Postnatal Fate of Cajal-Retzius Neurons in Mouse Neocortical Development

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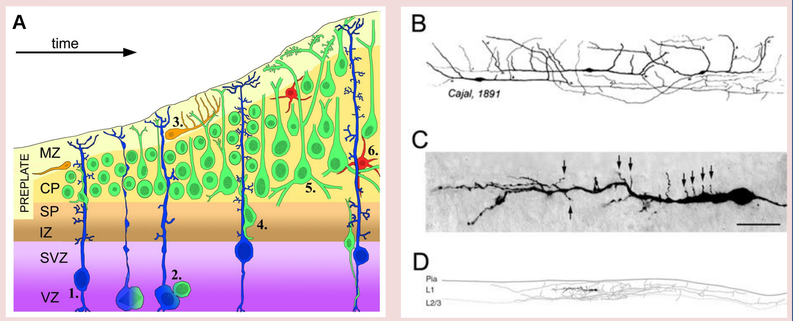
INTRODUCTION

- CR neurons are the first born neurons in the developing cortex where they play a crucial role in neuronal migration and cortical lamination.
- A majority of CR neurons gradually disappear during postnatal development.



Characteristics of Cajal-Retzius neurons. A: CR neurons (orange) guide neuronal migration through secretion of Reelin. **B:** CR neurons drawn by Ramón y Cajal. **C:** A typical CR neuron in developing rat neocortex (Scale bar is 20 µm). **D:** camera lucida drawing of the axon and dendrite of the CR neuron in C. (Radnikow et al., J Neurosci, 2002).

- What is the cause of CR neuron disappearance?
- Where do CR neurons come from?
- What is the role of surviving CR neurons?

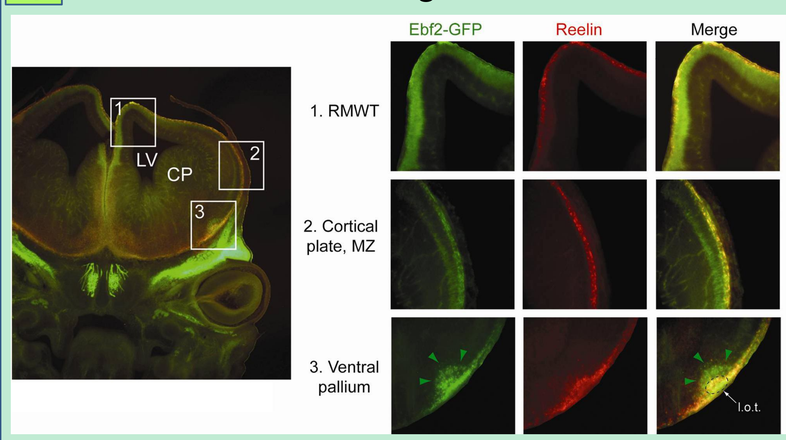
HYPOTHESIS: CR neurons may have additional functions in brain development, perhaps in the structural maturation of pyramidal neurons and their integration into functional cortical circuits.

MATERIALS AND METHODS

- Immunohistochemistry for Reelin and caspase3.
- In vivo two-photon microscopy in transgenic Ebf2-GFP mice line for chronic imaging of CR neurons.
- Patch-clamp electrophysiology in acute slices for whole-cell recordings.

