# Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases

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# *Cortical Migration Review*

- Neurogenesis in VZ (projection neurons), SVZ (other neurons, glia)
- Migration either by *somal translocation* or *locomotion*

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# *Outstanding Question:*

"…the dynamics of migration, as well as of neurogenesis, have not been directly observed in the context of the lineage relationship of individual cells"

- We can determine which cells are descended from a single progenitor and where they end up.
- We don't know how they got there.

# *Experimental Approach*

▶Direct observation: Label progenitors with fluorescent marker, watch them divide and migrate using time-lapse microscopy.

▶Inject GFP-encoding retrovirus at low titer (to restrict labeling to small % of cells) into ventricles of E16 rat embryos.

▶Wait 24h.

▶Dissect out brains, culture coronal slices in medium in an incubator.

▶Every few hours, photograph slices using confocal microscope.

▶To determine cell type of $GFP^+$ cells:

Characteristic shapes/migration patterns

Electrophysiology (whole-cell patch)

Immunohistochemistry

# *Findings*

- Division in VZ tends to be asymmetric; division in SVZ tends to be symmetric. (Consistent with *numb* results.)

- Migration to cortical plate from both VZ and SVZ often follows highly stereotyped pattern that includes *retrograde* (i.e. 'backwards') migration towards the ventricle.

- Radial cells undergo final division to give a presumptive astrocyte and an intermediate progenitor (that then divides to make 2 neurons.)

# *Neurons arise in symmetric (VZ) and asymmetric (SVZ) division zones*

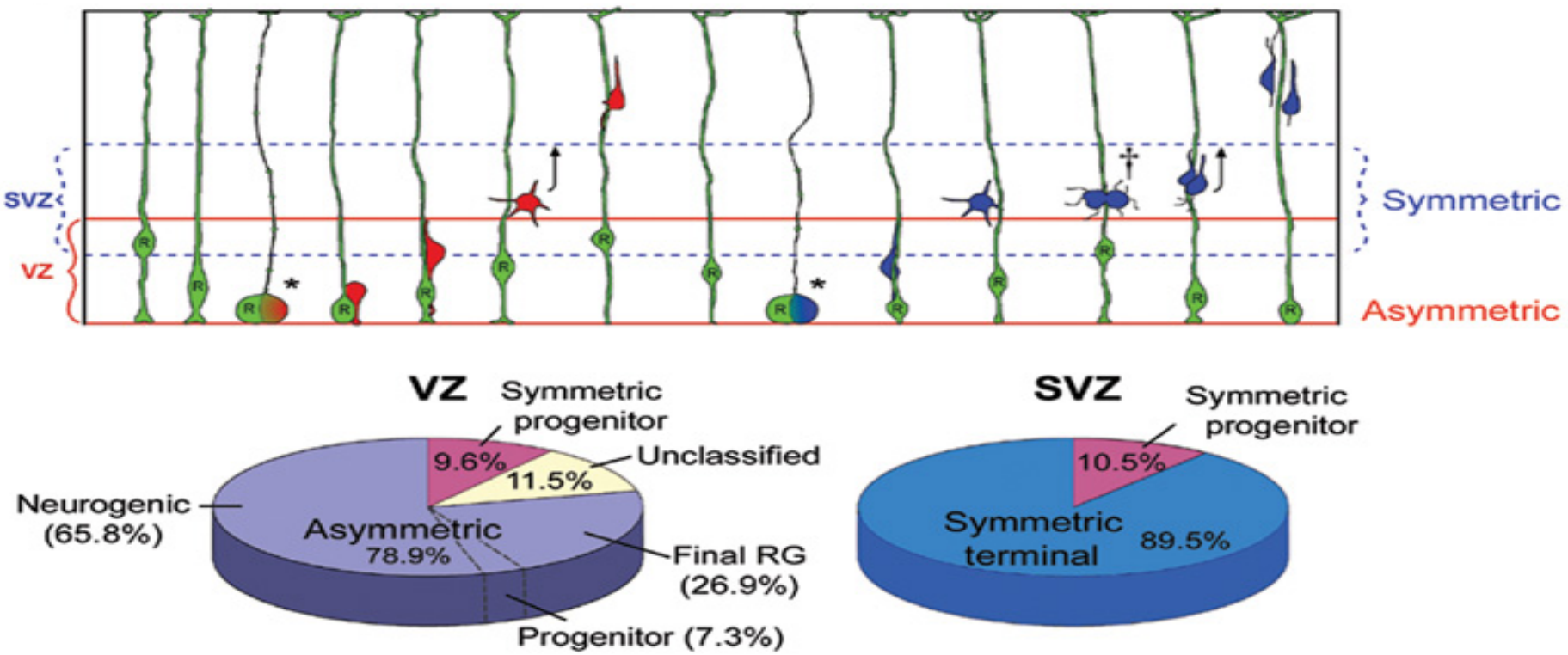
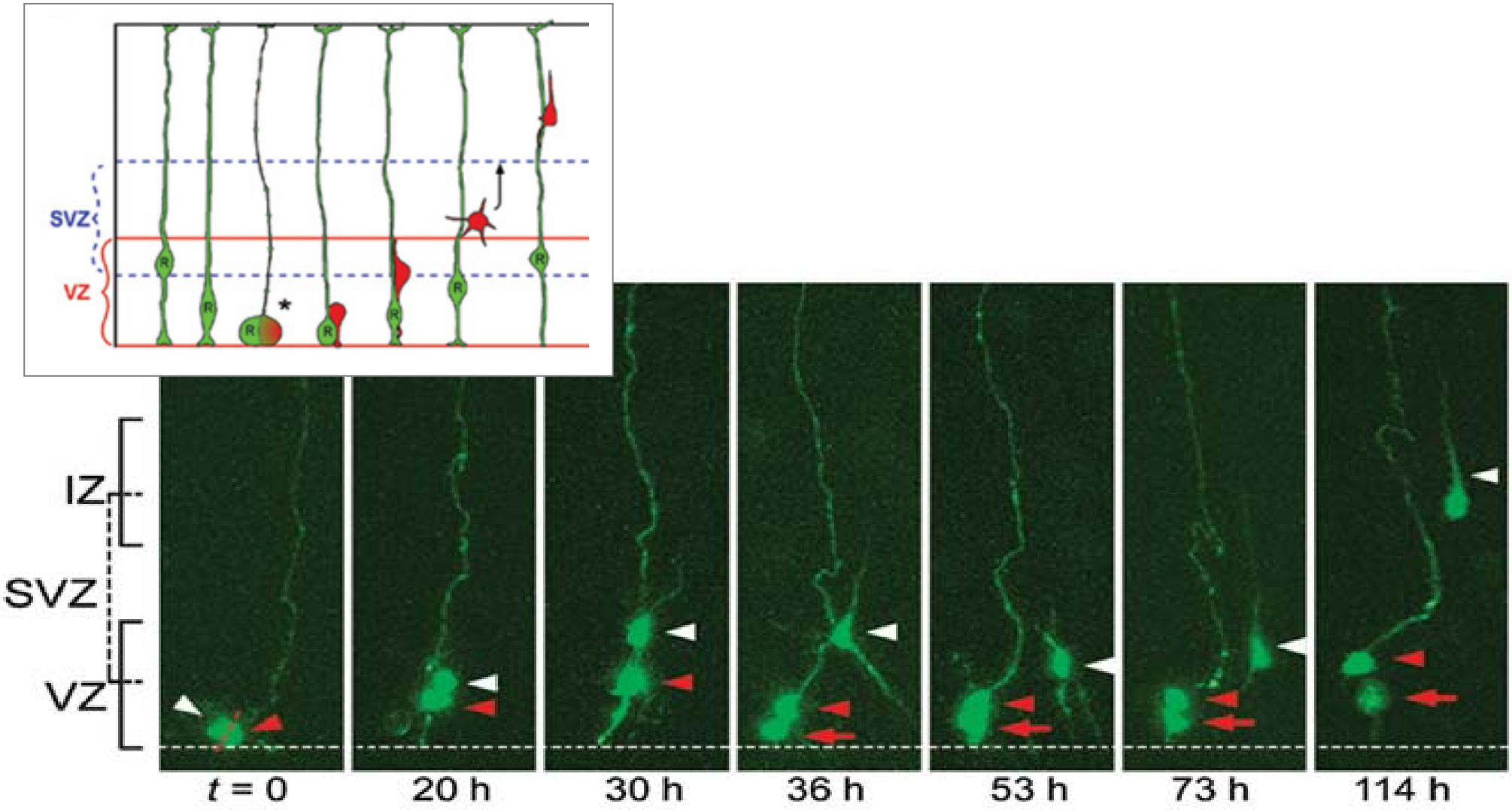





