Dear neuroscience students,

I enjoyed teaching you and our interactions in class. Feel free to contact me at any time during your ongoing career at UCSF.

Warm Regards,

Ben Cheyette

Signaling Themes in Neural Development

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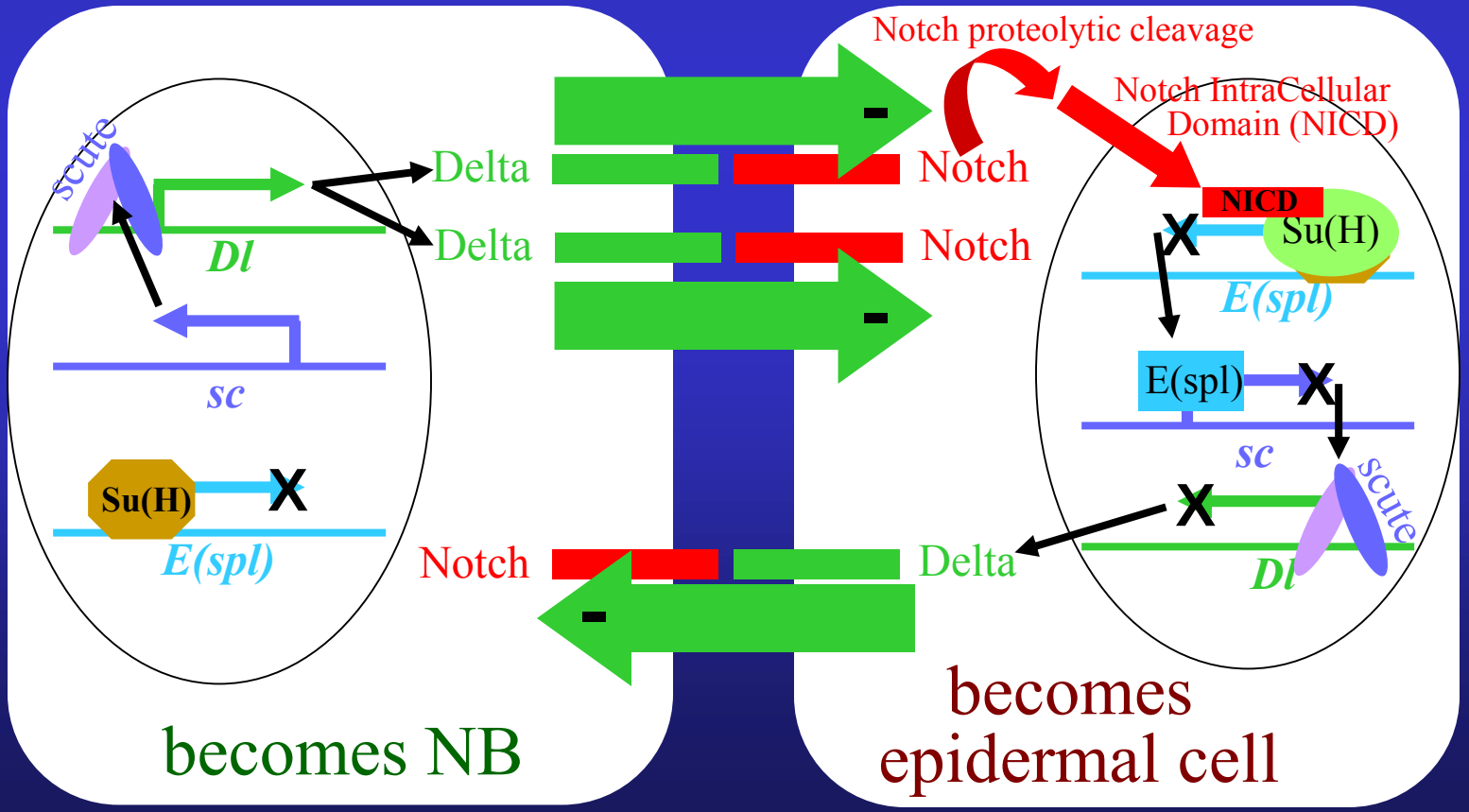
II

Secreted Signaling Molecules

Review from last week

The Notch signaling pathway is the main conserved molecular mechanism whereby lateral inhibition (lateral specification) is achieved during neural development



Notch is a transmembrane ligand

This is ideal for lateral specification (why)?

What about ligands (signals) that are not stuck to the membrane (i.e. secreted)?

