Comptox Community of Practice
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Computational Biology and Predictive Toxicology of Neurovascular Morphogenesis

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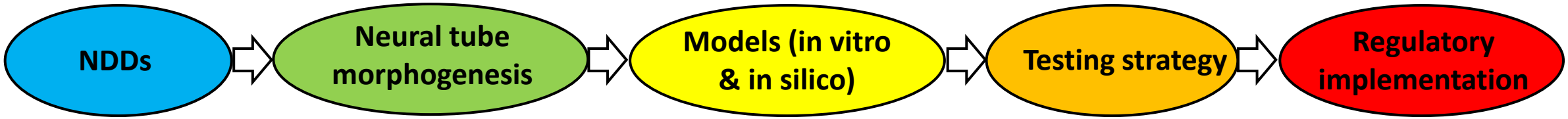
DISCLAIMER: The views expressed are those of the presenters and do not reflect Agency policy.

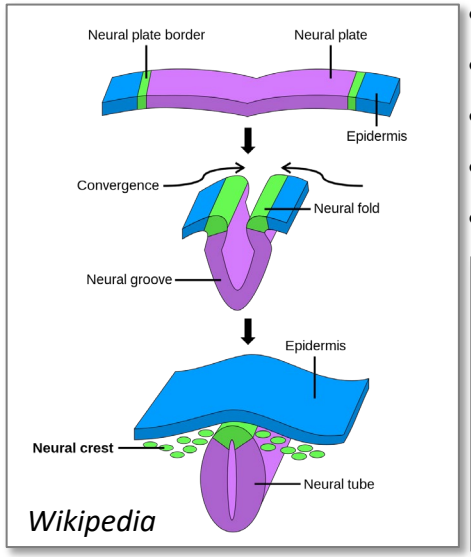
Context: *neurodevelopmental defects - basic embryology to predictive toxicology*

- Morphogenesis involves complex spatial organization of tissues through interplay between cellular networks and physical forces.
- A lot is known about the morphoregulatory signals and pathways that mediate neural tube morphogenesis over time and space.
- Reconstructing dysmorphogenesis from ToxCast HTS bioactivity data remains a challenge for modeling complex dynamical systems.
- Human cell-based synthetic microsystems (*in vitro*) and computational systems models (*in silico*) are emerging tools for predictive modeling.



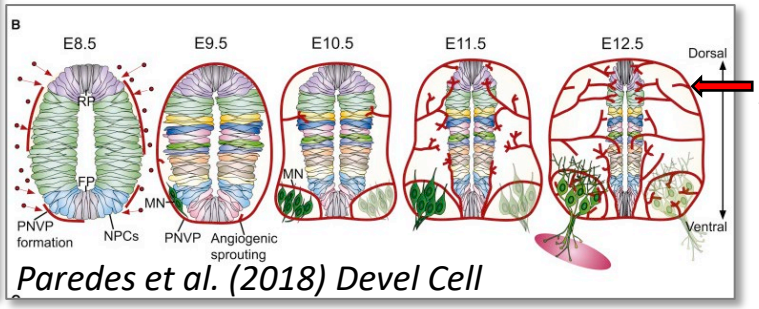
Neurovascular crosstalk

Vascular homeostasis is gaining increasing recognition in the pathogenesis of NDDs.



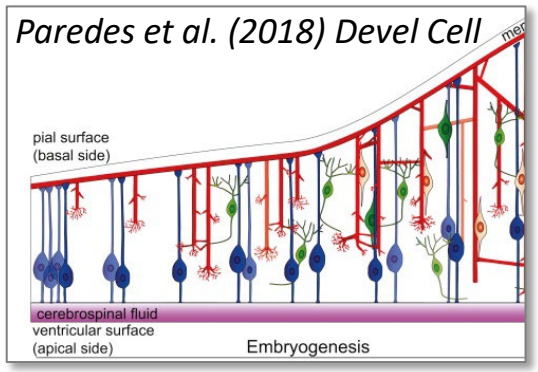
Wikipedia

- Neural plate (E6.5)
- Neurulation (E7.5-E8.5)
- Neural tube closure (E8.5-E9.5)
- Vascular ingression (E9.5-E11.5)
- Microvascular patterning (E11.5-E14.5)

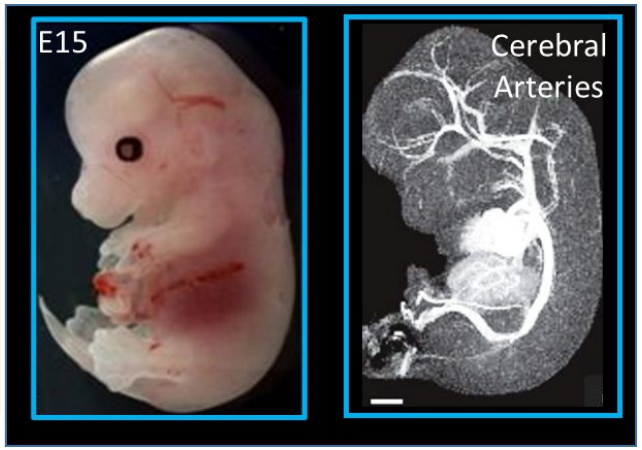


Paredes et al. (2018) Devel Cell

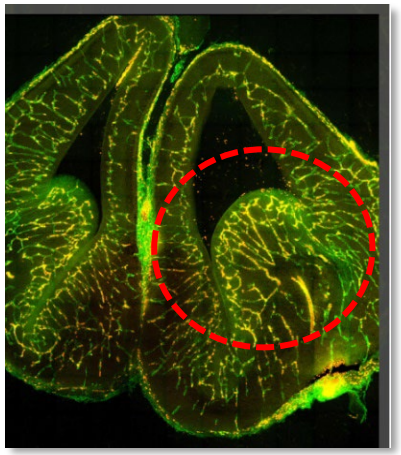
Perineural vascular Plexus (PNVP)

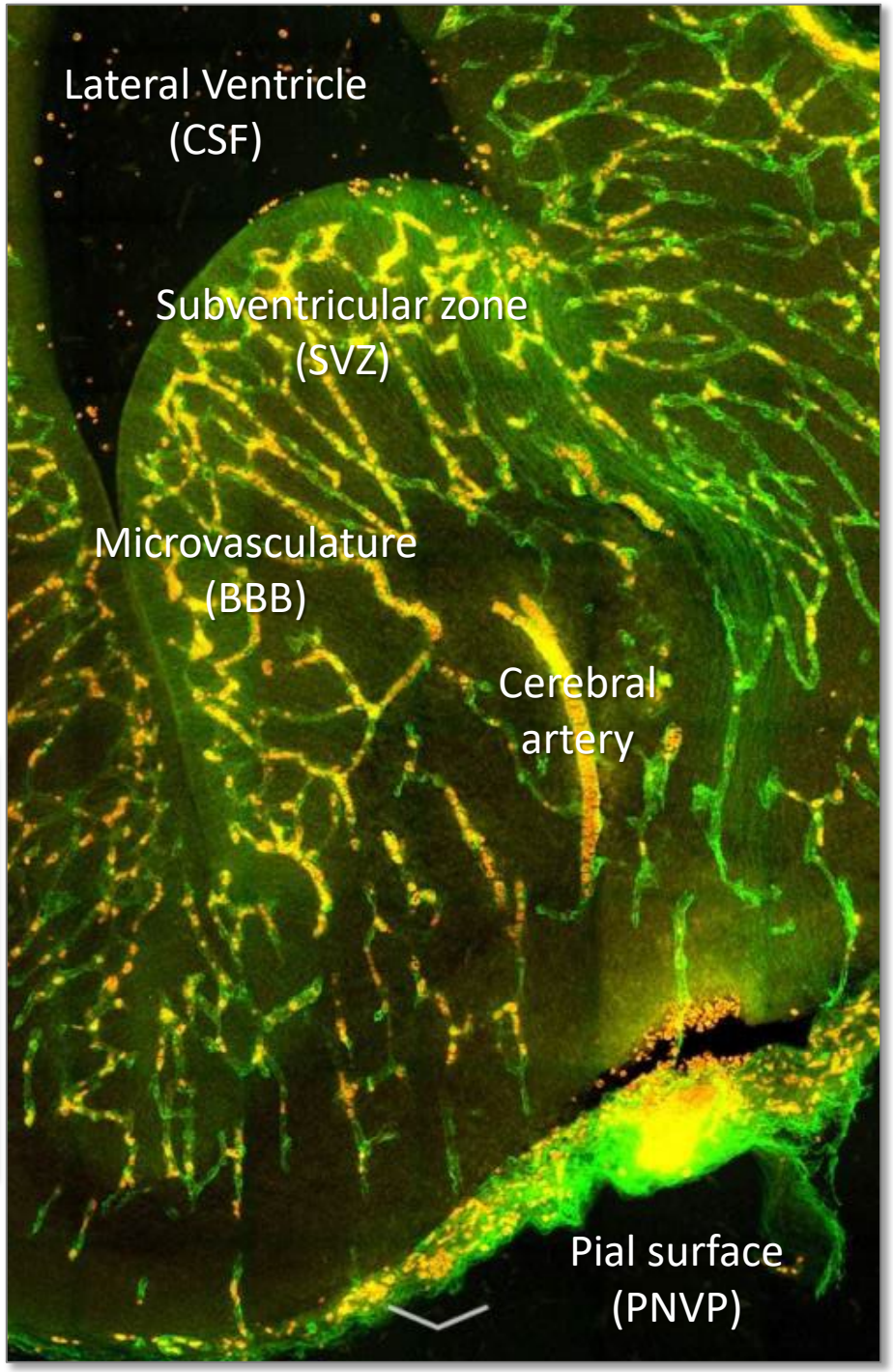


Paredes et al. (2018) Devel Cell



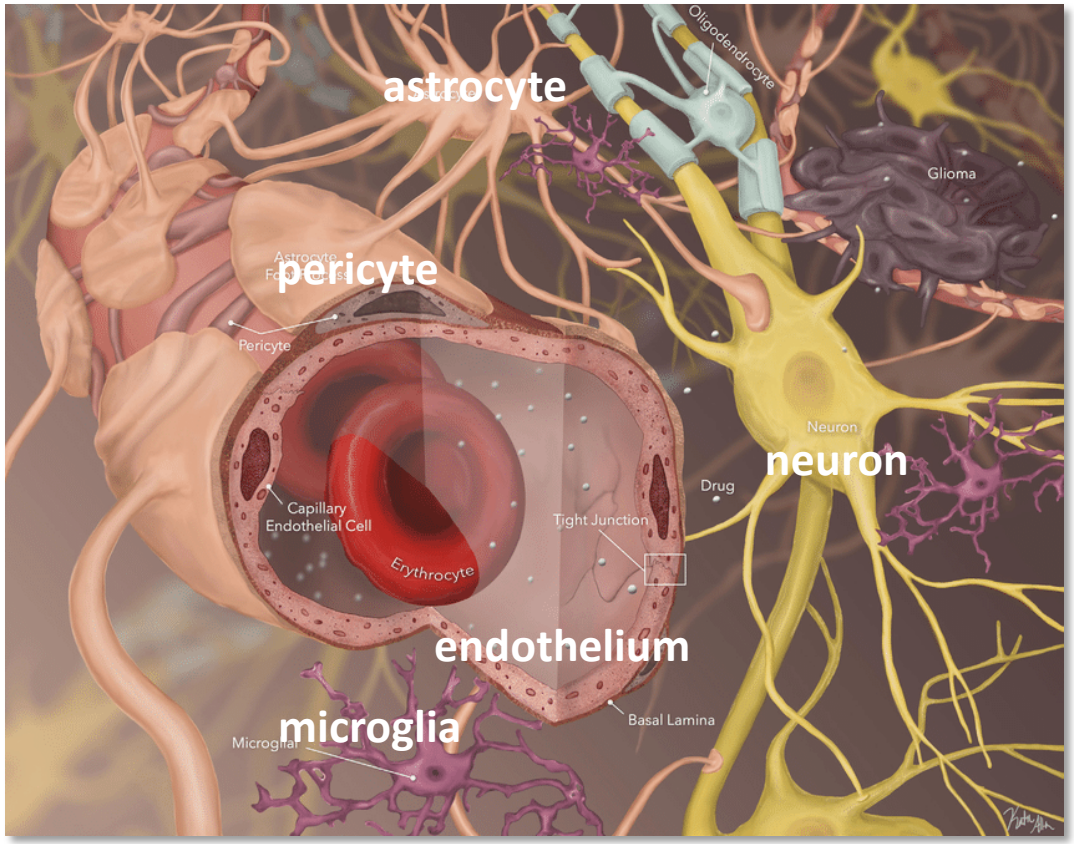
fetal brain





B. Sheikh, Max Planck Institute of Immunobiology and Epigenetics
https://www.mpg.de/14953239/0615-immu-110988-the-brain-and-its-blood-vessels

Neurovascular unit (NVU): microanatomy of the blood-brain barrier (BBB)



Researchgate.net

Endothelial cells: continuous tight junctions, no fenestrations, limited transcytosis.

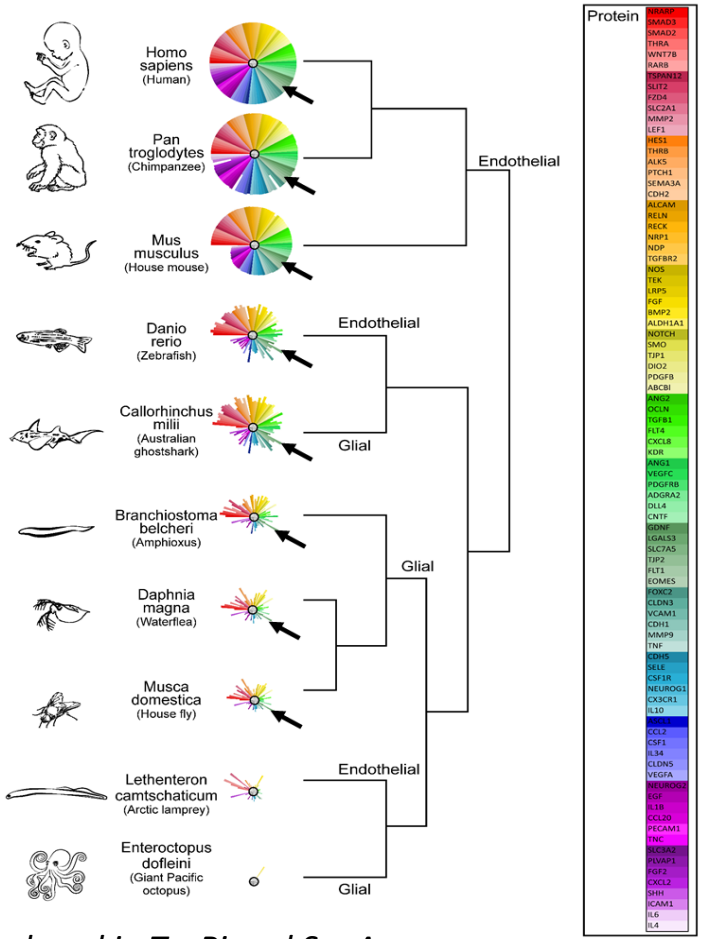
Pericytes: produce a basement membrane continuous with that produced by the endothelial microvasculature.

