Layering Defect in p35 Deficiency is Linked to Improper Neuronal-Glial Interaction in Radial Migration

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Presented by Suzanne Luther

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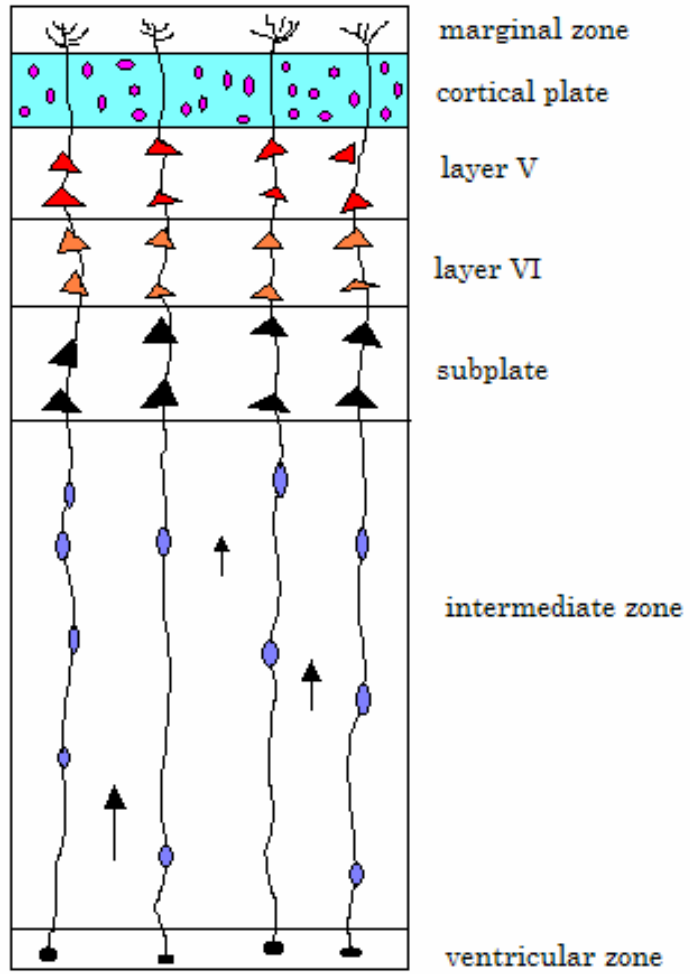
A bit of background…

- The neocortex of mice deficient in the protein p35 displays inverted layering.
- p35 is an activator of cyclin-dependent kinase 5 (Cdk5).
- Sought to identify the mechanisms which are the basis for this defect.
- Time-lapse imaging on cortical slices of wild-type (WT) and p35-null (*null)* mice used to determine influence of Cdk5 in neuronal migration.

Migration

- Neocortex formed by “waves” of neurons which migrate from ventricular zone
- First “wave” establishes preplate zone
- Second wave establishes marginal zone (MZ), cortical plate (CP), and ventricular zone (VZ)
- Layers generated by neurons migrating through intermediate zone (IZ) in “inside out” fashion



Adapted from Bear, Connor and Paradiso. *Neuroscience: Exploring the Brain*. Lippincott Williams & Wilkins, 2001.

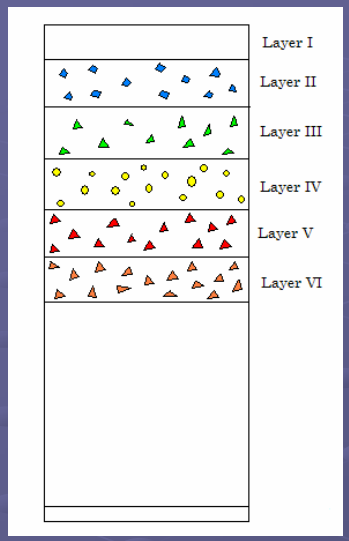
Migration, again

- Neurons migrate in two ways.
  - Locomotion – migration along radial glial fibers, in which cell body and leading edge move in unison.
  - Translocation – leading edge is attached to pial surface and ventricular zone.
- Neurons move in early stages via translocation, but in later stages by locomotion.

p35 and Cdk5

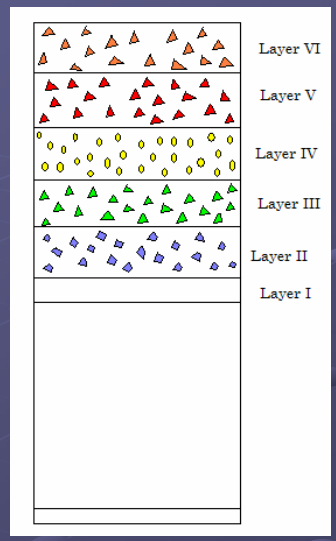
- p35 is an activator of cyclin-dependent kinase 5 (Cdk5)
- Cdk5 has been shown to be required for neuritic outgrowth and synaptogenesis in rat brain, among other functions.
- The Cdk5/p35/p39 signaling pathway has previously been shown to be crucial to neocortical layering regulation.

Inside-out and Inverted…



Normal layering – seen in WT

Adapted from Bear, Connor and Paradiso. *Neuroscience: Exploring the Brain*. Lippincott Williams & Wilkins, 2001.