Important Secreted Signaling Molecules and Pathways whose names and basic classification you should *recognize*

(once again, underlined words are those I'd hope you might remember)

Hedgehog (Hh, Shh)

Receptor Tyrosine Kinase Superfamily

e.g. Fibroblast Growth Factor (FGF)

[also: EGF, BDNF, VEGF, etc]

Transforming Growth Factor Beta (TGFß, Nodal, Activin)

[& Bone Morphogenic Proteins (BMP)]

Wnt (*wg* in Drosophila)

Six Principles of Secreted Signals

1. The ligand is secreted *from its source* but that does **NOT** mean it is "free-floating"!
   extracellular distribution is regulated
2. There is at least one (and not uncommonly more than one) specific cell-surface receptor complex on receptive cells
3. Periplasmic conduction downstream of an activated receptor complex is through protein-protein interaction domains (i.e. "scaffold proteins")
4. Regulation of phosphorylation is an important component of cytoplasmic transduction
5. The typical endpoint of signaling is target gene regulation in the nucleus, but other cellular outputs are also possible (i.e. regulation of cytoskeletal dynamics)
6. **ALL** steps are subject to static inhibitory and/or feedback inhibitory mechanisms:

   Static inhibition:

   A. dampens "noise"
      (creates a high signal-to-noise ratio)

   B. prevents ectopic activation
      (which otherwise spells trouble: malformations and cancer)

   Feedback inhibition:

   A. creates temporal fidelity (loss of ligand = end of signal transduction)

   B. preserves fidelity of signal strength down the cascade

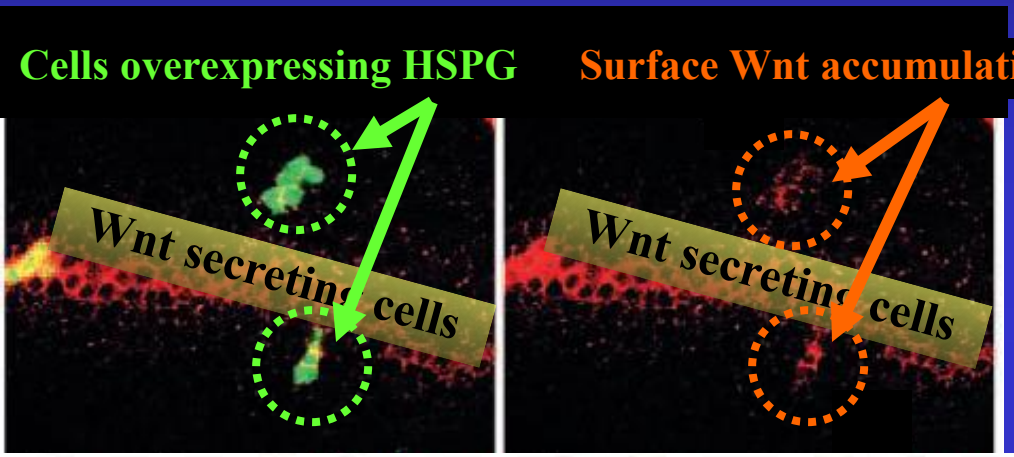
   (25% receptor occupancy = 25% kinase activity = 25% target activation)

without internal feedback inhibition, signal will tend to be magnified at each step – think of an enzymatic reaction

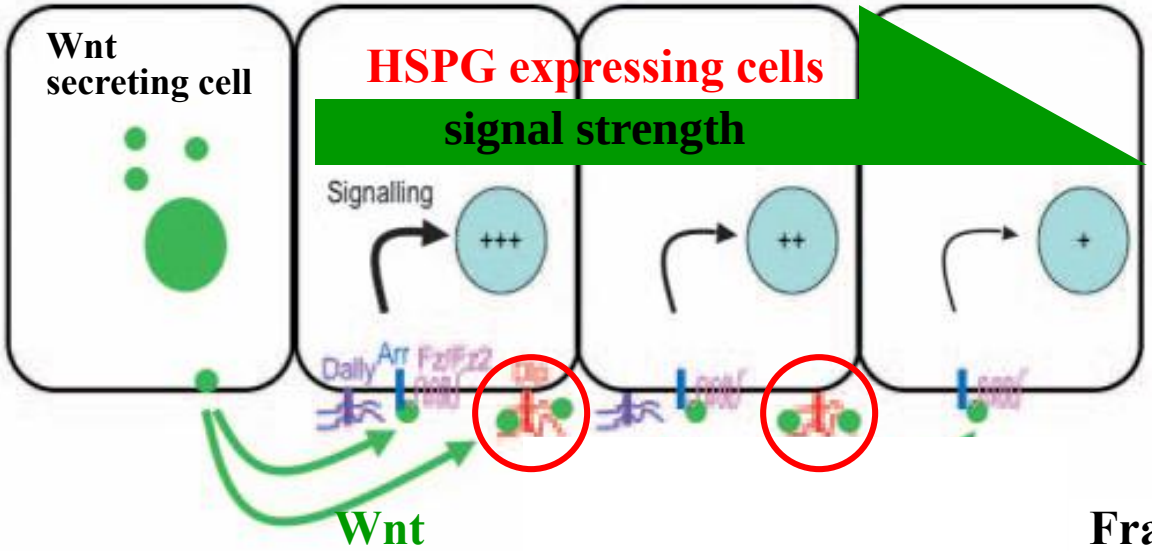
Illustration of principles: Wnt cascade

1. The ligand is secreted *from its source* but that does **NOT** mean it is "free-floating"!
   regulated extracellular distribution

Example: Heparin-Sulfate-Proteoglycans (HSPGs) "extracellular matrix proteins" regulate the intensity and distribution of Wnt (and other) signals away from their source.



