

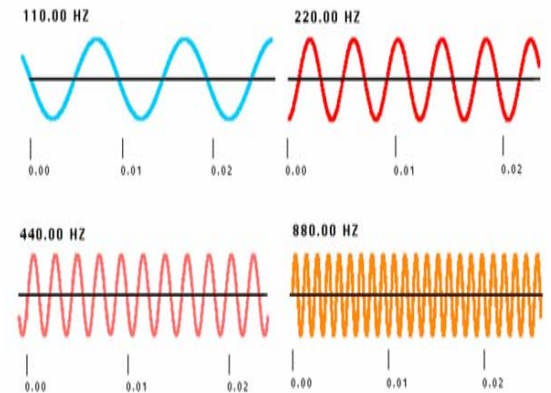
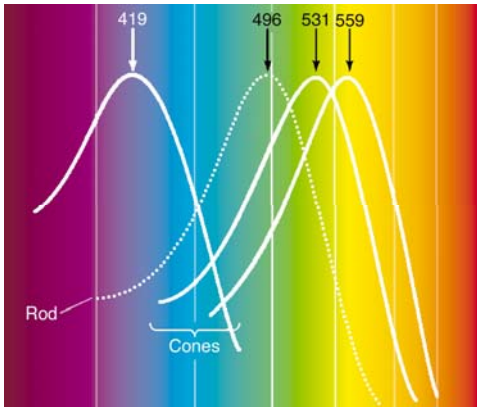
Ectopic Gene Expression in Bulbar Interneurons by Postnatal Electroporation

Dong-Jing Zou
Department of Biological Sciences
Columbia University

Making Sense

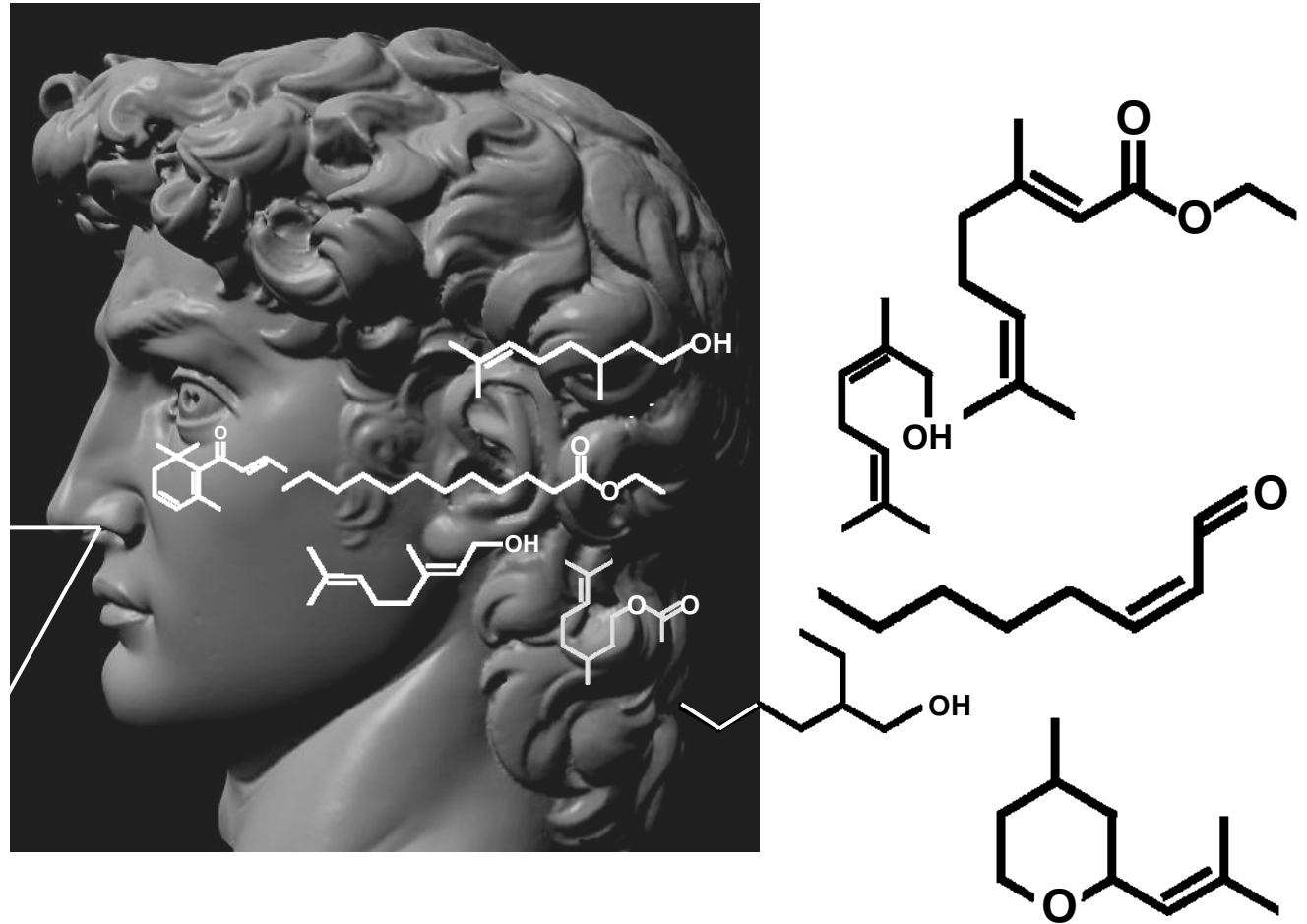


Making Sense



Making Sense of Scents

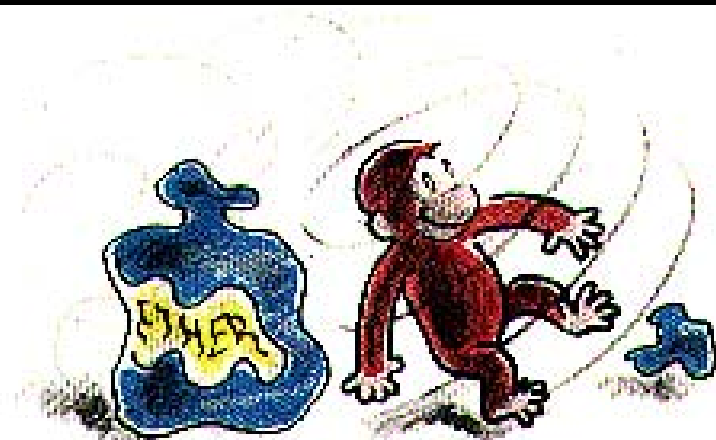




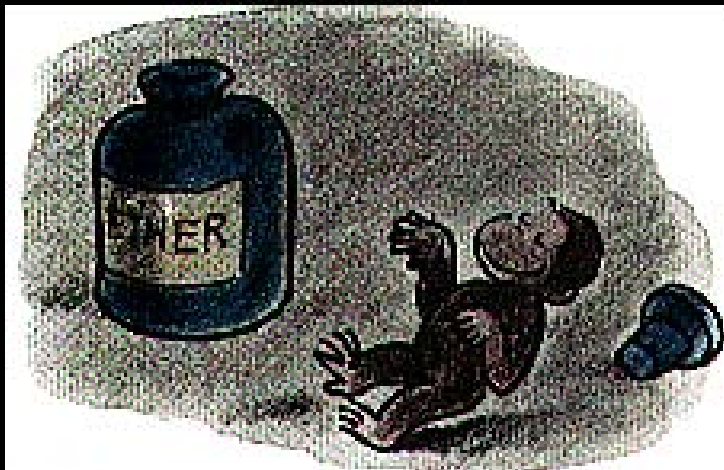
Curious George



It smelled funny!



Suddenly his head began to turn.



then everything went dark...



Cellular Organization of Olfactory Epithelium

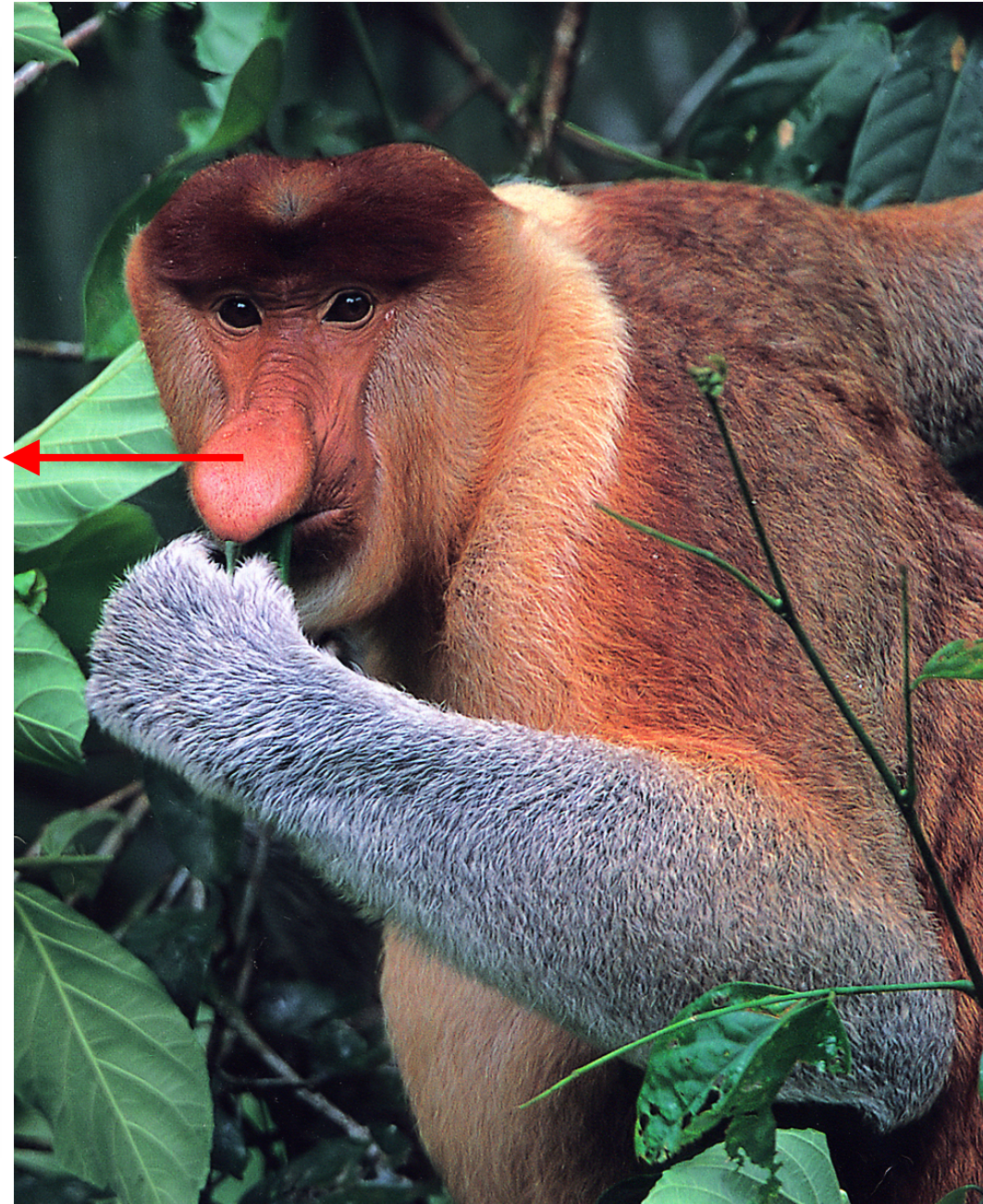
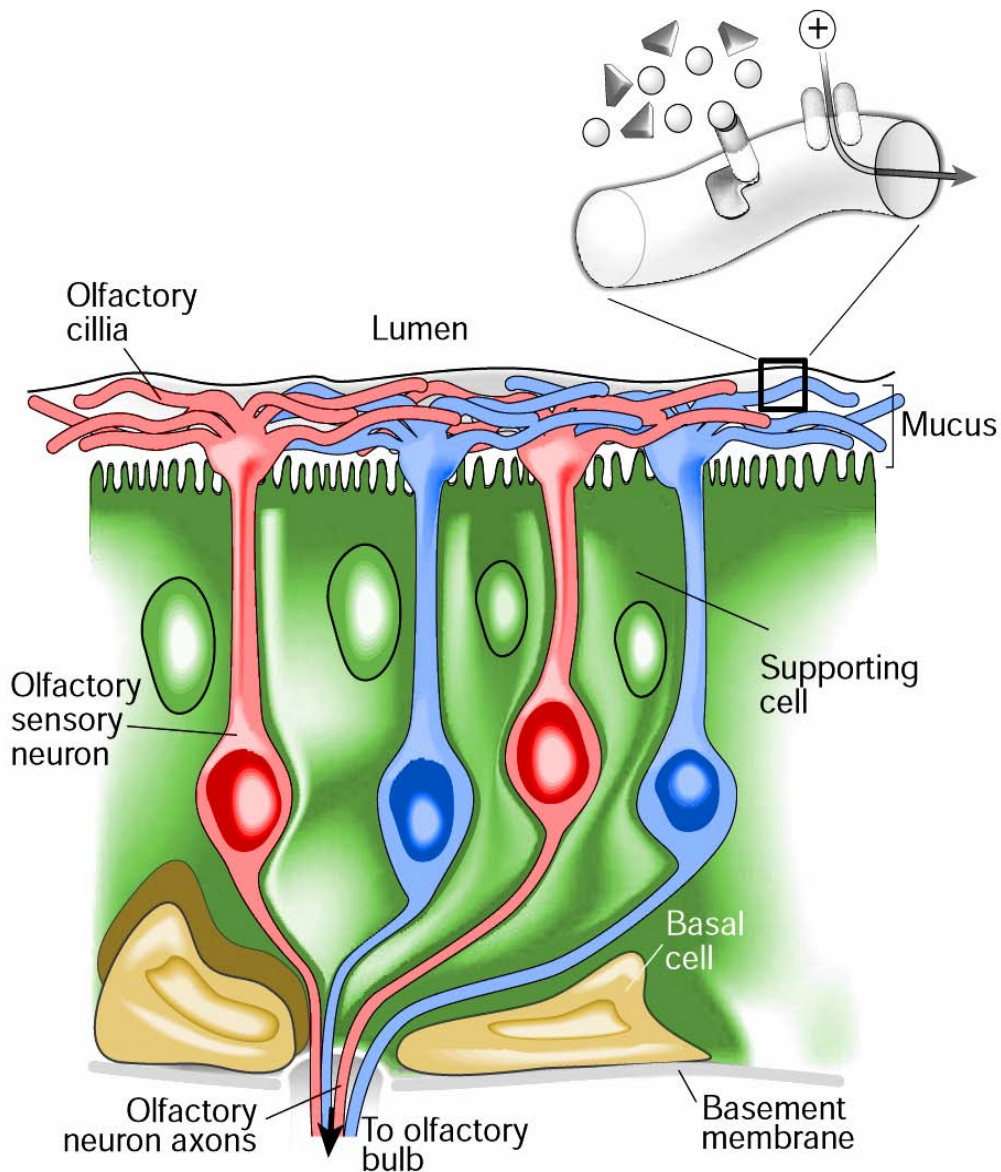
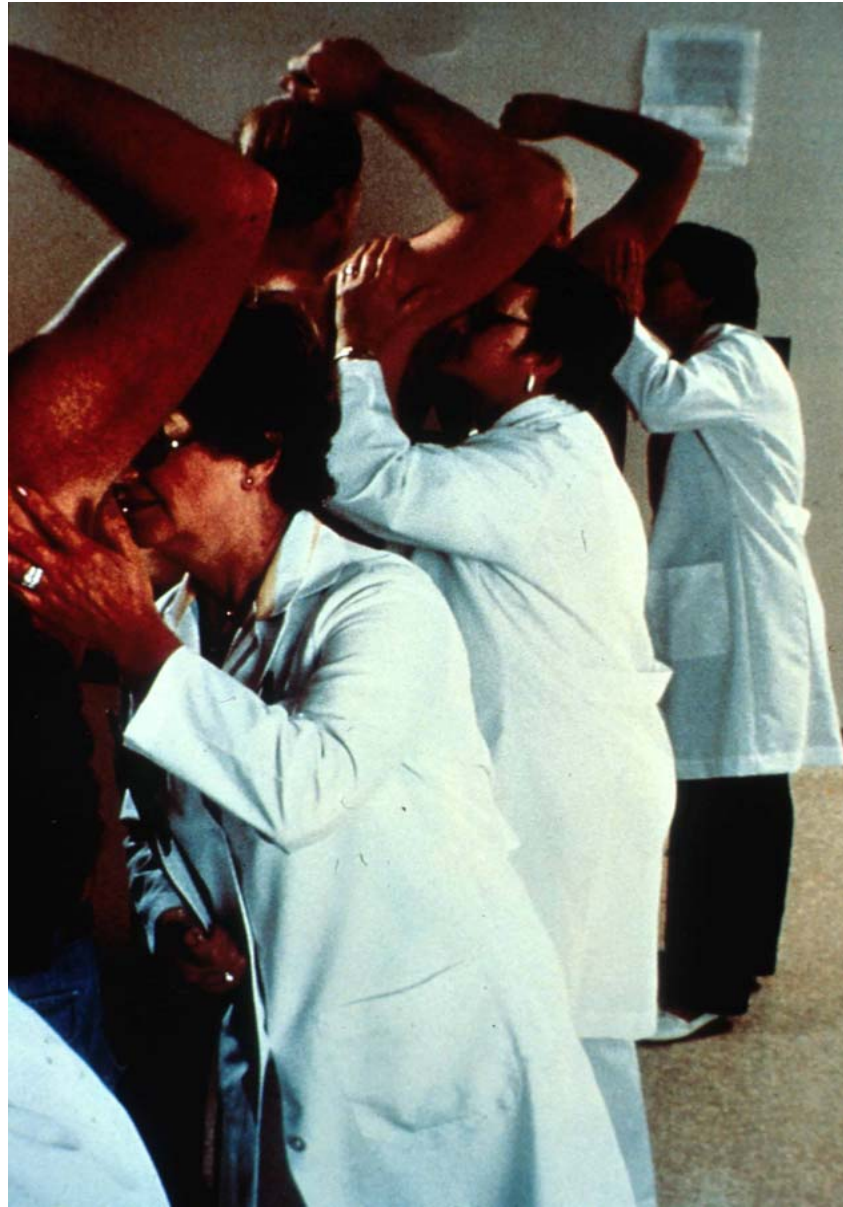


