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February 2-3, 2021

Neurovascular Unit Modeling and Blood Brain Barrier Function

Thomas B. Knudsen, PhD
Developmental Systems Biologist
Center for Computational Toxicology and Exposure
Research Triangle Park NC 27711

knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

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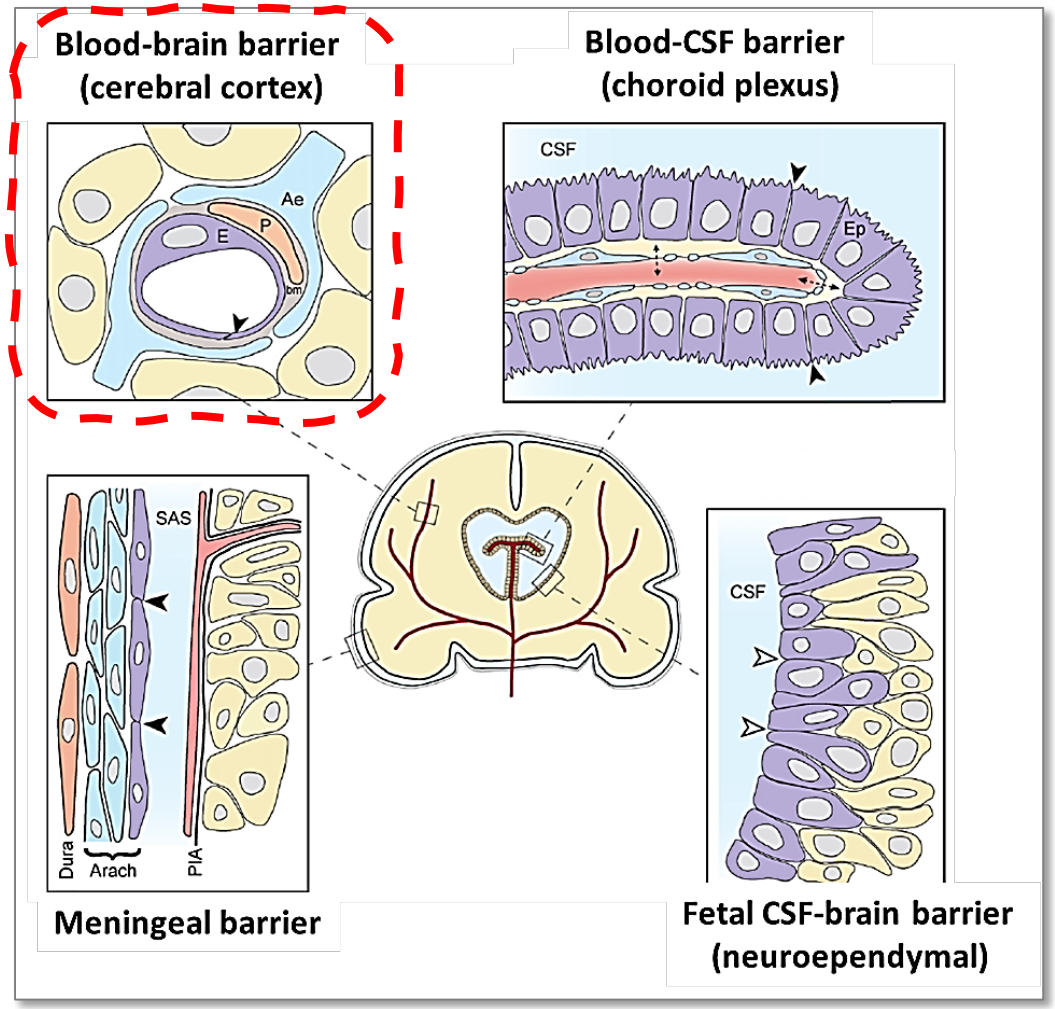
Fetal Brain Barriers

The Neurovascular Unit (NVU) is a relatively recent concept describing the relationship between neuronal and vascular compartments, particularly for two key processes:



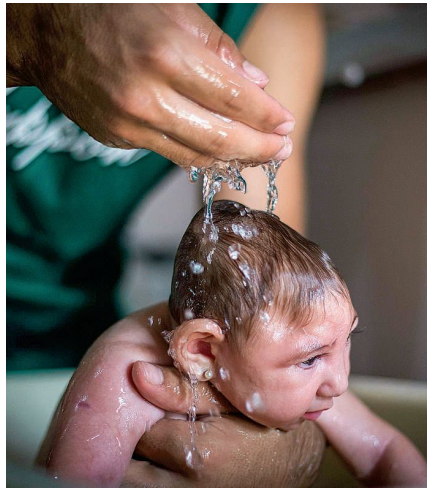
Ek et al. 2012. *NeuroToxicology*

- main driver of functional hyperemia, matching local blood supply to neuronal demand via glutamate (stimulates release of vasoactive signals from astrocytes and pericytes).

- development and regulation of the cerebral blood-brain barrier (BBB) that is fundamental as a selective transport barrier to maintain an optimal environment for brain function.

BBB pathophysiology

- Evidence linking BBB dysfunction with prenatal/antenatal pathophysiological states:



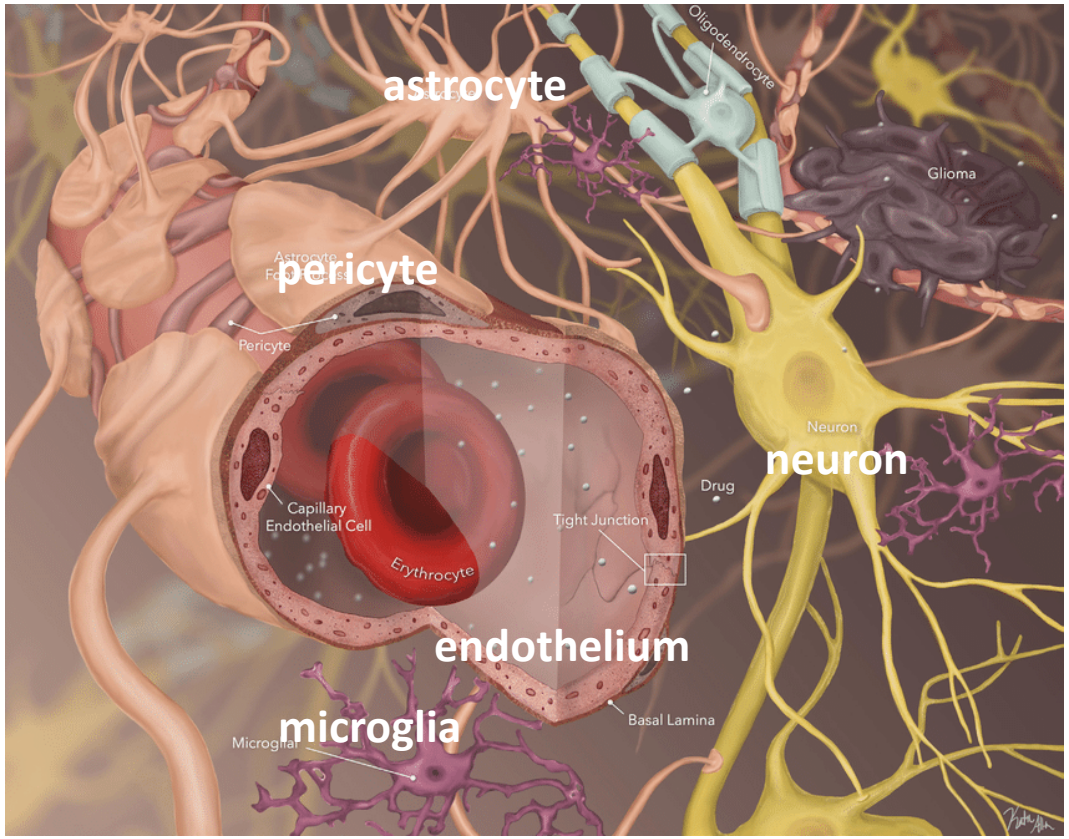
Vogel (2016) Science

  - defective brain transport of leptin (obesity)
  - reduced CNS insulin (baroreceptor deficiency in pregnancy)
  - microglial activation and neuroinflammation (Zika-microcephaly, FIRS)
  - GLUT1 deficiency syndrome (epilepsy, learning disabilities)
  - SL75A (LAT1) dysfunction (autism)
  - SL16A2 (MCT8) deficiency (altered thyroid delivery and neurological impairment)
  - DNT – hypoxia, metal toxicity, pesticide toxicity, …

- OECD Test No. 424: Neurotoxicity Study in Rodents – does not directly evaluate BBB function but can be influenced by a breakdown in the function in the various cell types.

- We know that chemicals interact with the BBB, but to what extent do chemicals of interest disrupt its development and function?

BBB microvasculature: *late fetal to adult lifestages*



Researchgate.net

<u>Endothelial cells</u>: continuous tight junctions, no fenestrations, limited transcytosis.

<u>Pericytes</u>: produce a basement membrane continuous with that produced by the endothelial microvasculature.

<u>Astrocytes</u>: processes (end-feet) interact directly with the basement membrane; appear after formation of the BBB.

<u>Microglia</u>: resident macrophages of the brain, are of hematopoietic origin in the early embryonic yolk sac.

- Microglia orchestrate neurovascular patterning through local signaling; however, when activated they can invoke a local neuroinflammatory response.