Fig. 1d-f

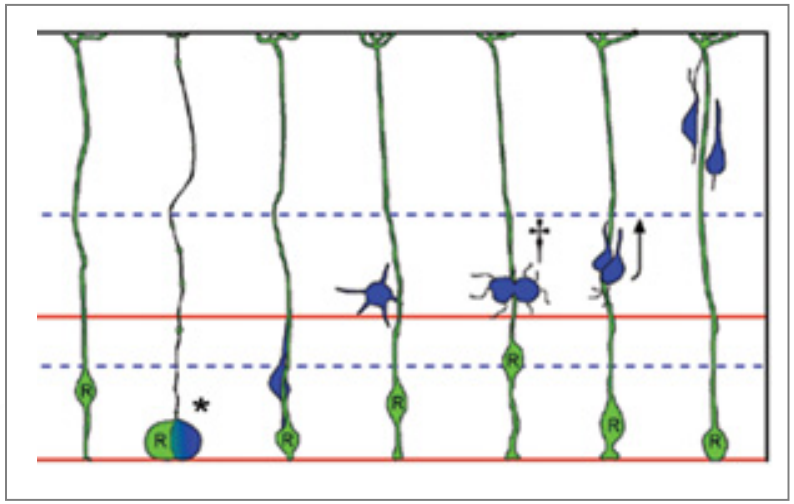
# *Asymmetric division in VZ*




Source: Noctor, S. C., V. Martinez - Cerdeno, L. Ivic, and A. R. Kriegstein. "Cortical Neurons Arise in Symmetric and Asymmetric Division Zones and Migrate Through Specific Phases." *Nature Neuroscience* 7 (2004): 136-144. Courtesy of the authors. Used with permission.

Fig. 1a

# *Symmetric division in SVZ*



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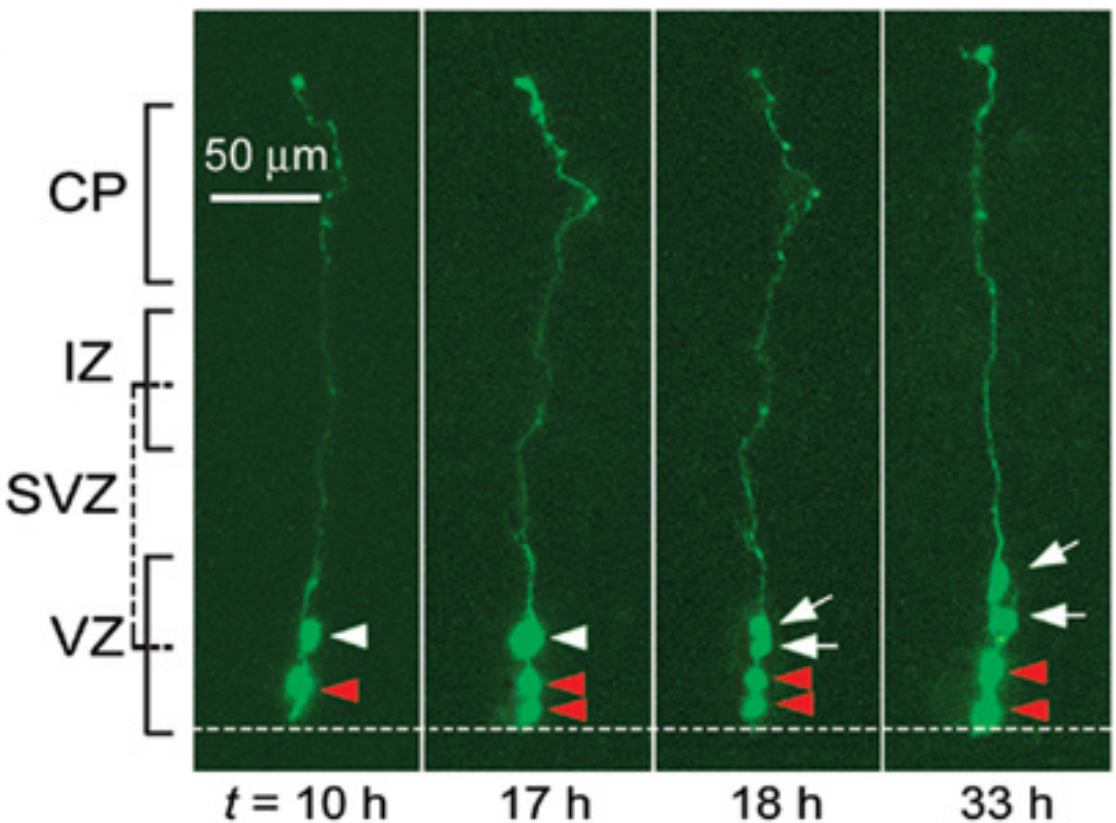


