



# Computational Modeling of the Neurovascular Unit to Predict Microglia Mediated Effects on Blood-Brain Barrier Formation

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*Models of Brain Disorders and Disease*

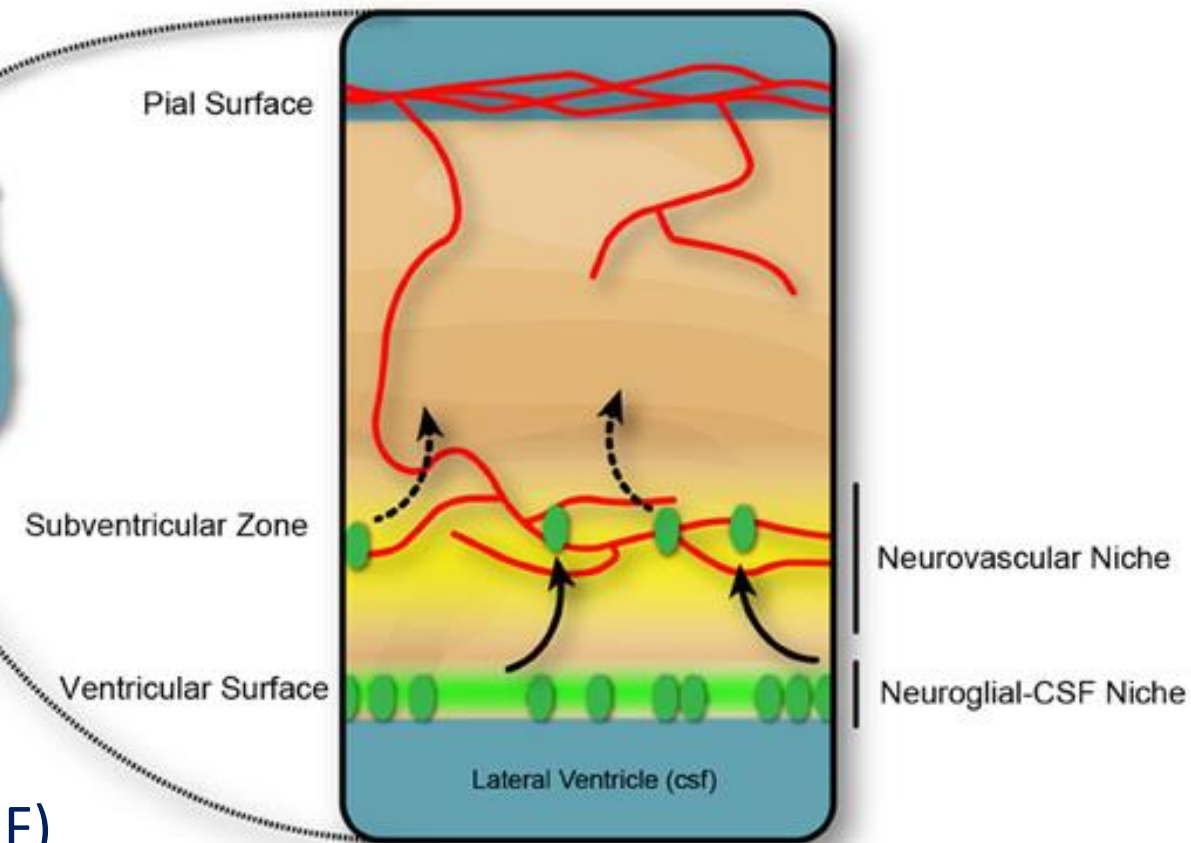
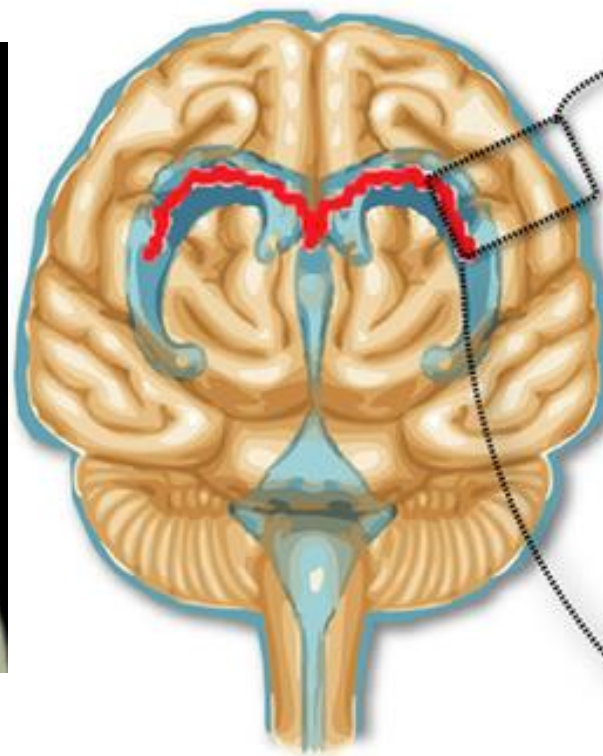
*10<sup>th</sup> World Congress: Alternatives and Animal Use in Life Sciences*