BBB phylogeny

DOI: 10.1002/bdr2.1180

REVIEW ARTICLE

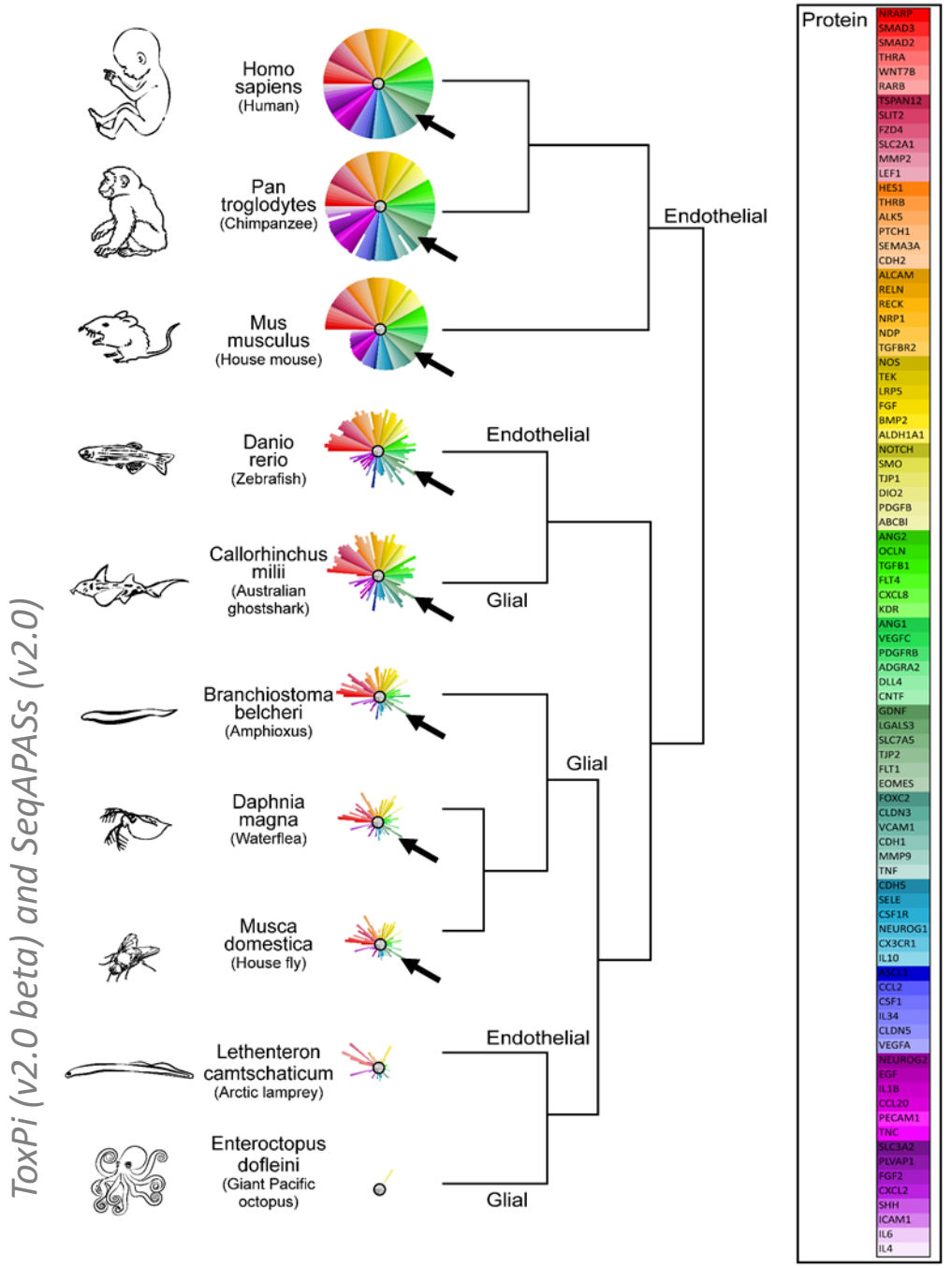
WILEY Birth Defects Research

Blood-brain barrier development: Systems modeling and predictive toxicology

Katerine S. Saili[1] | Todd J. Zurlinden[1] | Andrew J. Schwab[2] | Aymeric Silvin[3] | Nancy C. Baker[4] | E. Sidney Hunter III[2] | Florent Ginhoux[3] | Thomas B. Knudsen[1]

Key BBB transporters are conserved

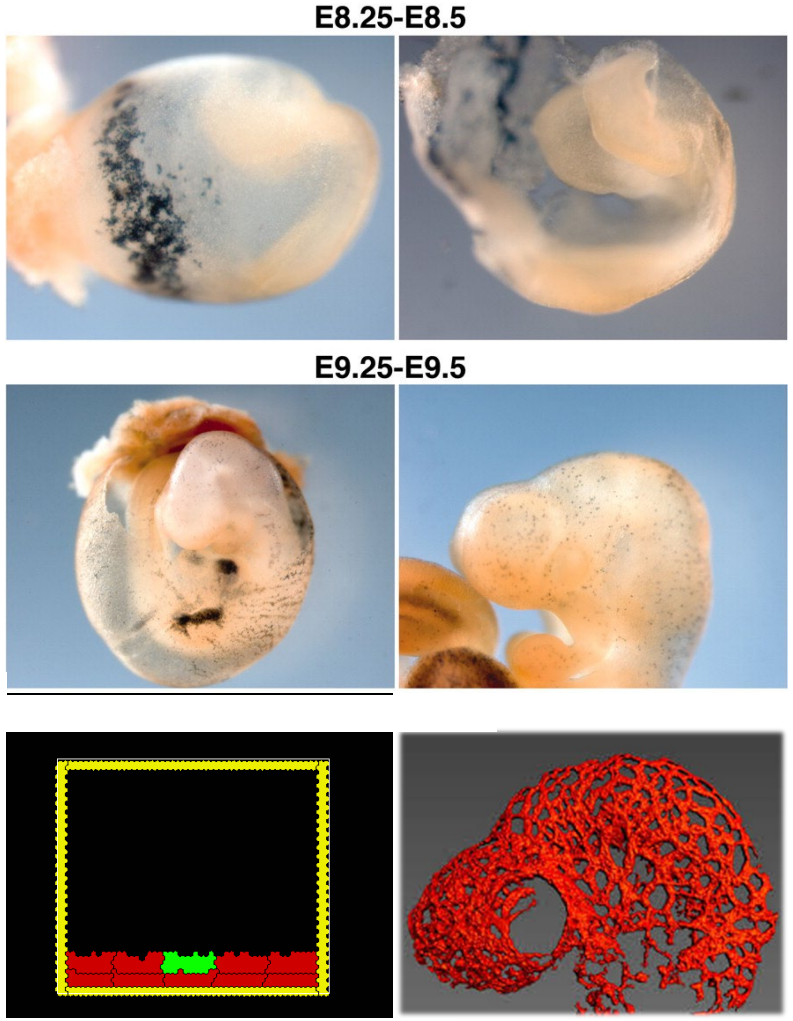
Species	GLUT1	P-gly	SLC7A5
Human	100	100	100
Chimpanzee	99.7	99.5	99.6
House mouse	97.3	87.1	81
Zebrafish	81.3	64.8	77.7
Australian ghostshark	82.7	65.7	72.7
Amphioxus	38	54.5	61.6
Waterflea	46.1	48.5	45.4
House fly	50	41.5	48.5
Arctic lamprey	64.4	---	---
Giant Pacific octopus	---	---	---



86 genes (of >400) play a role in BBB formation

Saili et al. 2017, Birth Defects Res

BBB ontogeny

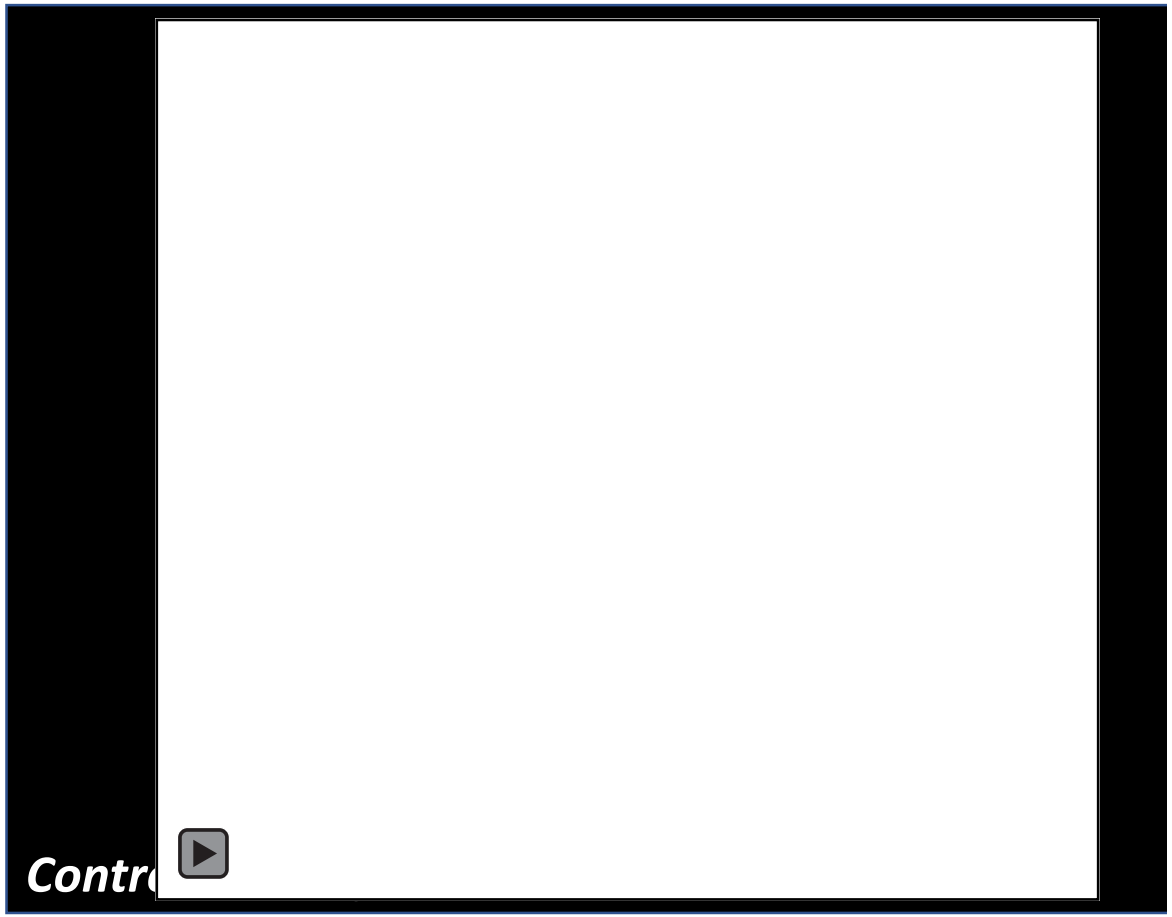


Ginhoux et al 2010, *Science*

- Different components emerge and mature at different stages of prenatal development.
- Commences with angiogenic sprouting from the perineural vascular plexus (PNVP).
- ECs + PCs invade the embryonic neural epithelium on E9-10 (mouse) and GD 26 (human).
- Circulating microglia from the yolk sac colonize the neuroepithelium → resident macrophages of the brain.
- BBB properties (tight junctions, GLUT1) and barrier function (TEER) evident by E11 and increases to birth.

