Neuroscience NS201B

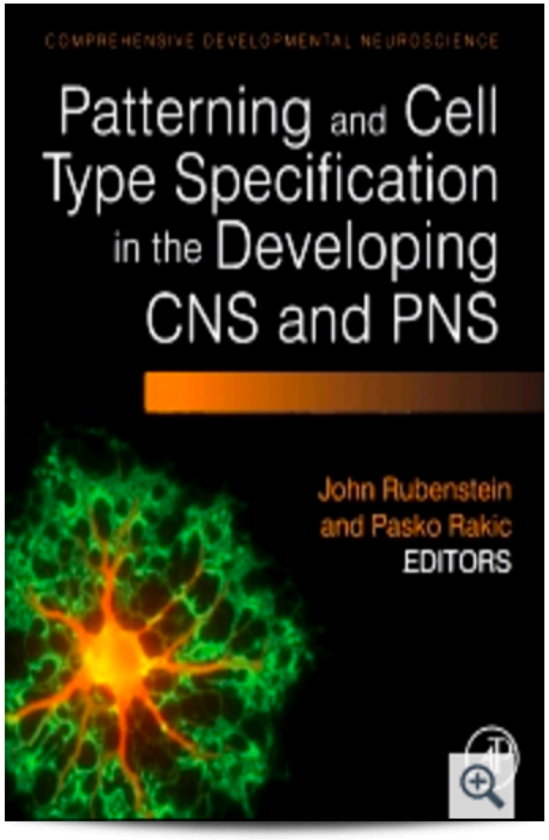
Patterning and Differentiation of the Embryonic Telencephalon Cortex and Basal Ganglia

John Rubenstein

December 2016

Patterning and Cell Type Specification in the Developing CNS and PNS, 1st Edition

Comprehensive Developmental Neuroscience



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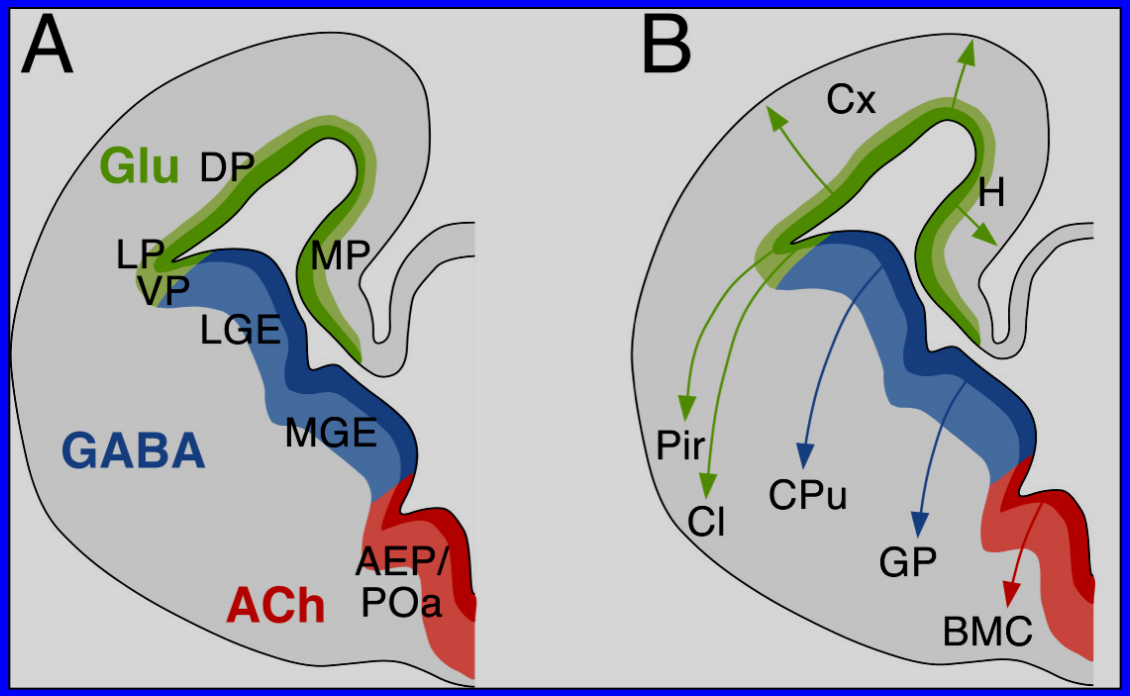
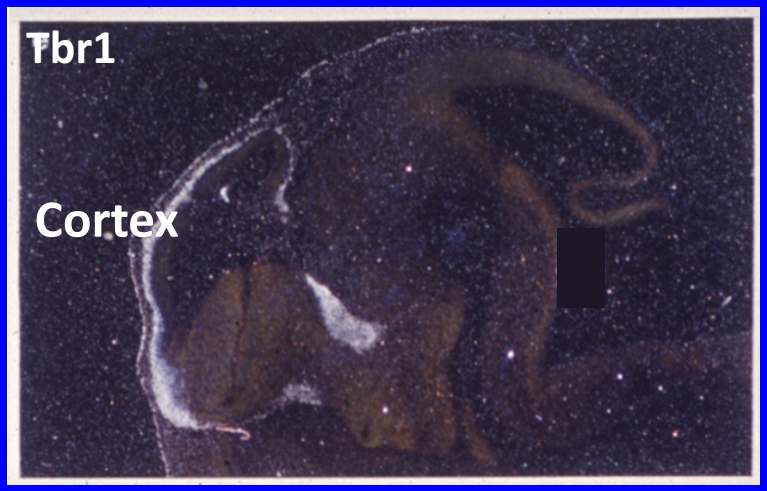
Part of a series offering the most comprehensive coverage of developmental neuroscience available, covering molecular development through to cognition and disease

Patterning of the Cortex and Basal Ganglia

Telencephalic Regions & Cell Types



Work Began 1987
Isolated Dlx2 and Tbr1
Regulate GABAergic and Glutamatergic Telencephalic Neurons



Rubenstein et al., 1990
Porteus et al., 1991
Bulfone et al., 1995

Tbr1 Is a High Confidence Autism Gene That Regulates Cortical Layer 6 Identity

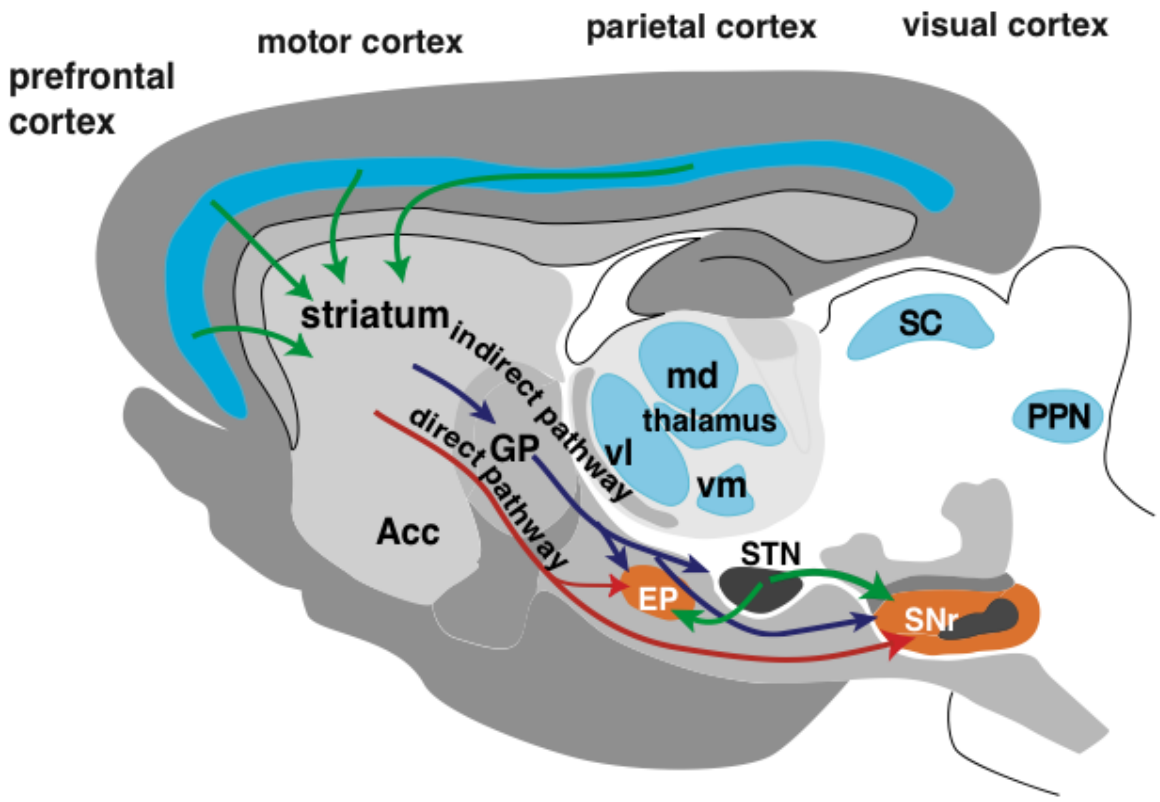
Hevner et al Neuron. 2001 Feb;29(2):353-66.

McKenna et al. J Neurosci. 2011 Jan 12;31(2):549-64

Dlx2 Controls Forebrain GABAergic Neuronal Development and Function

Cortex-Basal Ganglia

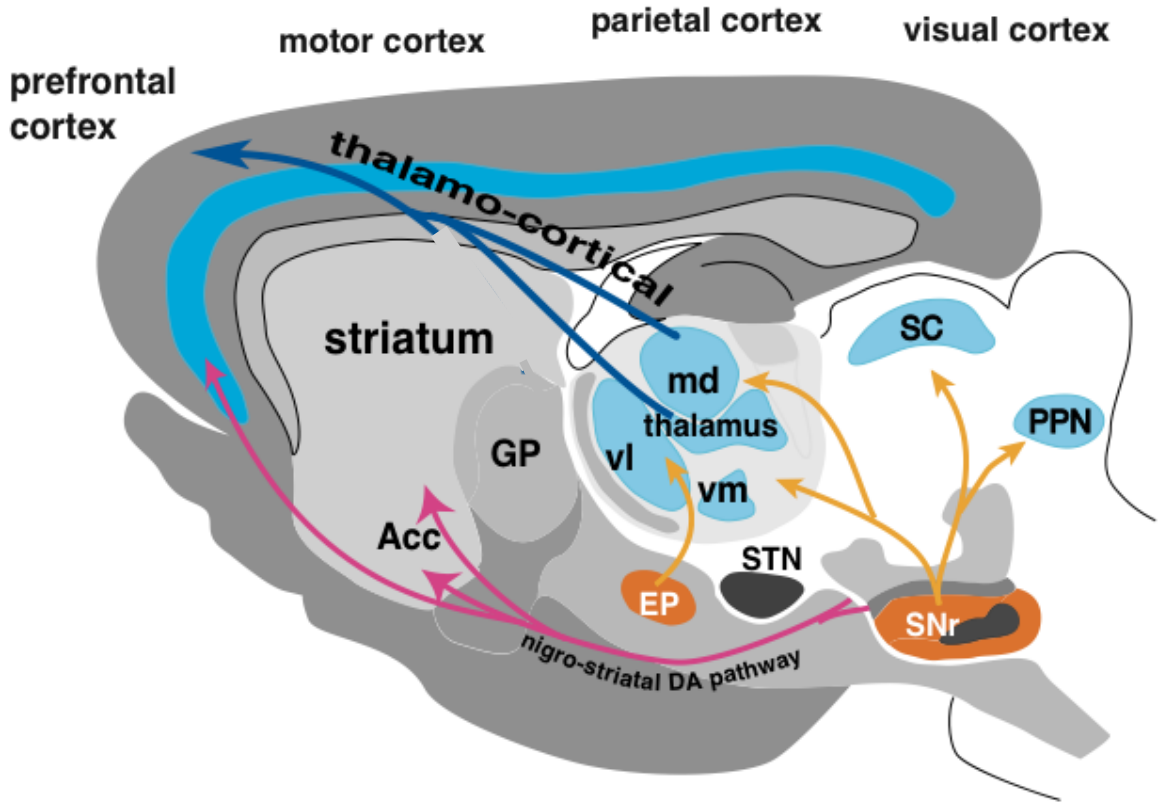
Descending Pathways



From: Charles Gerfen

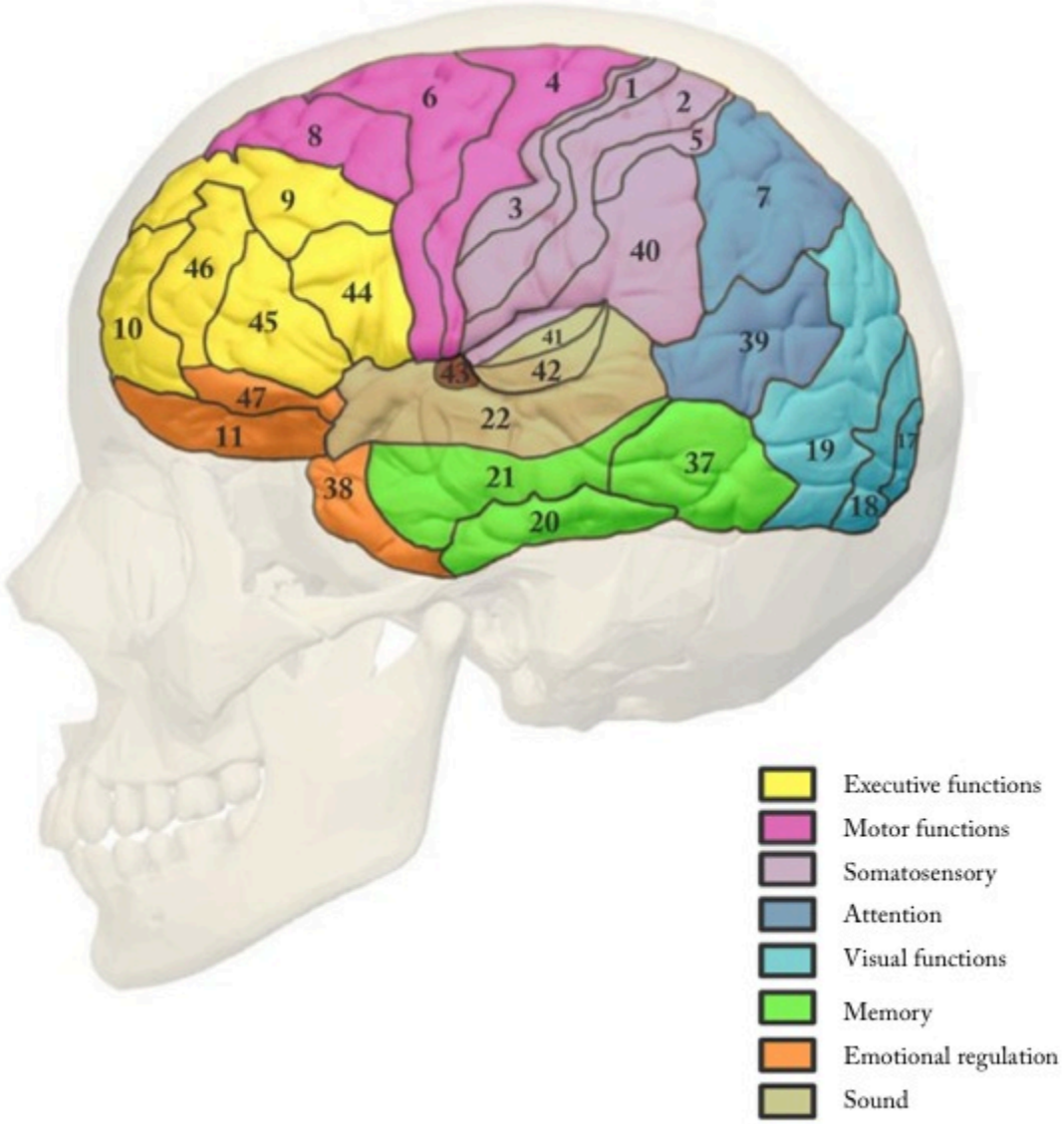
Basal Ganglia-Thalamus-Frontal Cortex

Ascending Pathways



Adapted from Charles Gerfen

Brodmann Cortical Areas



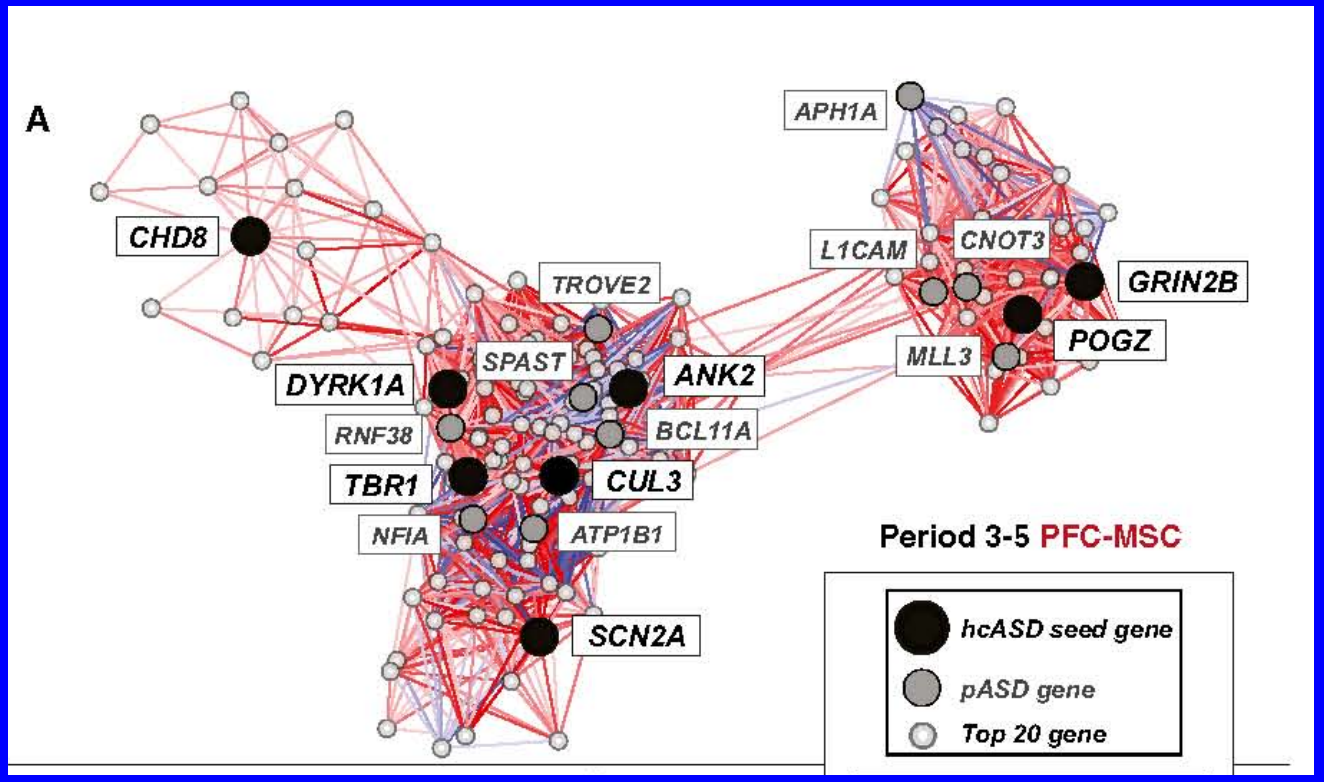
Frontal Cortex Function and Anatomy Is Abnormal in Neuropsychiatric Disorders

Hypoplasia and Hypofunction in Schizophrenia

Hyperplasia and Hypofunction in Autism

Autism Gene Network Dysfunction in Fetal Deep-Layer Frontal Cortex Projection Neurons

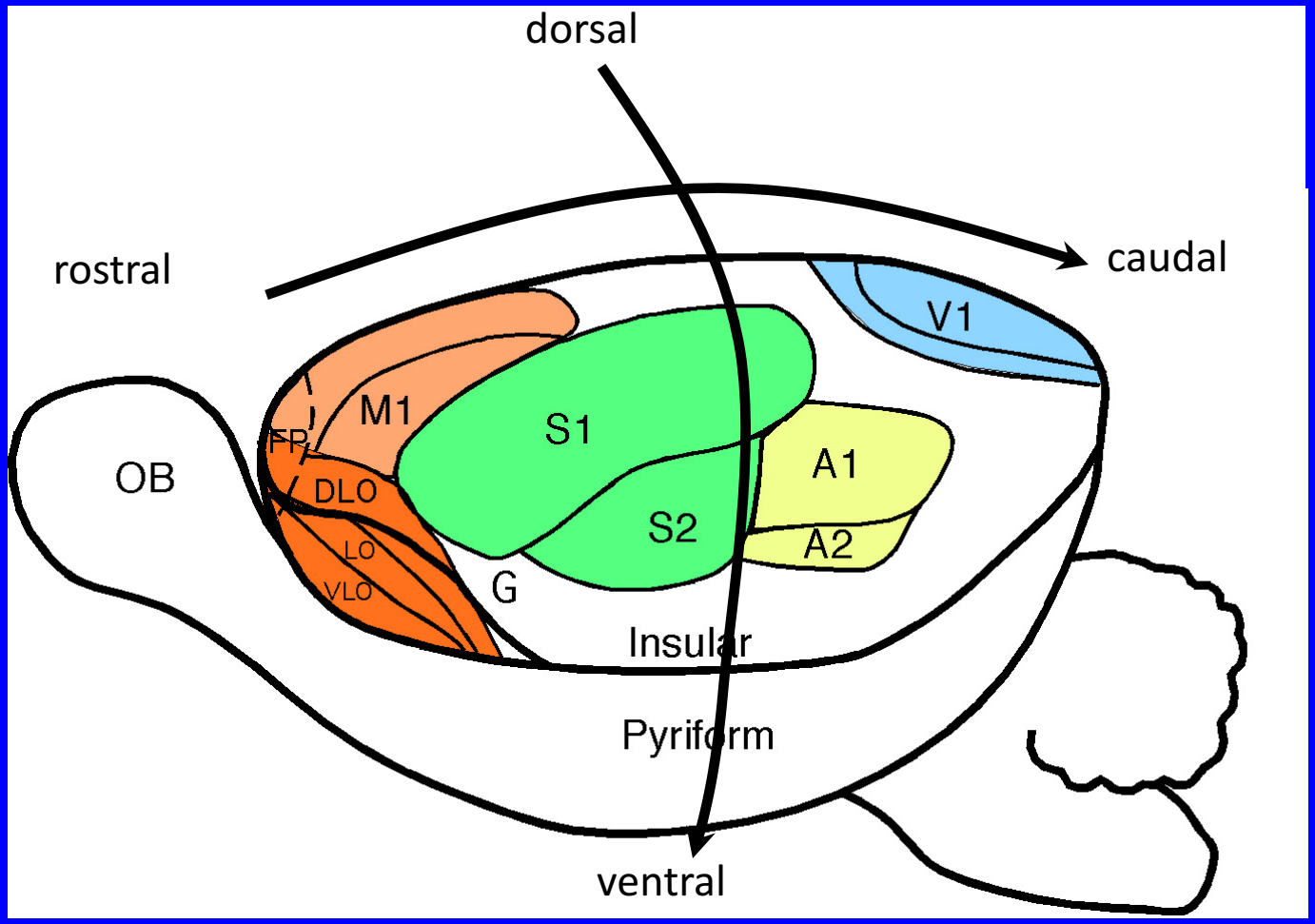
(Matt State and Nenad Sestan)



Willsey et al., 2013

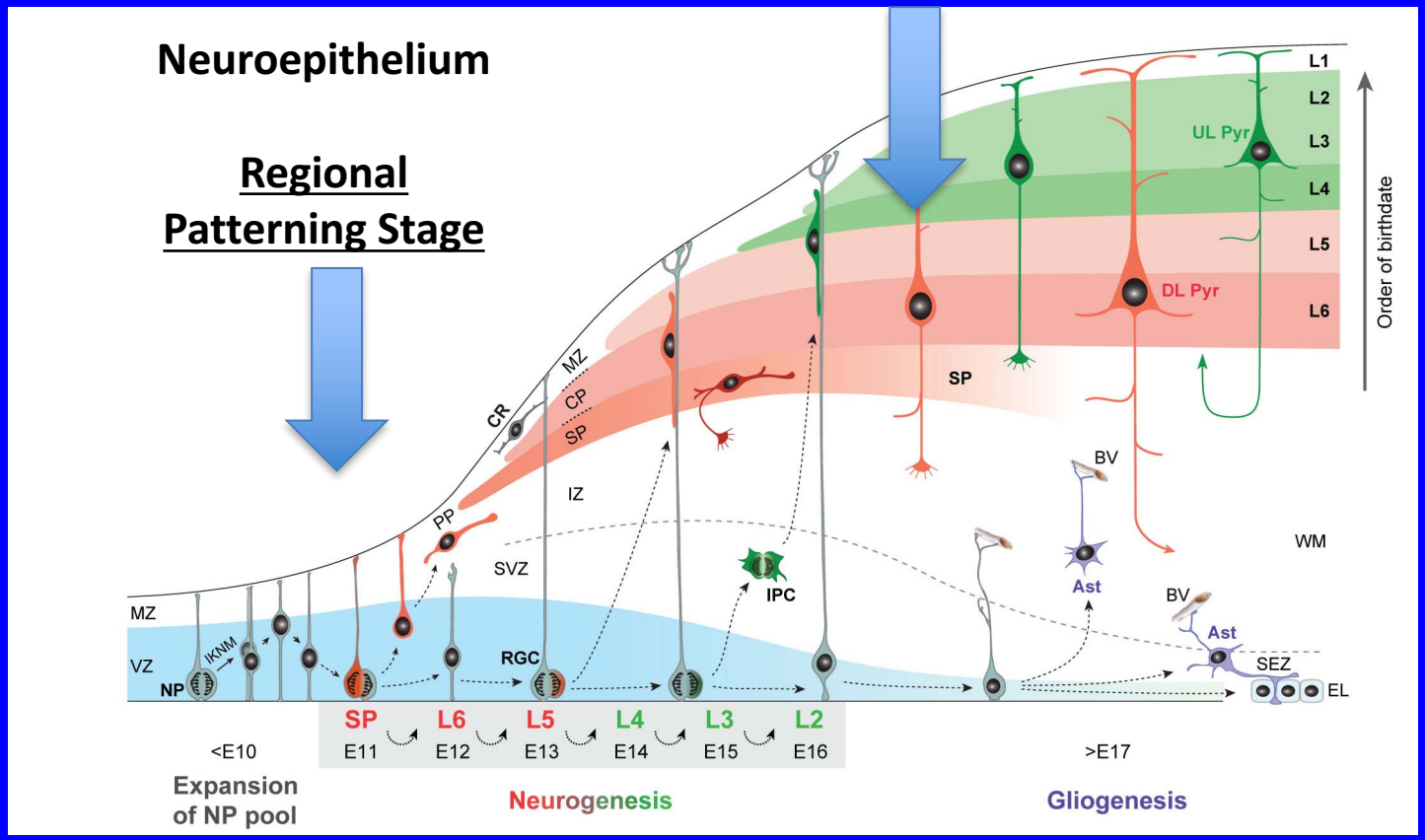
Focus on the Process of Cortical Regionalization in Building the Frontal Cortex

Subdivisions of the Cortex (Pallium)



Formation of 6 Layered Cortex

Neurogenesis and Laminar Patterning Stages

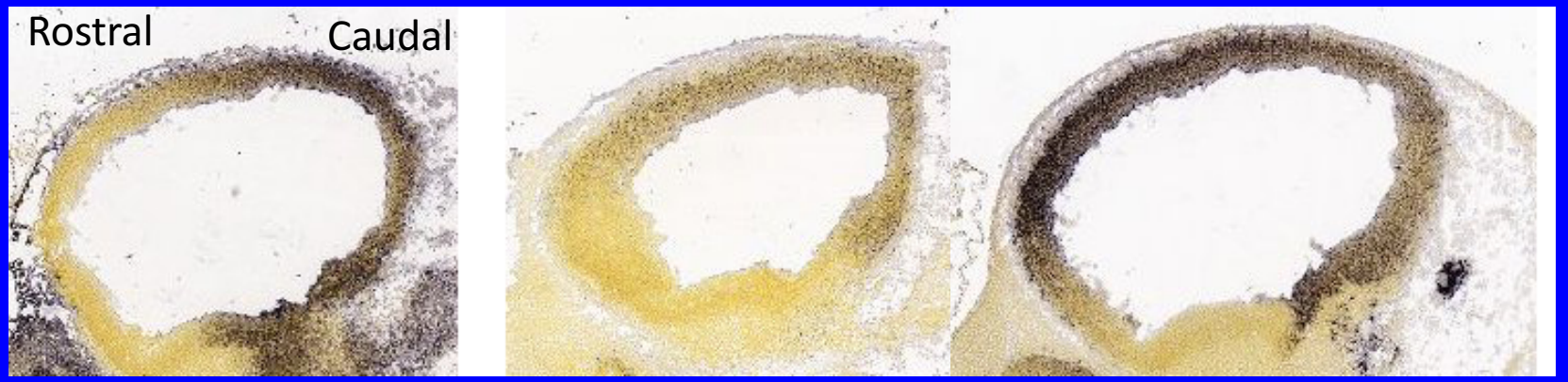


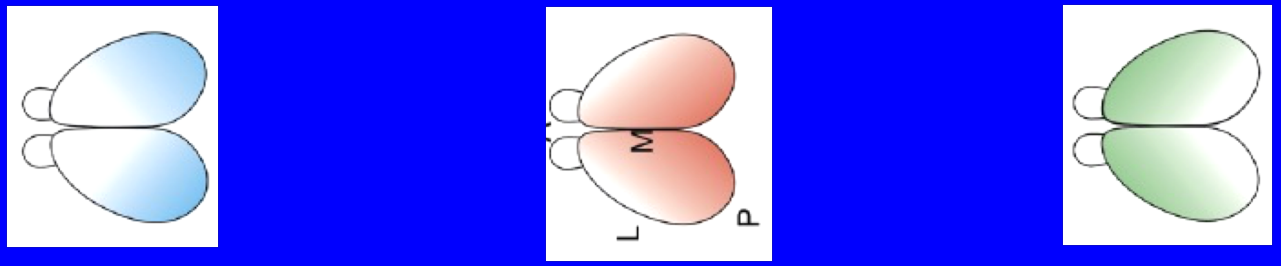
TIME

Kuang et al., 2012

Gradients of TF Expression

CoupTFI Emx2 Pax6





E11.5 Mouse

Four Short Stories About:
Molecular Components
Involved in Frontal Cortex Patterning

1. Fgf Signaling: Fate of $Fgf8^{+}$ Cells
2. Otx2 Controls Fgf-Signaling
3. Enhancer Elements Define Frontal Cortex Anlage
4. Pbx Controls Frontal Cortex Patterning