Inverted layering – seen in *null* mutants

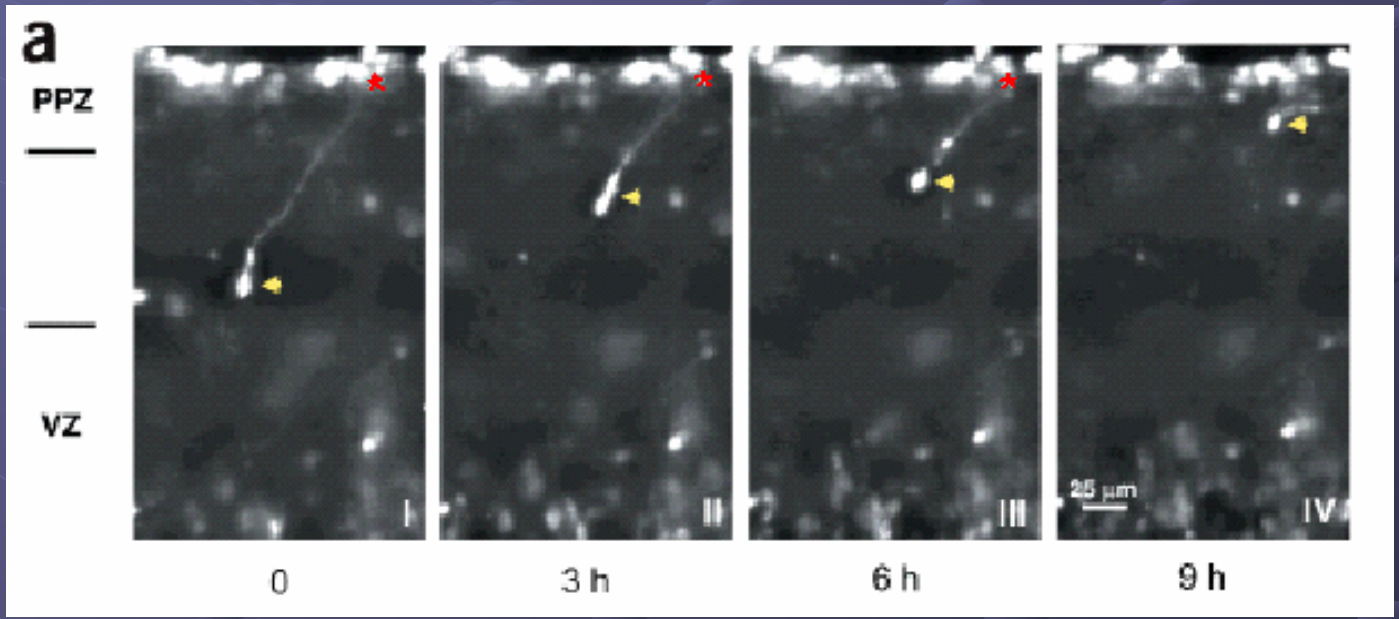
- In normal inside-out migration, each “wave” of cells migrates past the previous layer, so that the layer closest to the surface is formed last.
- In the *null* mutants, the “waves” of cells do not move past the previous layer, so that the layers are reversed.

Experiments

- Time-lapse recordings of migration *in vitro* in both WT and *null* mice at E13 and E15.
  - Cortical slices from the WT and *null* mice were compared
- *In vivo* analyses to determine glial guidance and p35 rescue abilities.
- Cortical slices from *null* mice compared with those from *Reelin* deficient mice, who also show neocortex layer inversion.

Is migration normal at E13?

- Most E13 WT neurons migrated by somal translocation into PPZ – shortens cell length
- Straight trajectory
- Figure a. – Red asterisk: leading process, attached to PPZ. Yellow arrowhead: cell soma.



Source: Gupta, A., K. Sanada, D. T. Miyamoto, S. Rovelstad, B. Nadarajah, A. L. Pearlman, J. Brunstrom, and L. Tsai. "Layering Defect in p35 Deficiency is Linked to Improper Neuronal - Glial Interaction in Radial Migration." *Nature Neuroscience* 6 (2003): 1284- 1291.
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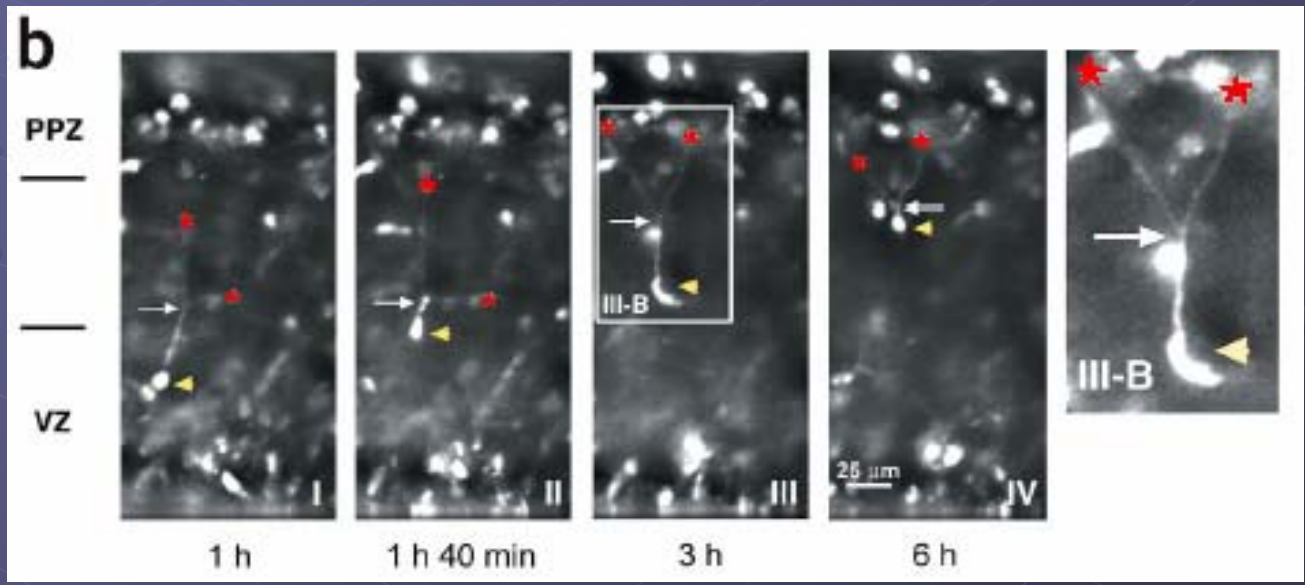
Null migration at E13

