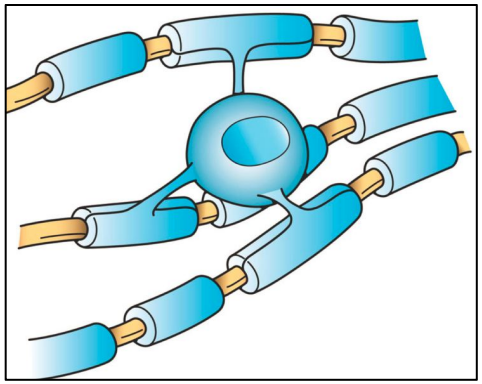
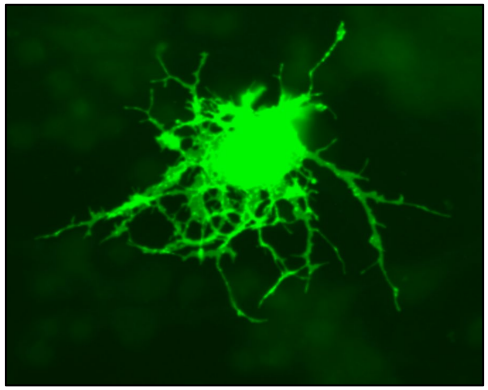
Oligodendrocyte precursors migrate along vasculature in the developing nervous system

章栩

2016/3/3





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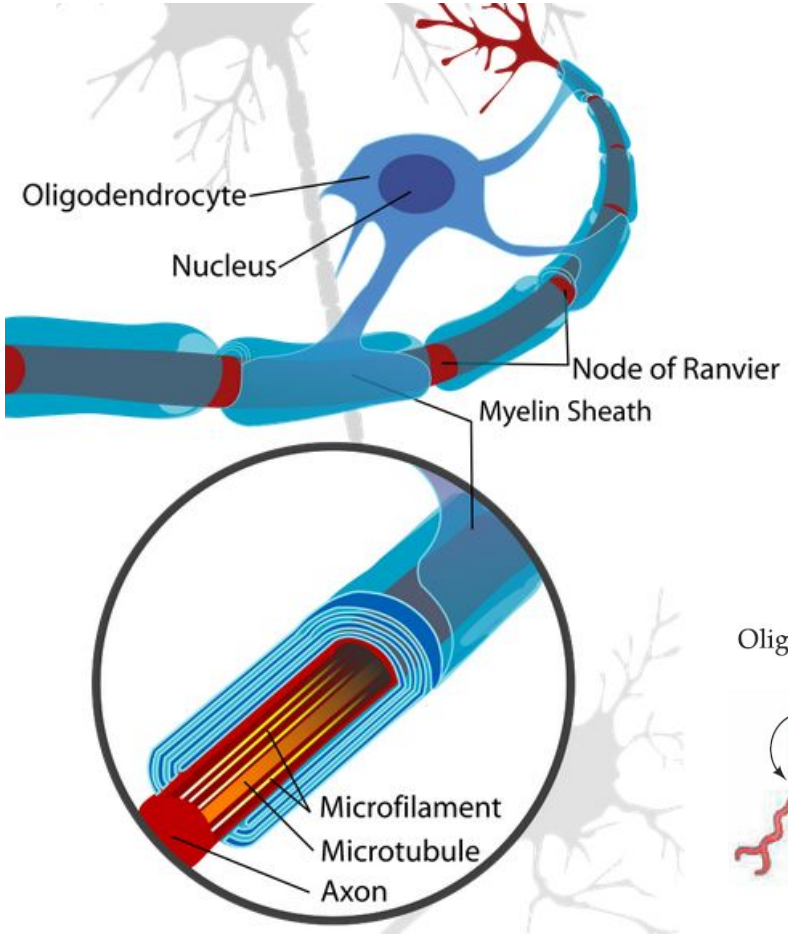
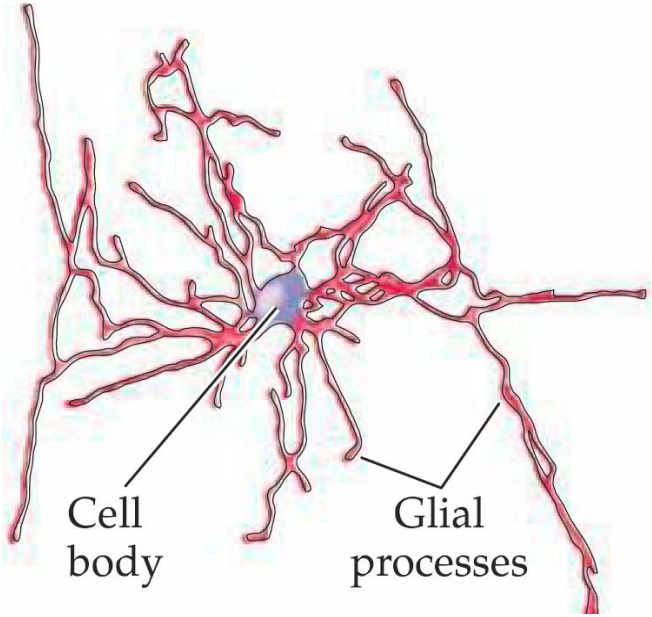
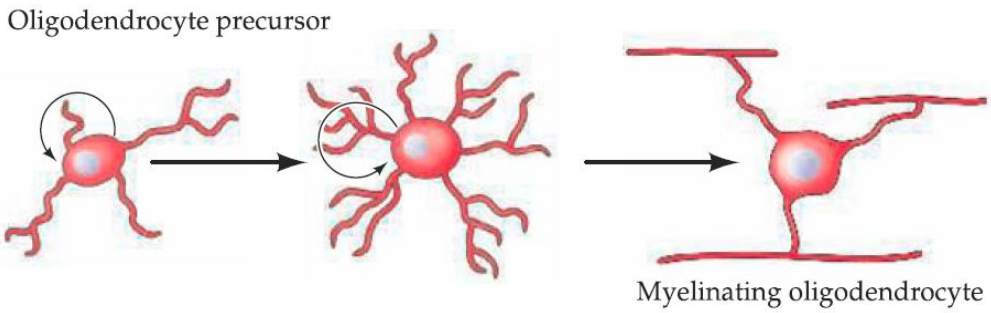
Background