Photo by Tim Laman, *National Geographic*

Olfactory Experiments in the Old Days



THE BREAKTHROUGH!

Cell, Vol. 65, 175–187, April 5, 1991, Copyright ©1991 by Cell Press

A Novel Multigene Family May Encode Odorant Receptors: A Molecular Basis for Odor Recognition

Linda Buck* and Richard Axel*†

*Department of Biochemistry and Molecular Biophysics

†Howard Hughes Medical Institute

College of Physicians and Surgeons

Columbia University

New York, New York 10032

Odorant Receptor (OR) Genes

the Largest Gene Family

Organism



worm



Fruit fly



Zebra fish

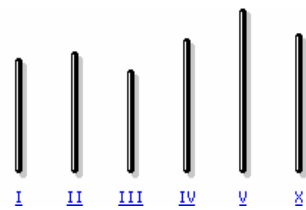


Mouse



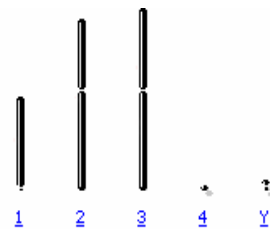
Human

Genome



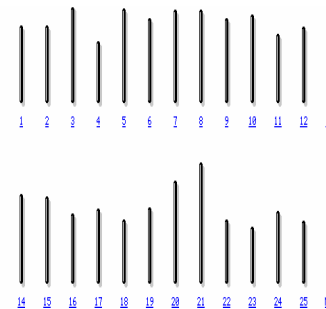
97 Mb

19,000 genes

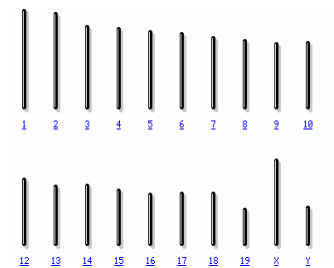


120 Mb

13,000 genes

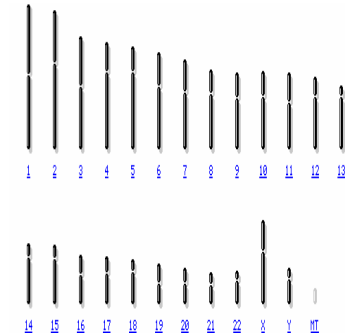


1.7 Gb



2.8 Gb

40,000 genes



2.9 Gb

40,000 genes

OR
genes

500 CRs
2.6%

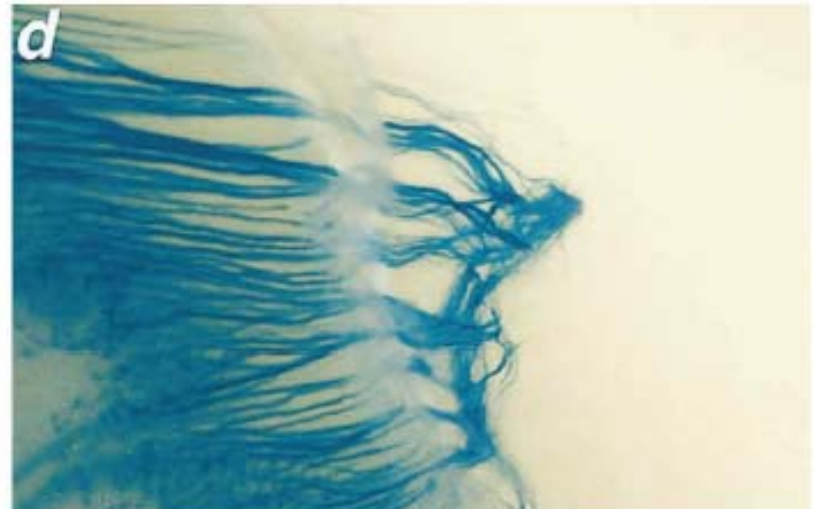
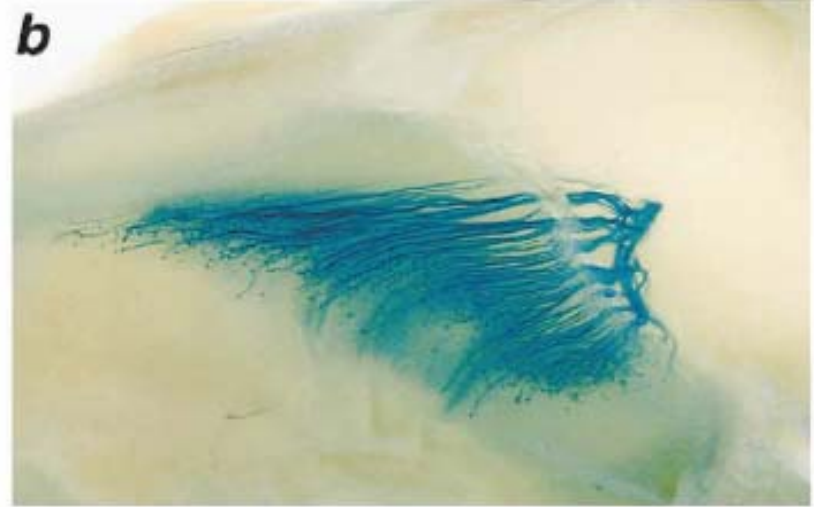
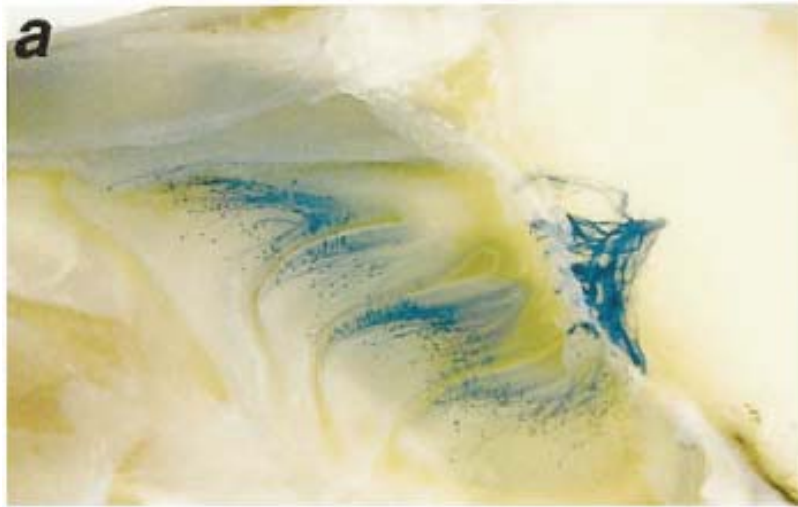
62 ORs, 69
GRs
1%