- Neurons show branched migration
- In branched migration, leading processes are branched, and are not fixed.
- Somata move toward branch points, which are stable – movement through two branch points leads to radial advancement.



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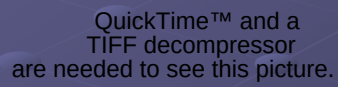
Branched Migration



Null migration, continued

- Branches are dynamic – change length and direction before fixing position.
- Trajectory is in a zig-zag sort of path.

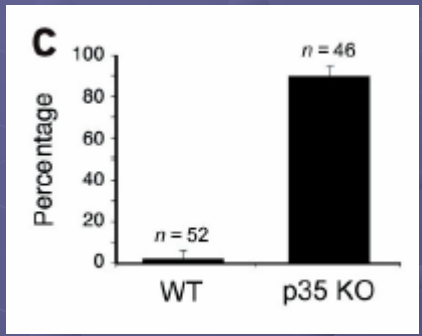
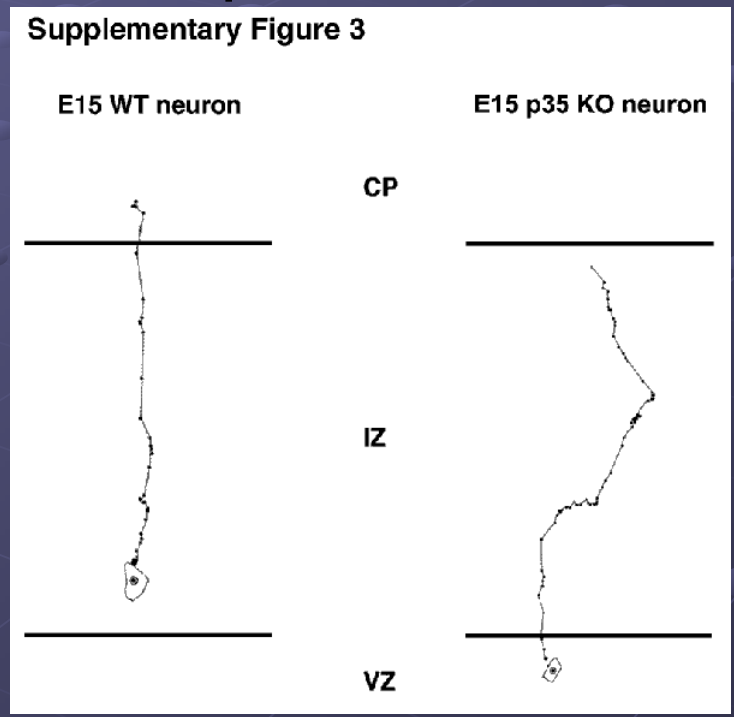


Figure c. Percentage of each type of neuron that changed direction by more than 30 degrees per hour during migration.



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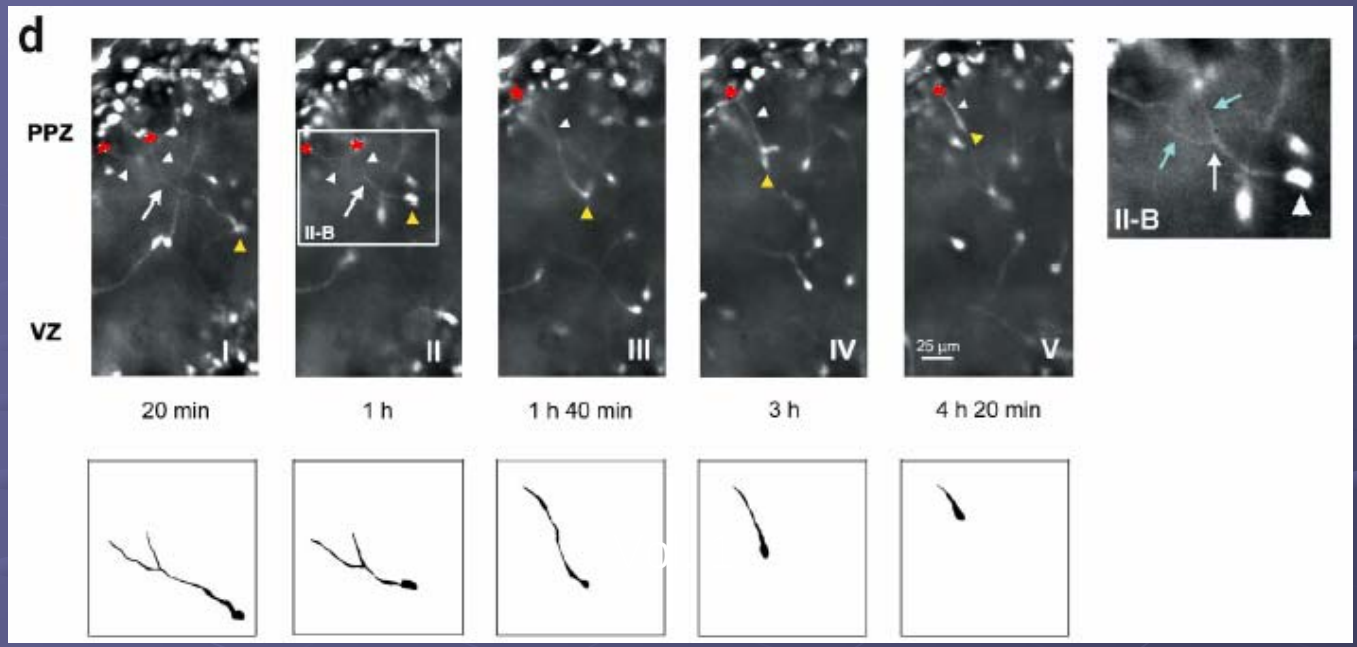
But the PPZ splits properly!