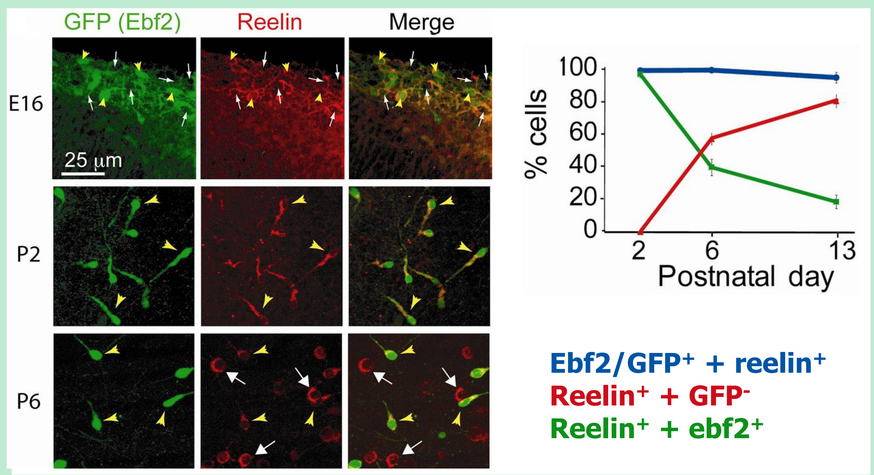
RESULTS

1 Ebf2-GFP+ cells emerge from Ventral Pallium



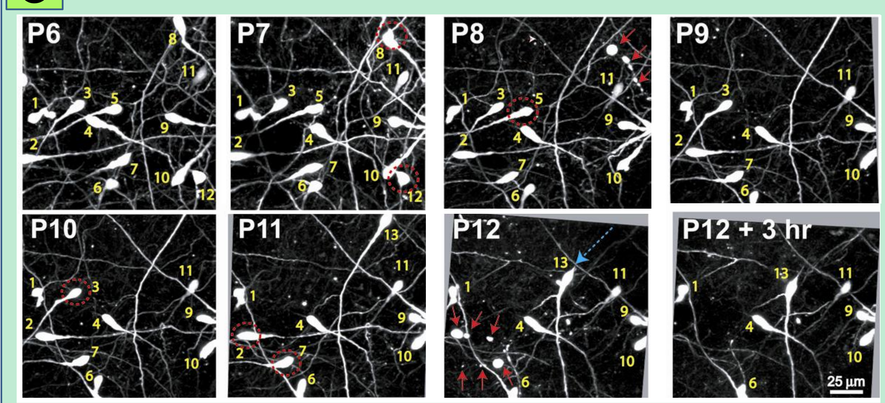
GFP+ cells in embryonic Ebf2-GFP mice are present in the ventral pallium. Immuno-staining for reelin (red) in the cortex of Ebf2-GFP embryos at E14. Note that GFP is highly expressed in the rostromedial wall of the telencephalic vesicle (RMWT; 1) and in the marginal zone of the cortical plate (2). It is also expressed in the ventral pallium (3), a progenitor zone that contains CR cell precursors that do not yet express reelin (green arrowheads). Double-labeled CR cells are present in the lateral olfactory tract (l.o.t.) and throughout the cortical plate.

2 Ebf2 is a marker of all postnatal CR neurons



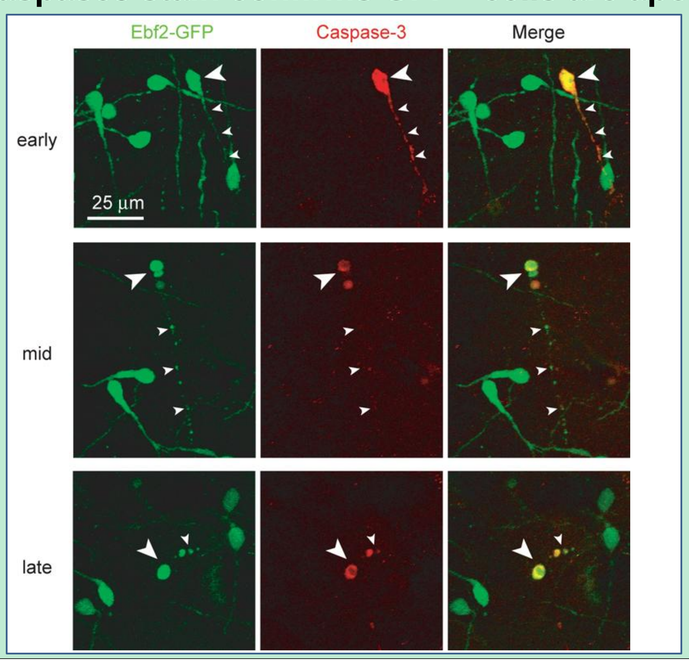
Reelin and GFP expression during embryonic and postnatal development Tangential sections cut through cortical layer 1 in fixed brains from Ebf2 mice at E16, P2, and P6, stained with antibodies to reelin (red), and then imaged with two-photon microscopy (green: native GFP fluorescence). Quantitative analysis of the correspondence of reelin immuno-reactivity and GFP throughout development.