>100 ORs

1000 ORs
2.5%

350 ORs
0.9%

Convergence of OR Specific Axons

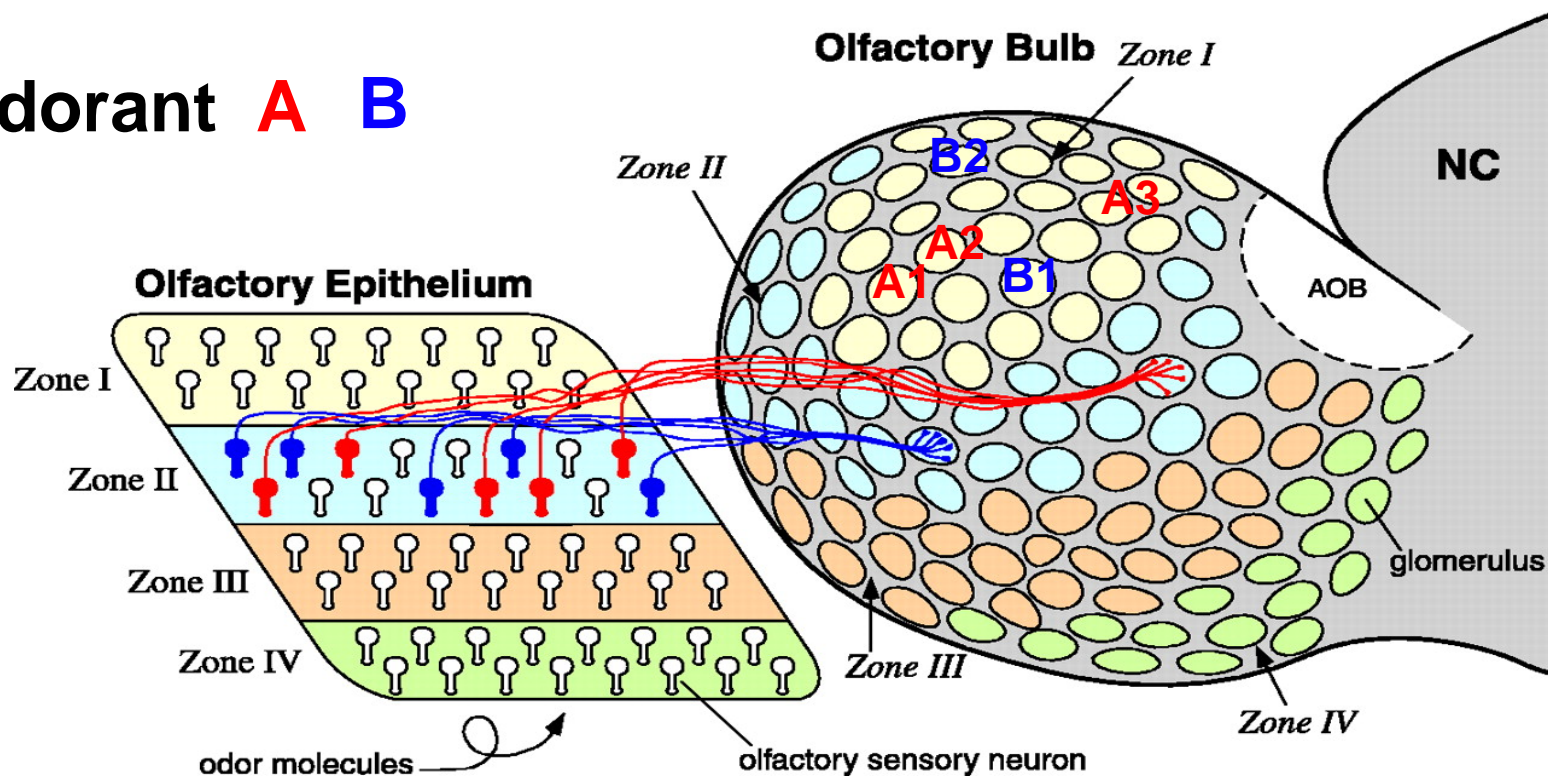


P. Mombaerts et al., Cell 1996

Olfactory Sensory Projections

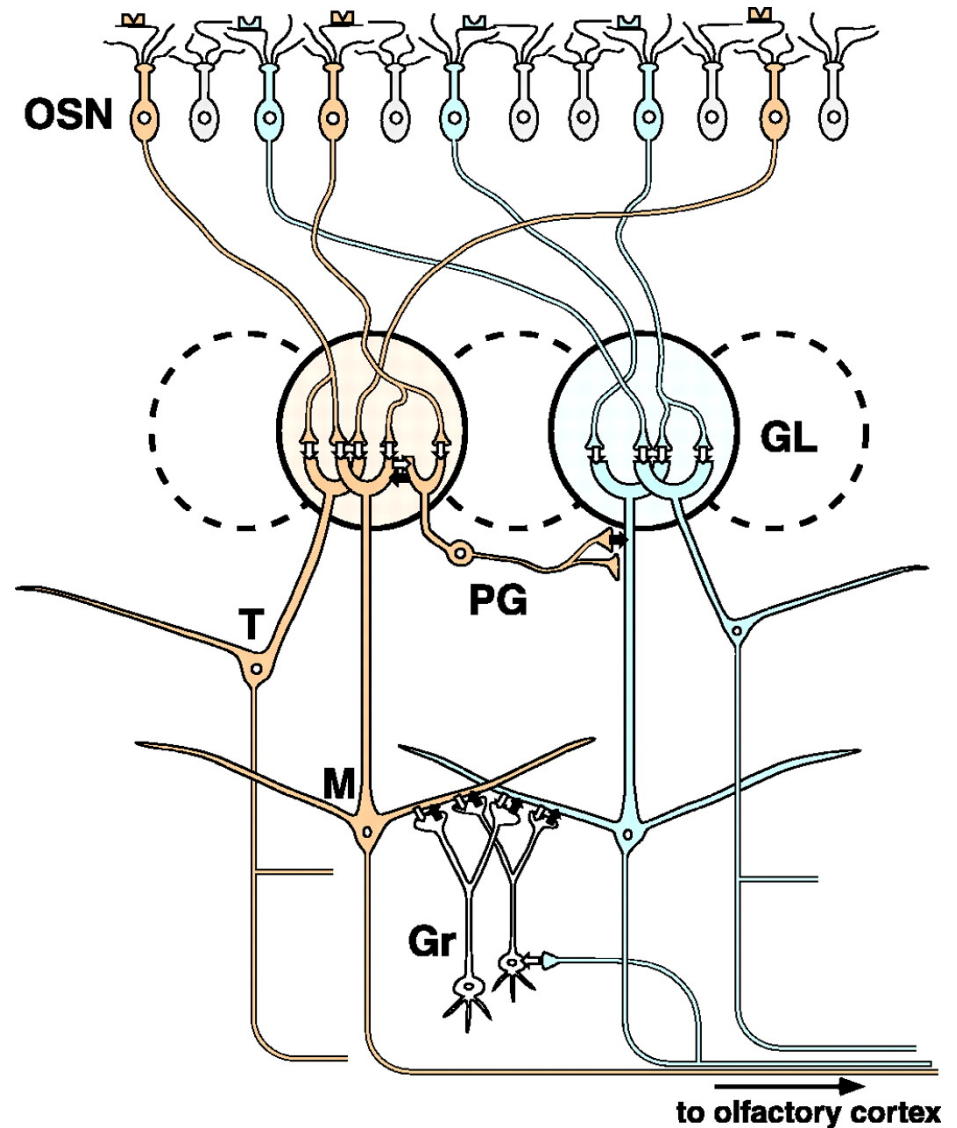
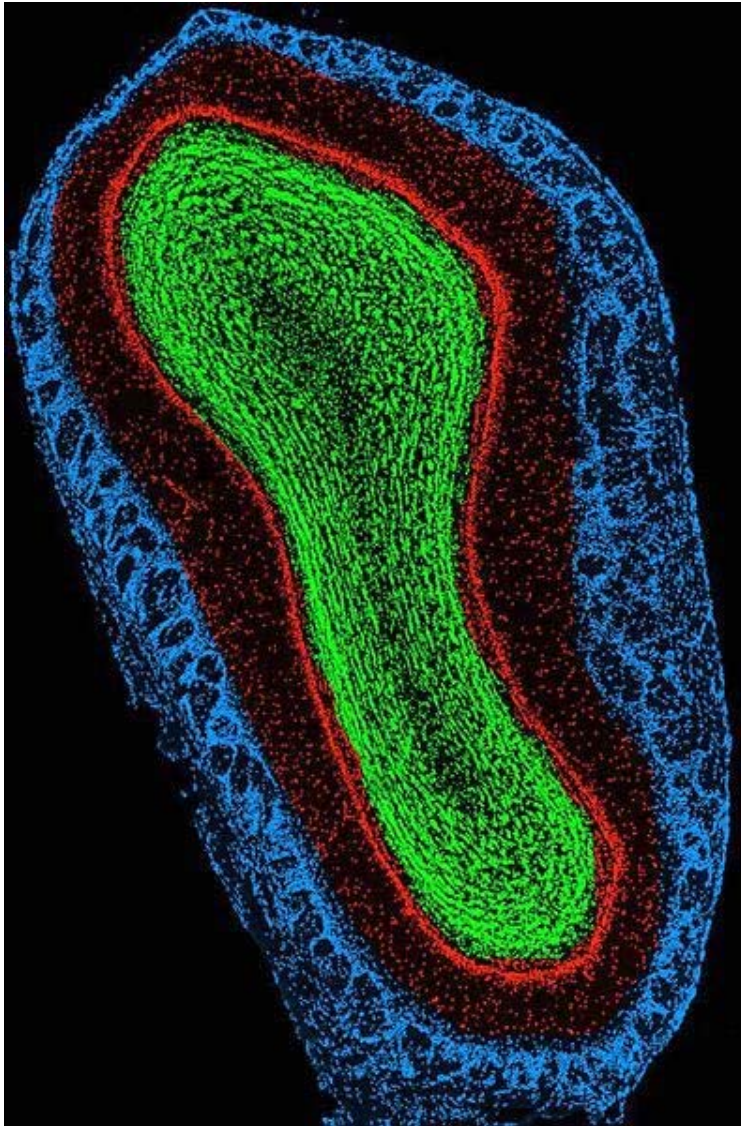
1. Single odorant receptor (OR) in each sensory neuron
2. Zonal distribution of sensory neurons
3. Axonal convergence into OR specific glomerulus
4. Glomerular activation patterns may code olfaction

Odorant **A** **B**

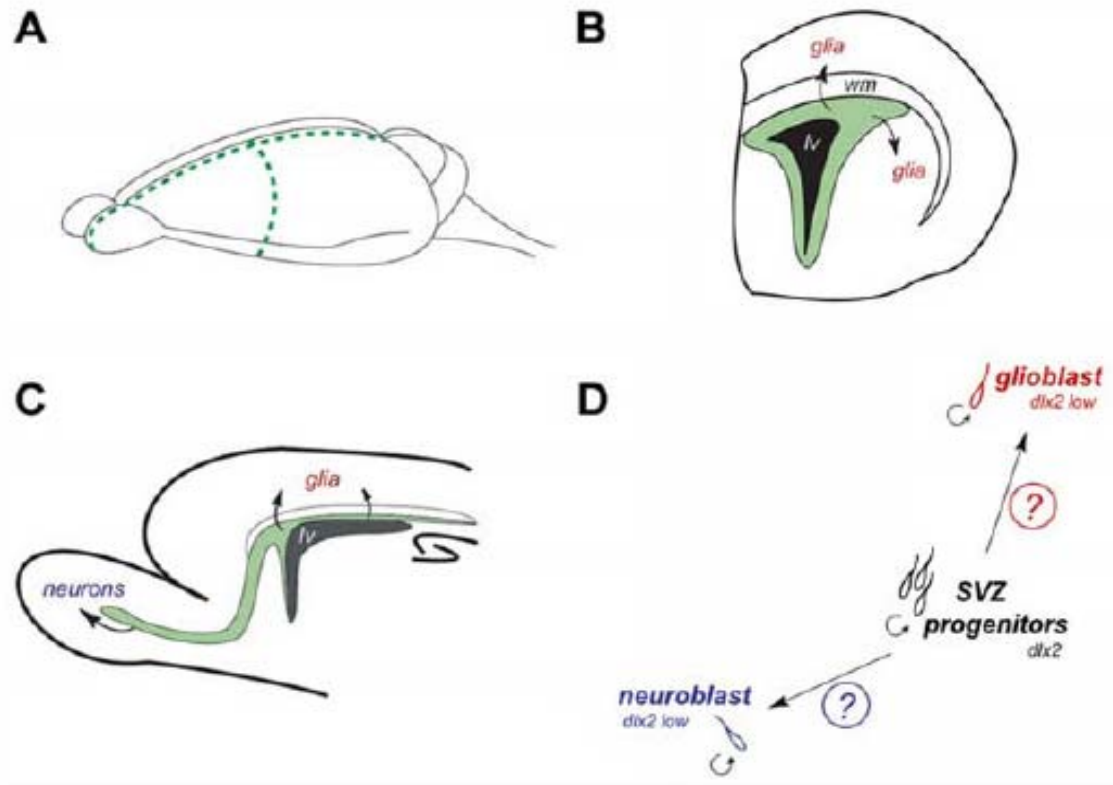


Modified from K.Mori et al., Science, 1999

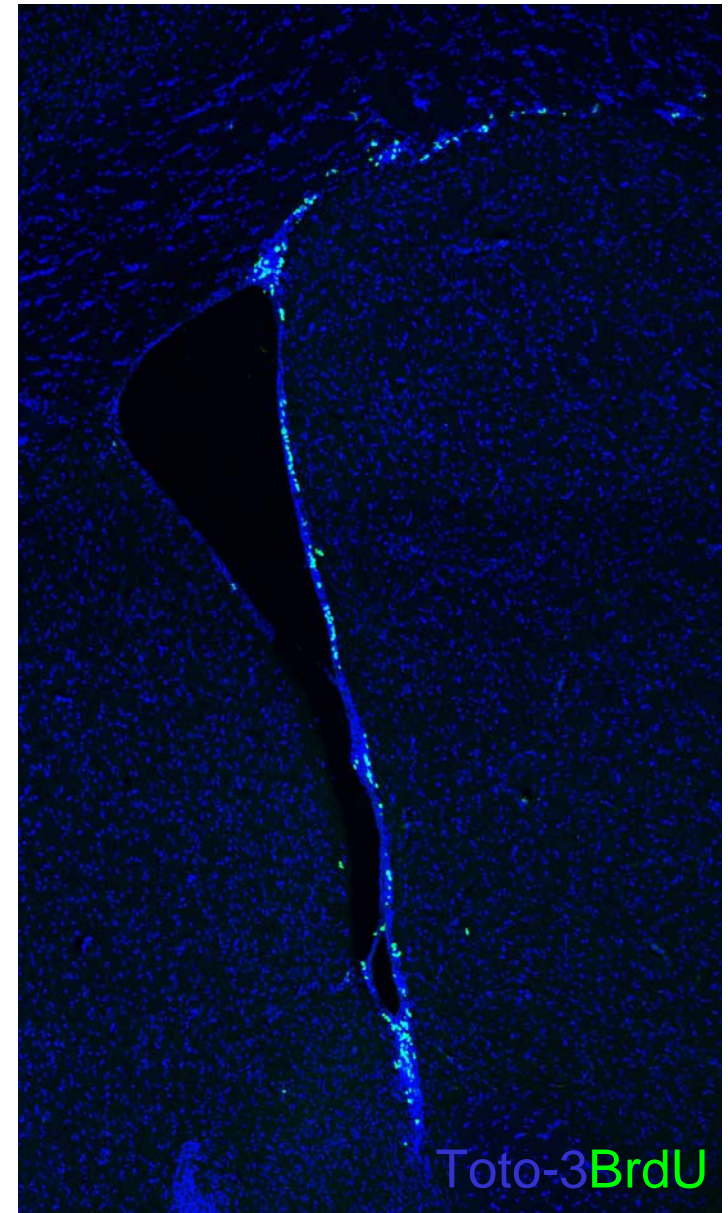
Cellular Organization of Main Olfactory Bulb



Postnatal Neurogenesis in Sub-Ventricular Zone



Marshall et al., 2005, J. Neurosci.



Postnatal Neurogenesis: differentiation, migration, integration, and function

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

Lledo and Lazarini, 2007, C.R.Biol.

Conventional Genetic Manipulations

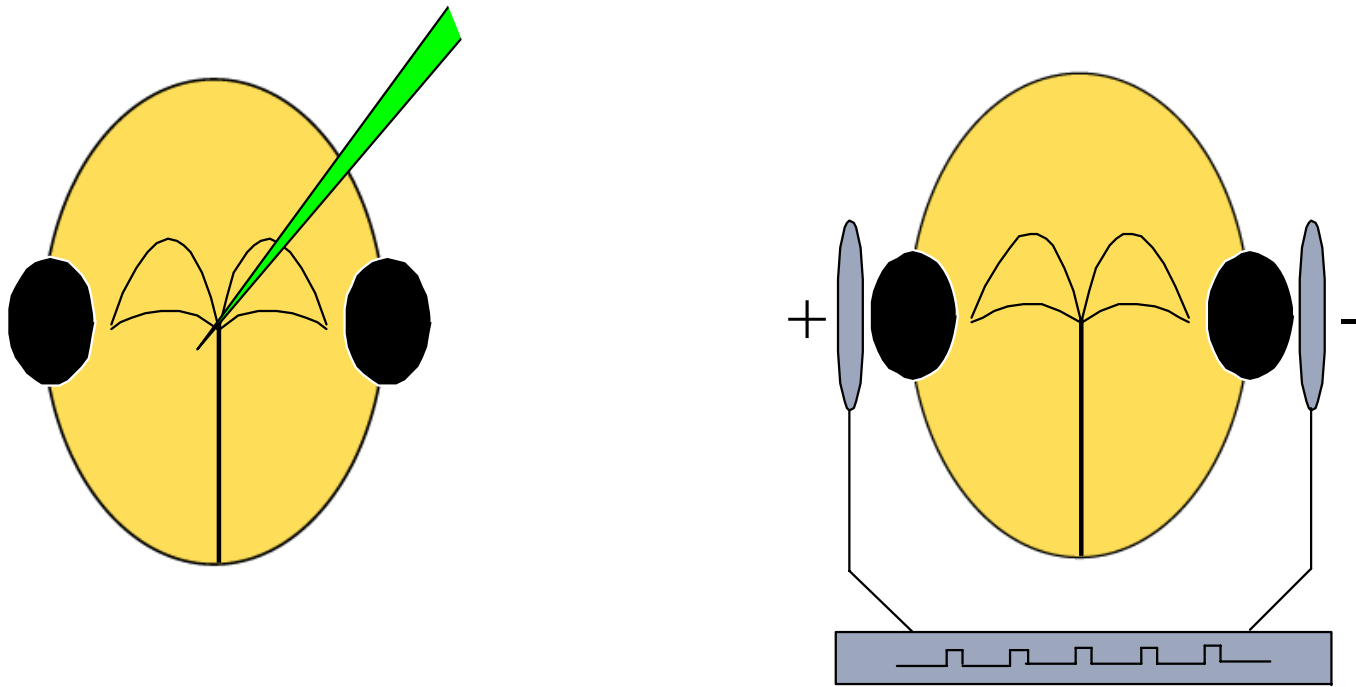
Transgenic and Gene Targeting

Technically demanding and time consuming
Spatial and temporal control

Recombinant Virus

Insert and promoter size
Manipulation of multiple genes
High titer
Biohazard concerns

Postnatal Electroporation



Potential Advantages:

Technically less demanding
Robust ectopic gene expression
Time and cost effective

Postnatal Electroporation: step by step

Anaesthetize pups

--P0 - P5

Pressure Injection of Plasmids

-- Promoter CMV, CAG, and ...