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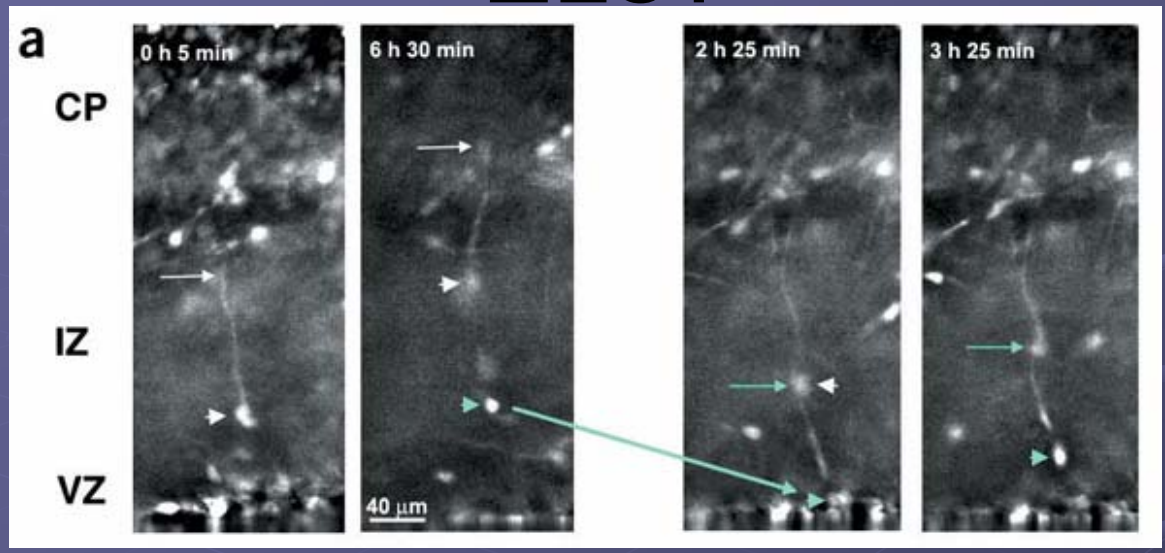
- Once *null* neurons reached the PPZ via branched migration, neurons moved along processes which were unbranched and attached to MZ.
- This looks identical to the migration of WT neurons at this stage.

What does migration look like at E15?



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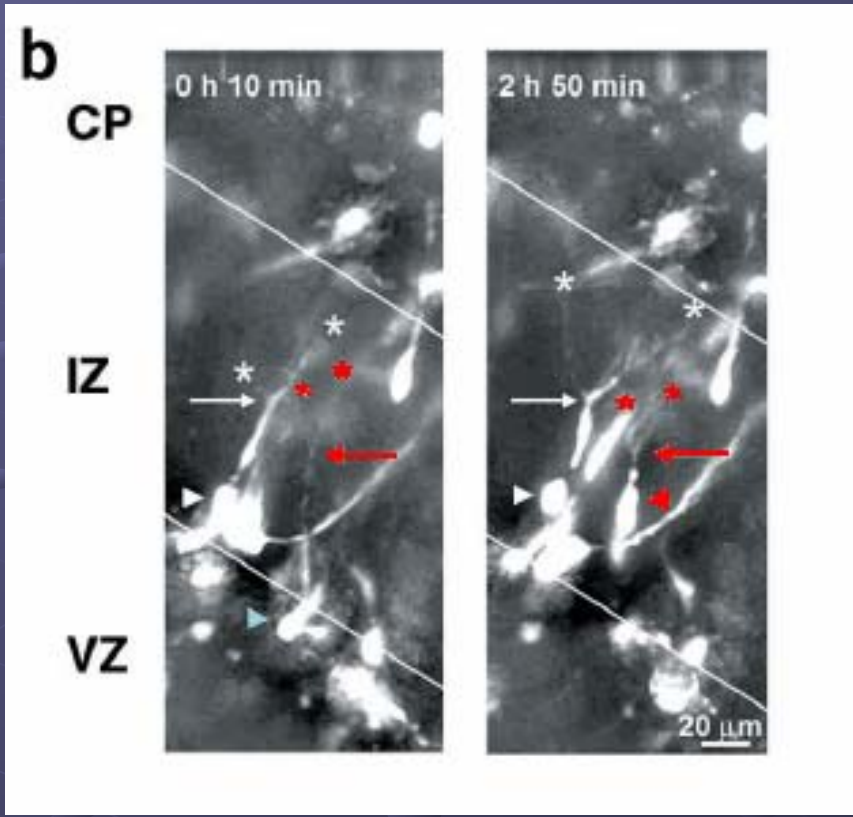
- Locomotion used by 66% of WT neurons
- In deeper layers of developing neocortex, 86% of WT neurons use locomotion
- Locomotion maintains constant neuronal length
- Locomotion is the predominant migration method in E15 neocortex to cross IZ