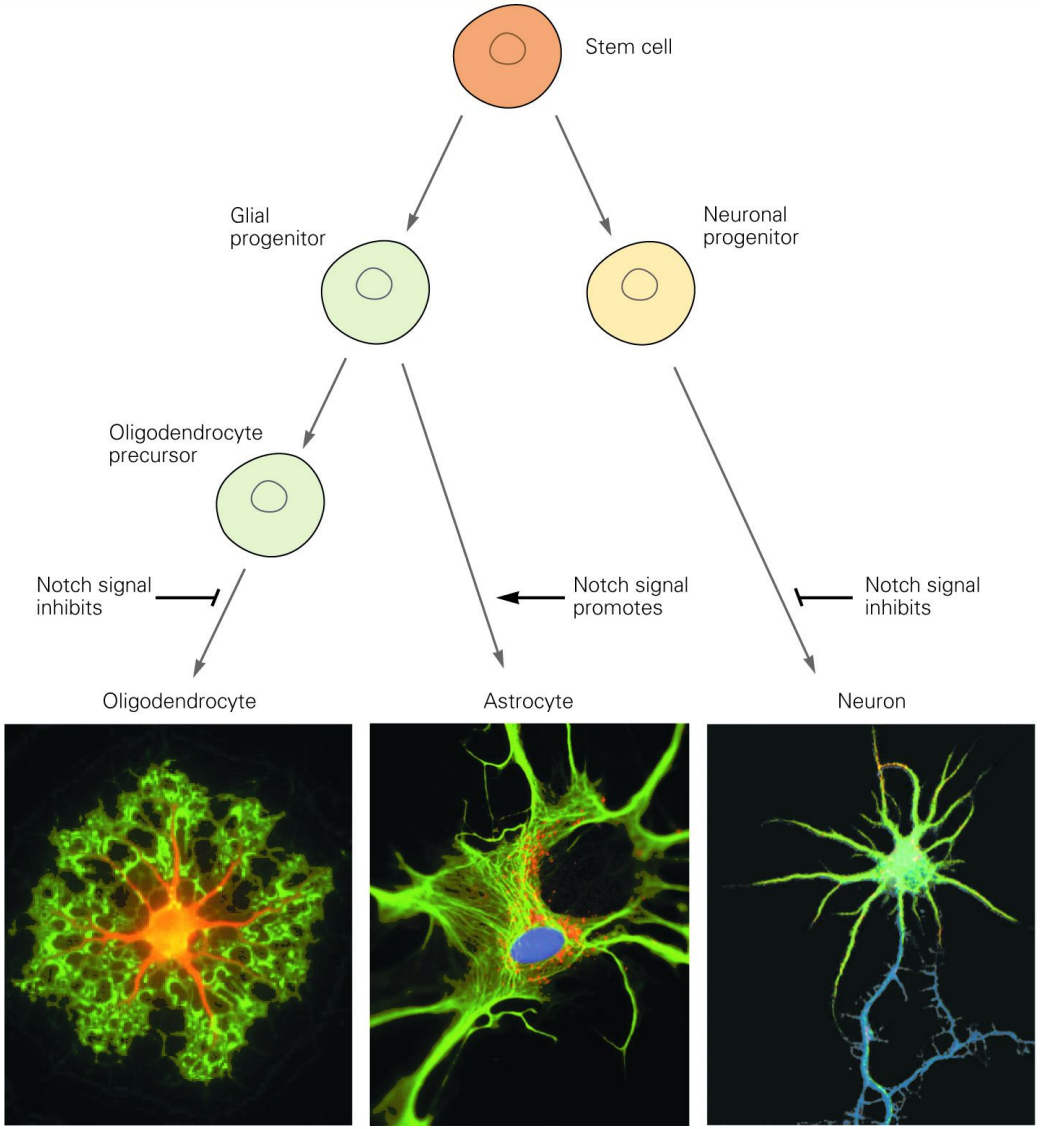
- A brief review of oligodendrocytes



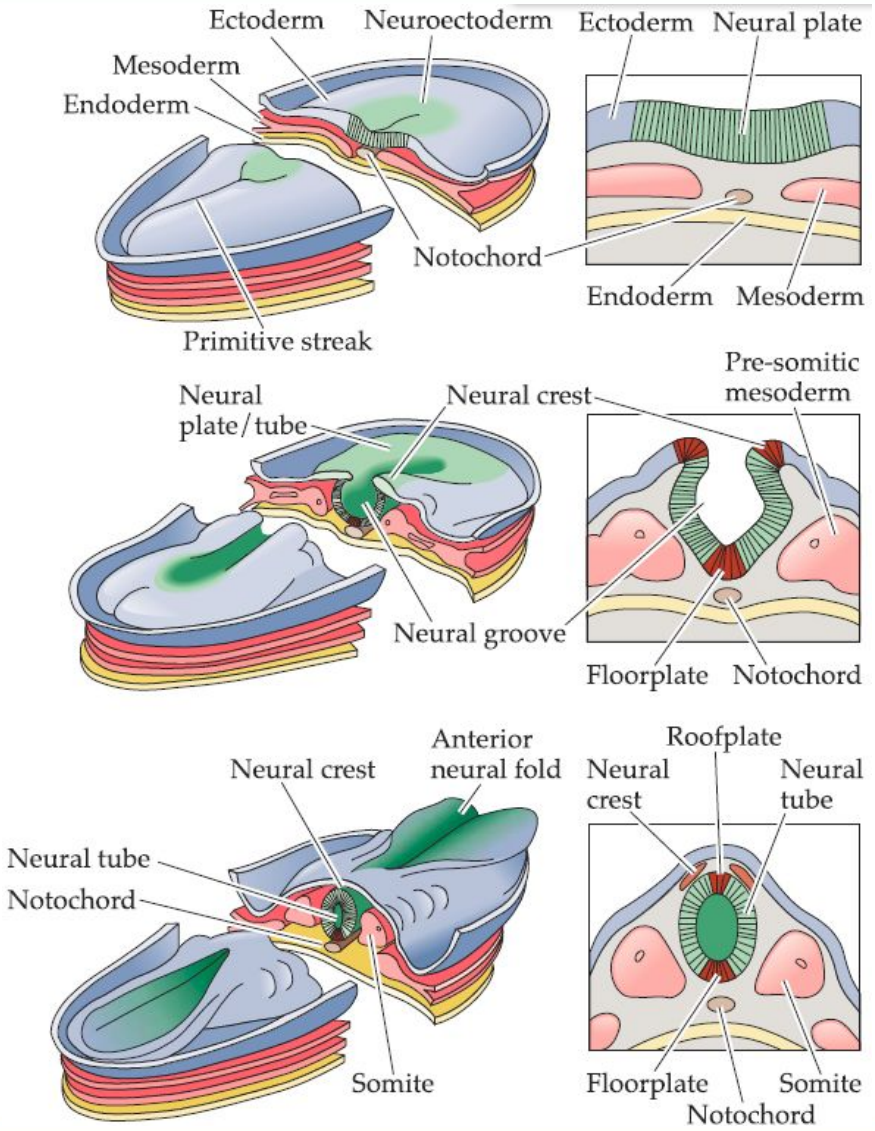




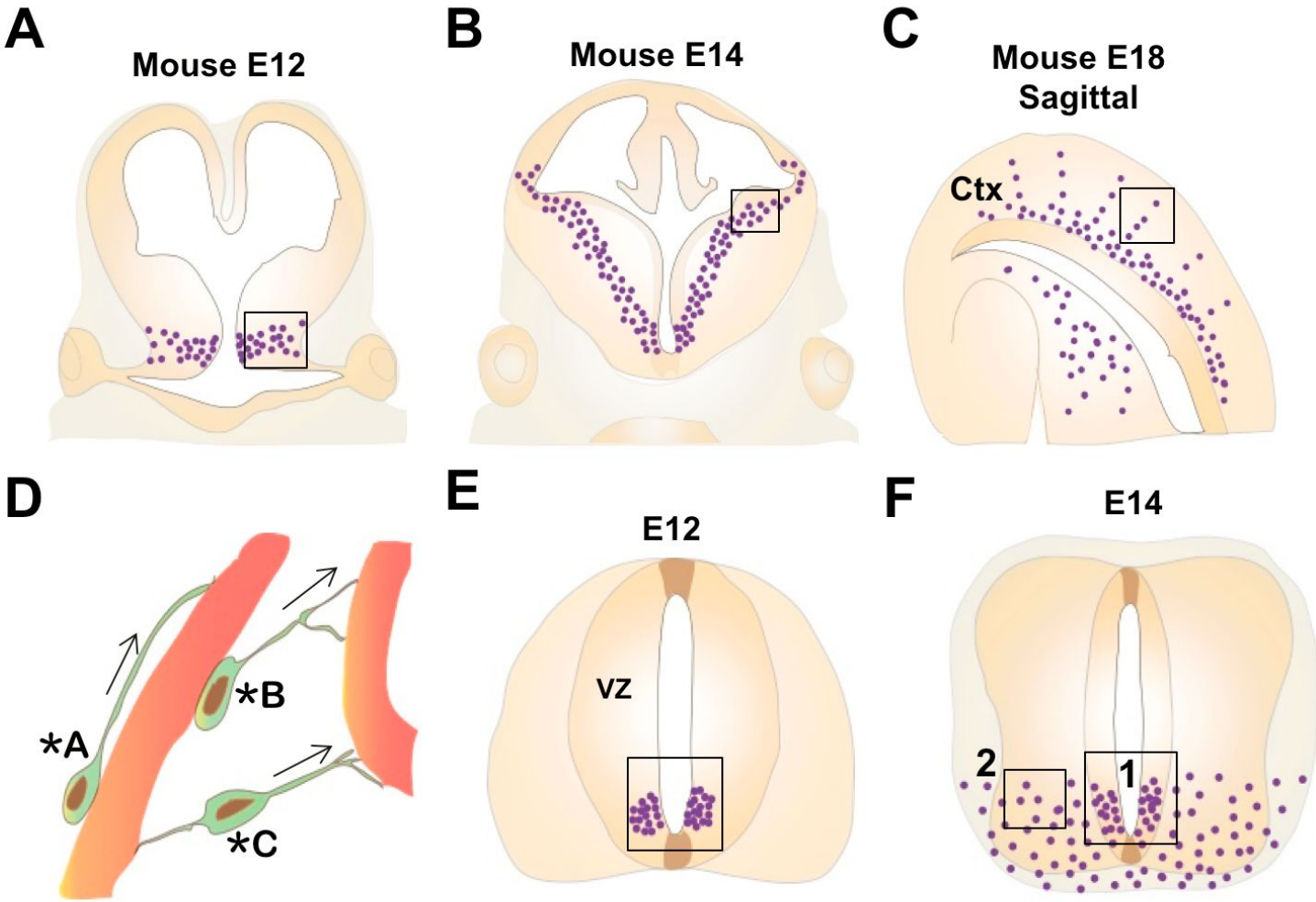
Background


Stem cell
Glial progenitor
Neuronal progenitor
Oligodendrocyte precursor
Notch signal inhibits
Notch signal promotes
Notch signal inhibits
Oligodendrocyte
Astrocyte
Neuron

Background


Ectoderm
Neuroectoderm
Ectoderm
Neural plate
Mesoderm
Endoderm
Notochord
Endoderm
Mesoderm
Primitive streak
Neural plate/tube
Neural crest
Pre-somitic mesoderm
Neural groove
Floorplate
Notochord
Anterior neural fold
Roofplate
Neural crest
Neural crest
Neural tube
Neural tube
Notochord
Somite
Floorplate
Somite
Notochord

Background


A
Mouse E12
B
Mouse E14
C
Mouse E18
Sagittal
Ctx
D
*A
*B
*C
E
E12
VZ
F
E14
2
1

Background

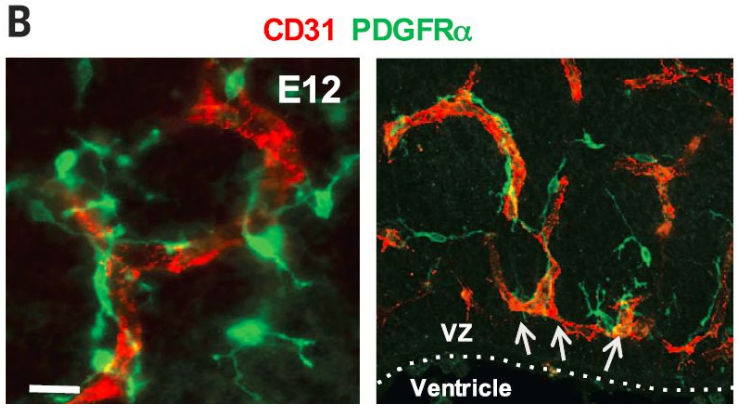
- This study tries to uncover
  - The substrate for OPC migration
  - Mechanisms directing OPC migration during development

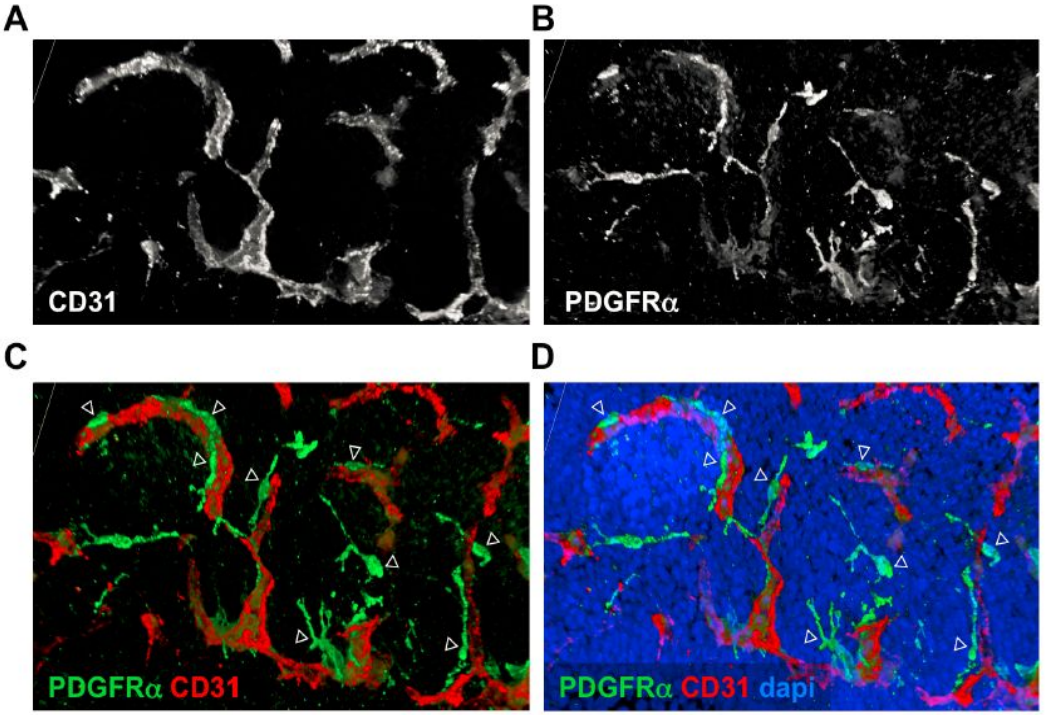
Experiment 1

- Aim:
  - Find the physical substrate for OPC migration
- Principles and methods in brief:
  - Immunostaining/Immunohistochemistry
  - Migratory OPCs express PDGFRα (labeled in green)
  - Vascular endothelial cell contain CD31 (labeled in red)

Experiment 1

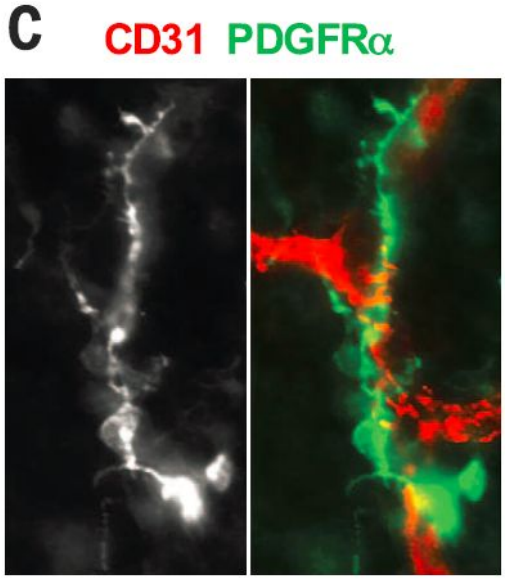
- Results:

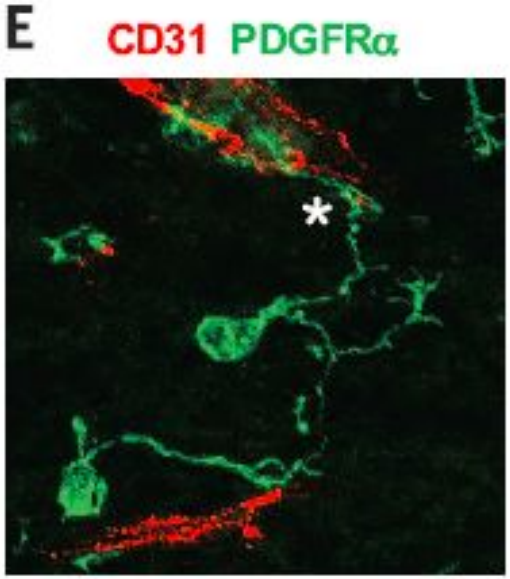


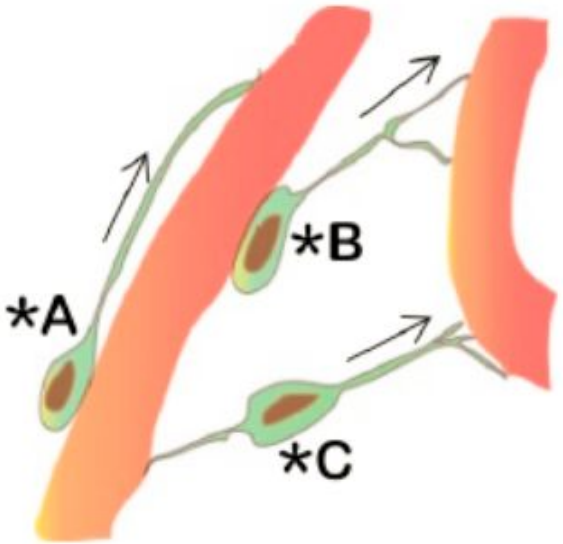


Experiment 1

- Results:
  - Many of these migratory OPCs are elongated along blood vessels, with their cell bodies directly on the abluminal endothelial surface and a single, long leading process along the vessel