Interestingly, the cells that overexpress the HSPG in this case (i.e. that we can see have more Wnt protein stuck to their surface) nonetheless have LOW levels of Wnt activation. But the cells immediately adjacent to them have HIGH levels of Wnt activation.



Model: This particular HSPG sequesters available Wnt *away* from active Wnt receptors on the cells that express it. But it serves to make the ligand *more* available to cells further away from the Wnt source.

(Other HSPGs behave differently & may act as Wnt co-receptors).

Franch-Marro et al, *Development* 2005

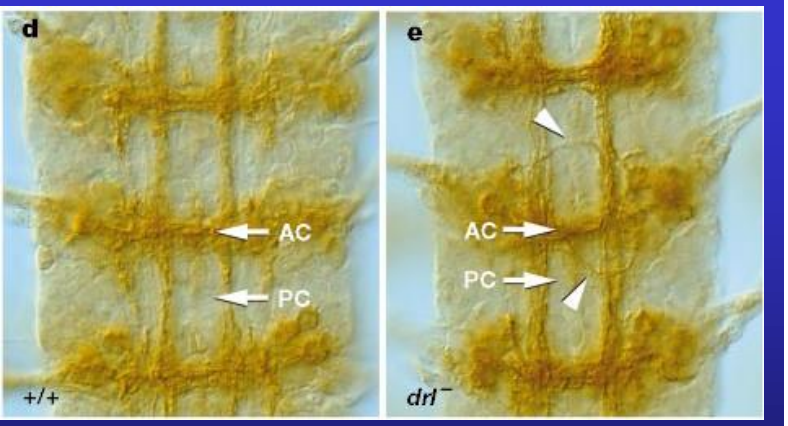
Illustration of principles: Wnt cascade

2. There is at least one (and not uncommonly more than one) specific cell-surface receptor complex on receptive cells

<u>Example:</u> The originally identified "Wnt receptor" is <u>Frizzled</u>, a 7-pass transmembrane receptor (i.e. a member of the superfamily that includes neurotransmitter receptors).

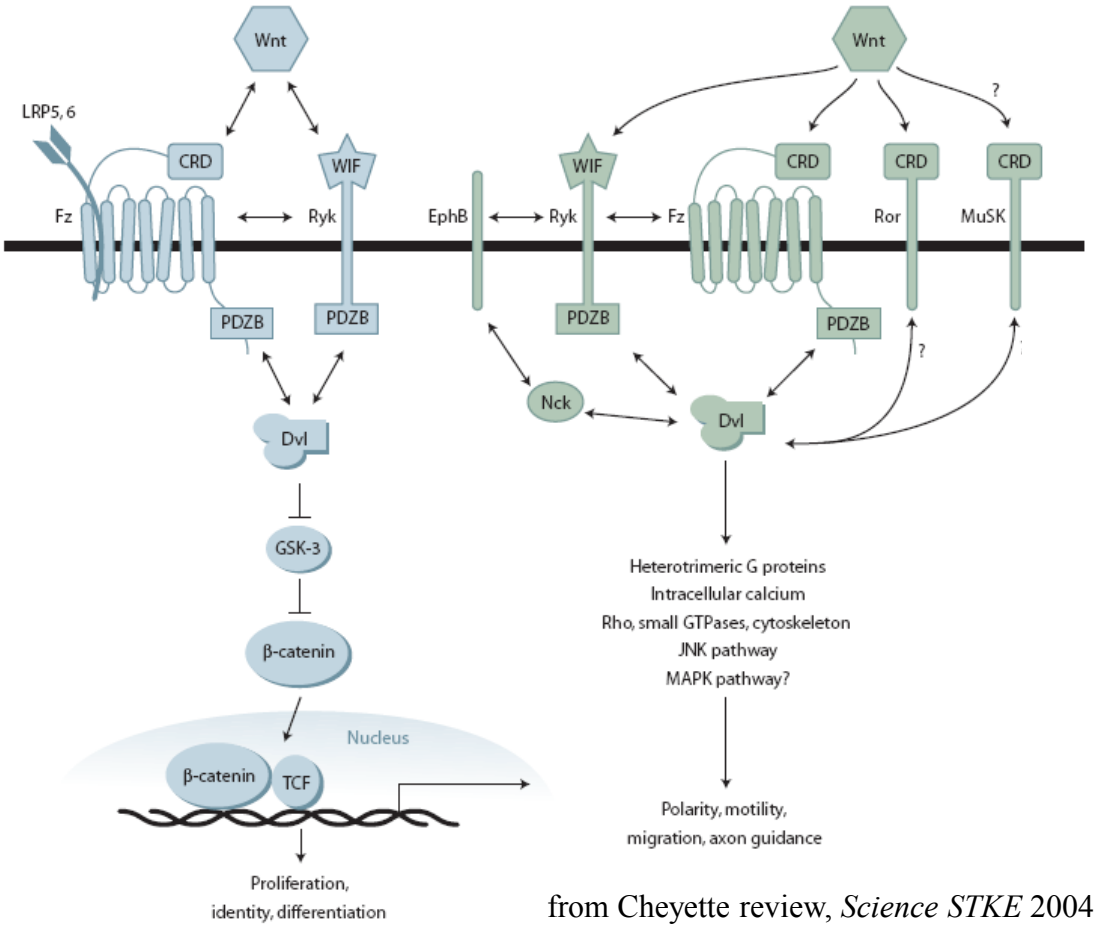
…then (c. 2000) LRP5/6 ("Arrow") was found to be an essential co-receptor for Wnt/ß-catenin-signaling…