Microglia are required to establish BBB/microvasculature

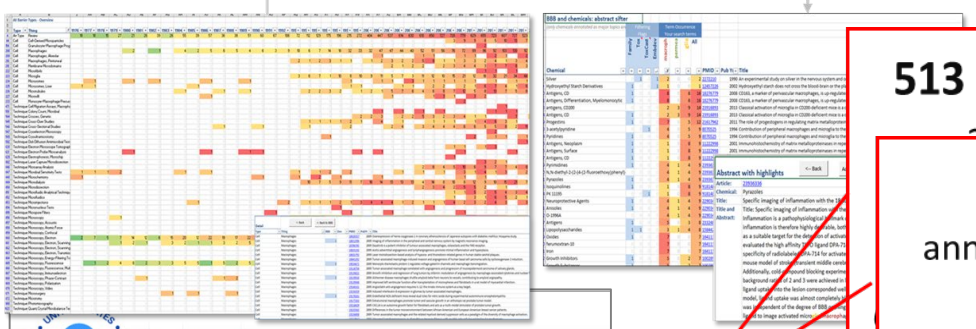
- promote vascular patterning and BBB barrier development in the embryonic forebrain.



*EPA-A*STAR collaboration with A Silvin, F Ginhoux – A*STAR/SIgN*

Hypothesis: *'microglial sensing' is a key event in BBB developmental toxicity*

BBB Knowledgebase sifter



513 records → main subject (MeSH) on BBB in an article annotated for development and a chemical effect

115 DNT chemicals → main subject (MeSH) in an article annotated for development/embryology AND neurological effects

82 chemicals → 5 with DNT effects on BBB

BPA, 5FU, Pb, paraquat, retinoic acid

Int J Dev Neurosci. 2004 Feb;22(1):31-7.

Mosquito repellent (pyrethroid-based) induced dysfunction of blood-brain barrier permeability in developing brain.

Sinha C[1], Agrawal AK, Islam F, Seth K, Chaturvedi RK, Shukla S, Seth PK.

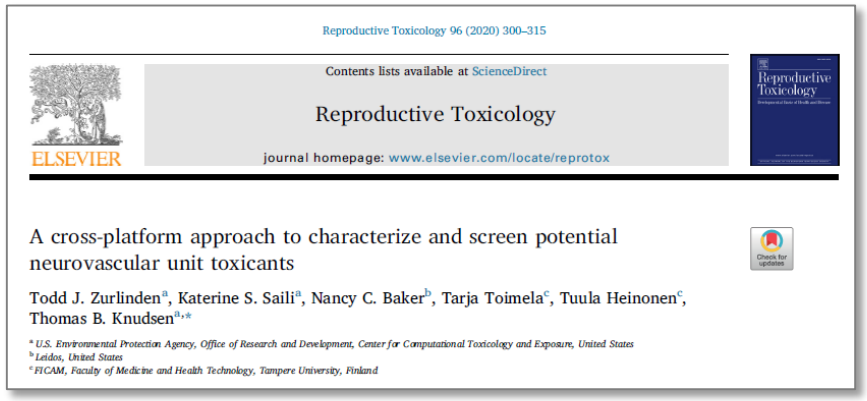
Author information

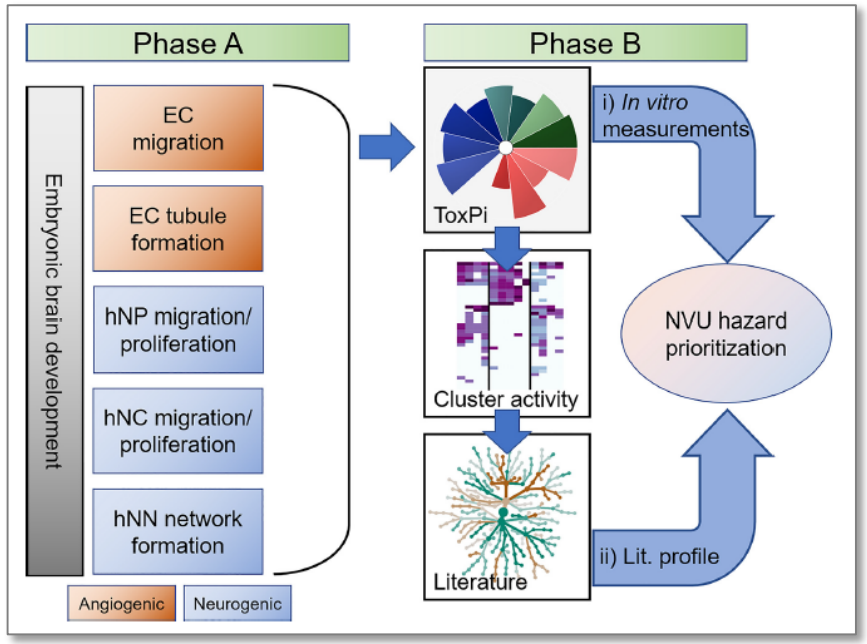
Abstract

Pyrethroid-based mosquito repellents (MR) are commonly used to protect humans against mosquito vector. New born babies and children are often exposed to pyrethroids for long periods by the use of liquid vaporizers. Occupational and experimental studies indicate that pyrethroids can cause clinical, biochemical and neurological changes, and that exposure to pyrethroids during organogenesis and early developmental period is especially harmful. The neurotoxicity caused by MR has aroused concern among public regarding their use. In the present study, the effect of exposure of rat pups during early developmental stages to a pyrethroid-based MR (allethrin, 3.6% w/v, 8h per day through inhalation) on blood-brain barrier (BBB) permeability was investigated. Sodium fluororescein (SF) and Evan's blue (EB) were used as micromolecular and macromolecular tracers, respectively. Exposure during prenatal (gestation days 1-20), postnatal (PND1-30) and perinatal (gestation days 1-20 + PND1-30) periods showed significant increase in the brain uptake index (BUI) of SF by 54% ($P < 0.01$), 70% ($P < 0.01$), 79% ($P < 0.01$), respectively. This increase persisted (68%, $P < 0.01$) even 1 week after withdrawal of exposure (as assessed on PND37). EB did not exhibit significant change in BBB permeability in any of the group. The results suggest that MR inhalation during early prenatal/postnatal/perinatal life may have adverse effects on infants leading to central nervous system (CNS) abnormalities, if a mechanism operates in humans similar to that in rat pups.

development.

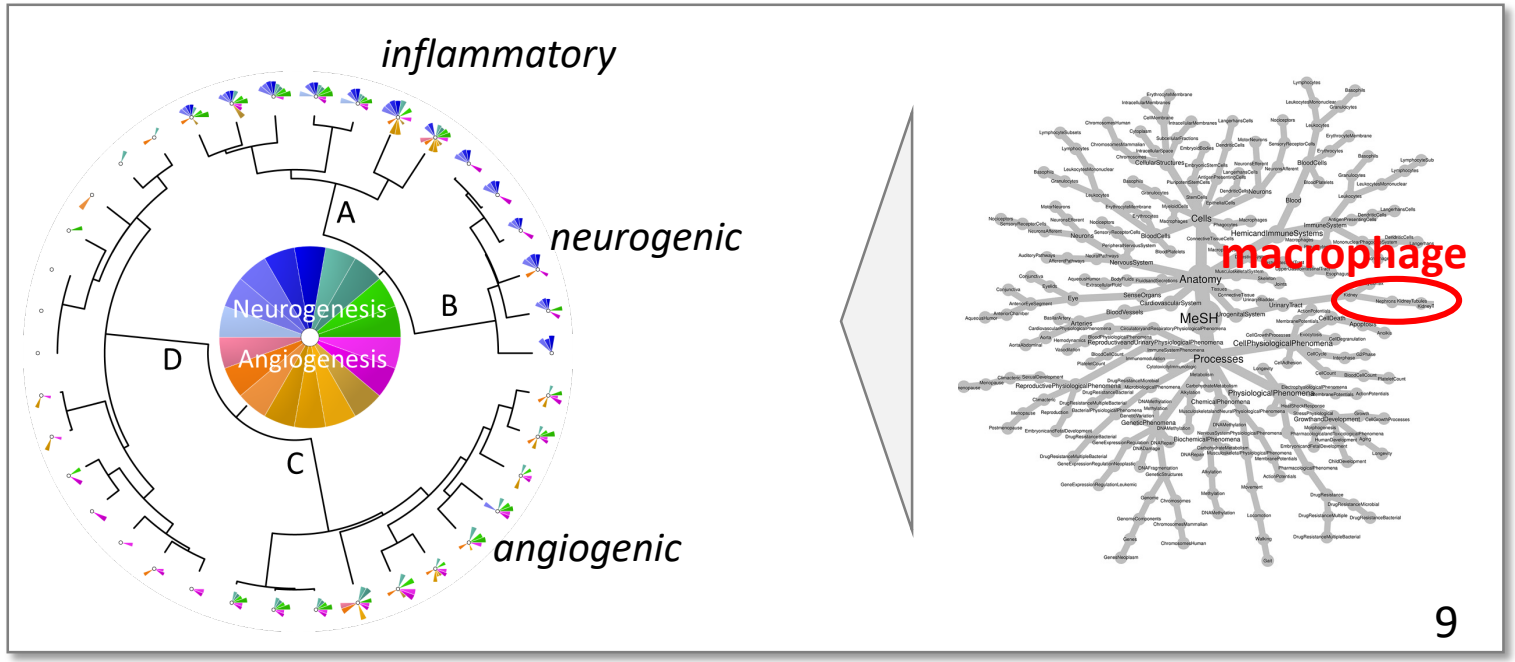
HTS profiling of angiogenic-neurogenic chemical bioactivity