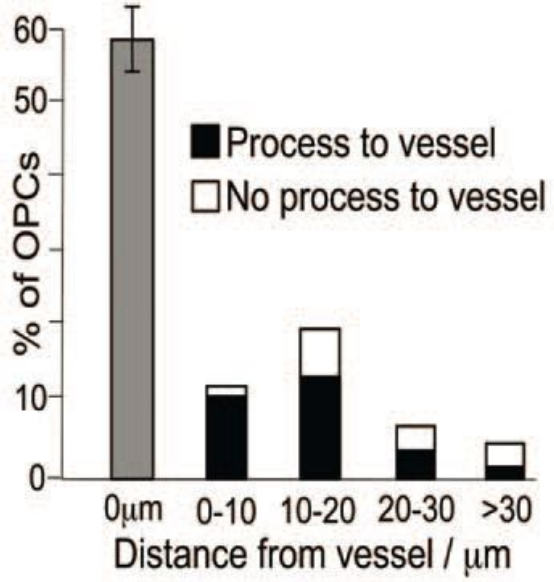






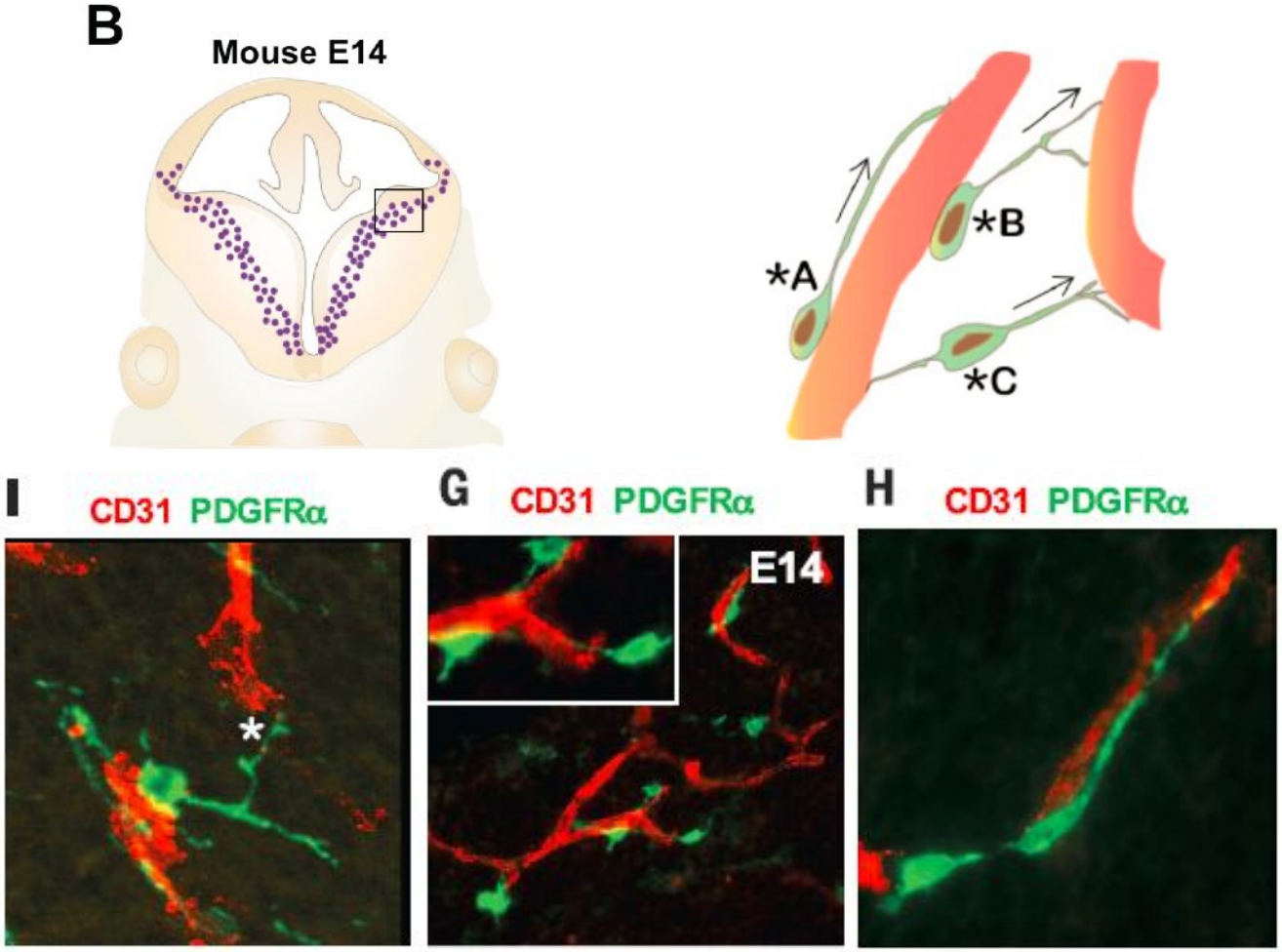
Experiment 1

- Results:
  - 58% (±4.4%) of OPCs have their cell bodies directly on a vessel wall
  - Of the remainder, 67% (±8.9%) display at least one observable process that engages a vessel



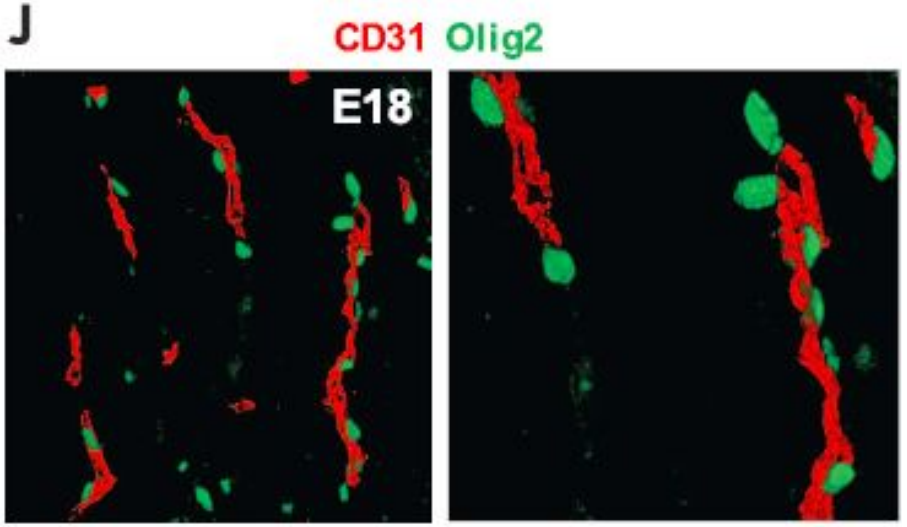
Experiment 1

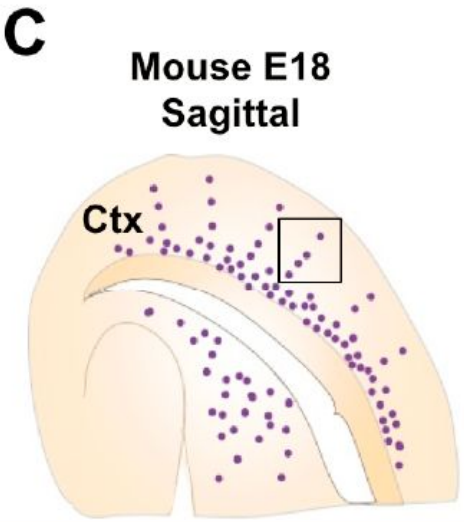
- Results:



Experiment 1

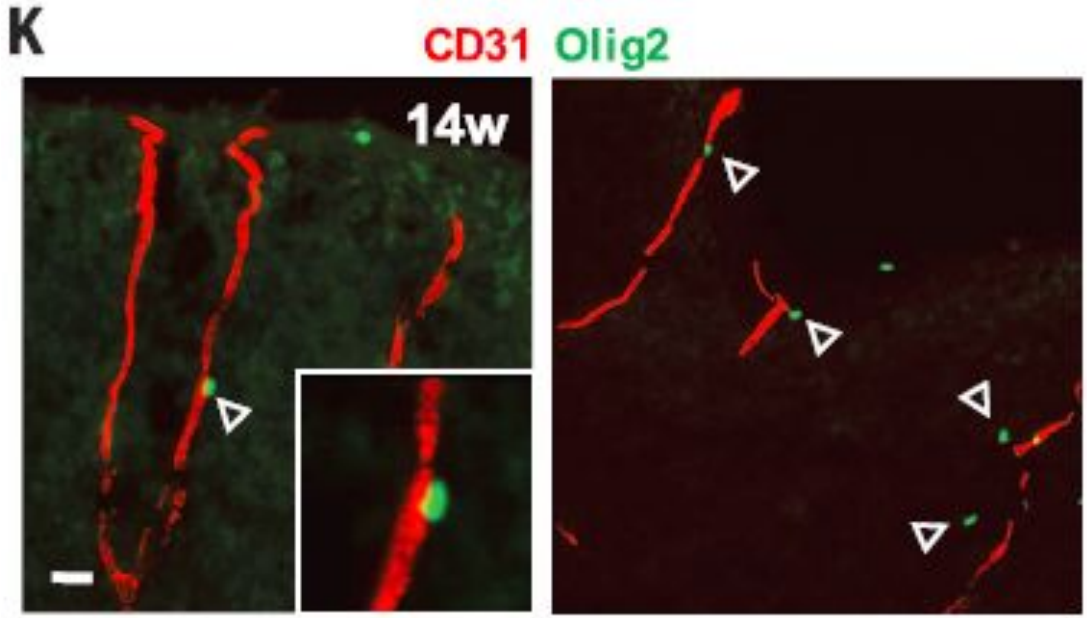
- Results:
  - The number of OPCs in the mouse cortex tripled between E16 and E18.
  - Olig2+ cells migrating from deep to superficial cortical layers palisade along the vasculature that penetrates the cortex at E18.





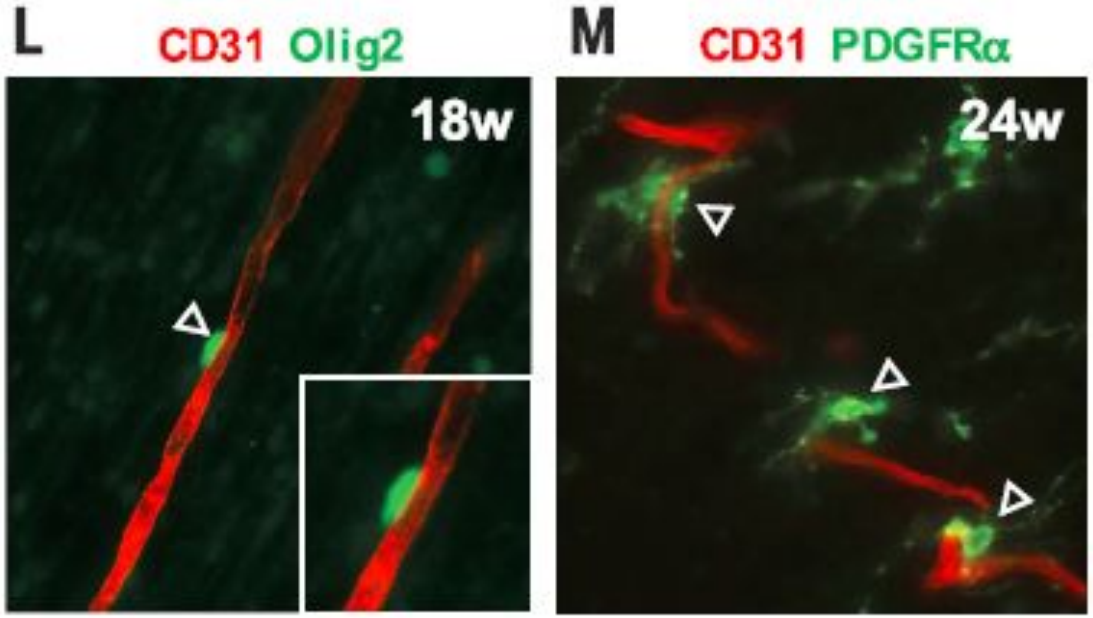
Experiment 1

- Supplementary experiment:
  Observations on the human cortex showed similar results
  - The first Olig2-expressing cells to arrive in the human outer cortex at gestational week 14 appose penetrating vessels



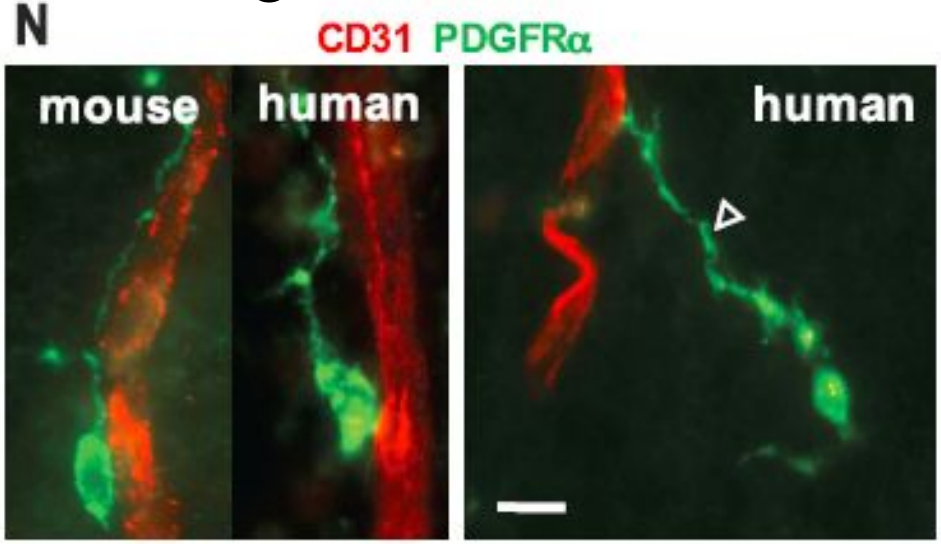
Experiment 1

- Supplementary experiment:
  Observations on the human cortex showed similar results
  - Association of Olig2+ and PDGFRα+ OPCs with blood vessels remains evident at gestational weeks 18 and 24



Experiment 1

- Supplementary experiment:
  Observations on the human cortex showed similar results
  - Migrating human OPCs, expressing PDGFRα, are morphologically similar to those of mice in that they extend single leading processes in the direction of movement along and toward vessels



Experiment 1

- Conclusion:
  - Modes of migration across mammalian species are common
- Comments on Experiment 1:
  - Revealed the intimate relationship between OPCs and the vasculature through immunostaining
  - Behaviors of OPCs on vessels remained unknown
  - Comparison with human cortex may be too early
  - Really necessary?