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

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

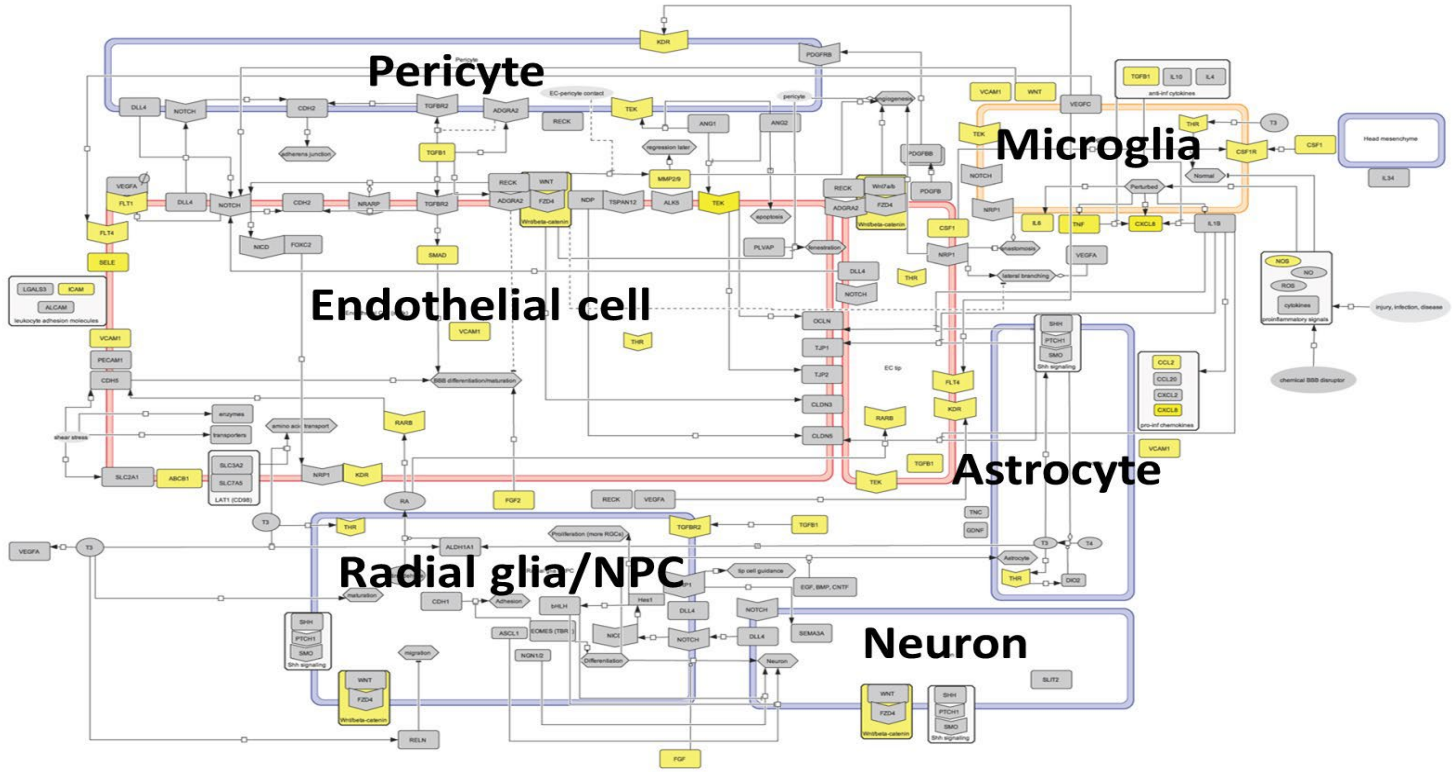
BBB phylogeny and ontogeny

86 genes in BBB evolution



Produced in ToxPi and SeqApass

Developmental Systems Network

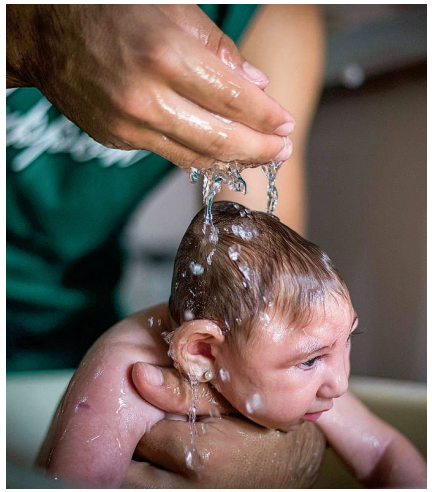


24 molecular targets
(99 ToxCast bioactivity assays)
https://comptox.epa.gov/dashboard/

Produced in CellDesigner v4.4

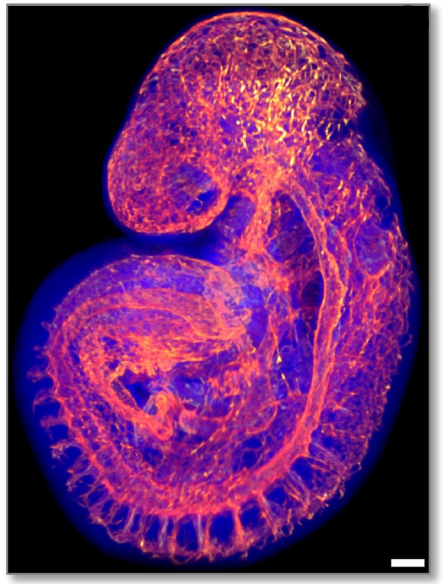
Saili et al. (2017) Birth Defects Res

BBB pathophysiology: dysfunction in prenatal/antenatal conditions.



Vogel (2016) Science

- NDDs - Rett Syndrome, Autism Spectrum Disorders
- defective brain transport of leptin (obesity)
- reduced brain insulin (baroreceptor dysfunction 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)
- chemicals (BPA, lead, pesticides, retinoic acid, …)



Walls et al. (2008) PLoS One

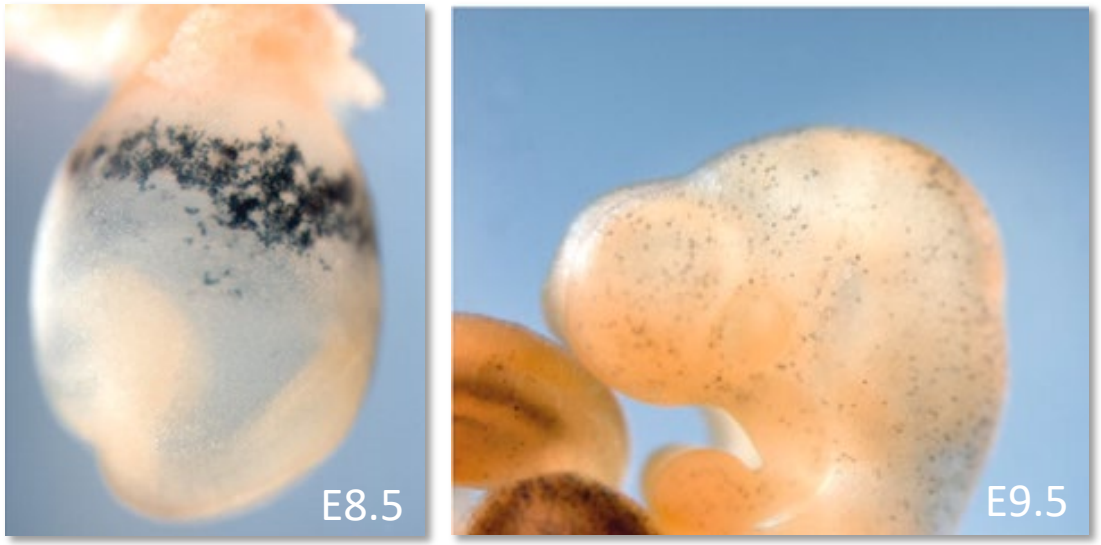
While we know that chemicals of interest may interact with the BBB, to what extent do they perturb the microvasculature?

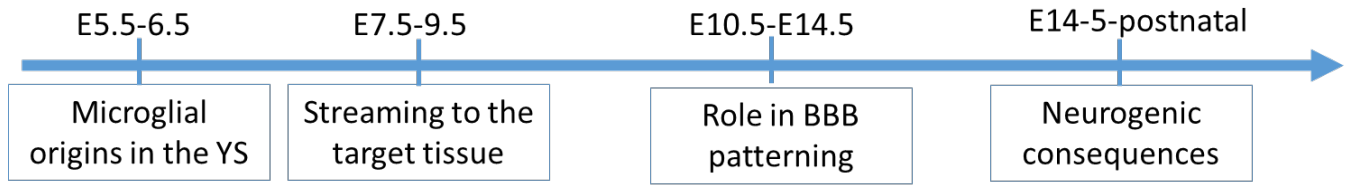
Hypothesis:

'Microglial sensing' is a key target for chemical effects on neurodevelopment through perturbation of BBB formation and physiology.

Microglial cell origins in the mouse embryo

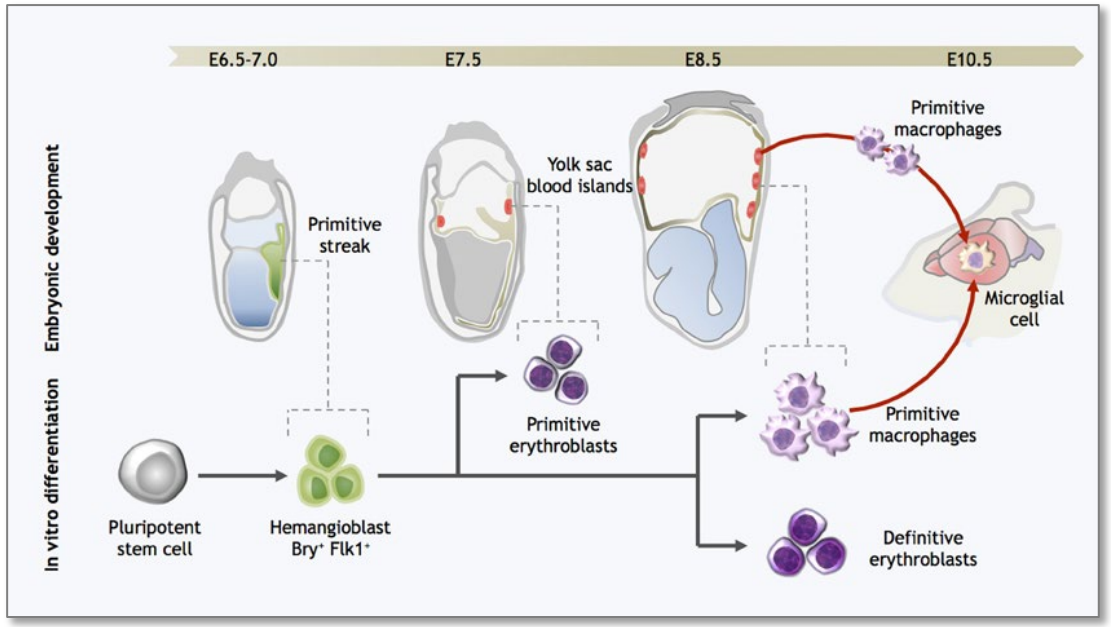
Ginhoux et al. (2010) Science

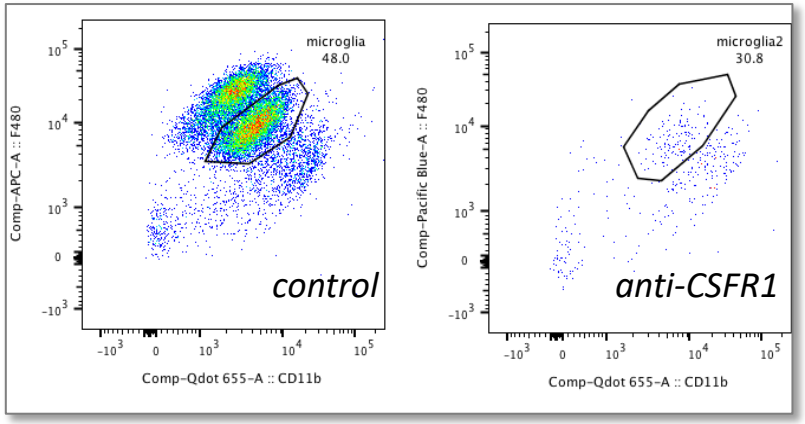




- Lineage arises in yolk sac blood islands by E7.5 and emigrate along vasculature E8.5.
- Yolk sac MGCs pepper the cranial neuroepithelium (E9.5) but depleted with anti-CSFR1 on E6.5 and E7.5.
- Local hotspots of microglial activation invoked by BBB damage, microbial infection, and neuroinflammation.