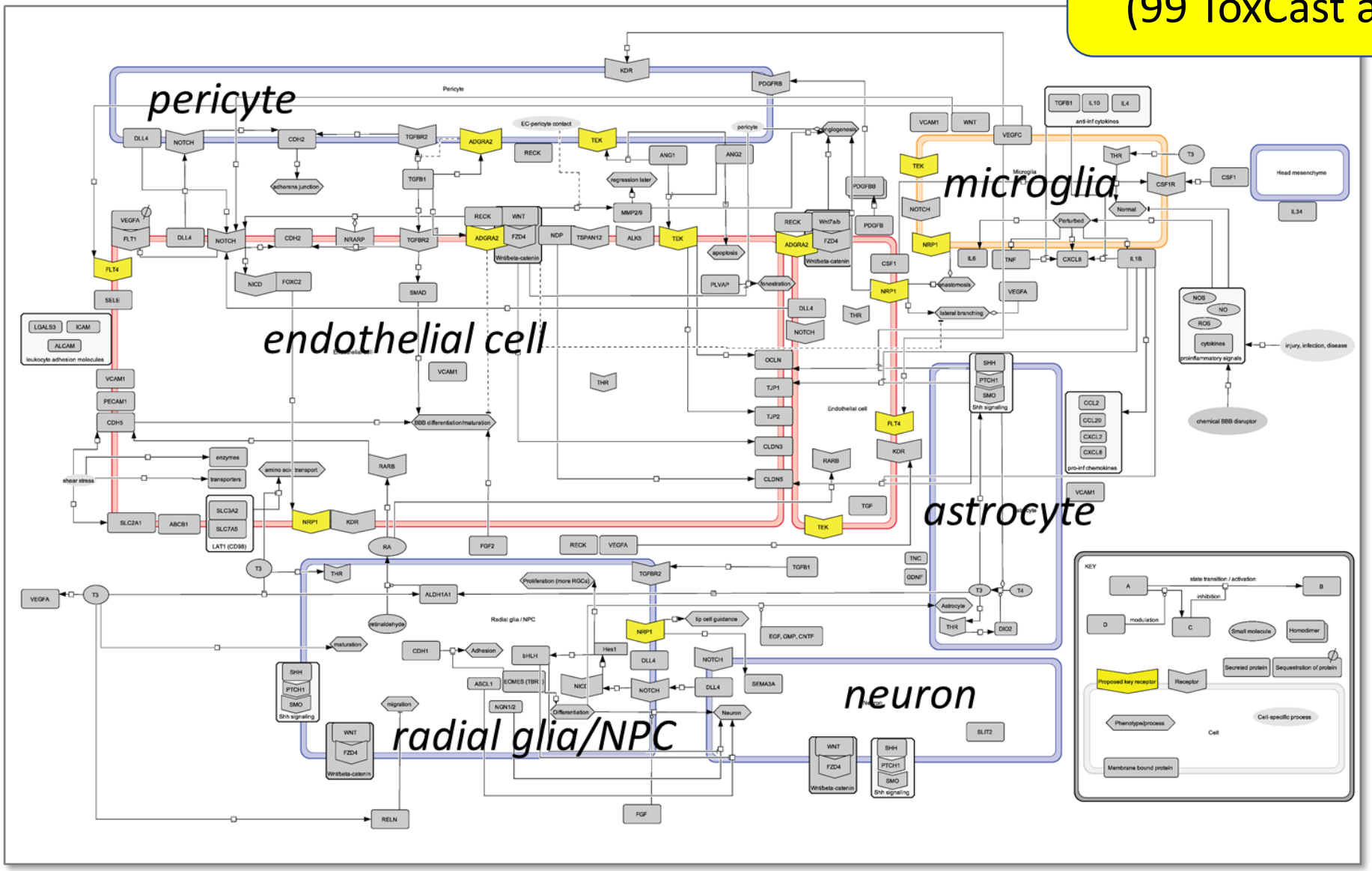
Zurlinden et al. 2020, Reprod Toxicol

- HTS data generated on up to 58 reference chemicals across 18 diverse cell-based angiogenic and neurogenic features.
- ToxPi bioactivity signatures used to train a logistic regression literature model to annotate clusters with PubMed MeSH.
- Chemical-specific pairwise mutual information score predicts NVU developmental hazard potential for advanced modeling.



BBB systems model for predictive toxicology

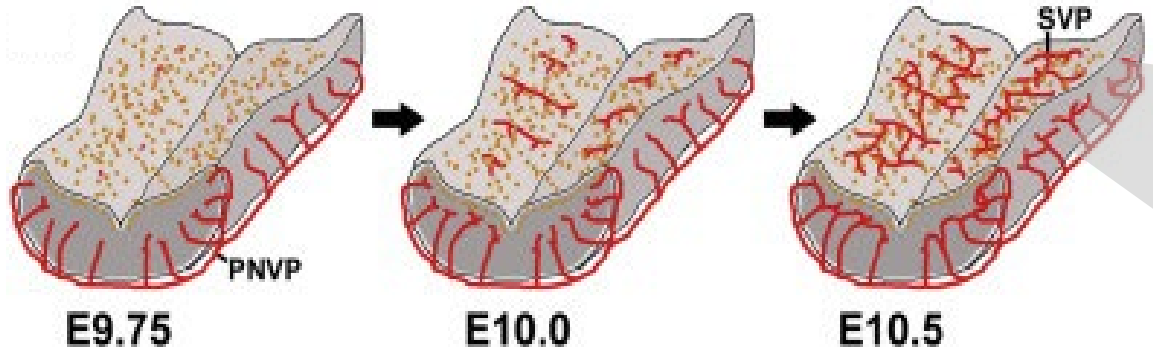
24 molecular targets
(99 ToxCast assays)

