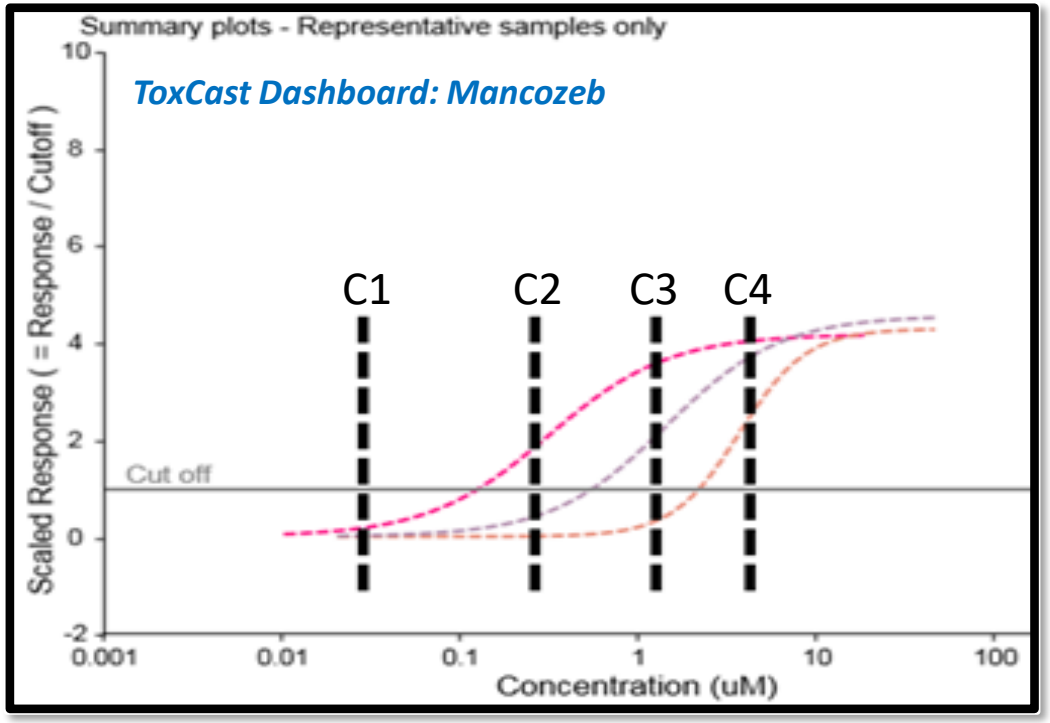


Modified from Saili et al. 2017. *Birth Defects Research. The Dynamic and Vulnerable Teenage Brain (special issue)*

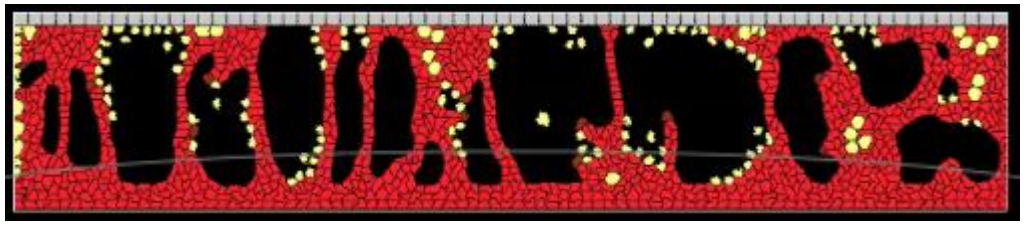
In silico CC3D model predicts perturbed BBB development