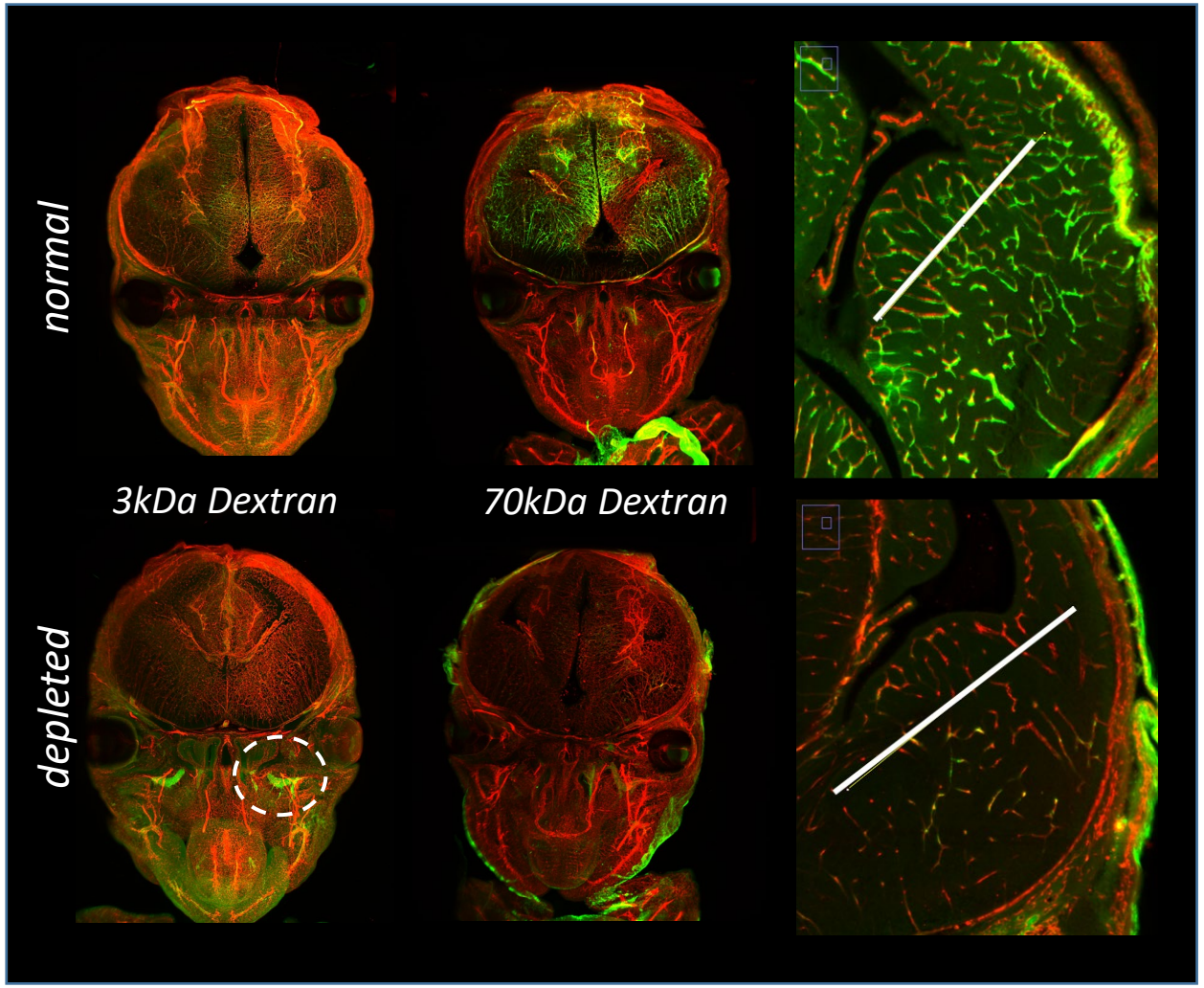
Ginhoux et al. (2013) Front Cell Neuro

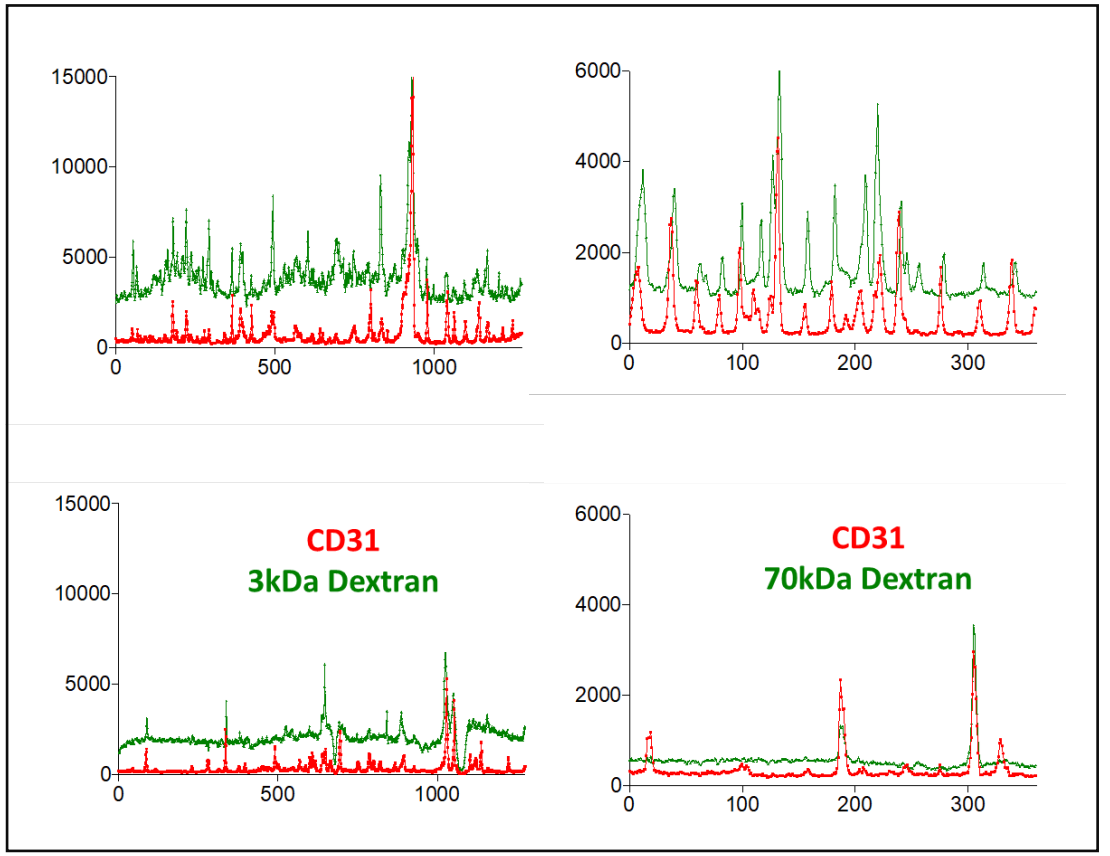




Consequences of microglial cell depletion

↓microvasculature, ↑permeability



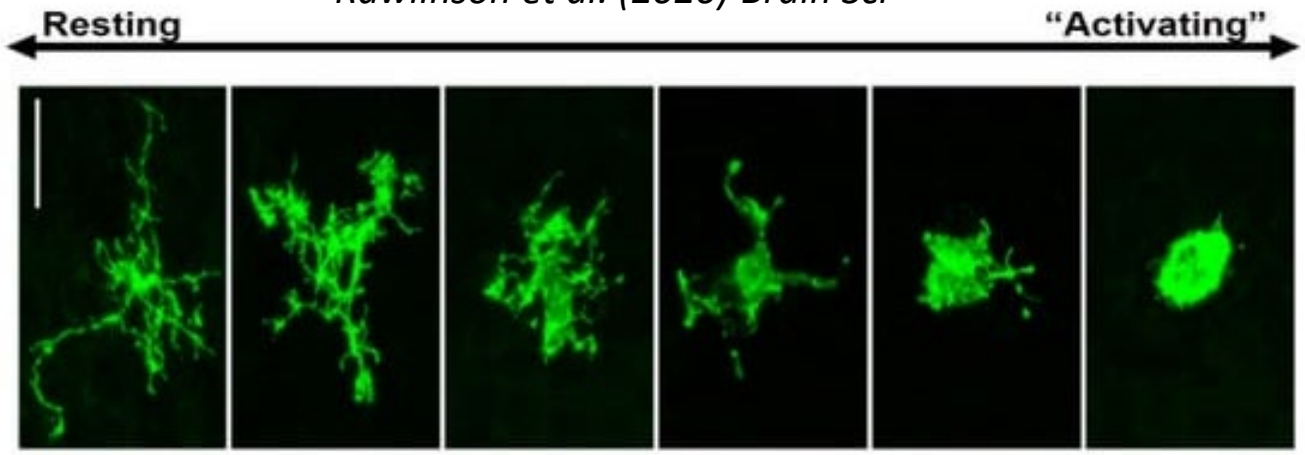


*SOURCE: A Silvin, F Ginhoux, Singapore Immunology Network (EPA-SIgN A*STAR collaboration)*

Focus on Microglia

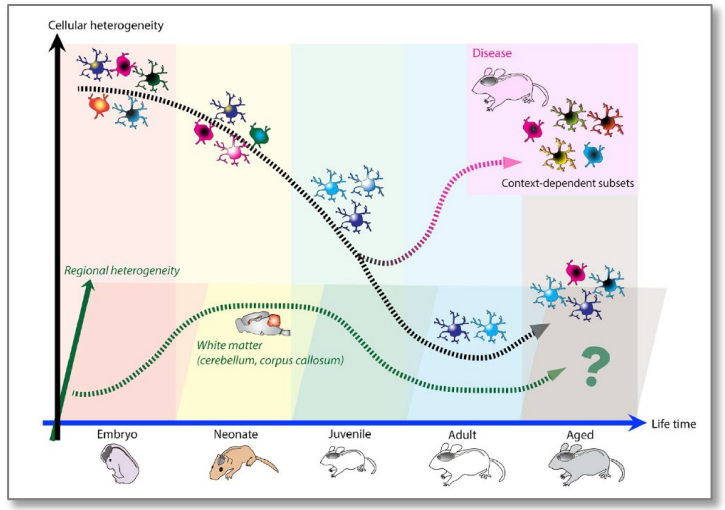
- Microglial cells are essential building blocks of microvascular development (angiogenesis) and BBB permeability (barriergenesis) in the embryonic brain.
- The recent microglial cell atlas of the brain (scRNA-seq) reveals peak phenotypic diversity during the embryonic period.
- Gestational BBB functions soon after emergence (<E14.5 mouse, <8th week human) driven by heterogeneous interactions (endothelial-pericyte-MCG).

Rawlinson et al. (2020) Brain Sci



M0 homeostatic state
(*ramified*)

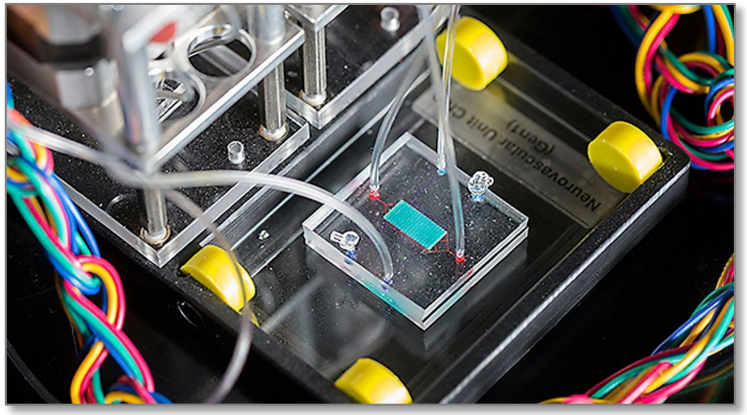
M1 activated state
(*amoeboid*)



Masuda et al. (2020) Cell Reports

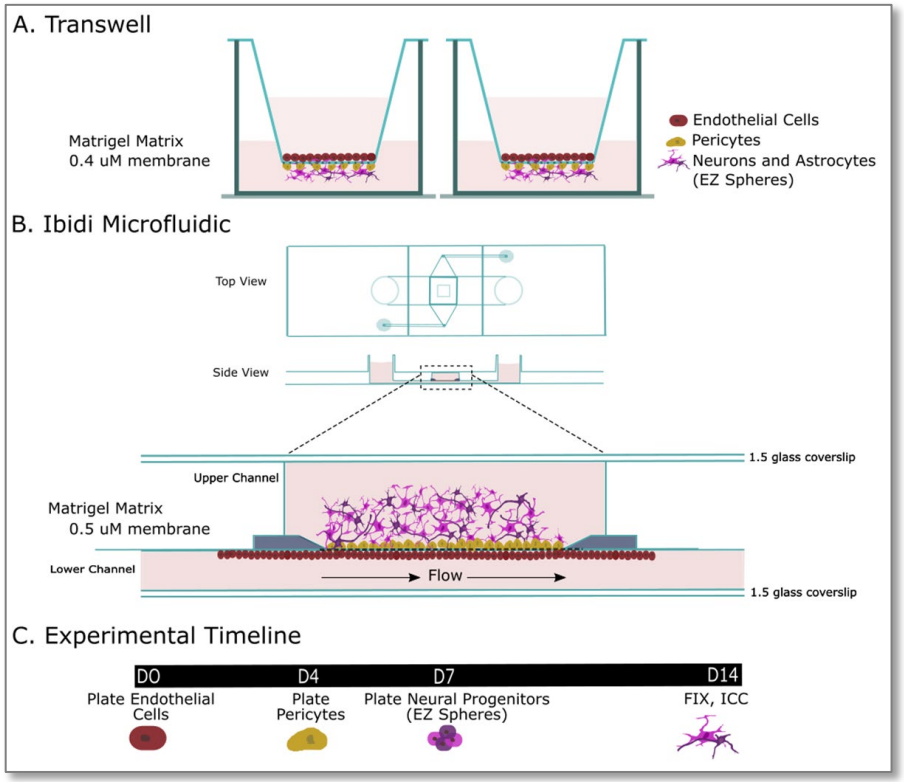
What underlies the microglial-depleted BBB phenotype?

Some literature suggests that microglial activation from M0 → M1 state can disrupt BBB function by MMP secretion; however, not a likely explanation for the depletion model.

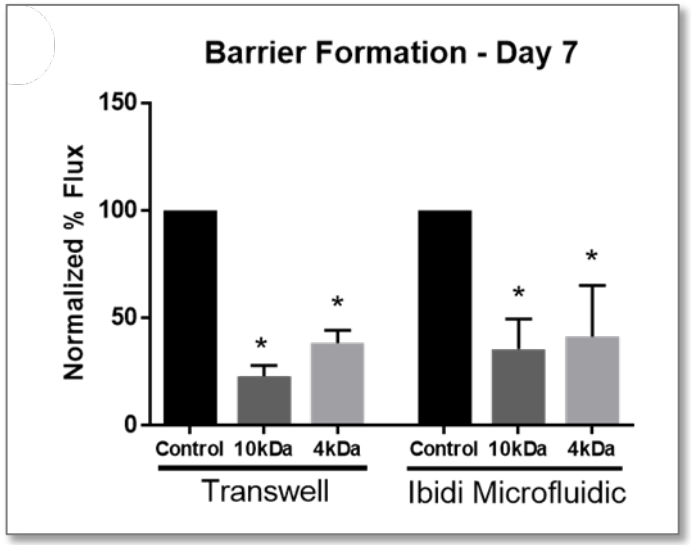


Barriergenesis is influenced by the molecular phenotype of endothelial cells, vascular flow (sheer-stress), and pericytes recruited to nascent capillaries.

Miller et al. (2021) Front Bioeng Biotechnol
(EPA STAR grant R839504 – D Cliffel)



Schwab et al. (submitted – in revision)



Test impact of chemicals on endothelial-pericyte barrier with FITC-dextran markers.