…meanwhile, studies in *Drosophila* identified a novel transmembrane protein (Derailed) involved in midline axon pathfinding…



…turns out Derailed (aka Ryk) is another Wnt co-receptor, that preferentially mediates a different type of downstream signaling…

A plethora of potential Wnt receptor complexes

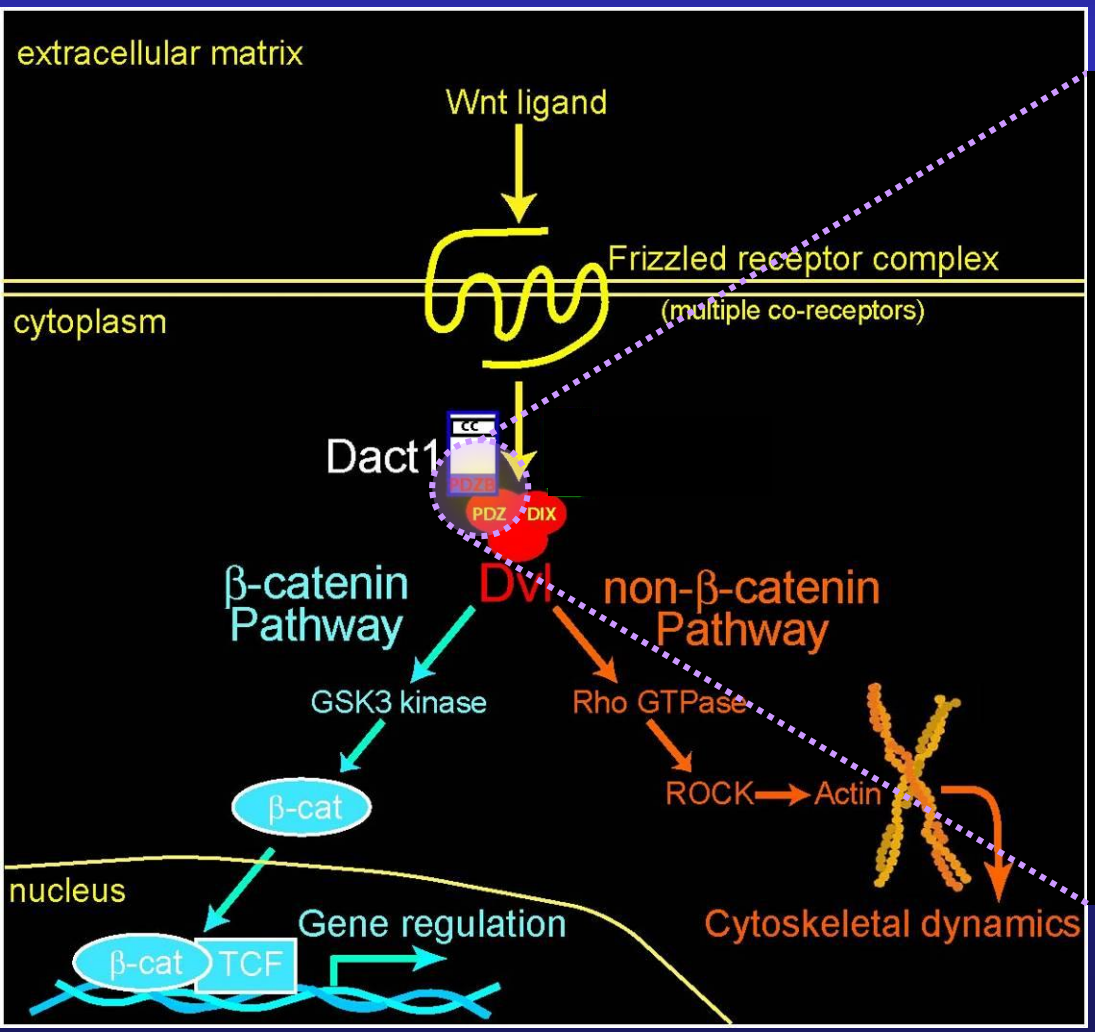


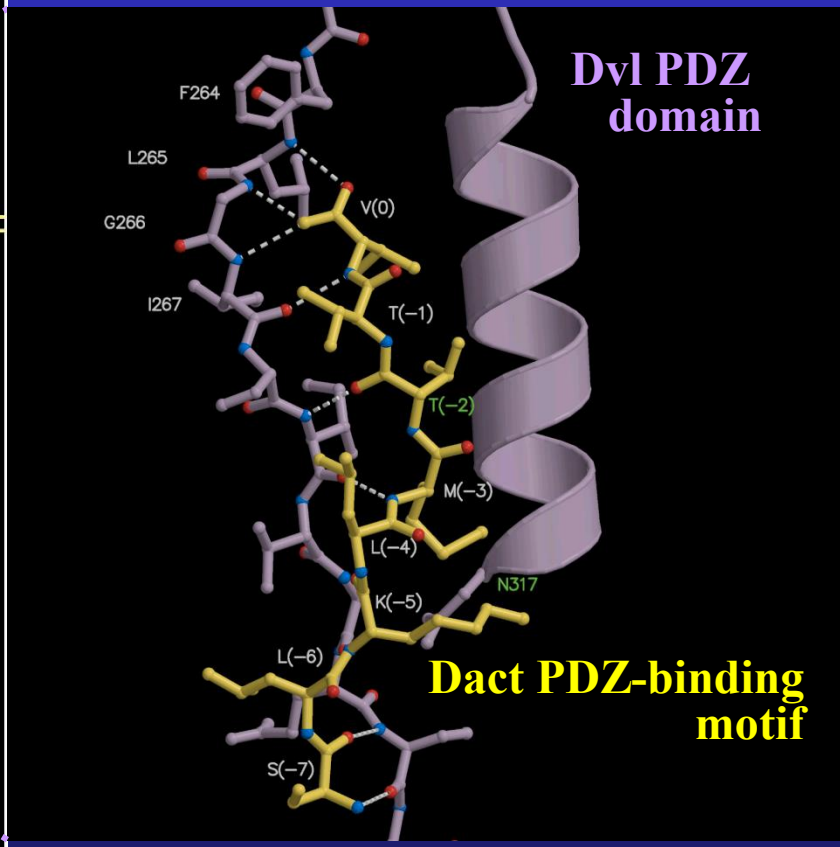
from Cheyette review, *Science STKE* 2004

Illustration of principles: Wnt cascade

3. Periplasmic conduction downstream of an activated receptor complex is through protein-protein interaction domains (i.e. "scaffold proteins")

<u>Example:</u> Dact (Dpr/Frodo) binds to the Dvl protein (central to Wnt signaling) via a conserved interaction between a PDZ-binding motif (in Dact) and a PDZ domain (in Dvl).