Locomotion


QuickTime™ and a
TIFF decompressor
are needed to see this picture.

Null migration at E15

- Branched migration was again found to occur
- 93% of migration occurred by branched migration
- Branch points occurred in IZ and CP only – never reached pial surface



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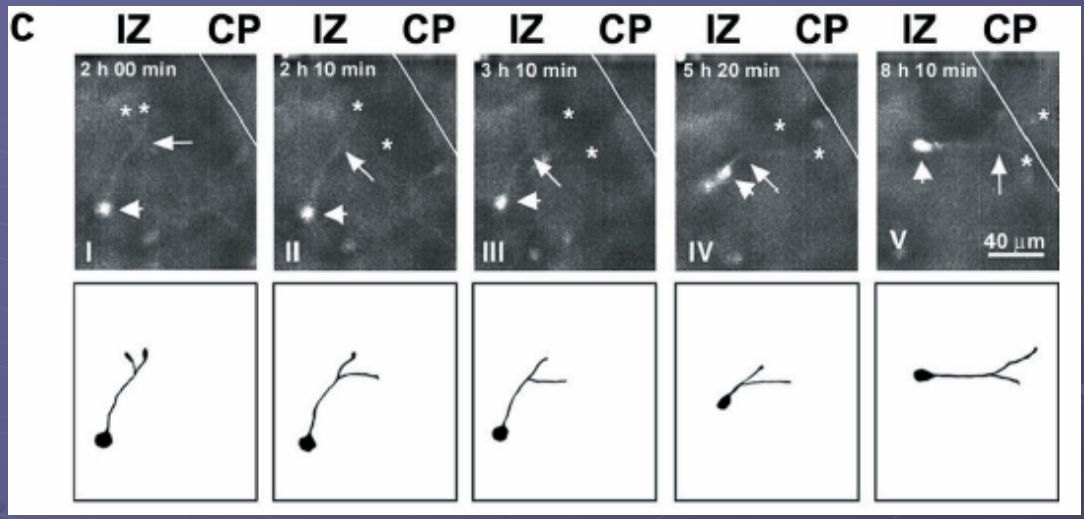
Null migration at E15



Source: Gupta, A., K. Sanada, D. T. Miyamoto, S. Rovelstad, B. Nadarajah, A. L. Pearlman, J. Brunstrom, and L. Tsai. "Layering Defect in p35 Deficiency is Linked to Improper Neuronal - Glial Interaction in Radial Migration." *Nature Neuroscience* 6 (2003): 1284- 1291.
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- Over longer time periods, migration resembles that seen in E13 neocortex
- Branching found to be dynamic
- Movement in somewhat zigzag trajectory

Might branched migration be glia-independent?

- WT radial glial cells give rise to daughter cells which migrate along mother process.
- Two indications that branched migration does not rely on glia
- Cells which are guided by glia do not show any branched leading processes
- Cells moving via branched migration demonstrated zig-zag trajectories, as opposed to guided cells, which demonstrated straight trajectories.

A test of glia-dependence

- Hypotheses:
  - Neurons from *null* mutants use one of their branches to migrate along glia.
  - Neurons from *null* mutants move completely independently of any glial guidance.
- Introduce GFP-carrying retrovirus *in utero* in *null* E12 embryos.
- Use GFP-immunostaining at E15 to determine behavior of clonal descendents.