S Hunter, CSS 405.1.6 (FY23-26)

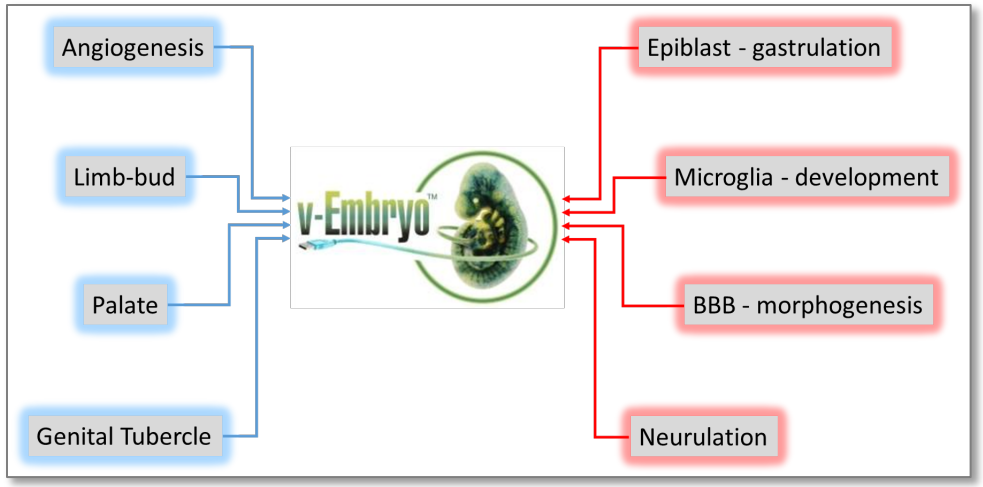
Multiscale Modeling and Quantitative Simulation

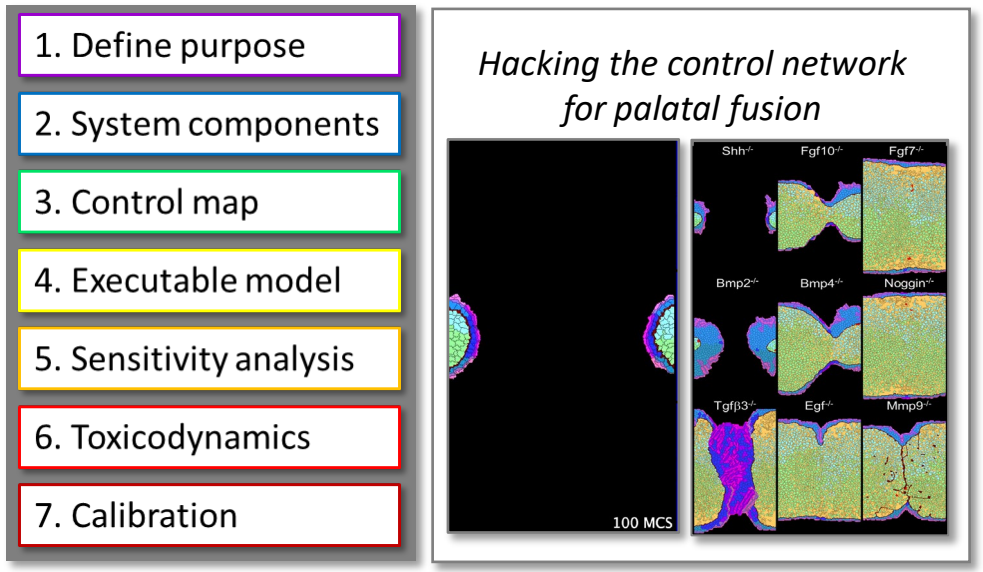
Goal *(CSS 405.2.3)*: build a fully computable system for *in silico* modeling and simulation that can: (i) titrate chemical effects from *in vitro* bioactivity profiles (ToxCast), and (ii) map to critical windows of neurovascular development in real time:

- microglial origins and emigration in the early embryo, leading to colonization of the anterior neural tube and subsequent vascularization;
- computational neurovascular unit (cNVU) implementing 'microglial sensing' and the molecular heterogeneity of the microglial pool; and
- interactive Cinematic Scientific Visualization (iCSV) of neurovascular development and developmental toxicity.

What we have learned across systems …

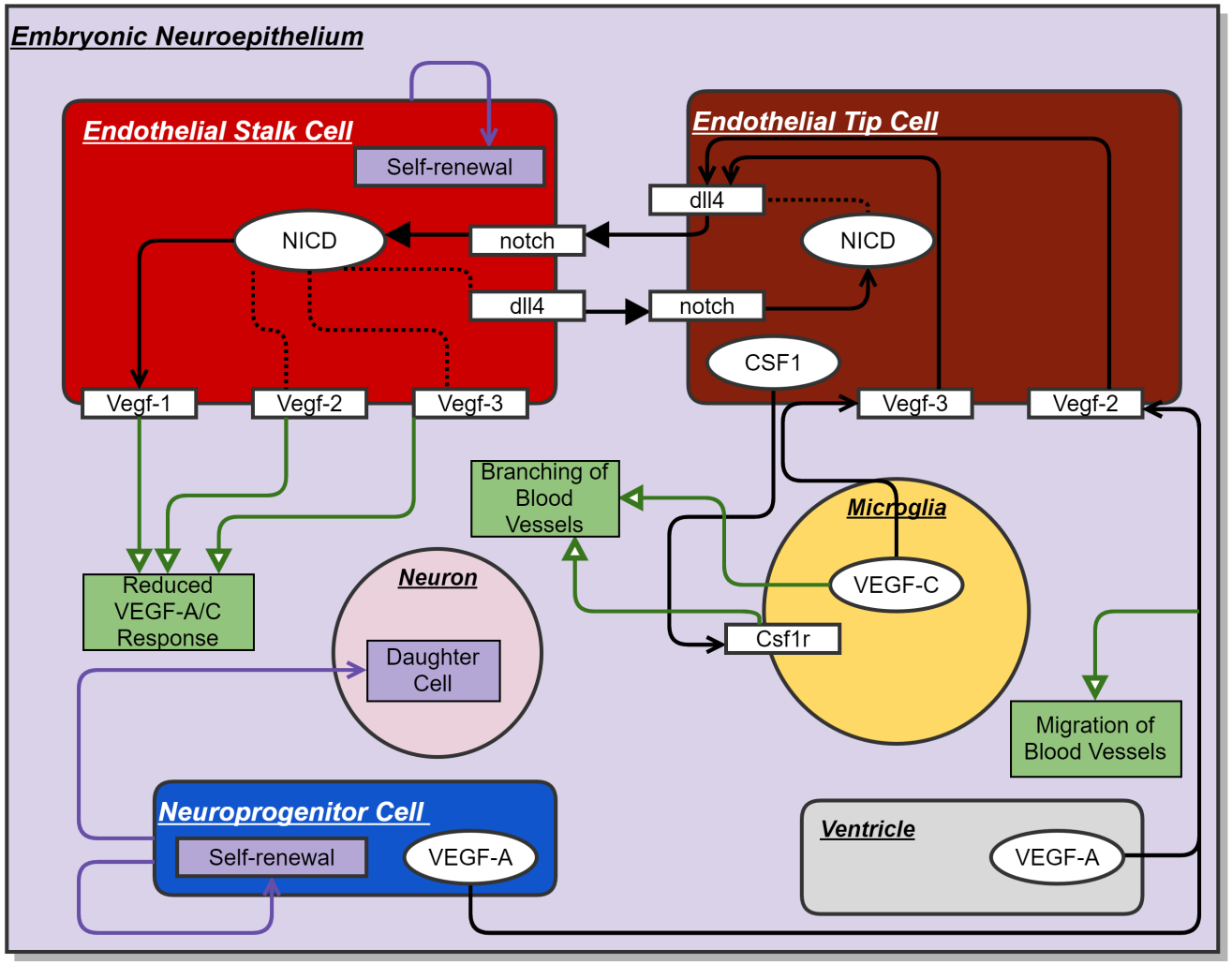
Computational models with sufficient biological intelligence can quantitatively simulate multiscale dynamics of biomolecular perturbation(s), predicting an adverse phenotype.

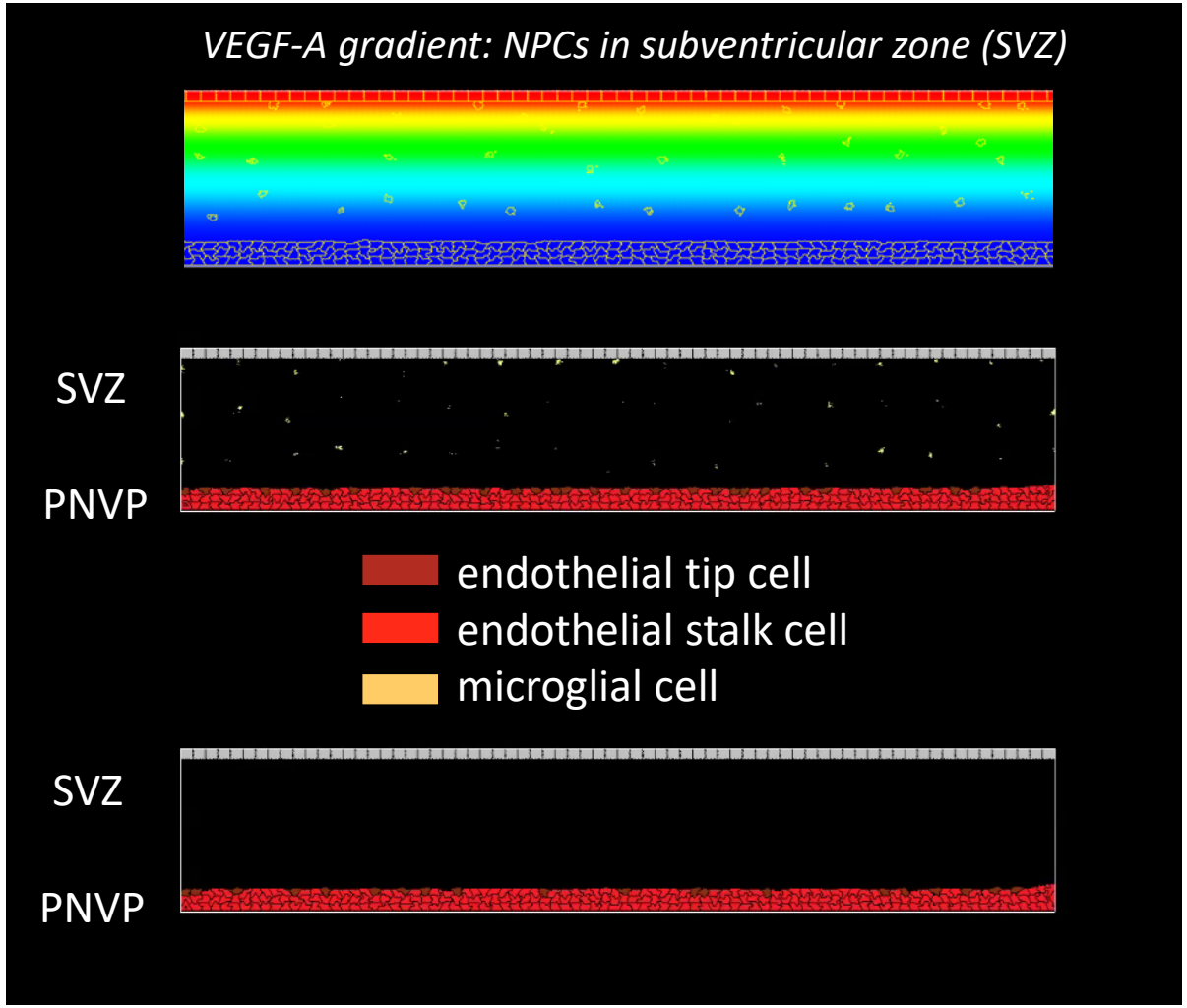




- Nature-inspired *agents* (cells) and *rules* (behaviors) set into motion as a self-organizing system, using an open-source modeling environment (**CompuCell3d.org**).
- Soft-computing (fuzzy logic) to simulate forces or properties governing cell activity where rules are inexact or knowledge incomplete (**computational intelligence**).
- Change course in response to a particular stimulus (genetic errors or environmental disruption) fed into the model from real world data (**mechanistic causation**).
- Probabilistic rendering of where, when and how a particular condition (or combination) might lead to an adverse developmental outcome (**cybermorphs**).