Experiment 2

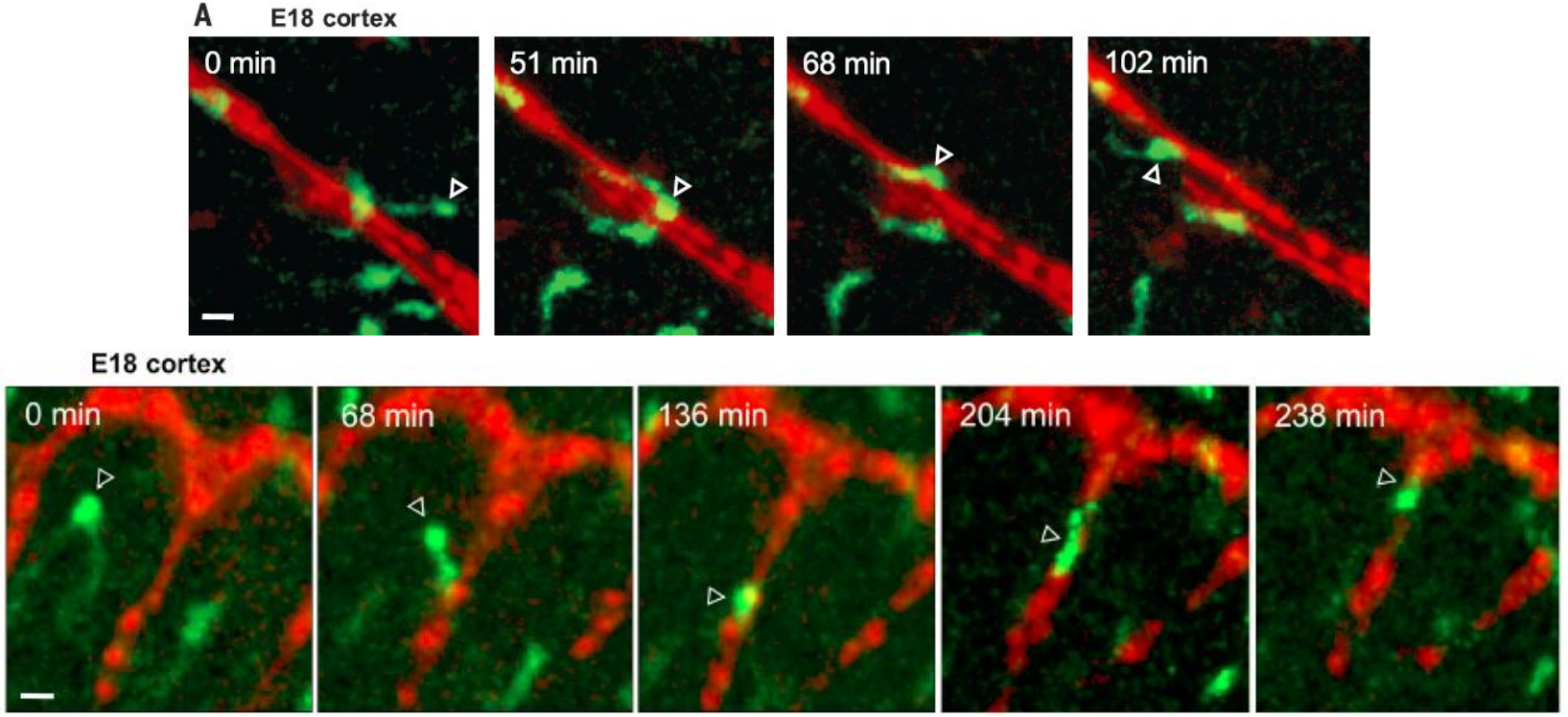
- Aim:
  - Live-image OPC migration to find its behaviors
- Principles and methods in brief:
  - Olig2-GFP reporter mouse intracardiacally infused with rhodamine-lectin for real-time observation
  - Select brain slices to observe regions with actively migrating OPCs

Experiment 2

- Results:
  - Two behaviors of OPCs were observed during migration on vessels: crawling & jumping

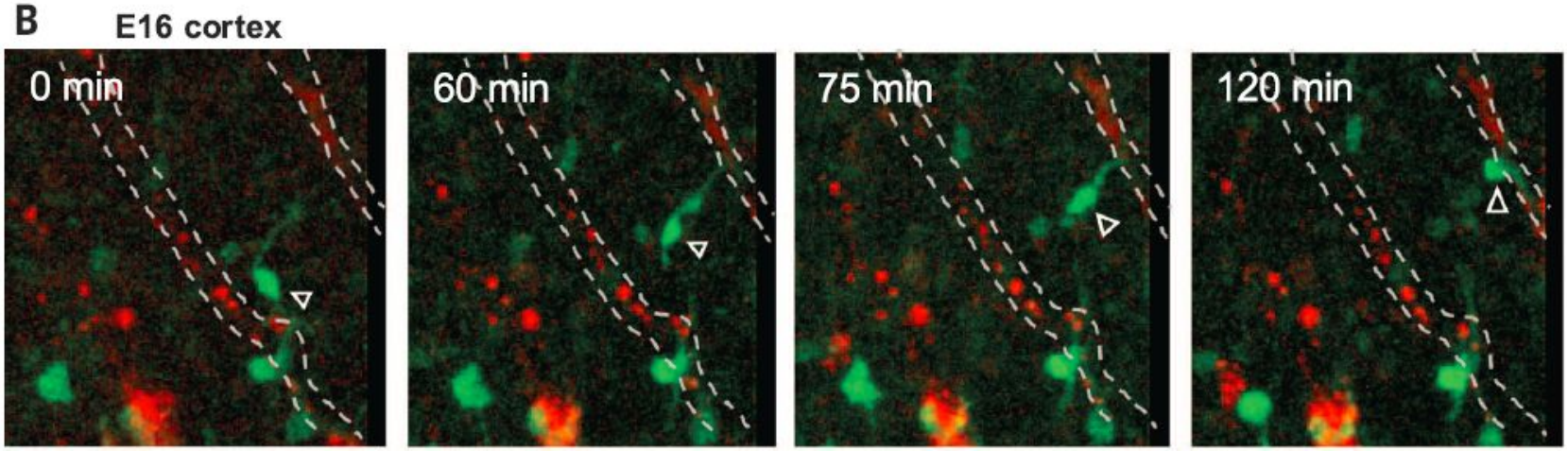
Experiment 2

- Results:
  - Crawling: Cell body maintaining contact with the abluminal endothelial surface



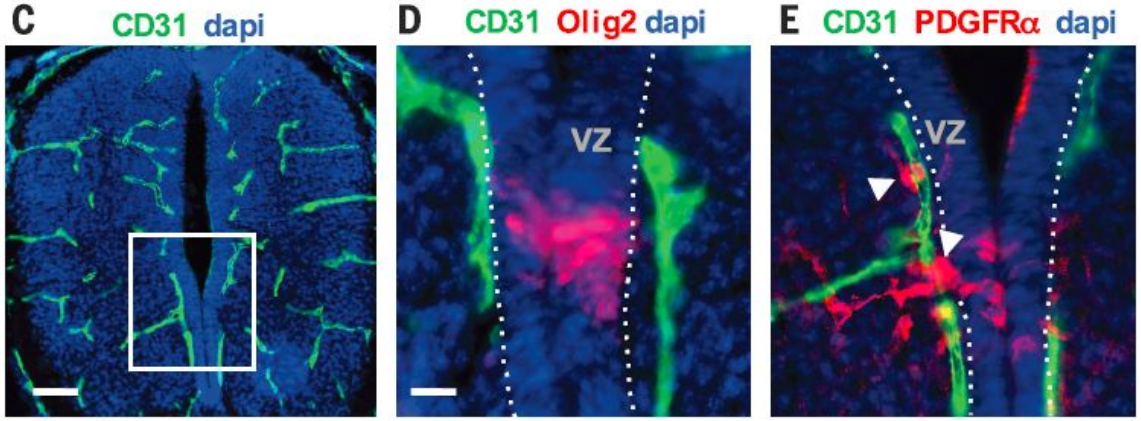
Experiment 2

- Results:
  - Jumping: OPC extending a leading process from one vessel toward another, followed by translocation of the cell body to make contact with the new vessel



Experiment 2

- Results:
  - Jumping is more rapid than crawling, presumably entailing fewer physical contacts with the endothelial surface
  - The association of migrating OPCs with the vasculature is also found in the spinal cord and at later postnatal times when OPCs are required to migrate



Experiment 2

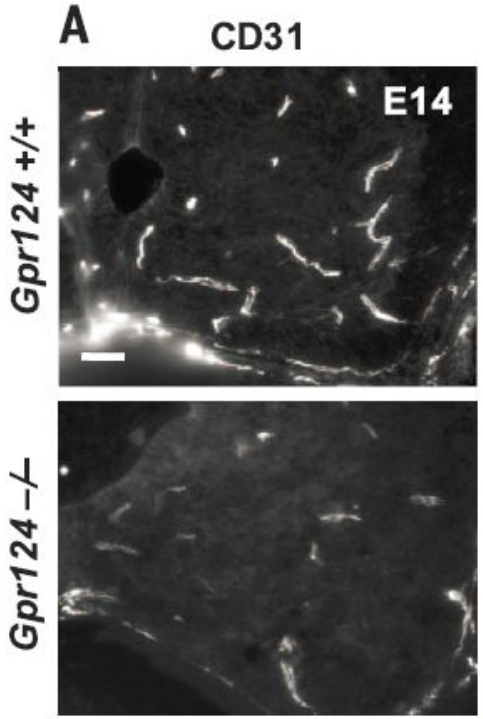
- Comments on Experiment 2
  - Revealed the dynamic behaviors of OPCs during migration on vessels
  - Requirements for such migration remained unknown
  - Could we perfrom this experiment at the very beginning?

Experiment 3

- Aim:
  - Find the requirement for OPC migration on vessels
- Principles and methods in brief:
  - Disrupt vascular development using both conventional and conditional transgenic knockout mice
  - GPR124 (expressed by endothelium and pericytes within the CNS) is essential for developmental vascular sprouting

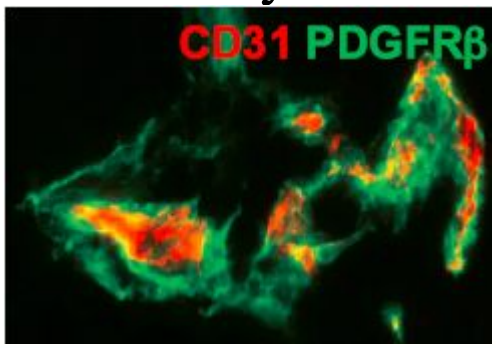
Experiment 3

- Principles and methods in brief (continued):
  - At E11, mice lacking GPR124 exhibit
    - CNS vascular patterning defects and reduced vascularization



Experiment 3

- Principles and methods in brief (continued):
  - At E11, mice lacking GPR124 exhibit
    - CNS vascular patterning defects and reduced vascularization
    - Glomeruloid vascular abnormalities
      - highly irregular, multilayered endothelial aggregates with peripheral PDGFRβ+ pericyte investment
      - lack of ventricularly directed endothelial filopodia

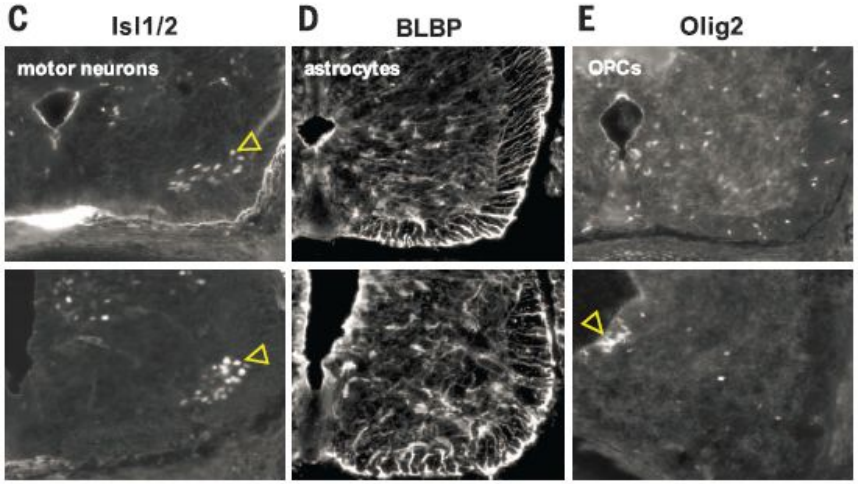


Experiment 3

- Principles and methods in brief (continued):
  - Hypothesis: OPC dispersal would be abnormal in E14 GPR124−/− embryos.