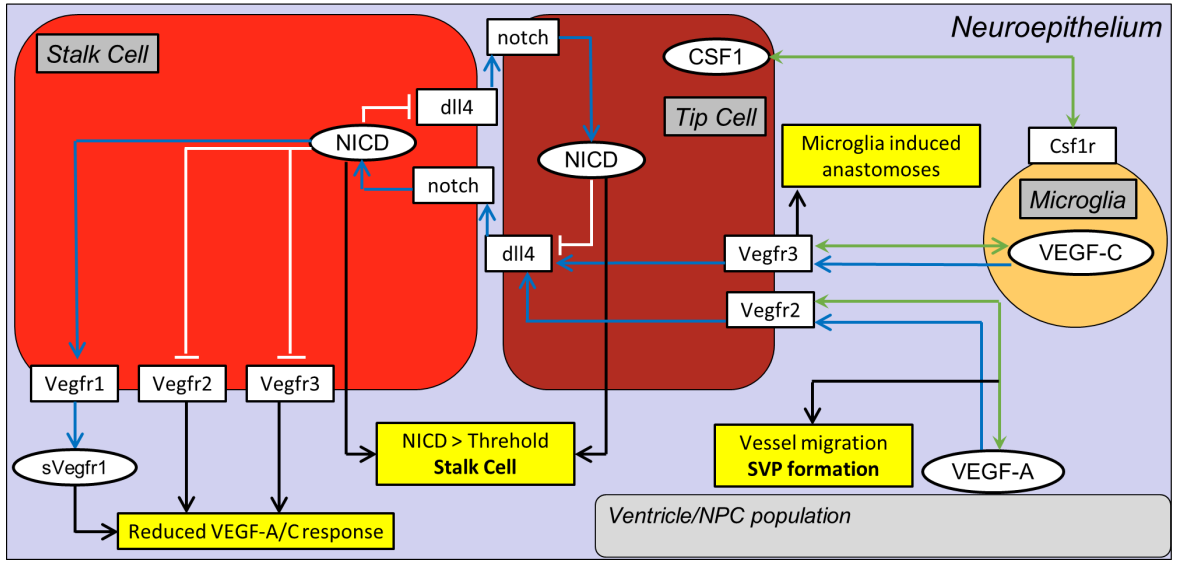


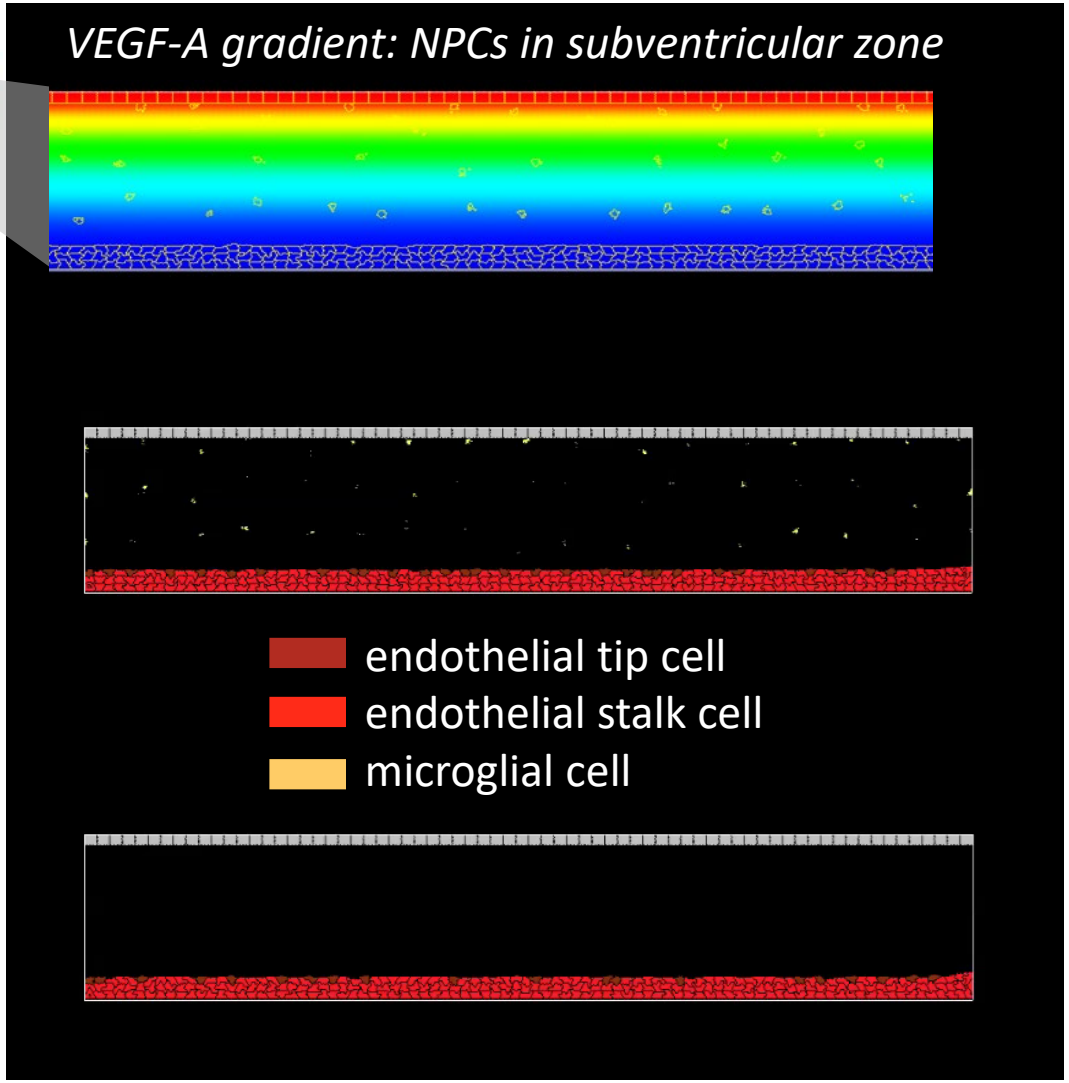
Saili et al. 2017, Birth Defects Res

Advanced Modeling: *neovascularization of the neural tube*

Tata et al. 2015, *Mech Dev*

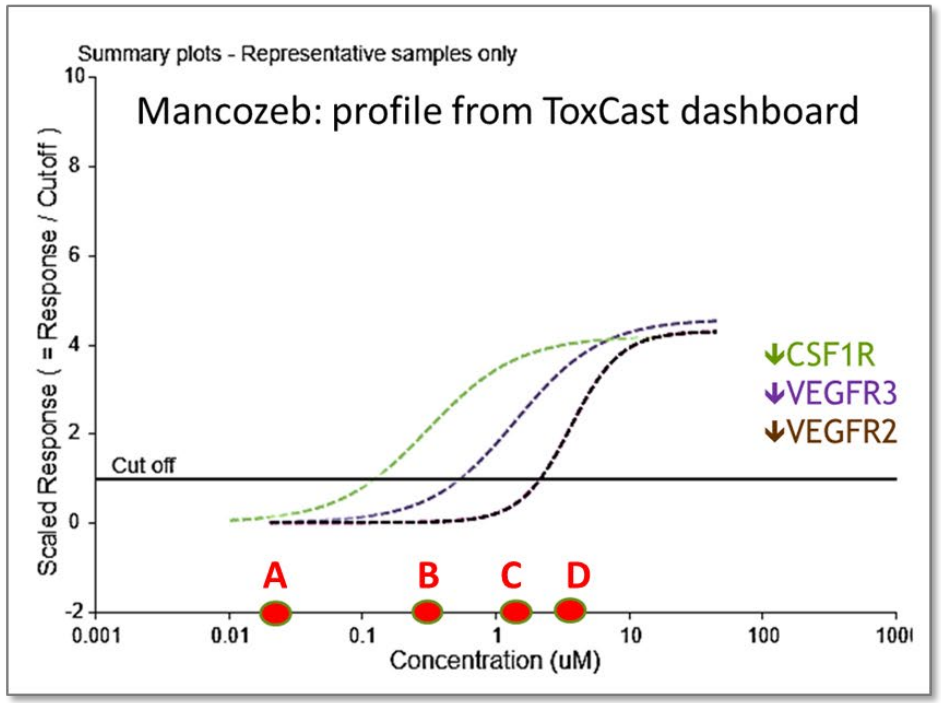


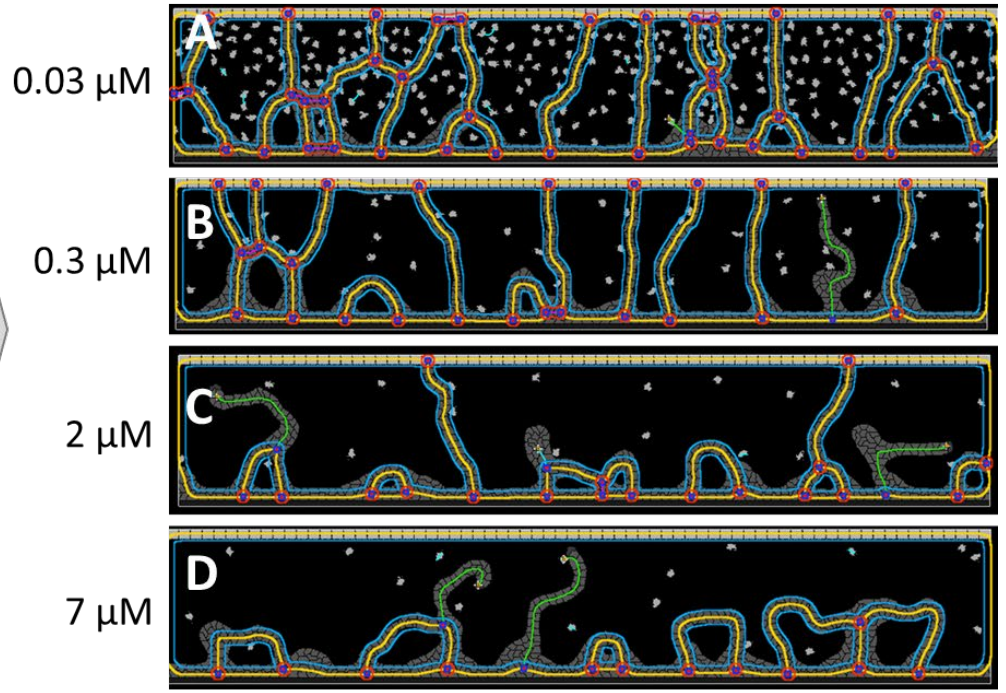


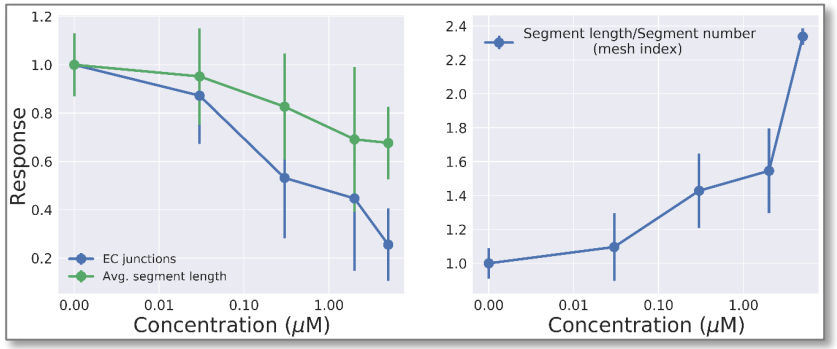


CCTE, work in progress

Executing a simulated concentration-response

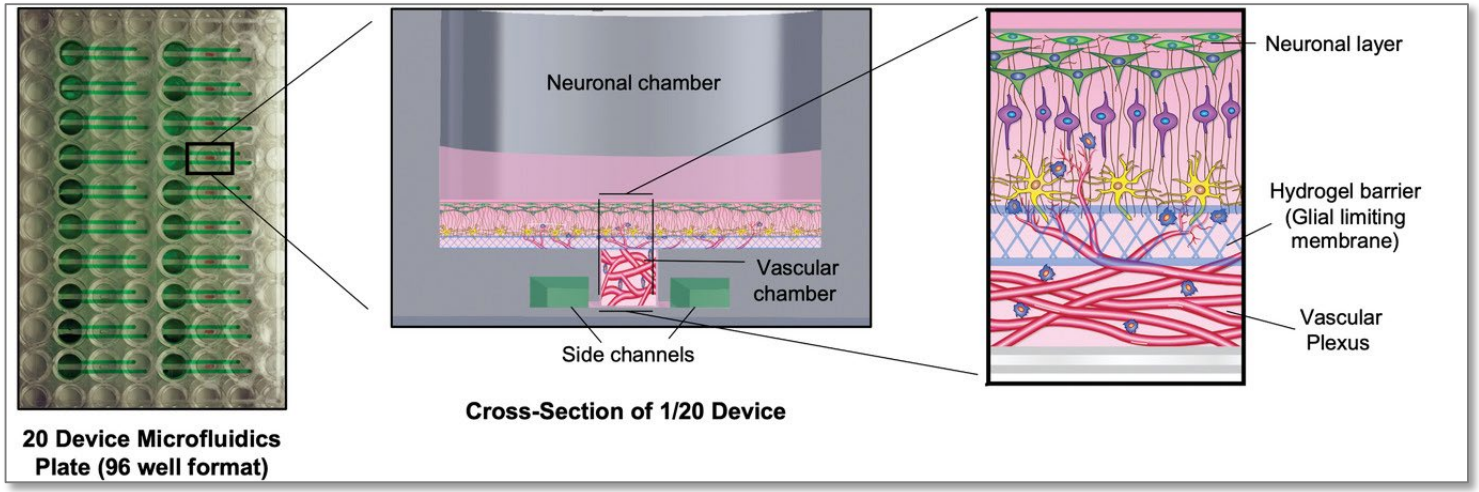






- Progressive bioactivity affects microglial-endothelial interaction (reduced tortuosity → deficiency of SVZ).
- Quantitative microvascular ‘cybermorphs’ predicts an AC50 for Mancozeb disruption at 0.5 µM.

Checking the prediction: *microglial integration in a synthetic microsystem*