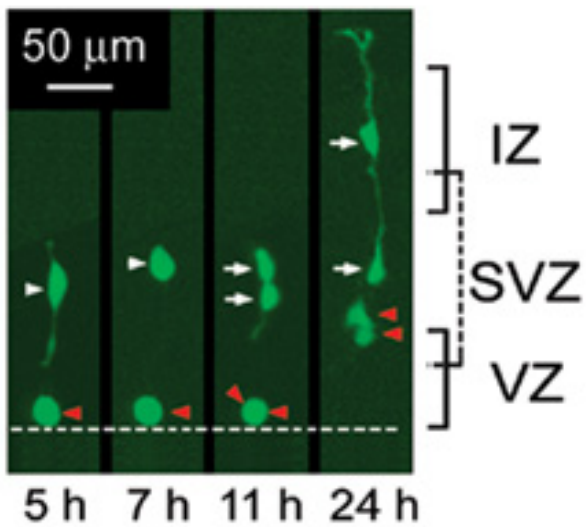
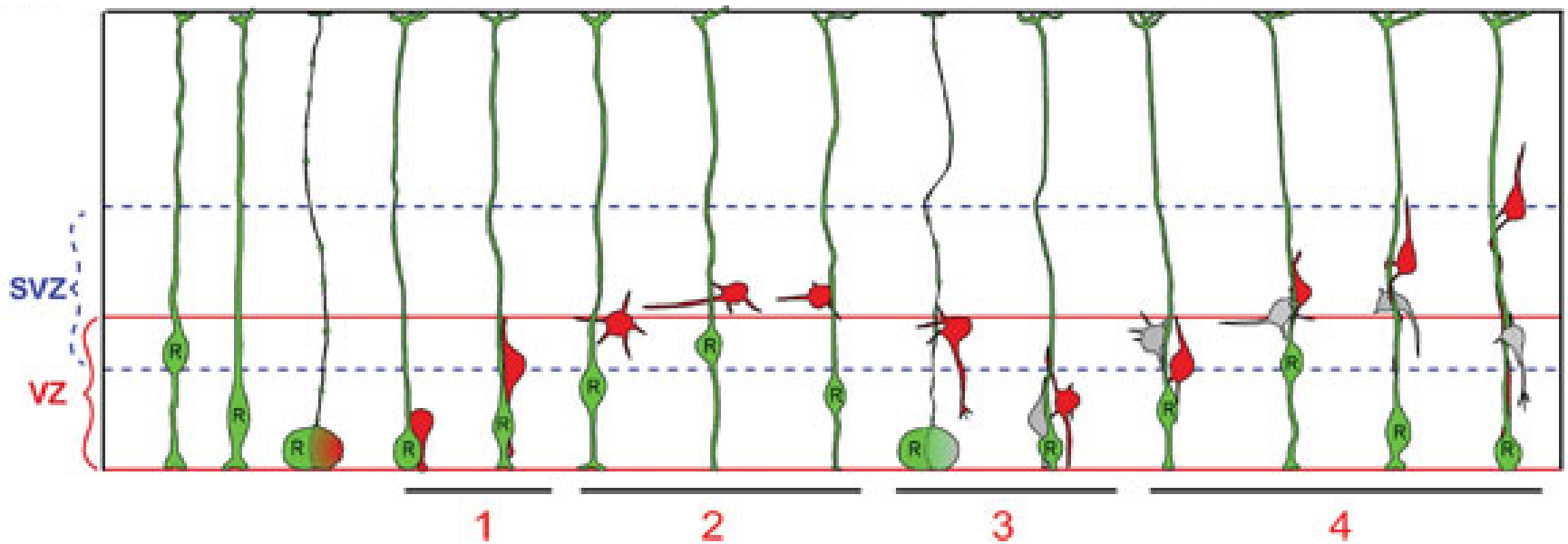




Fig. 2b-c

# *Neuronal migration has four distinct phases*




1. Bipolar; rapid ascent from ventricle to SVZ
2. Become multipolar; pause in SVZ for 24h
3. Extend process (axon?) towards ventricle; migrate back towards ventricle
4. After contact with ventricle, become bipolar again, locomote to cortical plate

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Fig. 2c

# *Neuronal migration has four distinct phases*

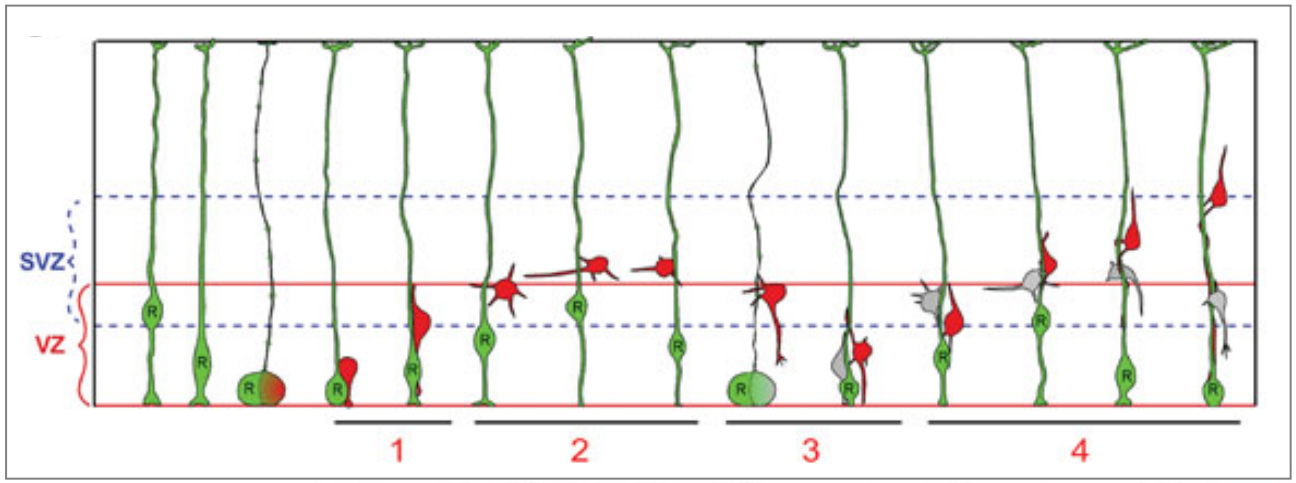
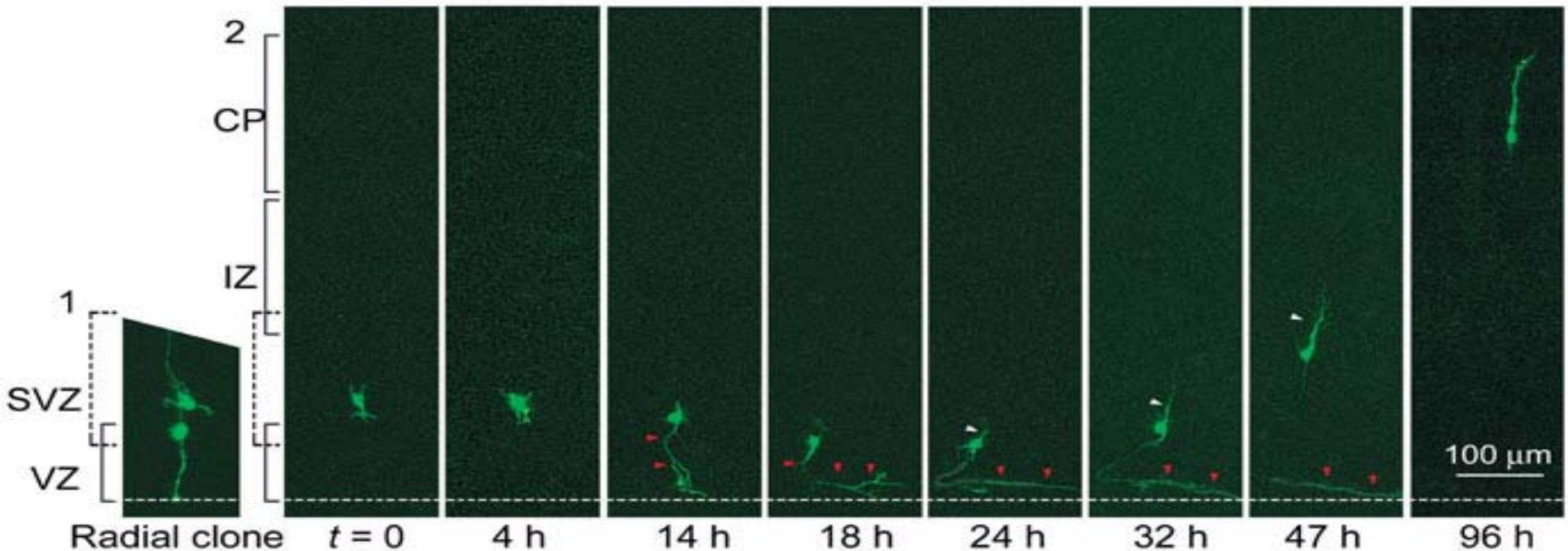