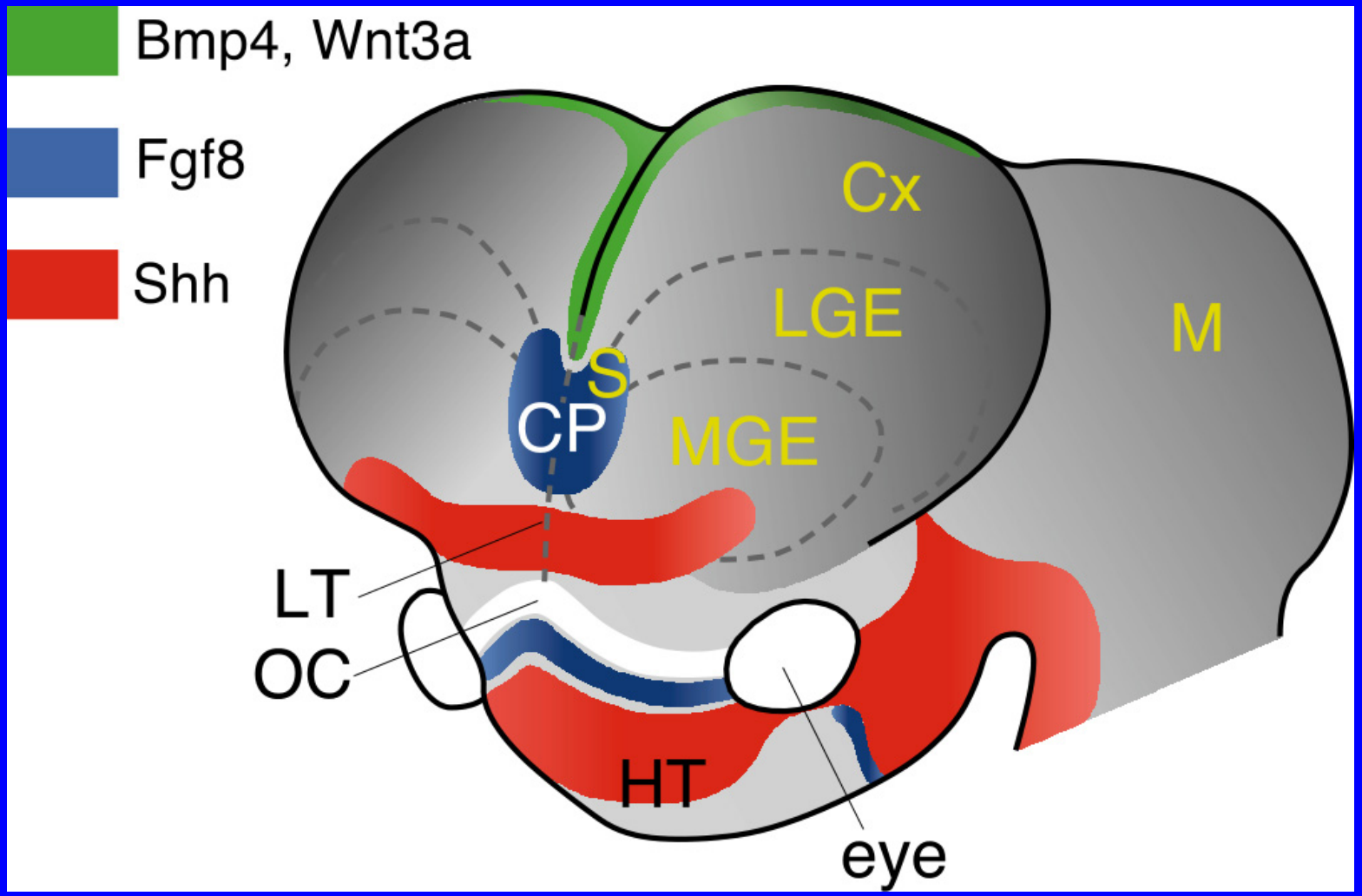
Part I

Fgf8 Patterning of the Frontal Cortex

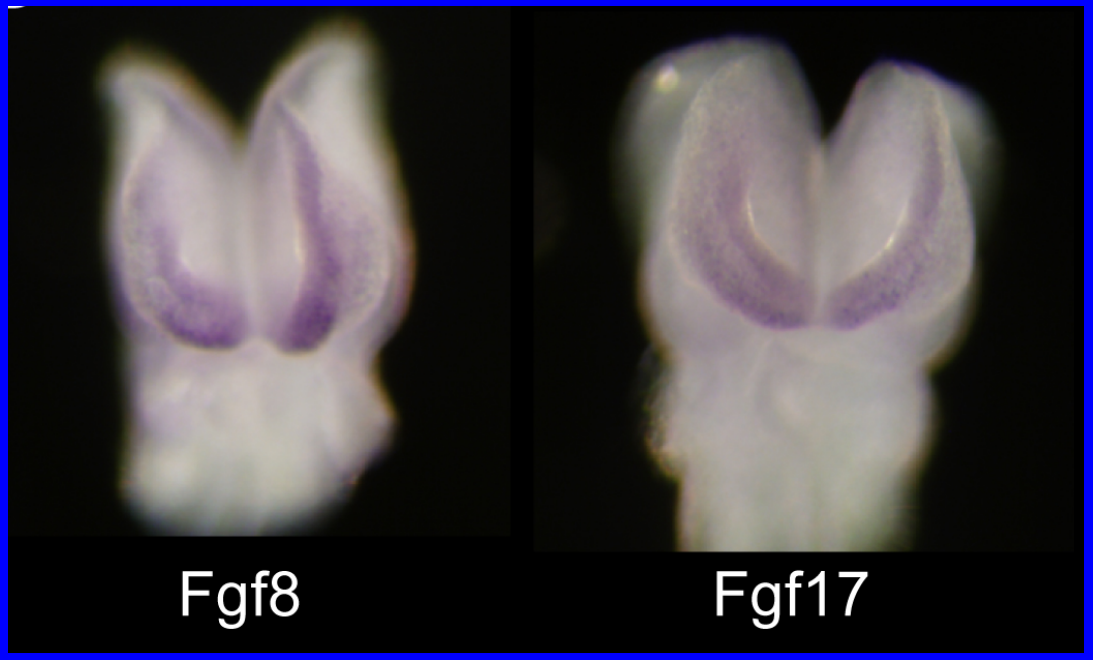
and

The Fate of $Fgf8^{+}$ Cells

Patterning Centers in the Forebrain



Fgf8 and Fgf17 Expression in the Anterior neural Ridge ~E8.75

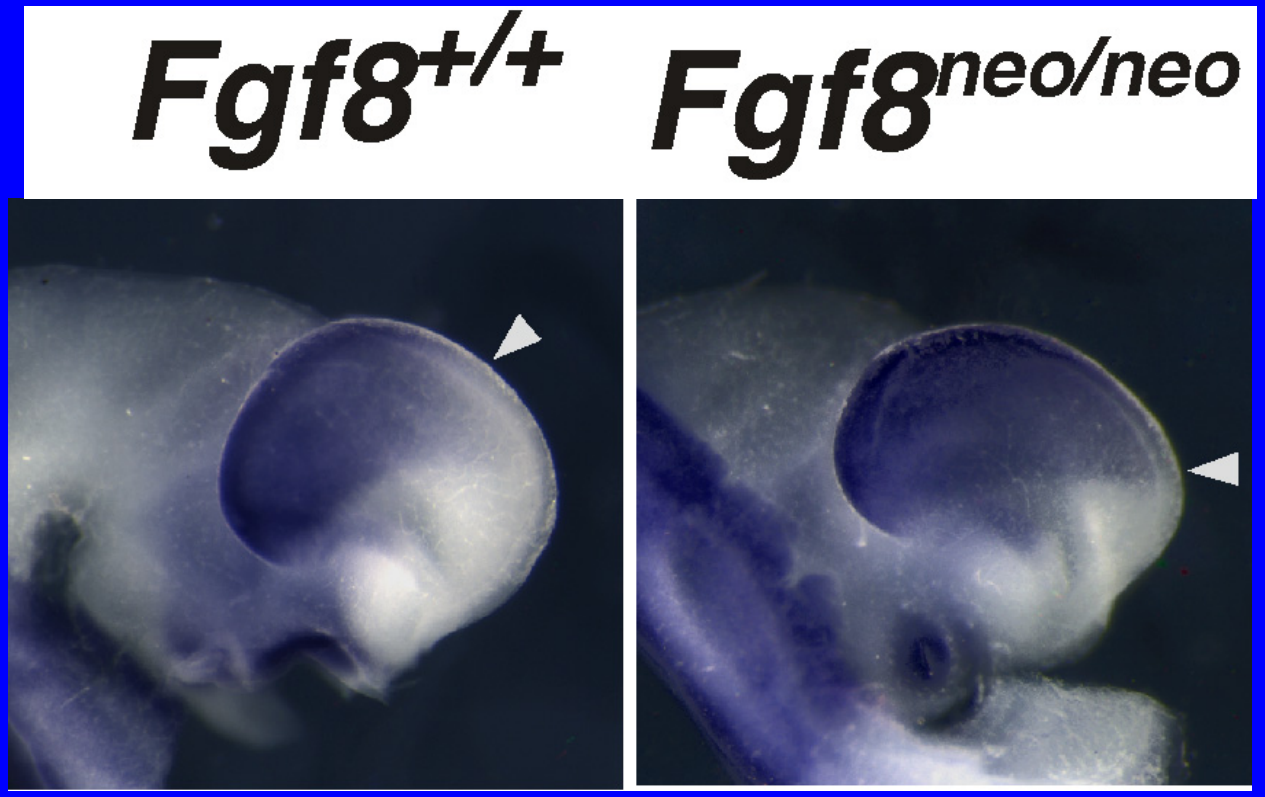


Fgf8 Regulates **Long-Range** Cortical Patterning.

Fgf17 Regulates **Short-Range** Cortical Patterning.

Fgf8 Hypomorphic Mutant

Rostral Expansion of CoupTFI Expression

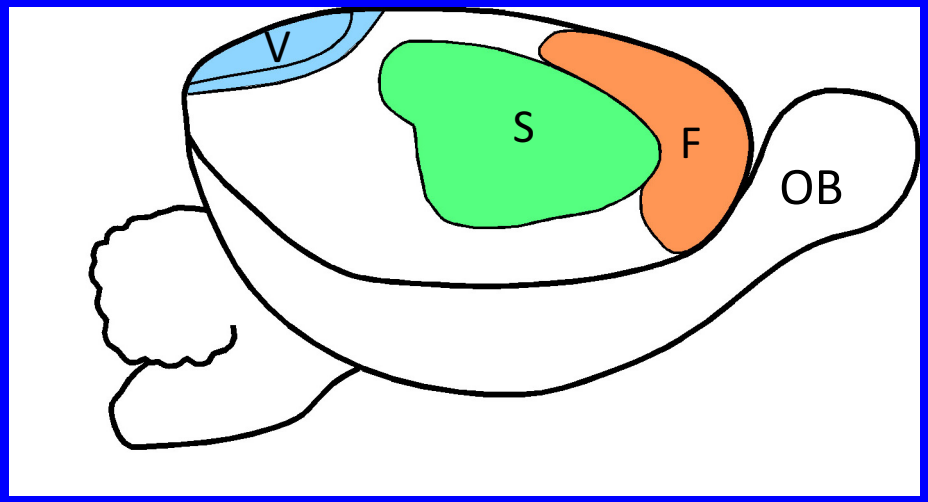


Garel et al., 2003

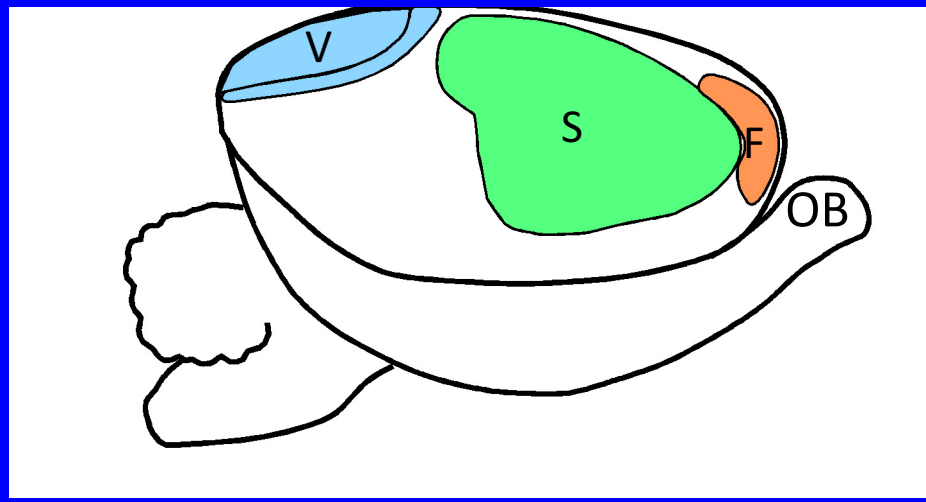
Fgf8 Hypomorphic Mutant

Small Frontal Cortex (F) and Expanded Somatosensory (S) Cortex

Normal



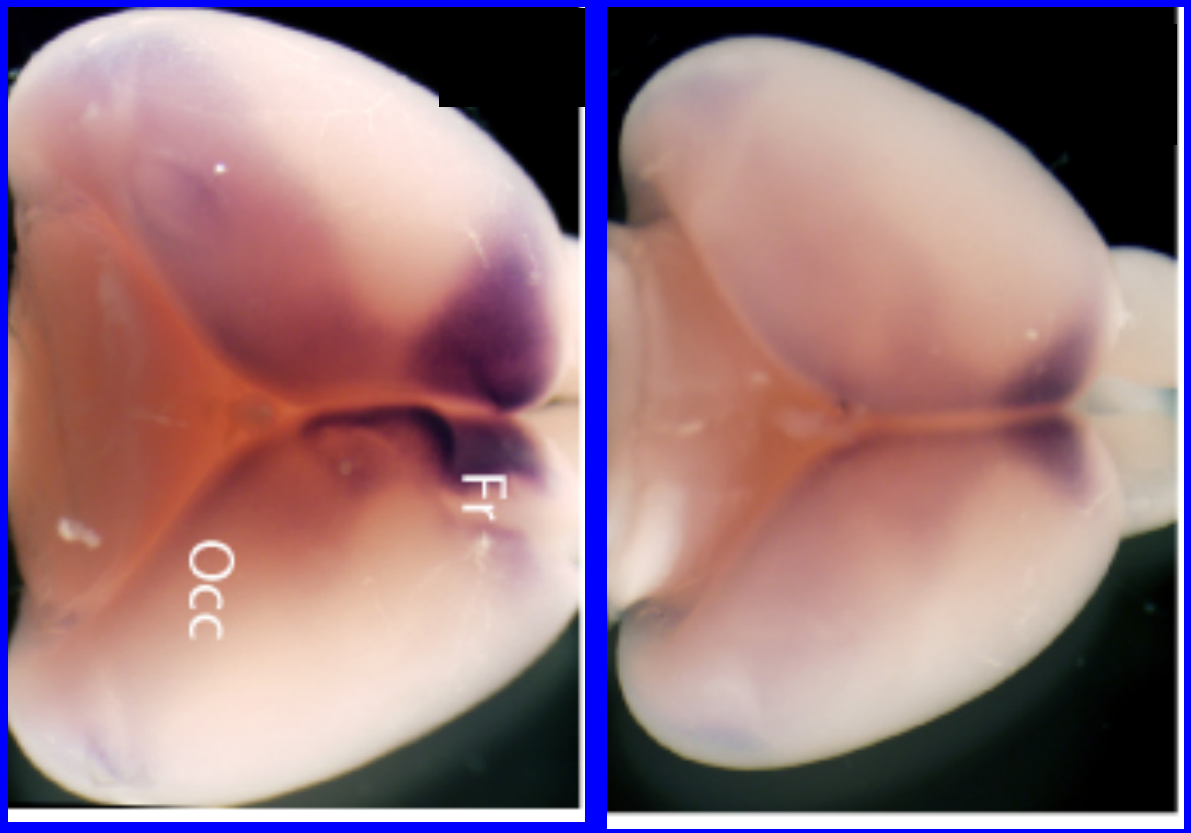
Fgf8$^{neo/neo}$



Fgf17 Null Mutant

WT

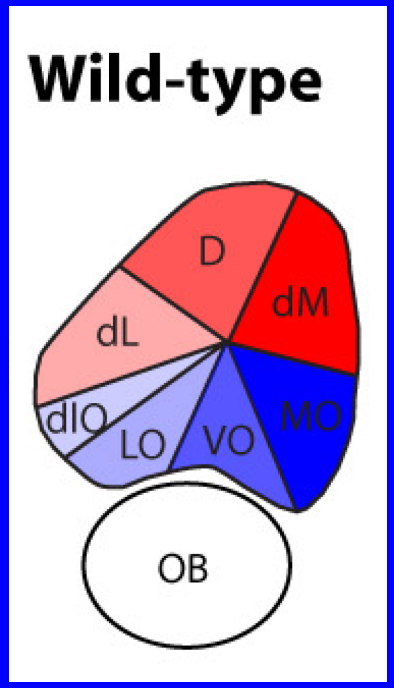
Null Mutant

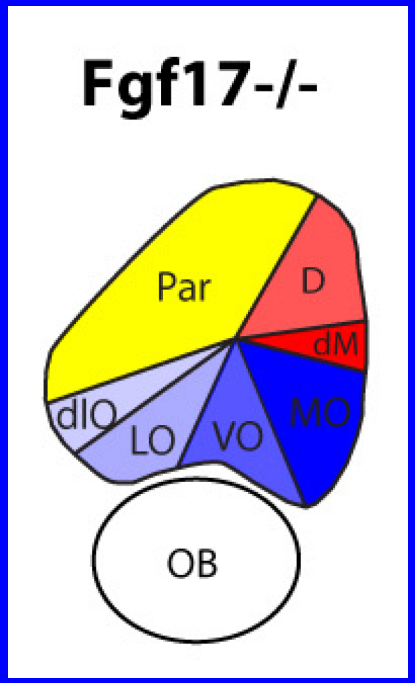


Reduced Frontal Cortex
(Lmo4^{+} Domain)

Cholfin and Rubenstein, 2007

Hypoplasia of the *Dorsomedial* Frontal Cortex in Fgf17$^{-/-}$ Mutants



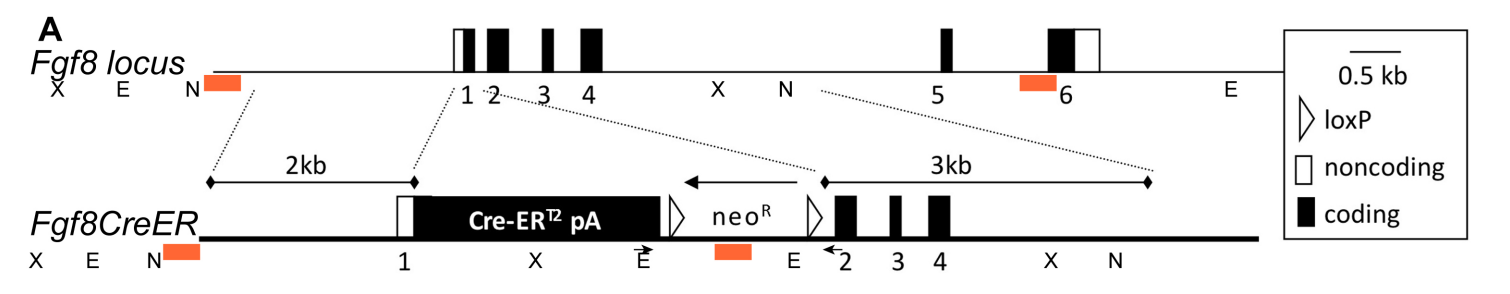


Does Fgf8 Only Affect Adjacent Regions
By Acting at a Distance (as a Morphogen)
from the Rostral Patterning Center?

and/or

Do $Fgf8^{+}$ Cells Contribute Neurons and Glia
to the Cortex and Basal Ganglia?

Knockin of CreER into Fgf8 Locus



Hoch et al., 2015

Fate Mapping from *Fgf8-CreER*

Tamoxifen:

E7.5

E8.5

E9.5

Analysis at P0 using Two Cre Reporters:

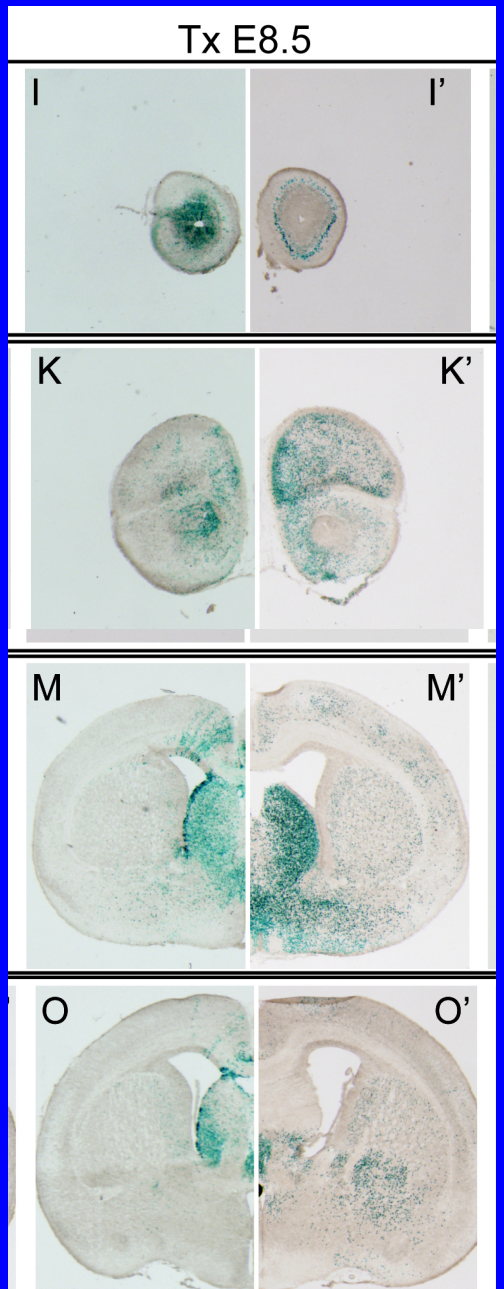
ROSA26R: progenitors (Left Column).

TauR: neurons (Right Column).

Two Cre Reporters:

.

ROSA26R ***Progenitors***	*TauR* ***Neurons***



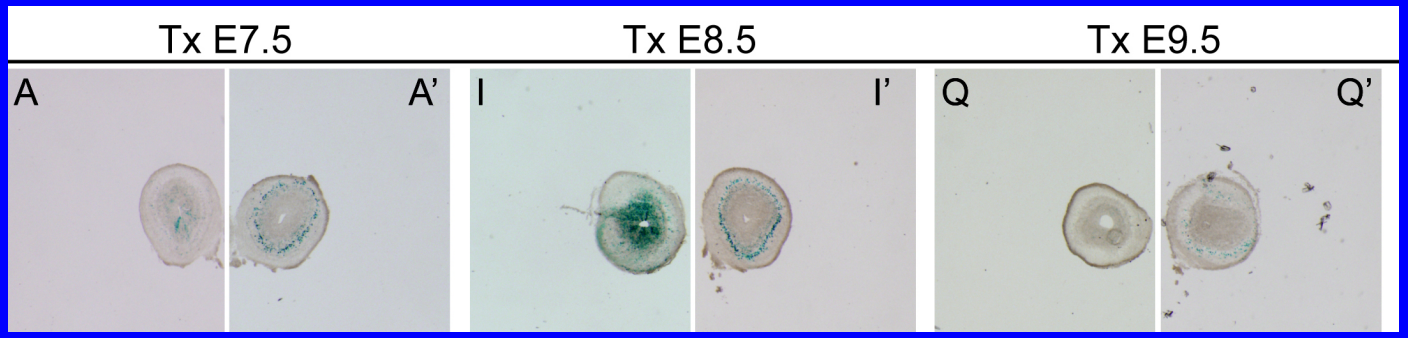
Olfactory Bulb

Prefrontal Cortex

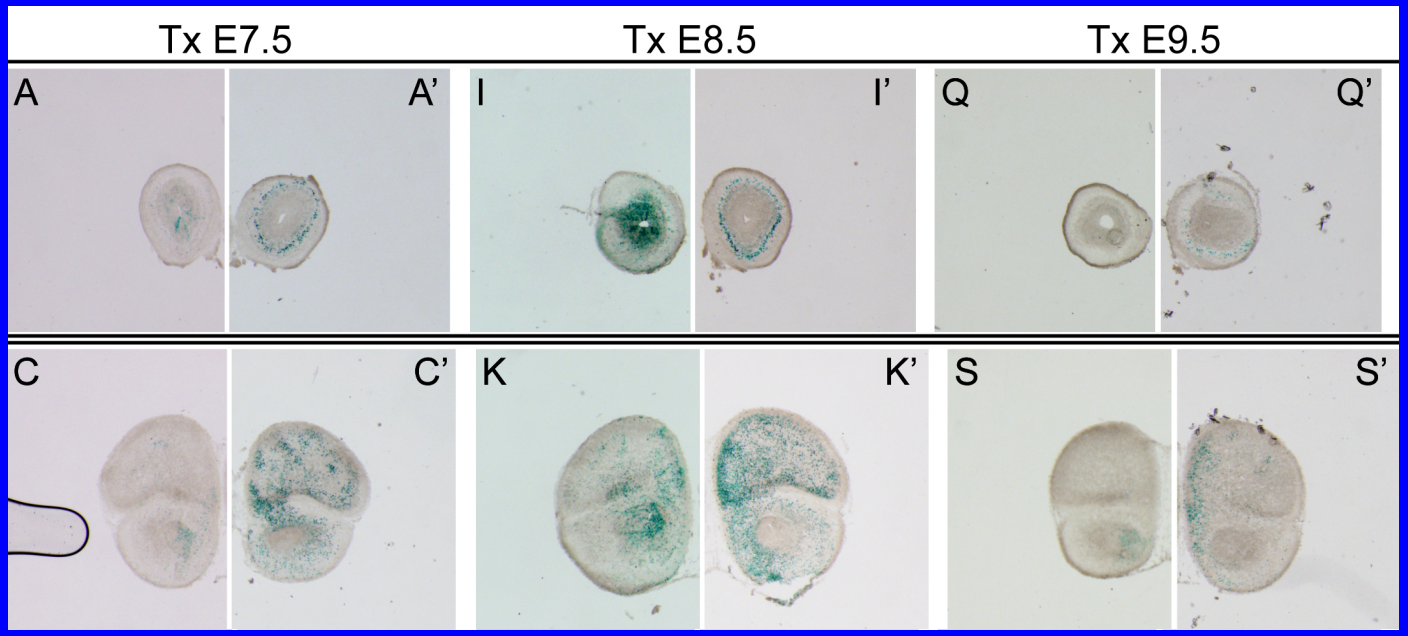
Septum

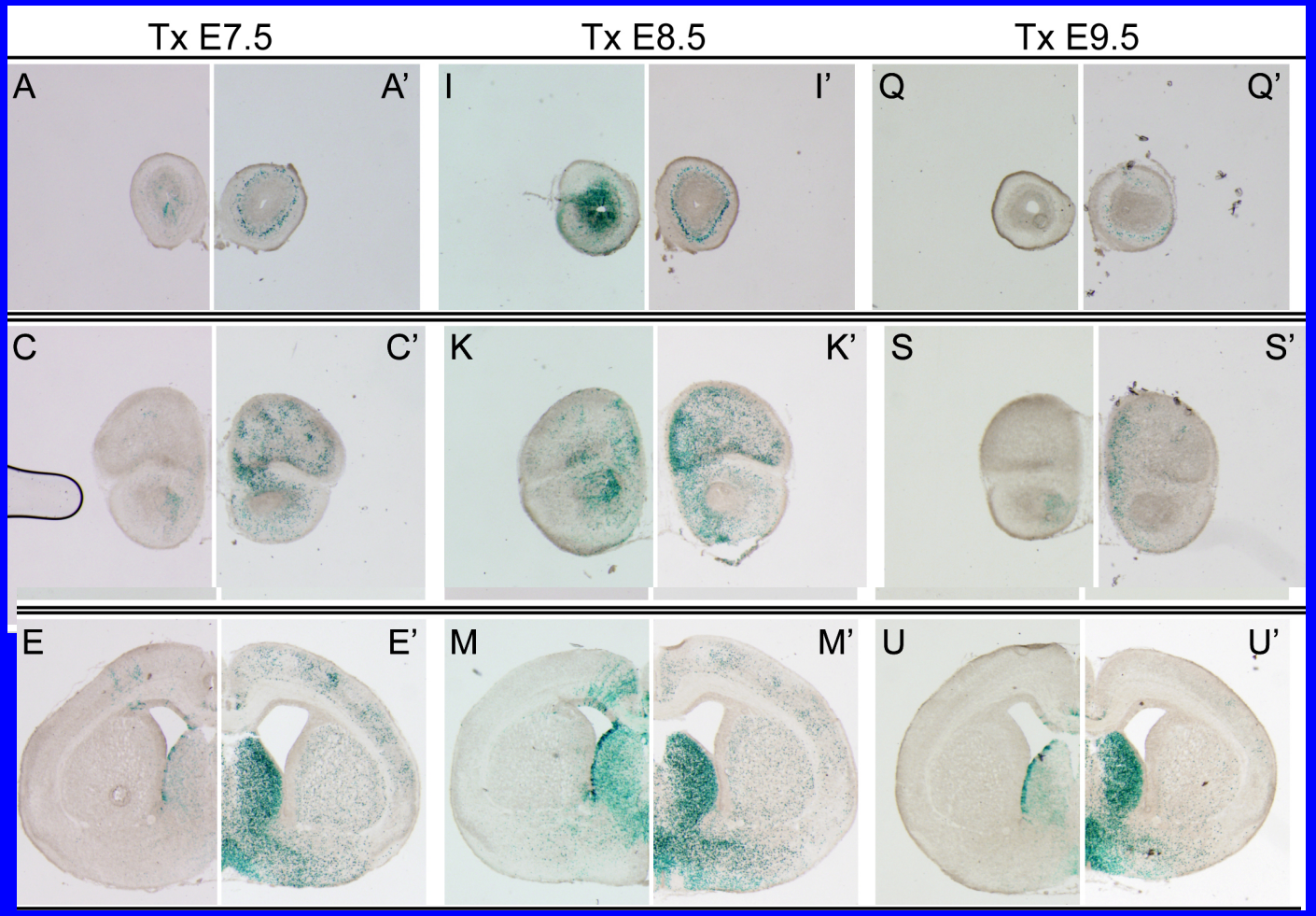
Cortex: Motor and Sensory

Globus Pallidus



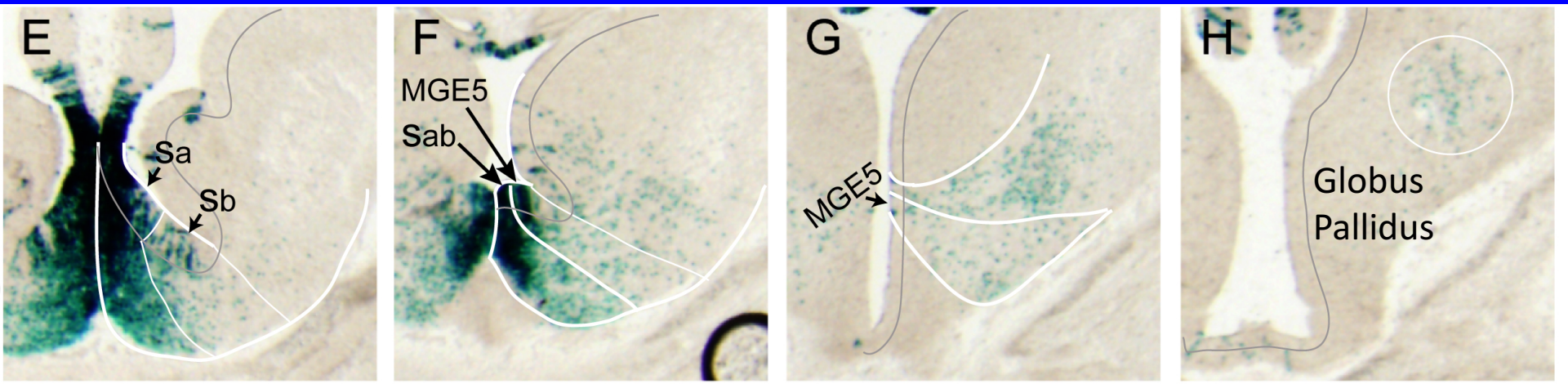
OB


Tx E7.5
Tx E8.5
Tx E9.5
A
A'
I
I'
Q
Q'
C
C'
K
K'
S
S'
OB
Prefrontal Cortex


Tx E7.5
Tx E8.5
Tx E9.5
A
A'
I
I'
Q
Q'
C
C'
K
K'
S
S'
E
E'
M
M'
U
U'
OB
Prefrontal Cortex
Septum