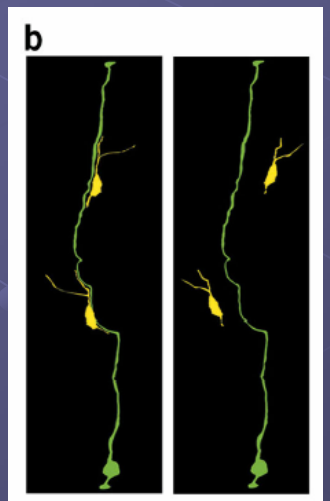


Fig. 3b

Illustrations of hypotheses

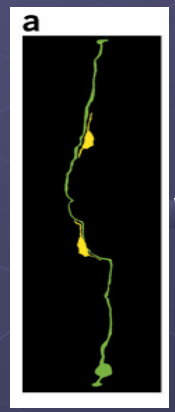


Fig. 3a

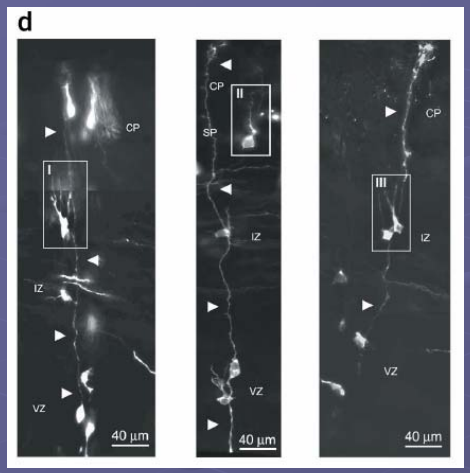
Wildtype migration

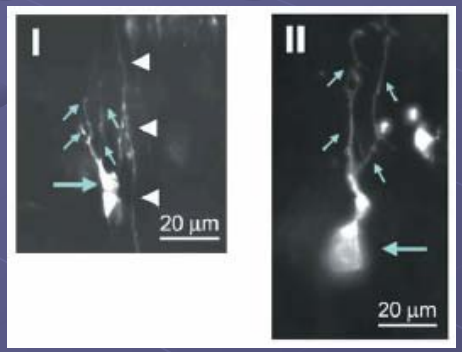
Glial Guidance in posterior *null* neocortex

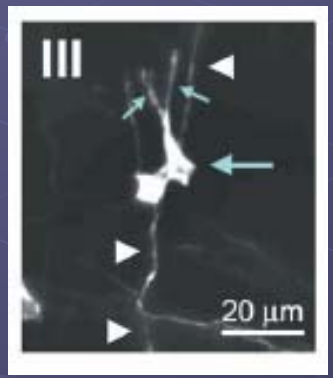
- *Null* posterior neocortex – relationship like that of WT cells.
- Elongated cell somata and unbranched leading processes noted.
- Translocation observed near pia (no glial guidance)

Source: Gupta, A., K. Sanada, D. T. Miyamoto, S. Rovelstad, B. Nadarajah, A. L. Pearlman, J. Brunstrom, and L. Tsai. "Layering Defect in p35 Deficiency is Linked to Improper Neuronal - Glial Interaction in Radial Migration." *Nature Neuroscience* 6 (2003): 1284- 1291.

Glial guidance in anterior *null* neocortex







- Extensive branching seen across IZ – no association with glial processes of mother cells.
- Branched neurons seen in subventricular zone – early detachment from mother cells

Source: Gupta, A., K. Sanada, D. T. Miyamoto, S. Rovelstad, B. Nadarajah, A. L. Pearlman, J. Brunstrom, and L. Tsai. "Layering Defect in p35 Deficiency is Linked to Improper Neuronal - Glial Interaction in Radial Migration." *Nature Neuroscience* 6 (2003): 1284- 1291.
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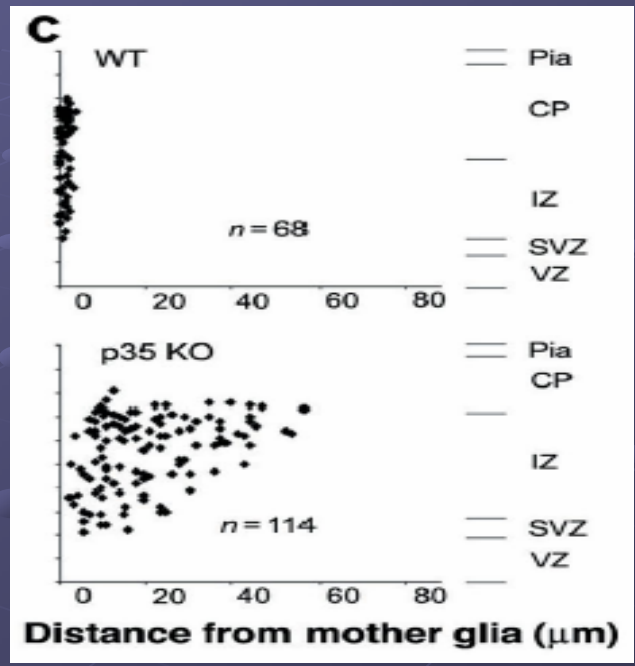
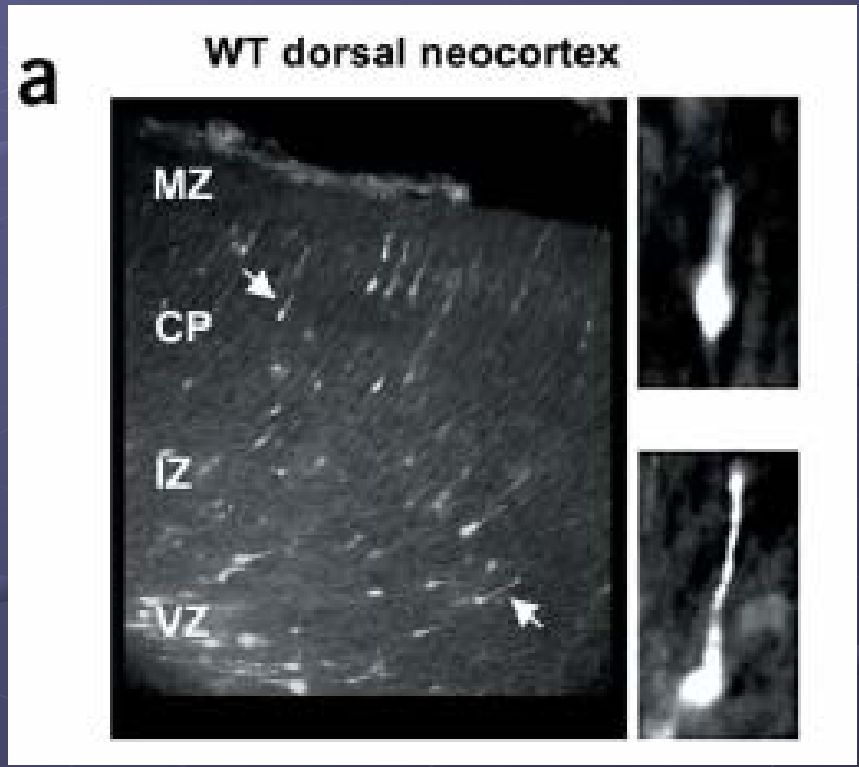
Abnormal neuronal-glial interaction