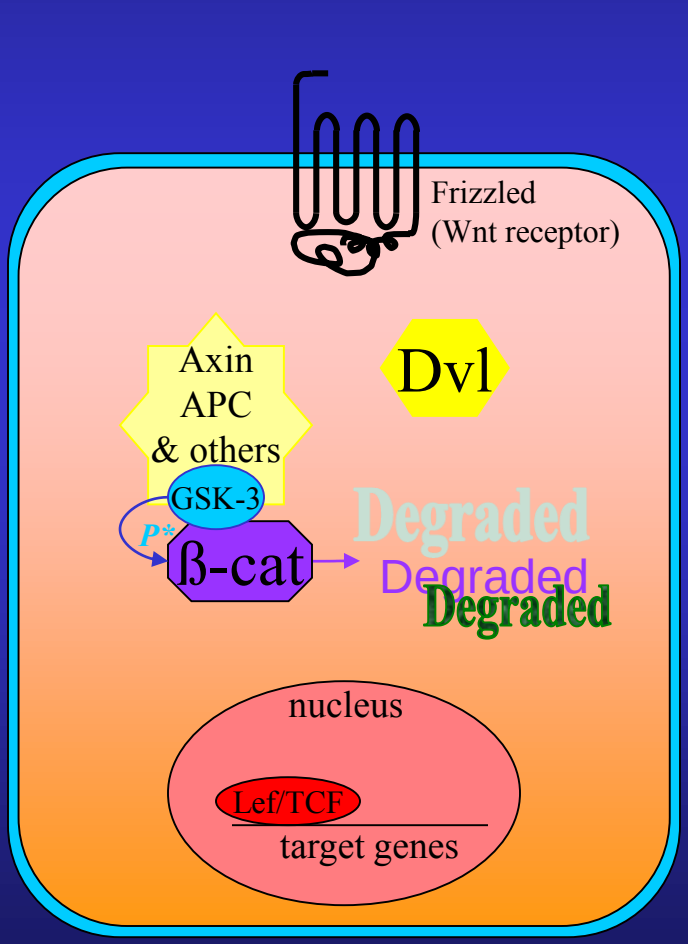


Cheyette et al, *Developmental Cell* 2002

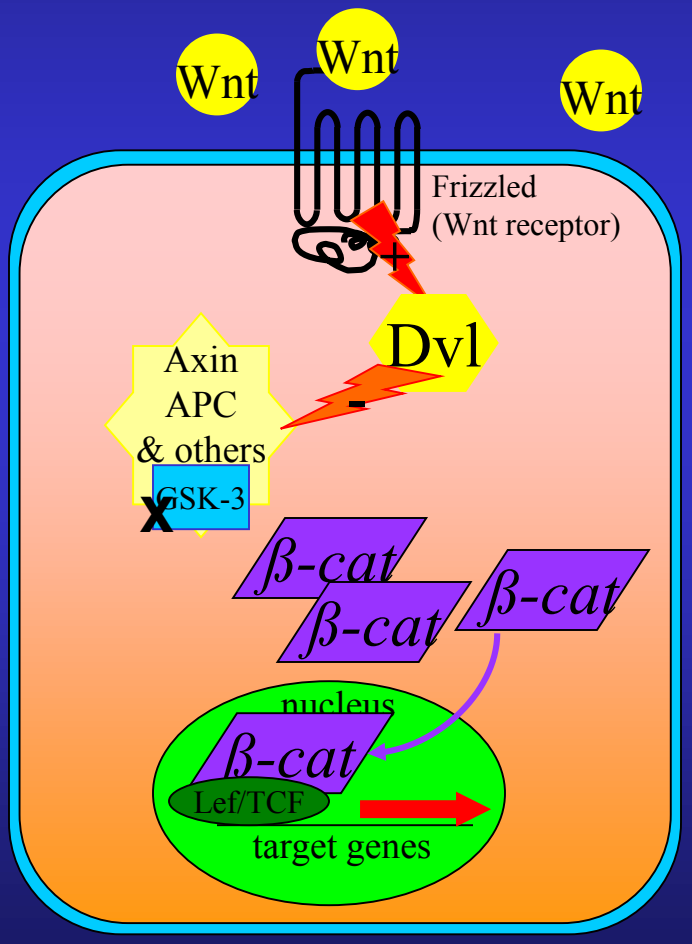
Illustration of principles: Wnt cascade

4. Regulation of phosphorylation is an important component of cytoplasmic transduction

Example: Wnt/ß-catenin ("canonical") signaling depends on *blockade* of a KINASE (GSK3ß), whose activity promotes phosphorylation-dependent degradation of free **ß-catenin**