Origin of Globus Pallidus and Cholinergic Neurons

Tx:8.5; Analysis E13.5



The $Fgf8^{+}$ Rostral Patterning Center Generates:

- The Septum

But also:

- Prefrontal Cortex
- Globus Pallidus,
- Cholinergic Neurons

Hoch et al., 2015

Part II:

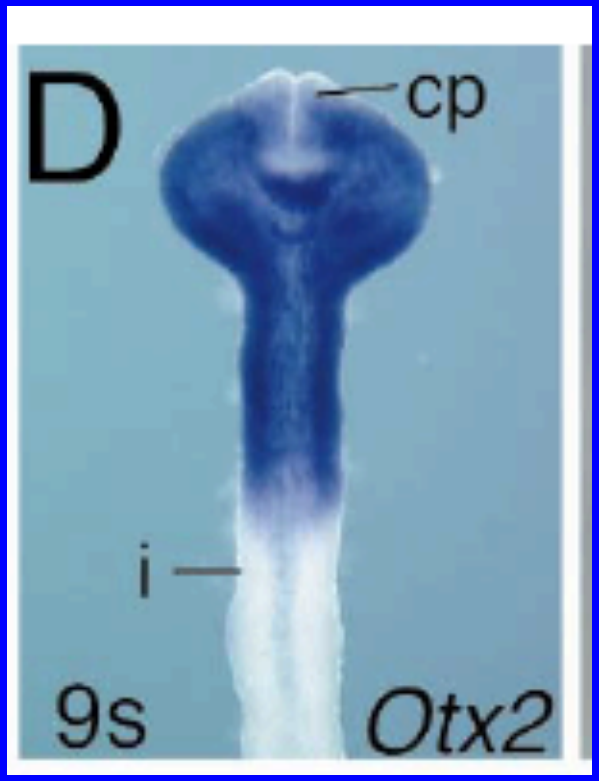
What Transcriptionally Regulates Fgf8 Expression and Signaling:

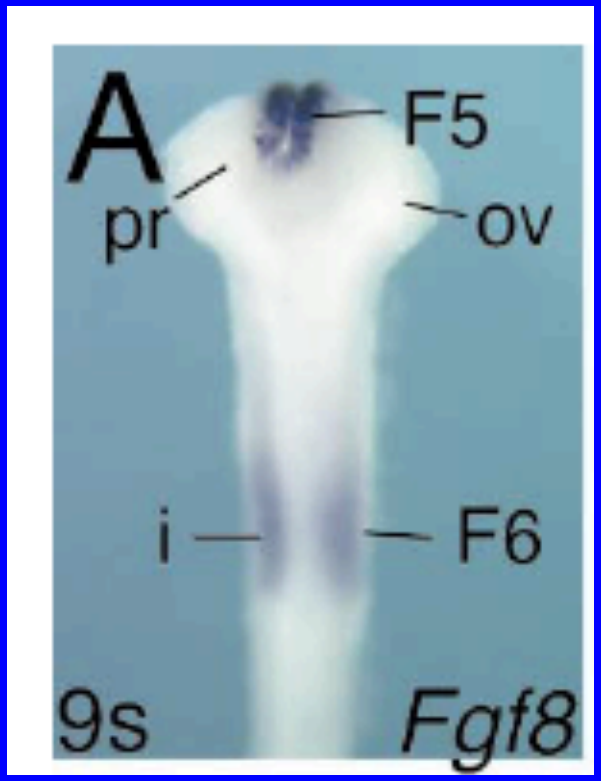
Otx2

Homeodomain Transcription Factor

Reciprocal Expression of Otx2 and Fgf8

Otx2 *Fgf8*

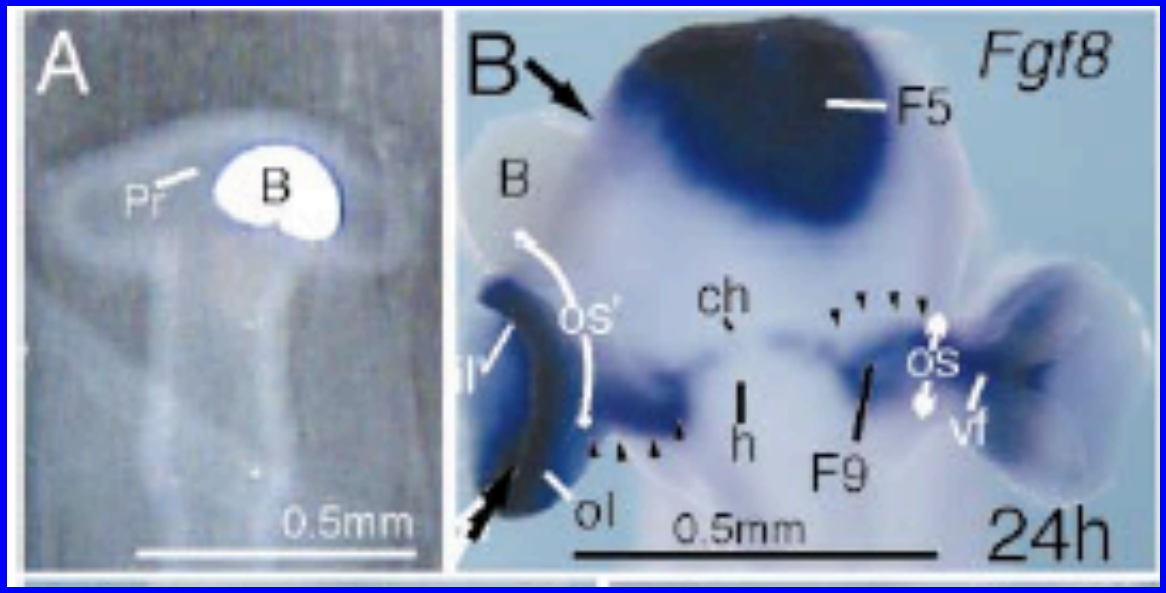




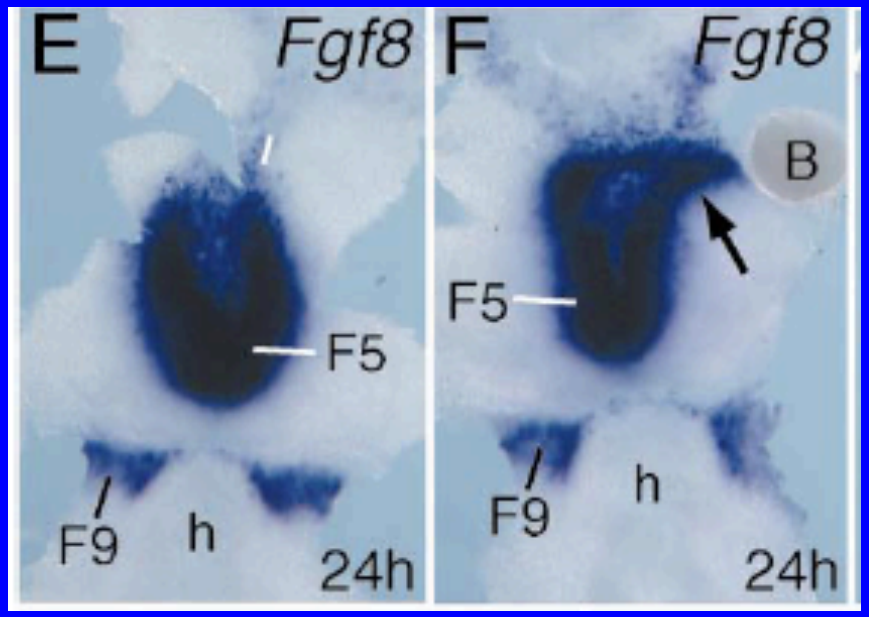
Anterior Neural Tube of Chicken Embryos

Crossley et al., 2001

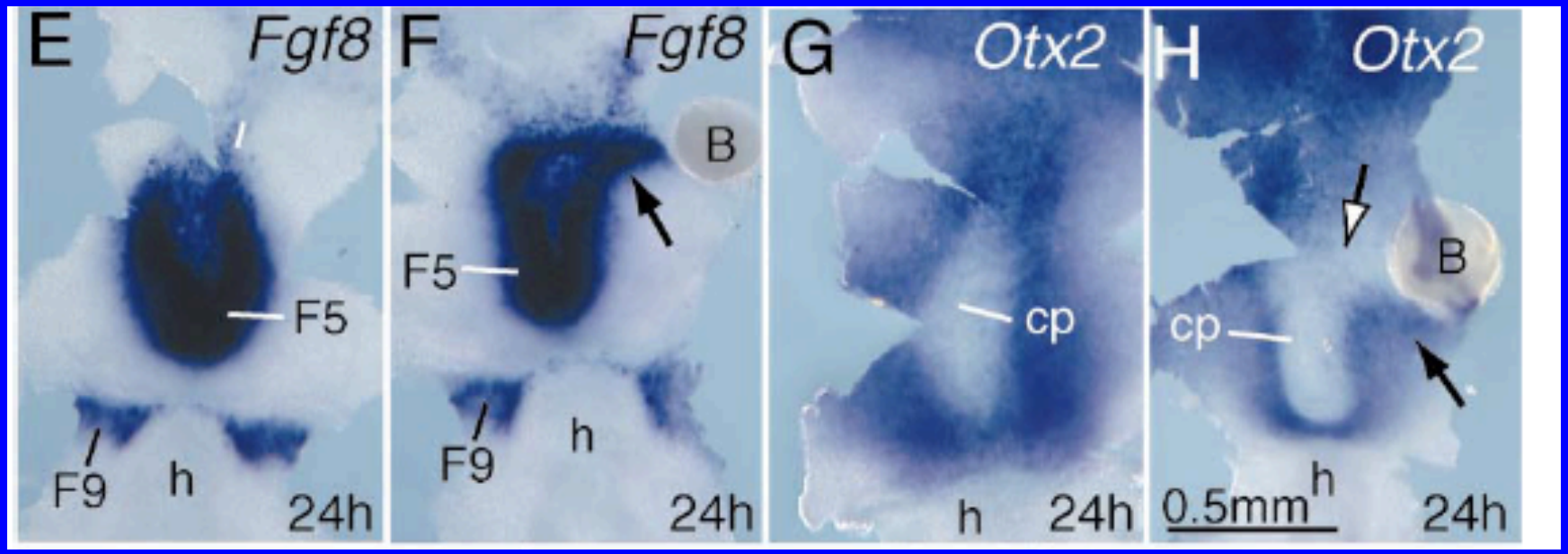
FGF8-Containing Beads (B) Locally Induce Fgf8



FGF8 Beads Induce Fgf8



FGF8 Beads Induce Fgf8
And
Repress Otx2



Otx2 Conditional Mutant

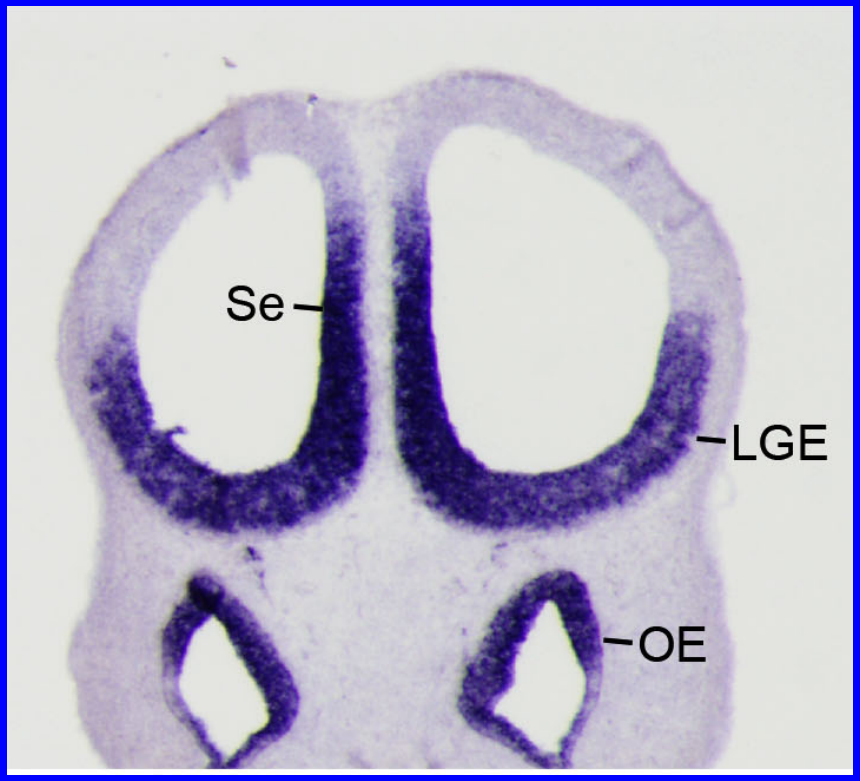
Use Rx-Cre to Delete Otx2

Hoch et al, 2015

Otx2 flox: from Shin Aizawa
Rx Cre from from E. Swindell

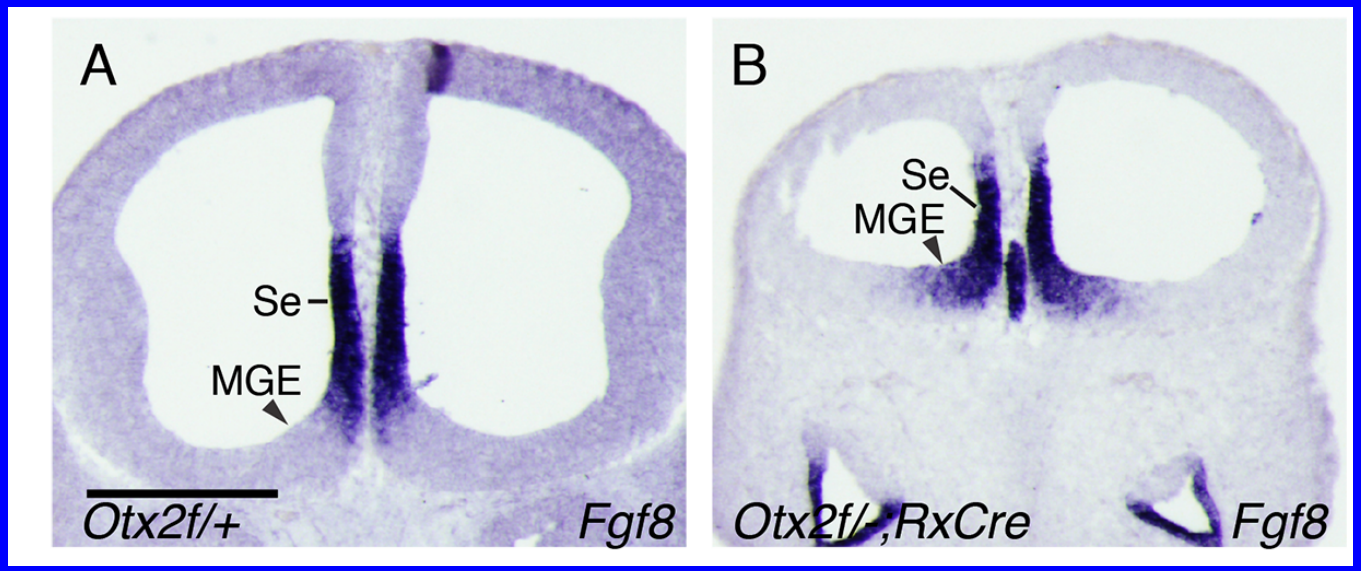
Otx2 Expression Becomes Restricted to the Subpallium

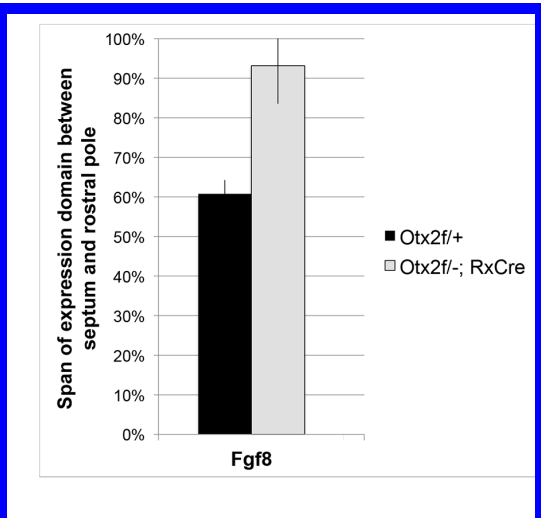
Septum (Rostral Patterning Center)
LGE



E12.5

Expansion of Fgf8^{+} Domain in Otx2 Mutant



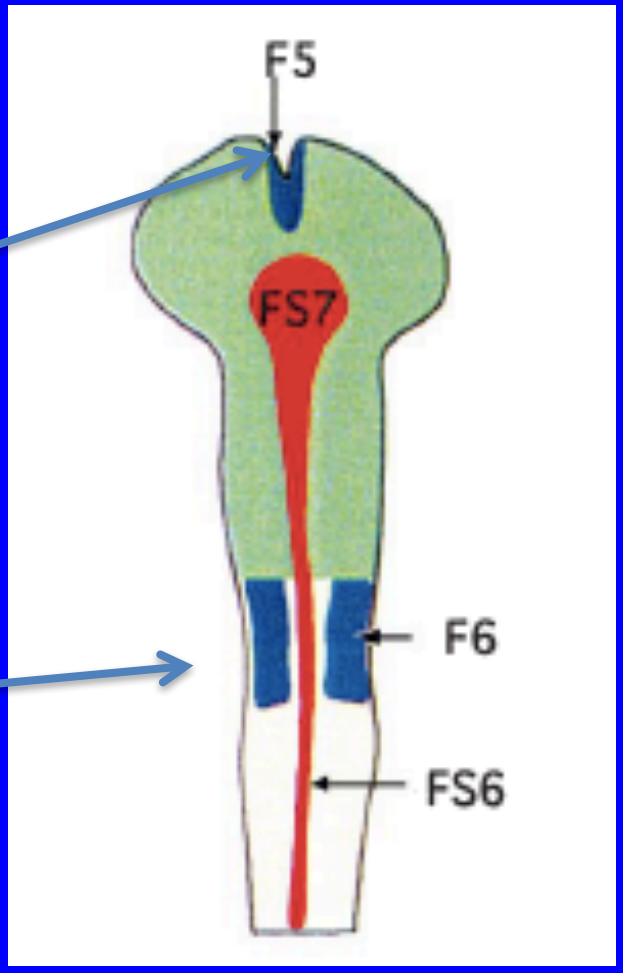


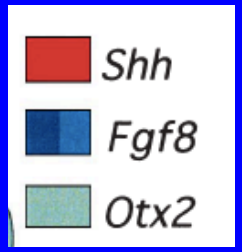
E12.5

Two Fgf8+ Patterning Centers

Rostral Patterning Center
RPC
Otx2+

Isthmic Patterning Center
Engrailed+, Pax3+





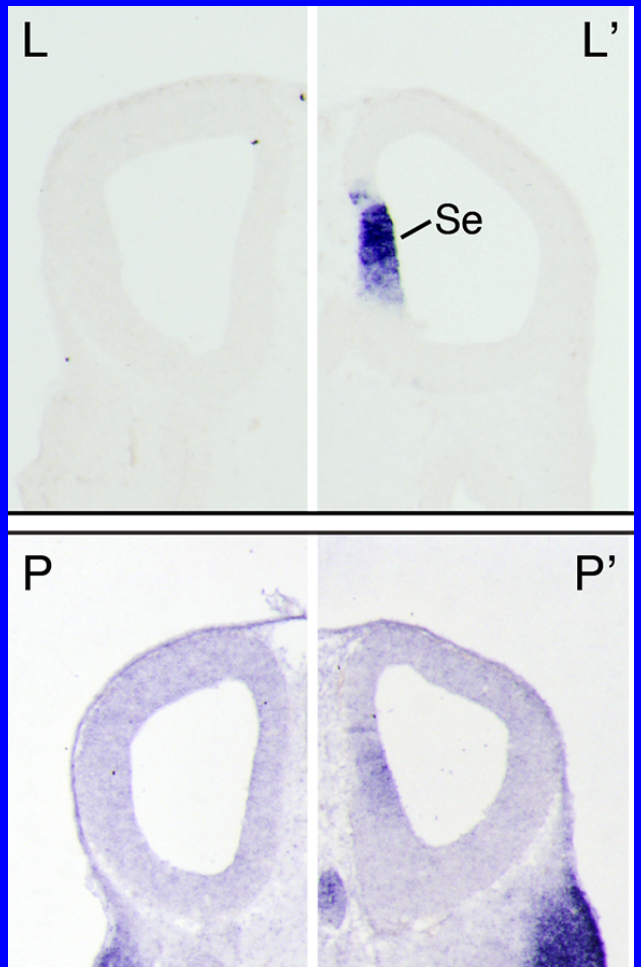
Otx2 Controls the Identity of the RPC

In Mutant: Ectopic Expression of Engrailed2 and Pax3

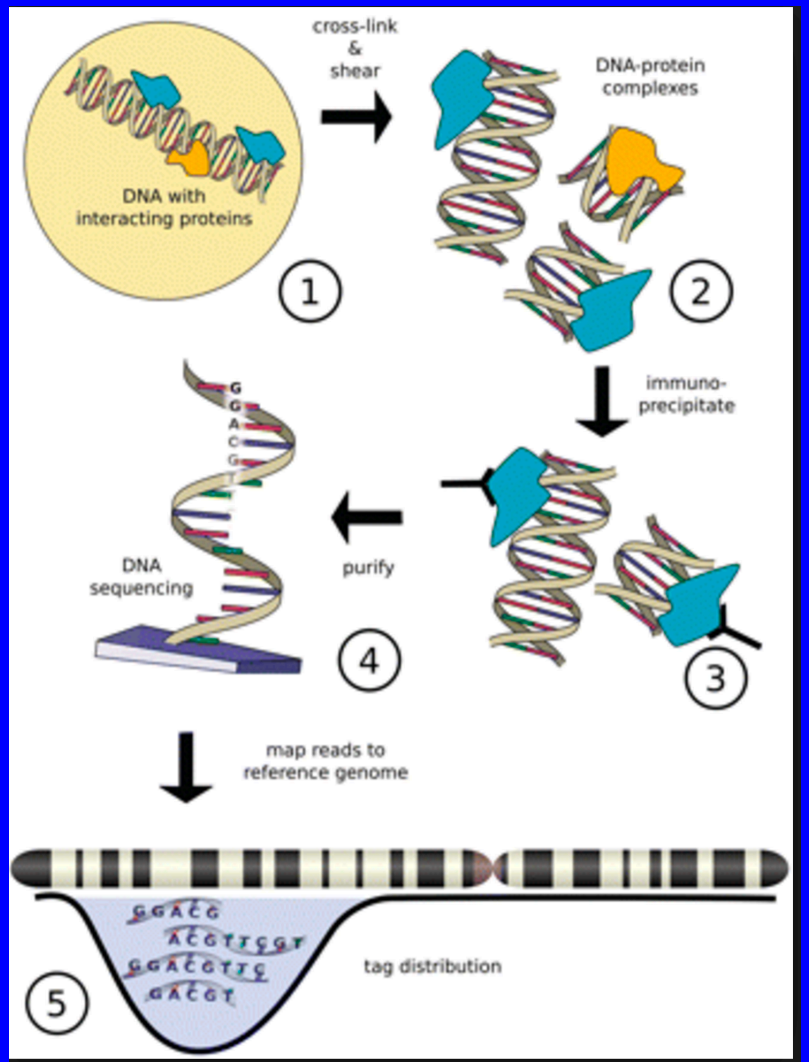
WT Otx2 Mutant

Engrailed2

Pax3



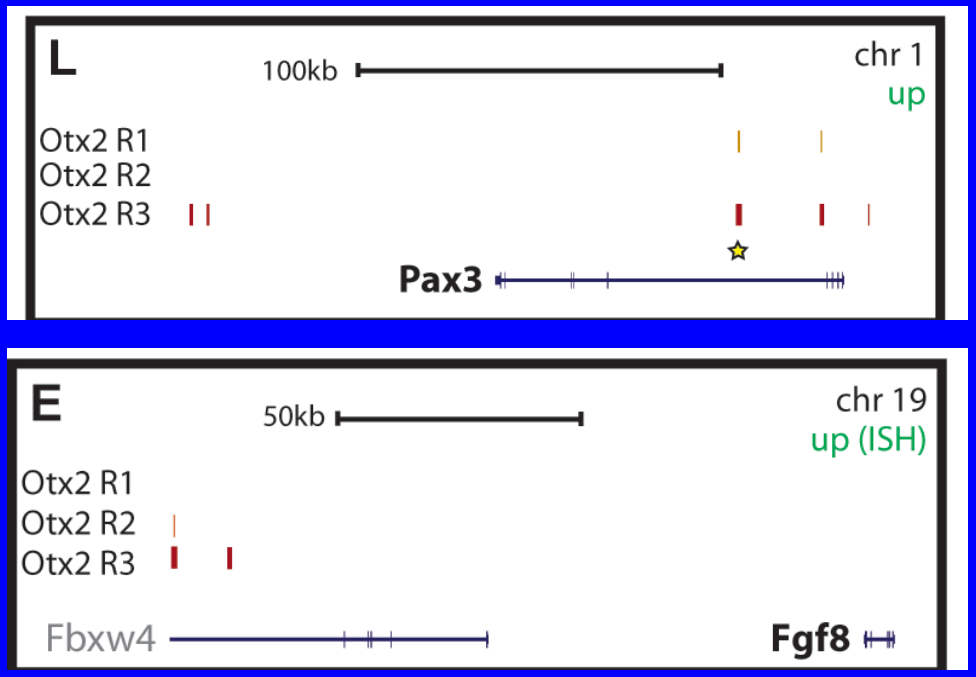
Chromatin Immunoprecipitation & DNA Sequencing Using Anti-OTX2



OTX2 ChIP-Seq (Triplicate)

OTX2 Binds to, or Near to,

Pax3 and Fgf8 Loci



E12.5 Subpallium

Otx2 Regulates the **Strength** and **Identity** of the Rostral Patterning Center

By

Directly Controlling
Expression of Fgf8 and Pax3

Fgf8 Induces Fgf8

Initiates Patterning Center In an $Otx2^{+}$ Domain

- Fgf8 Represses Otx2
- Otx2 Restricts the Size of Fgf8 Domain in the Rostral Patterning Center
- Otx2 Specifies Rostral Patterning Center; Represses Isthmic Patterning Center

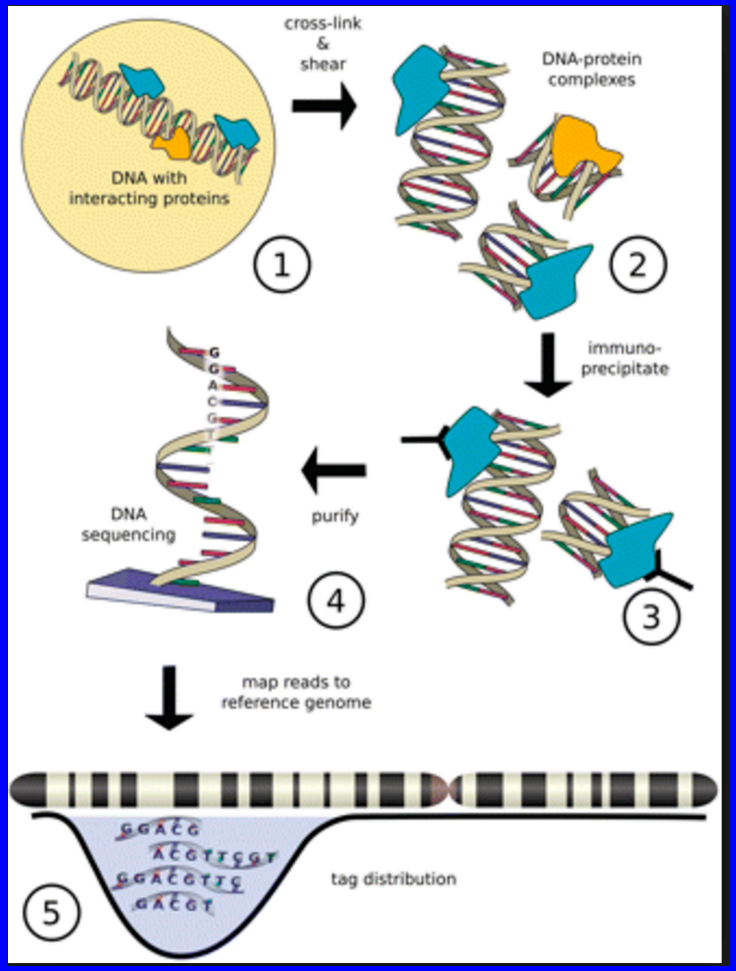
Part III:

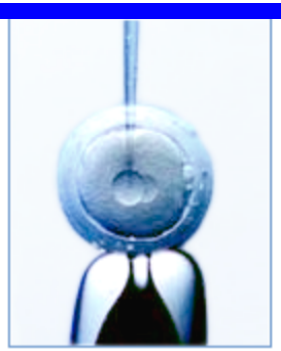
Enhancer Elements Define Frontal Cortex Anlage

Enhancer Discovery

With Axel Visel and Len Pennachio

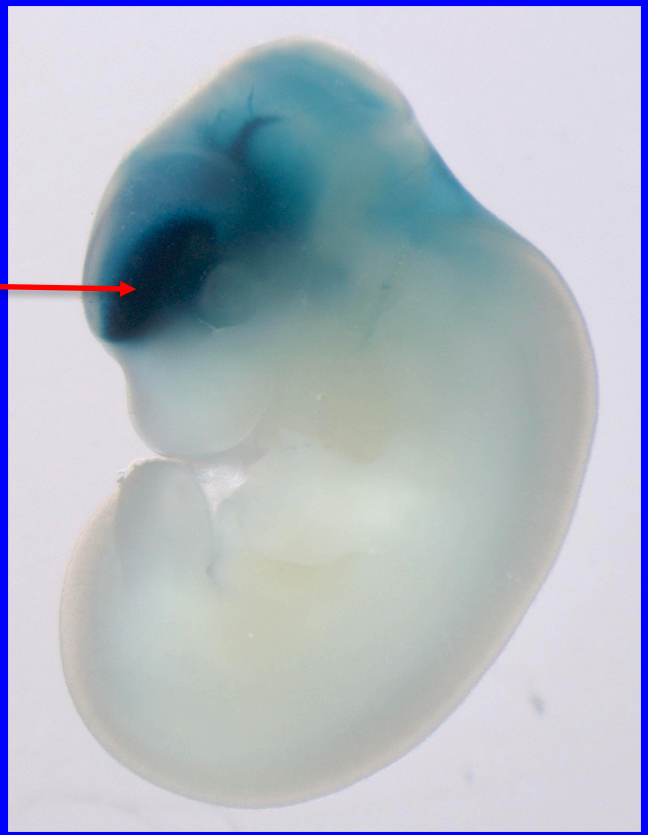
ChIP-Seq
Transcription Factor and/or Chromatin Proteins