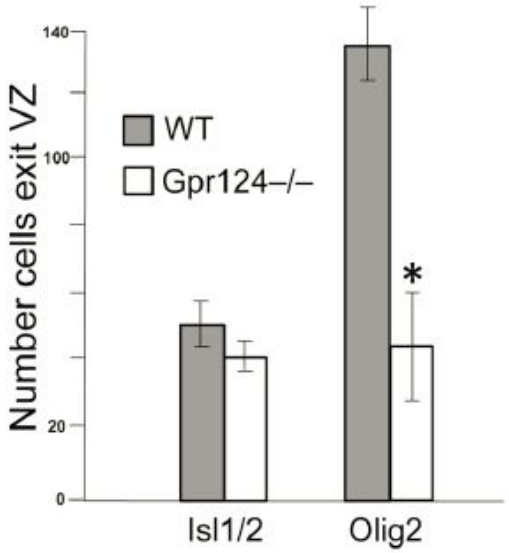
Experiment 3

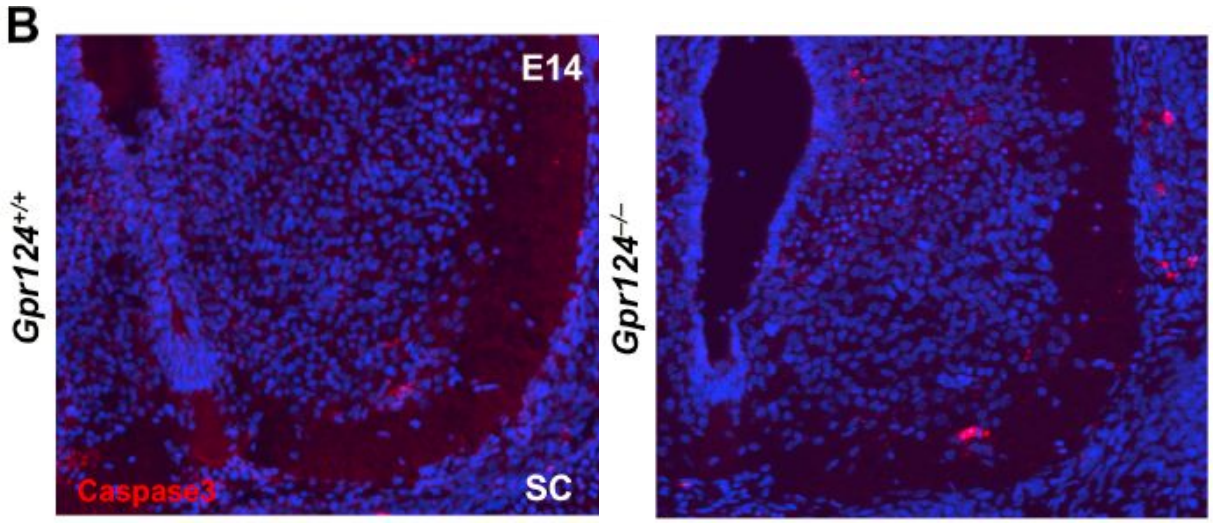
- Results:
  - Migration of Isl1/2-expressing motor neurons, Glast+ radial glial fibers and BLBP–expressing astrocytes all appeared normal
  - OPCs abnormally accumulated in the pMN and failed to egress normally from the **spinal cord** ventricular zone



Experiment 3

- Results:
  - 70% fewer OPCs dispersed into the surrounding gray matter
  - Rates of OPC cell death were unchanged



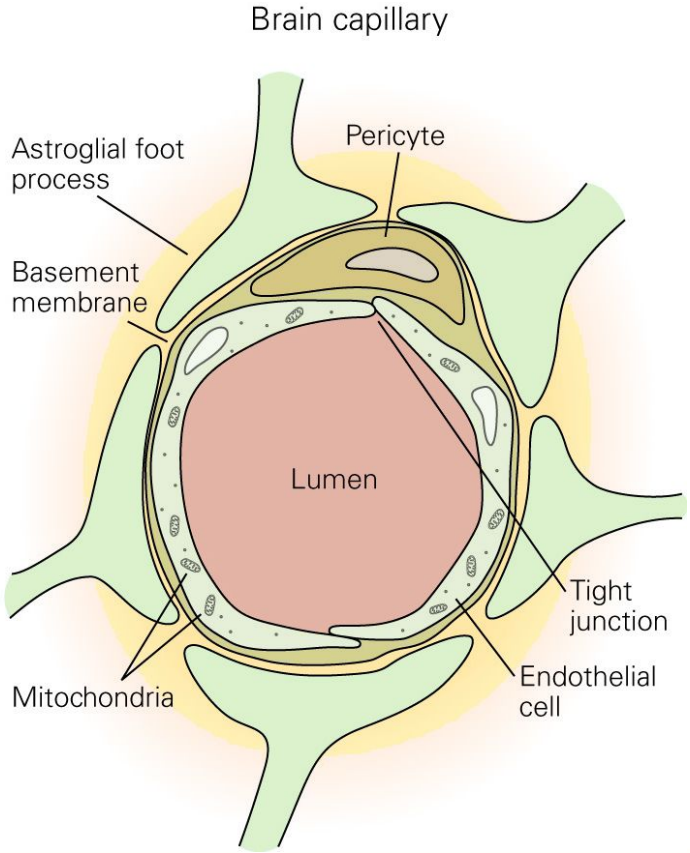


Experiment 3

- Conclusion:
  - The problem is in **migration** (rather than differentiation, cell death, etc.)
  - GPR124 is required for normal migration of OPCs

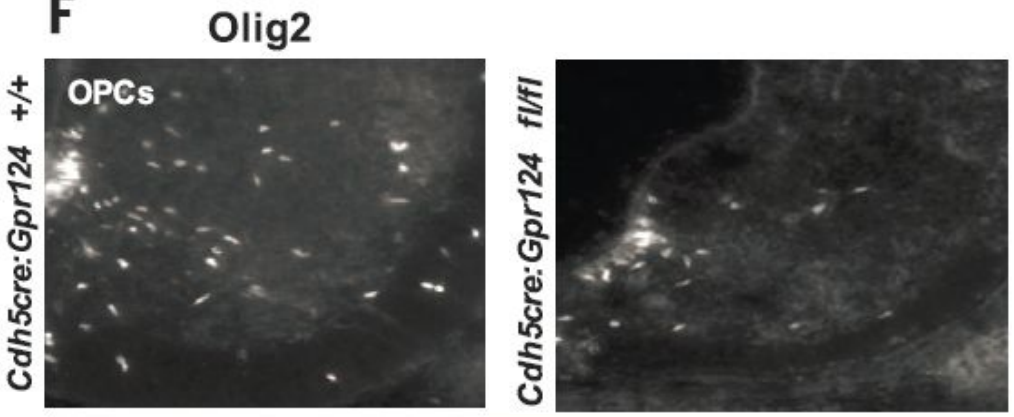
Experiment 3

- Both **endothelial cells** and **pericytes** express GPR124, which one is really required?



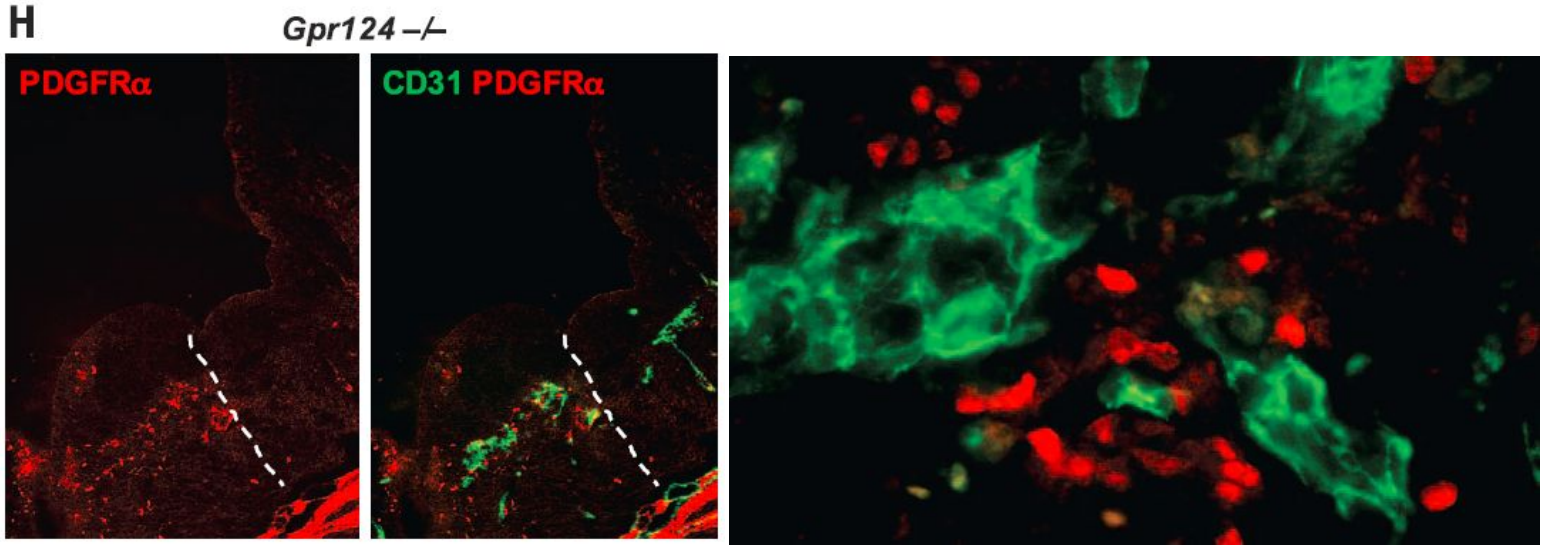
Experiment 3

- Supplementary Experiment:
  - Use Cdh5-cre:Gpr124(fl/fl) mice (Cdh5cre is vascular endothelium–specific VE-Cadherin-cre) to target loss of function to the **vascular endothelium**
  - The same OPC migration deficit was observed
    - GPR124 function in the **endothelium** is required to regulate OPC migration



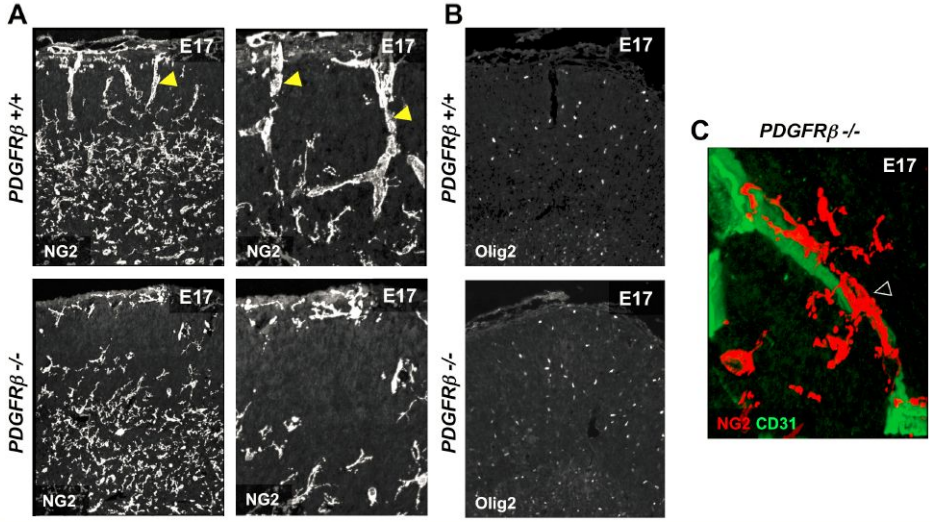
Experiment 3

- Supplementary Experiment:
  - Vascular development is also deficient in the brains of E14 GPR124−/− mice with associated severe OPC migration deficits