Fig. 2a

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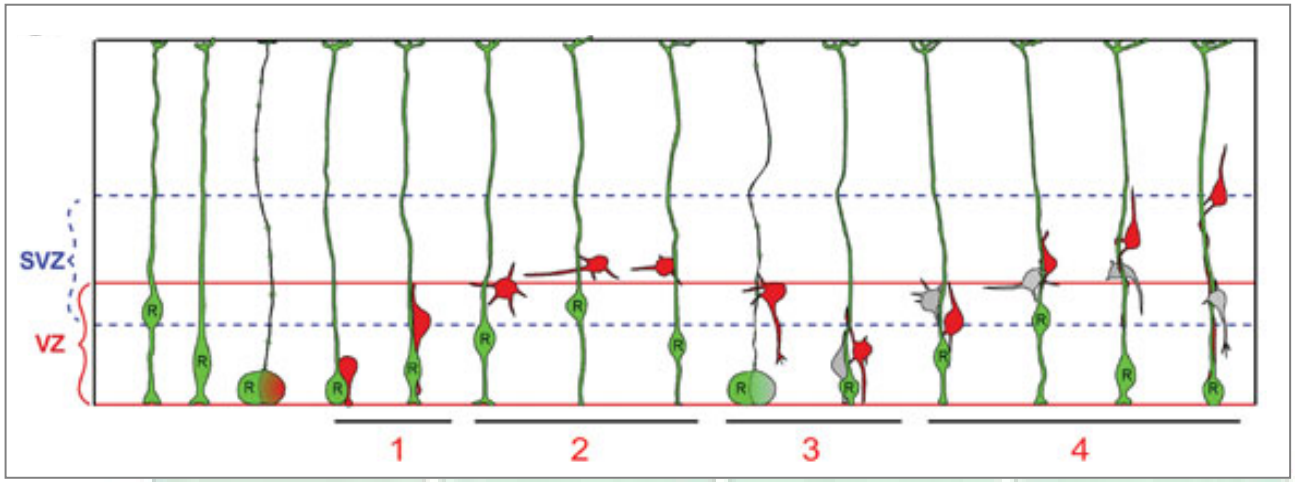
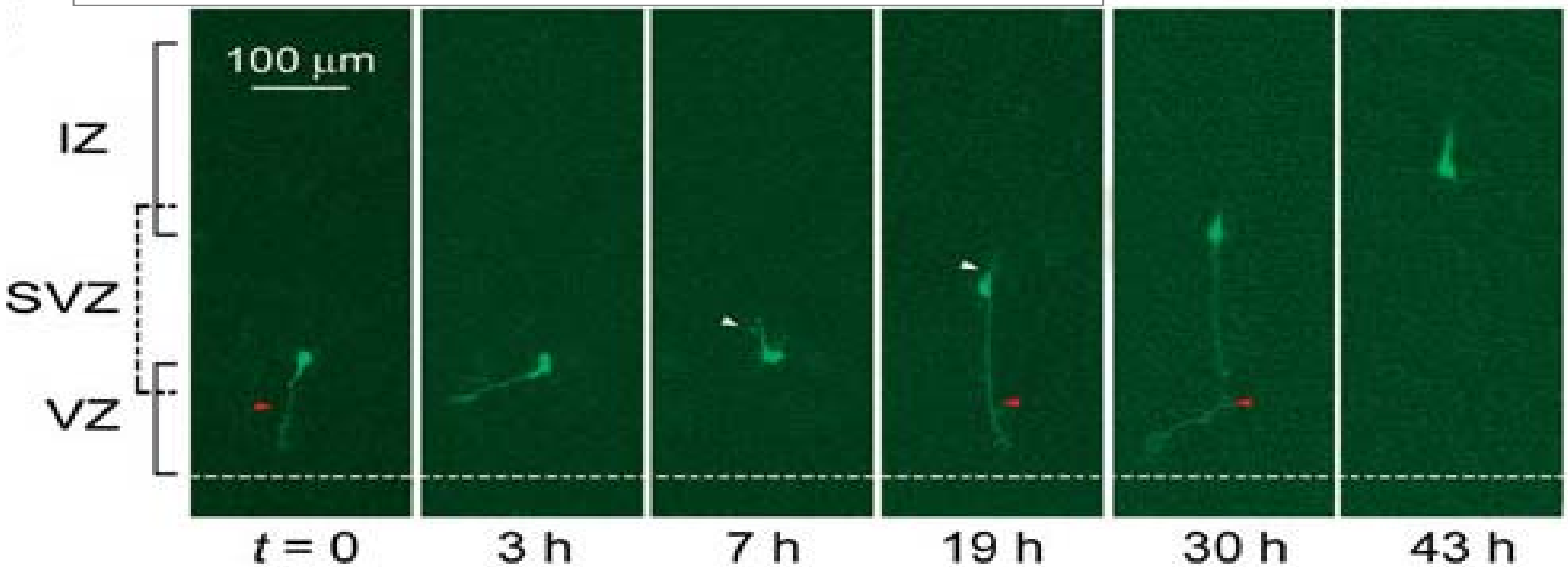



Fig. 2b

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# *Dividing cells in SVZ also migrate retrogradely*