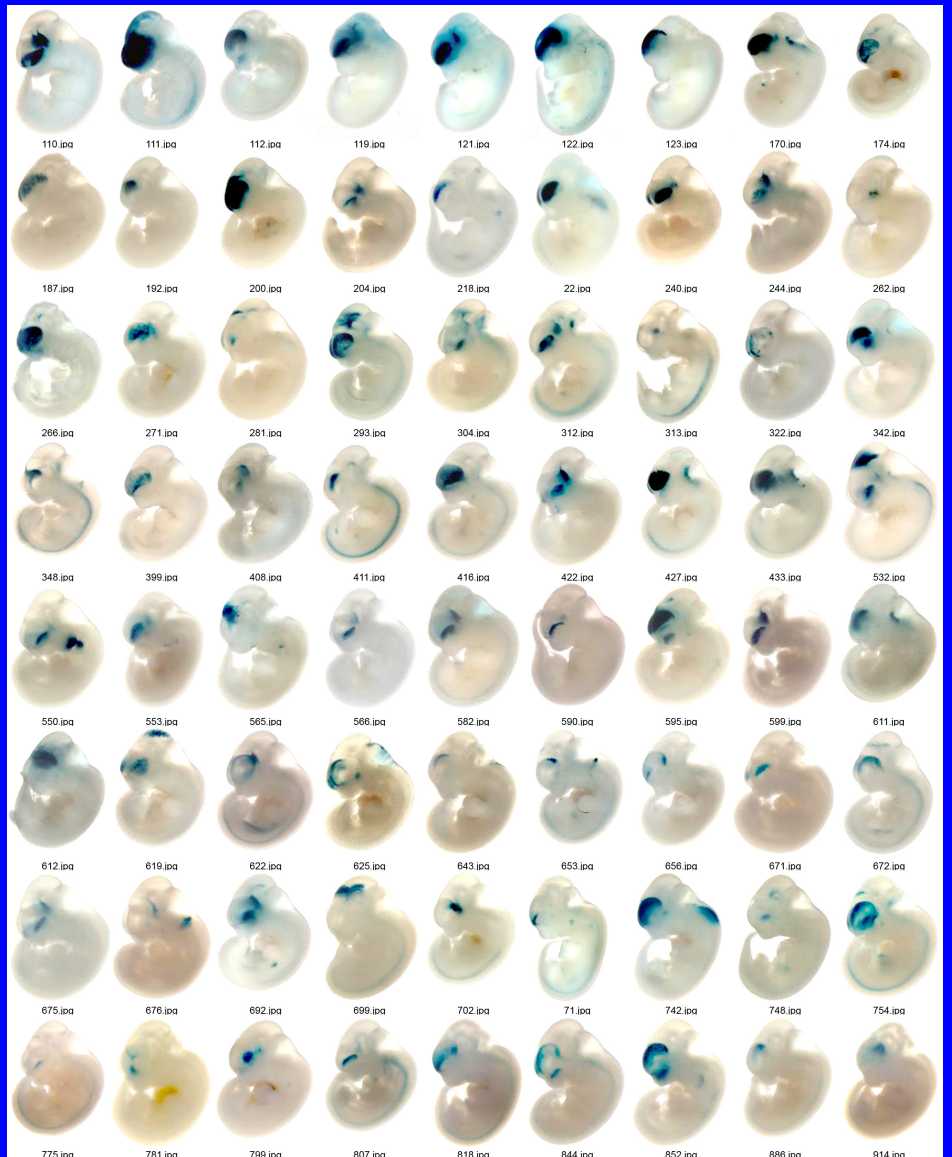


Mouse Egg Microinjection

This enhancer is active in the embryonic basal ganglia (ganglionic eminences; Ges)

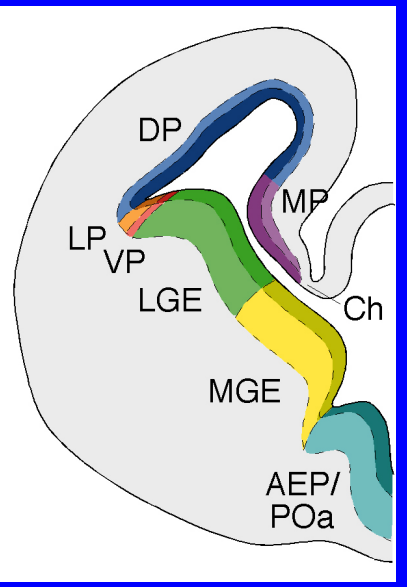


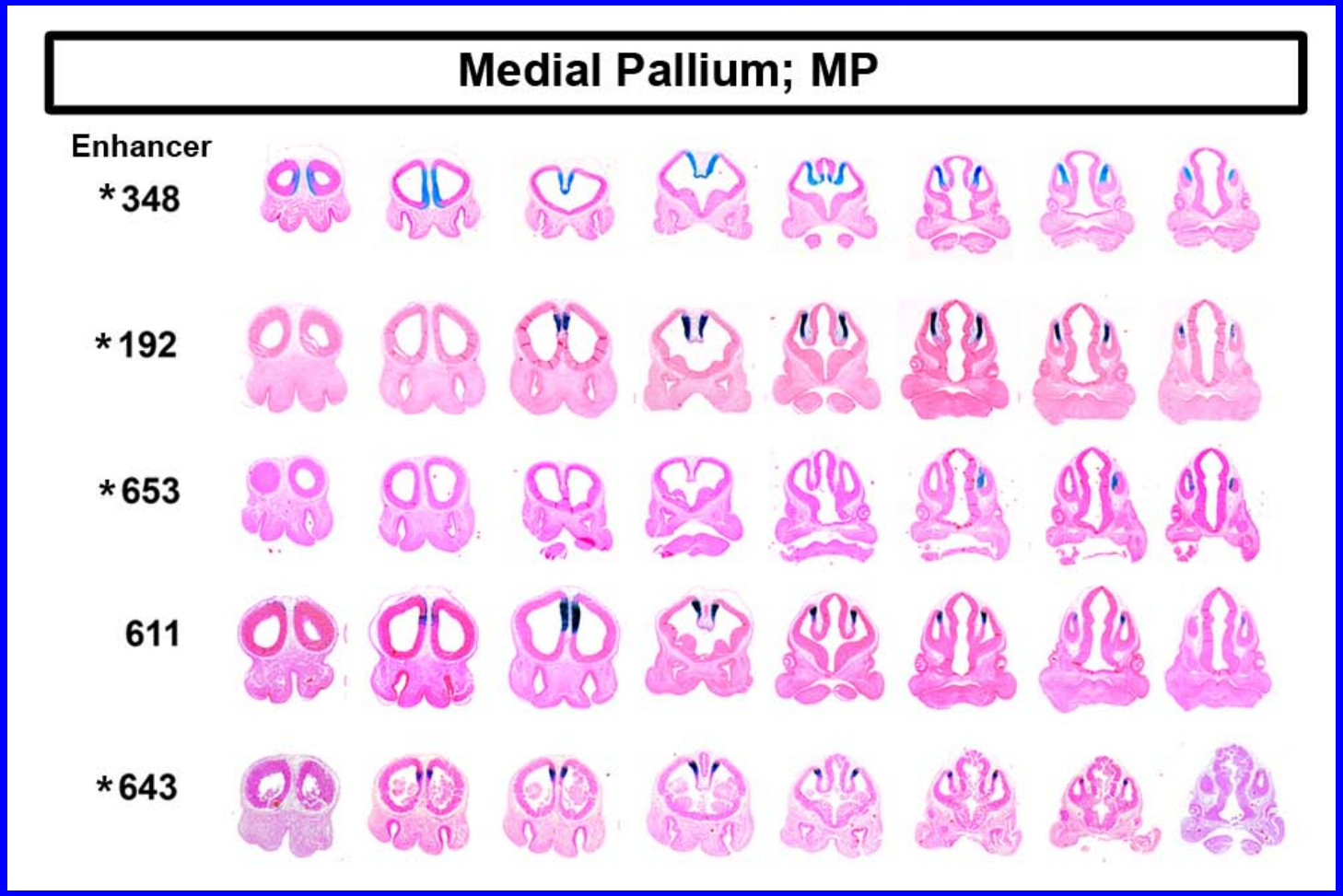
Human Enhancers Have Regionally Restricted Activity in the Mouse E11.5 Forebrain



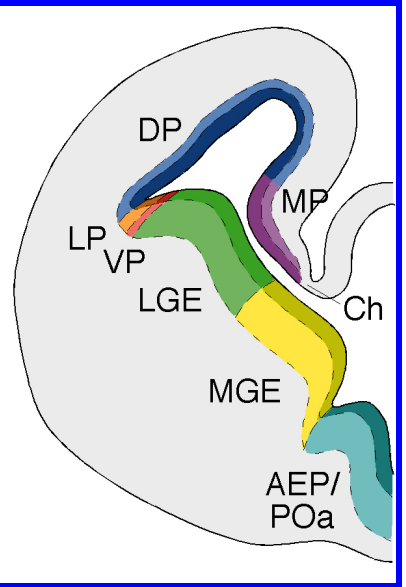
Visel et al, 2013

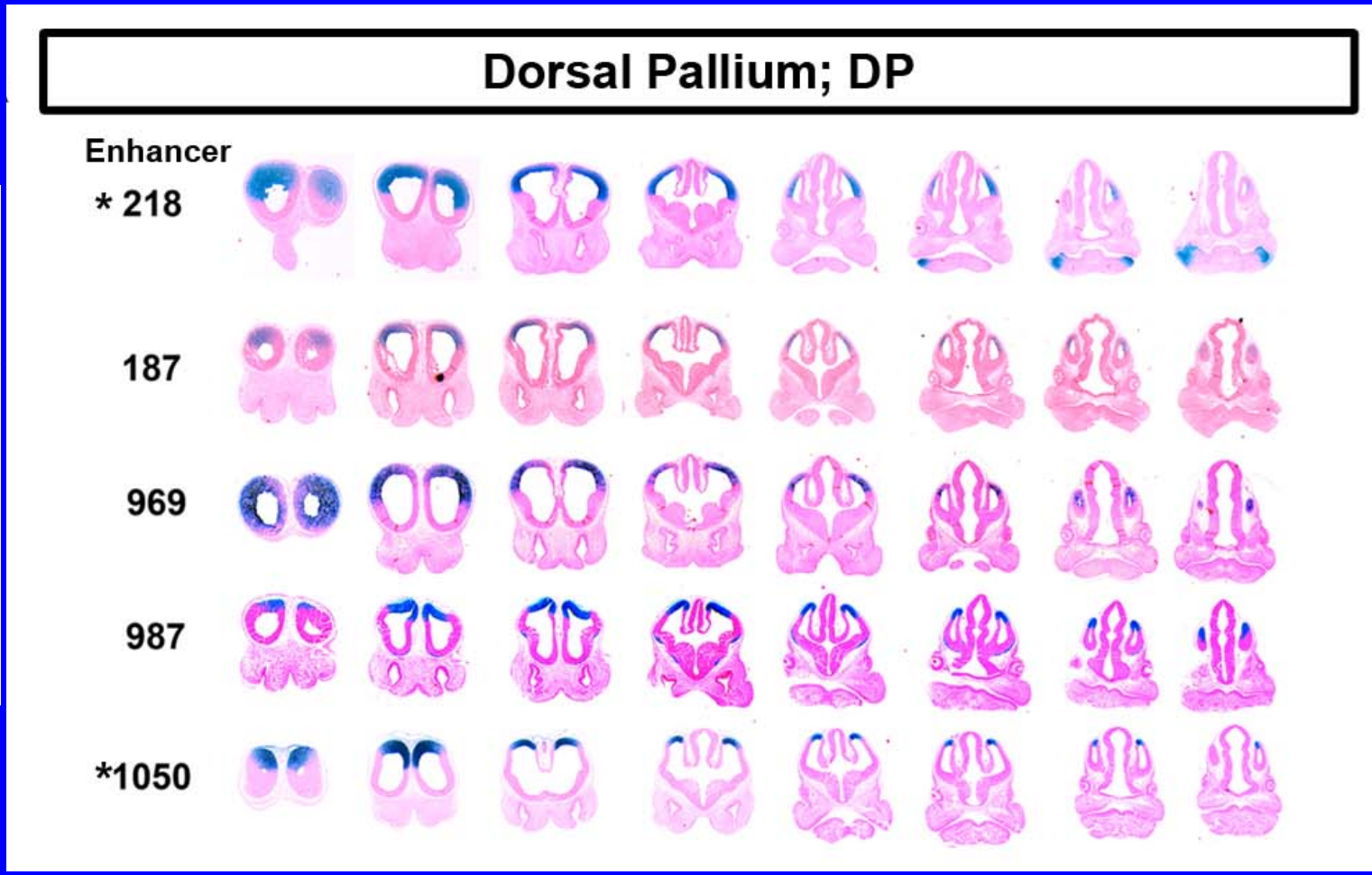
Medial Pallial Enhancers



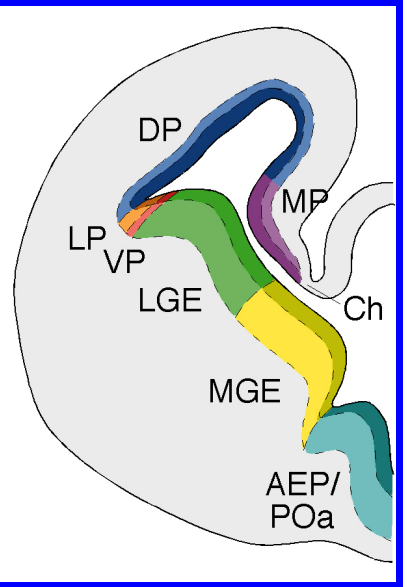


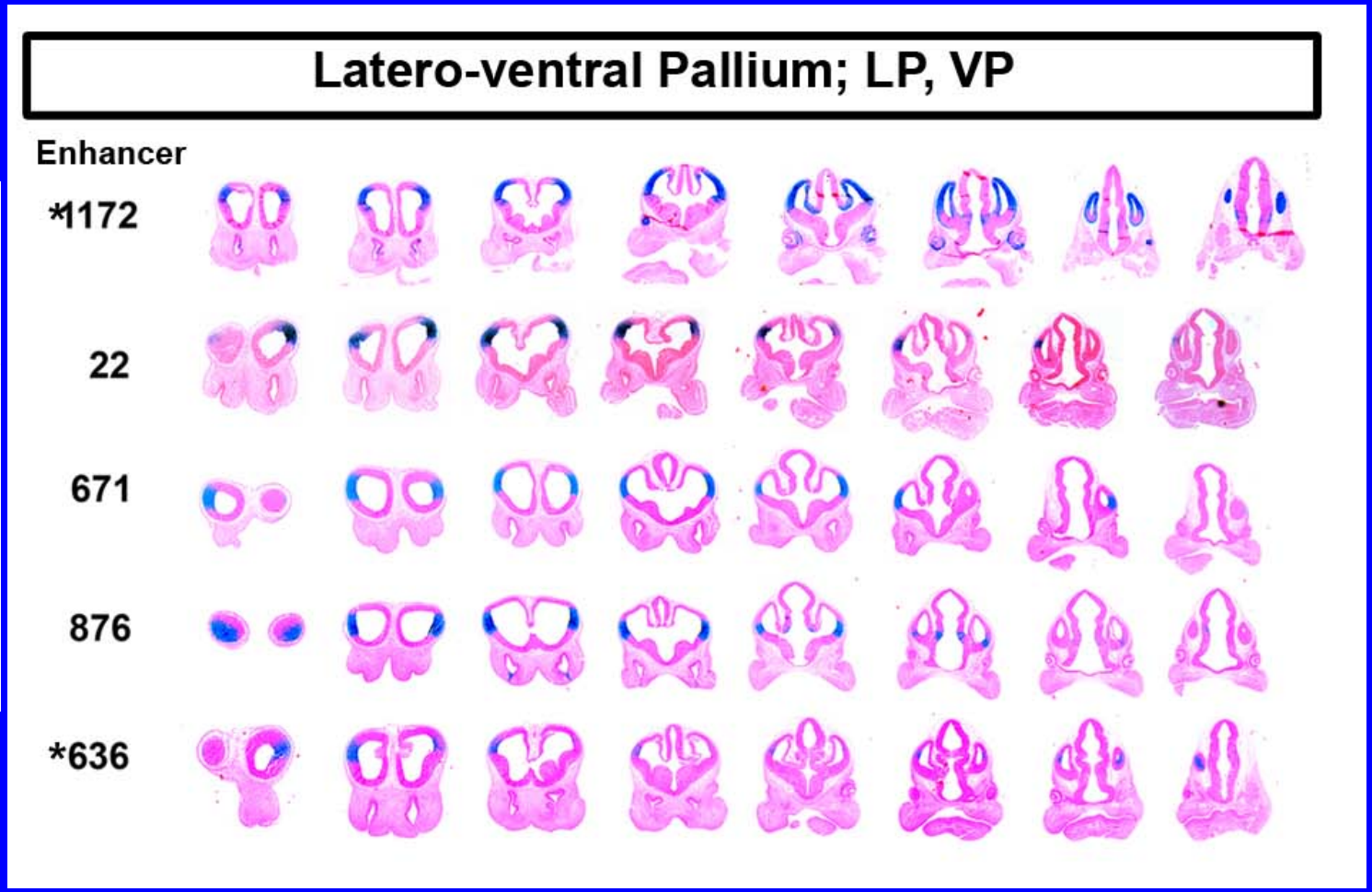
Dorsal Pallial Enhancers





Latero-Ventral Pallial Enhancers





These Boundaries Suggest That Cortical Primordium is Subdivided At a Resolution Greater Than Suggested by the Broad Gradients of TF Expression

Use the Enhancers to Determinate Fate Map of Cortical Progenitor Domains

Work of Kartik Pattabiraman and Olga Golonzkha

Anatomical Analysis with Luis Puelles

Pattabiraman et al., 2014

Generate 14 Stable Transgenic Mouse Lines That Have Distinct Pallial Enhancers

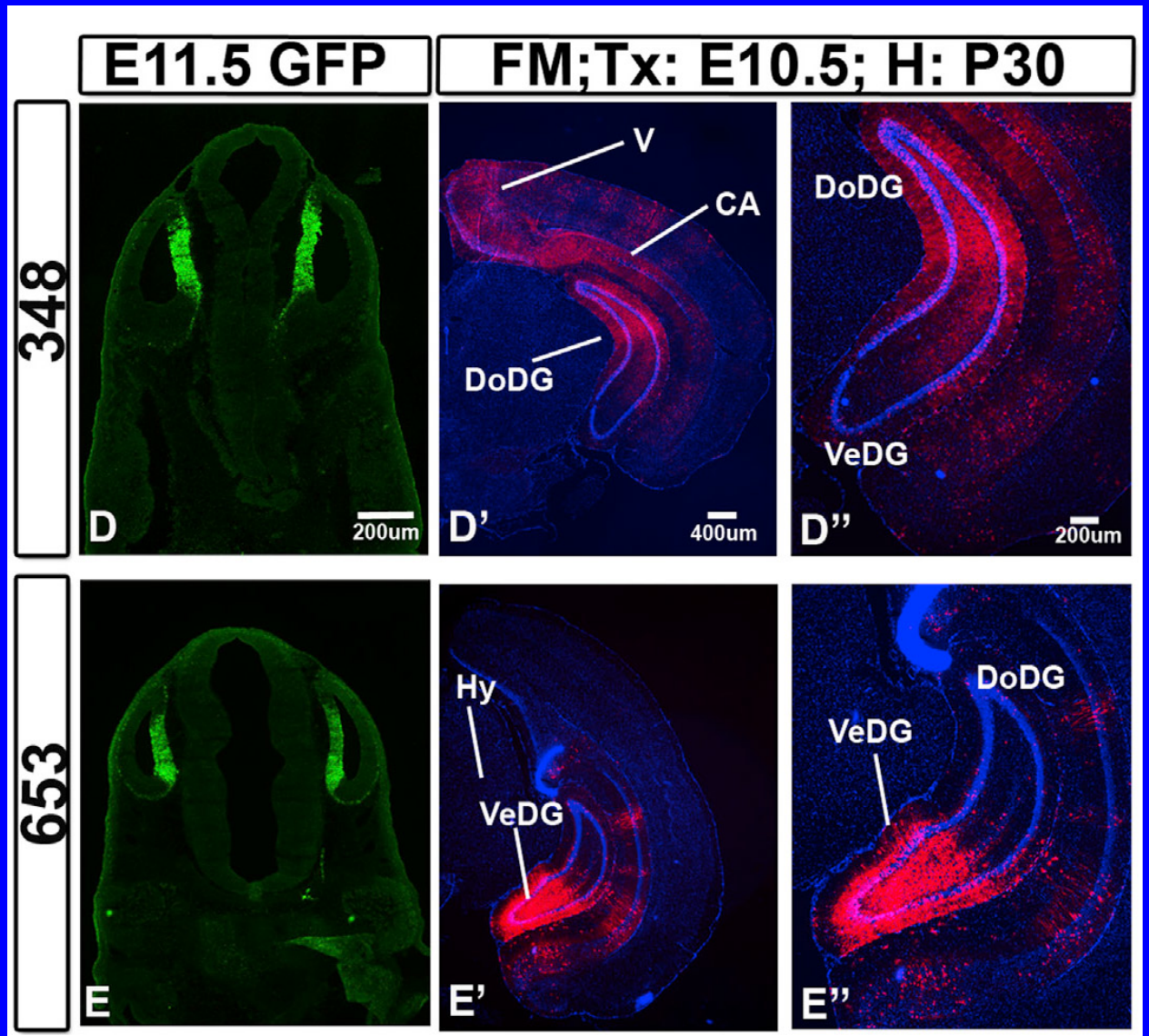
Transgenic Construct

Cre Fate Mapping (Tamoxifen-Induced)

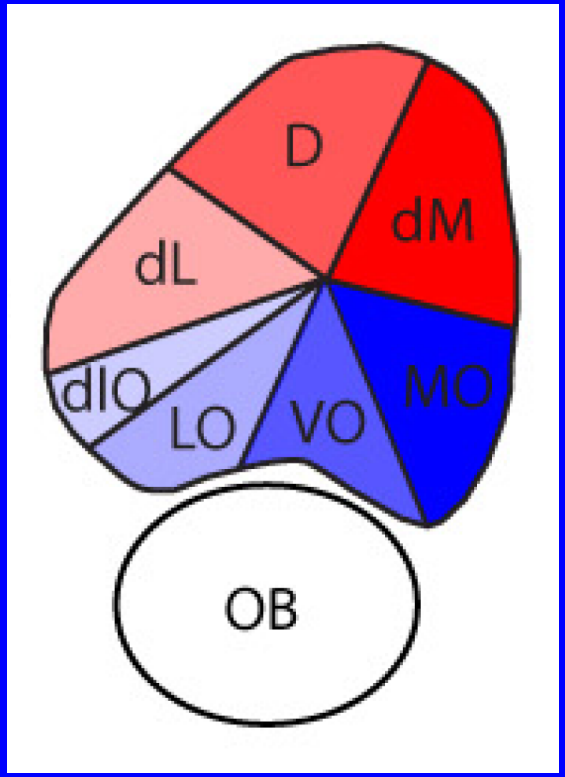
GFP Expression

Renee Hoch

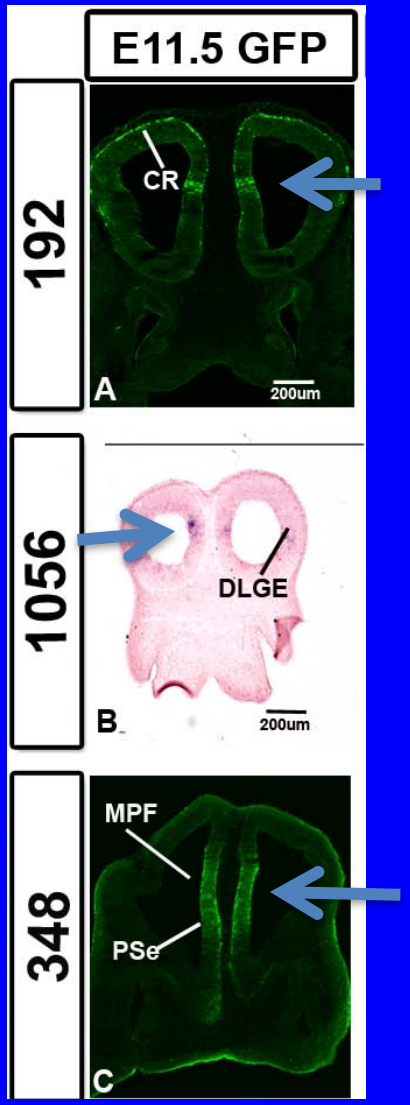
Dorsal (348) and Ventral (653) Hippocampus Enhancers



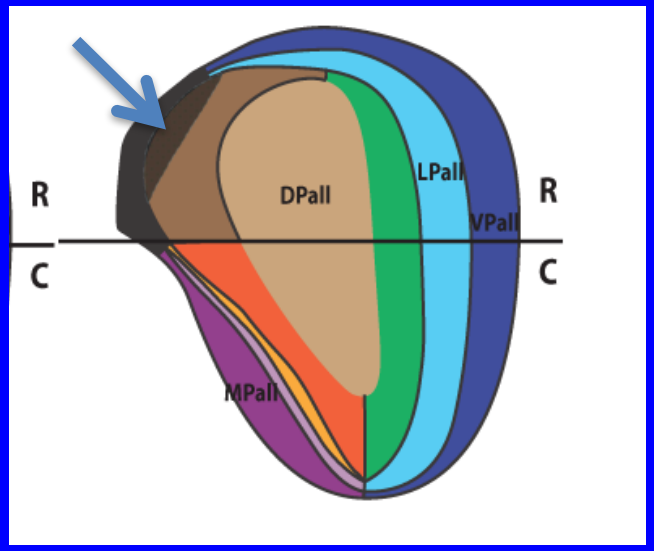
Fate Mapping of Frontal Cortex Subdivisions



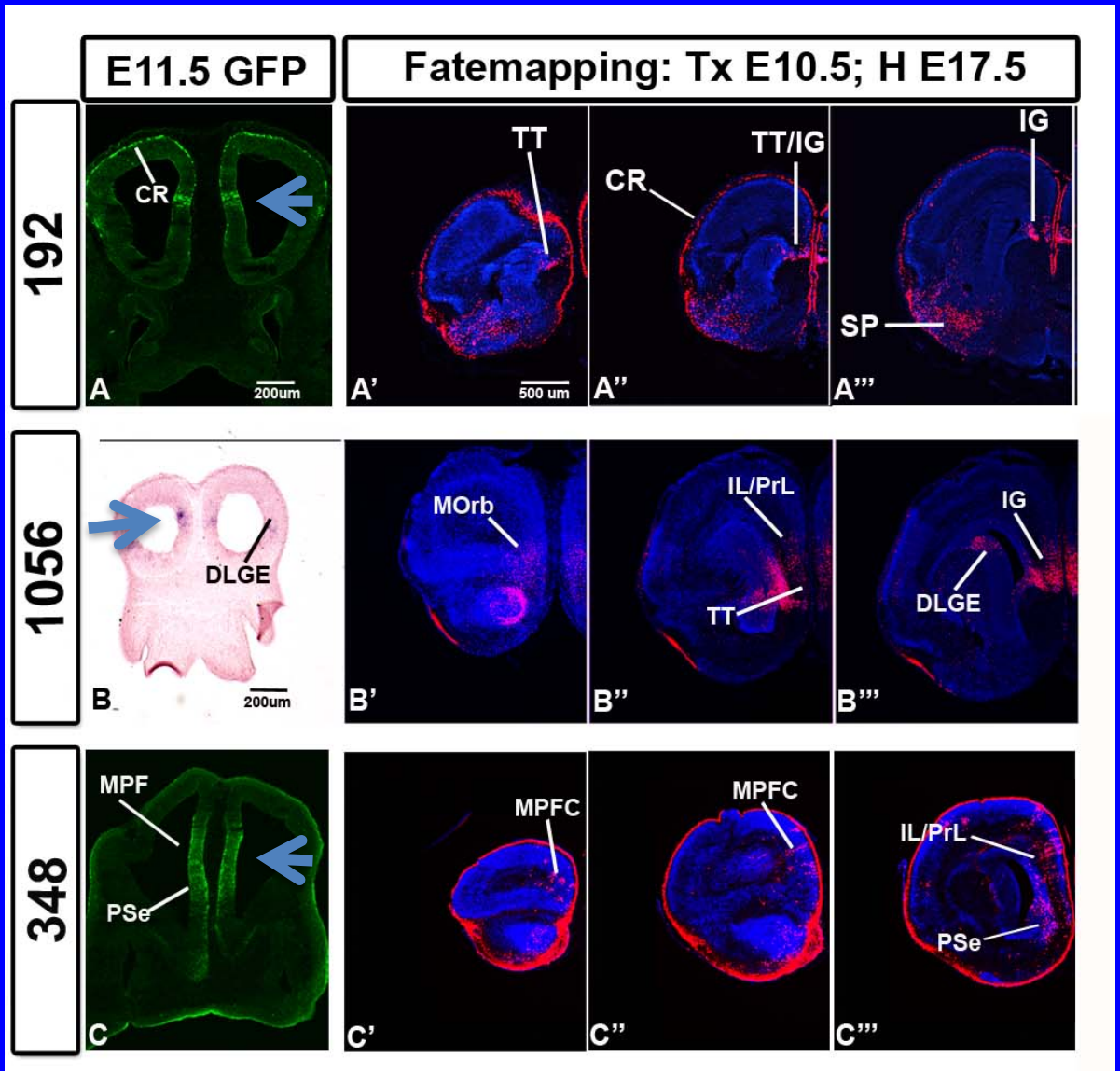
Medial Prefrontal Cortex Enhancers E11.5 Activity