-- 0.2 - 2 μ l with a concentration of 1-2 μ g/ μ l

Electroporation with BTX CM830

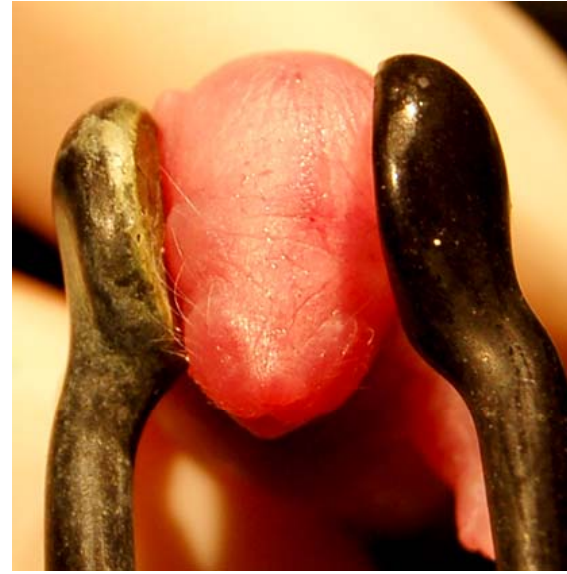
-- 5X 50 ms pulses with 1s intervals

-- 150 Volts (depending on animal age)

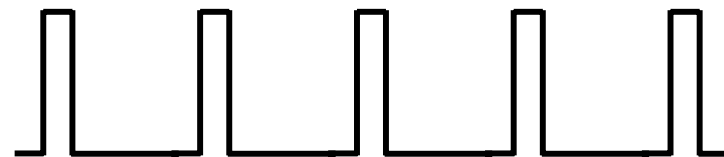
Observations

-- A few hours to more than a year post electroporation

Postnatal Electroporation

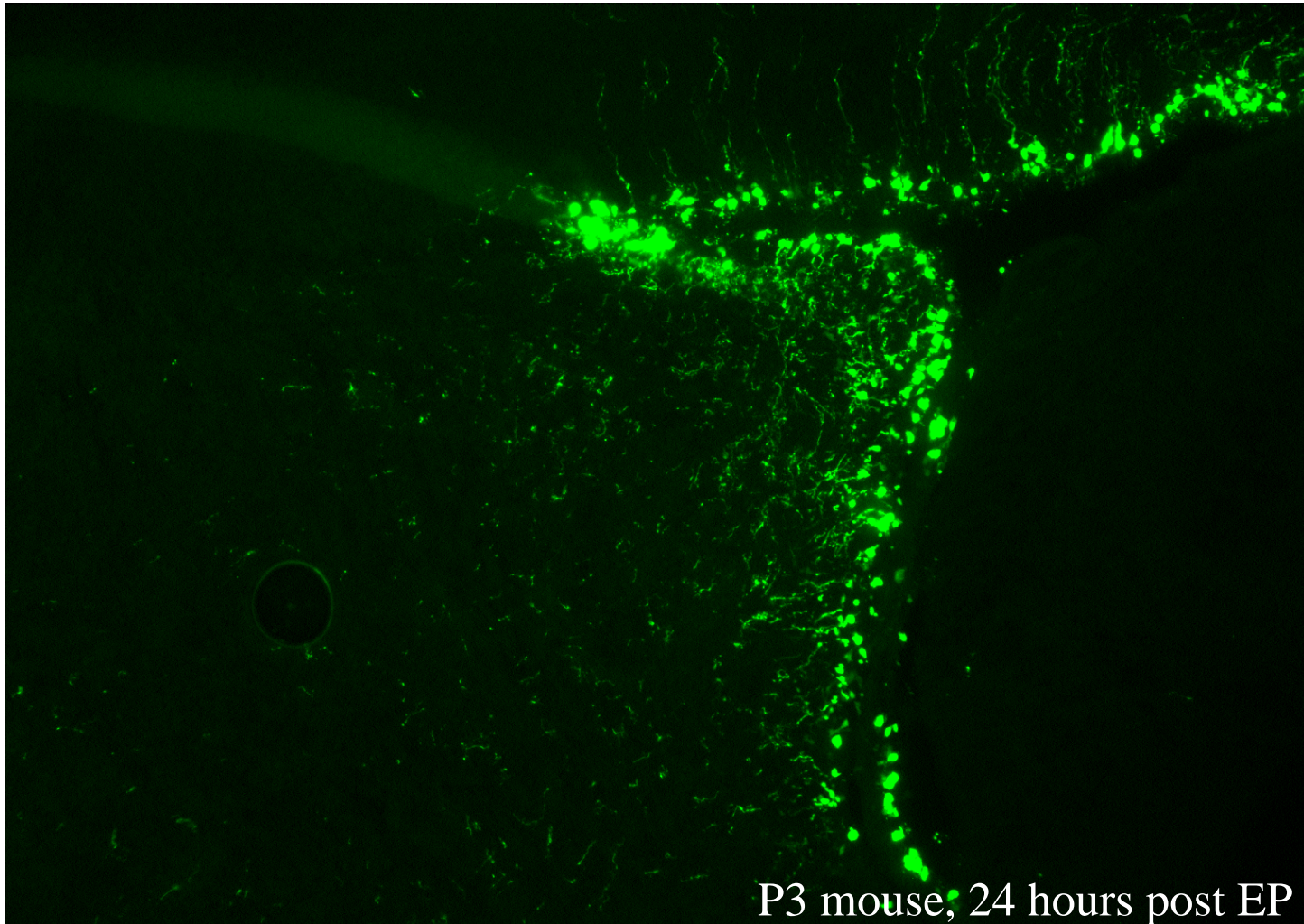


QuickTime™ and a
decompressor
are needed to see this picture.

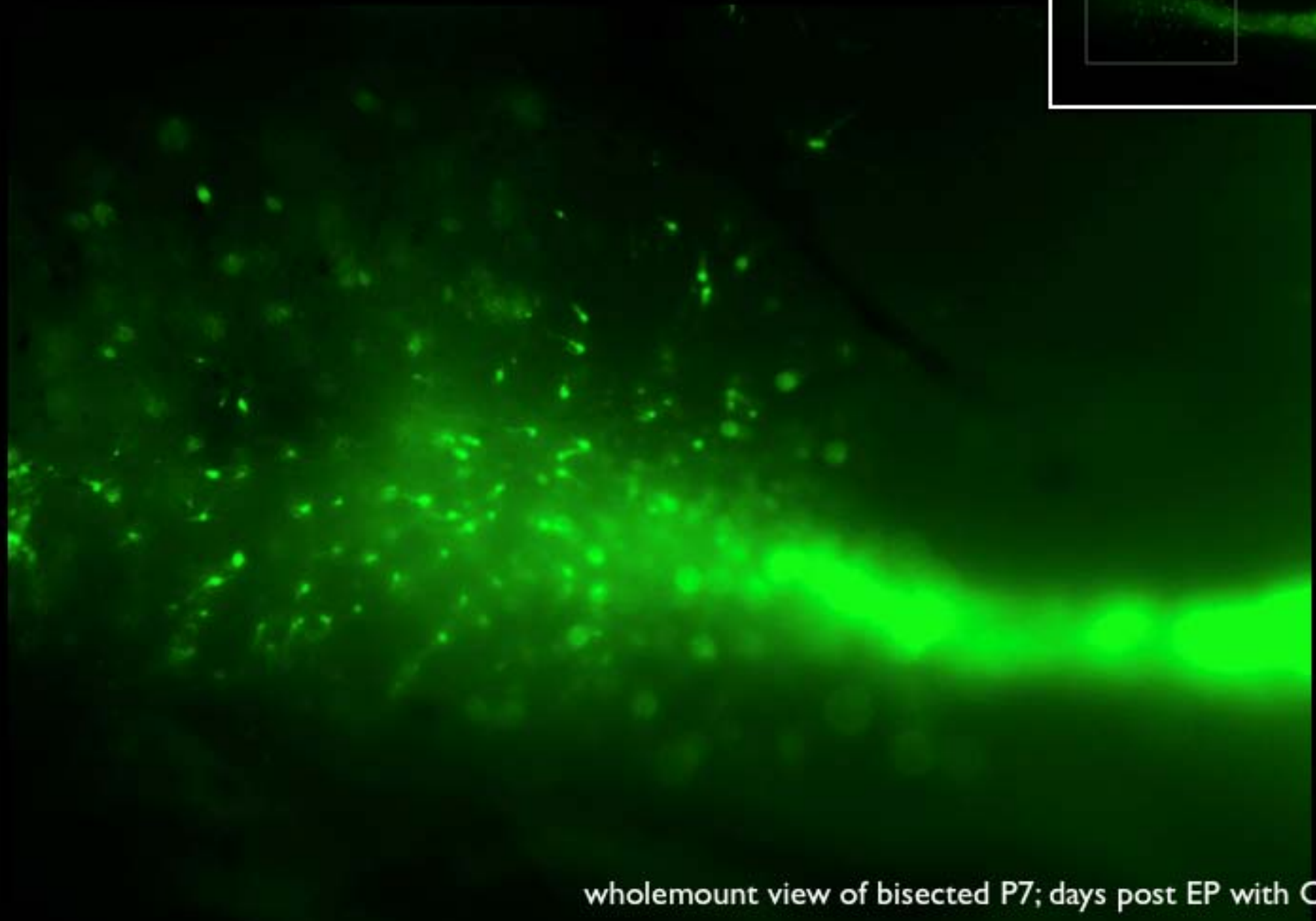
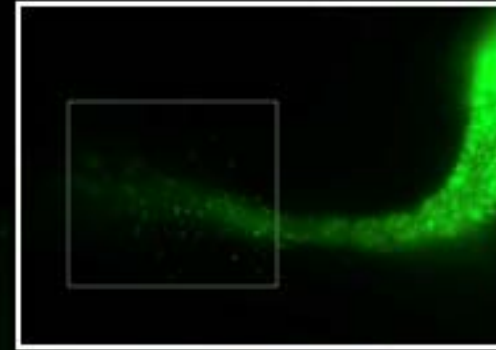


5 pulses, 150 Volts
50 ms, 1 s interval

Postnatal Electroporation: Rapid and Wide spread expression of GFP in SVZ

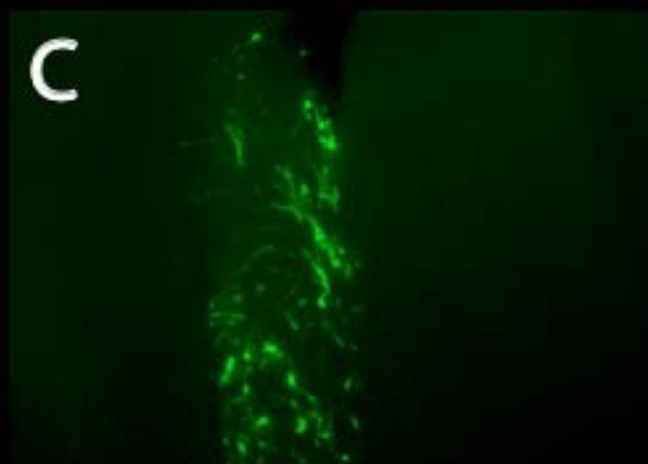
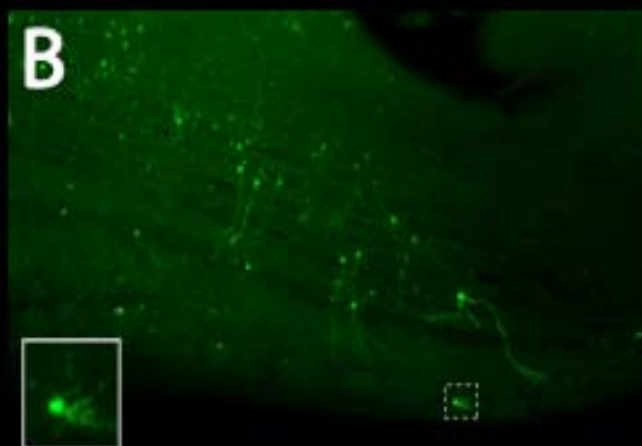
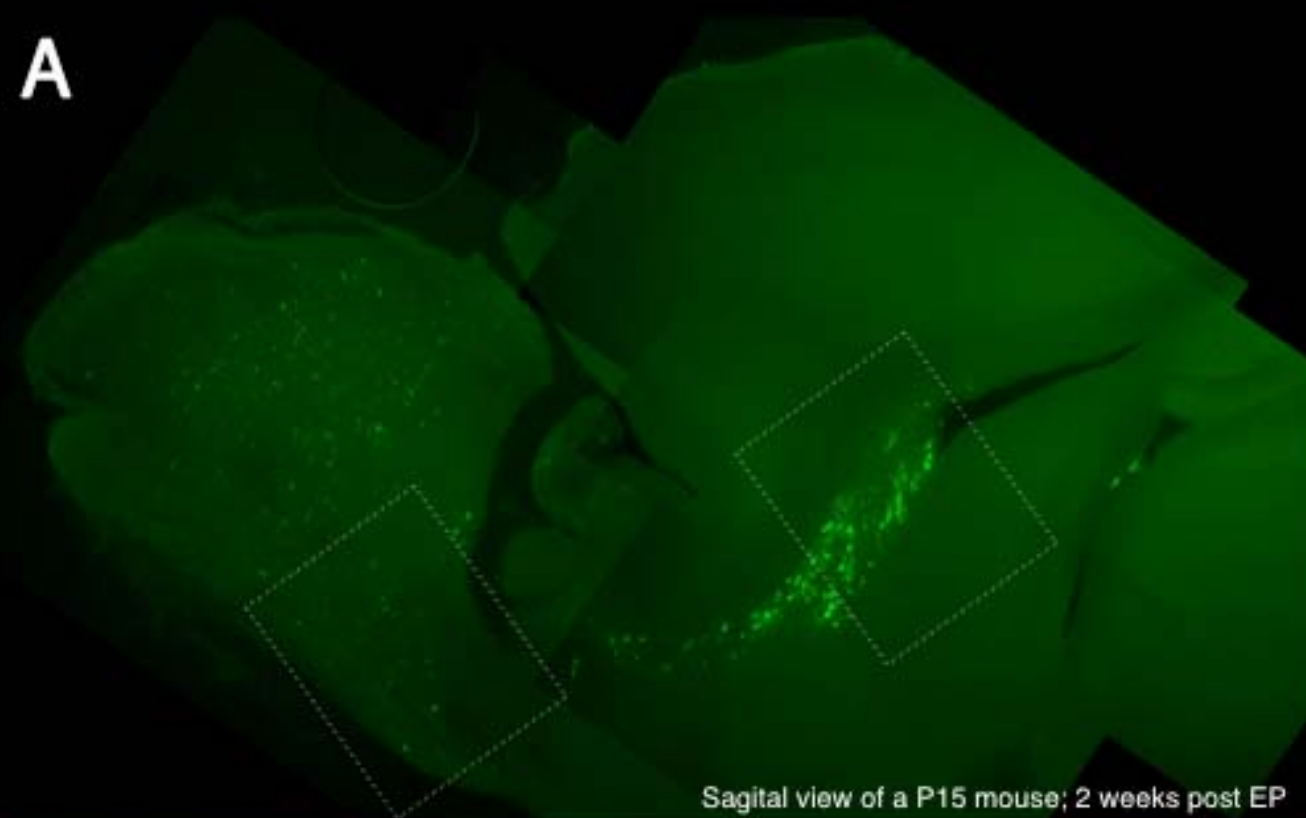