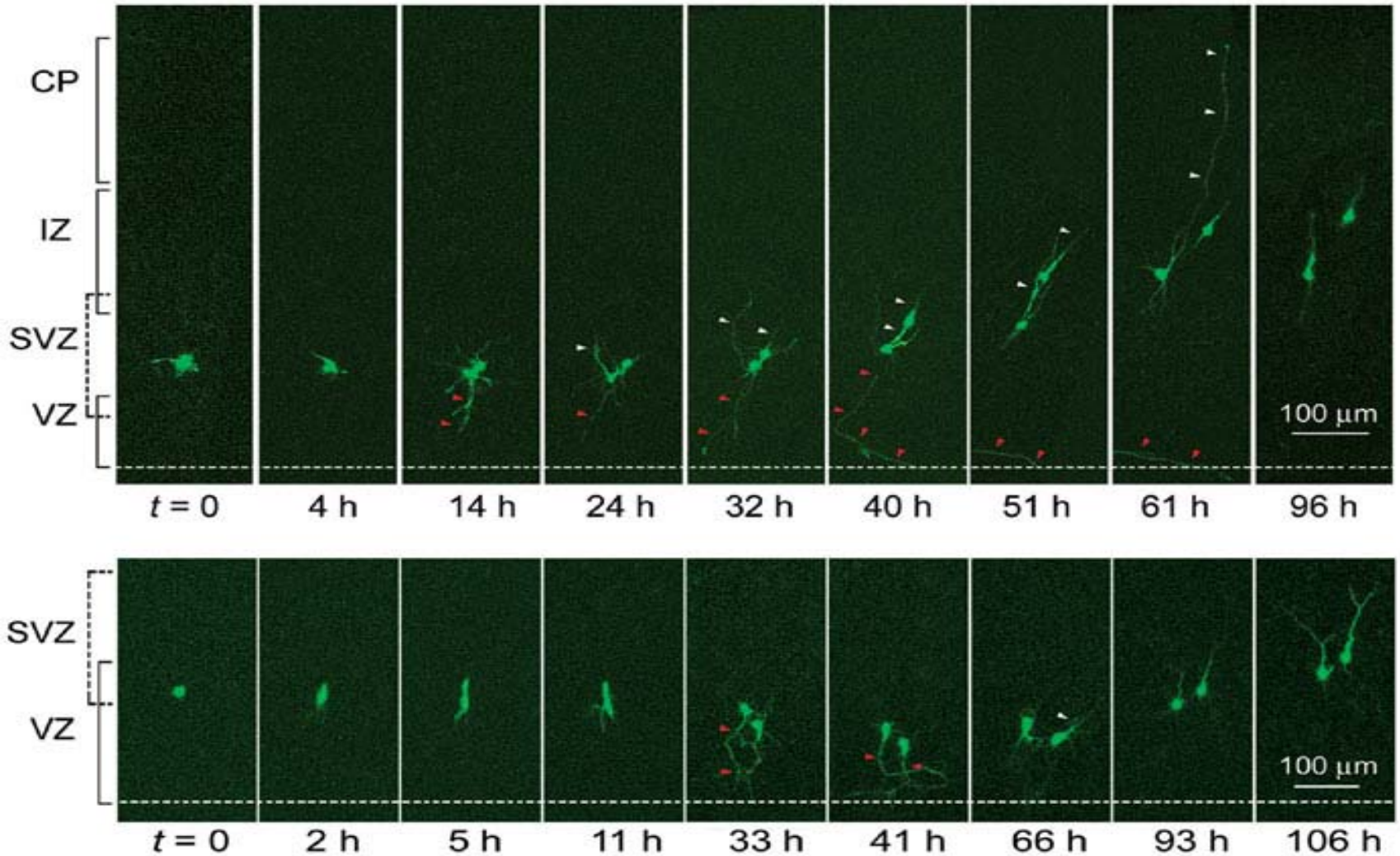
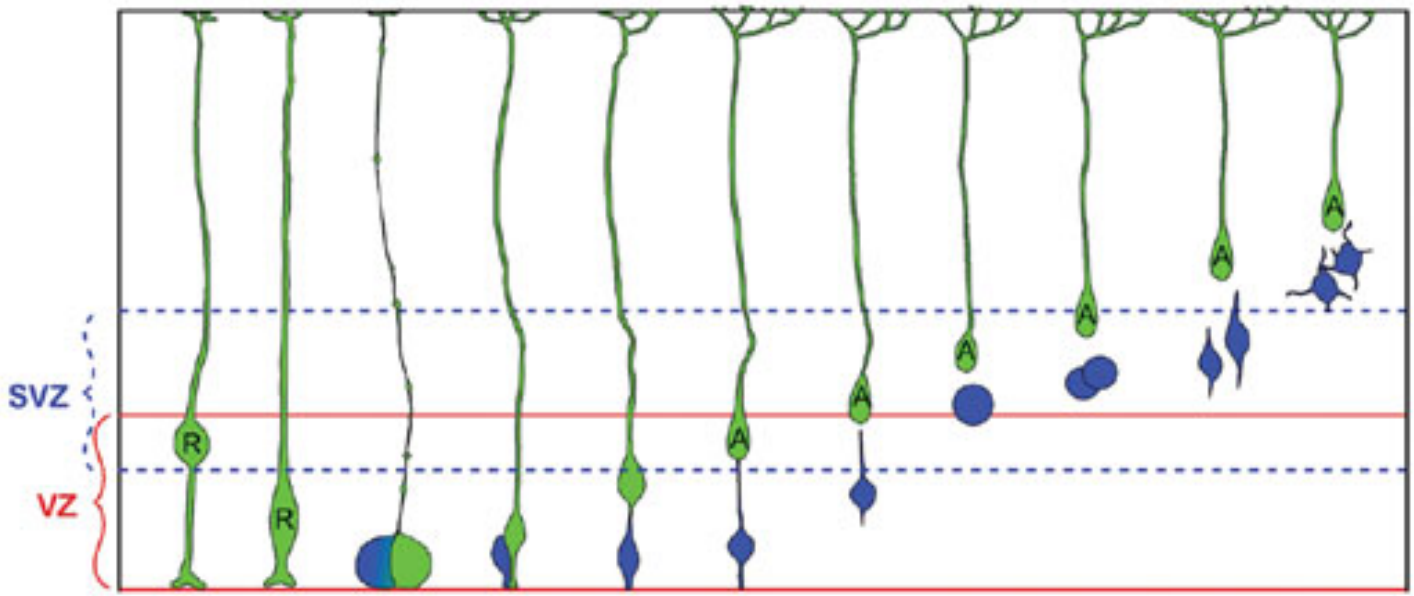





Fig. 3a-b

# Supplementary video 2

# *Radial glial cell final divisions*

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- RGC daughter cell (green) 'inherits' the pial fiber, translocates to CP. Putative astrocyte.
- Other daughter cell (blue) is an intermediate progenitor; undergoes symmetric division. Putative neurons.