Prototype of microvascular development



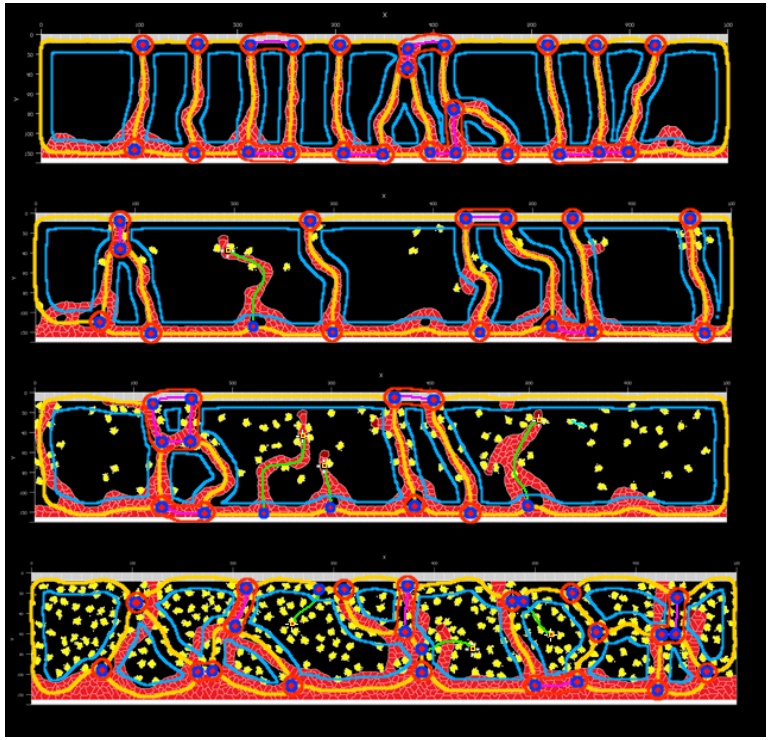


CompuCell3D.org modeling environment

O Naphade, EPA and Brown University - work in progress

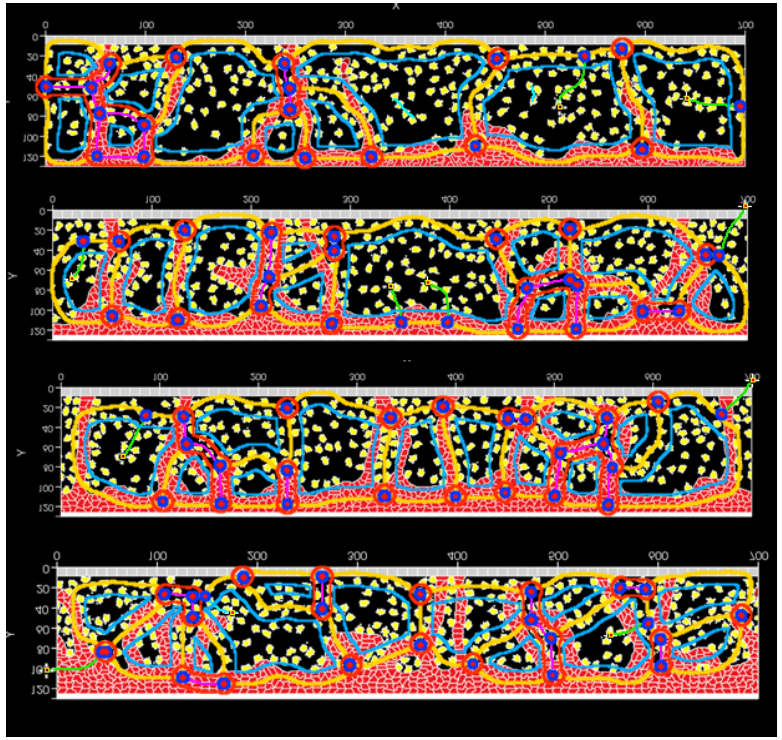
Sensitivity Analysis

Input: MGC number



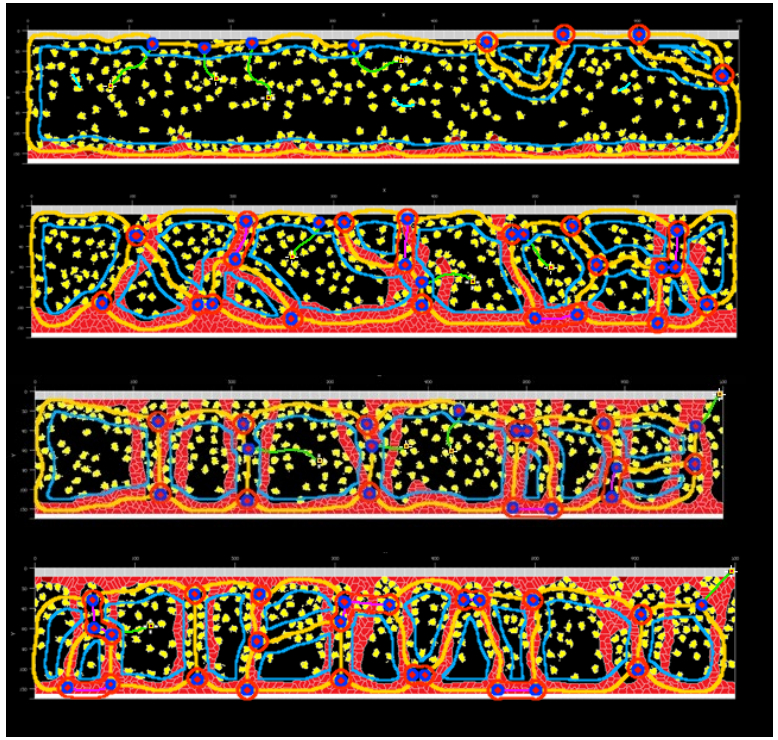
Output: vascular branching

Input: PNVP sprouting density



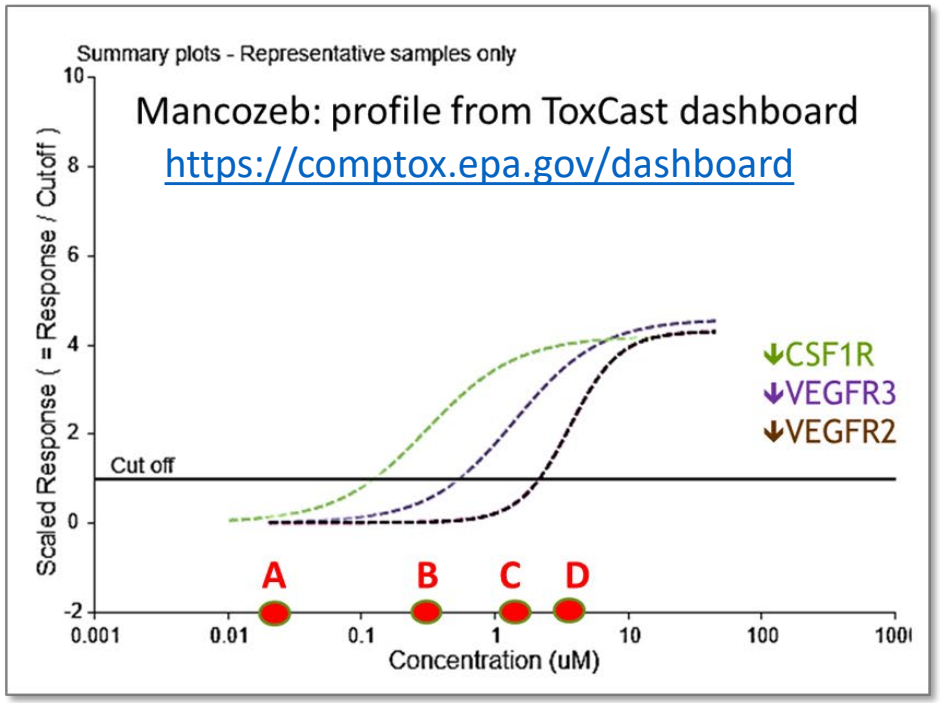
Output: sprouts reaching the SVZ

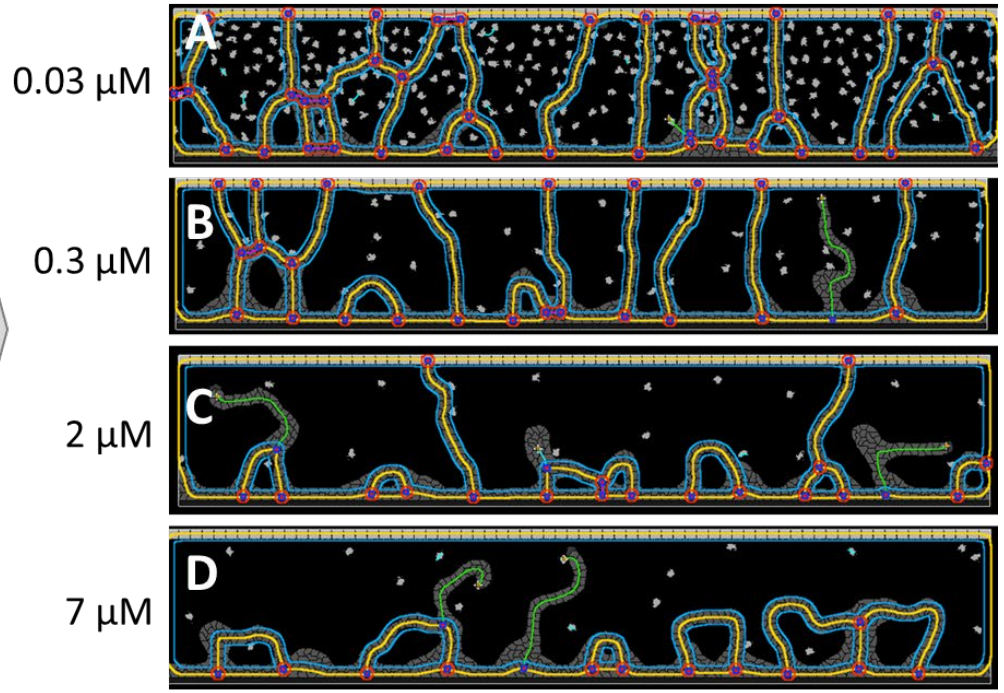
Input: VEGF-A levels



Output: sprout elongation

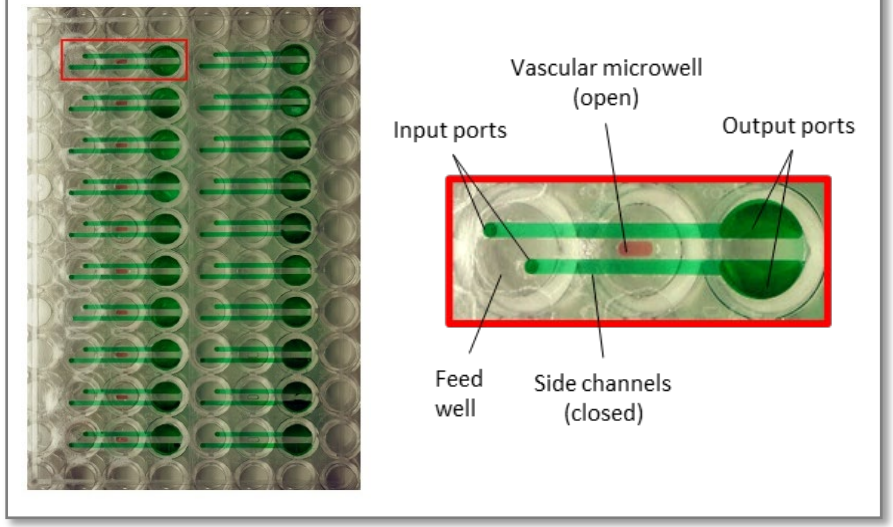
Executing a simulated concentration-response

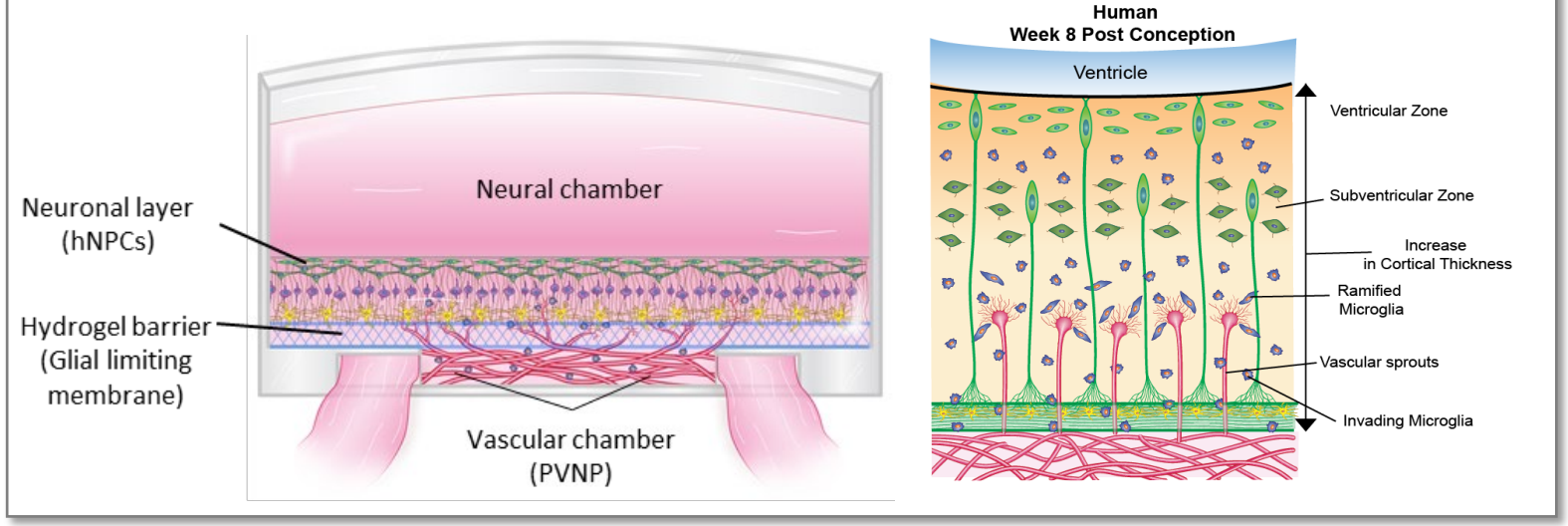




- Mancozeb's concentration profile (ToxCast_NVS) for key signals in microglial-endothelial interplay showed a rank order effect on CSF1R → VEGFR3 → VEGFR2.
- Simulation predicted a progressive reduction of angiogenic sprouts, vascular nodes, and branches reaching the SVZ with a critical effect at ~0.5 µM.
- Holding microglial cell number constant, the simulated effect became significant by 2.0 µM for vascular nodes (P=0.04) and by 20 µM for branches reaching the SVZ (P< 0.001).

Synthetic microsystem: *human PNVP development 6-8 weeks gestation*





- pumpless, tubeless
- 96 well plates (20 devices per plate)
- short production time
- optically transparent
- closed format for vascularization
- open format for organoid assembly
- realistic fluid budgets

Microfluidics device: David Beebe, Brian Johnson

Input cell types:
- Endothelial cells, pericytes
- Microglia
- Radial glia, NPCs

Output processes:
- Vascular network assembly (d5)
- Endothelial & Microglial migration (d14)
- Integration with neuronal layer (d21)

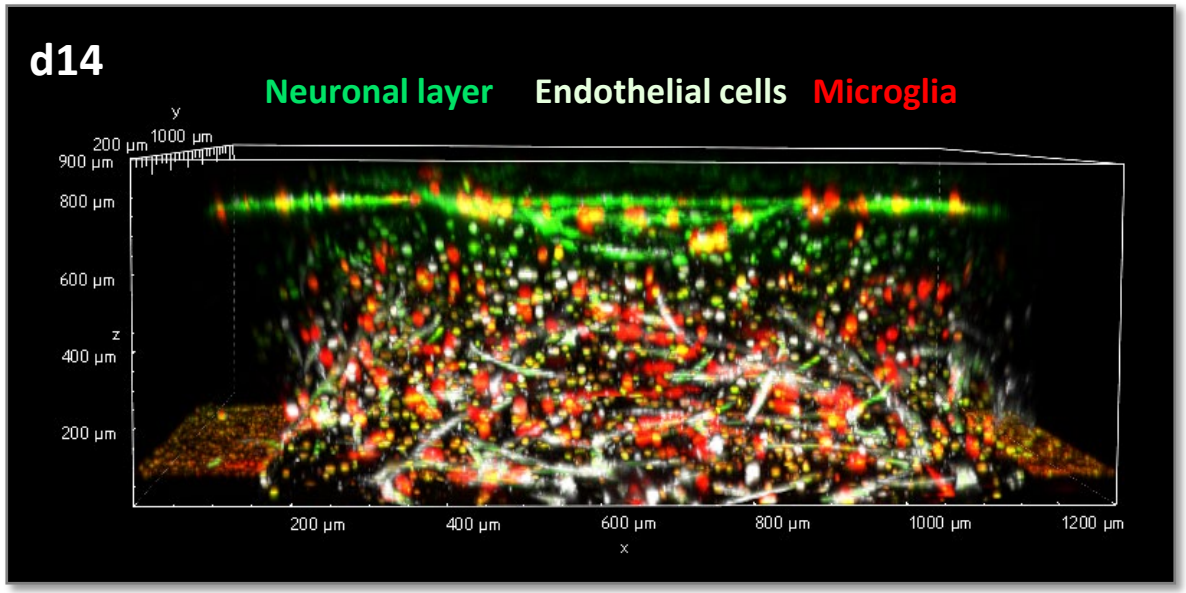
Engineered Perineural Vascular Plexus for Modeling Developmental Toxicity

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**

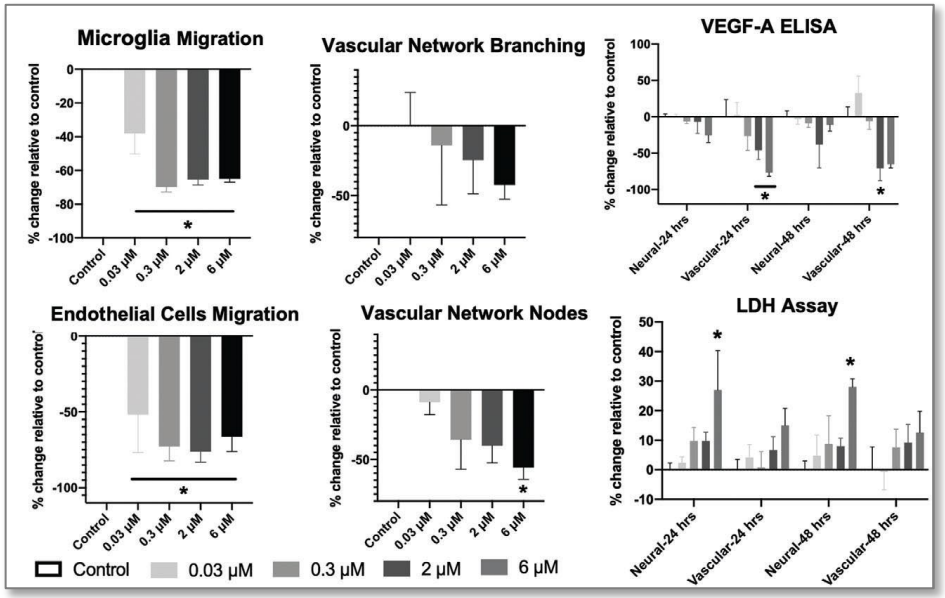
PNVP platform: Kauschik et al. (2020) Adv Healthcare Mater 2000825

Synthetic microsystem: *human PNVP development 6-8 weeks gestation*

Microglial and endothelial cell migration into neural layer



Effect of Mancozeb (two-day exposure, analyzed d16)