*August 21, 2017*

U.S. Environmental Protection Agency

*This work does not necessary reflect EPA policy*

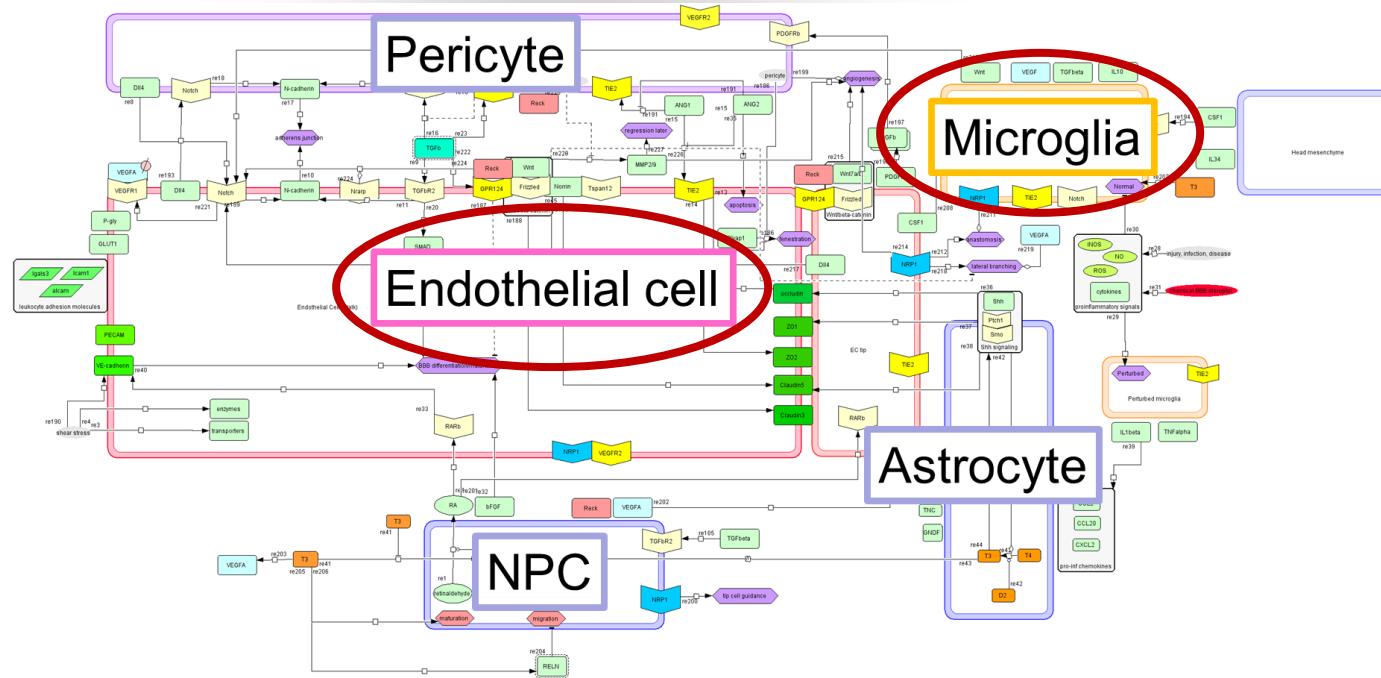
# Computational neurovascular unit (cNVU) focus



Chemical signals from the neuroepithelium (eg, VEGF) initiate brain angiogenesis via sprouting from the PNVP.

Stolp wt al., *Front. Integr. Neurosci.* 2013

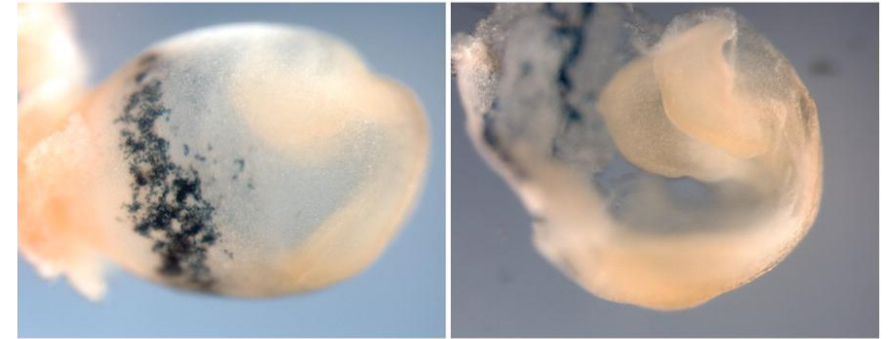
# Cell-Cell interactions of the NVU



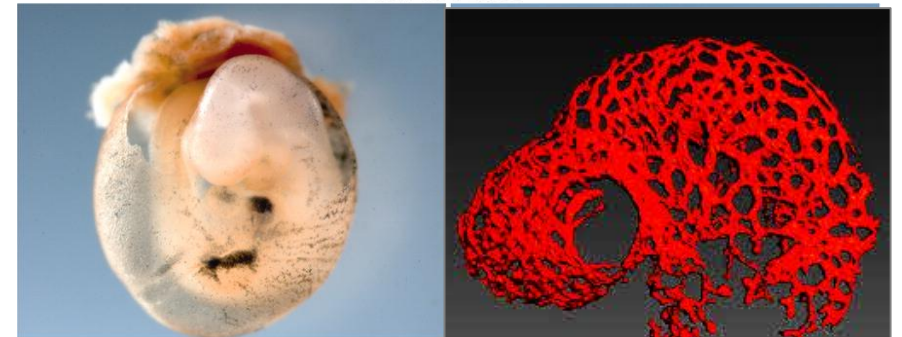
NVU systems map (K Sali, NCCT)

- Microglia, resident macrophages of the brain.
- During development...
  - Orchestrate neurovascular ramifications, surveillance of local injury where hyperactivation can invoke an adverse neuroinflammatory response
  - Are they mediators of developmental toxicity?

E8.25-E8.5



E9.25-E9.5



Ginhoux et al., *Science*, 2010

# Cell Agent-Based Modeling

- **Agent-Based Modeling and Simulation (ABMS):** a heuristic approach to reconstruct tissue dynamics using knowledge of biochemistry and cell-by-cell interactions.
  - Program each *agent* (cell) to follow specific rules
  - Interactions of agents gives rise to *emergent features* (phenotypic outcomes)
  - Qualify emergent feature with experimentally derived phenotypes (tissue level morphology)
  - Make toxicodynamic predictions by integrating biological knowledge & high throughput data
- **CompuCell3D\*:** open source modeling environment
  - Rules (steppables) for distinct cell behaviors (growth, proliferation, apoptosis, differentiation, polarization, motility, ECM, signal secretion, ...);
  - Rules coded in Python for cell-autonomous ‘agents’ that interact in shared microenvironment and self-organize into emergent phenotypes.
  - Methodology applied to past systems: vasculogenesis, genital tubercle, palate fusion, etc.