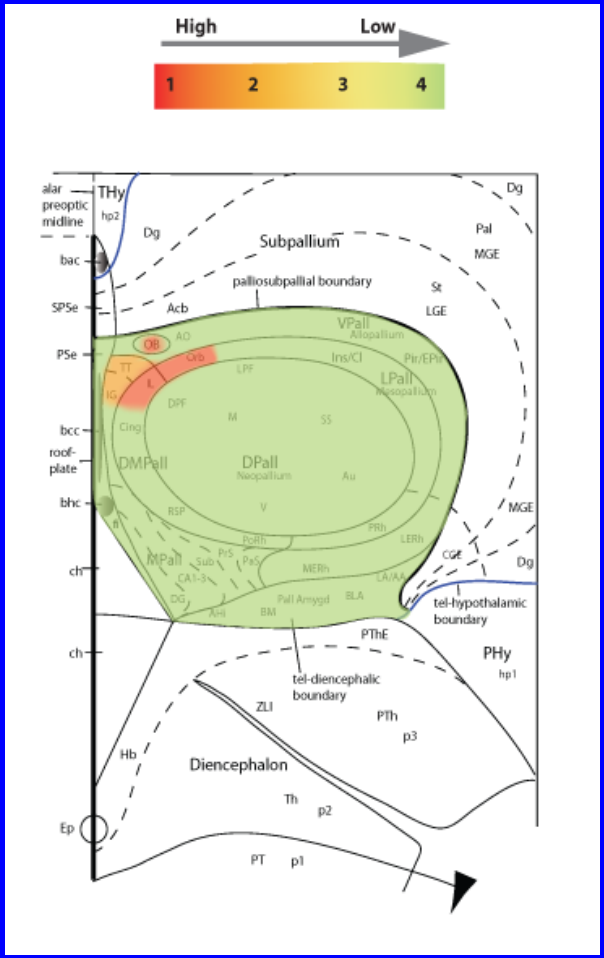
1056 Activity in the Rostromedial Pallium



Medial Prefrontal Cortex Enhancer Fate Mapping



1056 Fate Maps to Medial Prefrontal Cortex



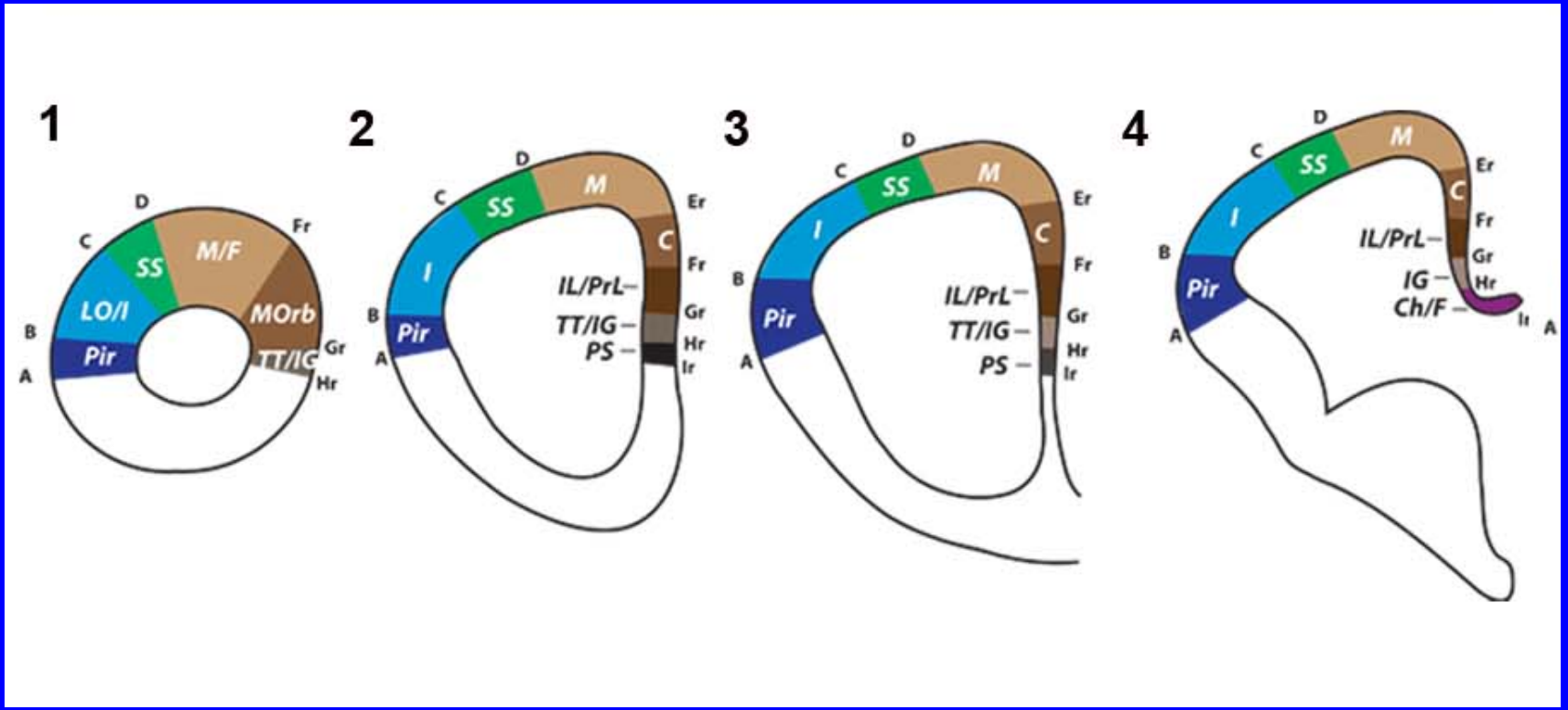
Other Enhancers Are Active in the Dorsal, Lateral and Orbital Prefrontal Cortex

Define Frontal Cortex Subdivisions
Based on:

1) Activities of
Enhancer Elements in the E11.5 VZ.

2) Fate Maps

Proposal for the Fate Map of Rostral Cortex



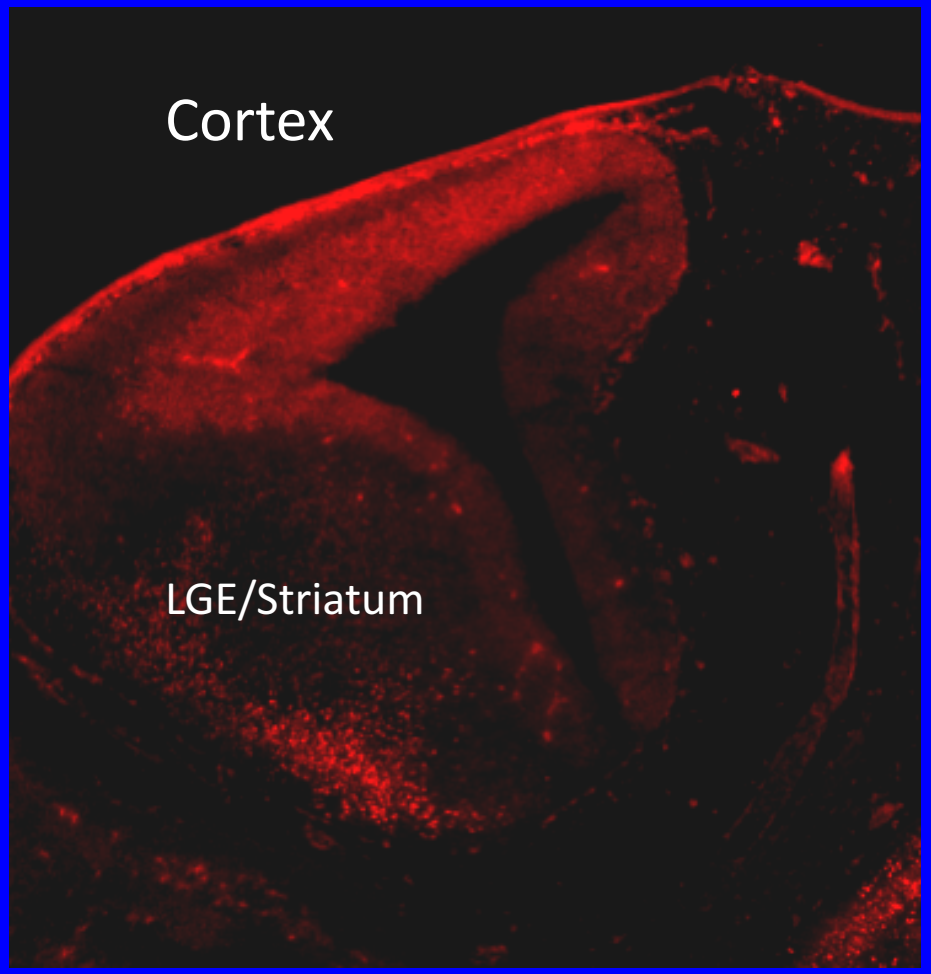
Pattabiraman et al, 2014

Part IV:

Pbx1 Homeodomain Transcription Factor Regulates Frontal Cortex Patterning Through Its Activity in Cortical Progenitors

Golonzkha et al., 2015

Pbx1 Expression



Slight Caudo-Rostral Cortical VZ Gradient; E12.5

Delete $Pbx1^{F/F}$ In Cortical Progenitors with Emx1-Cre

Use $Pbx2^{+/-}$ to Sensitize the Background

Pbx1 and Pbx2 Share Very Similar Expression

Assay Effect of Pbx Mutation on Patterning of the Cortical Progenitor Zone

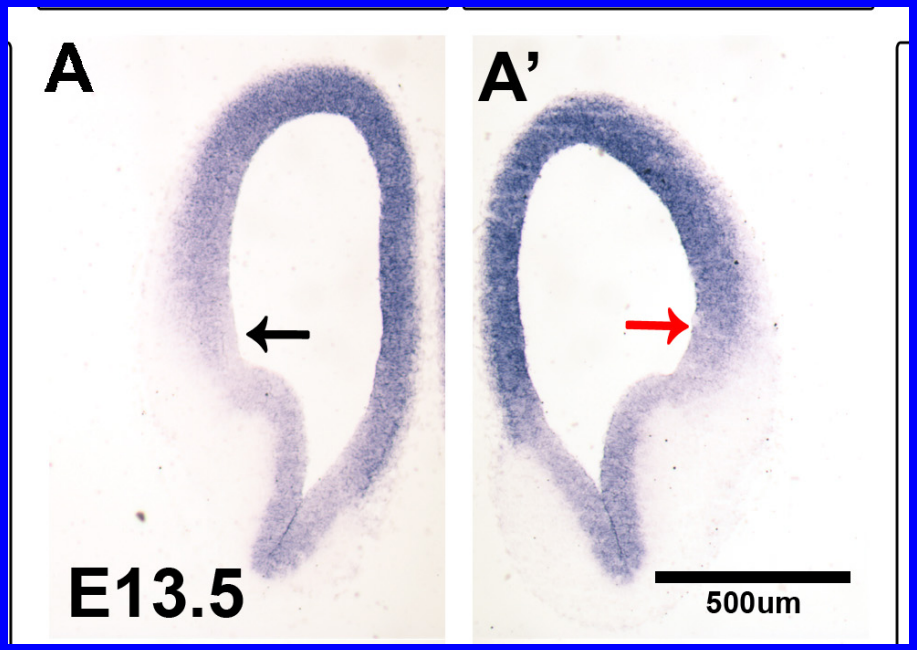
Lhx2 Expression

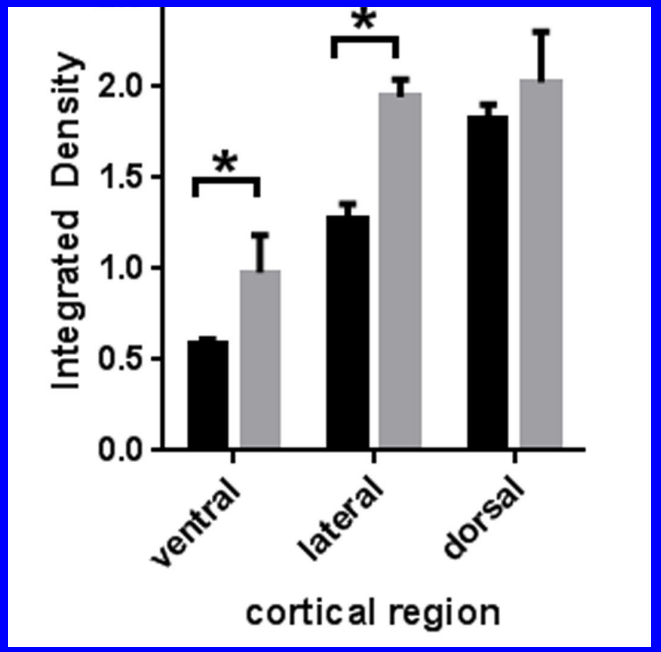
(Increased in Pbx Mutant)

In Situ Hyb.

E13.5

Control **Pbx Mutant**



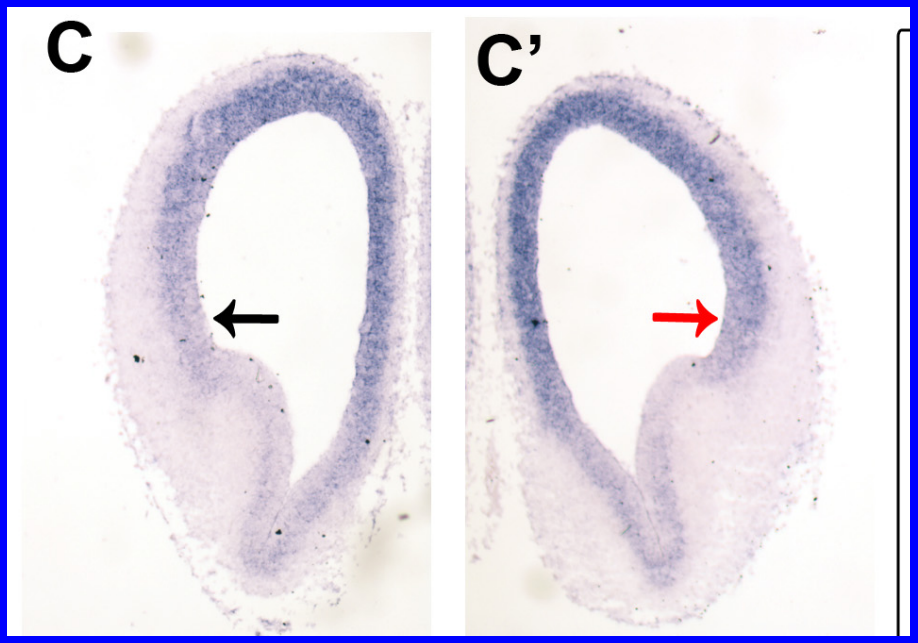


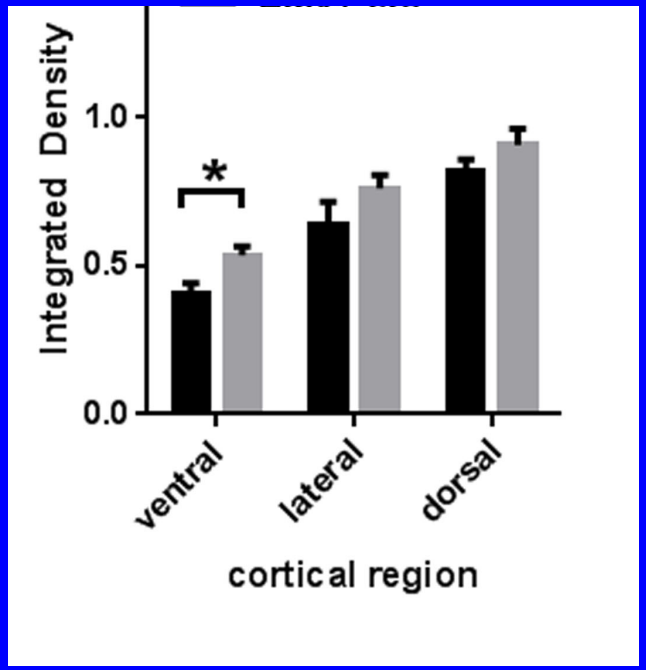
Emx2 Expression
(Increased in Pbx Mutant)

In Situ Hyb.

E13.5

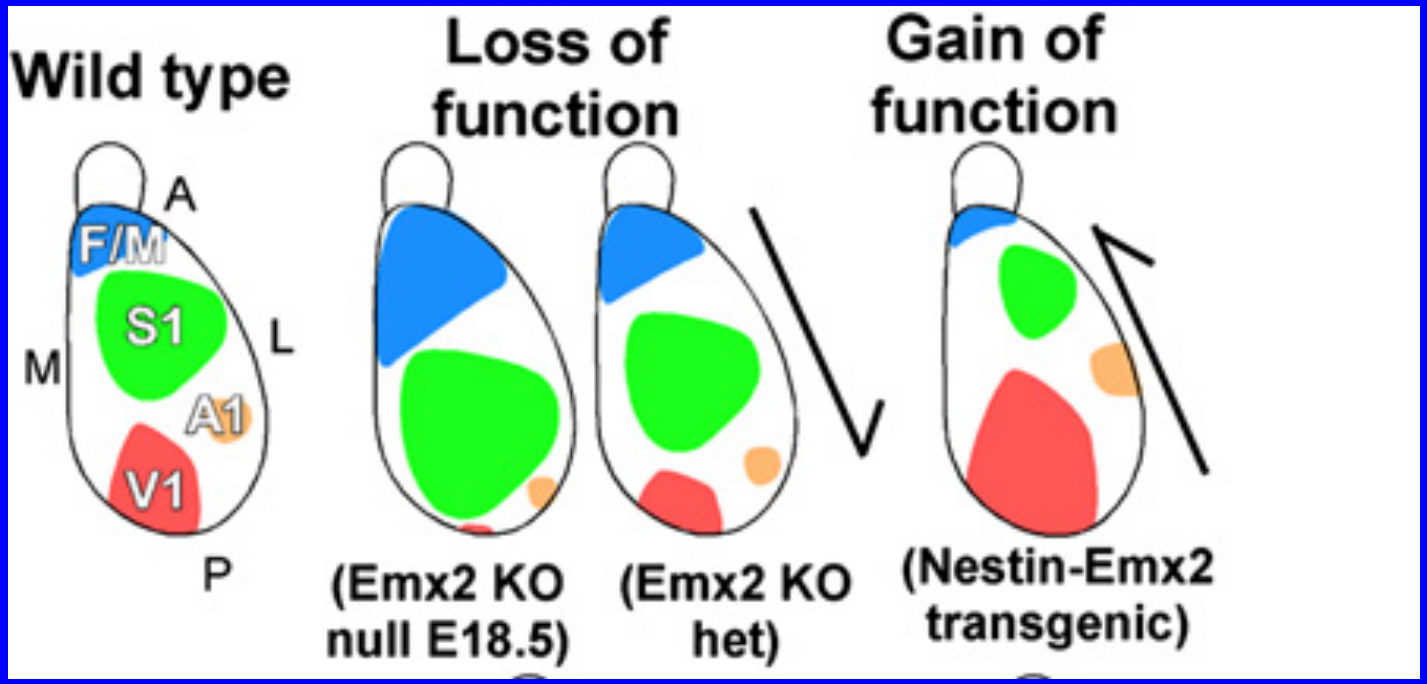
Control **Pbx Mutant**





Increased
Ventro-Rostral Expression
of Emx2 and Lhx2
in the VZ
at E13.5

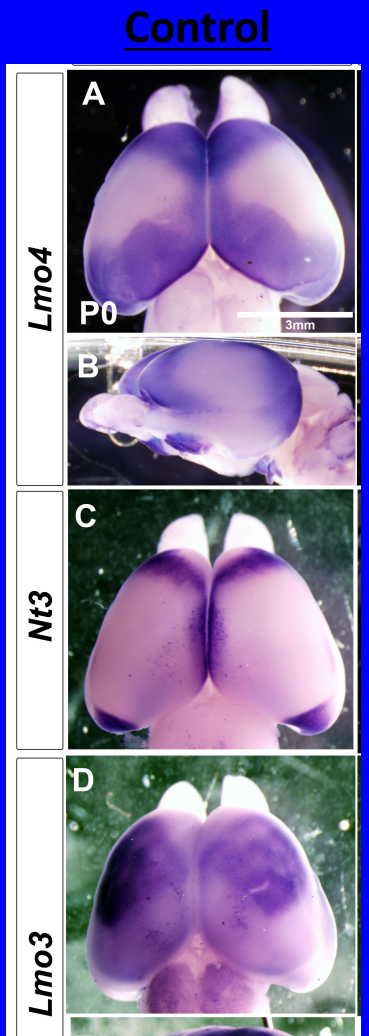
Emx2 Patterns the Neocortex



O'Leary, Malamacci, Boncinelli

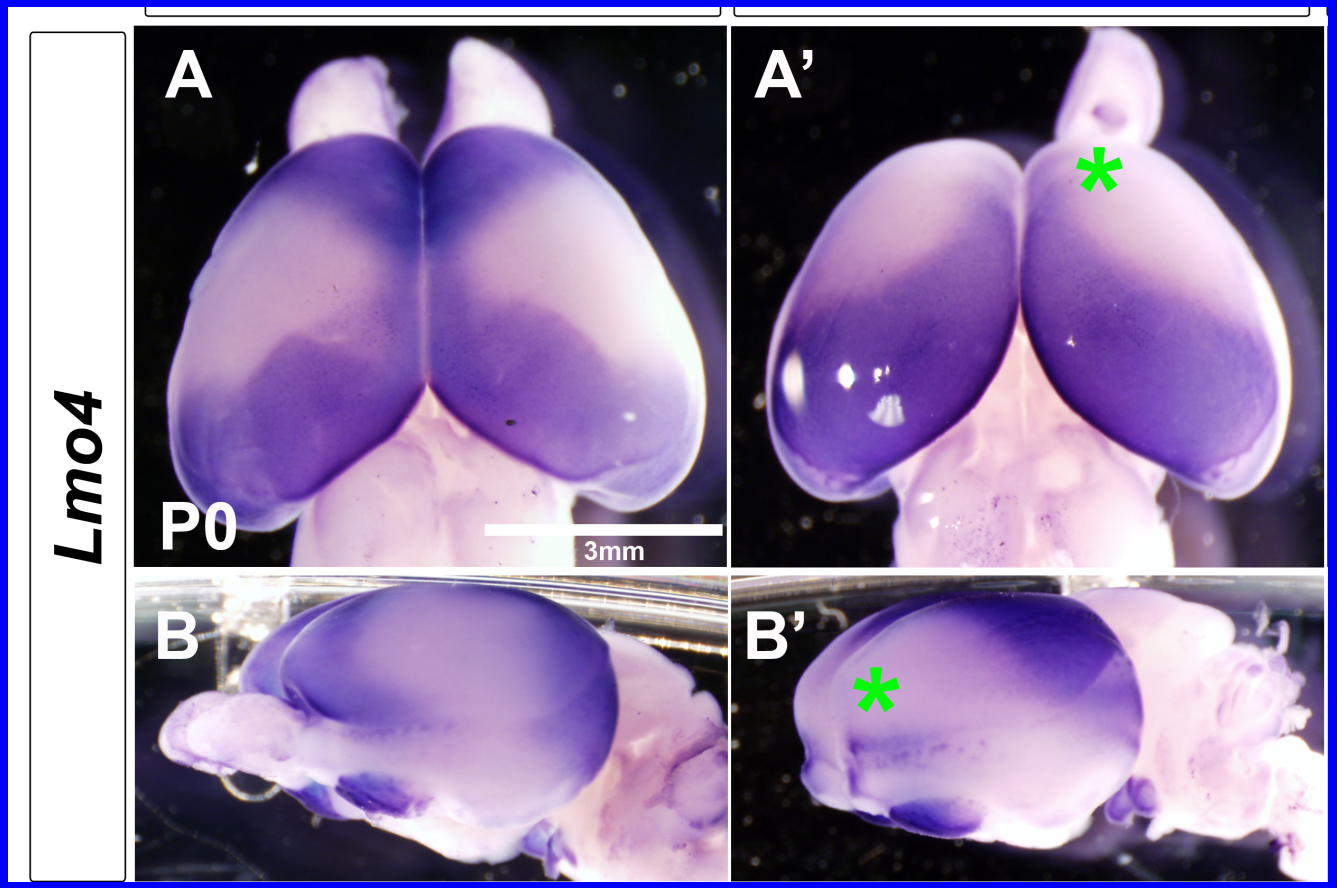
Assay of Cortical Regionalization

Lmo4, Nt3, Lmo3
Whole Mount
In Situ Hybridization
Neonatally (P0)

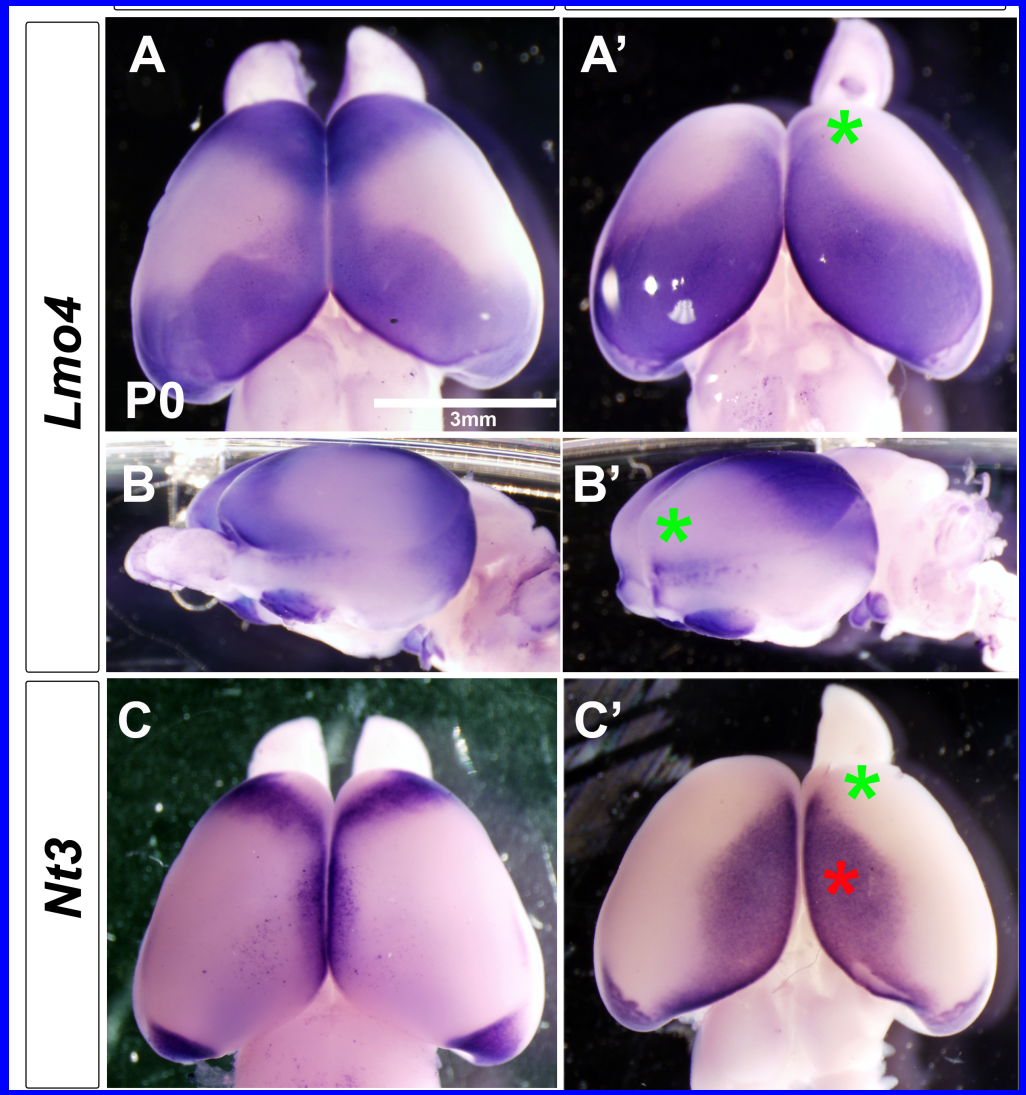


Control

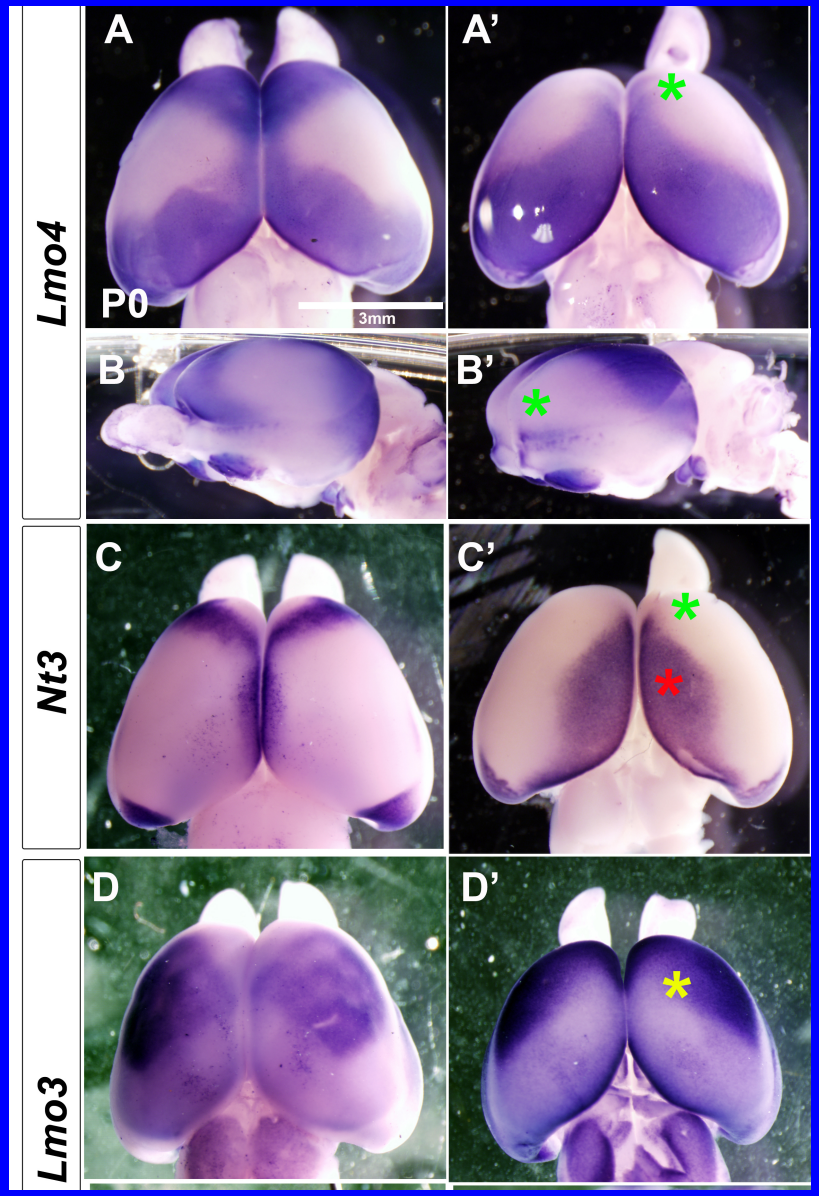
Pbx Mutant



Control Pbx Mutant



Control Pbx Mutant



Loss of frontal cortex expression (Lmo4 and NT3).

Ventral expansion of dorsal properties (NT3).