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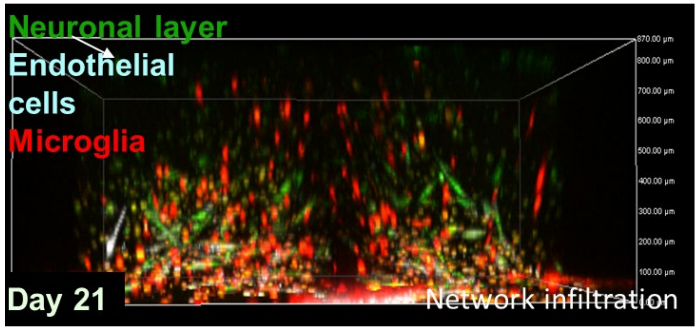
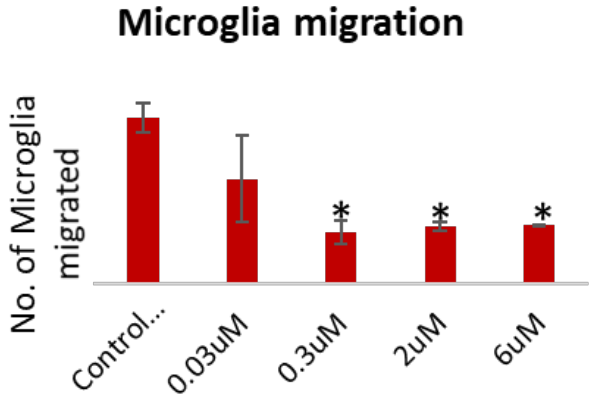
Engineered Perineural Vascular Plexus for Modeling Developmental Toxicity

Gaurav Kaushik, Kartik Gupta, Victoria Harms, Elizabeth Torr, Jonathan Evans, Hunter J. Johnson, Cheryl Soref, Suehelay Acevedo-Acevedo, Jessica Antosiewicz-Bourget, Daniel Mamott, Peyton Uhl, Brian P. Johnson, Sean P. Palecek, David J. Beebe, James A. Thomson, William T. Daly, and William L. Murphy**



Microglia Migration into Neural Layer

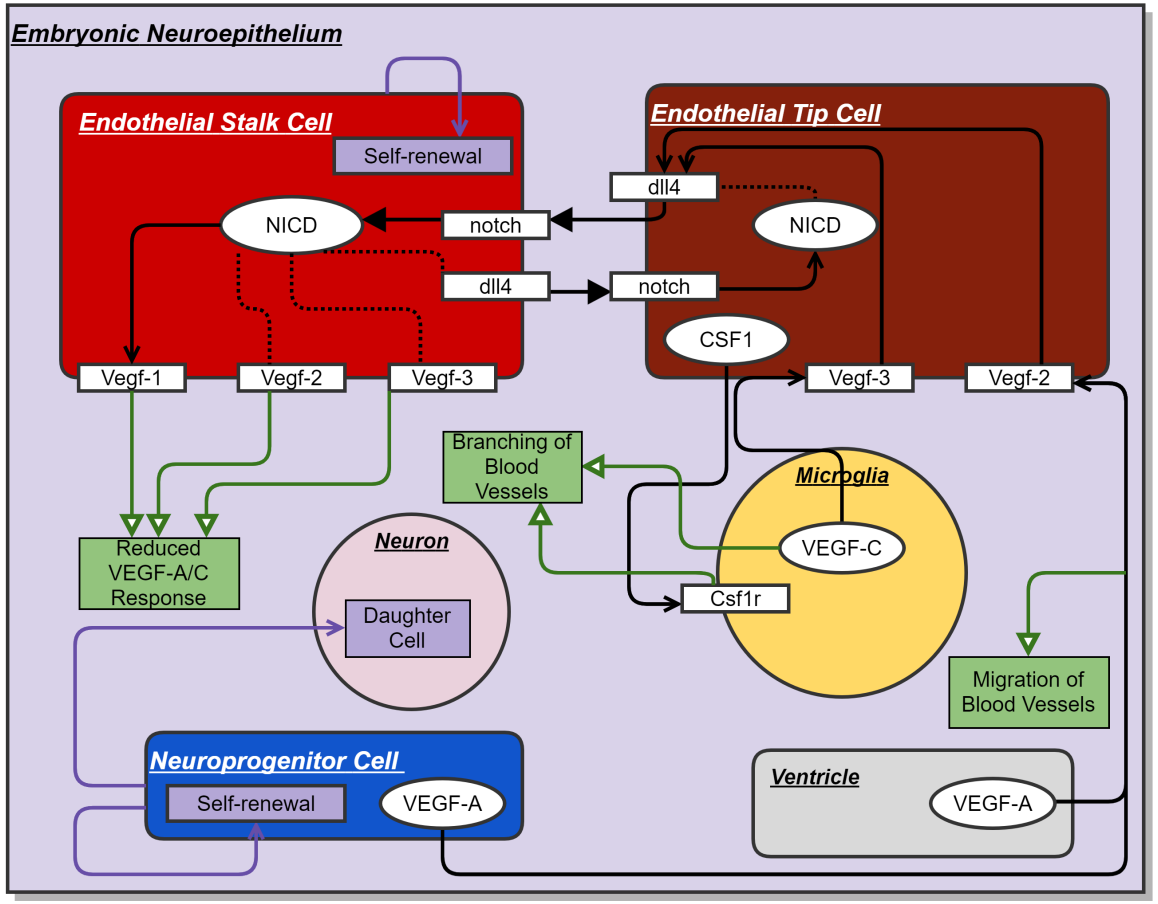


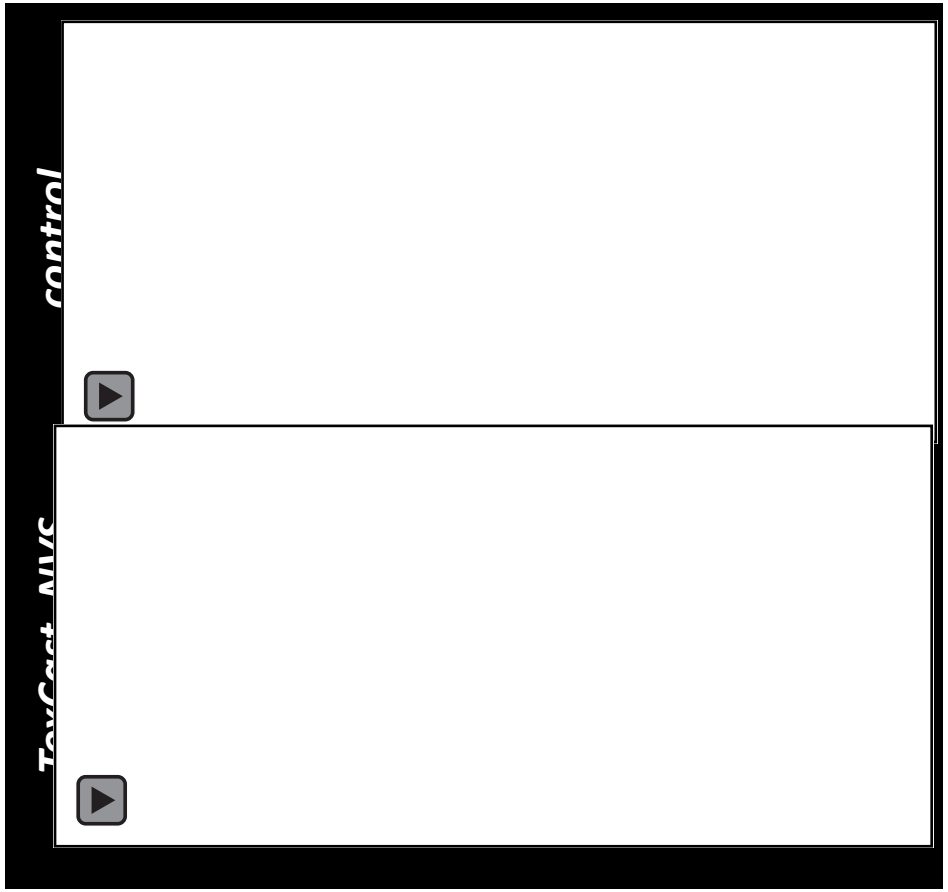
Critical concentration (PoD) for Mancozeb on neural tube vascularization:

- ***predicted*** *by in silico cNVU = 0.5 µM*
- ***observed*** *in organotypic culture = 0.3 µM.*

EPA STAR Center Co-operative grant #835737 , University of Wisconsin (W Murphy)

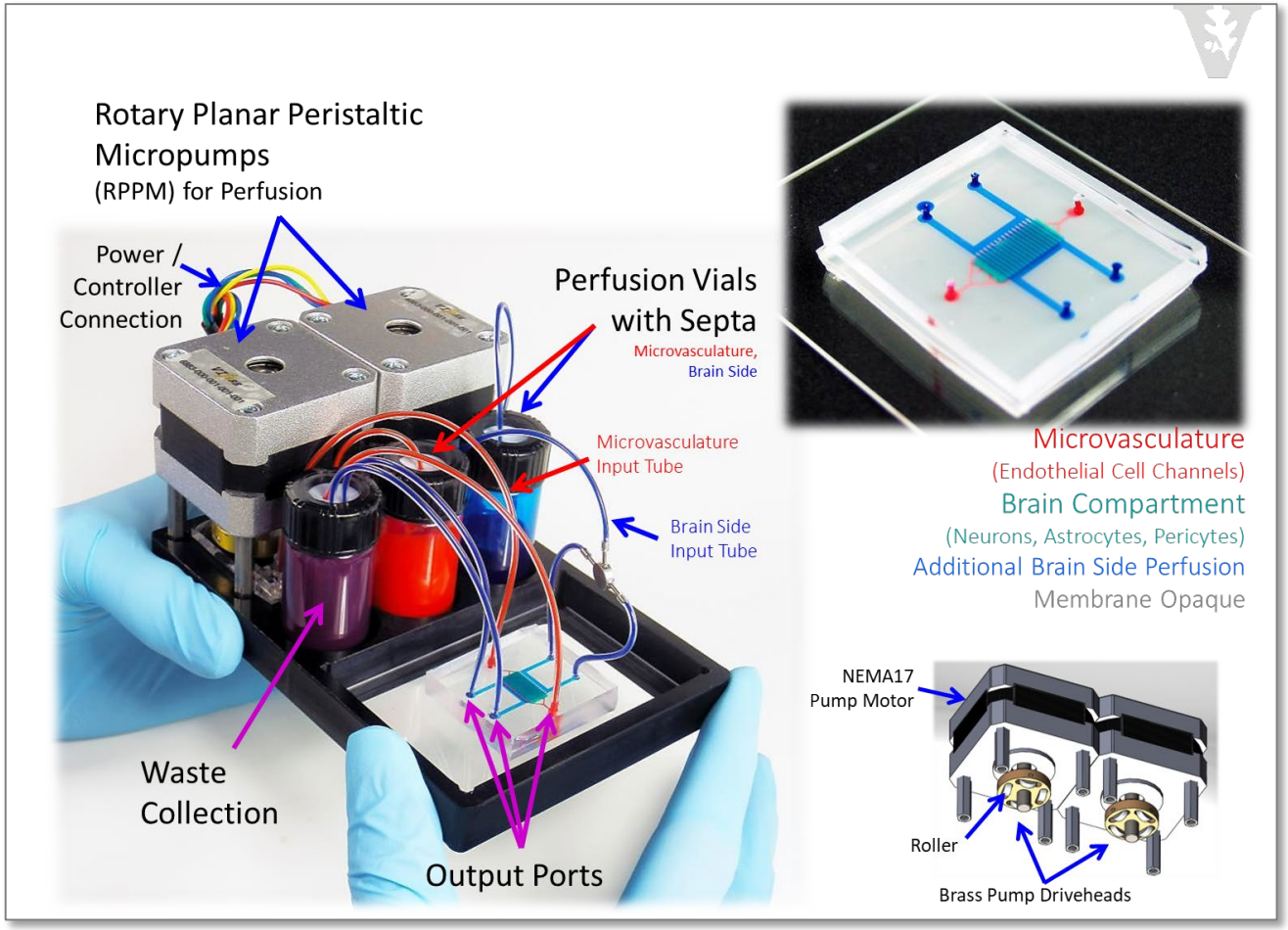
Incorporating the neurogenic domain (preliminary)





O Naphade, work in progress

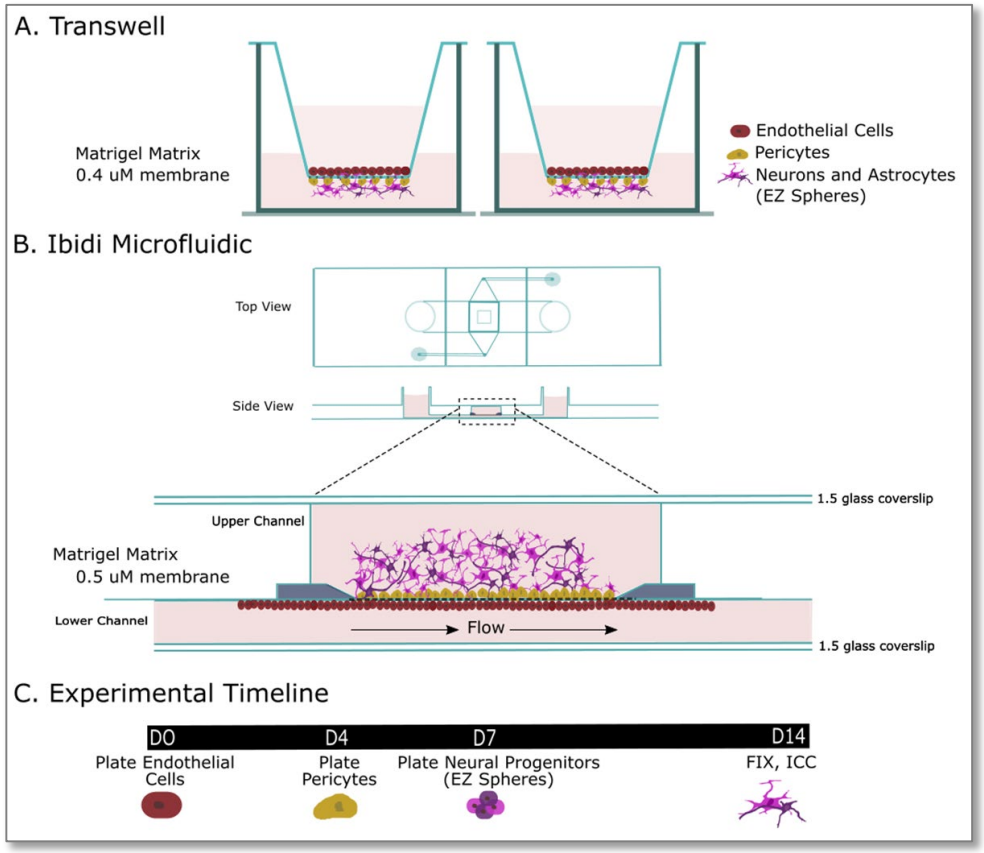
Neurovascular Unit-on-a-Chip Module

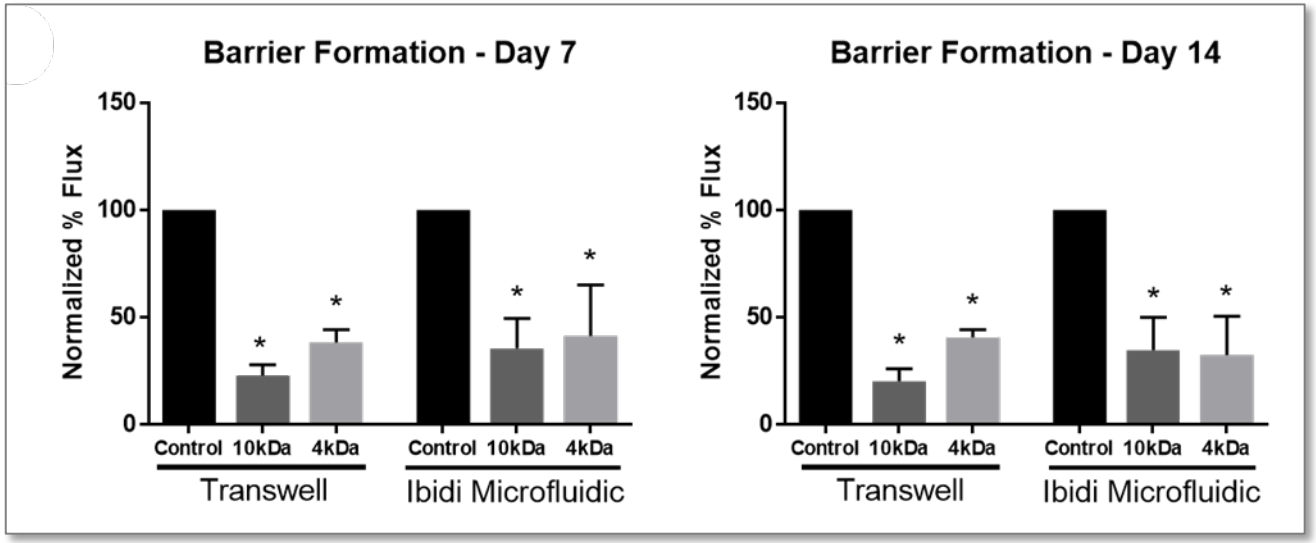


- Human endothelial cells, astrocytes, pericytes, and neurons.
- Microanalyzer for real time data (glucose, lactate, oxygen, pH, and 4 neurotransmitters).
- Testing neuroinflammation (LPS) and neurotoxicity (CPF) pathways.