# Cell-signaling network

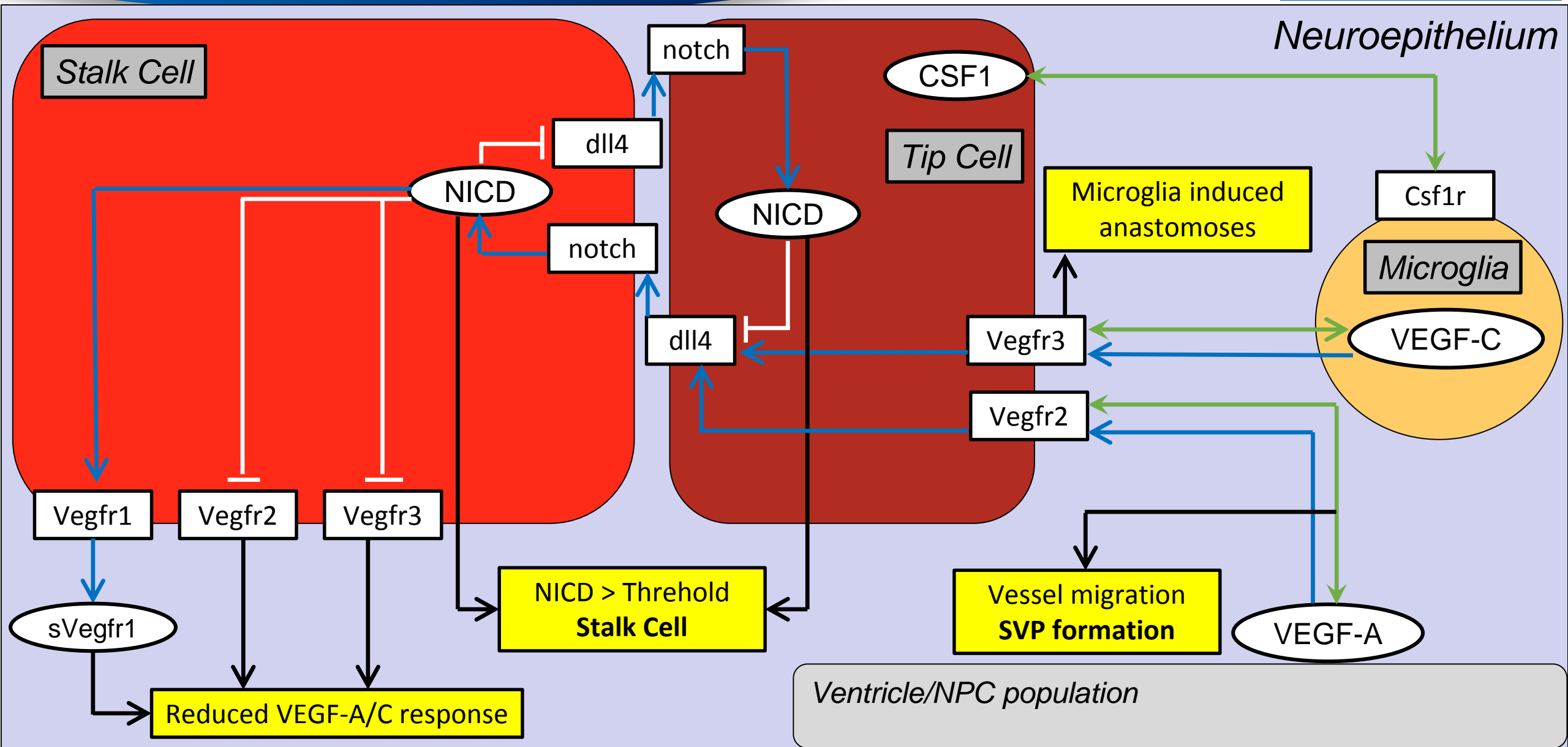
Cell type

Ligand

Chemotaxis  
Agonist  
Antagonist

Phenotype

Receptor



# Tip/Stalk Cell Selection

Cell type

Ligand