- Posterior *null* neocortex displays glial association similar to WT
- Anterior *null* neocortex does not show normal neuronal-glial interaction



A p35 “rescue” test via electroporation

- Is branching cell-autonomous?
- Electroporation of p35 into *null* mice at E13
  - Inject plasmid into ventricle of embryonic brain *in utero*.
  - Cells lining ventricle will take up plasmid after exposed to an electric field.
- Can branching be rescued in cells which end up expressing p35?



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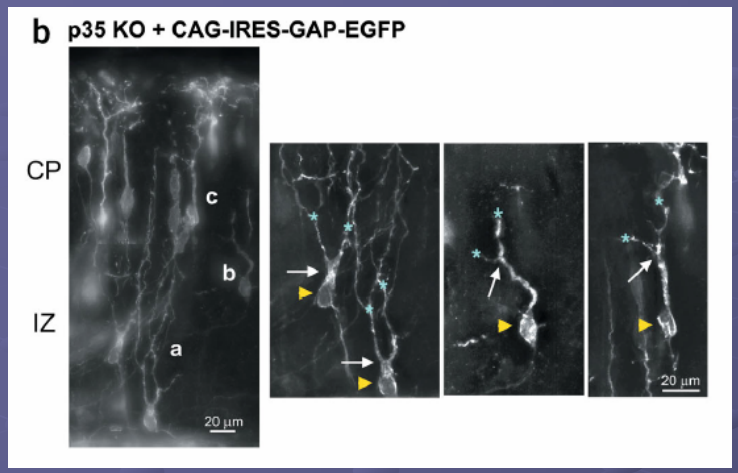
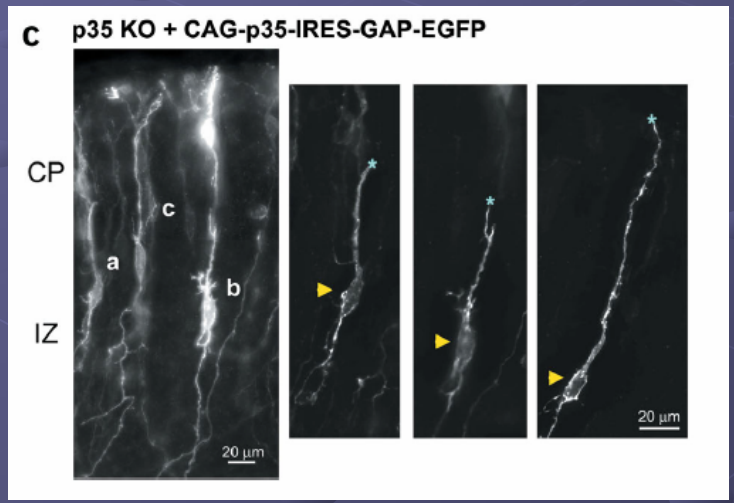
Rescue experiment results





- *Null* mice electroporated at E13 with either GFP or GFP/p35
- GFP-alone neurons showed leading-process branching
- GFP/p35 neurons showed unbranched leading processes, independent of location

Source: Gupta, A., K. Sanada, D. T. Miyamoto, S. Rovelstad, B. Nadarajah, A. L. Pearlman, J. Brunstrom, and L. Tsai. "Layering Defect in p35 Deficiency is Linked to Improper Neuronal - Glial Interaction in Radial Migration." *Nature Neuroscience* 6 (2003): 1284- 1291.
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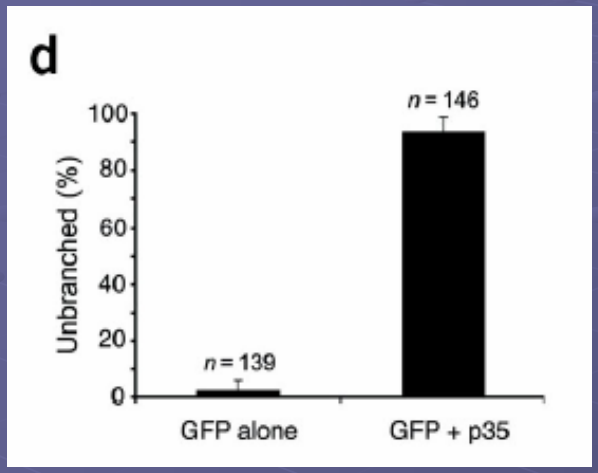
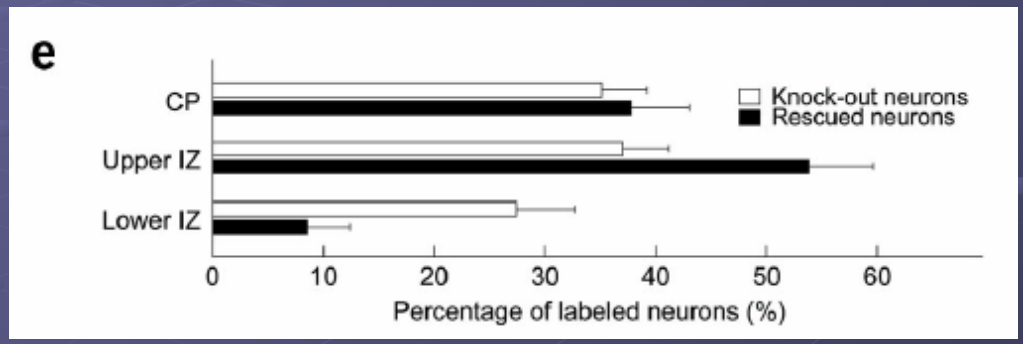
Rescue results, continued