unstimulated

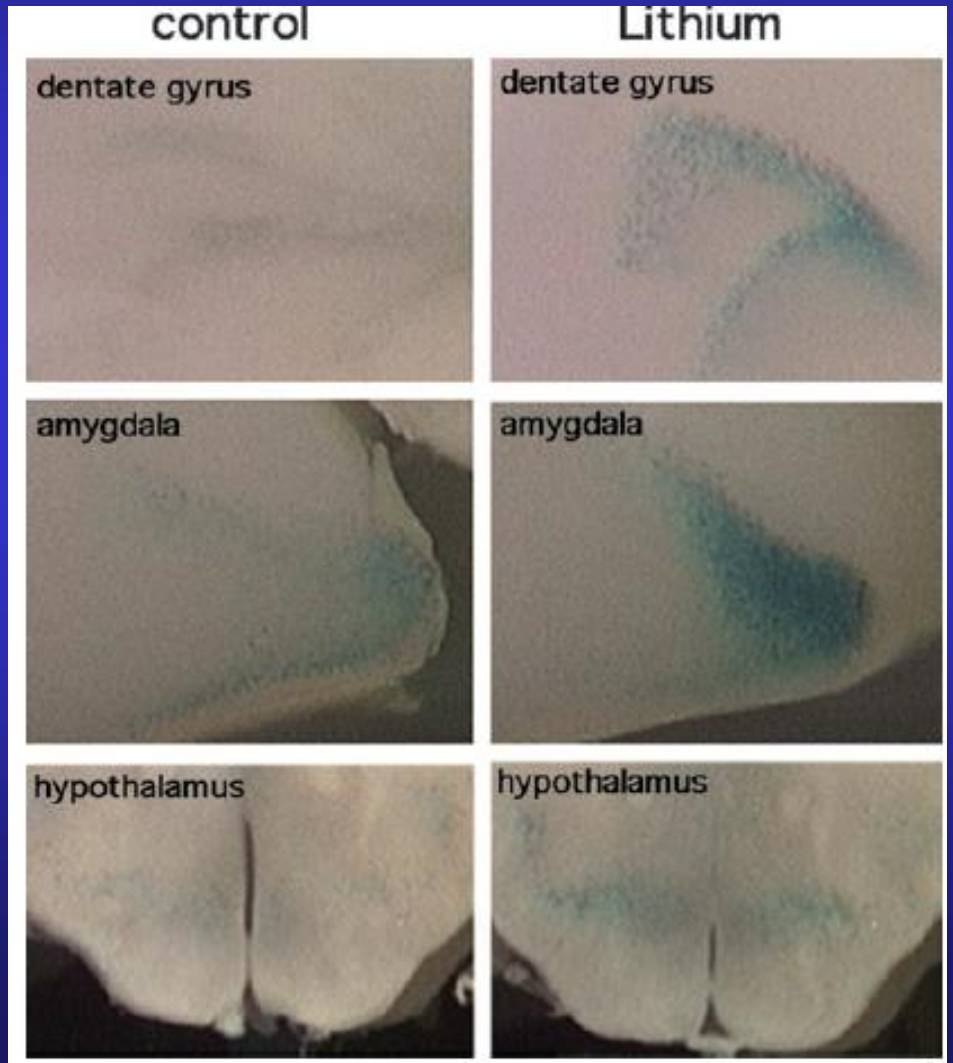


stimulated

Illustration of principles: Wnt cascade

5. The typical endpoint of signaling is target gene regulation in the nucleus, but other cellular outputs are also possible (i.e. regulation of cytoskeletal dynamics)

Example: Lithium, which pharmacologically inhibits the GSK3ß kinase (mimicking Wnt/ß-catenin pathway activation), up-regulates Wnt target gene activity in the mammalian forebrain.



Brains of mice carrying a transgene in which a Wnt-responsive promoter drives the *LacZ* gene. These were sectioned and stained for ß-galactosidase activity (blue color). Lithium treated animals had increased Wnt-target gene activity in the dentate gyrus, amygdala, and hypothalamus.

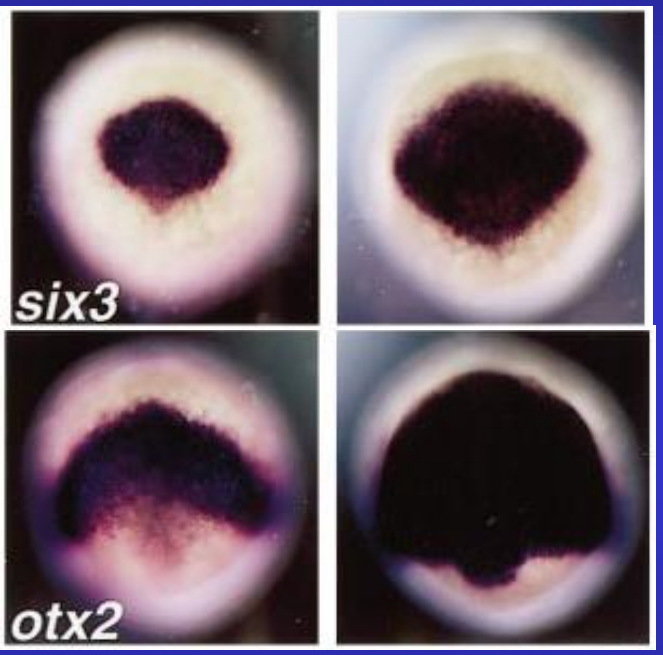
O'Brien et al (2004) J. Neurosci *24*, 6791-6798

Illustration of principles: Wnt cascade

6. **ALL** steps are subject to static inhibitory and/or feedback inhibitory mechanisms

Example: The secreted Wnt inhibitor Dikkopf regulates brain patterning in zebrafish embryos