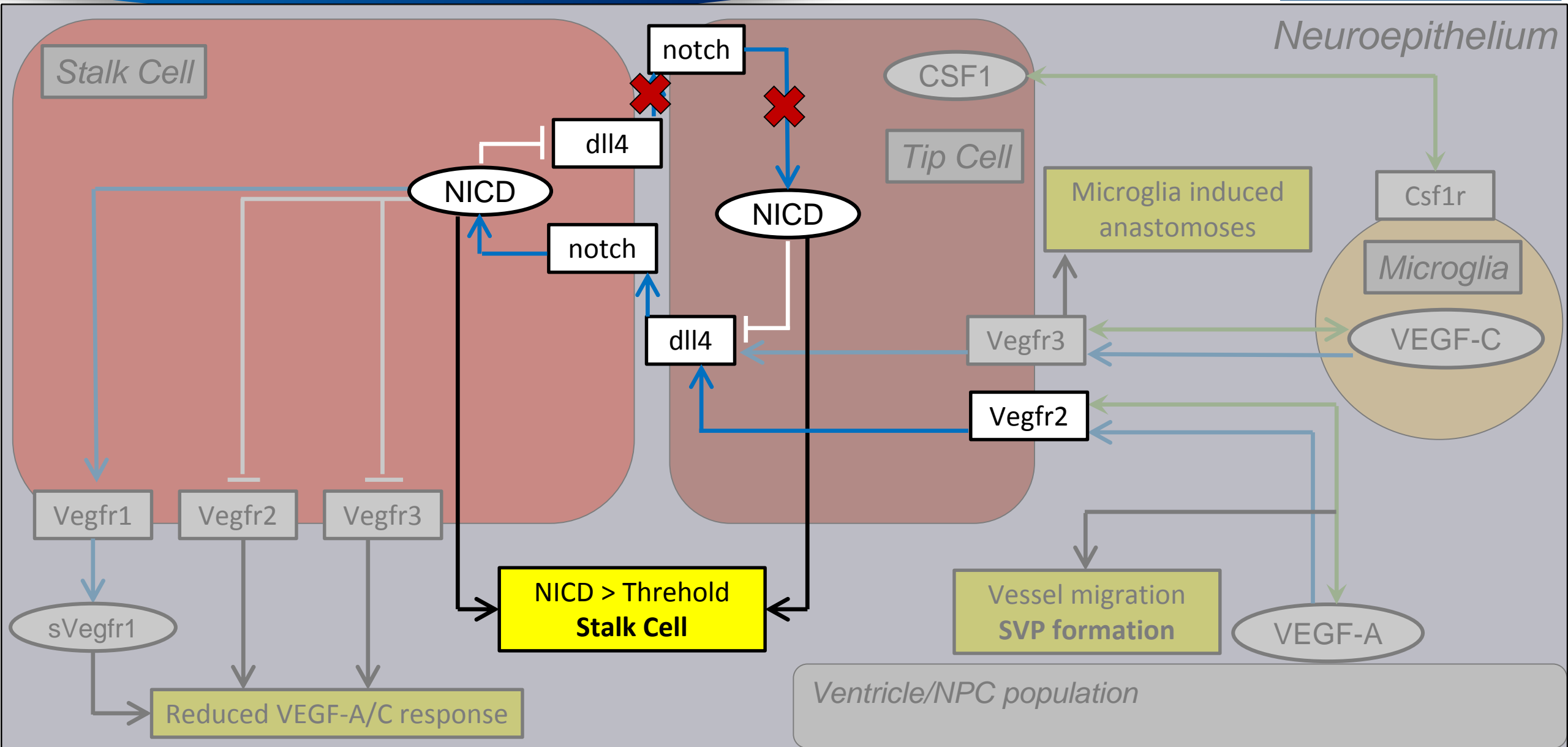
Chemotaxis

Agonist

Antagonist

Phenotype

Receptor



# Cortical Angiogenesis

Cell type

Ligand

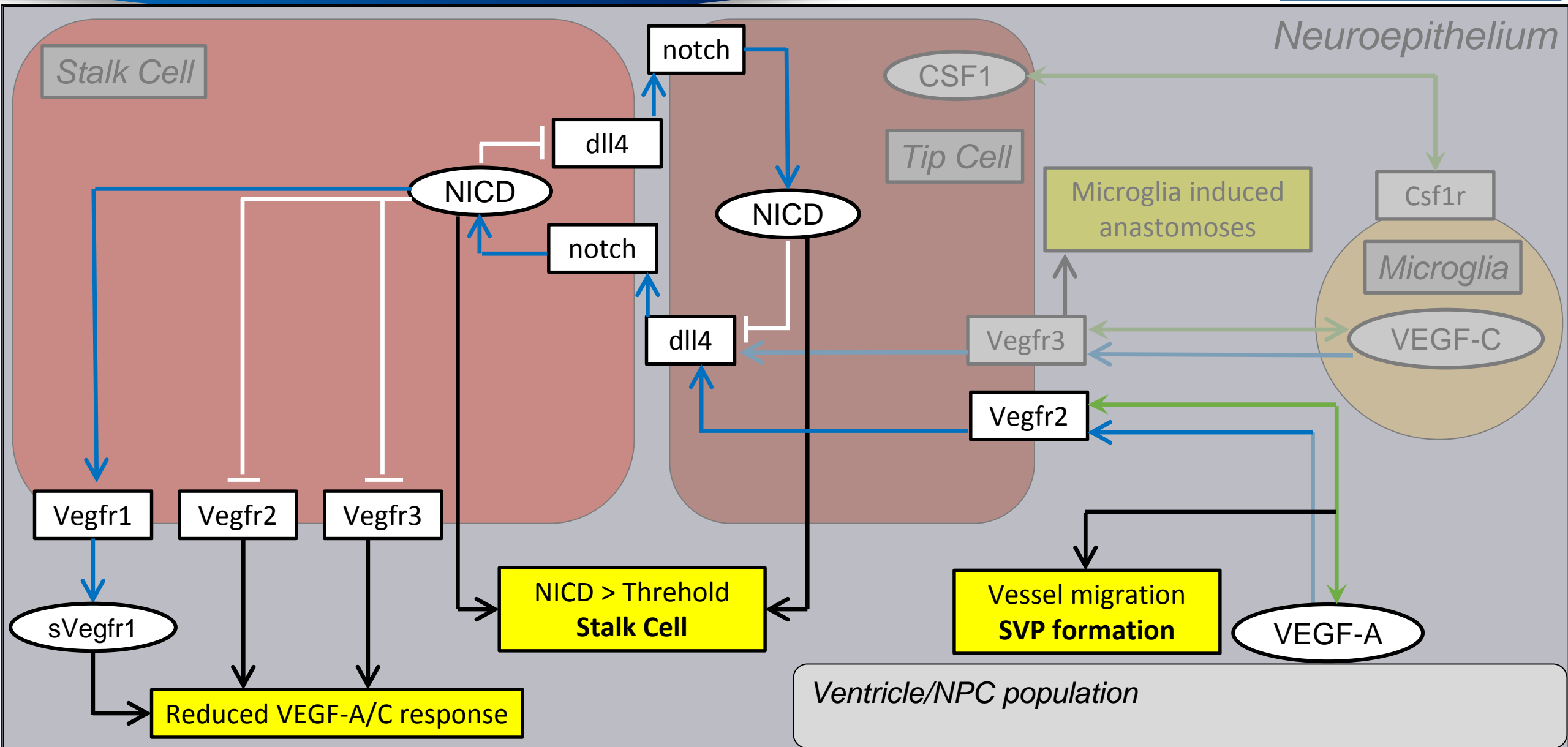
Chemotaxis

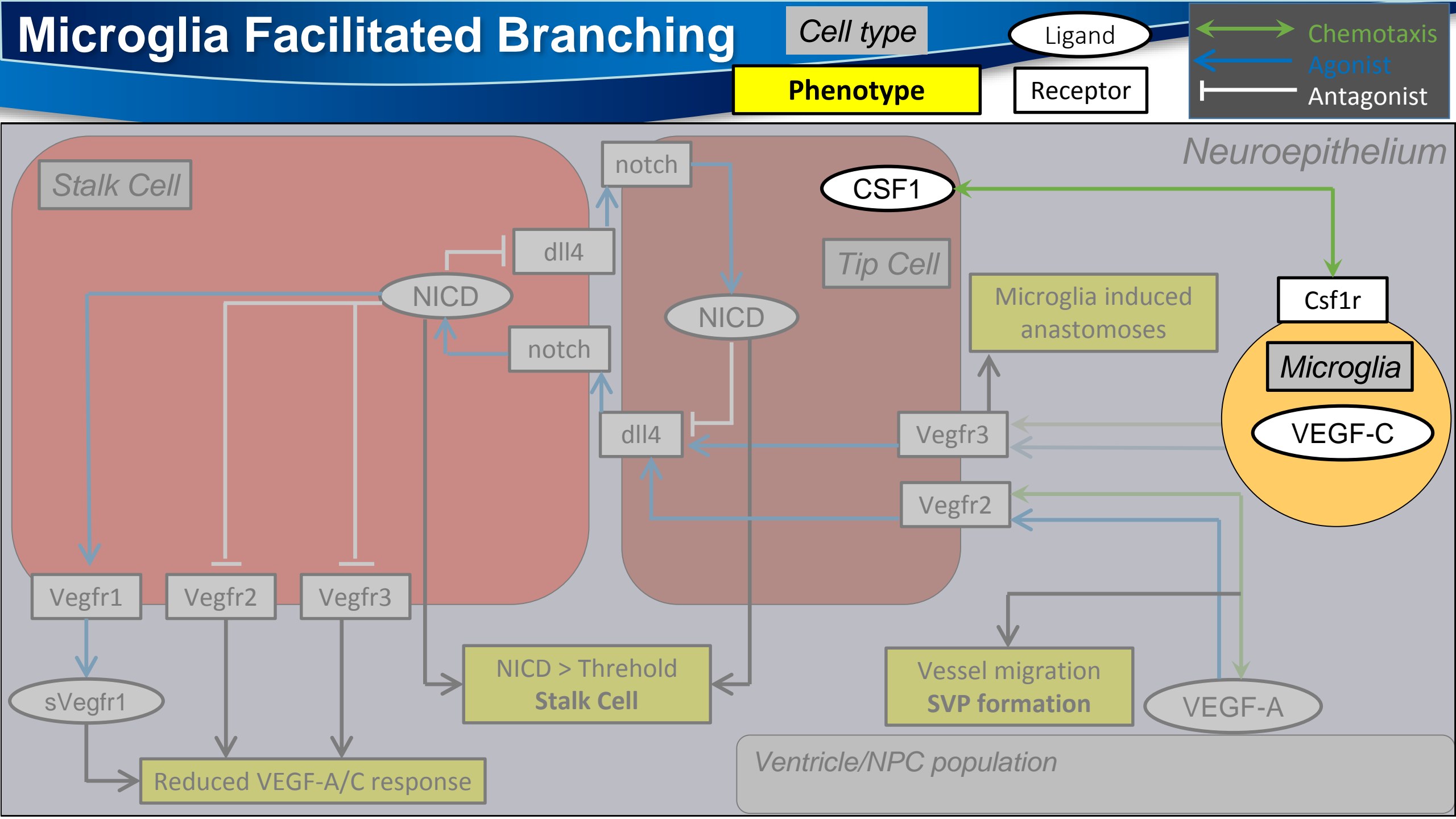