EPA STAR grant (new), Vanderbilt University, D Cliffel and J Wikswo

Embryonic Human Neurovascular Unit (hNVU): *quantitatively assess the impact of chemical-induced disruption of neural morphogenesis and function.*

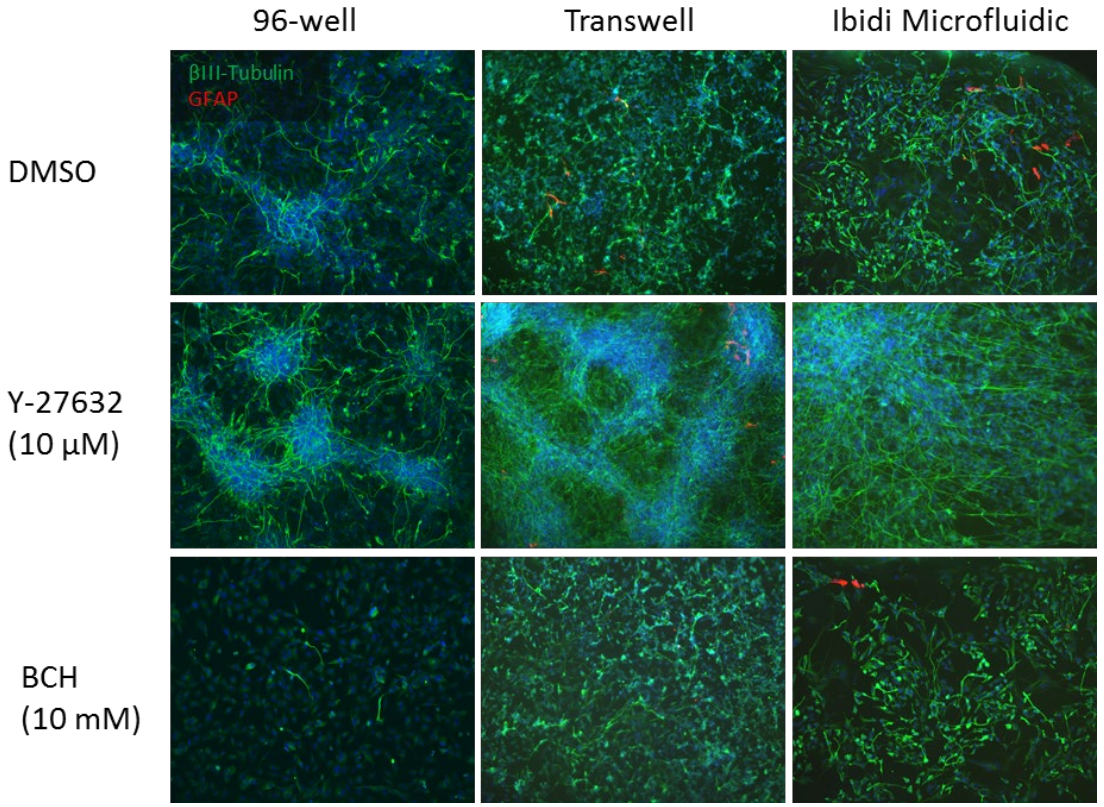


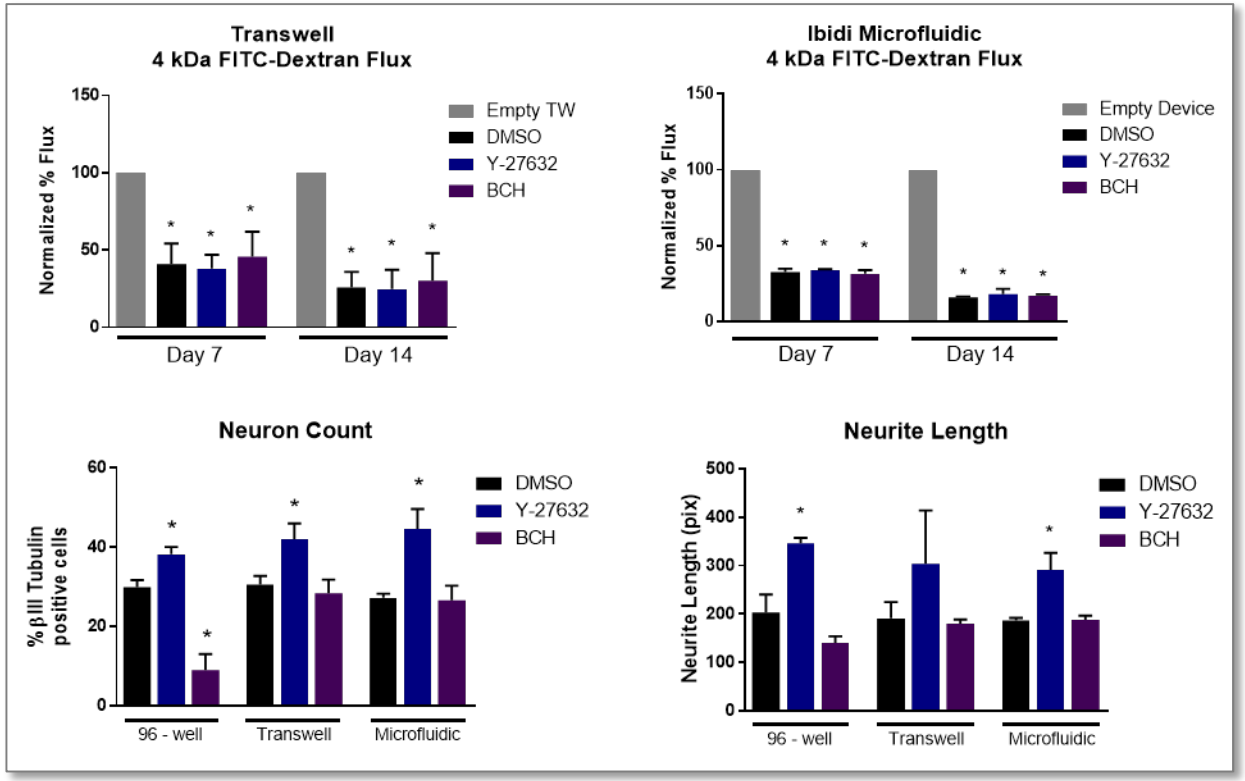


- Impact of the endothelial-pericyte barrier on developmental neurotoxicity,
- Assess chemical effects on barrier function in a human cell-based *in vitro* system(s).

S Hunter, work in progress

Qualification of barrier function in the hNVU

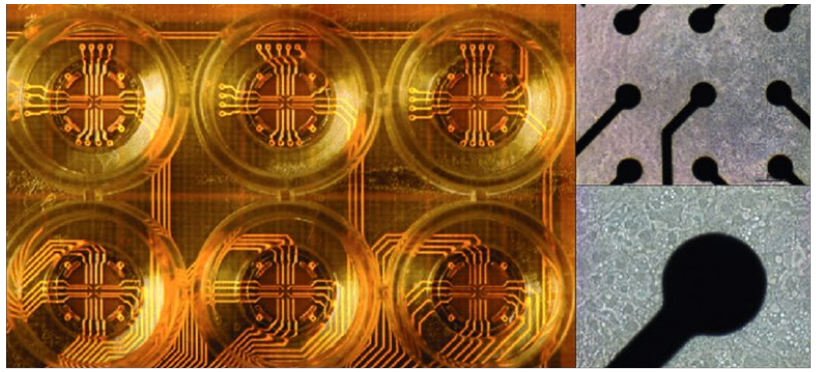


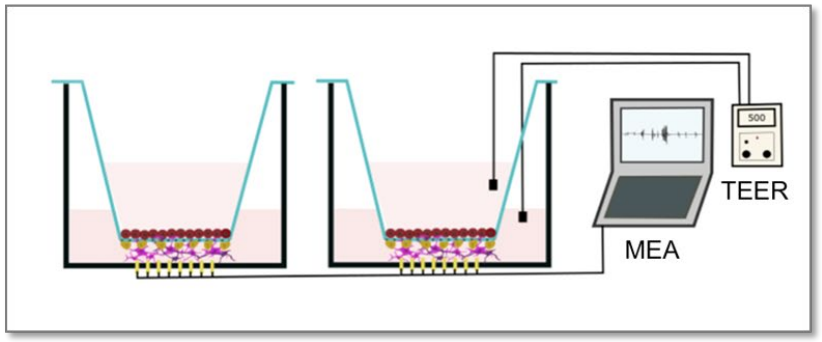


- Y-27632 is a cell permeable rock inhibitor that cross the NVU barrier – increases proliferation and differentiation of human ‘EZ neurosphere’ cells to neural and astrocytic phenotypes (green fluorescence) in all models.

- BCH is a LAT-1 transporter inhibitor that does not cross the barrier; with BCH there are few NPCs cells, little differentiation (bright green neural structure) and almost no red astrocytes. In the MPS devices, proliferation and differentiation are similar to control cultures.

Embryo-fetal NVU Barrier: *application to developmental neurotoxicity*





- Microelectrode array (MEA) assay platform developed in Tim Shafer's lab.
- Monitors rat cortical neuronal network formation and electrochemical activity.
- Used to profile ToxCast chemicals for direct effects on neuronal networks.
- Rat cortical MEA system has been integrated with the transwell hNVU.

T Shafer, S Hunter - work in progress

Summary

- NVU composed of multiple cells types and >400 genes, at least 90 of which play important roles in BBB development and function.
- BBB becomes functional soon after it forms during organogenesis (6-14 weeks in human gestation).
- Development and function is perturbed by multiple pathophysiological conditions and may underlie neurodevelopmental disorders linked to chemical exposure.
- Dynamics of the system modeled *in silico* and *in vivo* focusing on microglial sensing as potential roles in neurodevelopmental toxicity linked to their activation.

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Bill Daly
Eric Nguyen (now NIH/NEI)
Gaurav Kaushik

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David Cliffel, PI
John Wikswo

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