Widespread expression in RMS 5 days post EP

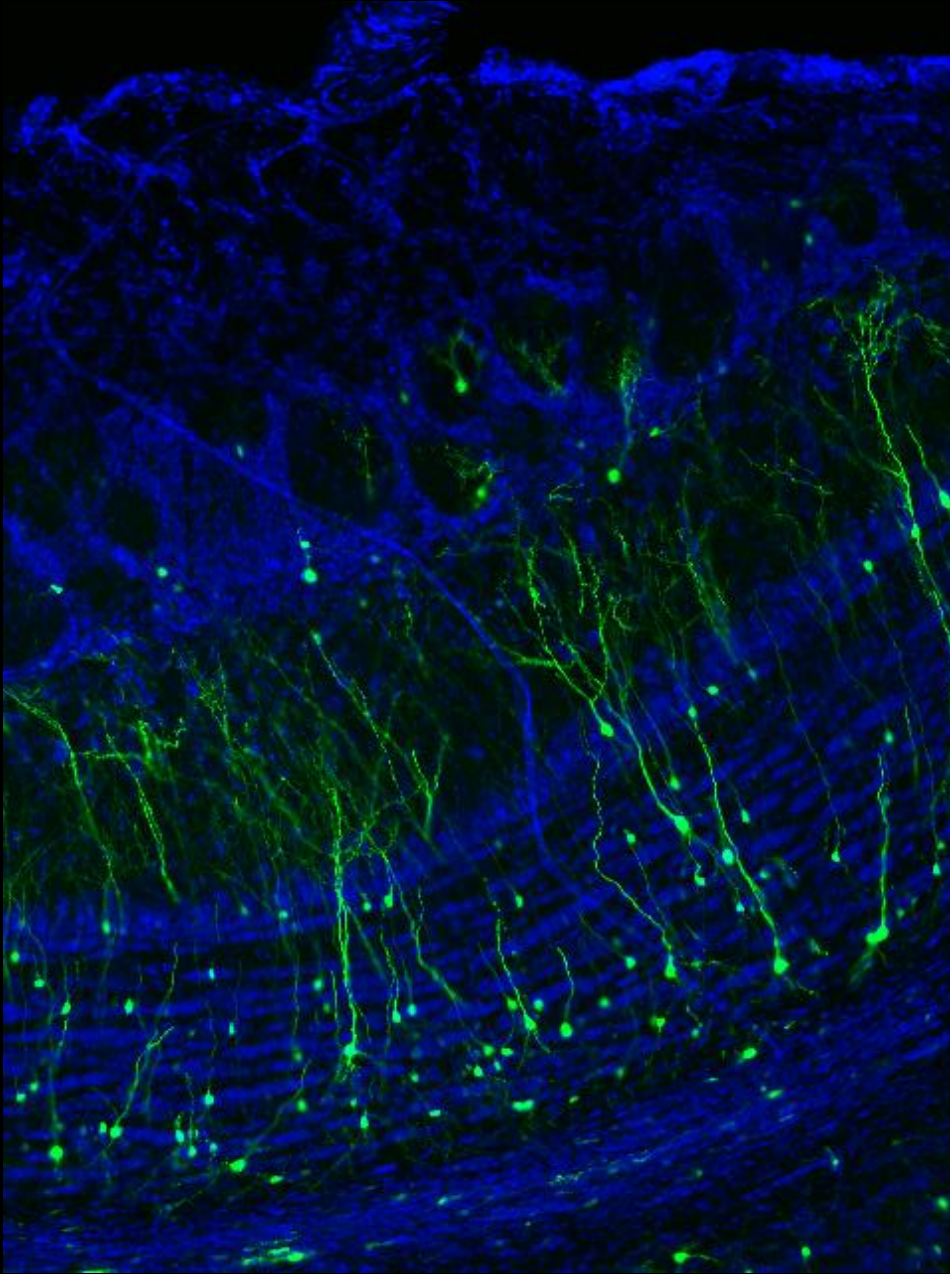


wholemount view of bisected P7; days post EP with CGLH

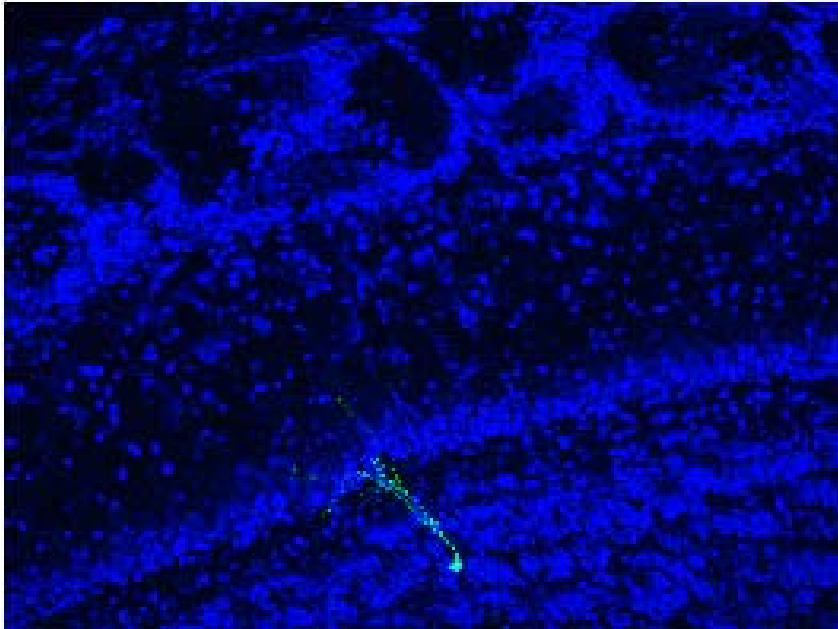
Widespread GFP expression throughout OB 2 weeks post EP



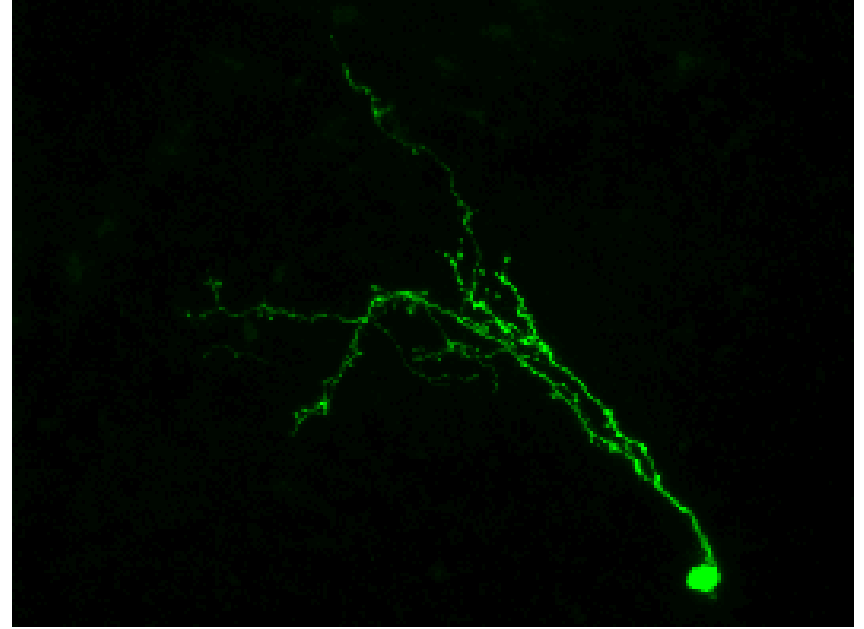
Widespread GFP expression in the bulb



Persistent GFP expression post electroporation

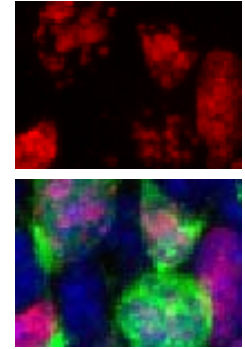
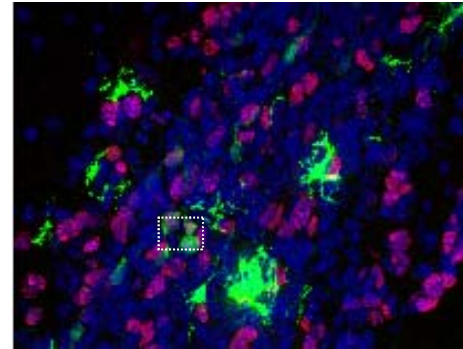
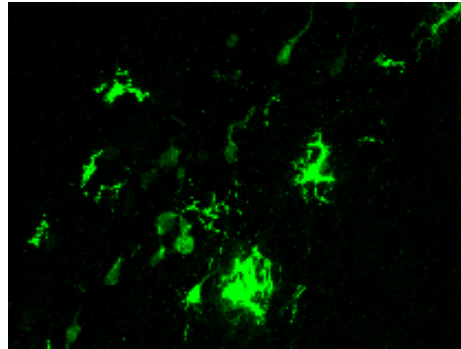
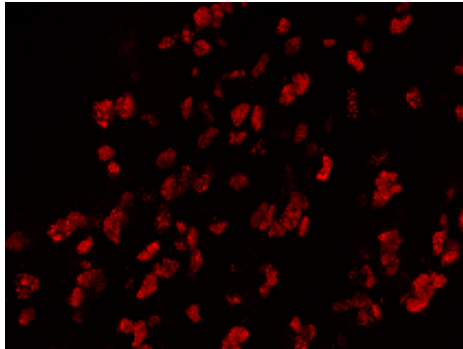


P430 mouse



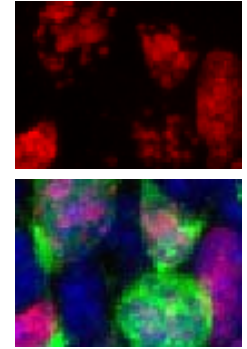
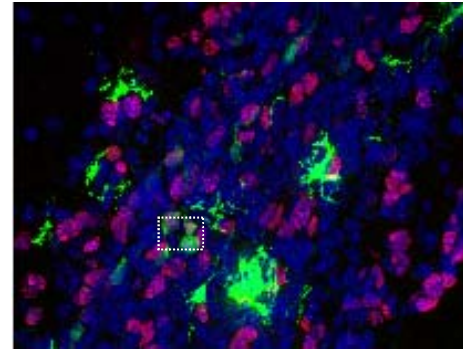
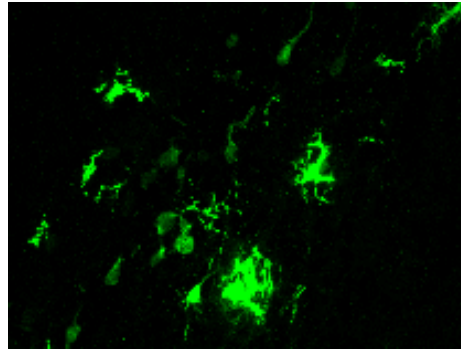
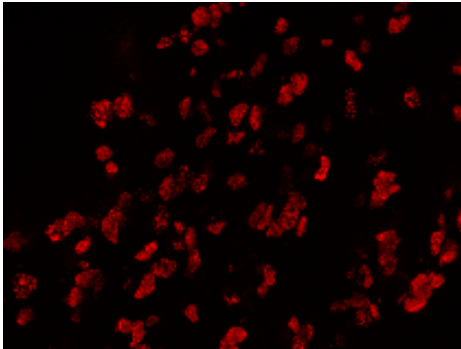
Normal Cell Differentiation post electroporation

BrdU (dividing cells)

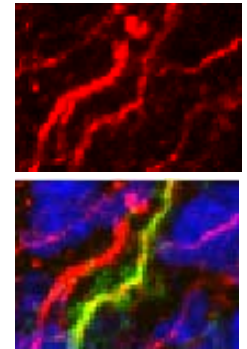
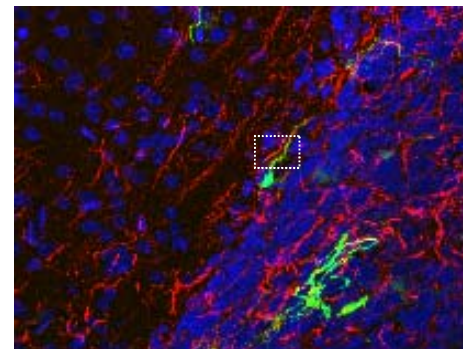
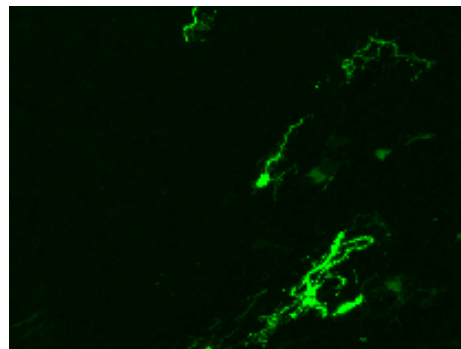
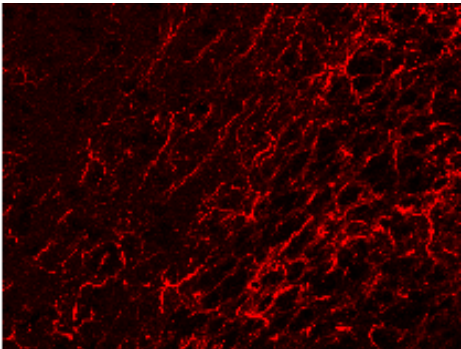


Normal Cell Differentiation post electroporation

BrdU (dividing cells)

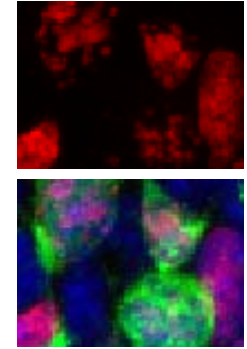
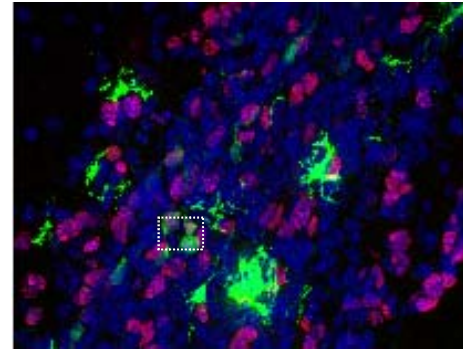
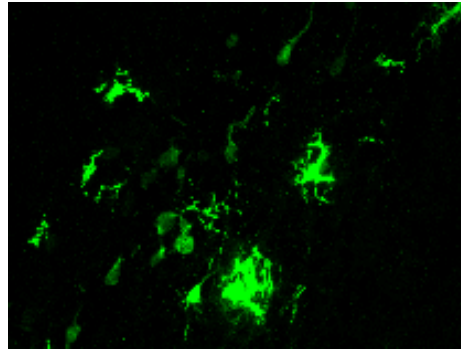
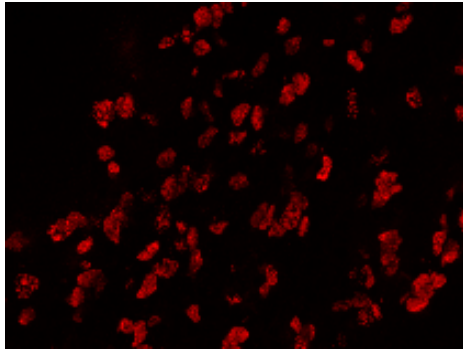


GFAP (progenitors)