Agonist

Antagonist

Phenotype

Receptor

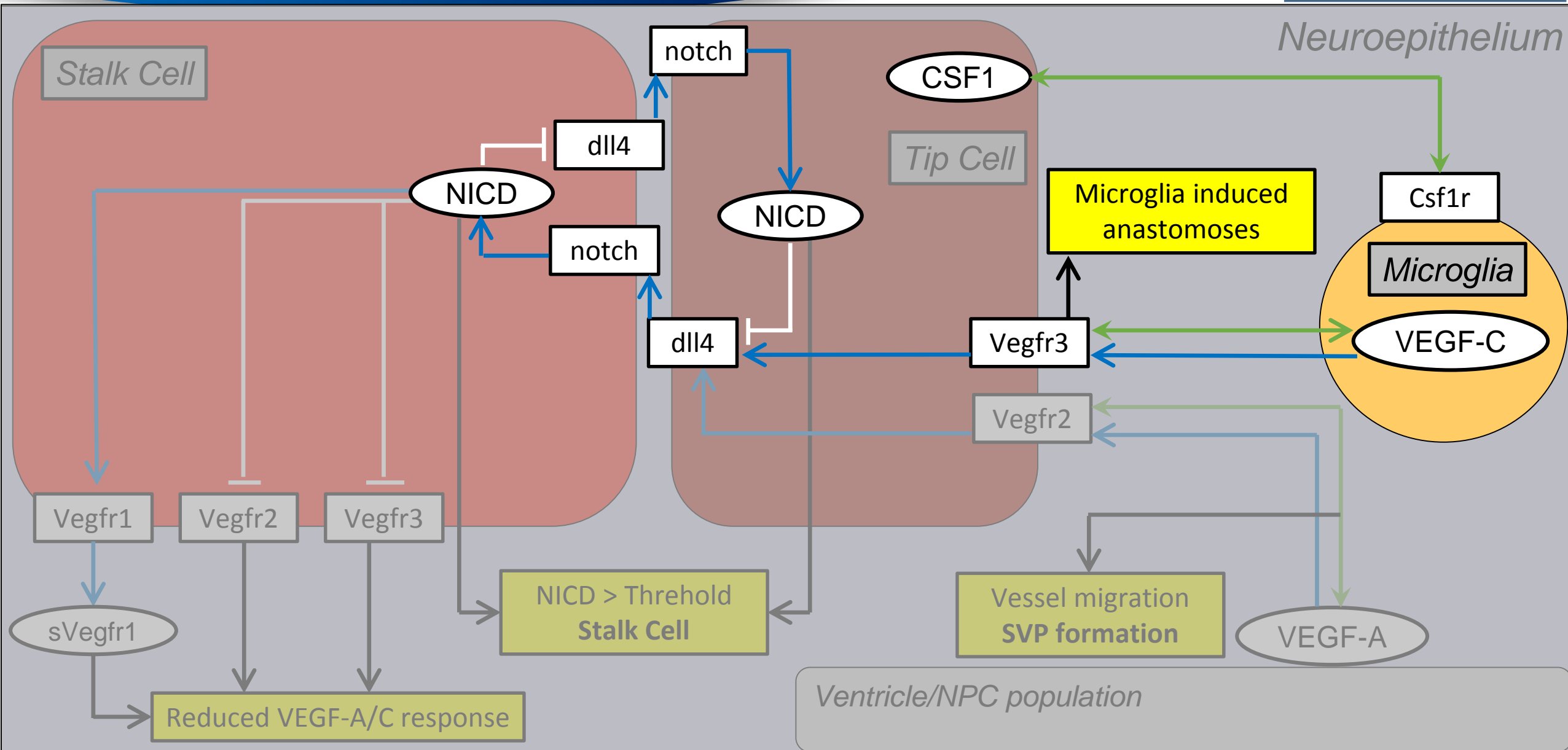




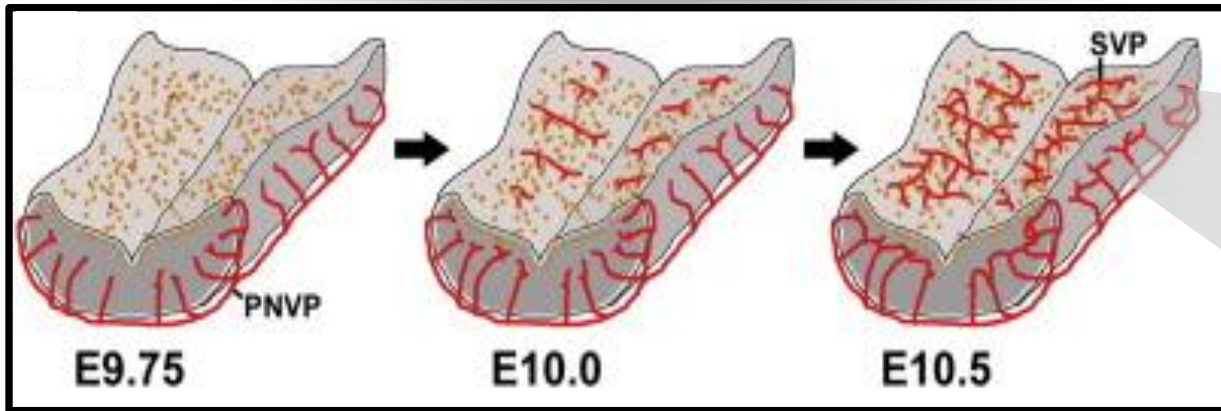


# Chemotaxis

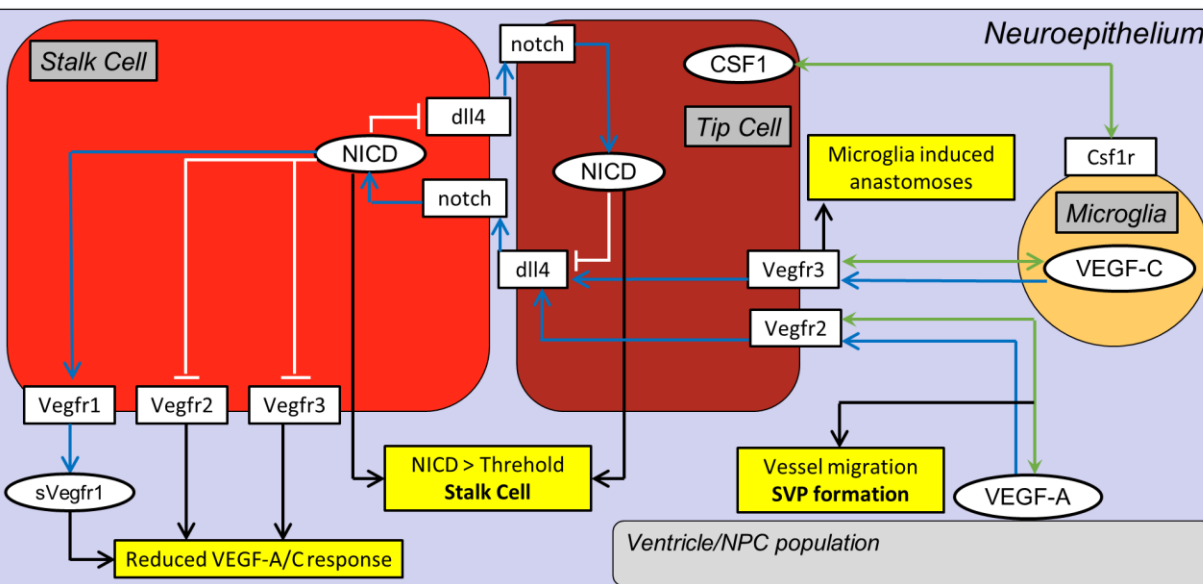