Fig. 6c

# *Radial glial cell final divisions*

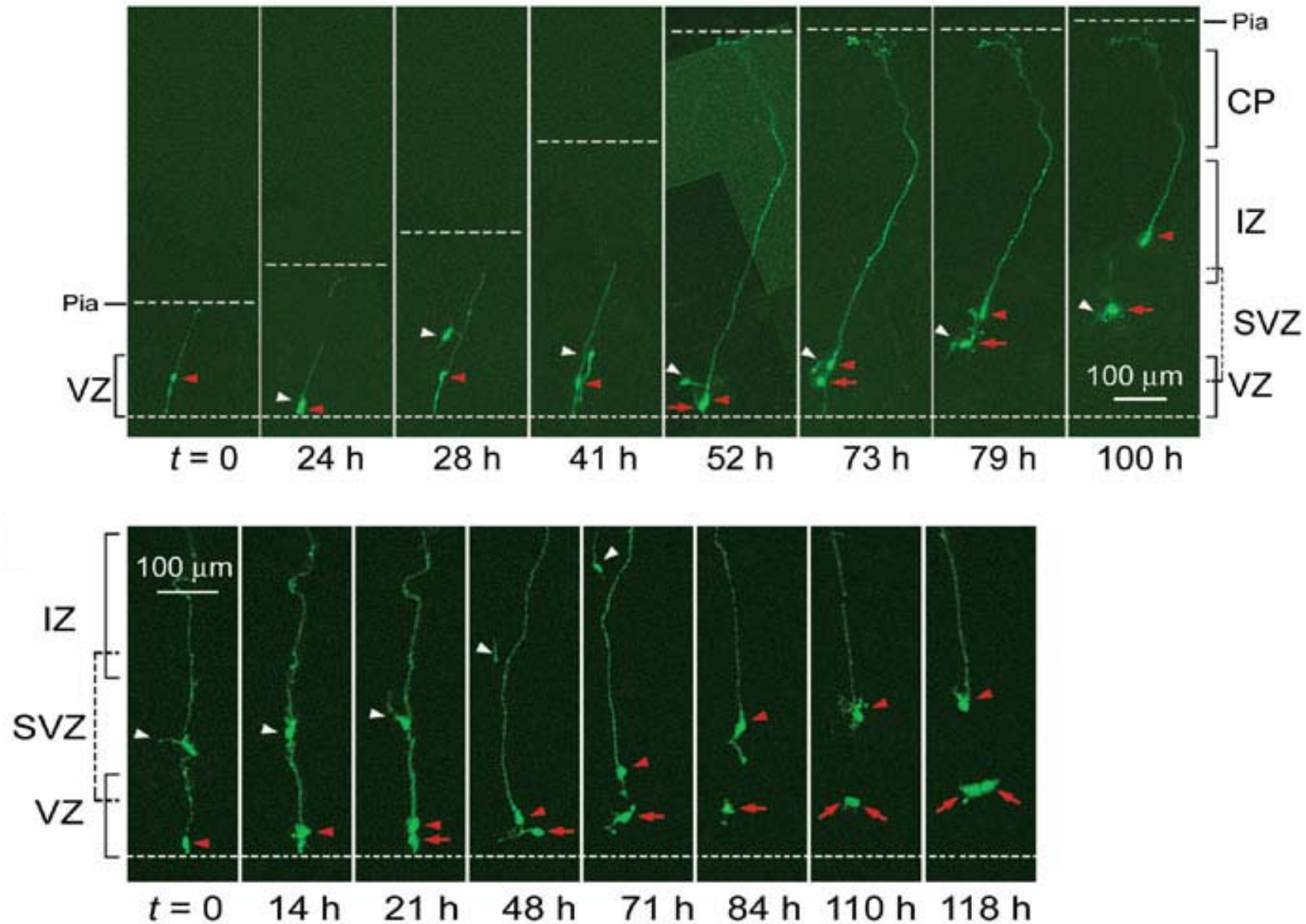


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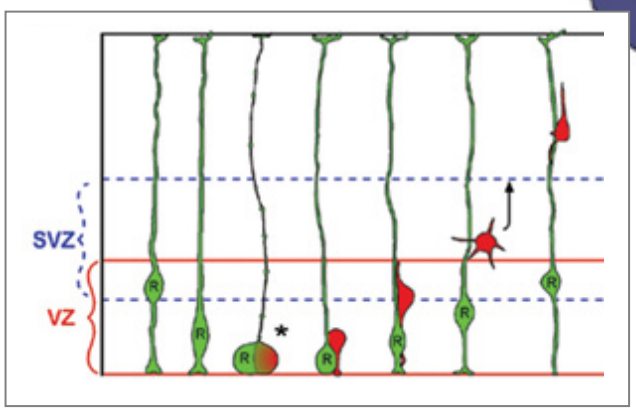
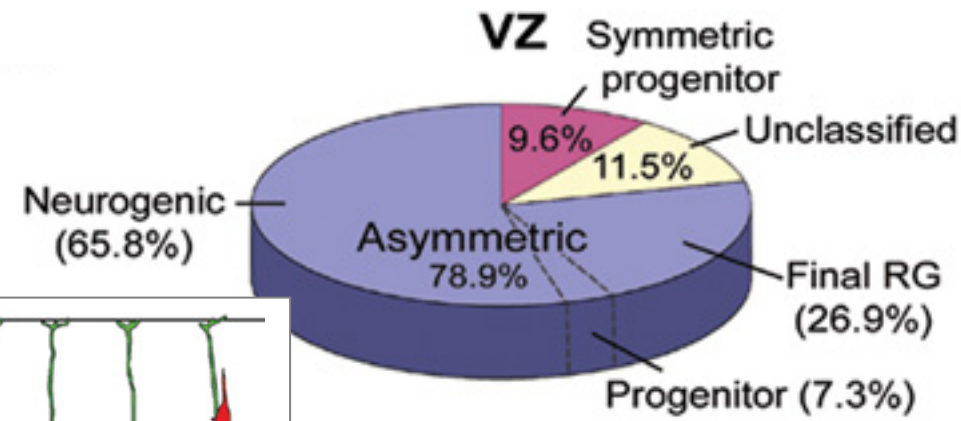
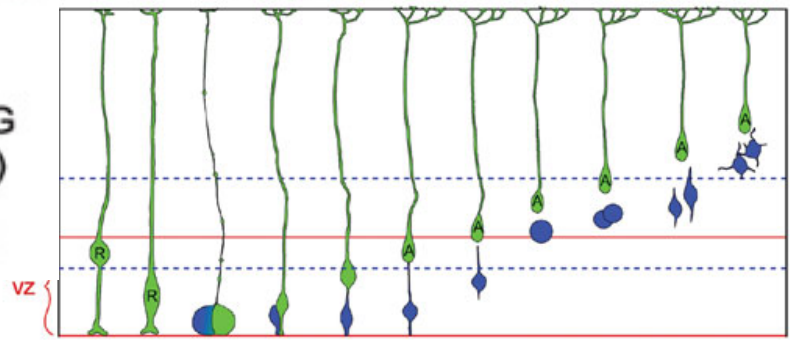
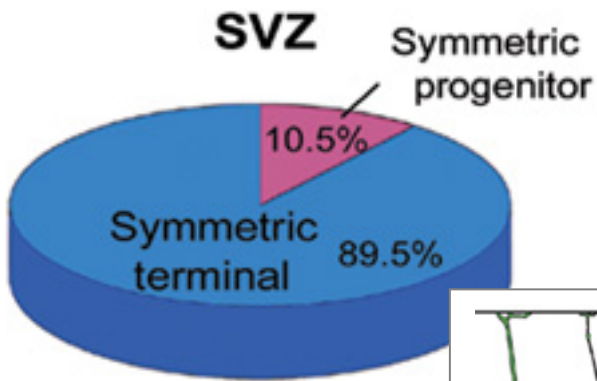
Fig. 6a-b

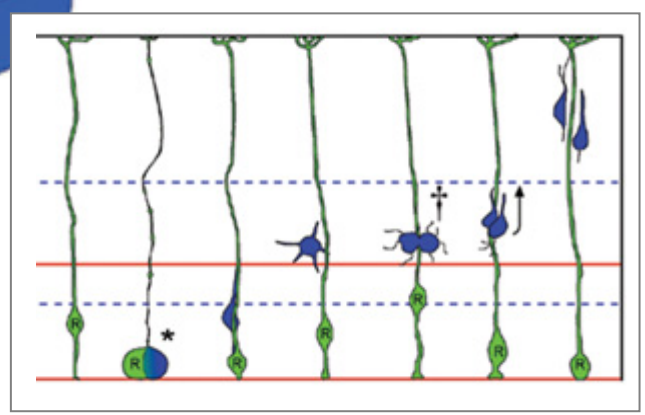
# *symmetric (VZ) and asymmetric (SVZ) division*



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# *Cell identities*

- OK, so we have shown how they divide and migrate. But what kinds of cells are these? Neurons? Glia?
- In particular, are we sure they're of neocortical origin?

- Electrophysiology

Neurons (even immature ones) have unique electrical properties which can be measured using patch clamp.

- Immunostaining

We can use markers for neurons (TuJ1), progenitors (Nestin), neuronal progenitors (Sox1), astrocytes (GFAP), oliodendrocytes (NG2; Olig2), radial glia (Vimentin), and inhibitory interneurons (GABA).