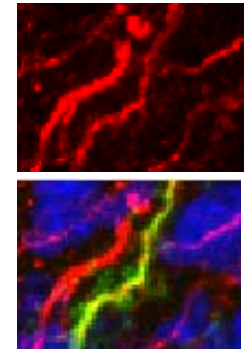
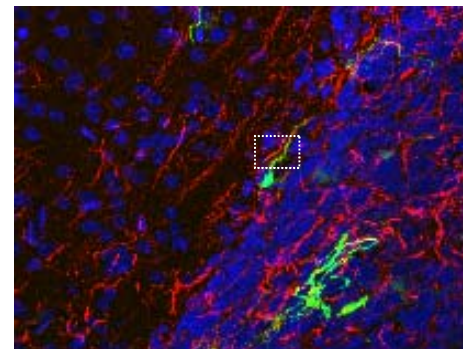
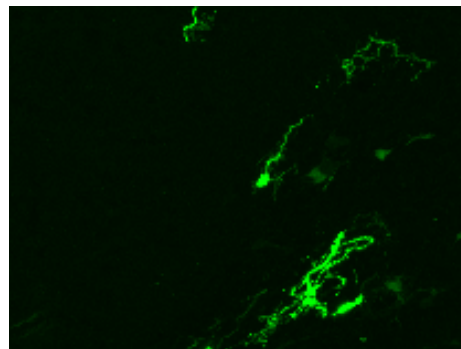
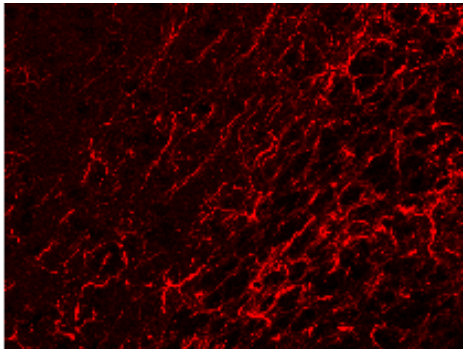


Normal Cell Differentiation post electroporation

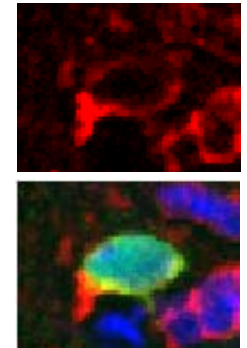
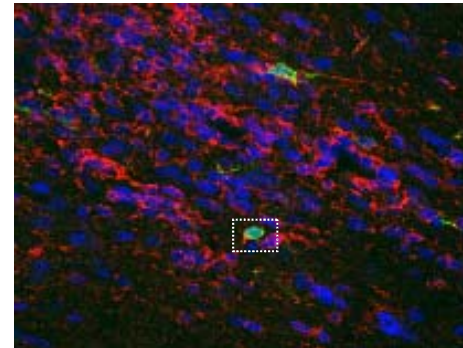
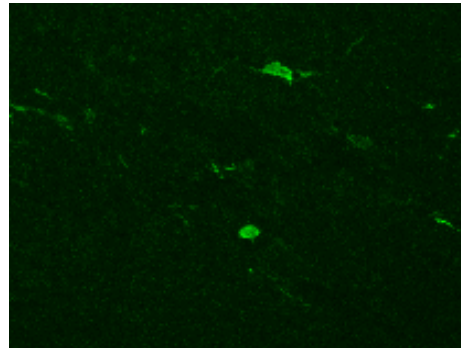
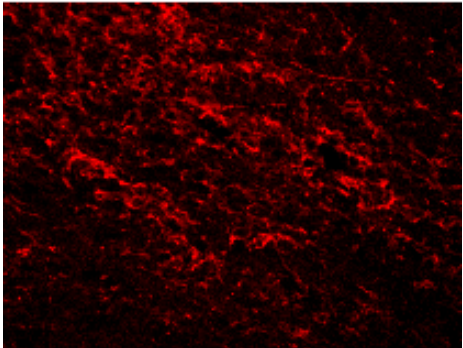
BrdU (dividing cells)



GFAP (progenitors)

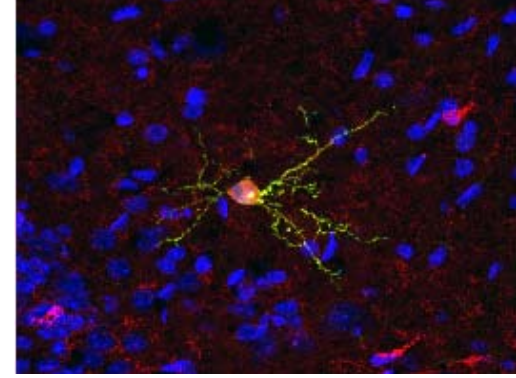
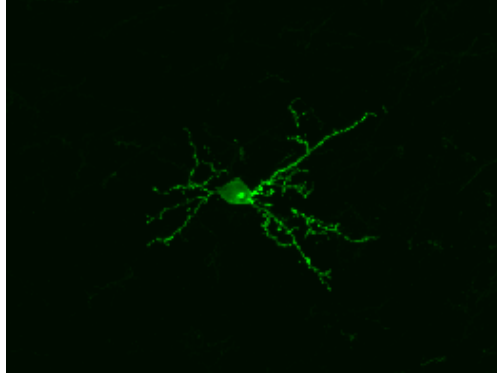
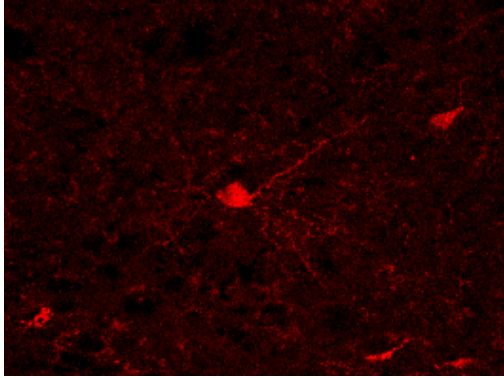


DCX (migrating cells)



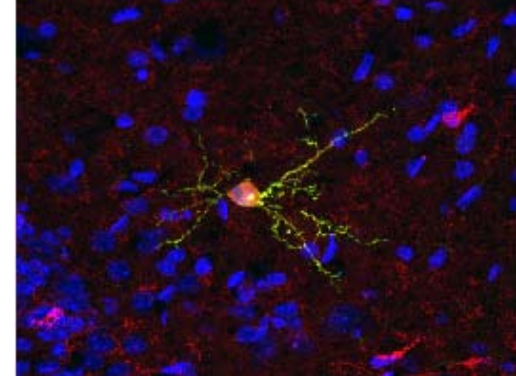
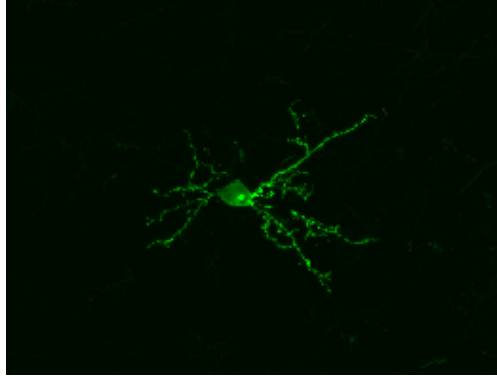
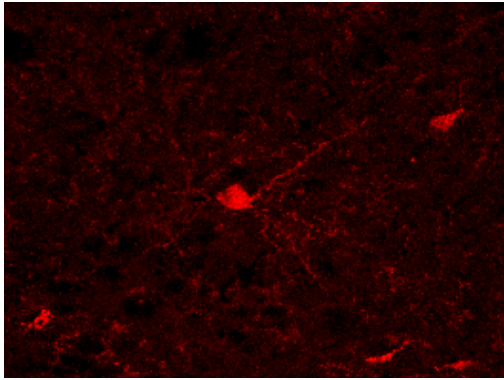
Normal Cell Differentiation post electroporation

parvalbumin (subset of EPL cells)

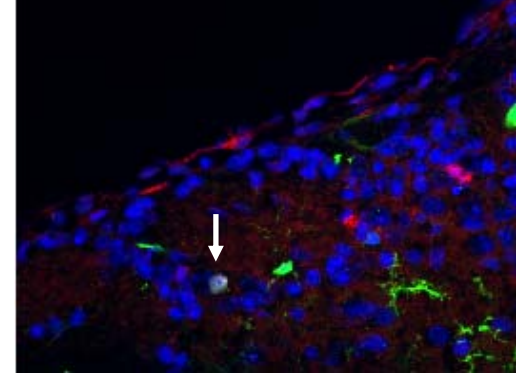
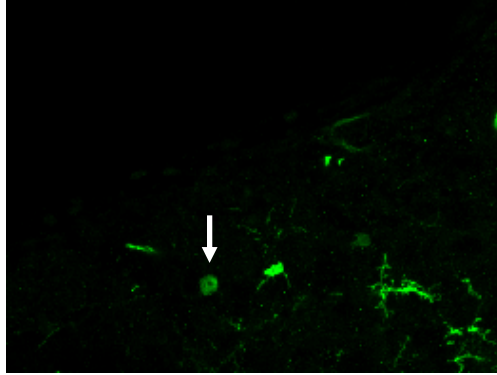
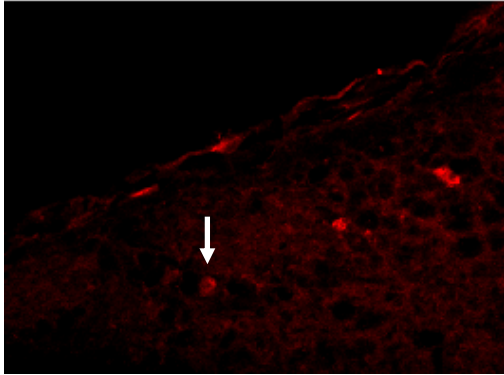


Normal Cell Differentiation post electroporation

parvalbumin (subset of EPL cells)

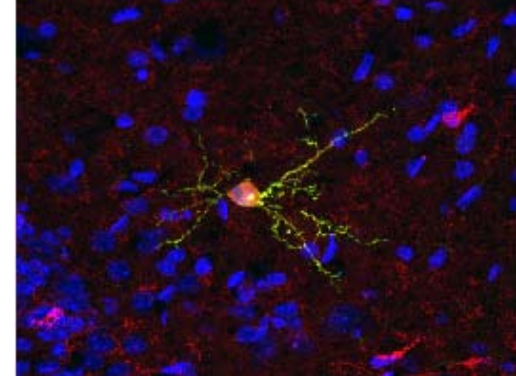
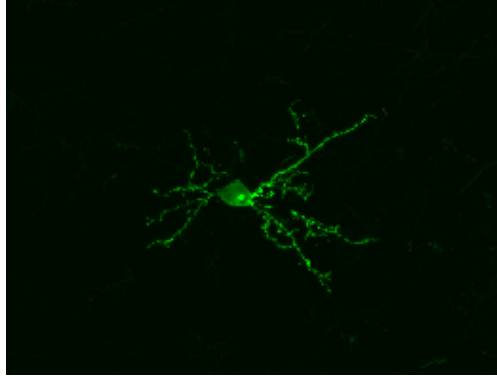
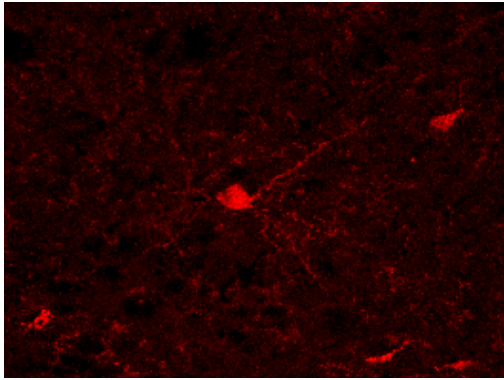


calbindin (subset of PGs)

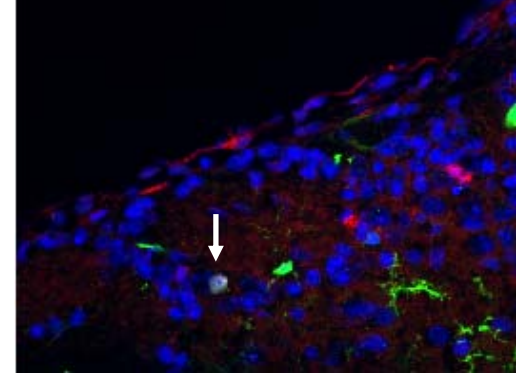
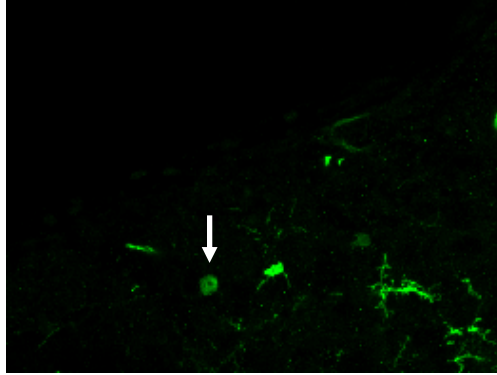
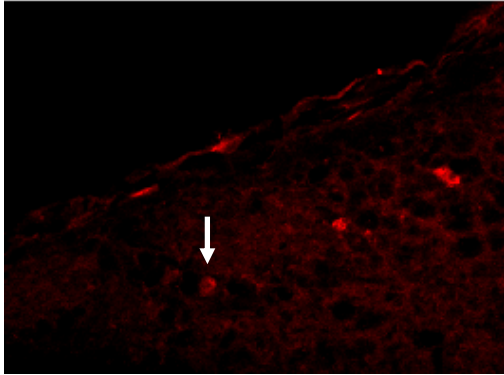


Normal Cell Differentiation post electroporation

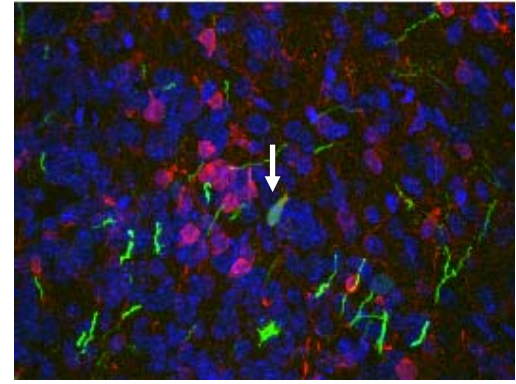
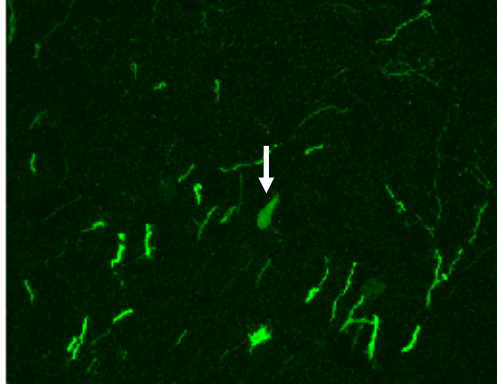
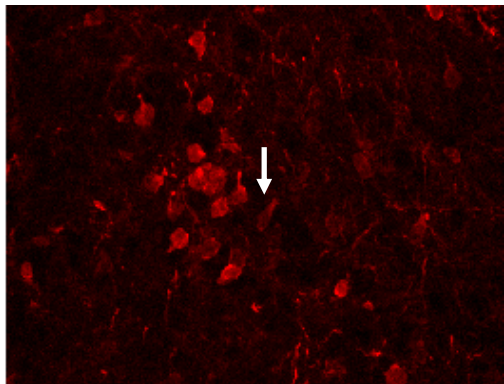
parvalbumin (subset of EPL cells)