Critical effect concentrations for Mancozeb:

- ***predicted*** *in silico: ~0.5 µM (MGC infusion), 2.0 µM (vascular nodes), <20 µM (branches reaching SVZ)*
- ***observed*** *in PNVP culture: 0.3 µM (EC/MG cell migration), 6 µM (vascular network nodes)*

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

Parameter wish list for cNVU simulation (* *modeled to date*)

BRAIN COMPARTMENTS
- vascular (PNVP*, microvasculature*)
- neuroepithlelium (SVZ*, radial glia)
- ventricle (ependymal layer*, CSF*)

CELL TYPES
- Microglia (M0*, M1, M2)
- Endothelia (tip*, stalk*)
- Pericytes
- Neuroprogenitors (NPC, neurons)

CELLULAR PROCESSES
- angiogenic sprouting*
- stalk cell proliferation*
- NPC proliferation*
- MGC growth*
- MGC migration

SIGNALS/RECPTORS
- VEGFA/VEGFR1*
- VEGFC/VEGFR3*
- CSF1/CSFR1*
- DELTA-4/NOTCH*
- IL-8/CXCR1
- FKN/CX3CR1
- NPY/CXCR4
- TGF-beta
- P2RY12

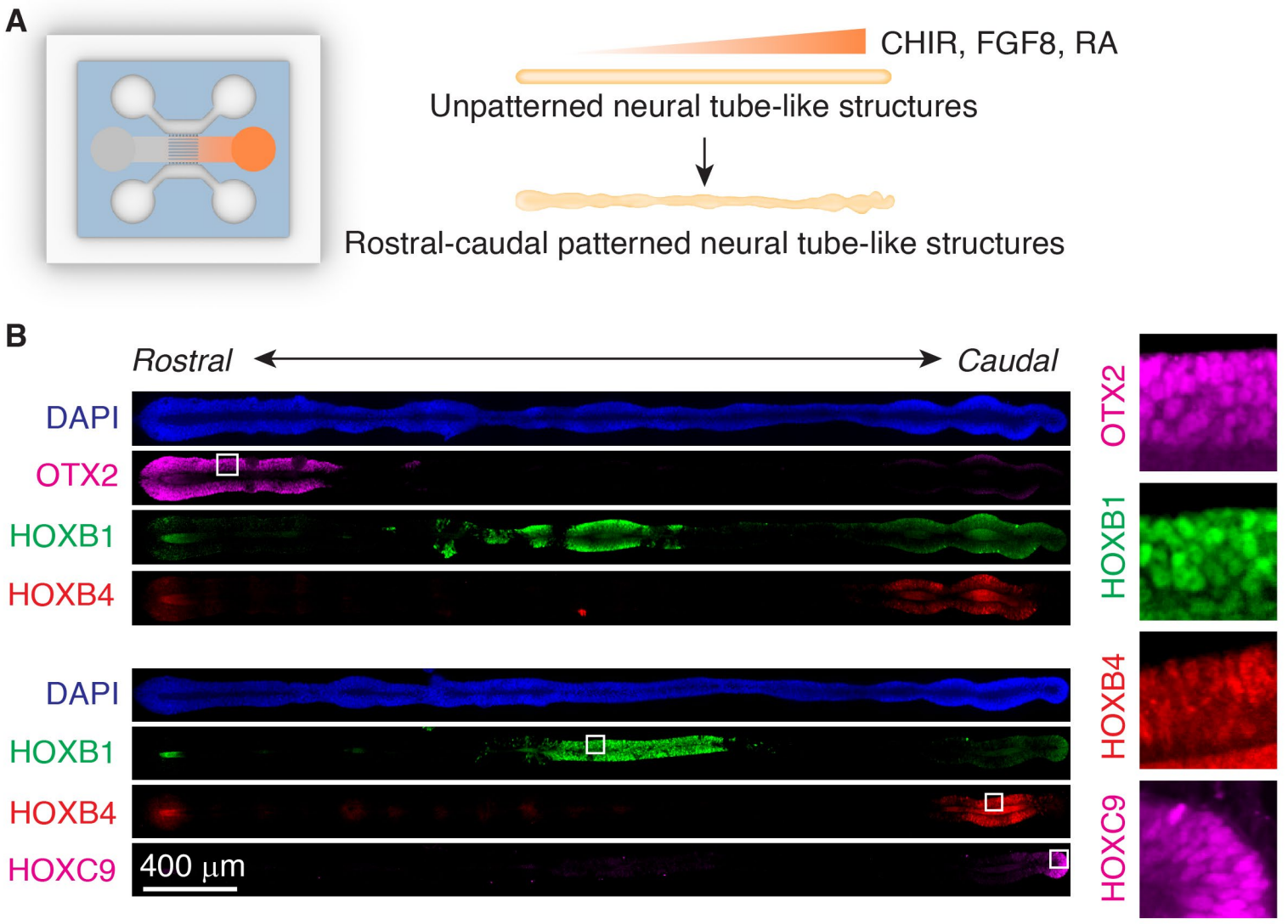
TRANSDUCERS
- AKT/IP3K
- SRC
- FOXO1
- FOXO3

BARRIERGENESIS
- vascular flow / shear force
- molecular transporters
- microvascular adhesion

EMERGENT PROPERTIES
- SVZ vascularization*
- microvascular tortuosity*
- MGC bridging*
- NPC mass
- NN numbers
- NN networks
- brain mass

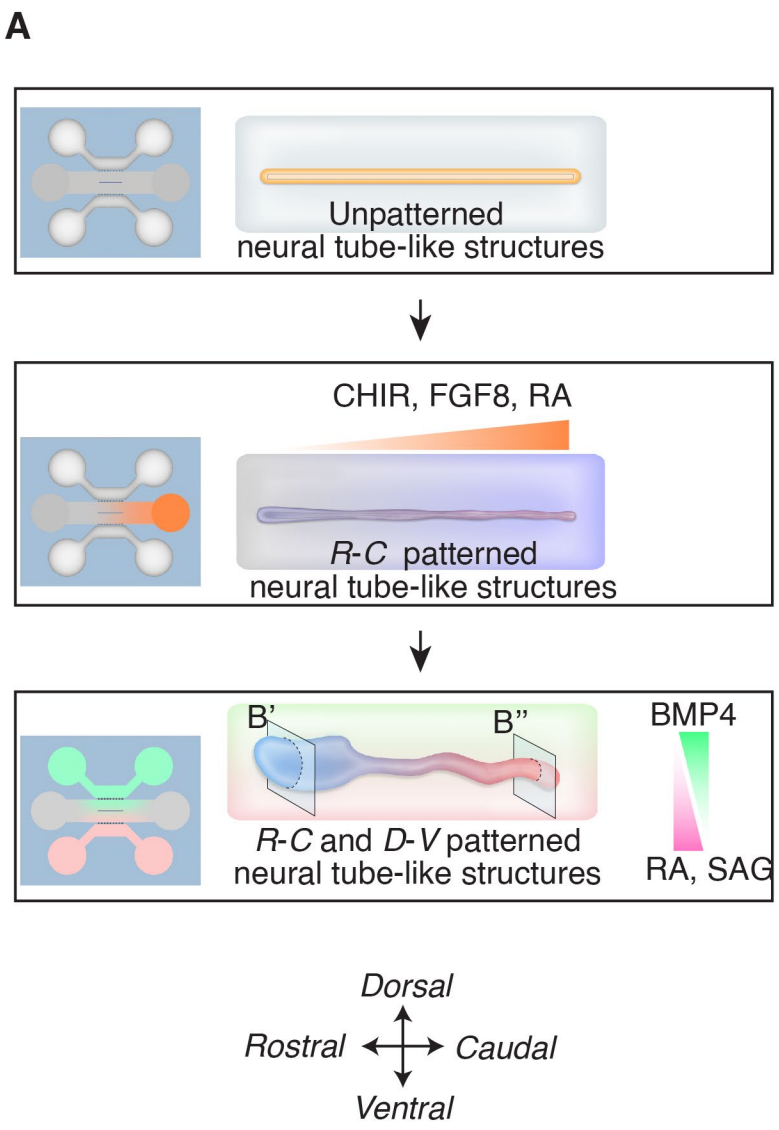
CASE STUDIES
- Mancozeb*
- Maneb*
- Oxytetracycilne*
- 1,5-Naphthalenediamine*
- PFOS*
- ATRA
- TNP470
- 5HPP-33
- other?

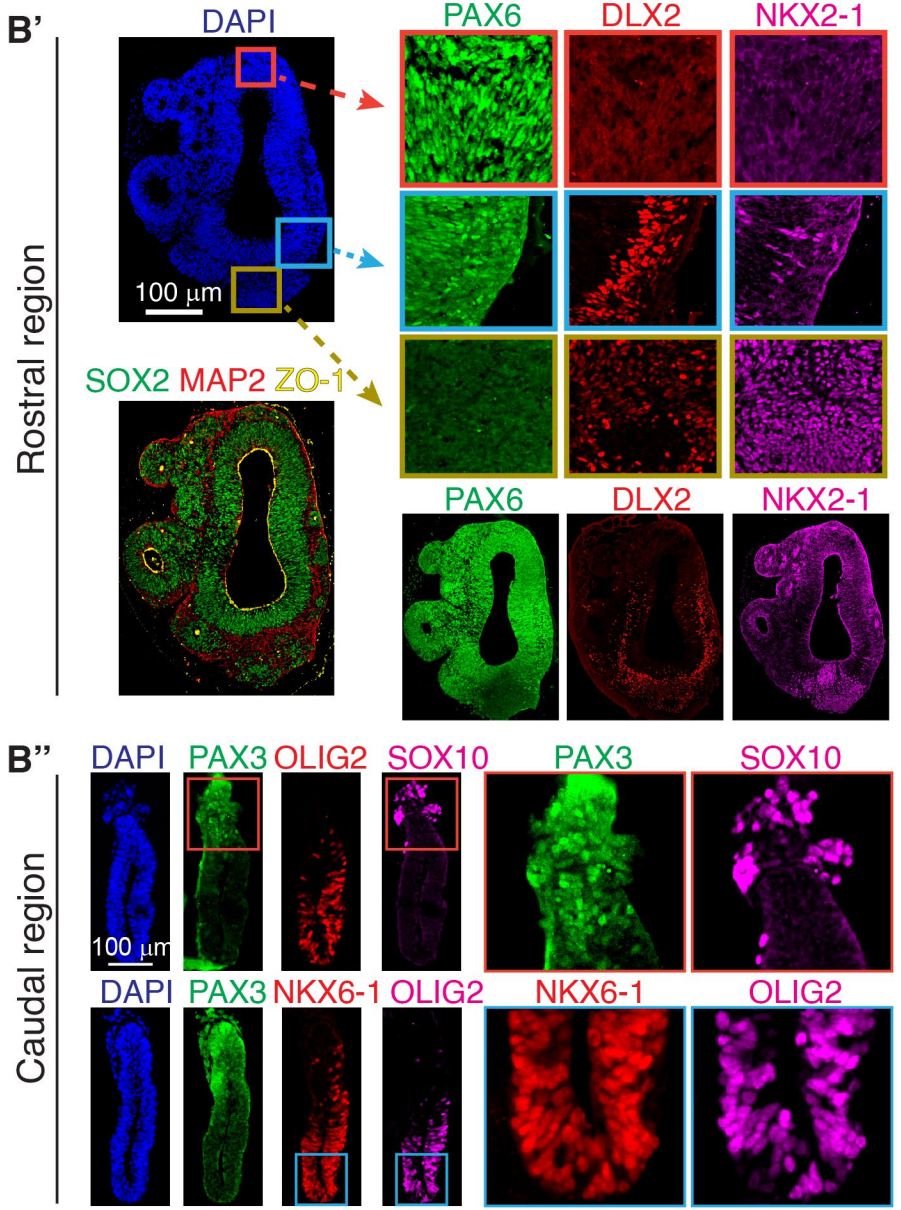
Patterning: *self-organization of a neural tube-like microsystem with microfluidics*

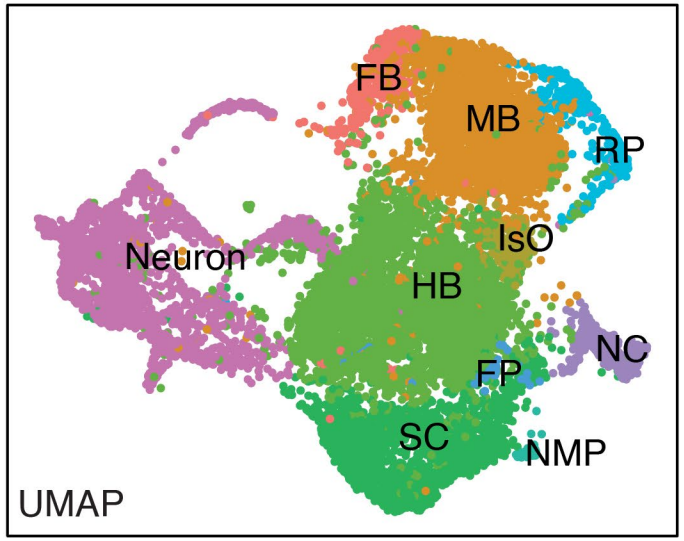


Xue et al., manuscript in revision

Specification: *rostral-caudal and dorsal-ventral patterning*





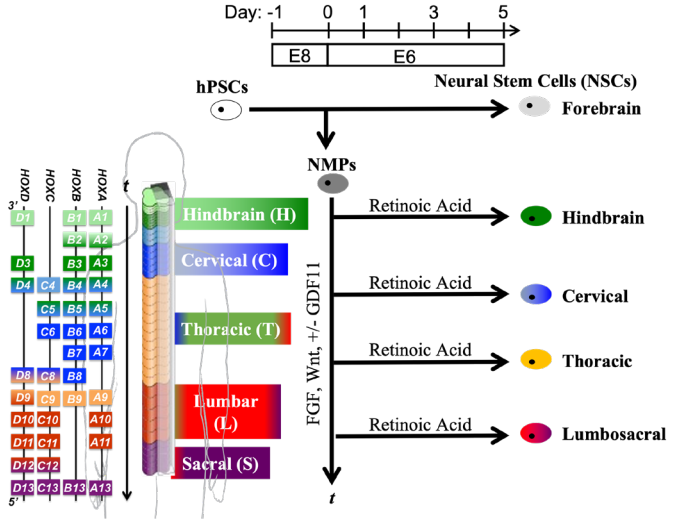


FB, forebrain;
MB, midbrain;
IsO, isthmic organizer;
HB, hindbrain;
SC, spinal cord;
NMP, neuromesodermal progenitors;
RP, roof plate;
FP, floor plate;
NC, neural crest.