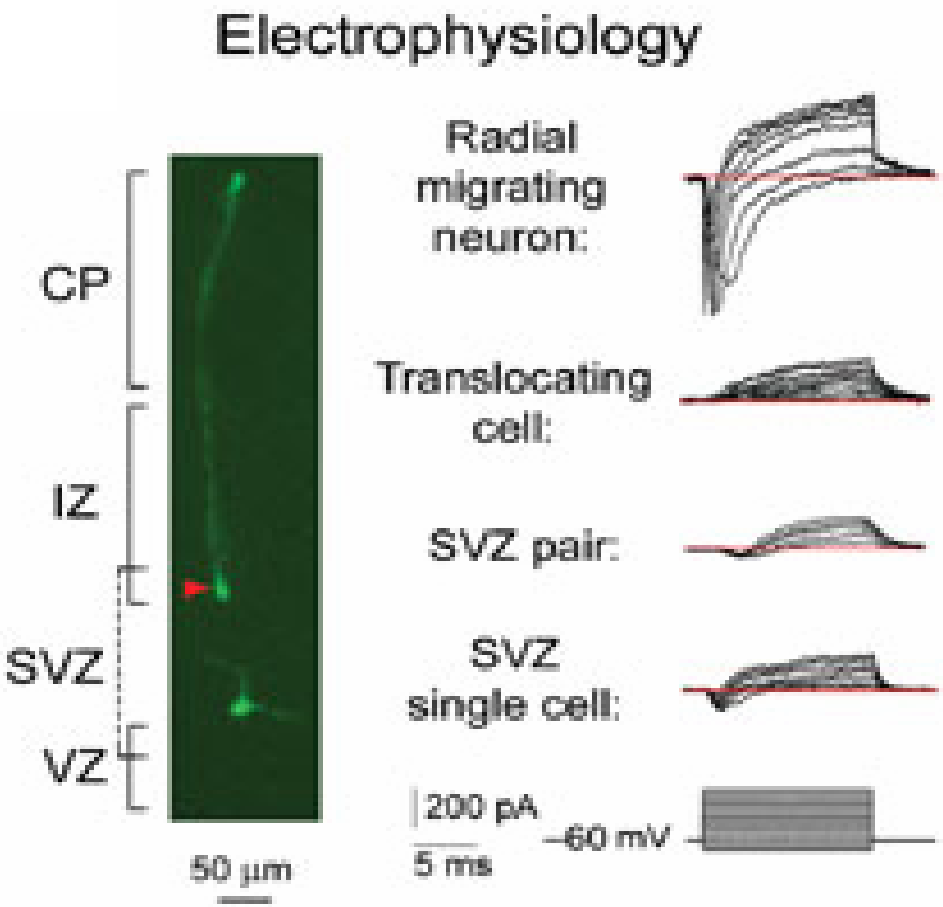


Fig. 7g


Fig. 2b

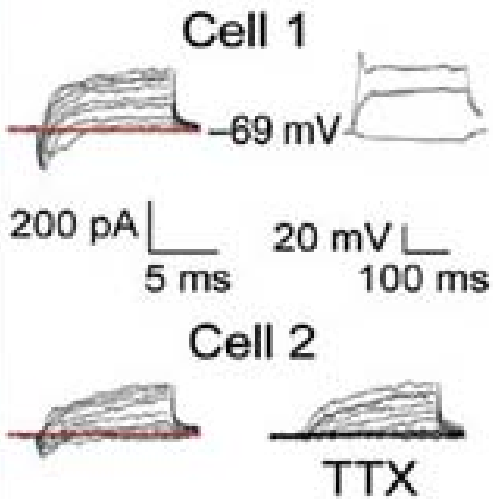


Fig. 3c

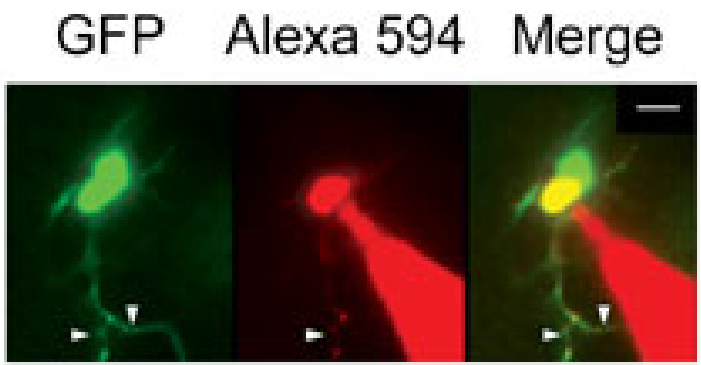


Fig. 5d

# *Are single-cell clones GABA-ergic cells or glial precursors that have migrated tangentially?*

- No: RGCs (4a) are Sox1$^{+}$(neuronal progenitor), give rise to daughters that are, TuJ1$^{+}$ (immature neuron), nestin$^{-}$, and GABA$^{-}$.
- Also, no staining in VZ/SVZ cells with oligodendrocyte precursor antibodies.

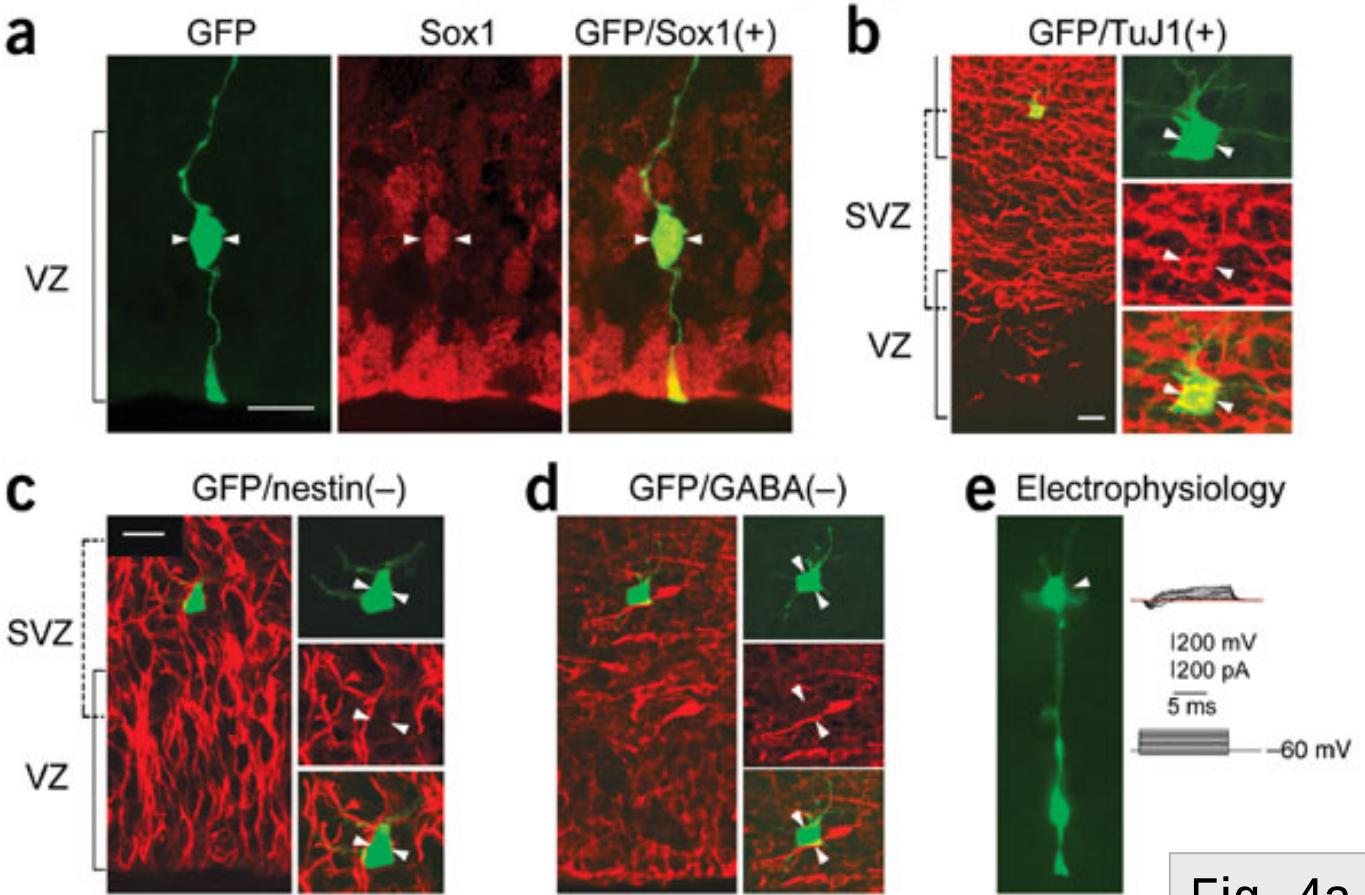


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Fig. 4a-e

# *Same goes for double-cell clones*

- No: Nestin- (not progenitors), TuJ1$^{+}$ (immature neuron), GABA$^{-}$.