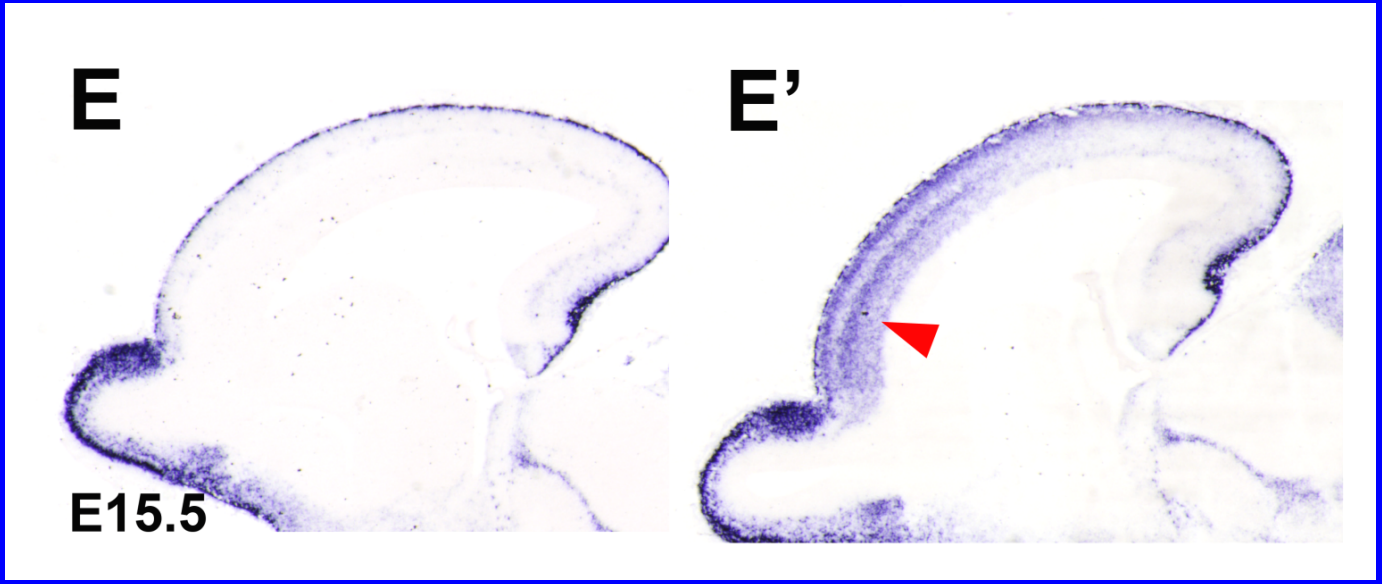
Rostral expansion of caudal properties (Lmo4 and Lmo3).

Big Surprise:

Ectopic Reelin Expression
in the Frontal Cortex

Ectopic Reelin in the Rostral Cortical Plate

Control **Pbx Mutant**

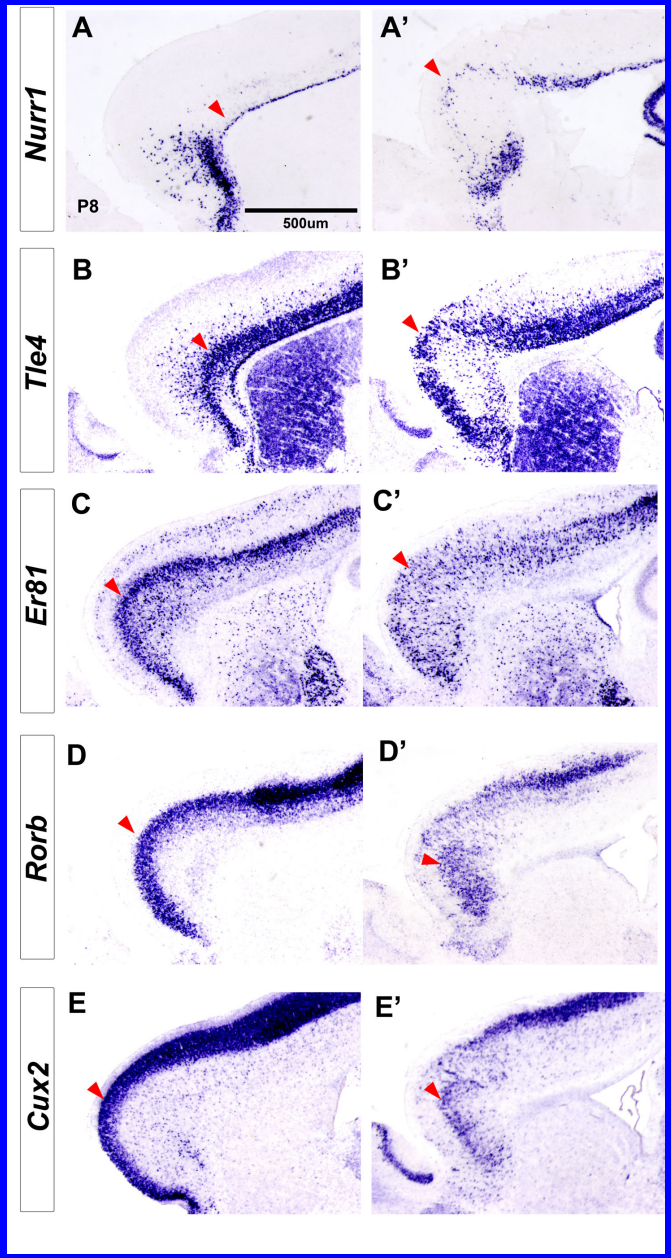


Ectopic Reelin

Find:

Laminar Inversion
of the Rostral Cortex

Control Pbx Mutant



Subplate

Layer 6

Layer 5

Layer 4

Layers 2/3

P7

Pbx1/2 Required for Frontal Cortex

In Progenitors

Repress Dorsocaudal Cortical Fates
(Emx2, Lhx2)

Represses Reelin
Associated in Rostral-Specific
Laminar Inversion

Pbx1/2 Required for Frontal Cortex

In Neurons (not shown; Nex-Cre)

Directly Promote Expression of Molecular Features of the Rostral Cortex (ChIP-Seq Data)

Frontal Cortex Early Development Requires

Fgf-Signaling from the Rostral Patterning Center

Reciprocal Repression of **Fgf8 and Otx2**

Frontal Cortex Early Development Requires

- **Enhancer** Elements to Integrate Transcriptional Information to Generate Protomap of the Frontal Cortex

- **Pbx1/2** Regulate Regional and Laminar Patterning of the Frontal Cortex

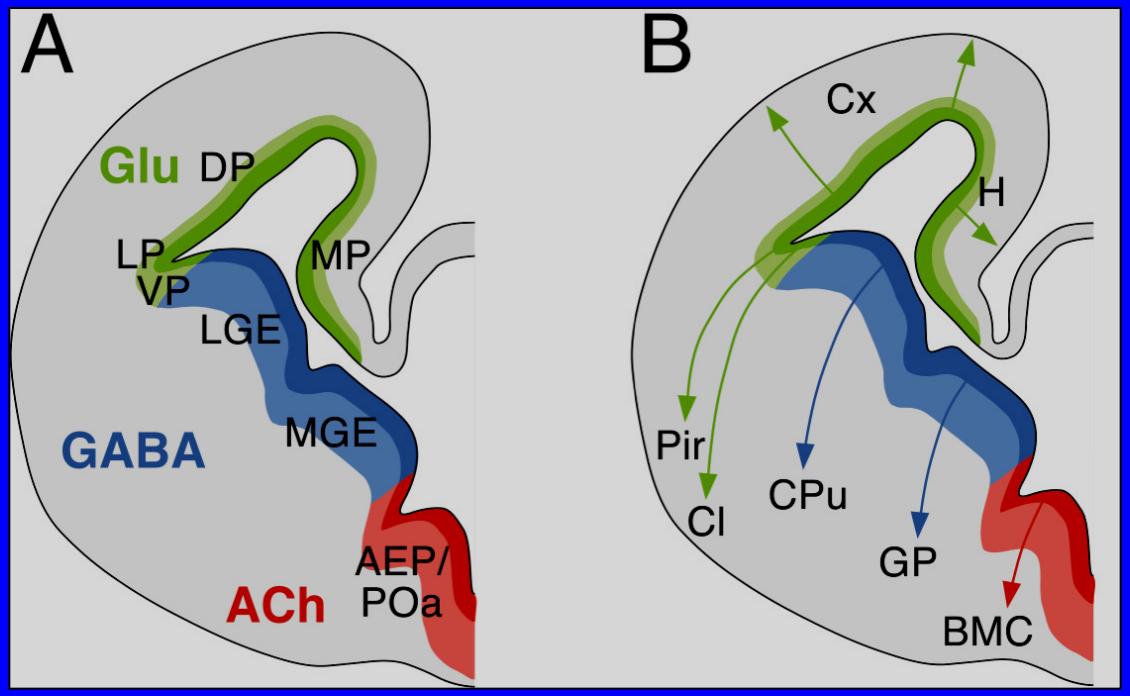
Transcriptional Regulation of Telencephalic GABAergic Regions & Cell Types

Transcription Factors

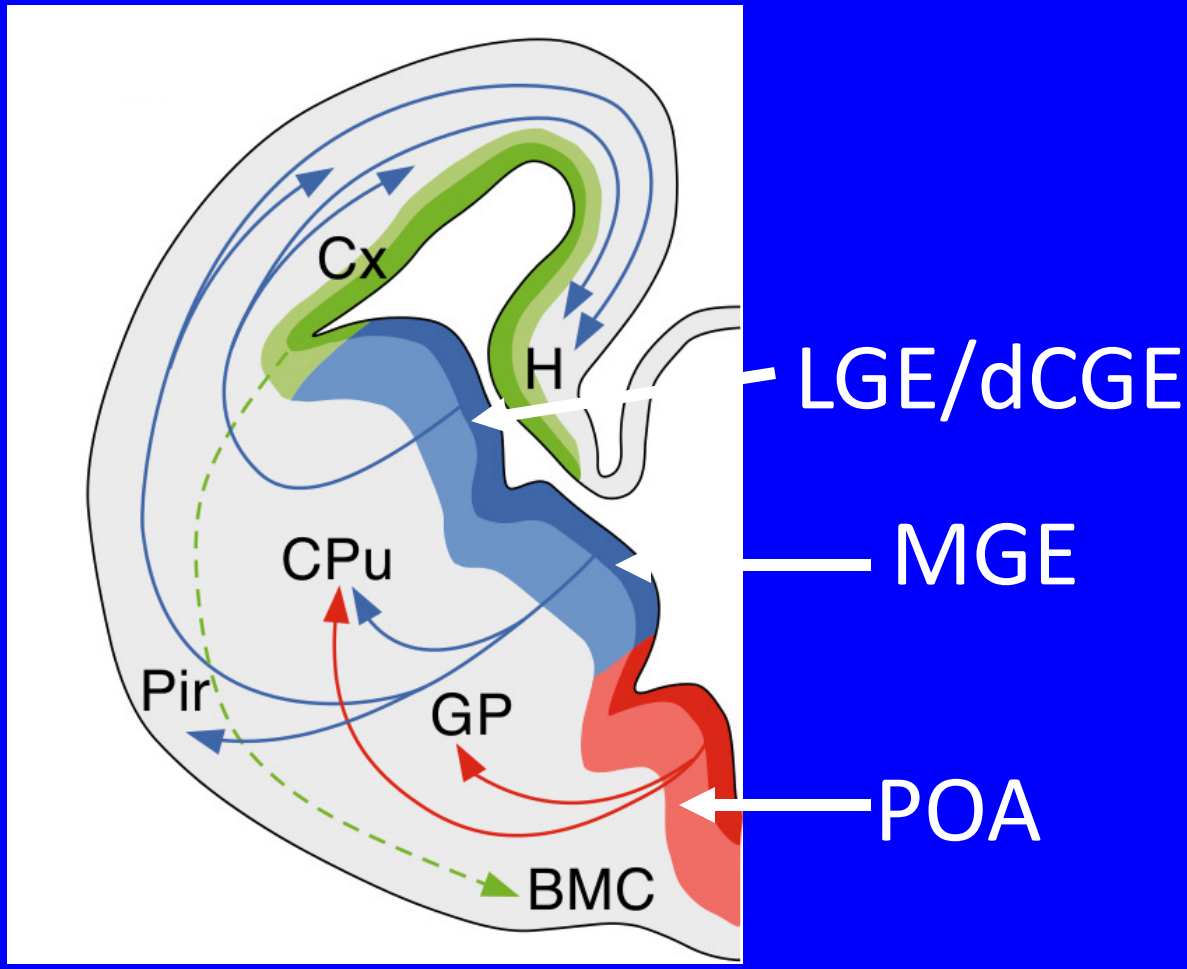
Enhancers

and the Epigenome

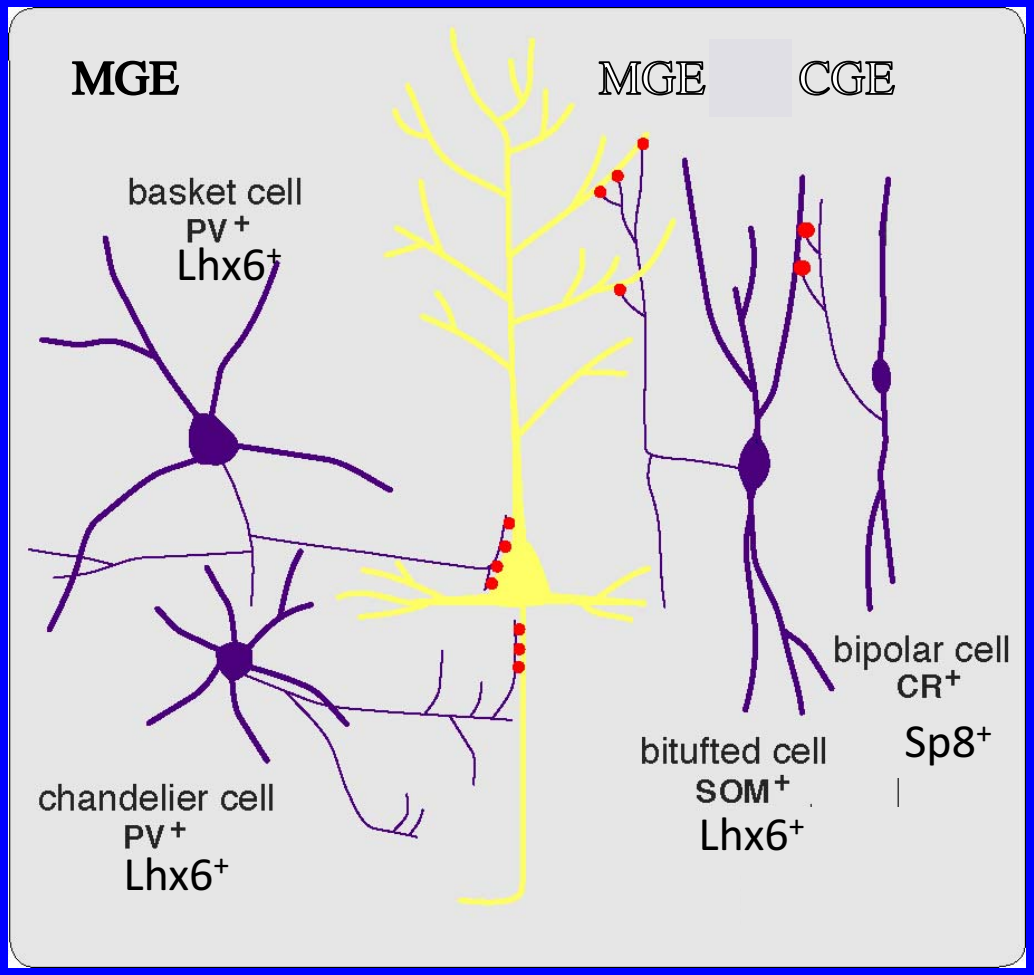
Telencephalic Regions & Cell Types



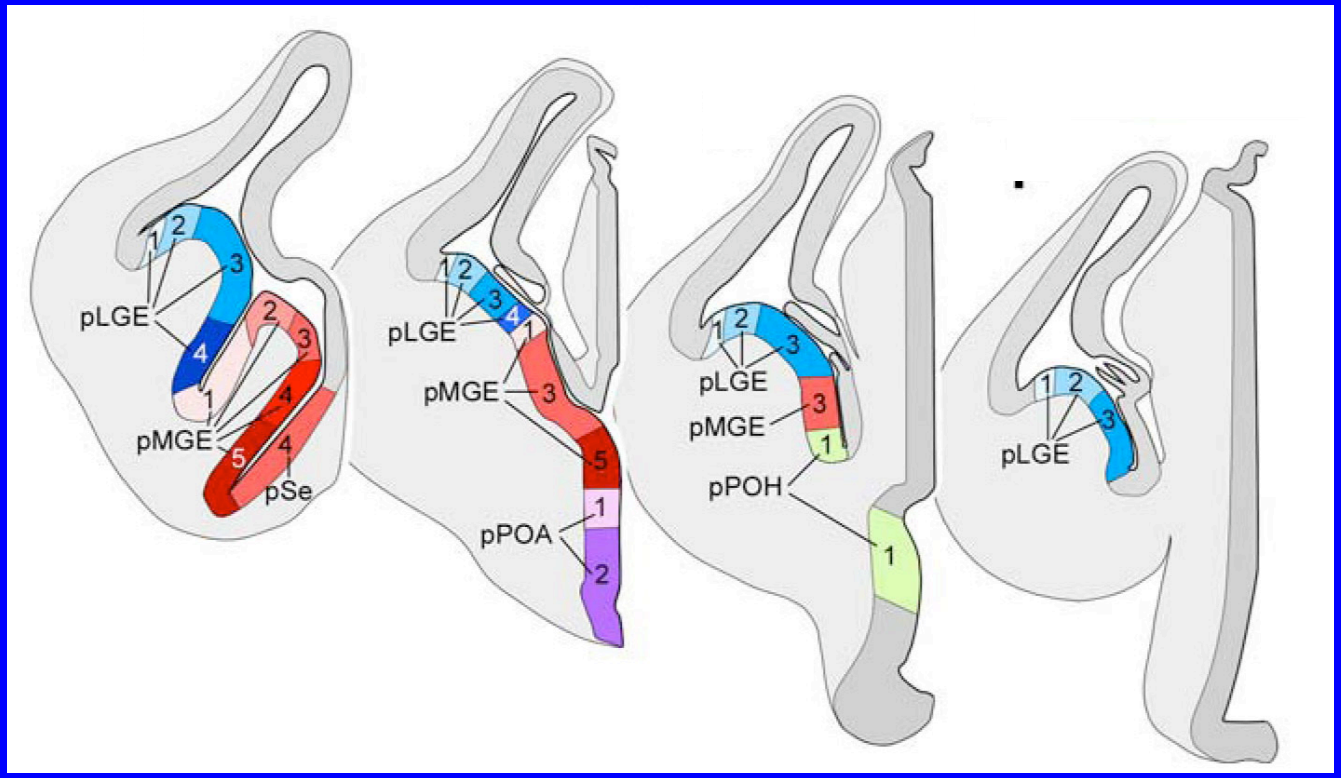
Interneurons (Cortex & Striatum) Originate in the Embryonic Basal Ganglia (Subpallium)



Cortical Interneurons



Model of Subpallial Progenitor Domains

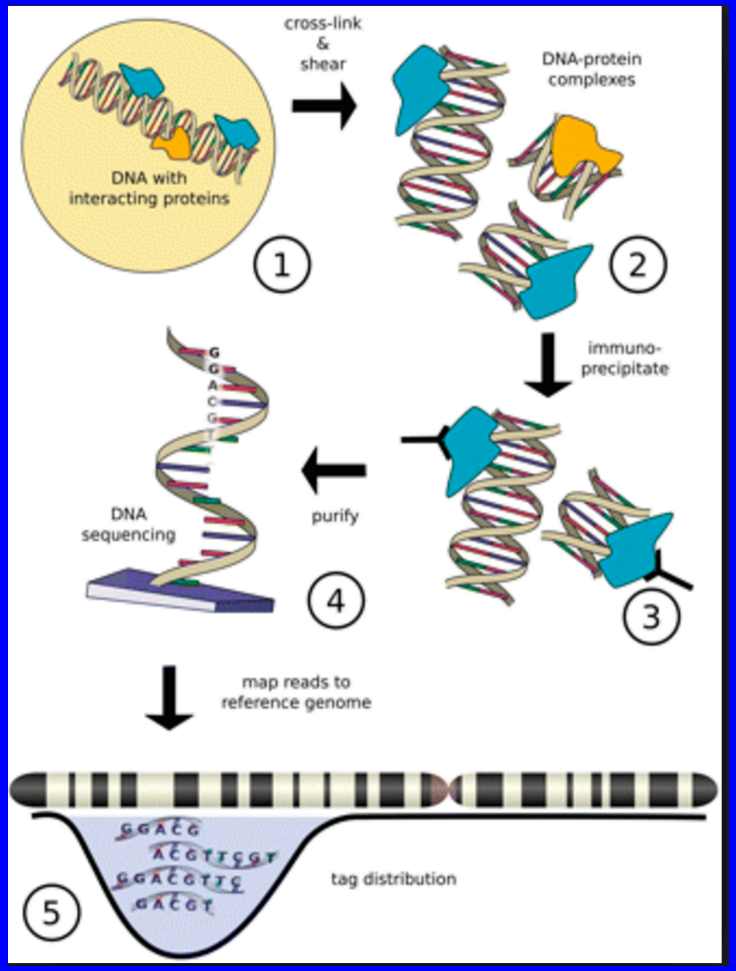


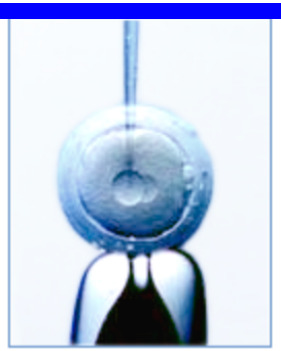
With Oscar Marin and Luis Puelles --- Flames et al., 2007

Hypothesis

Enhancers That Integrate
Transcription Factor Binding
Mark Embryonic Subdivisions and Cells Types

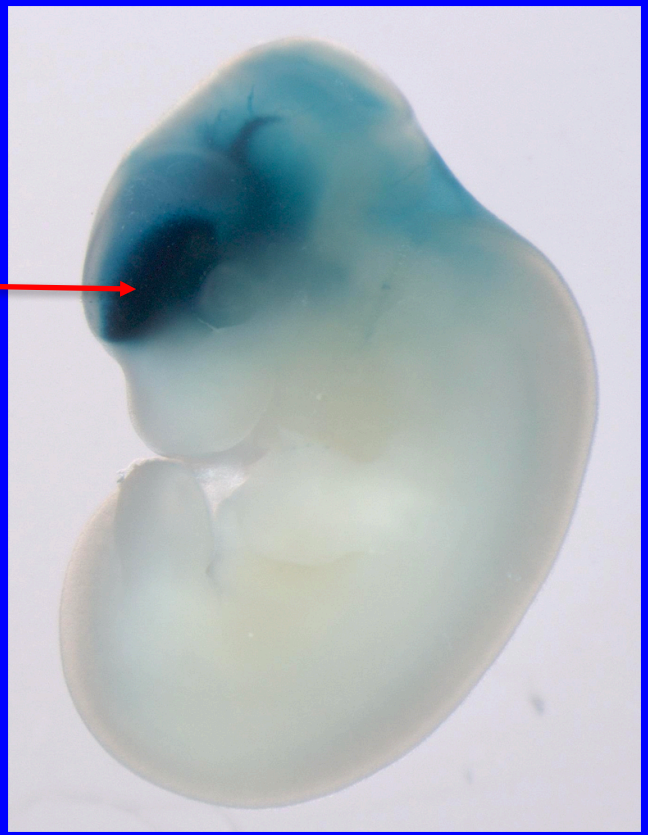
ChIP-Seq
Transcription Factor and/or Chromatin Proteins



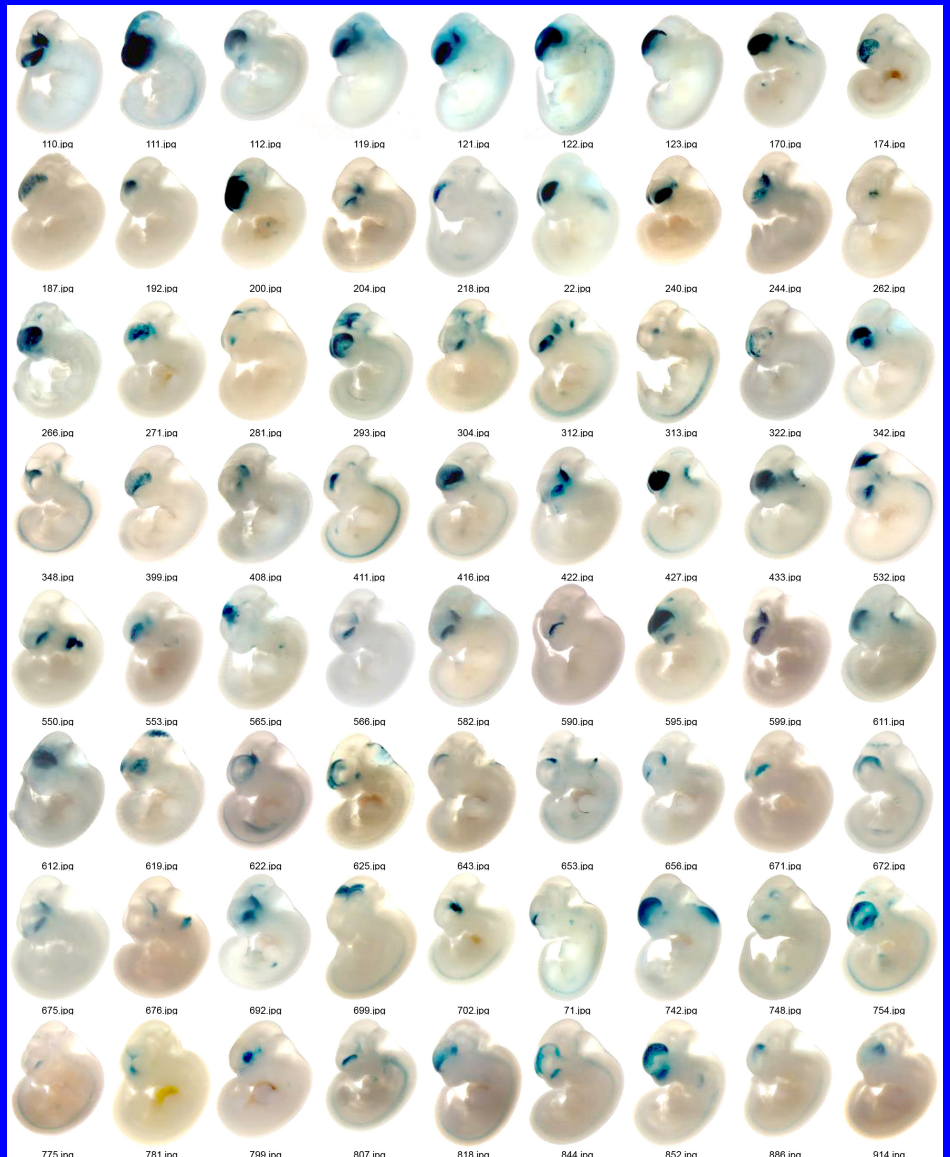


Mouse Egg Microinjection

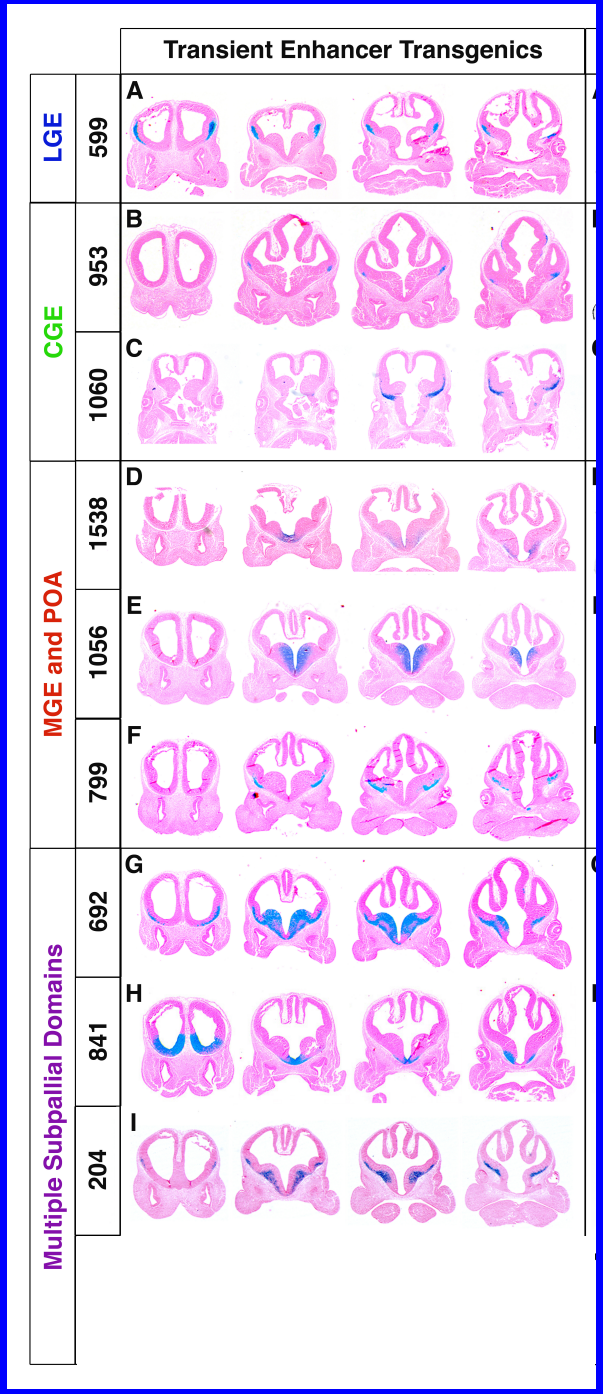
This enhancer is active in the embryonic basal ganglia (ganglionic eminences; Ges)