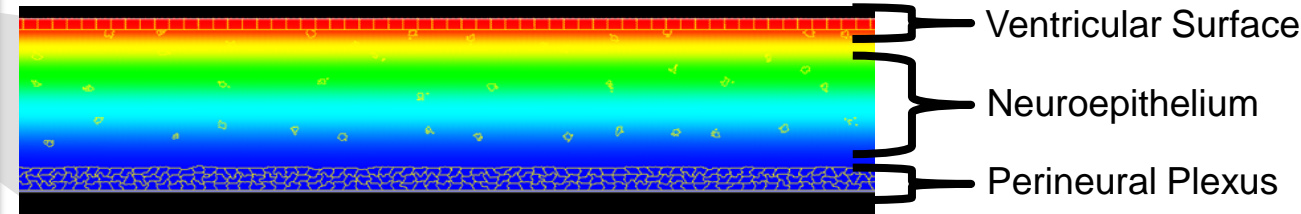
# Neuroepithelium



# Modeling Brain Angiogenesis

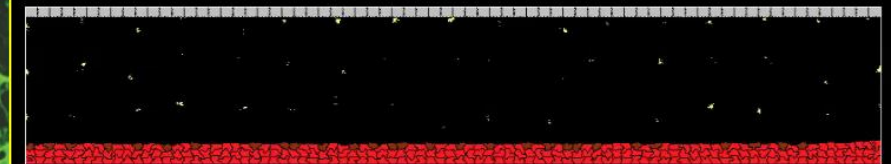
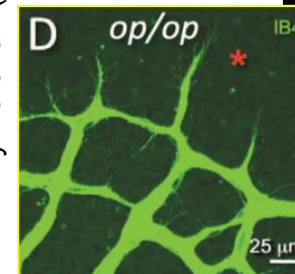
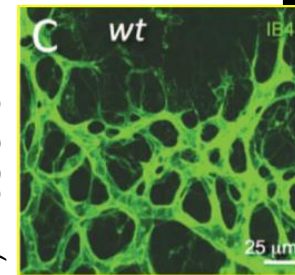