Experiment 3

- Supplementary Experiment:
  - In PDGFRβ-null mice, which lack all pericytes, OPC migration was maintained
  - Thus, OPCs require an **endothelial vascular scaffold**, but **not pericytes**, as a physical substrate for migration throughout the developing CNS



Experiment 3

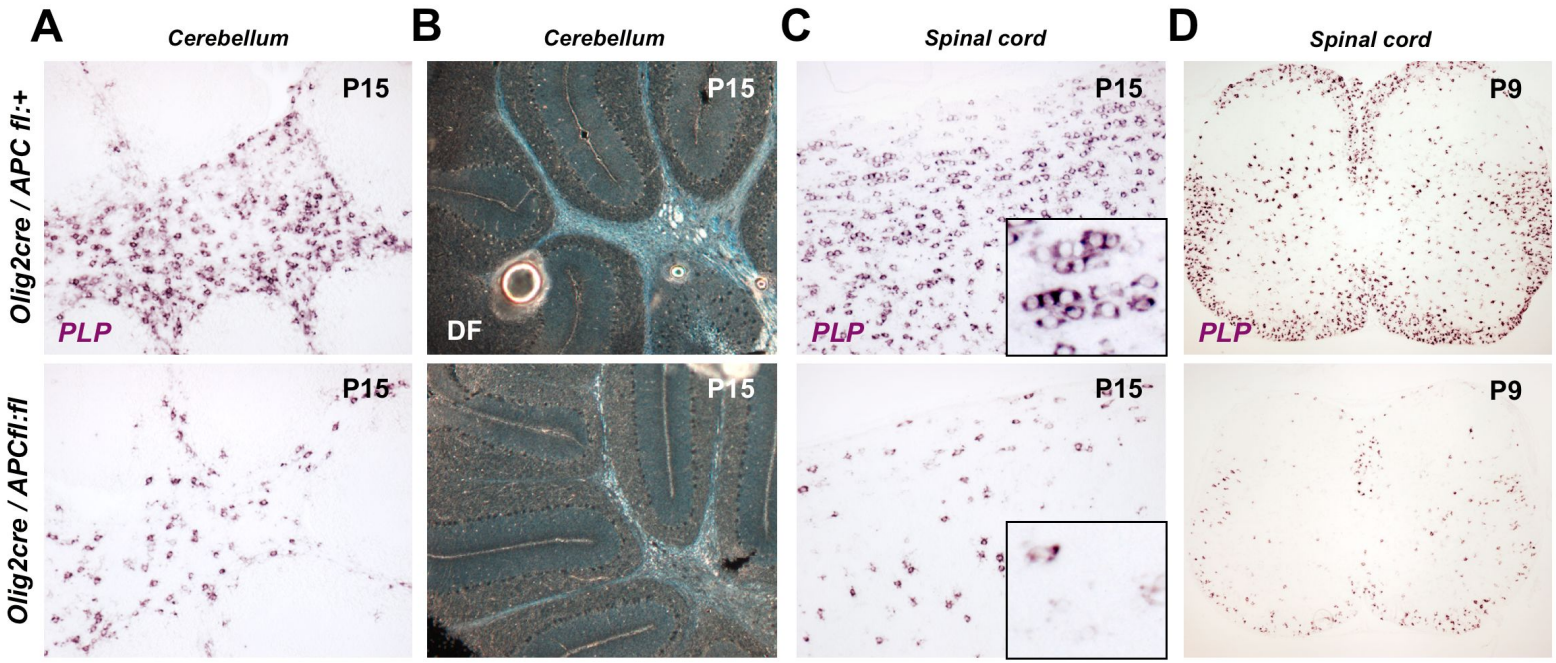
- Comments on Experiment 3:
  - Revealed the required physical scaffold of OPC migration on vessels
  - Relationship between migration and differentiation remained unknown (When and how to happen & stop)

Experiment 4

- Aim:
  - Find the pathway influencing migration and differentiation (differentiation after migration)
- Principles and methods in brief:
  - (Previous studies have shown) the Wnt pathway inhibits OPC differentiation
  - Olig2-cre:Apc(fl/fl) mice lack the obligate Wnt repressor APC and thus have high levels of Wnt signals

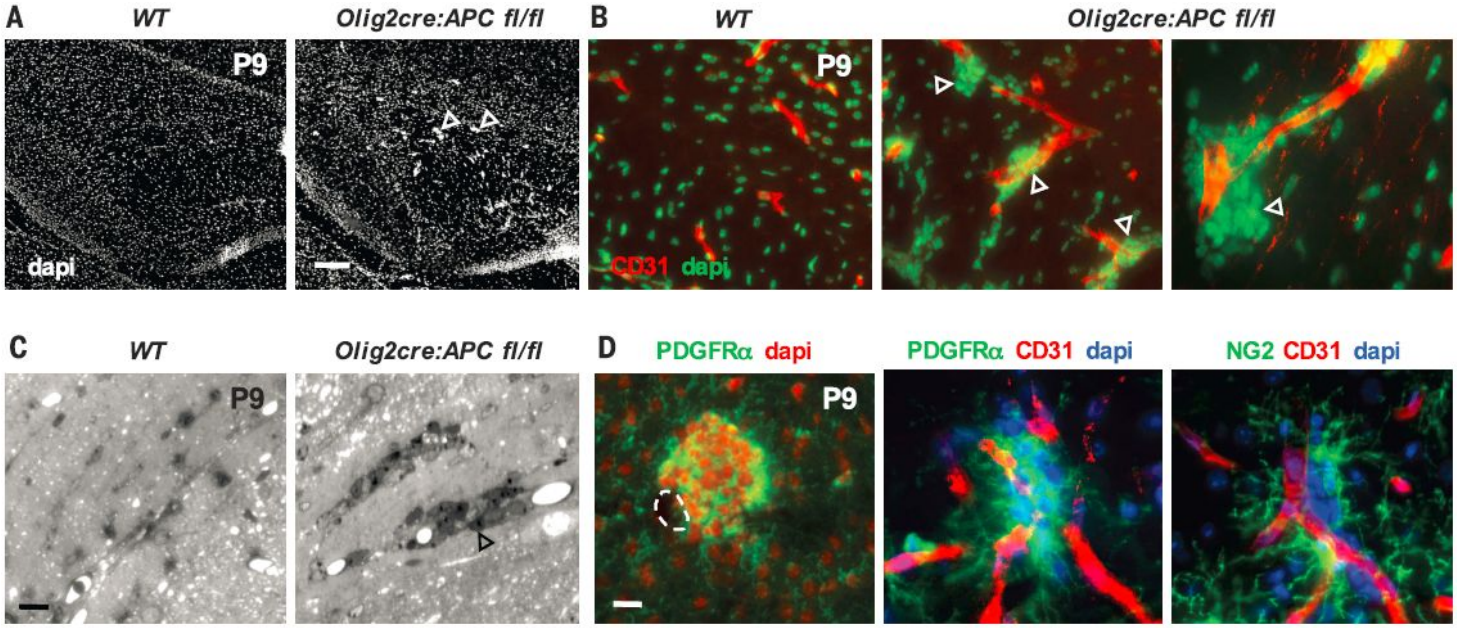
Experiment 4

- Results:
  - Delays in OPC differentiation with resulting hypomyelination



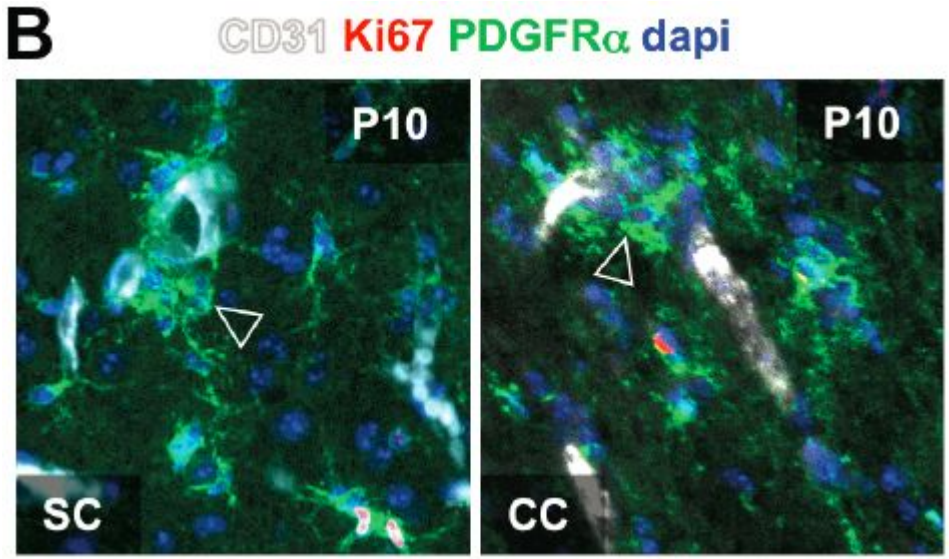
Experiment 4

- Results:
  - Aberrant clusters of OPCs associated with vasculature throughout the brain and spinal cord at early postnatal times



Experiment 4

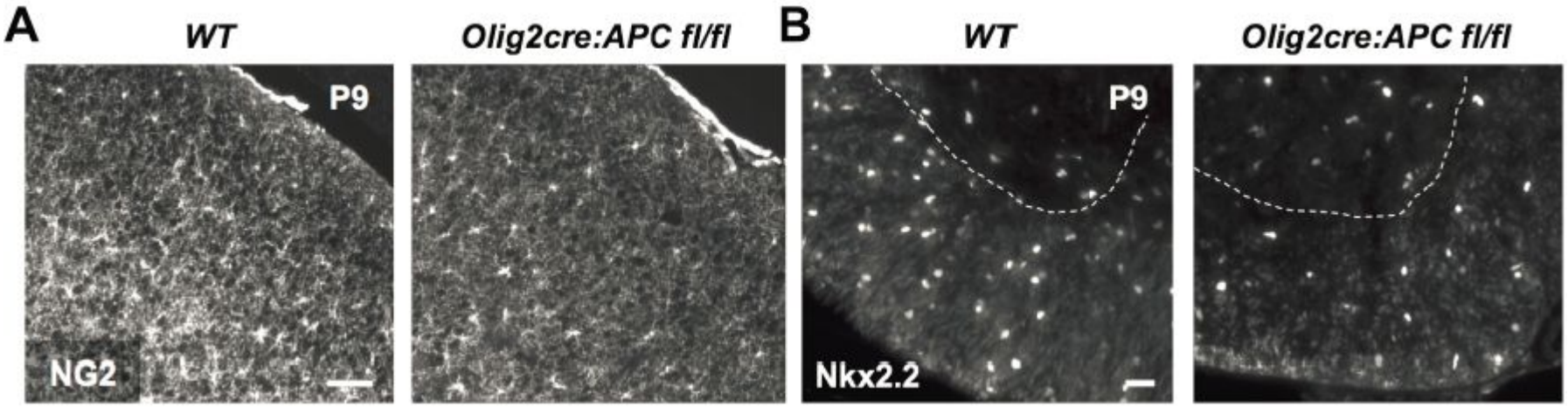
- Results:
  - OPC aggregation around vessels and absence of increased proliferation
  - Wnt activation in OPCs drives their attraction to the vascular scaffold



Experiment 4

- Results:
  - OPC aggregation around vessels and absence of increased proliferation
  - Wnt activation in OPCs drives their attraction to the vascular scaffold
  - High Wnt tone in OPCs in Olig2-cre:Apc(fl/fl) mice leads to an inability to dissociate from the vasculature and disperse normally into CNS parenchyma