Enhancers Have Regionally Restricted Activity in the Mouse E11.5 Forebrain



Visel et al, 2013

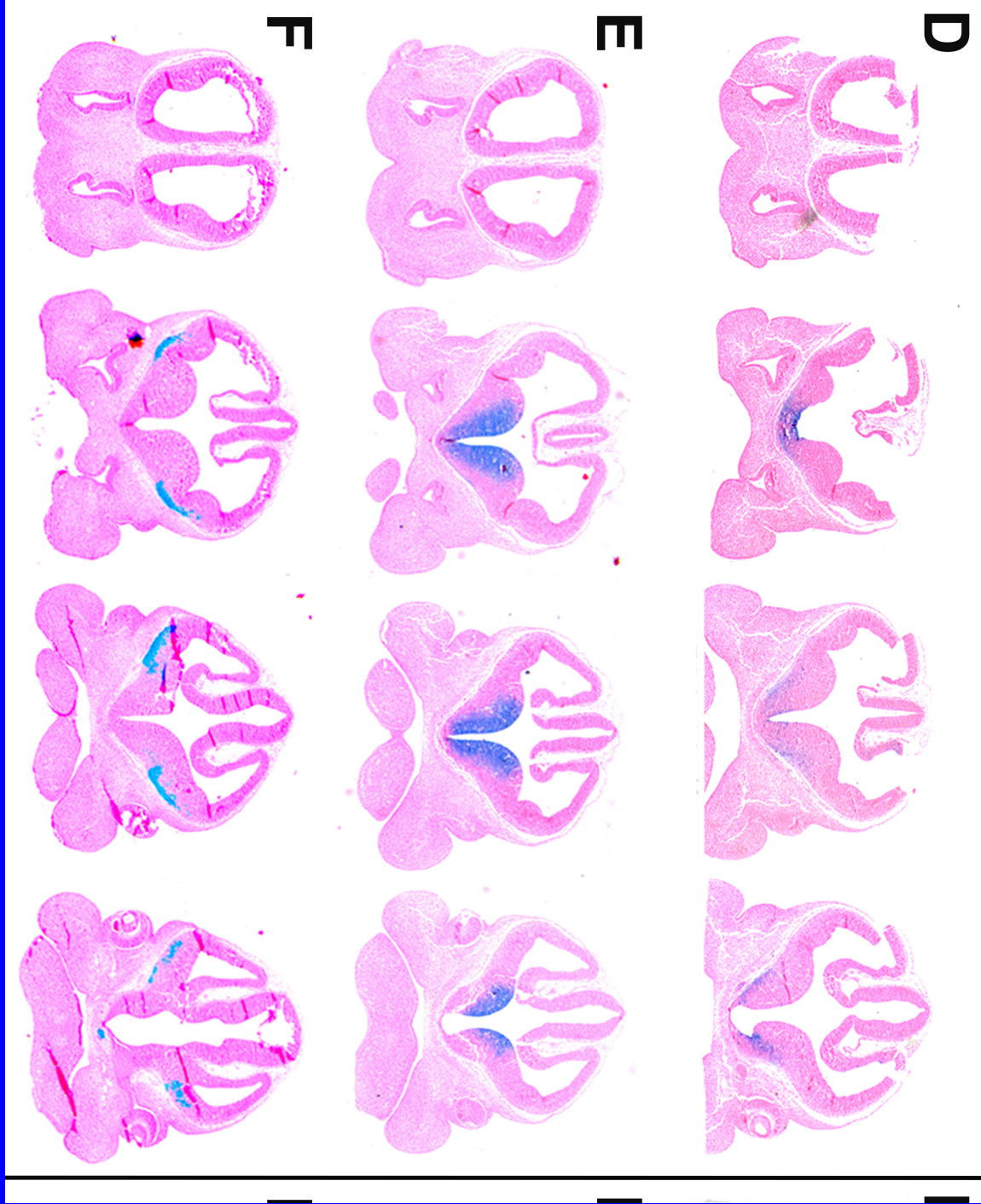


Silberberg et al 2016

LGE
599
A
CGE
953
B
1060
C

MGE and POA

799	1056	1538



Made Stable Transgenic Lines with 10 Enhancers

Shanni Silberberg

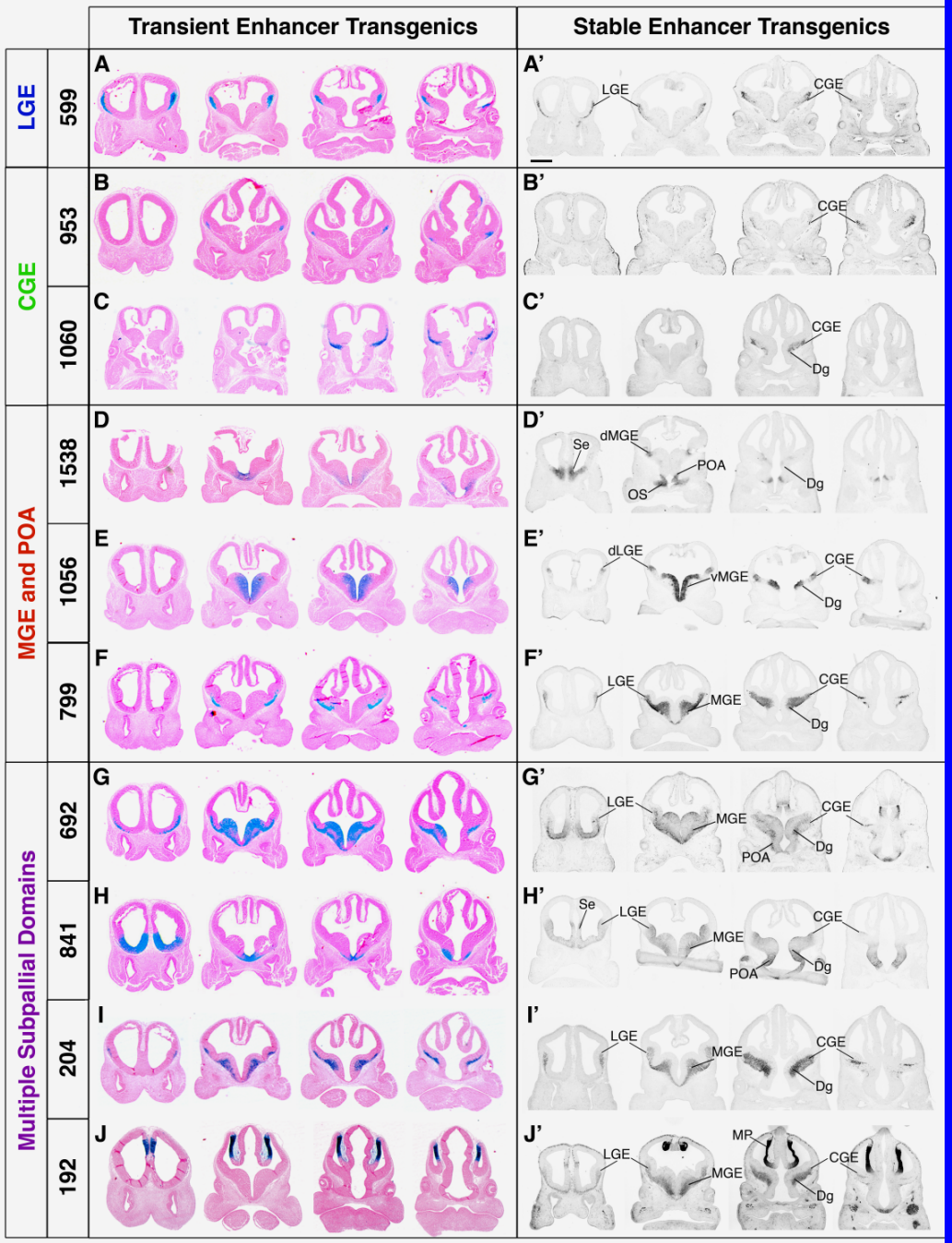
Silberberg et al., 2016

Transgenic Construct

Cre Fate Mapping (Tamoxifen-Induced)

GFP Expression

Renee Hoch


Transient Enhancer Transgenics
Stable Enhancer Transgenics
LGE
CGE
MGE and POA
Multiple Subpallial Domains
599
953
1060
1538
1056
799
692
841
204
192
A
A'
B
B'
C
C'
D
D'
E
E'
F
F'
G
G'
H
H'
I
I'
J
J'
LGE
CGE
Dg
Se
dMGE
POA
OS
dLGE
vMGE
MGE
MP

Fate Map

Tamoxifen: E10.5

Ai14 tdTomato Reporter

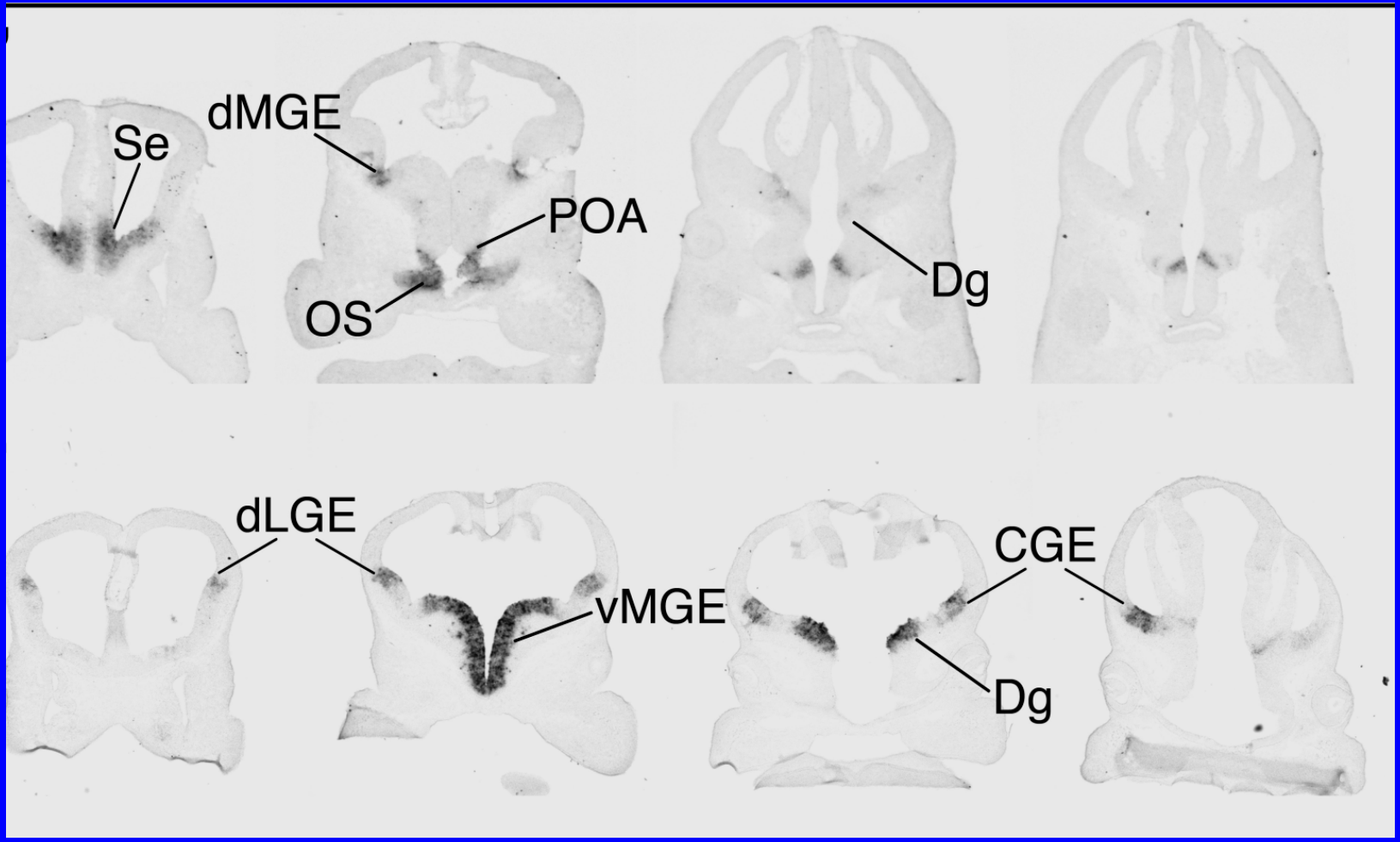
Analyze at E17.5 and P30

Fate Mapping of Enhancer Transgenics Using the Ai14 Reporter (tdTomato+)
(Tamoxifen E10.5; Analysis E17.5)
LGE
CGE
MGE and POA
Multiple Subpallial Domains
599
953
1060
1538
1056
799
692
841
204
192
A
B
C
D
E
F
G
H
I
J

Focus on Three MGE Enhancers

1538

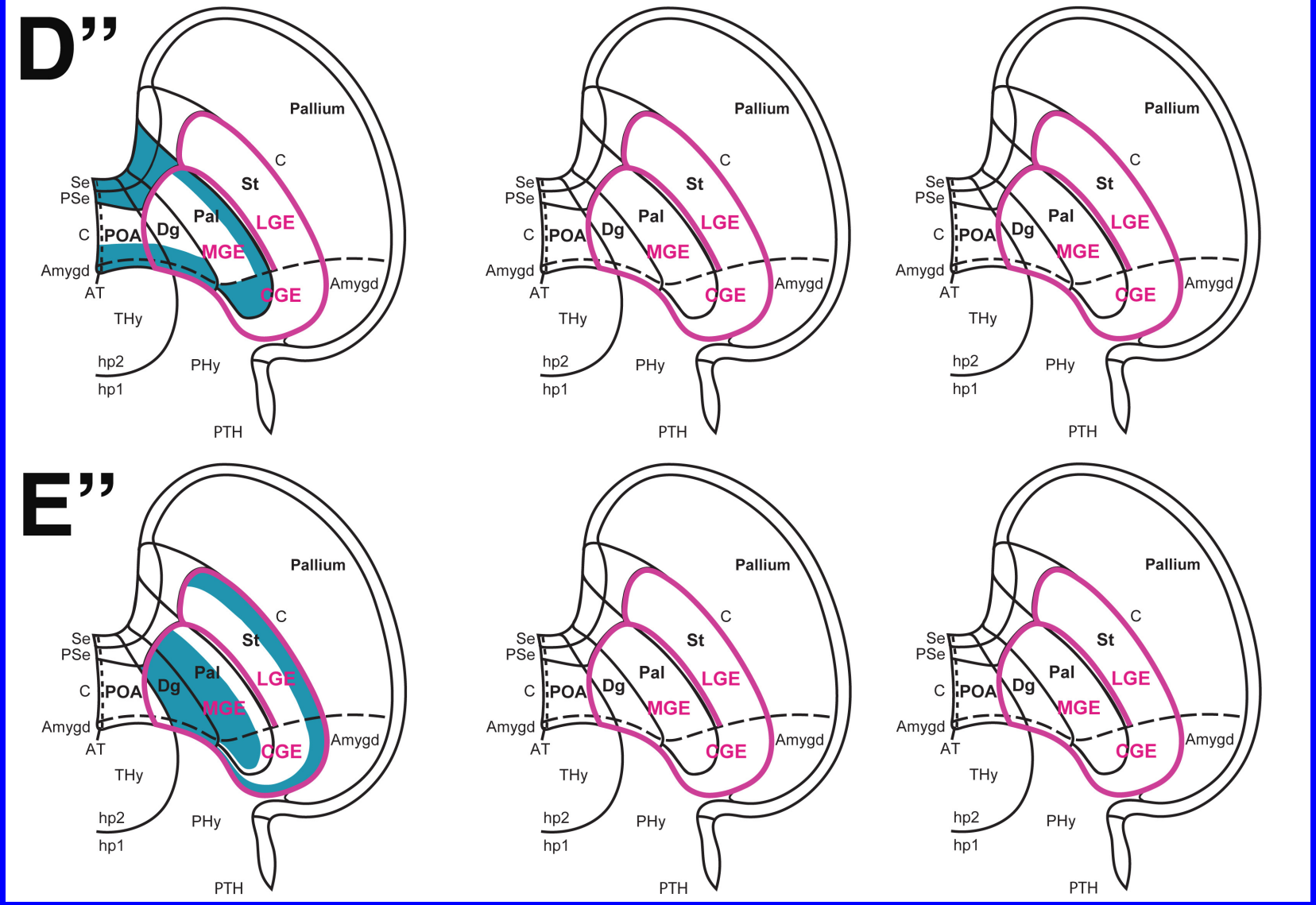
1056



VZ SVZ MZ

1538

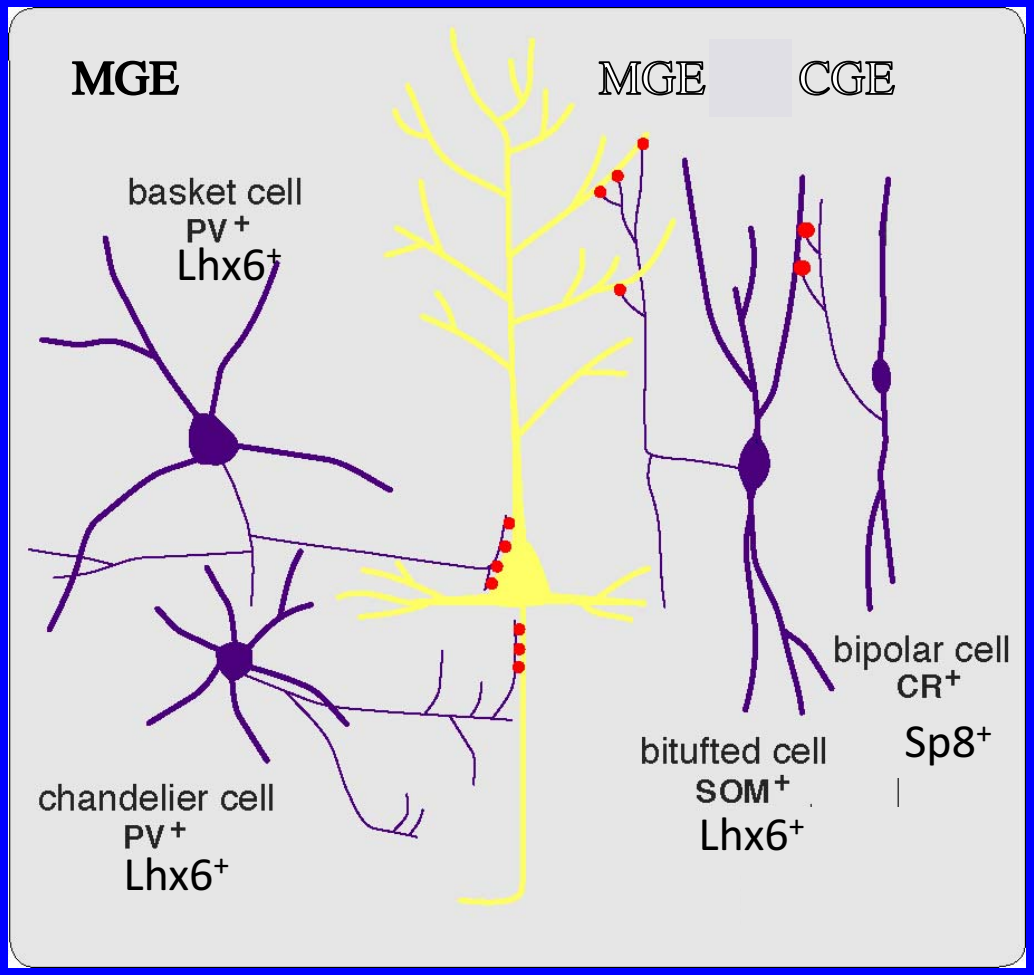
1056



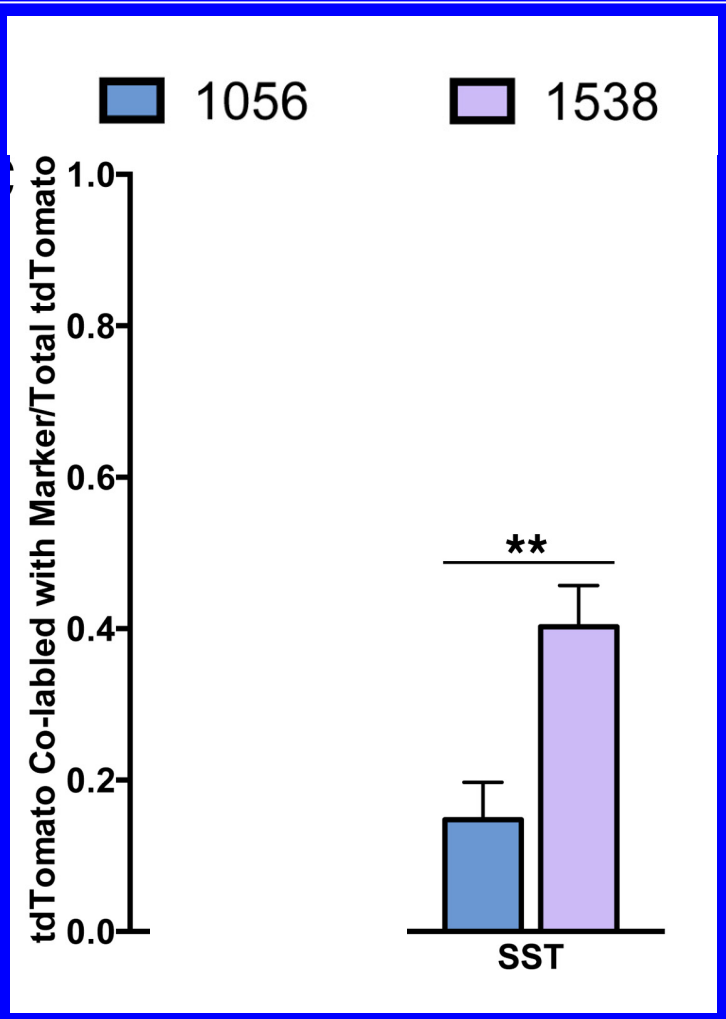
Interneurons Generated From the 1538 and 1056 Enhancer Mice

Tamoxifen: E10.5
Analysis: P30

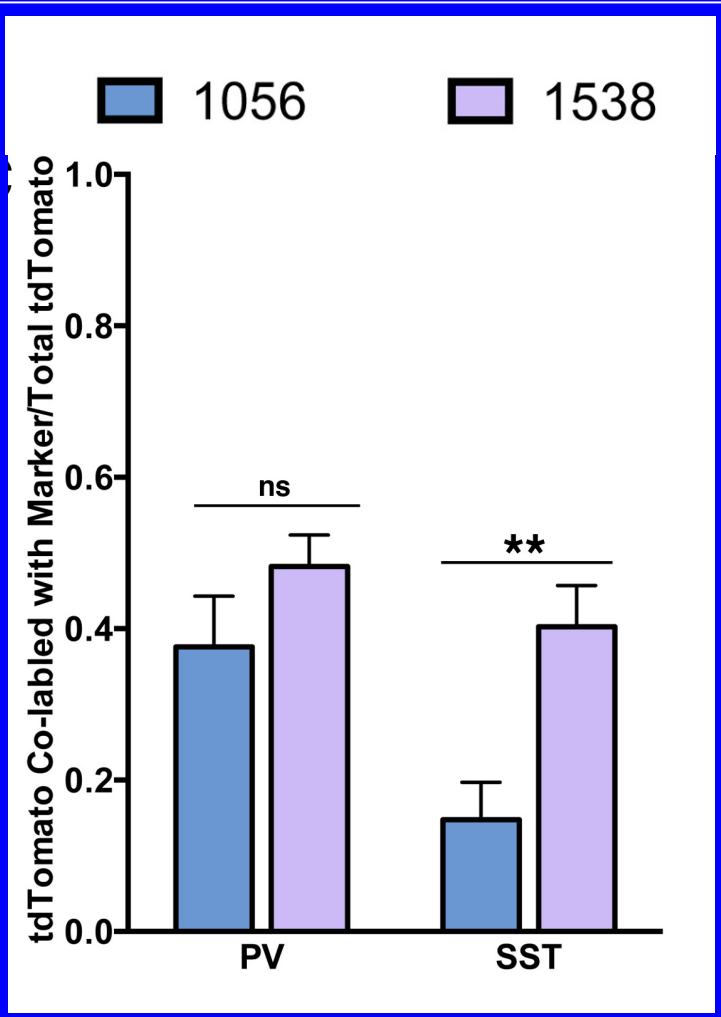
Cortical Interneurons

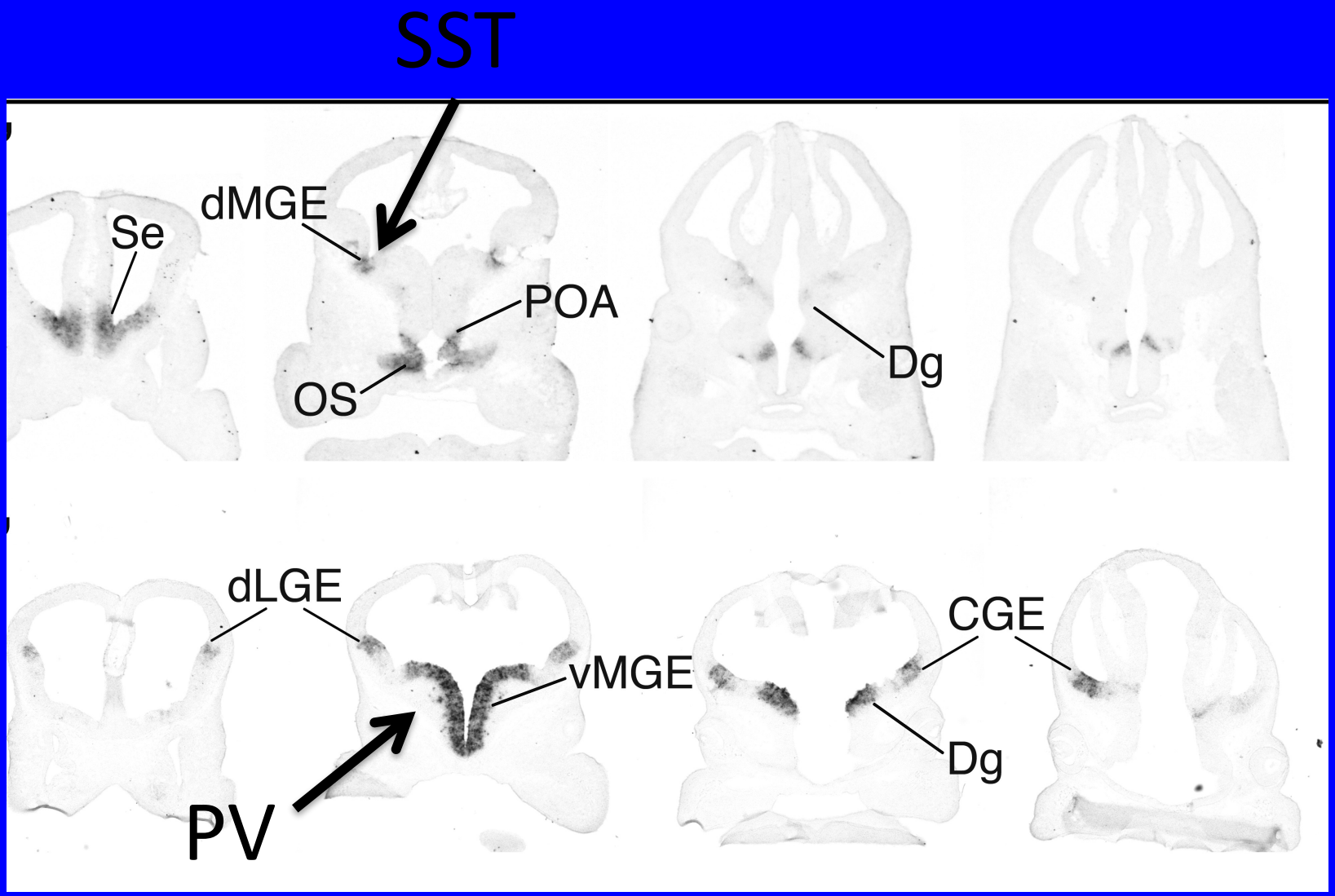


1538 Generates More SST Than 1056



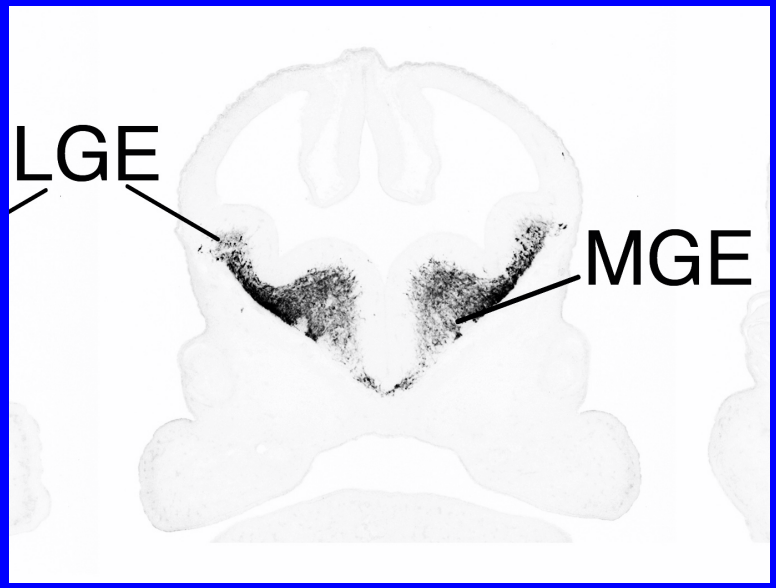
1056 Generates More PV Than SST




SST
Se
dMGE
POA
OS
Dg
1538
dLGE
vMGE
CGE
Dg
PV
1056

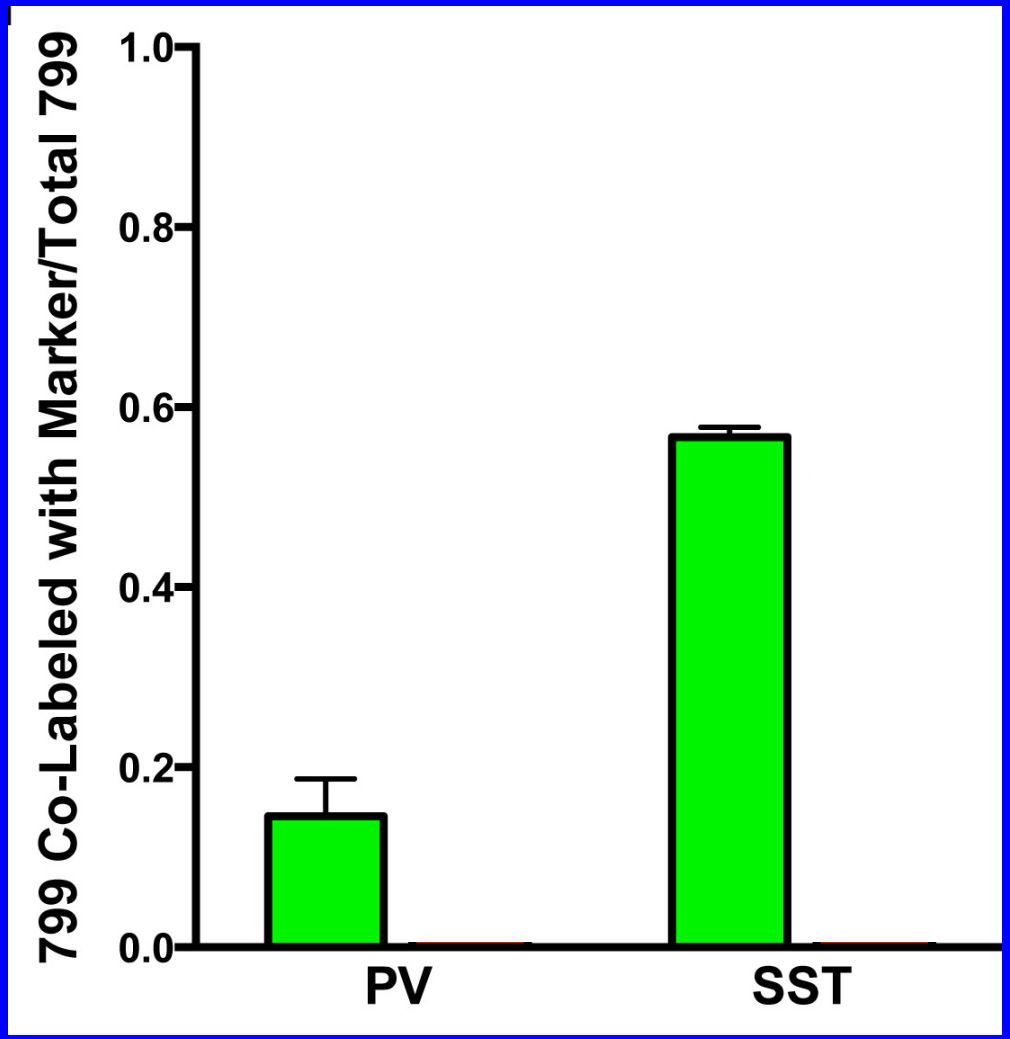
Another MGE Enhancer (799)

Active in the SVZ and Neurons



Maintains Activity (GFP) in Adult Interneurons

799 GFP Activity in Adult Cortex



Enhancer Activity in ***Progenitors***

1538 > 1056: SST

1056: PV > SST

Enhancer Activity in ***Neurons***

799: SST > PV

What Controls the Cell-Type Specificity of Enhancer Activity?