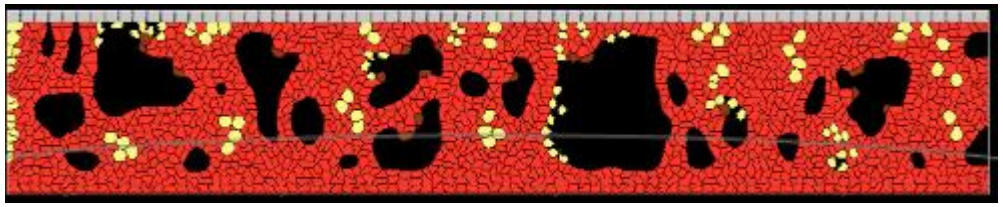


C1 pNEL

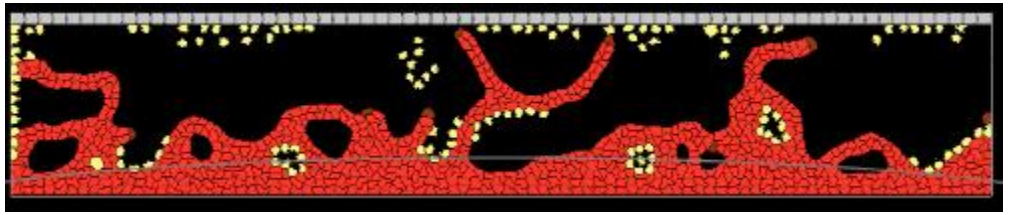
C2 ↓ CSF1R



C3 ↓ VEGFR3



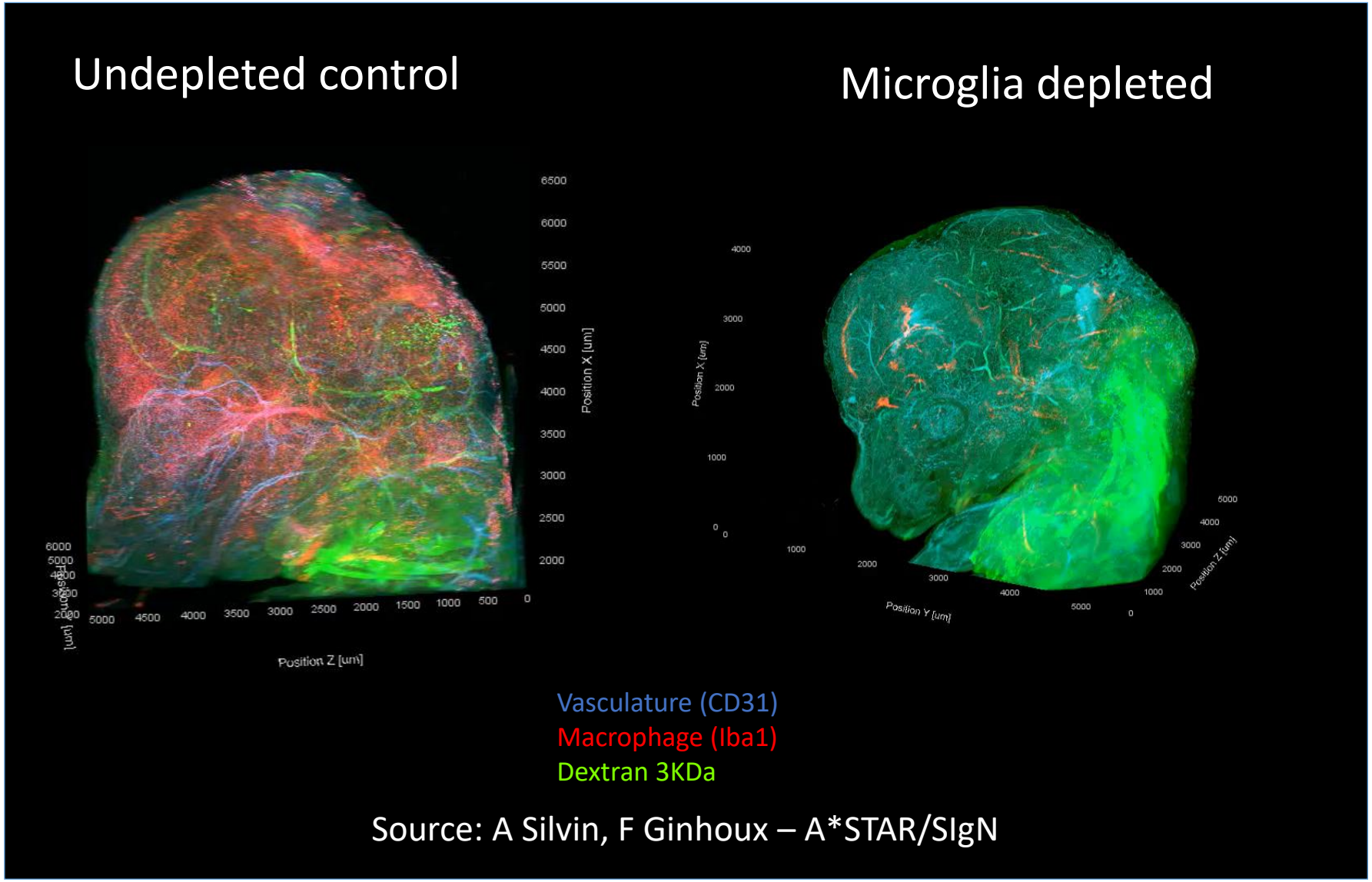
C4 ↓ VEGFR2



T. Zurlinden

Microglia are required to establish fetal BBB integrity

1. α-CSF1R at E6.5 & E7.5
2. Harvest embryo at E14.5
3. Perfuse Dextran-FL
4. Fix, immunostain
5. Optical clearing
6. Ultramicroscope imaging



Summary

1) Perturbed BBB development may lead to neurodevelopmental disorders (hypothesis)
2) The BBB is functional as soon as it forms (e.g., tight junctions)
3) Changes in susceptibility during gestation may reflect differences in transporter activity
4) Model species have conserved BBB features (e.g., transporters), but different timelines for BBB formation compared to humans
5) Human BBB develops between gestational weeks 6 and 14
6) *In silico* and *in vivo* models focusing on microglia suggest a key role for brain-resident macrophages in mediating developmental neurotoxicity via BBB disruption

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Nancy Baker (Leidos)

Sid Hunter (NHEERL-ISTD)

Andrew Schwab (NHEERL-ISTD/ORISE)

Virtual Tissue Models group (EPA)

Richard Spencer (ARA)

Florent Ginhoux (A*STAR/SIgN)

Aymeric Silvin (A*STAR/SIgN)

Tuula Heinonen (FICAM/U Tampere)

Tarja Toimela (FICAM/U Tampere)

David Reif (NCSU)

Carlie LaLone (NHEERL/ORD/EPA)

Molly Windsor (CSRA)

Kevin Crofton (NCCT/ORD/EPA, retired)

John Cowden (NCCT/ORD/EPA)