Xue et al., manuscript in revision

RosetteArray™: *screening region-specific vulnerabilities of the human neural tube*

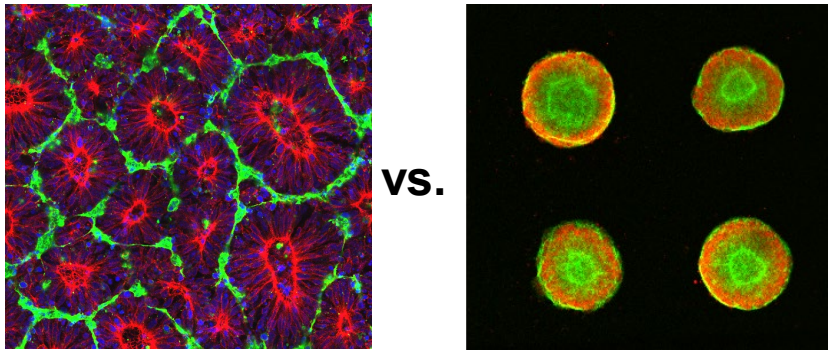
Region-specific Neural Stem Cell Derivation



Lippmann et al. Stem Cell Reports 2015

+

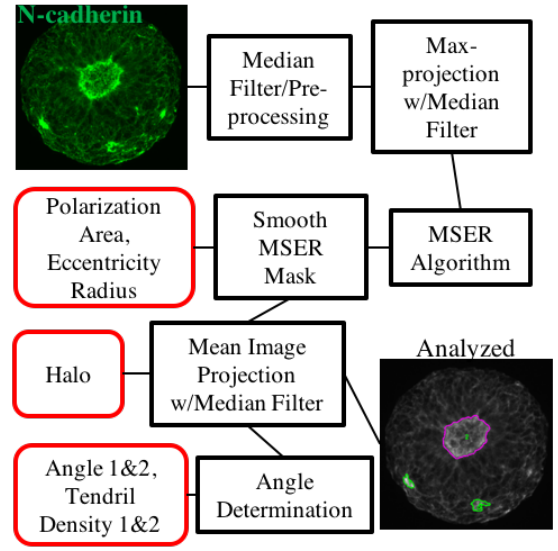
Micro-Patterned Neural Stem Cell Culture



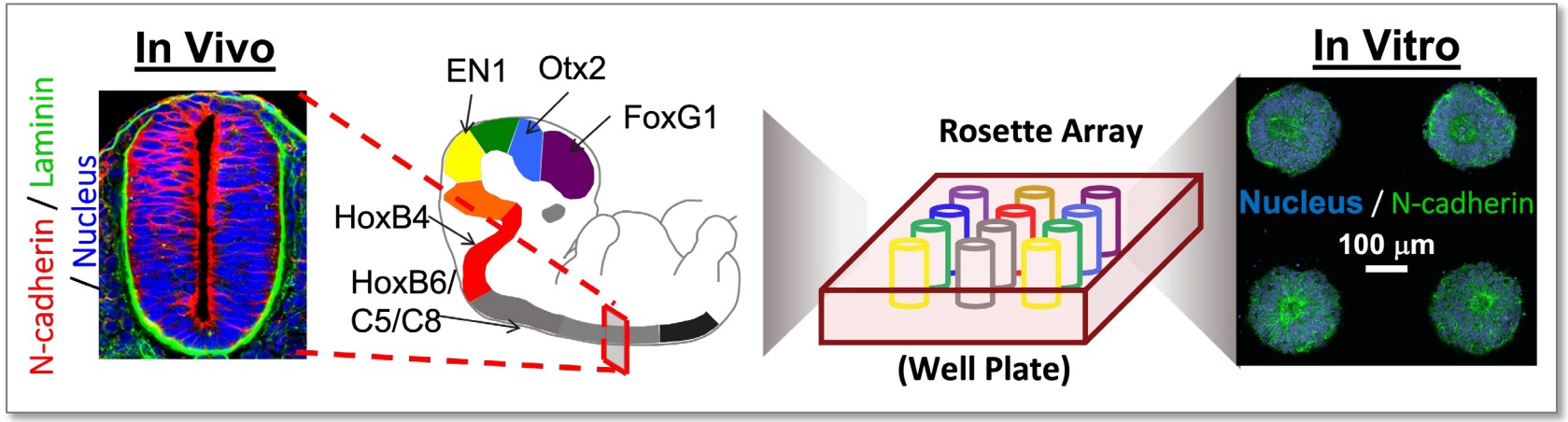
Knight et al. eLife 2018

+

AI-based Image Analysis



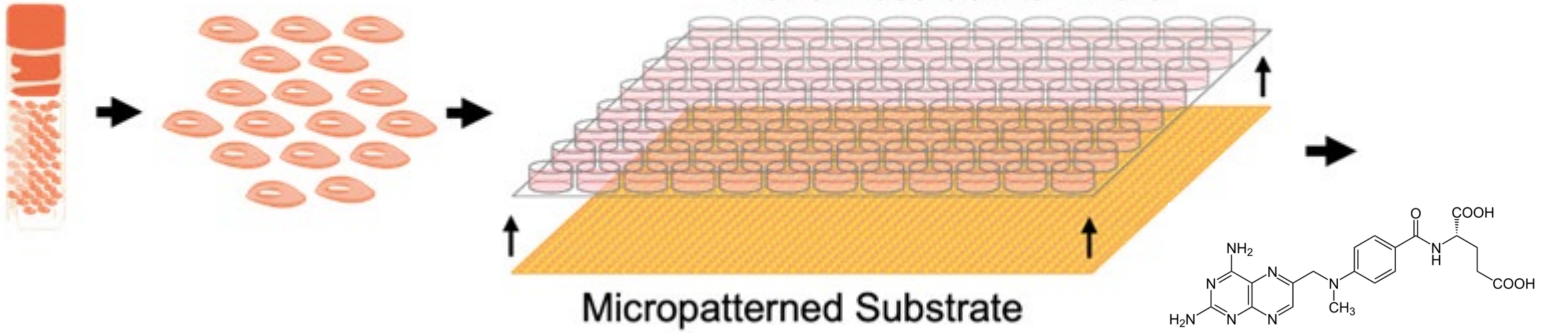
Knight et al. eLife 2018

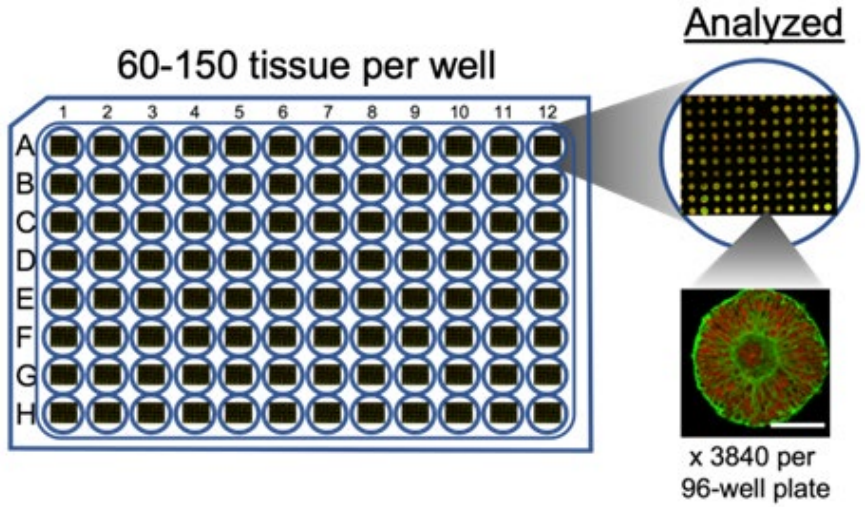


Randolph Ashton, co-founder of Neurosetta LLC

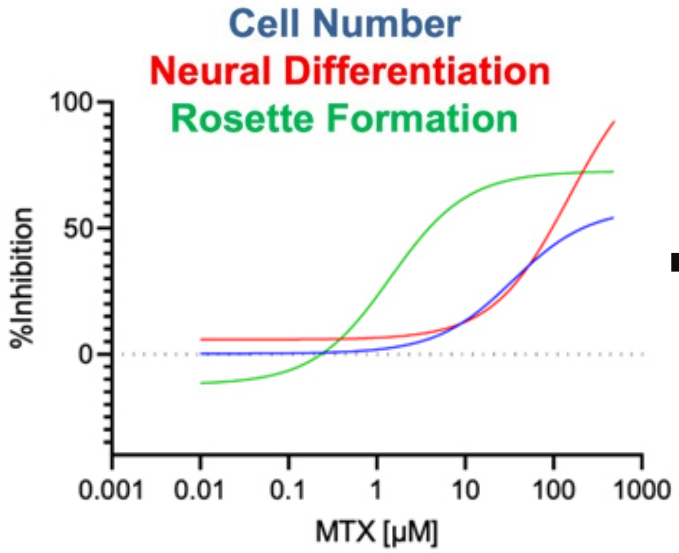
DNT screening: *scalable and off-the-shelf developmental neurotoxicity screening*

Cryopreserved Human Pluripotent Stem Cells or Spinal Progenitors





Neurosetta, LLC
Tissue detection image processing software



Mechanistic Insight
(beyond toxicity)

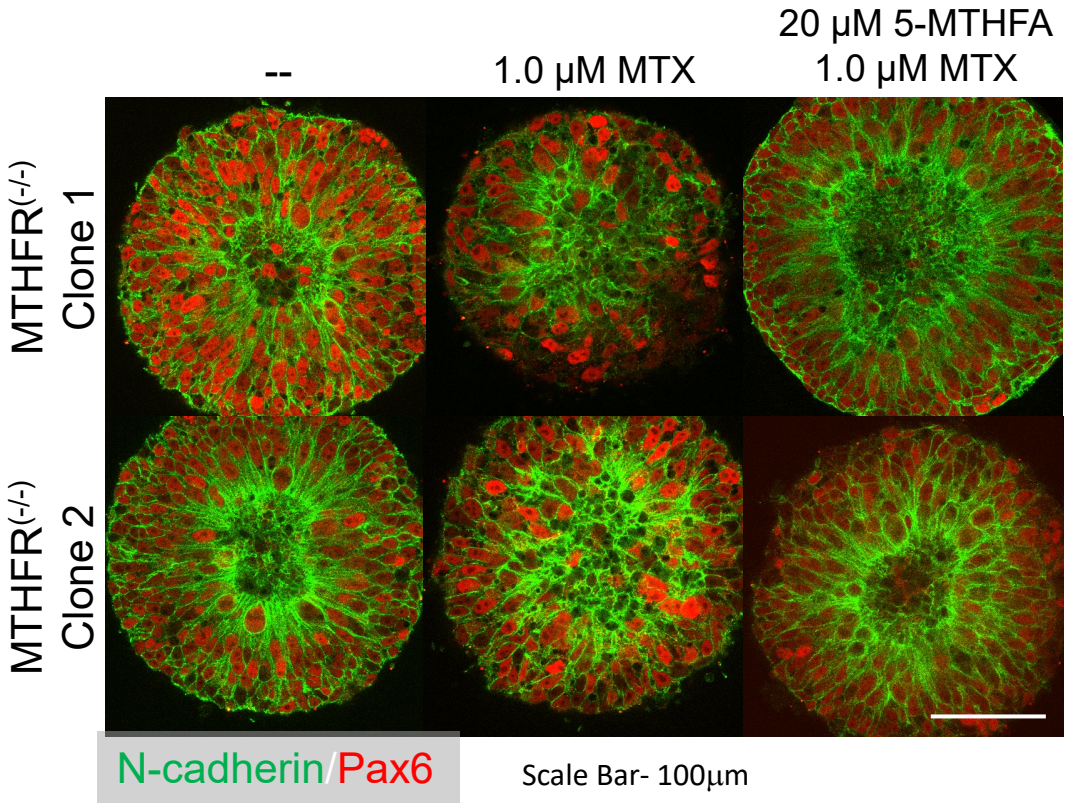
HTS platform being commercialized in biotech startup (Neurosetta LLC)

SCBRM
www.scbrm.bme.wisc.edu

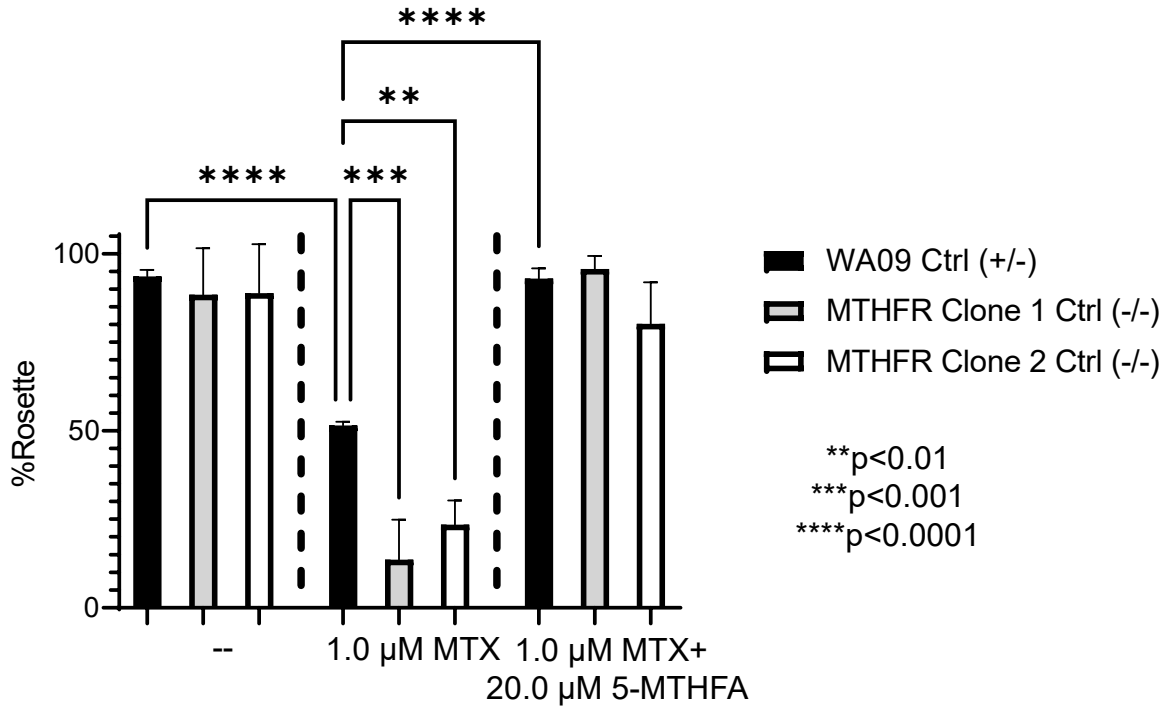
RosetteArray™: *modeling risk factors underlying Neurodevelopmental defects (NDDs)*

Neural Tube Defects:

- Multifactorial etiology, i.e., caused by genetic plus environmental factors
- $MTHFR^{C667T(-/-)}$ mutation decreases folic acid metabolism and is a risk factor for Spina Bifida.
- Methotrexate (MTX) is an anti-cancer drug that inhibits folic acid metabolism.
- 5-Methyltetrahydrofolic acid (5-MTHFA) can rescue a decrease in folic acid metabolism.