*VEGF-A gradient: NPCs in the subventricular zone*

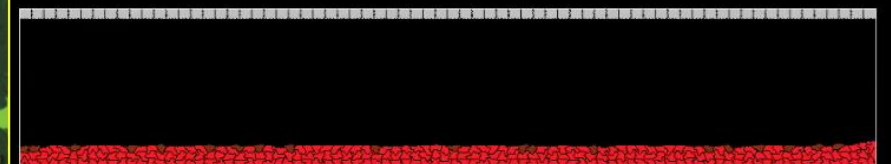


Cell agent Based model of microglia-endothelial interaction

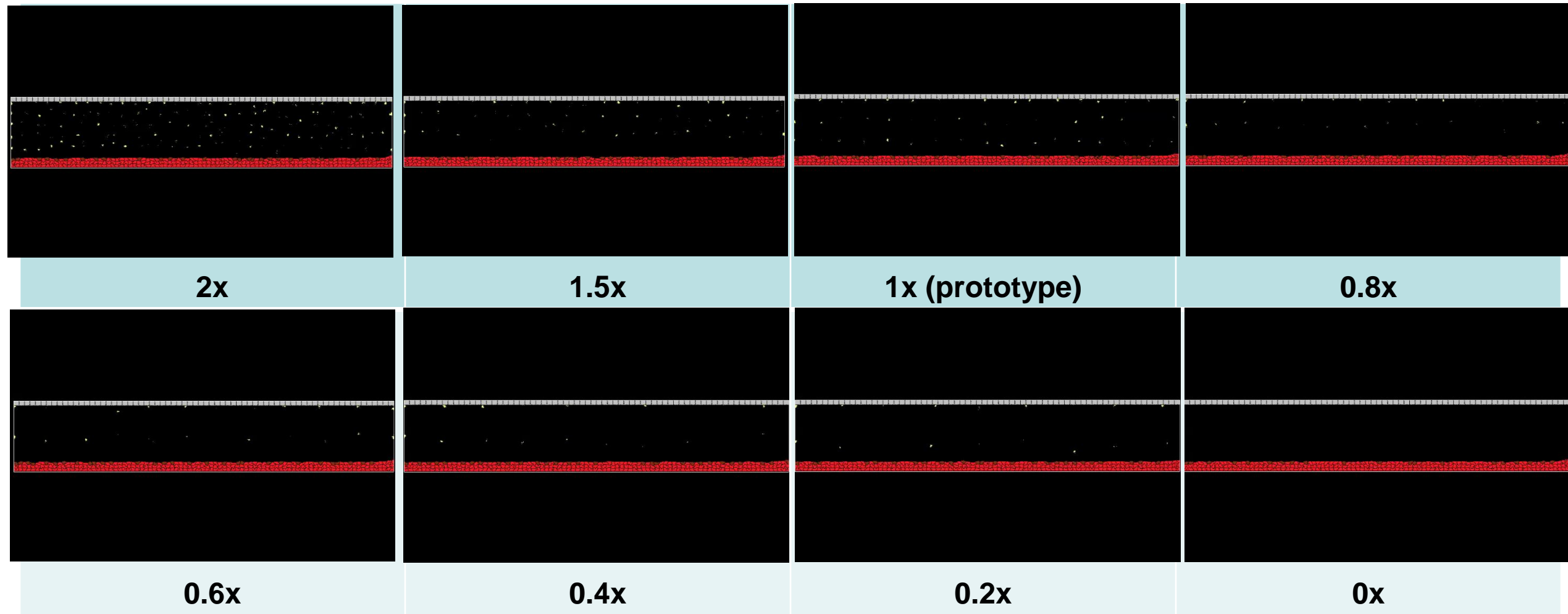
Rymo et al. (2011) PLoS One



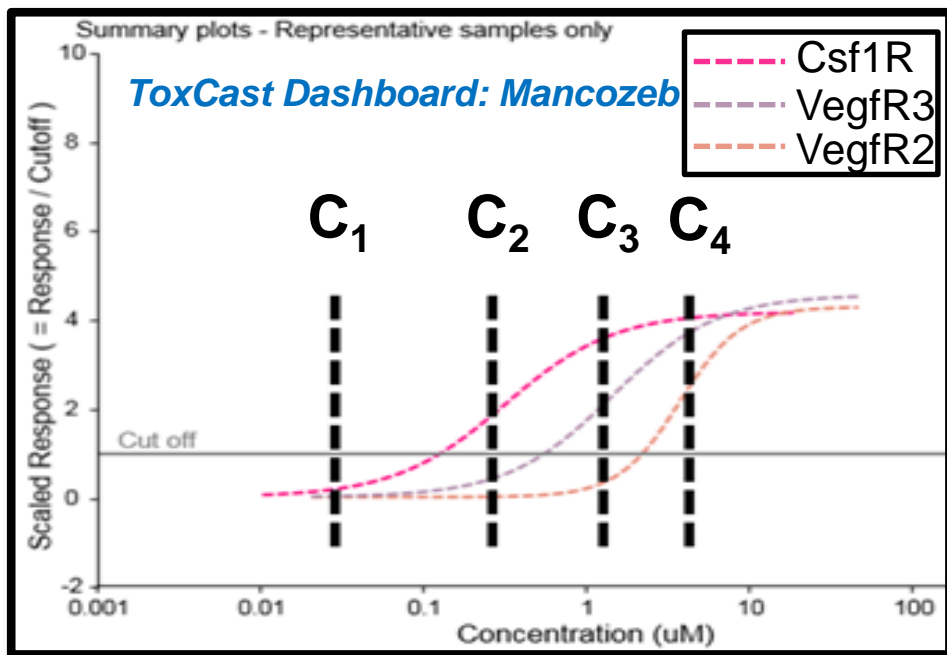
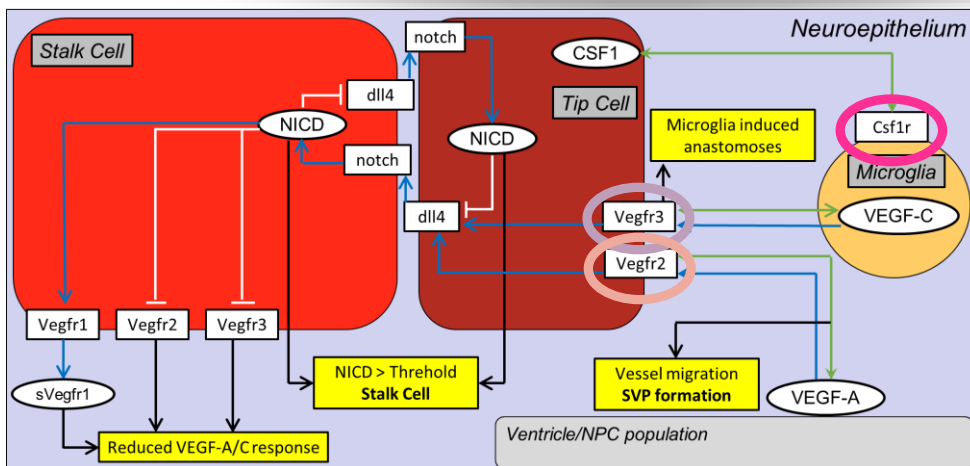
- endothelial tip cell
- endothelial stalk cell
- microglial cell