Experiment 4



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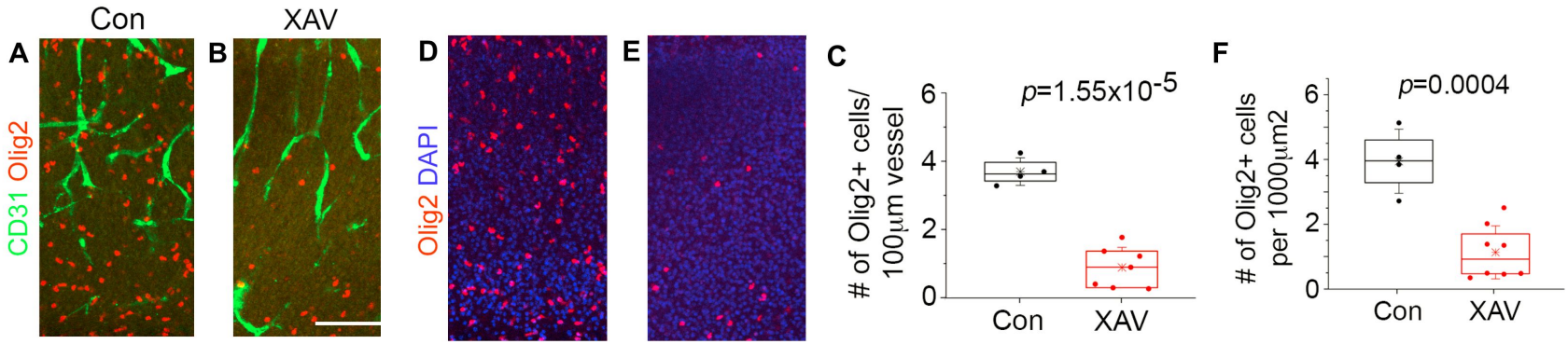
Experiment 4

- Results:
  - OPC aggregation around vessels and absence of increased proliferation
  - Wnt activation in OPCs drives their attraction to the vascular scaffold
  - High Wnt tone in OPCs in Olig2-cre:Apc(fl/fl) mice leads to an inability to dissociate from the vasculature and disperse normally into CNS parenchyma

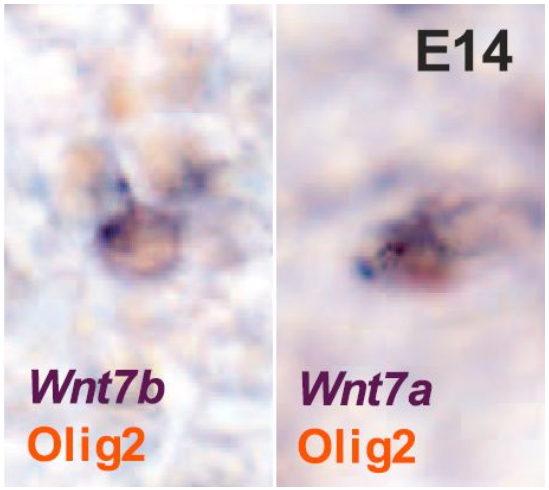
Experiment 4

- Results:
  - A loss of Wnt tone in OPCs, in cortical slice cultures treated with the small-molecule Wnt inhibitor XAV939, results in a 76% reduction in OPC recruitment to the microvasculature at postnatal day 1 (P1) and a 71% reduction in their migration to the outer cortex



Experiment 4

- Results:
  - OPCs are a source of the ligands Wnt7a and Wnt7b during embryonic migration in the brain and spinal cord
  - These ligands act cell-autonomously to activate the Wnt pathway in OPCs at later postnatal times



Experiment 4

- Conclusion:
  - The Wnt signal mediates the interaction with the endothelium during earlier OPC migration
    - High Wnt tone hampers OPC dissociation from the vasculature
    - Low Wnt tone reduces OPC migration
  - Ligands Wnt7a and Wnt7b are candidates for the source of Wnt

Experiment 4

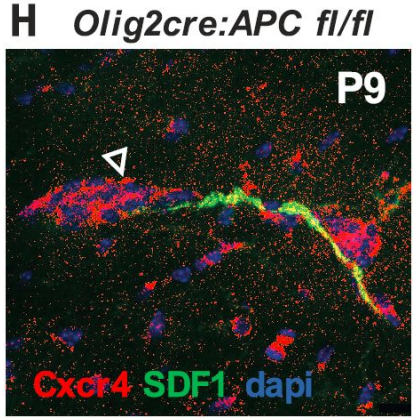
- Comments on Experiment 4:
  - Demonstrated that high and low levels of Wnt signals lead to two different results
  - In the normal case, how the Wnt pathway controls the process remained unknown

Experiment 5

- Aim:
  - Identify how Wnt pathway activation in OPCs promotes their attraction to the endothelium
- Principles and methods in brief:
  - Analyze mRNA transcripts up-regulated in mouse Wnt-activated OPCs

Experiment 5

- Principles and methods in brief (continued):
  - Chemokine receptor Cxcr4
    - One of the most highly up-regulated factors in Wnt-activated OPCs
    - A direct Wnt target in other systems
    - Binds the ligand Sdf1 (expressed by the endothelium throughout OPC developmental migration)

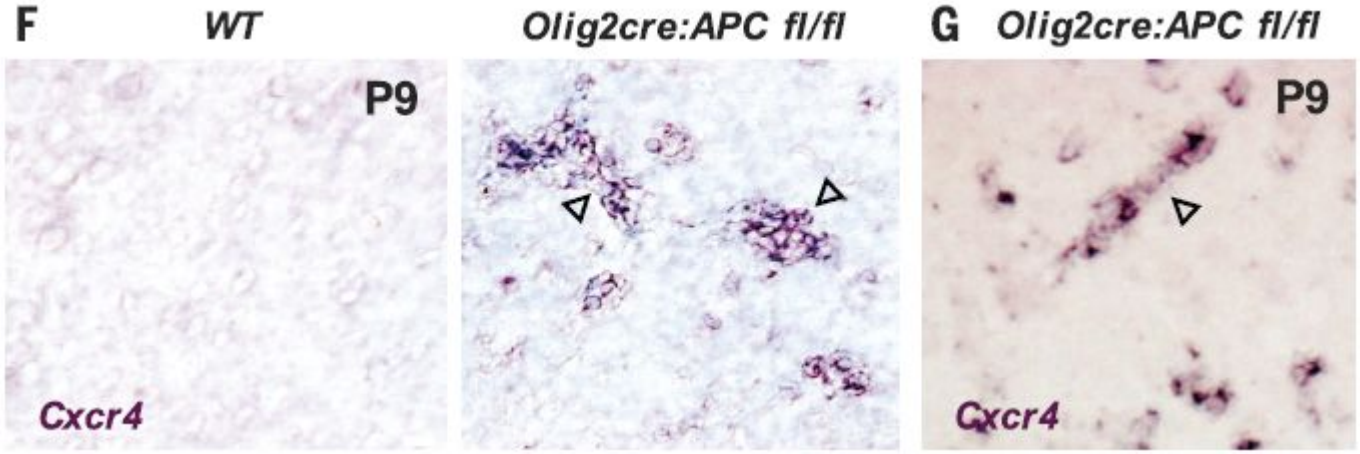


Experiment 5

- Principles and methods in brief (continued):
  - Chemokine receptor Cxcr4
    - One of the most highly up-regulated factors in Wnt-activated OPCs
    - A direct Wnt target in other systems
    - Binds the ligand Sdf1 (expressed by the endothelium throughout OPC developmental migration)
    - Has been implicated in OPC migration, but not in connection with the Wnt pathway or the vasculature

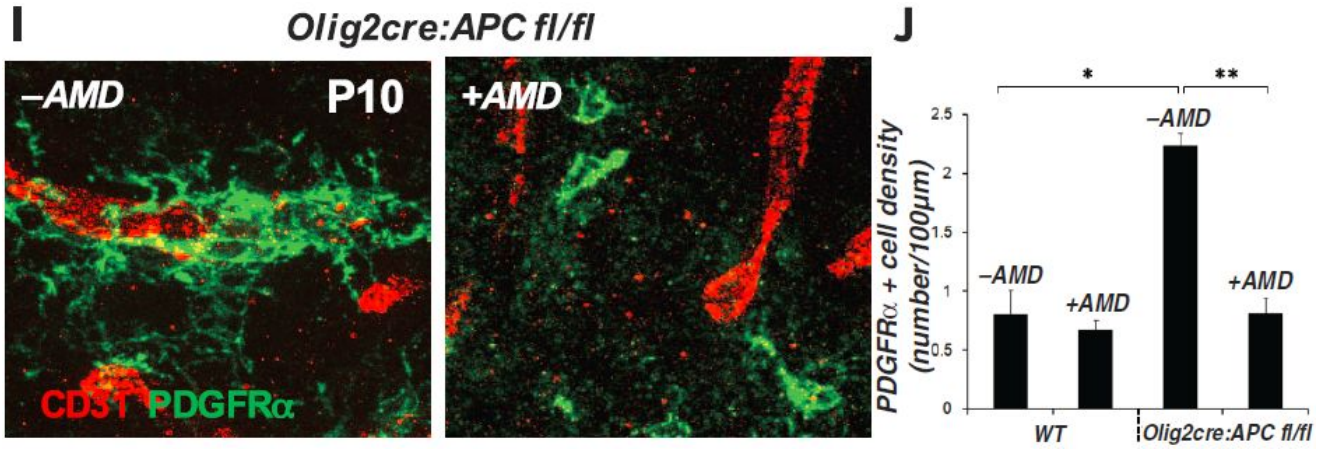
Experiment 5

- Results:
  - Up-regulation of Cxcr4 mRNA in the clustered Wnt-activated OPCs associated with vessels in the brain and spinal cord of Olig2-cre:Apc(fl/fl) mice



Experiment 5

- Results:
  - Treatment of these mice in vivo with the Cxcr4/Sdf1 antagonist AMD3100 between developmental ages P3 and P10 leads to a reversal of vessel-associated OPC clustering throughout the CNS

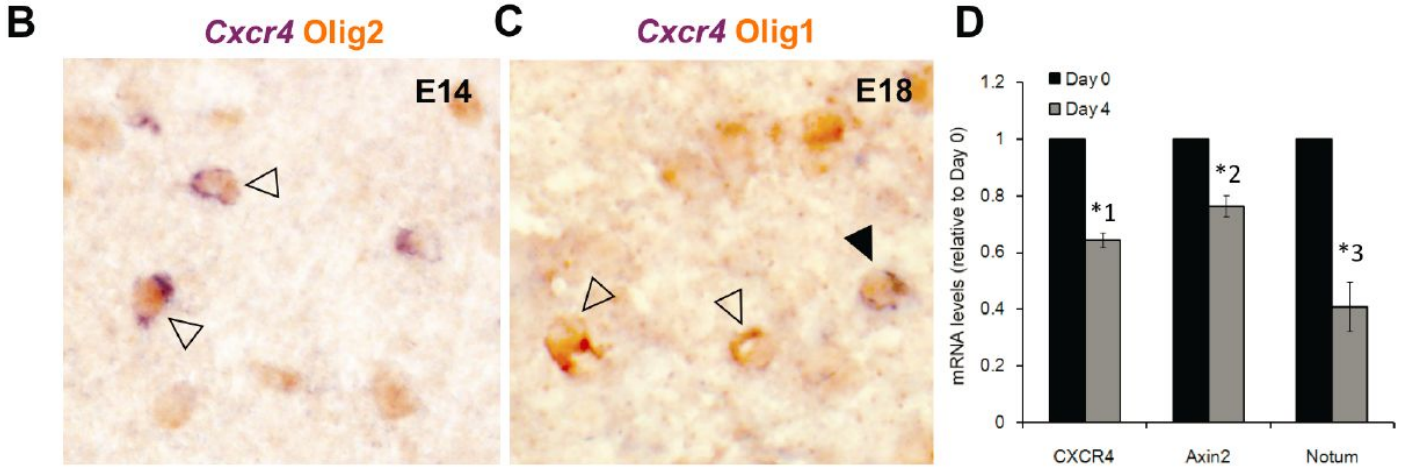


Experiment 5

- Results:
  - Treatment of these mice in vivo with the Cxcr4/Sdf1 antagonist AMD3100 between developmental ages P3 and P10 leads to a reversal of vessel-associated OPC clustering throughout the CNS
  - A Wnt-activated, Cxcr4- dependent mechanism drives attraction of OPCs to the vascular scaffold