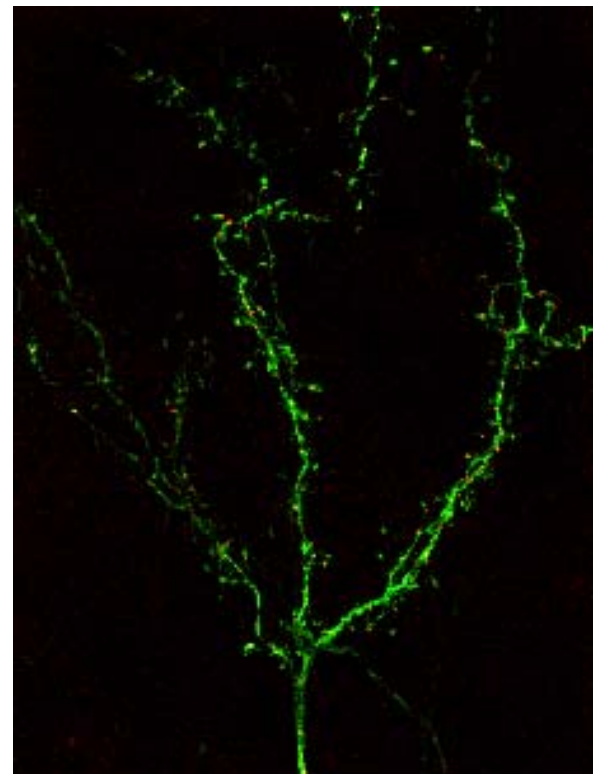
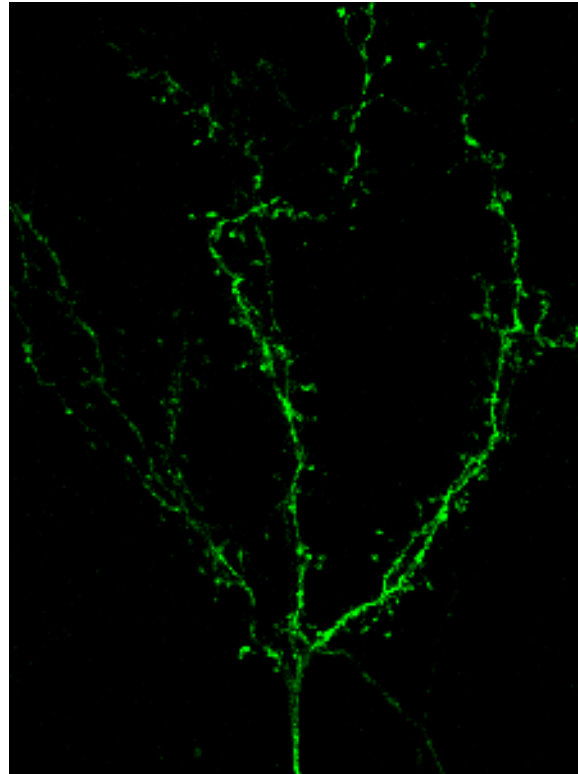
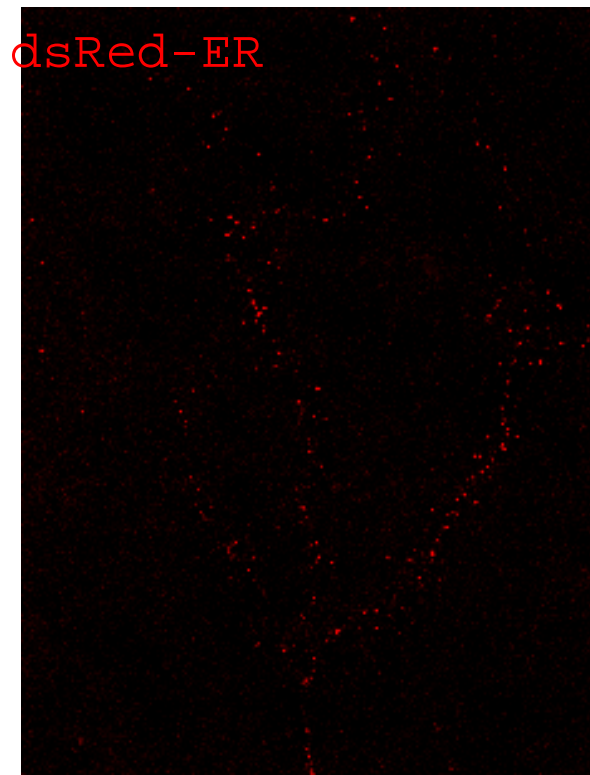
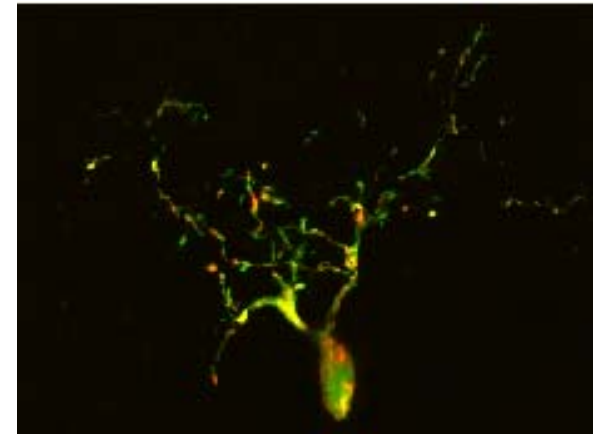
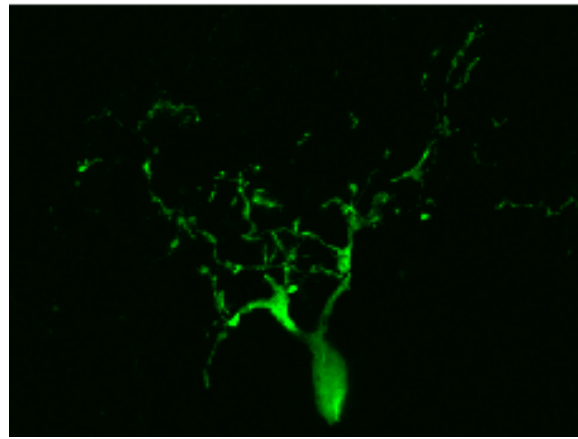
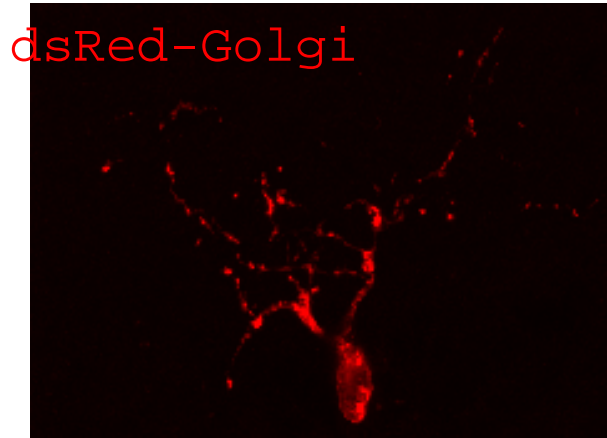
calbindin (subset of PGs)



calretinen (subset GCs/PGs)

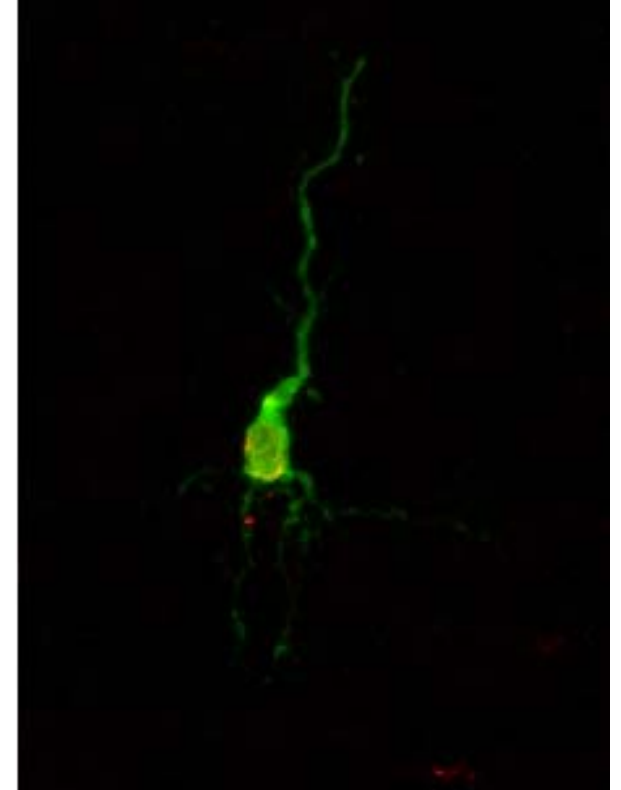
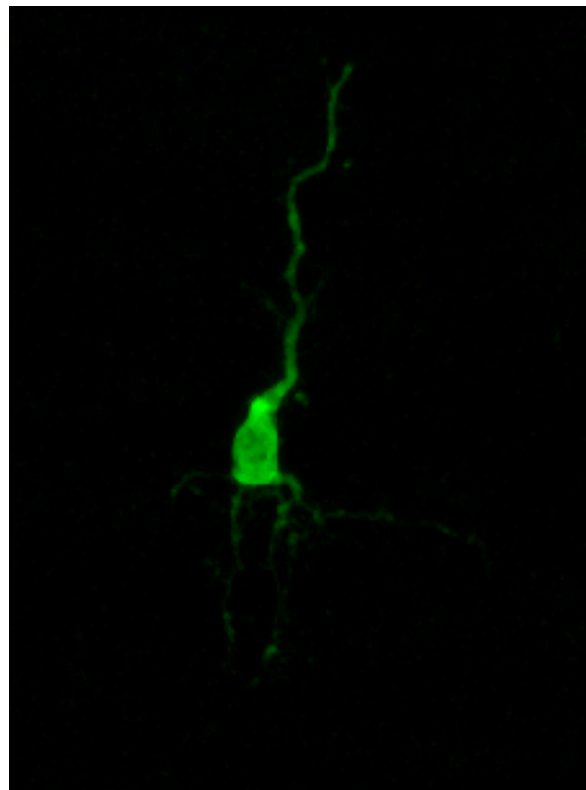


Co-expression of cellular markers by electroporation: mixture of two plasmids



Co-expression of cellular markers using IRES

Cre-IRES-GFP

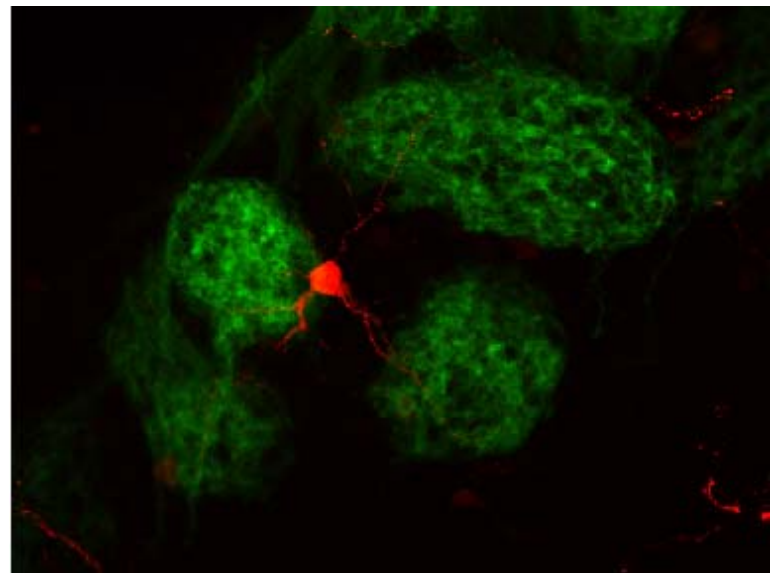
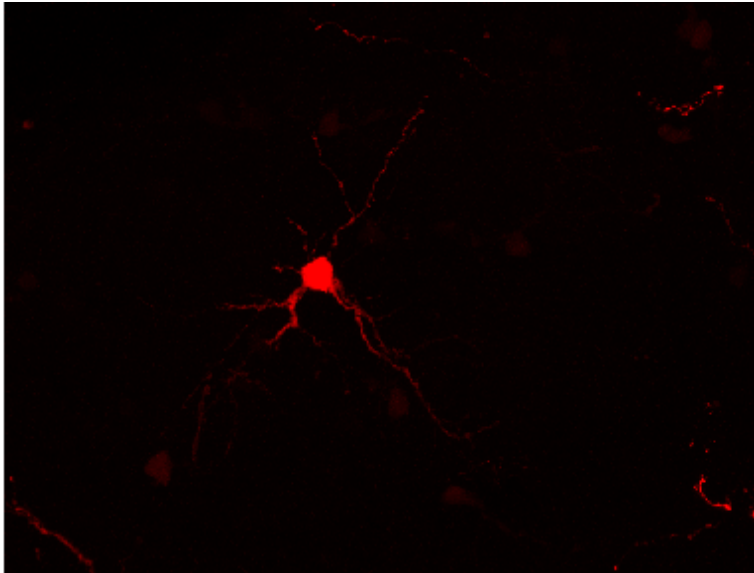


Ectopic expression in transgenic mice by electroporation

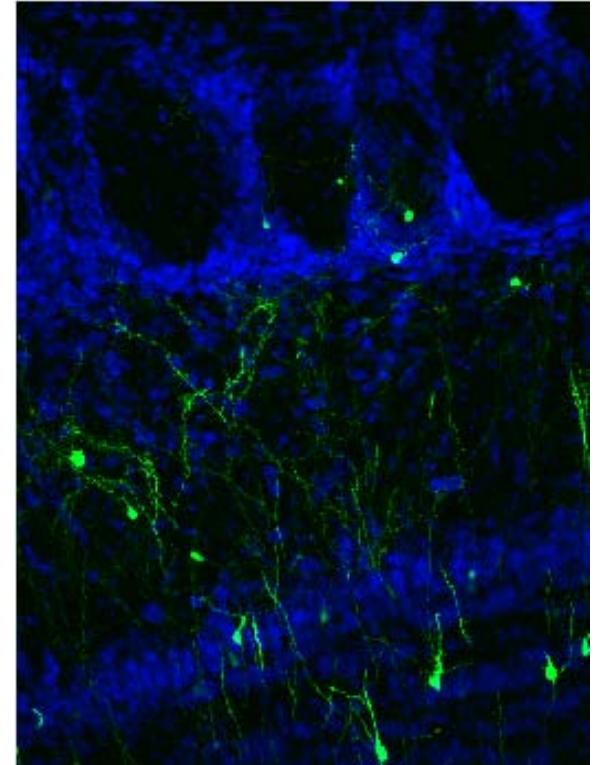
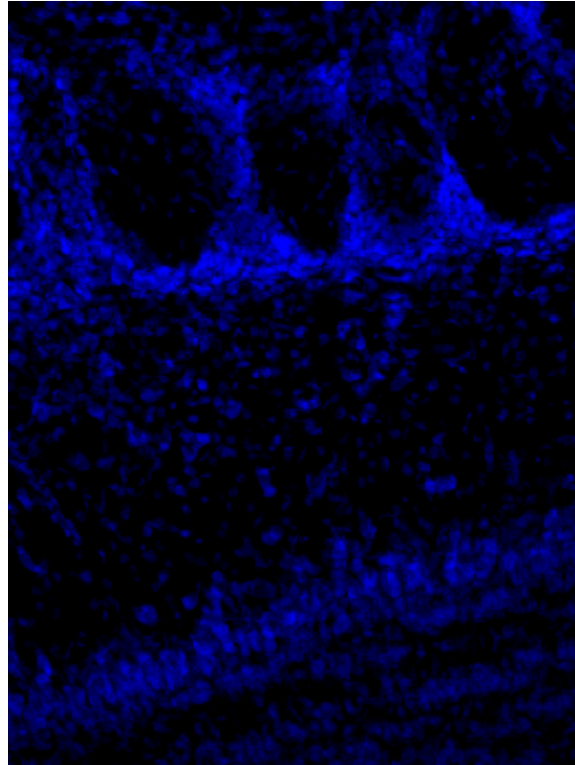
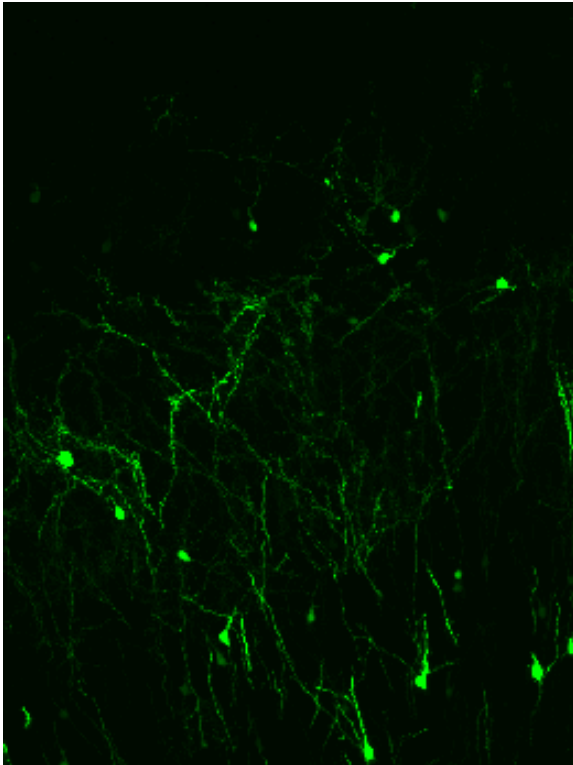
GFP->OMP mice