# Qualitative response: microglia abundance



# Translating HTS Data



**0.03  $\mu$ M**

No significant  
reduction in any  
receptor

**0.3  $\mu$ M**

50%  $\downarrow$  CSF1R

**2  $\mu$ M**

50%  $\downarrow$  VEGFR3  
80%  $\downarrow$  CSF1R

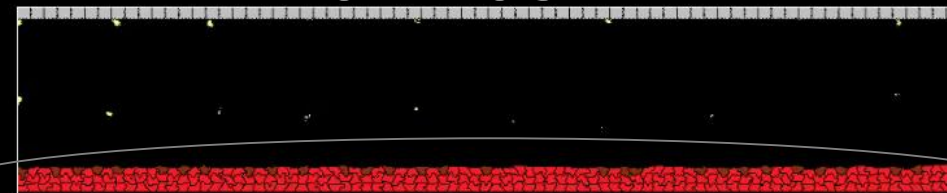
**6  $\mu$ M**

50%  $\downarrow$  VEGFR2  
85%  $\downarrow$  VEGFR3  
95%  $\downarrow$  CSF1R

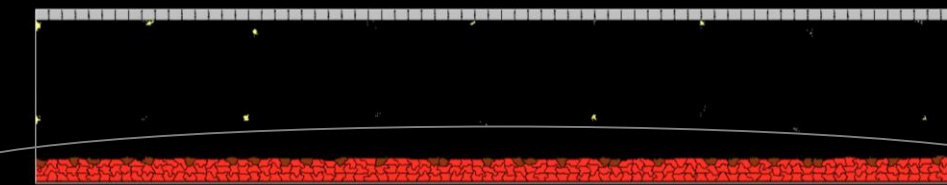
**C1 pNEL**



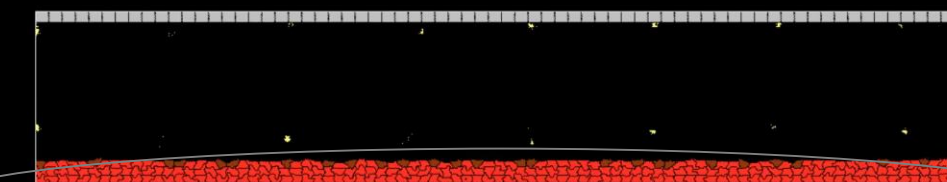
**C2  $\downarrow$  CSF1R**



**C3  $\downarrow$  VEGFR3**



**C4  $\downarrow$  VEGFR2**



# Experimental validation

## HTS Cell-based Assays

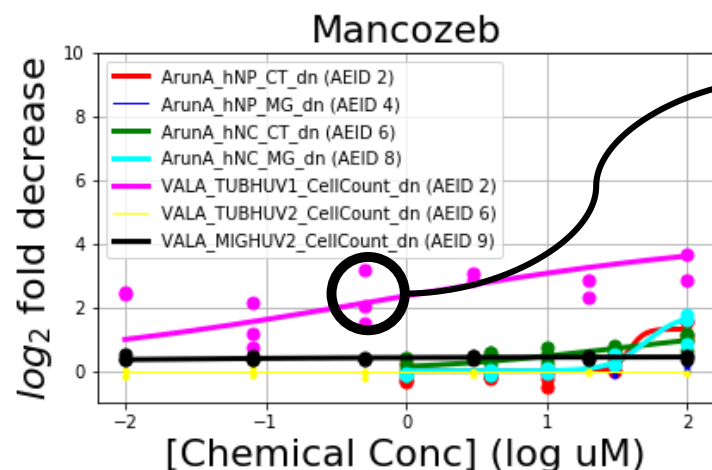
ArunA:

Migration/Proliferation  
hNP/hNC/hNN cells

VALA:

Tubulogenesis/Proliferation  
HUVEC cells

## Cell-based assay to validate model

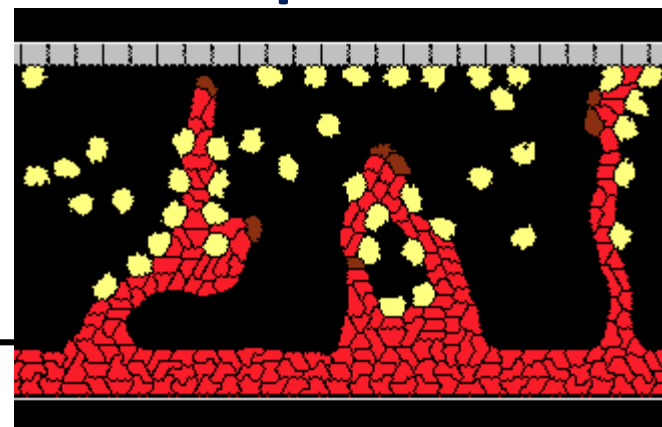


0.6  $\mu$ M

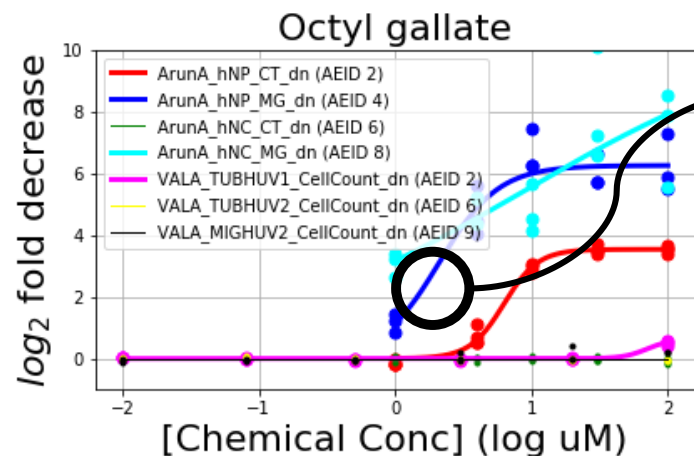
0.3  $\mu$ M

Decreased endothelial viability

pLEL



## Cell-based assay to inform model



2.12  $\mu$ M

?  $\mu$ M

Decreased neurogenic endpoints

ABM including  
neurogenic  
features

Develop corresponding  
control network with  
ToxCast assays to describe  
neurogenic component of  
NVU

Wednesday August 23, 4:15pm  
Best practices for Modeling Data

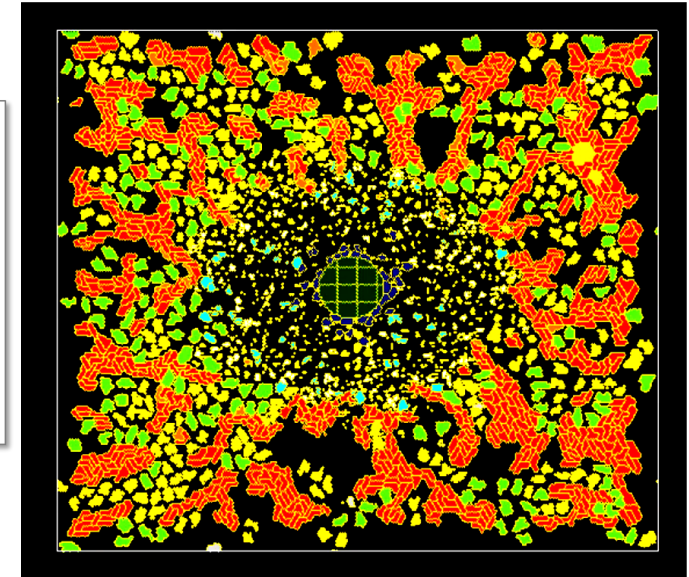
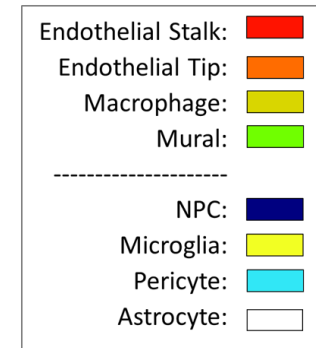


# Towards a functional cNVU model

- Biological pathway perturbations
  - ‘Cybermorphs’ for investigating single pathway knockouts
  - Continuum response following chemical exposure

- Neurogenesis submodel

- Proliferation of radial glia cells (neuroprogenitor cells)
- Differentiation/migration to neurons and astrocytes
- Utilize signaling pathways from microcephaly AOP
- Endothelial network interacting with neural network (3D)



Initial model comprising NVU cell types

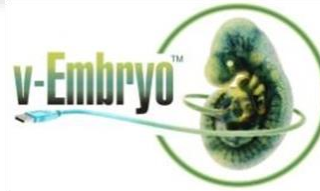
- Phenotype quantitation

- Microglia abundance, vessel branch points, network complexity (cortical angiogenesis)
- Neuron proliferation/differentiation (neurogenesis)
- Barrier permeation for chemical distribution to neural compartment (barriergenesis)



# Acknowledgements

- Tom Knudsen (mentor)
- Kate Saili (NCCT)
- Sid Hunter (NHEERL-ISTD)
- Andrew Schwab (NHEERL-ISTD)
- Nancy Baker (Leidos)
- Richard Spencer (ARA-EMVL)
- Florent Ginhoux (A\*STAR)
- Aymeric Silvan (A\*STAR)
- Virtual Tissue Modeling





# Thank You

## Questions?