Search for Nucleotide Motifs that Might Confer MGE vs LGE Activity

Analyzed 89 Enhancers with Either MGE or LGE Activity.

Unable to Find a Nucleotide Signature Predictive of MGE vs LGE Activity (So Far)

Shanni Silberberg and Leila Taher

Tried A Biochemical Approach

Performed Transcription Factor ChIP-Seq

13 Transcription Factors

ChIP-Seq
E13.5 Telencephalic Tissue

Either

LGE+MGE
or
Cortex

Annotated

Transcription Factor (TF) *in vivo*
Binding to the
89 Enhancers Which Had
Differentiation LGE and MGE Activity

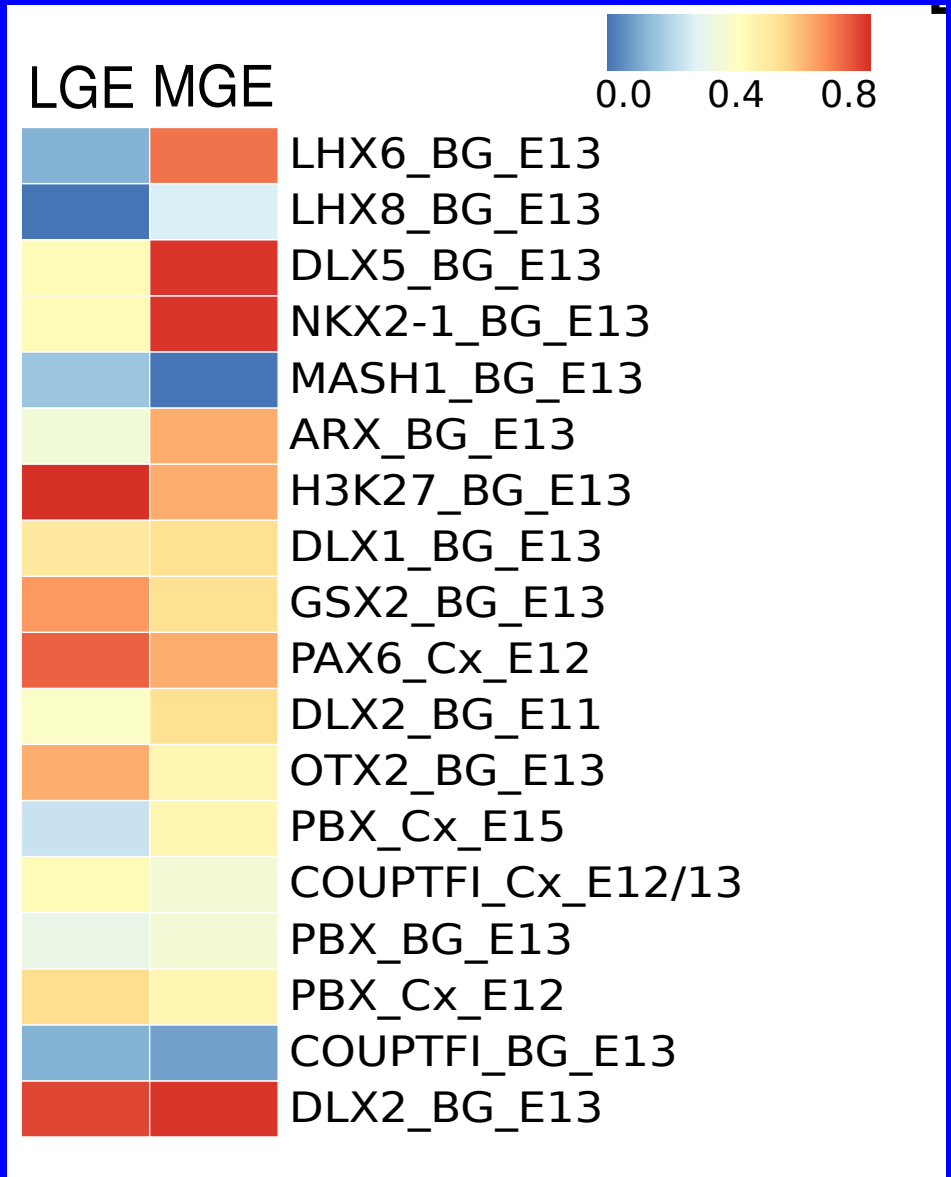
Machine Learning Analysis

Determined Which Transcription Factors Bind to the 89 Enhancers That Have Differential MGE vs LGE Activity

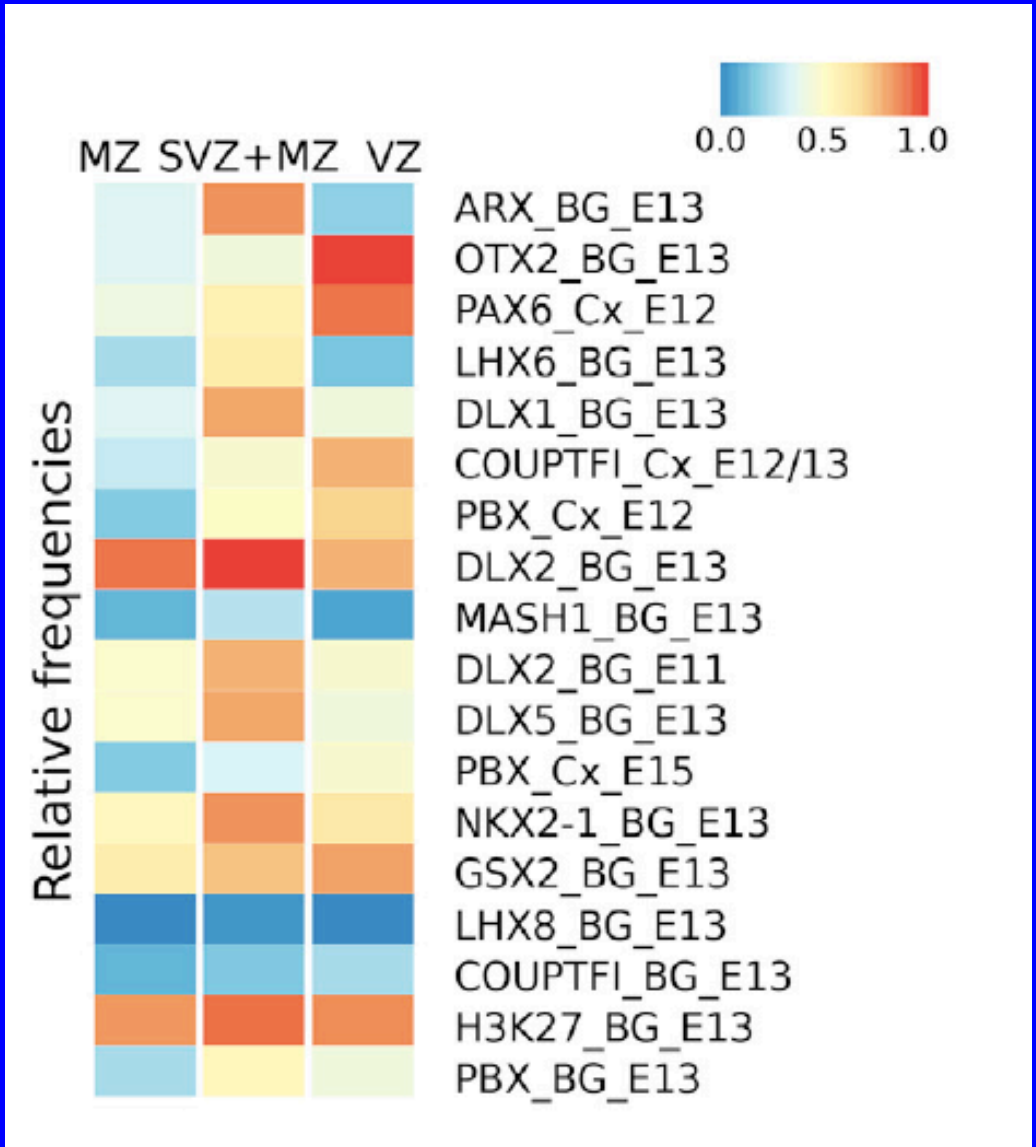
Leila Taher

Silberberg et al., 2016

Regional Activity: LGE vs MGE



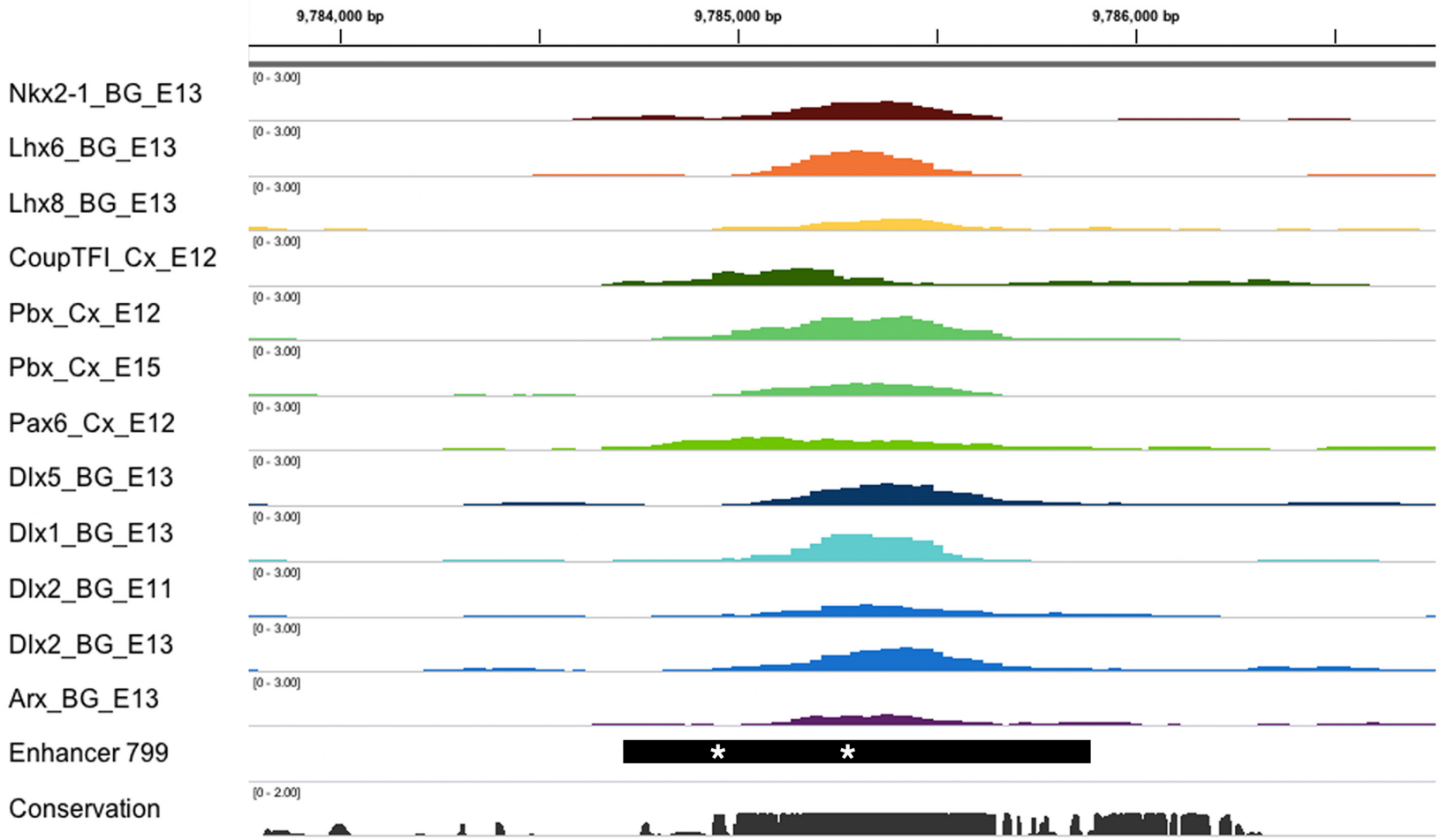
Differentiation Axis: VZ, SVZ, MZ



We then Focused on Regulation of an Enhancer with Activity in the SVZ/MZ of the MGE

Enhancer 799

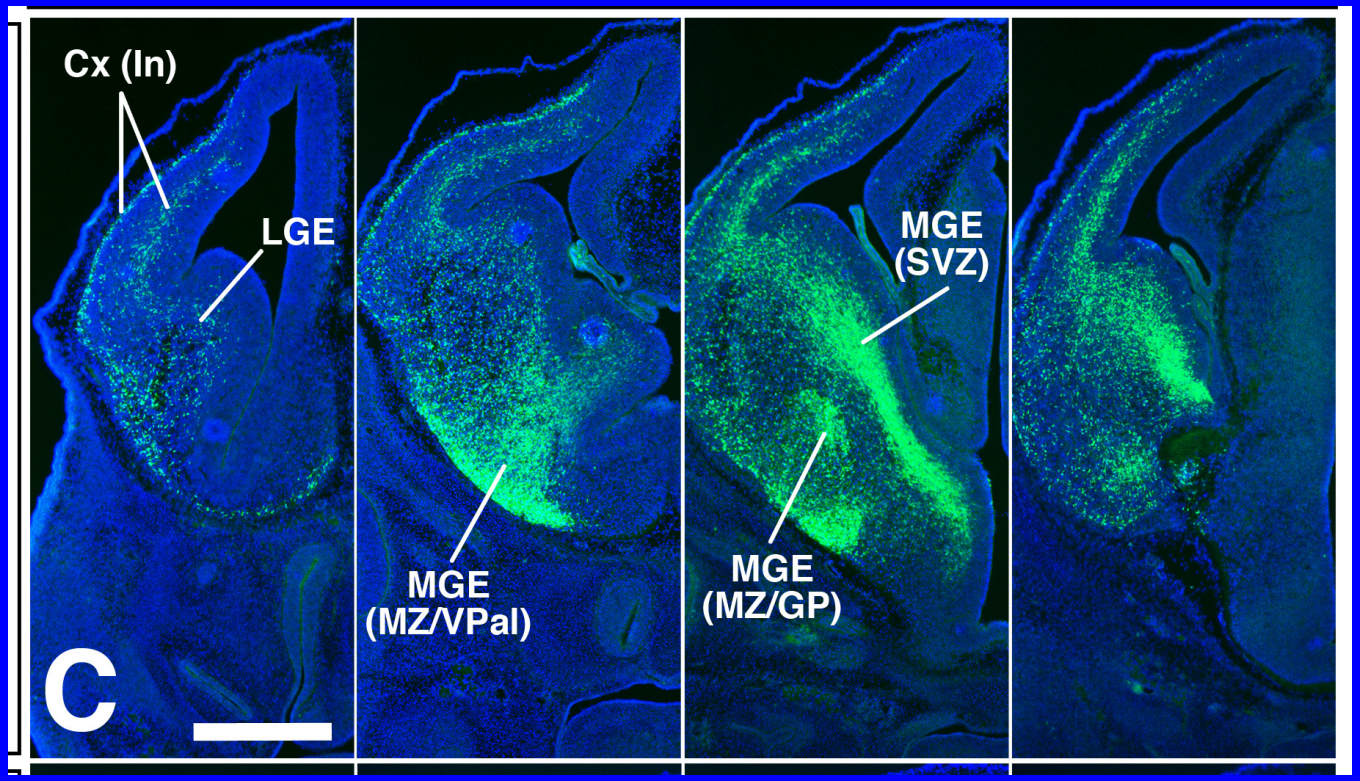
TF Binding to Enhancer 799



799 is Bound by Multiple TFs in the Same Region of the Enhancer

Test Regulation of Enhancer 799 *in vivo by Lhx6*

Enhancer 799 Activity at E13.5 (GFP)



Wild Type
Cx (In)
LGE
MGE
(MZ/VPal)
MGE
(SVZ)
MGE
(MZ/GP)
C
Lhx6 PLAP/PLAP
MGE
(MZ/GP)
F

Summary

Identified 89 Enhancers with Preferential Activity in:

MGE vs LGE

and

Progenitors vs Neurons

Stable Transgenic Lines Had Enhancer Activity In Specific Subdivisions of the LGE and MGE

Fate Mapping Defined Relationship of Progenitor Domains to Basal Ganglia Nuclei and Interneuron Subtypes

- *In Vivo* Transcription Factor Enhancer Binding Predicted MGE vs LGE Activity

Included TFs such as

LHX6 and NKX2-1

- *In Vivo* Transcription Factor Enhancer Binding Predicted MGE vs LGE Activity

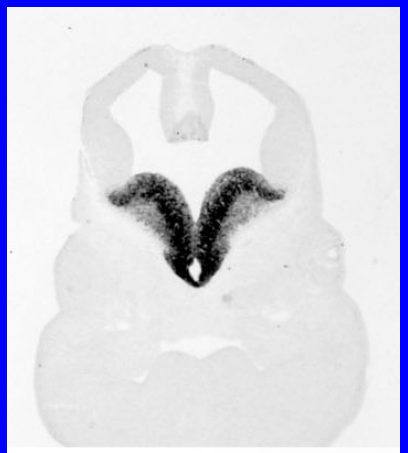
- LHX6 Binding Was Associated with MGE Activity of Multiple Enhancers

- *Lhx6* Promoted Enhancer 799 Activity

Function of NKX2-1 and LHX6 in Regulating MGE Enhancers and Development

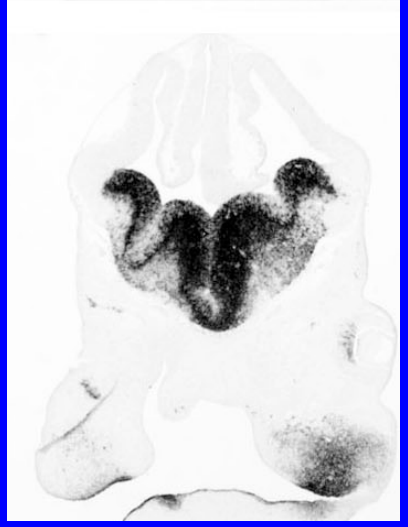
Homeodomain Transcription Factors Expressed in the MGE

Nkx2.1

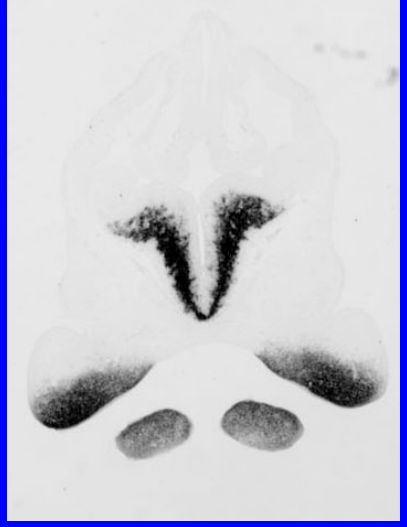


Lhx6

Dlx2



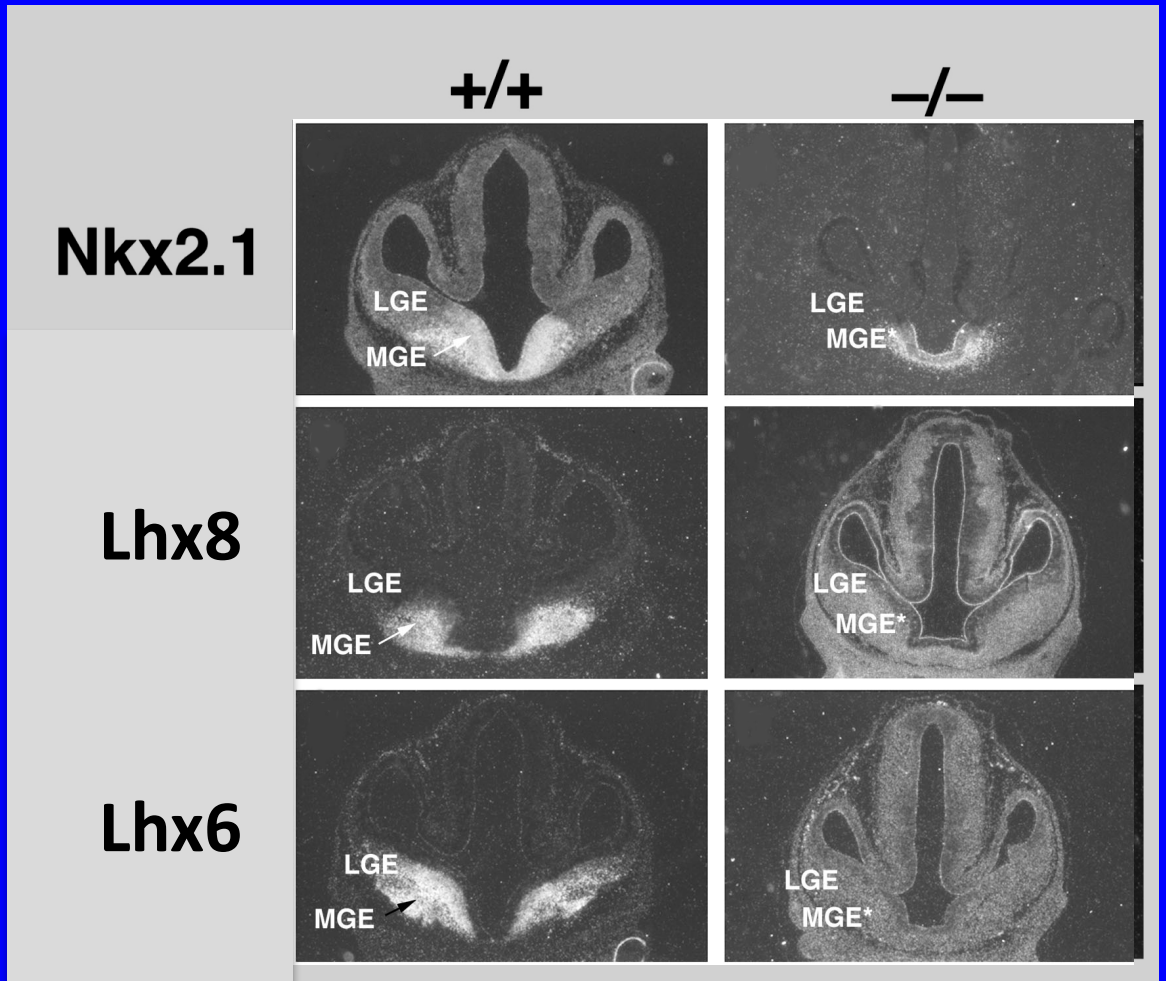
Lhx8



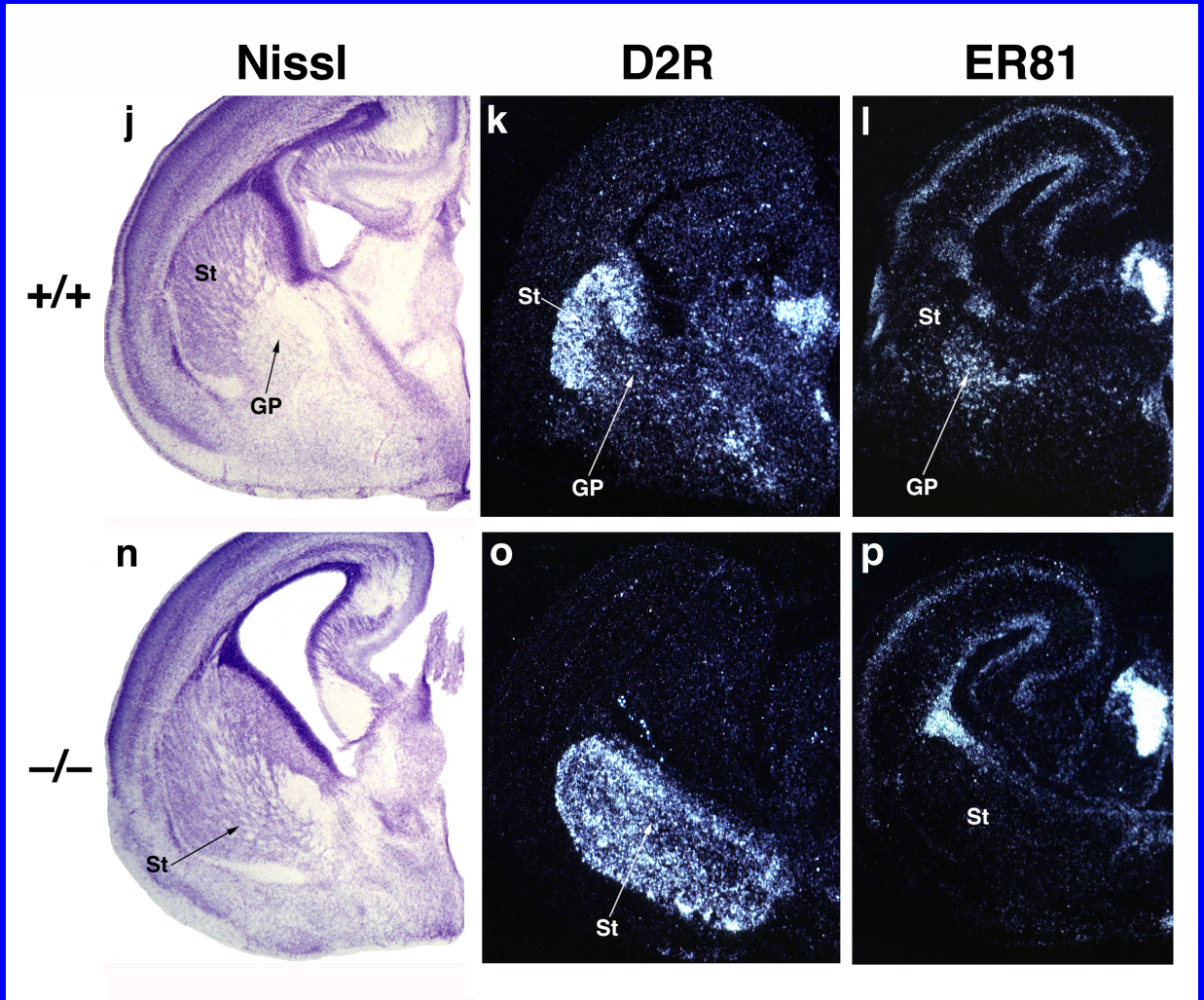
(E11.5)

Phenotypes of *Nkx2.1* and *Lhx6* in Mouse Mutants

Nkx2.1$^{-/-}$ Lacks *Lhx6* & *Lhx8* Expression



Nkx2-1$^{-/-}$
Enlarged Striatum
Loss of Globus Pallidus



Nkx2-1

1. Specifies MGE Identity.

2. Induces *Lhx6&8*.

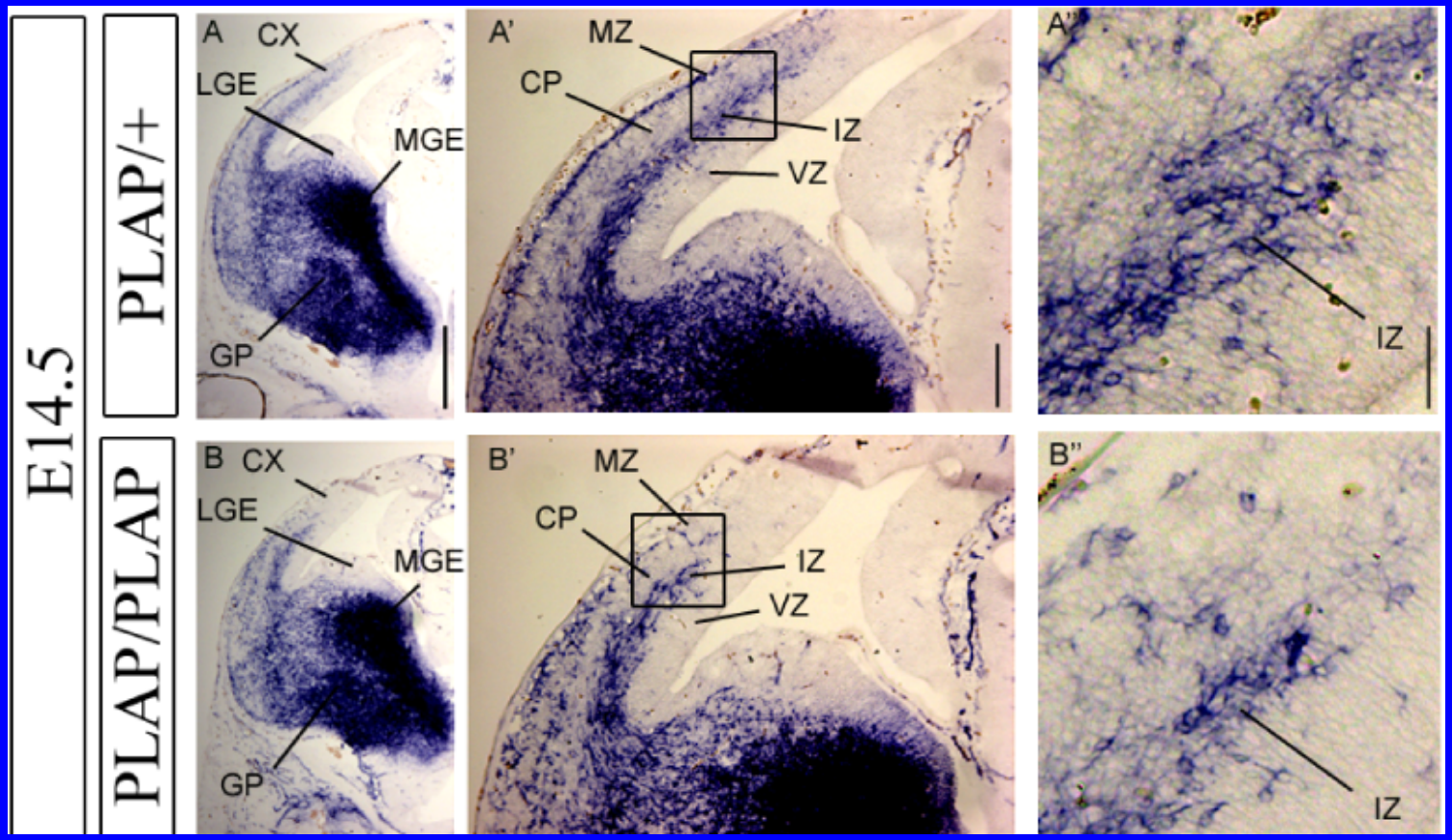
3. Required to Make Globus Pallidus & MGE-derived Interneurons.

Lhx6 (LIM-Homeodomain) Is Required for *Parvalbumin & Somatostatin* Interneurons

Liodis, Denaxa, Grigoriou, Akufo-Addo, Yanagawa, Pachnis, 2007

Zhao, Flandin, Long, Westphal and Rubenstein, 2008

Lhx6$^{PLAP/PLAP}$ Interneurons Migrate More Slowing to the Cortex (E14.5)



Zhao, Flandin, Long, Westphal and Rubenstein, 2008

Lhx6$^{PLAP/PLAP}$

Loss of

Parvalbumin & Somatostatin

Interneurons