mRFP plasmids

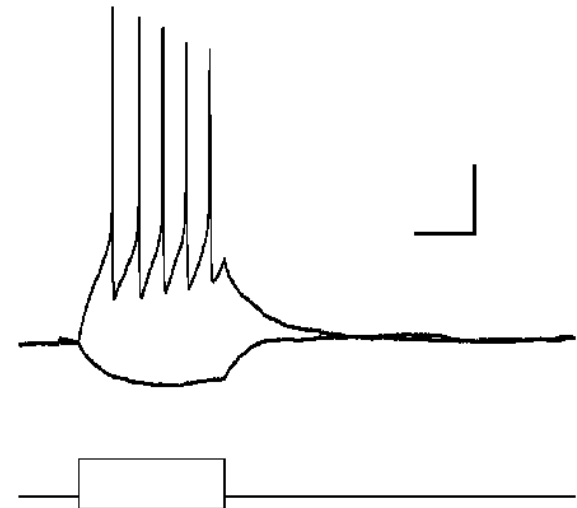
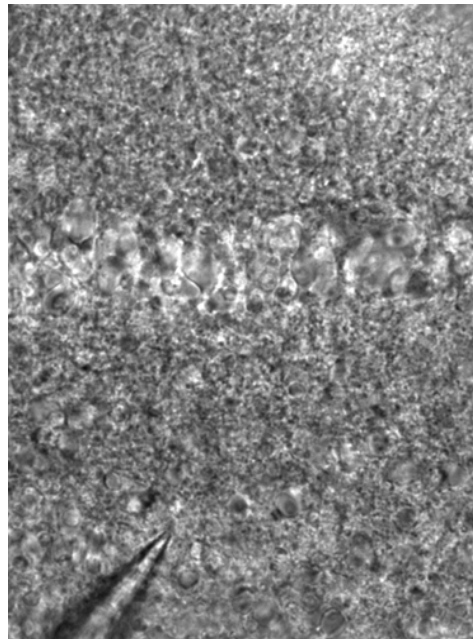
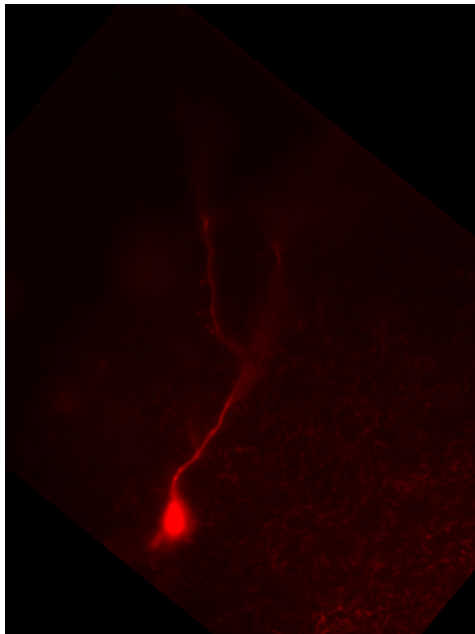
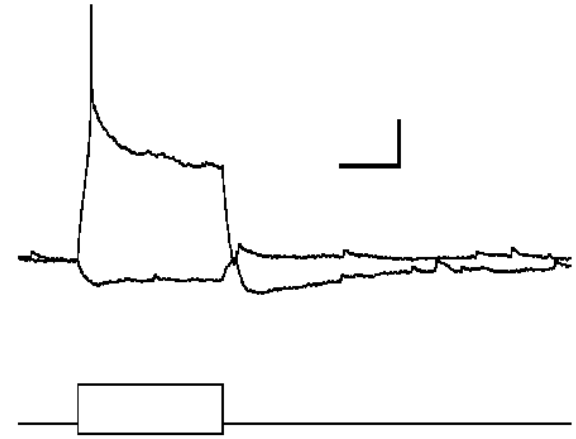
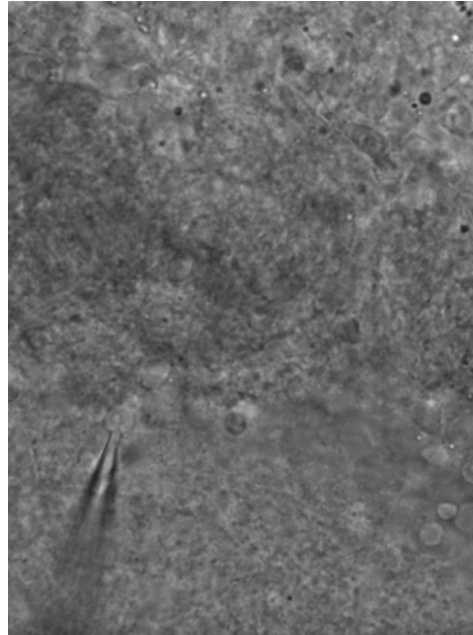
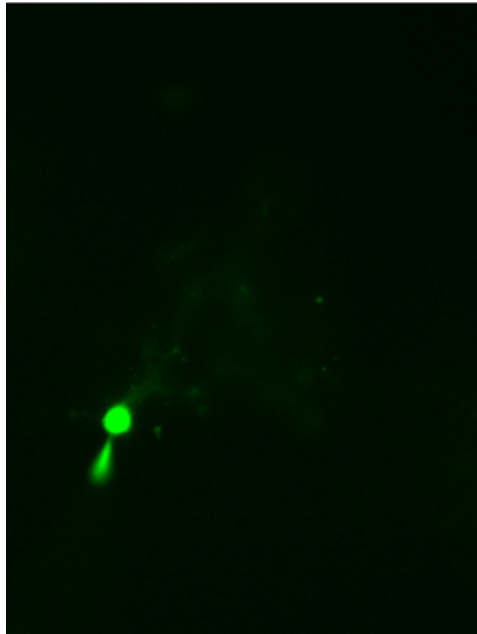
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.



Ectopic expression in rat by electroporation



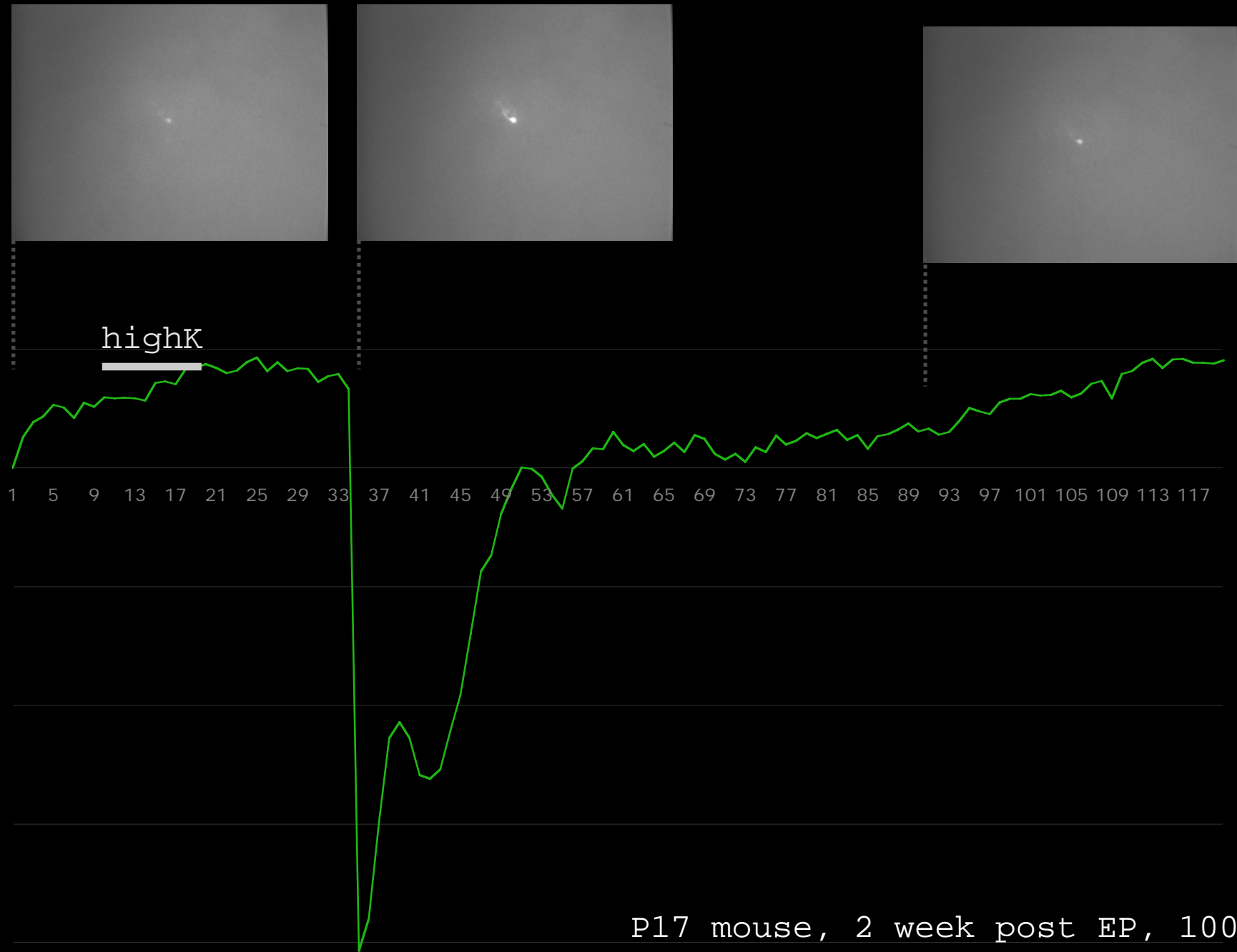
Recordings of labeled fluorescent neurons



GCamp2 expression in a PG cell



Robust Ca^{2+} responses in a GCamp2 labeled PG cell



P17 mouse, 2 week post EP, 100mM KCl
4sec/Frame

Future directions

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

Duan et al., 2008, Curr Opin Neurobiol

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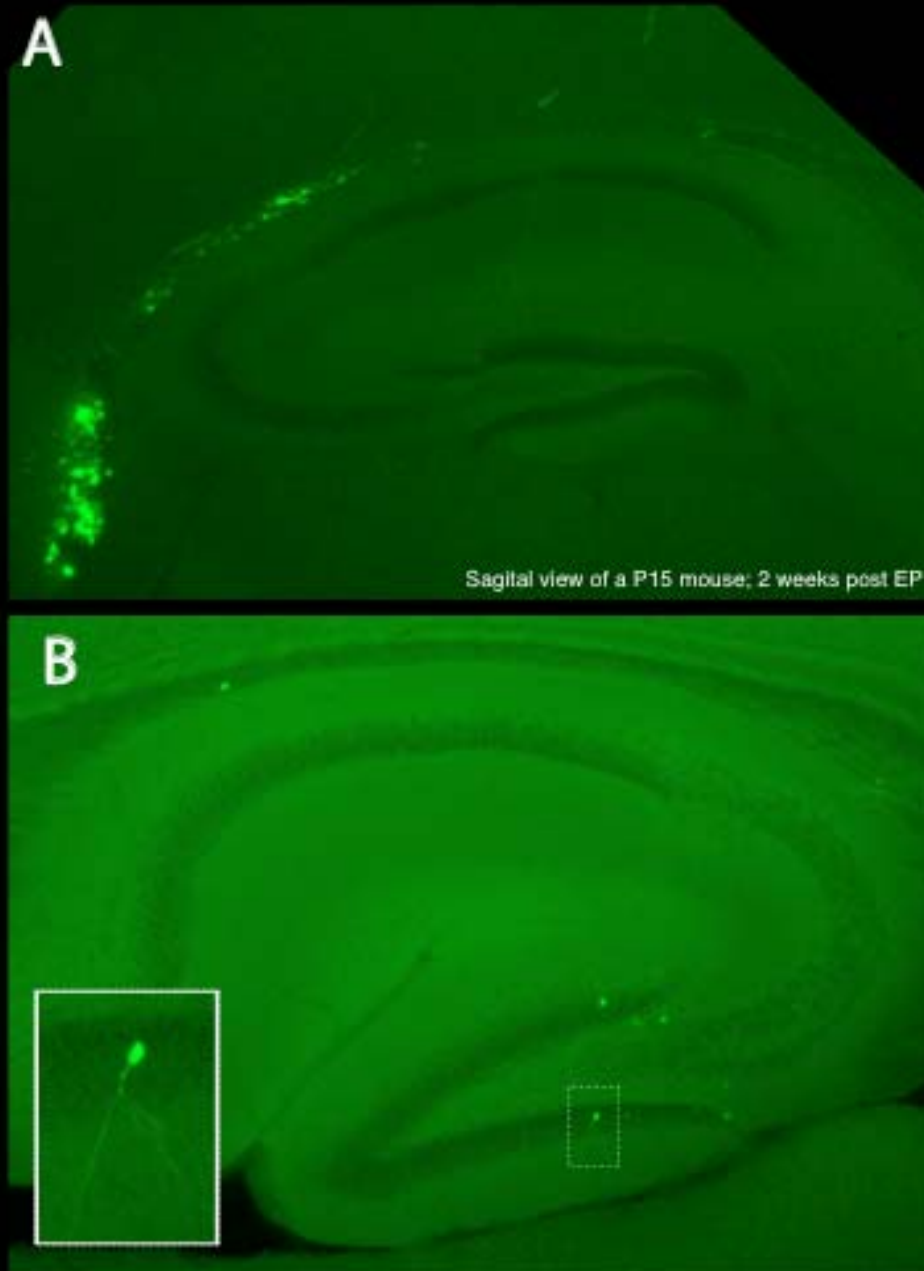
Nenad Stestan

Junichi Nakai

BTX

Derek Palmer

Expression occurs in other neurogenic areas as well



High levels of expression are achieved within 24 hours post EP