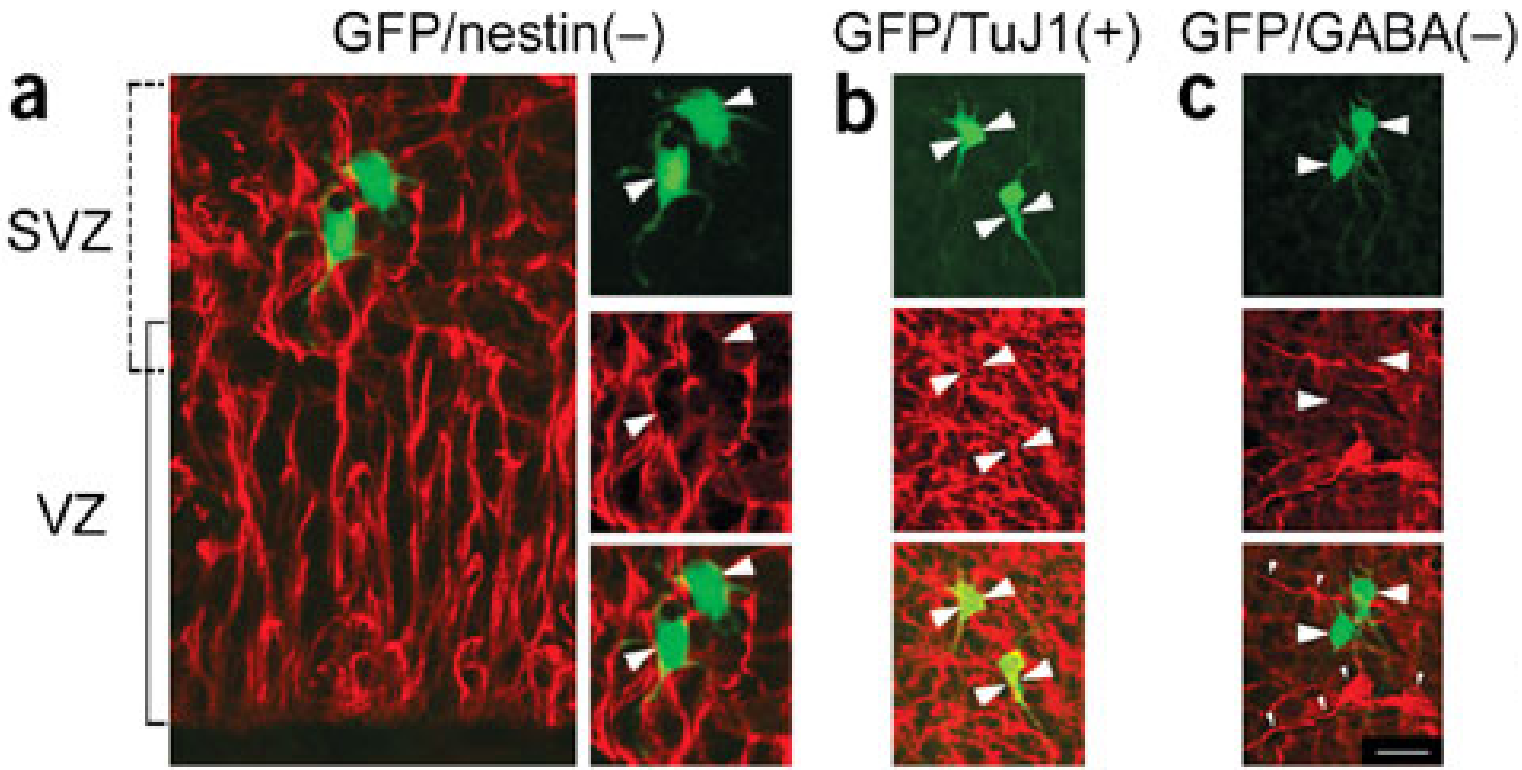
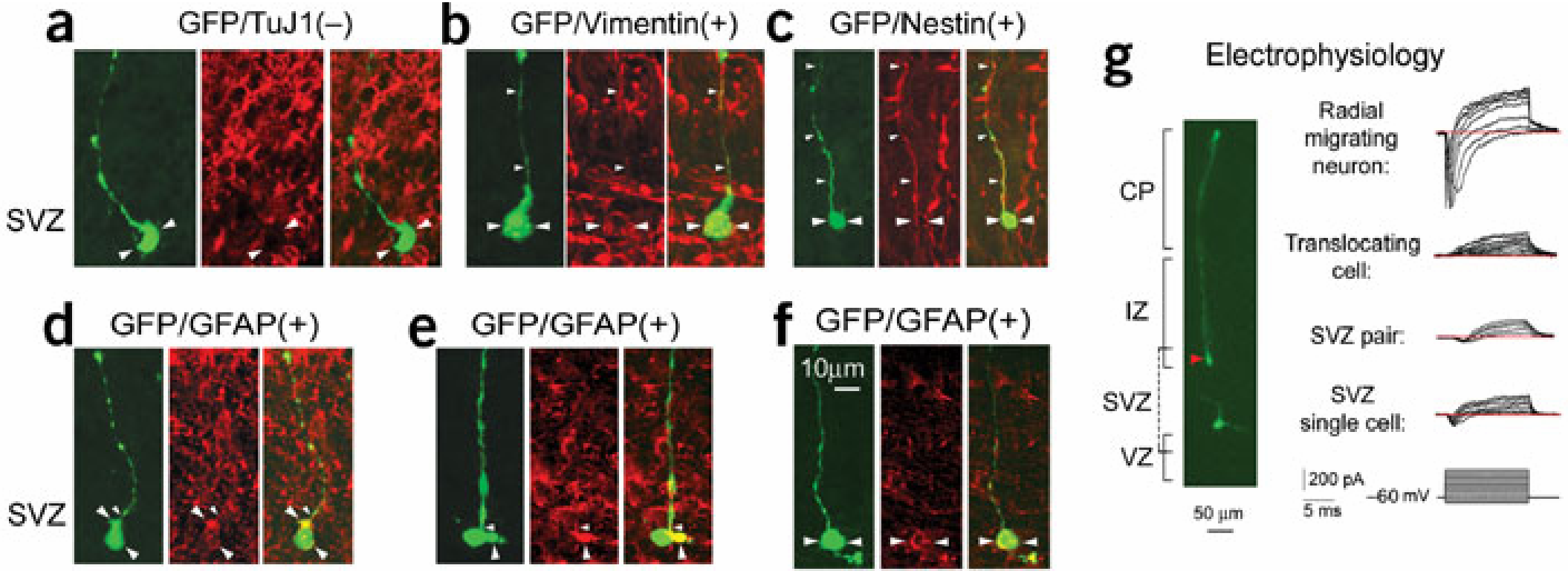





Fig. 5a-c

# *Translocating RGCs after final division are likely astrocytes*

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- TuJ1- (not neurons), Vimentin+, Nestin+, GFAP+ (astrocyte-specific).
- Also, ephys agrees these aren't immature neurons.

Fig. 7

# *Summary*

- Time-lapse monitoring revealed a stereotyped migration, featuring an unexpected ‘retrograde’ step
- Cleared up old question of what happens to RGCs after cortical development (become astrocytes)
- Showed that VZ/SVZ are specialized for asymmetrical/symmetrical division (consistent with *numb* distribution in these areas)

# *Concerns*

- Slice is not brain. Many effects were only seen after *days* in culture. Needs to be replicated in vivo (but how?)