Scale Bar- 100µm



Knight GT, et. al. (submitted)

Charting neurulation

Literature review

- Physiological information
- Cellular compartments
- Biochemical gradients
- Critical cell fate and behavior
- Reduced to the essence

Landscape View

Update Article Counts | More stuff | Heat Map by column | Heat Map by row

		Surface Ectoderm	Paraxial Mesoderm	Neural Crest	Notochord	(neural tube defects OR	retinoic acid OR ATRA OR tretinoic	BMP OR FGF OR NOG OR CHRD OR	FGF OR ZIC1 OR ZIC3
Subject queries:									
Preferred Name	**Chemical / Entity query**	**Surface Ectoderm**	**Paraxial Mesoderm**	**Neural Crest**	**Notochord**	**Neural Tube Defect**	**ATRA**	**Diff / migration**	**Growth and body axis extensi**
bmp	BMP OR Bone morphogenic protein OR BMP1 OR BMP3 OR BMP-3	69	1362	508	125	69	378	21324	1207
shh	SHH OR Sonic hedgehog	60	708	237	317	223	356	923	557
fgf	FGF OR Fibroblast Growth Factor	98	2380	587	153	139	1007	20313	19747
wnt	WNT protein OR wnt factor OR wingless	112	1713	677	147	188	563	2547	1133
Valproic Acid	Valproic Acid	1	22	30	3	352	189	43	11
Arsenic	Arsenic	2	8	10	0	62	1190	26	5
methyl cellosolve	methyl cellosolve	0	0	1	0	4	1	0	0
Methanol	Methanol	0	13	5	2	15	100	59	9
fumonisin B1	fumonisin B1	0	1	1	0	27	4	3	3
valnoctamide	valnoctamide	0	0	0	0	8	0	0	0
Ethanol	Ethanol	3	38	100	12	115	391	220	47
2,4-Dichlorophenoxyacetic Acid	2,4-Dichlorophenoxyacetic Acid	0	0	0	0	5	0	1	0
2,4,5-Trichlorophenoxyacetic Acid	2,4,5-Trichlorophenoxyacetic Acid	0	0	0	0	6	0	0	0
2-ethylhexanoic acid	2-ethylhexanoic acid	0	0	2	0	5	1	0	0
2-propyl-4-pentenoic acid	2-propyl-4-pentenoic acid	0	0	1	0	5	0	0	0
Carbamazepine	Carbamazepine	2	0	0	0	124	17	10	0
ethyl-p-hydroxybenzoate	ethyl-p-hydroxybenzoate	0	0	0	1	1	0	0	0
butylparaben	butylparaben	0	0	0	1	1	0	0	0
benzyl salicylate	benzyl salicylate	0	0	0	0	1	0	0	0

AbstractSifter: *deep dive into complex relationships among genes, pathways, and chemicals.*
[Baker et al. (2017) F1000]

Reprod Toxicol. 2021 Jan;99:160-167. doi: 10.1016/j.reprotox.2020.09.002. Epub 2020 Sep 11.

An ontology for developmental processes and toxicities of neural tube closure

Harm J Heusinkveld [1], Yvonne C M Staal [2], Nancy C Baker [3], George Daston [4], Thomas B Knudsen [5], Aldert Piersma [2]

Heusinkveld et al. (2021) Reprod Toxicol

Simulation: *developmental ontology-based computational model of neurulation*

Literature review

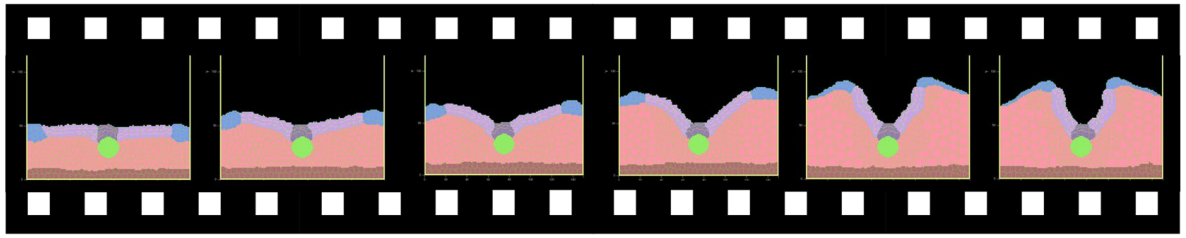
- Physiological information
- Cellular compartments
- Biochemical gradients
- Critical cell fate and behavior
- Reduced to the essence

Systems biology map

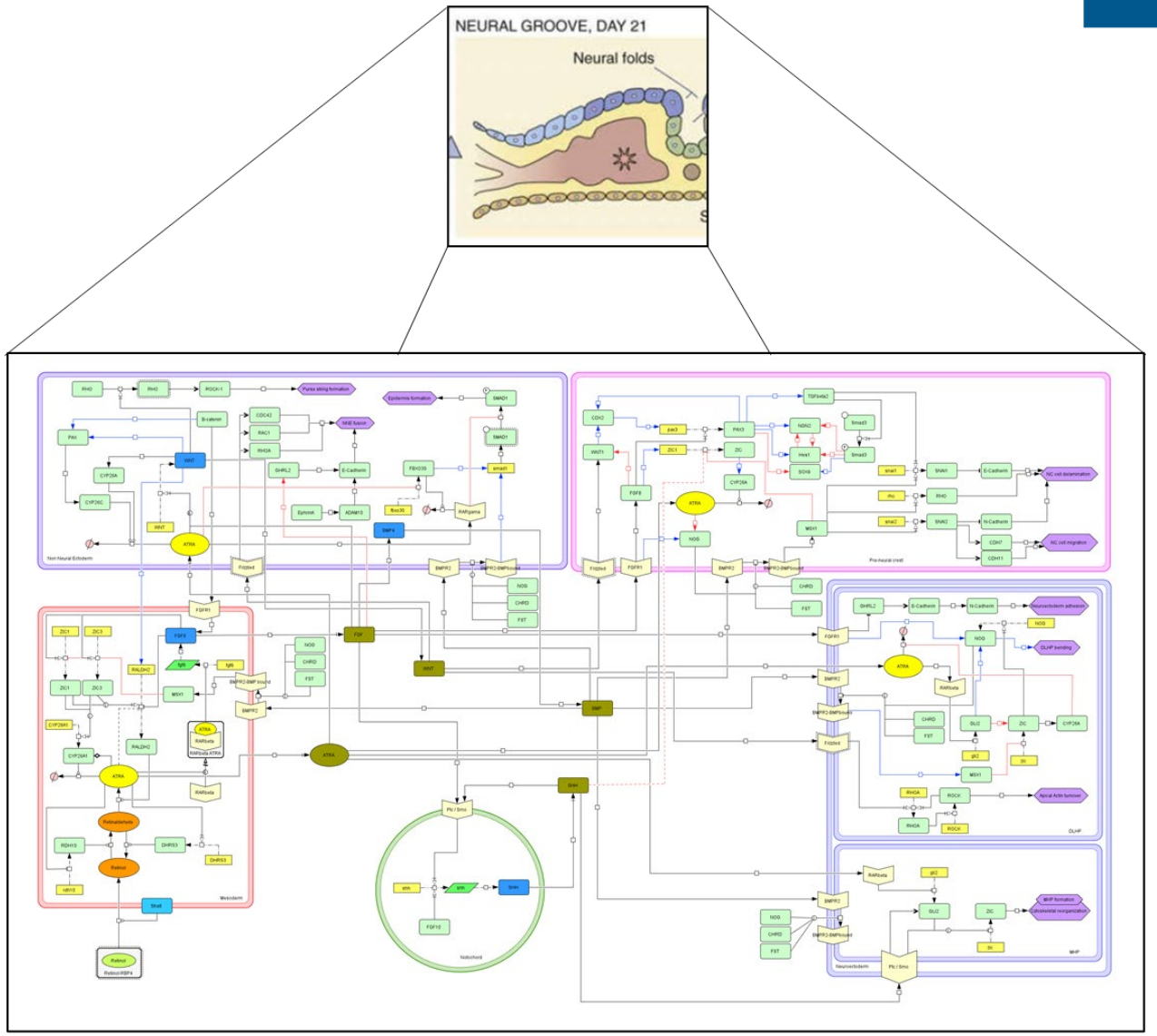
- Useful for AOP elucidation
- Starting point for *in silico* simulation

Organotypic culture and kinetic models

- Assessing toxicity *in vitro* (C Wolf, CSS 405.1.5)
- Pregnancy toxicokinetics (H El-Masri, CSS 405.2.2)



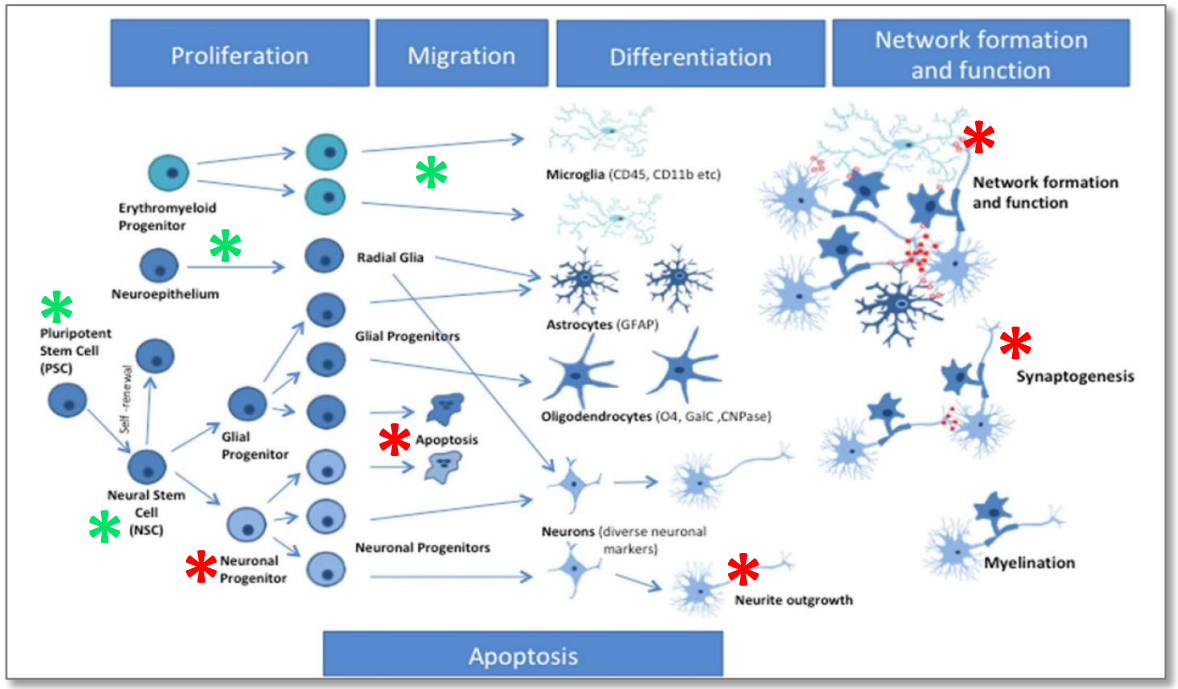
Job Berkhout, RIVM (work in progress)



Heusinkveld et al. (2021) Reprod Toxicol

DNT integration: *integrating NAMs data across diverse neurogenic processes*

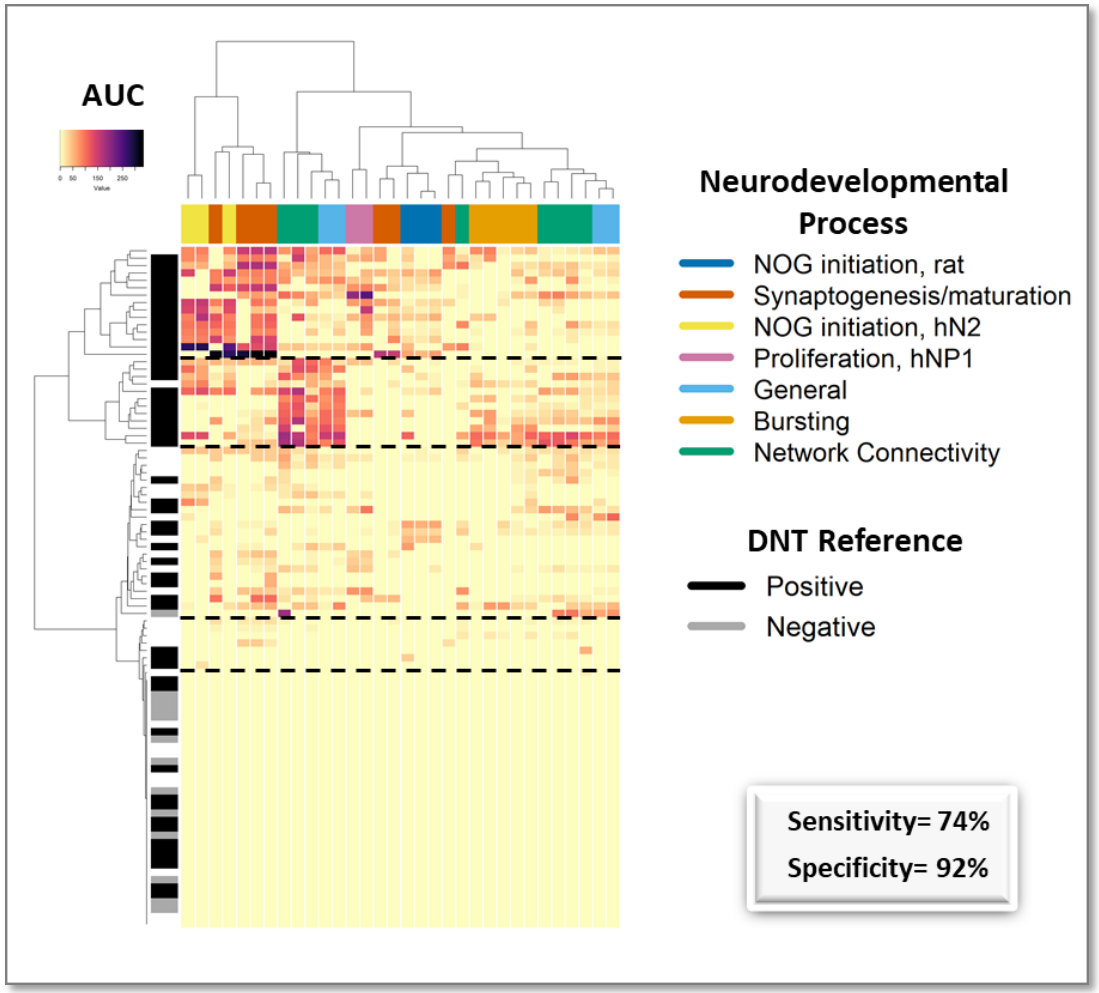
Processes essential for nervous system development



Bal-price et al. (2018) Altex

DNT toxicants likely disrupt one or more of these process:

- (*) processes under current modeling;
- (*) disruptions in PNVP formation and BBB function should be mapped so as not to overlook their potential role.



Carstens et al. (2022) Tox Sci

Summary: *synthetic microsystems, computational intelligence, and artificial life*

- Human cell-based synthetic microsystems (in vitro) and computational systems models (in silico) are emerging tools for predictive modeling of complex morphogenetic behaviors.
- Unraveling this complexity remains a challenge for predictive toxicology of complex self-organizing tissues such as early neural tube morphogenesis and vascularization.
- Biomimetic microsystems, together with chemical profiling data and knowledge of the relevant embryology, can begin to address this challenge for human development.
- A community of practice will be necessary to orchestrate fully executable computational models patterning neurovascular morphogenesis for predictive toxicology.

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Sid Hunter (CSS 405.1.6)
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