3 CR Neuron disappearance caused by apoptosis



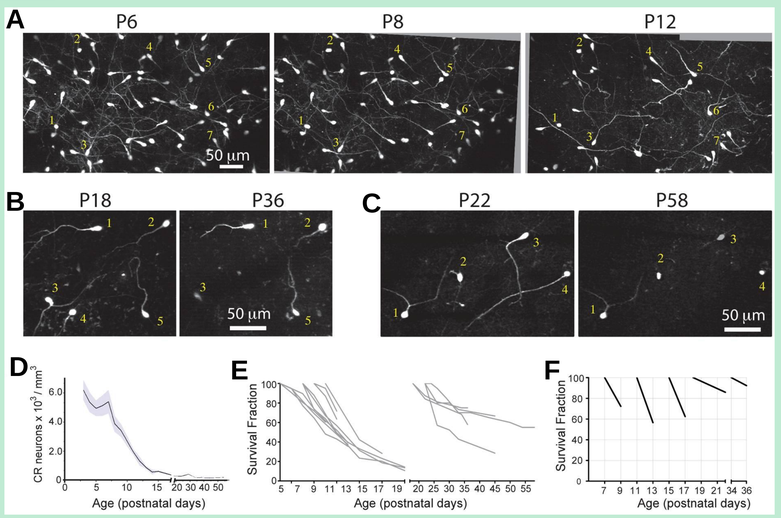
Time-lapse imaging in Ebf2-GFP mice confirms the death of CR neurons by apoptosis. Serial imaging of Ebf2-GFP mice with in vivo two-photon microscopy to re-identify the location of individual CR neurons over time. All images are max intensity projections of 30 slices, 2 µm apart. In many cases we "caught" the death of CR neurons by apoptosis (red arrows at P8 and P12).

4 Caspase3 stain confirms GFP+ cells are apoptotic



Caspase3 immunohistochemistry revealed that the vast majority of CR cells are apoptotic. Coronal slices of a fixed brain from a P8 Ebf2 mouse, stained with antibodies for caspase3 (red), revealed that many GFP+ CR neurons (green) were apoptotic. Panel shows examples of varying stages of apoptosis.

5 Some CR Neurons persist into adulthood



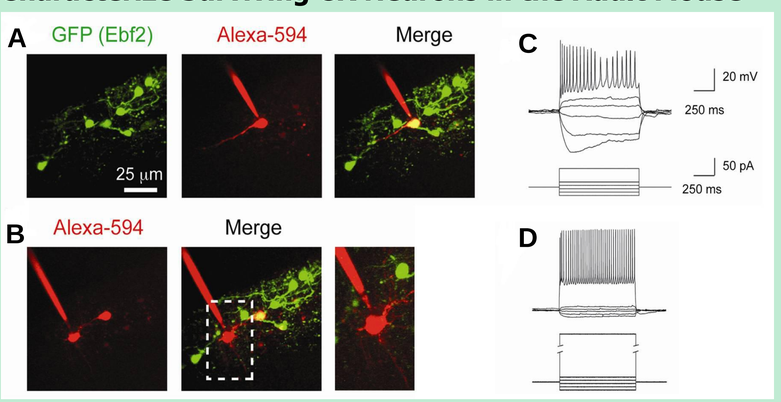
Quantitative analysis of CR cell counts and disappearance rates A,B,C: Time-lapse in vivo two-photon imaging of GFP+ CR neurons in Ebf2 mice at different ages. Images are max intensity projections of ~30 slices, 3 µm apart. **D:** CR cell density throughout postnatal development. **E:** Survival fraction (% CR neurons retained over subsequent imaging sessions) throughout postnatal development. Gray lines represent data from a single animal imaged chronically. Data for this graph came from 17 different mice. **F:** 2- or 4-day survival fractions at P7-P9 (3 mice), P11-P13 (3), P15-P17 (3), P18-P22 (2), P22-P26 (5), and P34-P36 (2).

CONCLUSIONS

- Postnatal CR neurons originate from the ventral pallium and express Ebf2.
- The vast majority of CR neurons die during early postnatal development by apoptosis.
- CR numbers are relatively stable after the 3rd postnatal week.
- ~2-3% of CR neurons present at birth survive to adulthood.

FUTURE DIRECTIONS

Characterize Surviving CR Neurons in the Adult Mouse



A: GFP+ cells in a P8 Ebf2 mouse were targeted for patch-clamp recordings in acute coronal brain slices and filled with Alexa-594 (red). **B:** We also recorded from a GFP- interneuron. **C:** Current clamp recording of a typical GFP+ cell showing broad action potentials with adaptation and a sag of in the hyperpolarizing response, characteristic of CR neurons. **D:** Recording of a fast spiking interneuron in L1. Current injection scale: -25 pA, -15 pA, -5 pA, +5 pA, +15 pA, and +55 pA (C) or +255 pA (D).

ACKNOWLEDGEMENTS

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