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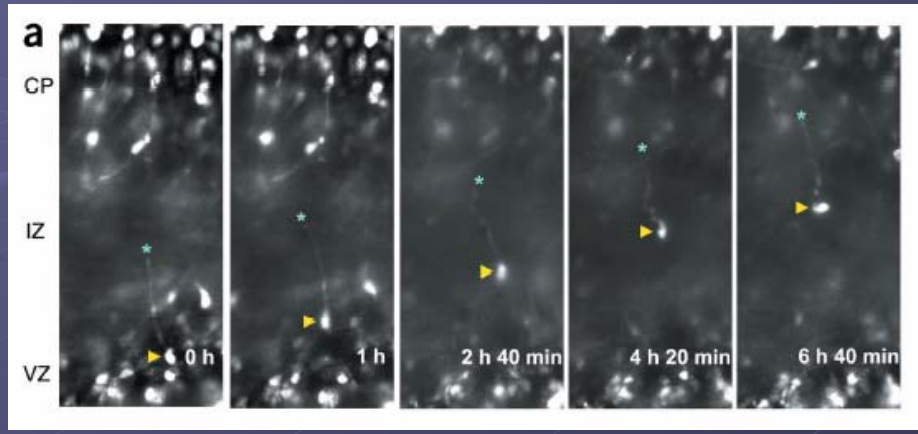
- A highly significant portion of *null* neurons electroporated with p35 displayed non-branching leading processes.
- Neurons expressing p35 were shifted toward pial surface
- Branching appears to be cell-autonomous. Cell positioning may be also, but not enough evidence yet.

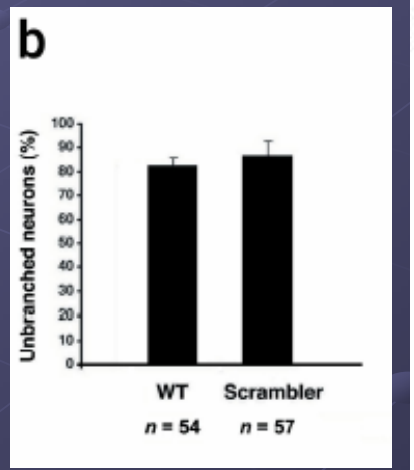
Is branched migration unique to p35 *null* mutants?

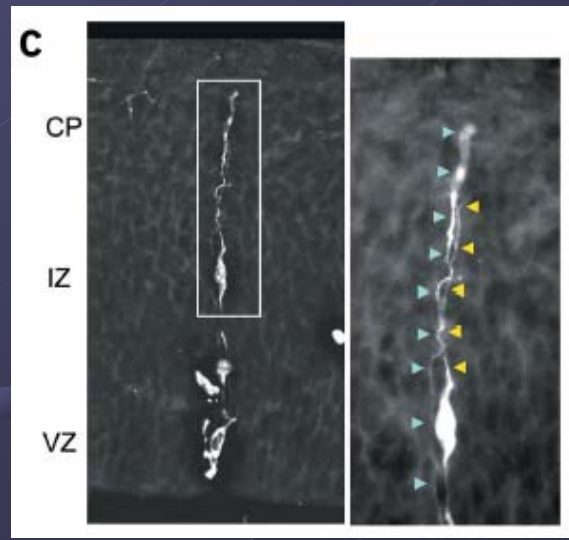
- Mice with a defective *Reelin* signaling pathway also display inverted neocortical layering
- Dab1 binds *Reelin* receptors within the cell
- *Scrambler* mice are Dab1 deficient
- Imaging studies at E15 were used on *Scrambler* neocortex to mimic defective *Reelin* signaling
- Clonal studies identical to those performed on p35 *null* mutants explored glia-guided migration

No branched migration in *scrambler* mutants

- No branching morphology apparent in *scrambler* neurons
- Clonal analysis showed that migrating *scrambler* neurons moved along mother cell glial process
- Branched migration the result of p35/Cdk5 signaling, not *Reelin* signaling.







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Experimental conclusions

- Loss of p35 signaling leads to abnormal methods of migration
- Abnormal migration does not necessarily lead to abnormal formation
- Altered migration still may play a role in inverted layering
- Branched neurons do not use glial guidance
- Branched migration a result of p35 deficiency in neurons themselves and is cell-autonomous
- Inverted layering does not require branched migration
- Glial guidance not sufficient for normal inside-out layering

Overall conclusions

- Normal inside-out layering dependent on parallel p35/Cdk5 and *Reelin* pathways
- In p35 *null* neocortex, normal radial migration is replaced with branched migration
- p35 appears to be important in glia-guided migration