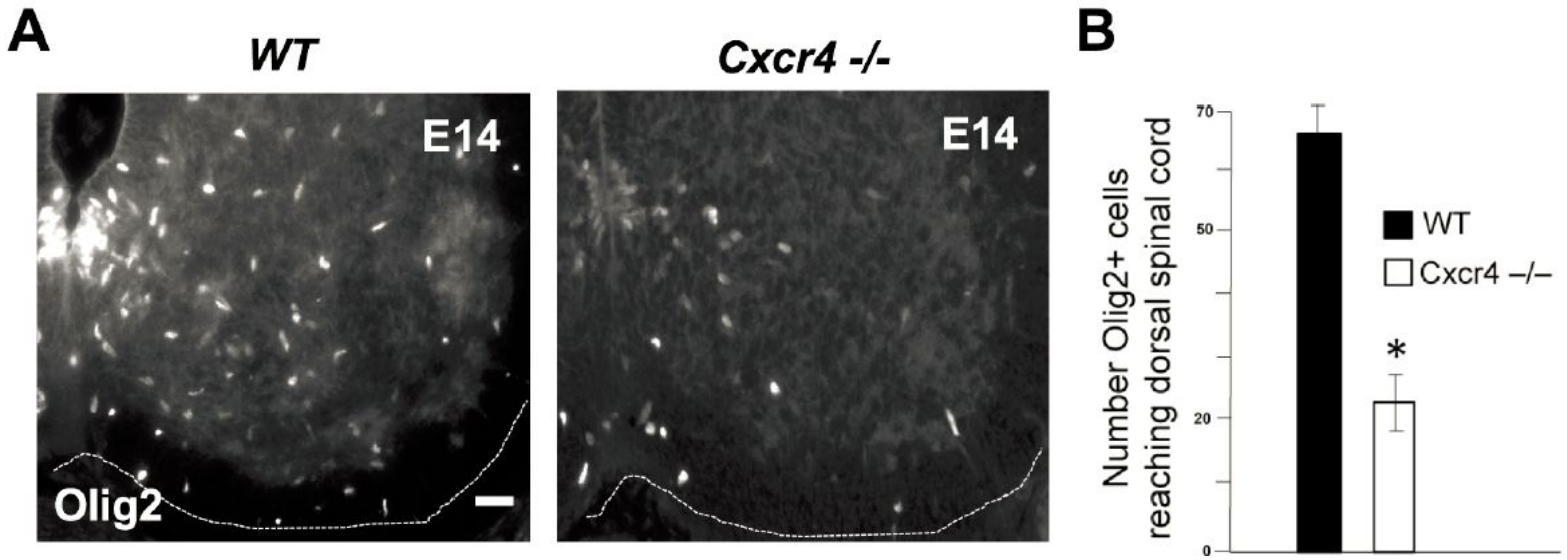
Experiment 5

- Results:
  - Cxcr4
    - Expressed by OPCs during embryonic developmental migration
    - Down-regulated along with Wnt pathway down-regulation in differentiating mature oligodendrocytes



Experiment 5

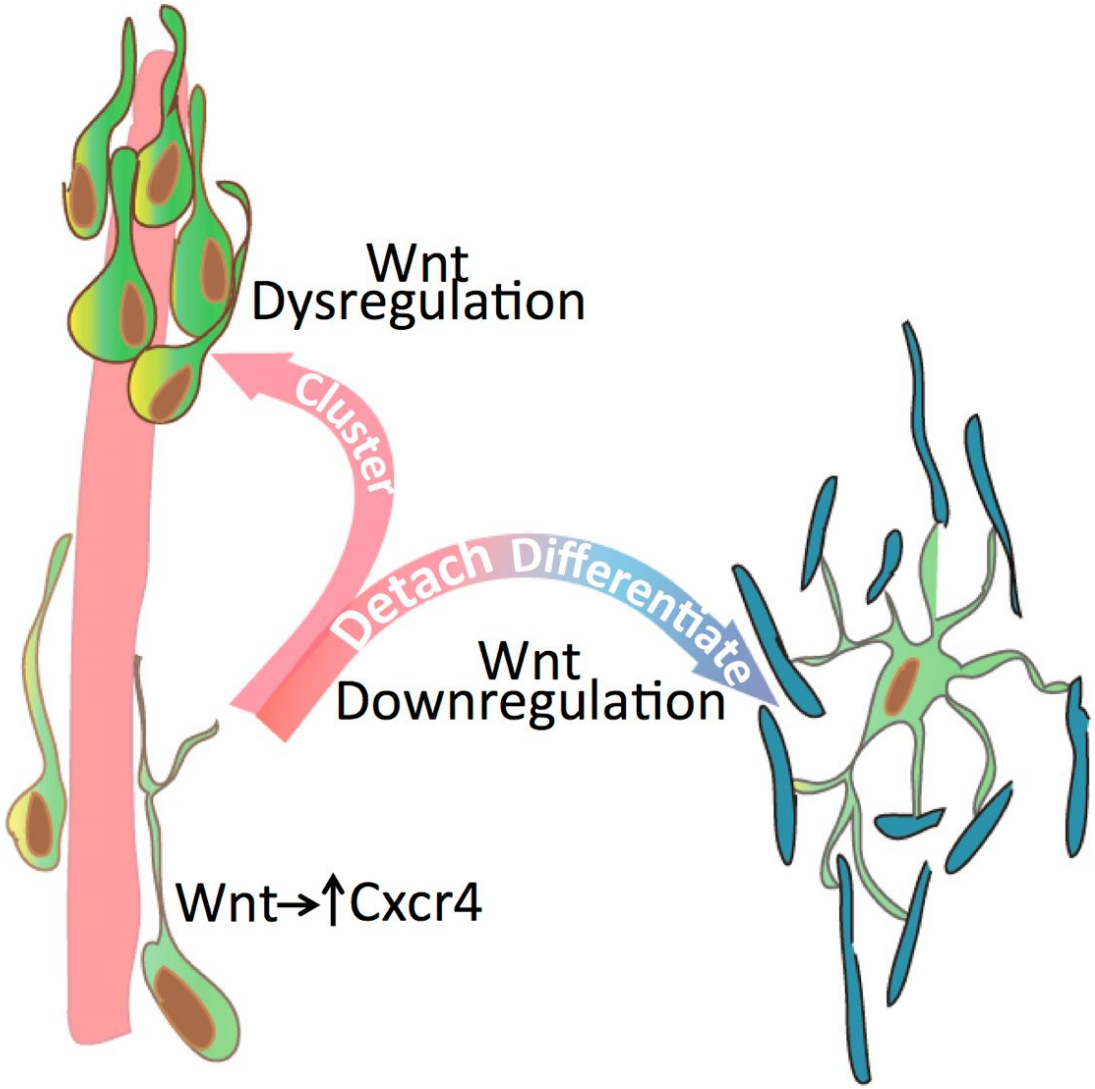
- Results:
  - Loss of Cxcr4 function leads to a diminished migratory ability of OPCs in the developing CNS



Experiment 5

- Conclusion:
  - Wnt activation in OPCs
    - Mediates their attraction to the vasculature and also blocks their differentiation during migration
    - The timing is coupled with Wnt down-regulation required for appropriate endothelial dissociation and subsequent differentiation

Experiment 5

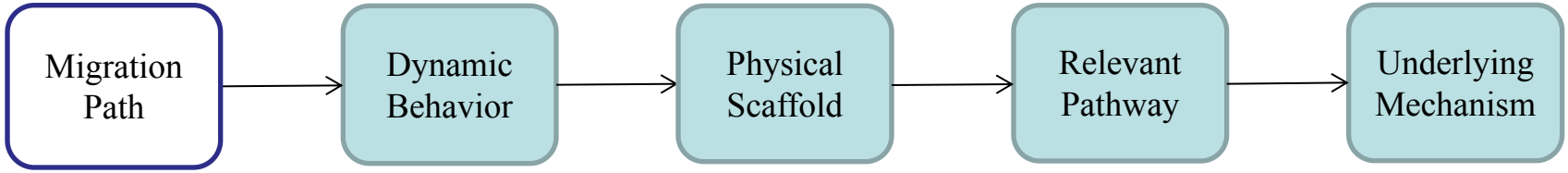


Experiment 5

- Comments on Experiment 5:
  - Uncovered the Wnt pathway that regulates both OPC migration and differentiation
  - Is the pathway common across mammalian species? Is it the same for humans?
  - Is Cxcr4 the only factor in the Wnt pathway involving in this process?

Summary

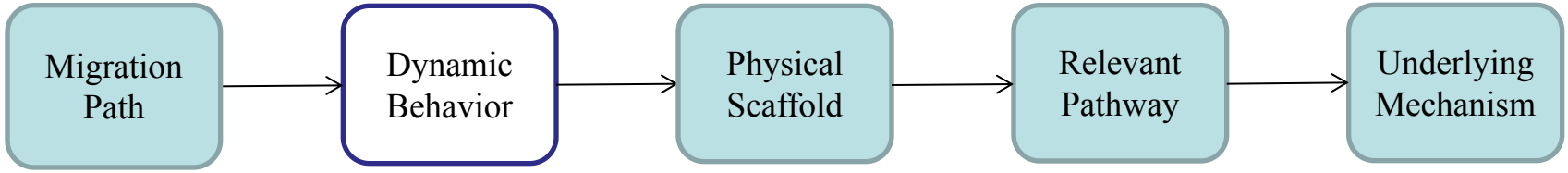
- This study shows



  - 1. OPCs migrate along the vesculature
    - Common modes of migration across mammalian species

Summary

- This study shows



  - 2. A physical interaction that brings migrating OPCs into intimate contact with the endothelium
    - Crawling
    - Jumping

Summary

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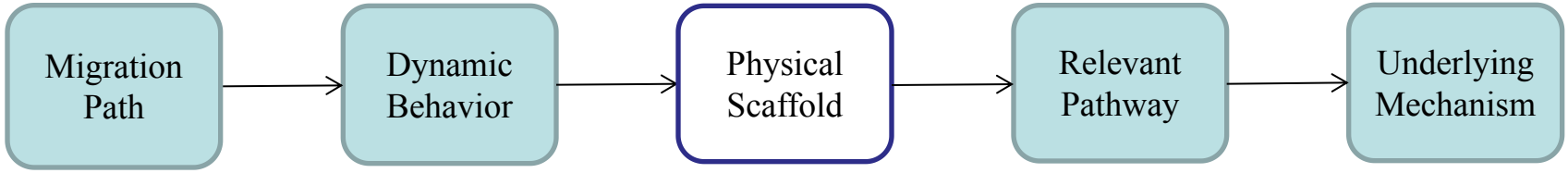
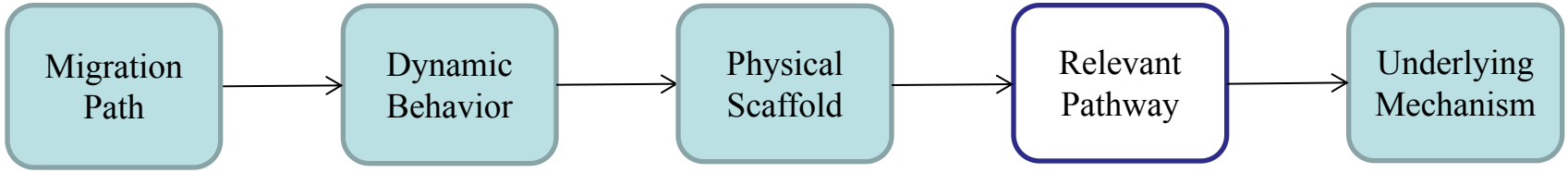


  - 3. OPCs require an endothelial vascular scaffold, but not pericytes, as a physical substrate for migration throughout the developing CNS
    - GPR124 expressed by endothelium is required for normal migration of OPCs

Summary

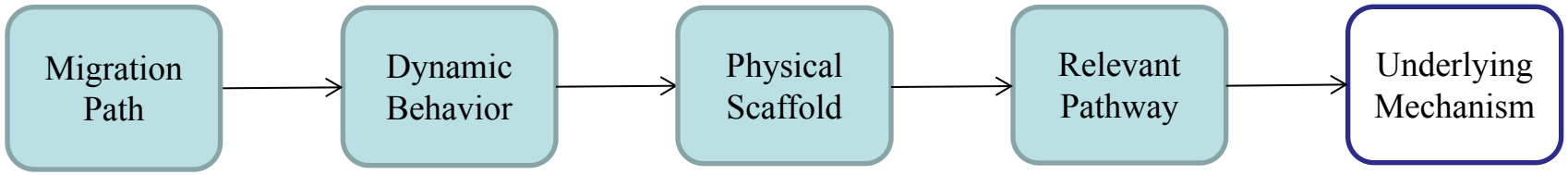
- This study shows



  - 4. The Wnt signal mediates the interaction with the endothelium during earlier OPC migration
    - Ligands Wnt7a and Wnt7b are candidates for the source of Wnt

Summary

- This study shows



  - 5. Wnt pathway activation of Cxcr4 in OPCs mediates their attraction to the endothelium
    - Most likely via the endothelial-expressed Sdf1 ligand
    - Prevents these cells from differentiating while associated with the vasculature during migration

Summary

- Potential applications:
  - OPC migration in injured or diseased nervous system (into demyelinated areas) may have similar mechanisms
    - Critical in human diseases (multiple sclerosis, hypoxic injury of the newborn brain, etc.)
    - Dysfunction may contribute to disease progression in these debilitating human conditions

Follow-up questions

- Have we fully understood the process of OPC migration?
  - 1. How to prove the similarity between OPC migration during development and in injured or diseased nervous system? Can we carry out the same procedures?
  - 2. What is the biomechanical mechanism that cotrols the physical interaction between OPCs and the vesculature?
  - 3. What potential factors may affect OPC migration? Are there other pathways also participating in this process?

Thank You