control *+ dkk1*

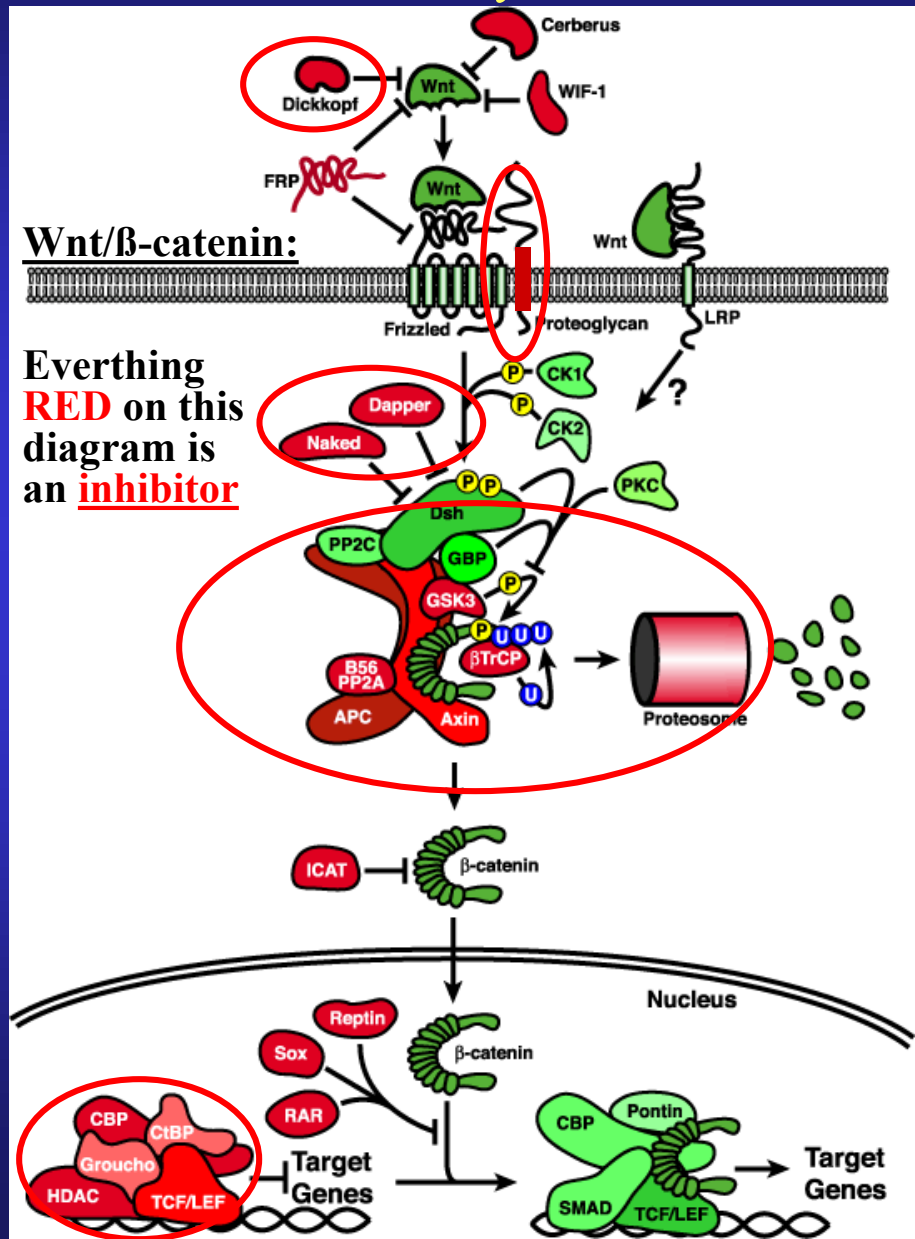


Molecular markers for the forebrain domain of the developing neuroectoderm

Hashimoto et al *Dev Bio*, 2000

For Wnt/ß-catenin signaling there are:

-secreted inhibitors (e.g. Dickkopf)
-membrane-tethered inhibitors (e.g. Dlp HSPG)
-scaffold protein inhibitors (e.g. Naked, Dapper)
-nuclear inhibitors (e.g. Groucho)
-the crux of the pathway is literally built around default inhibitory degradation of ß-catenin

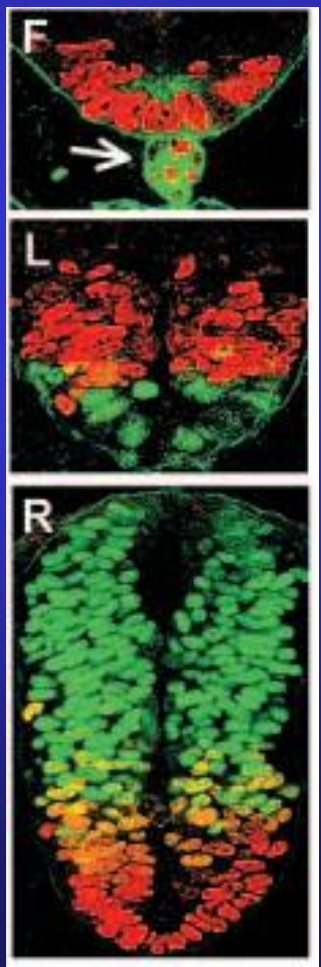


OK – now let's talk in a bit more detail about just one neurodevelopmental story where these signaling principles are in evidence and have been fairly well characterized…

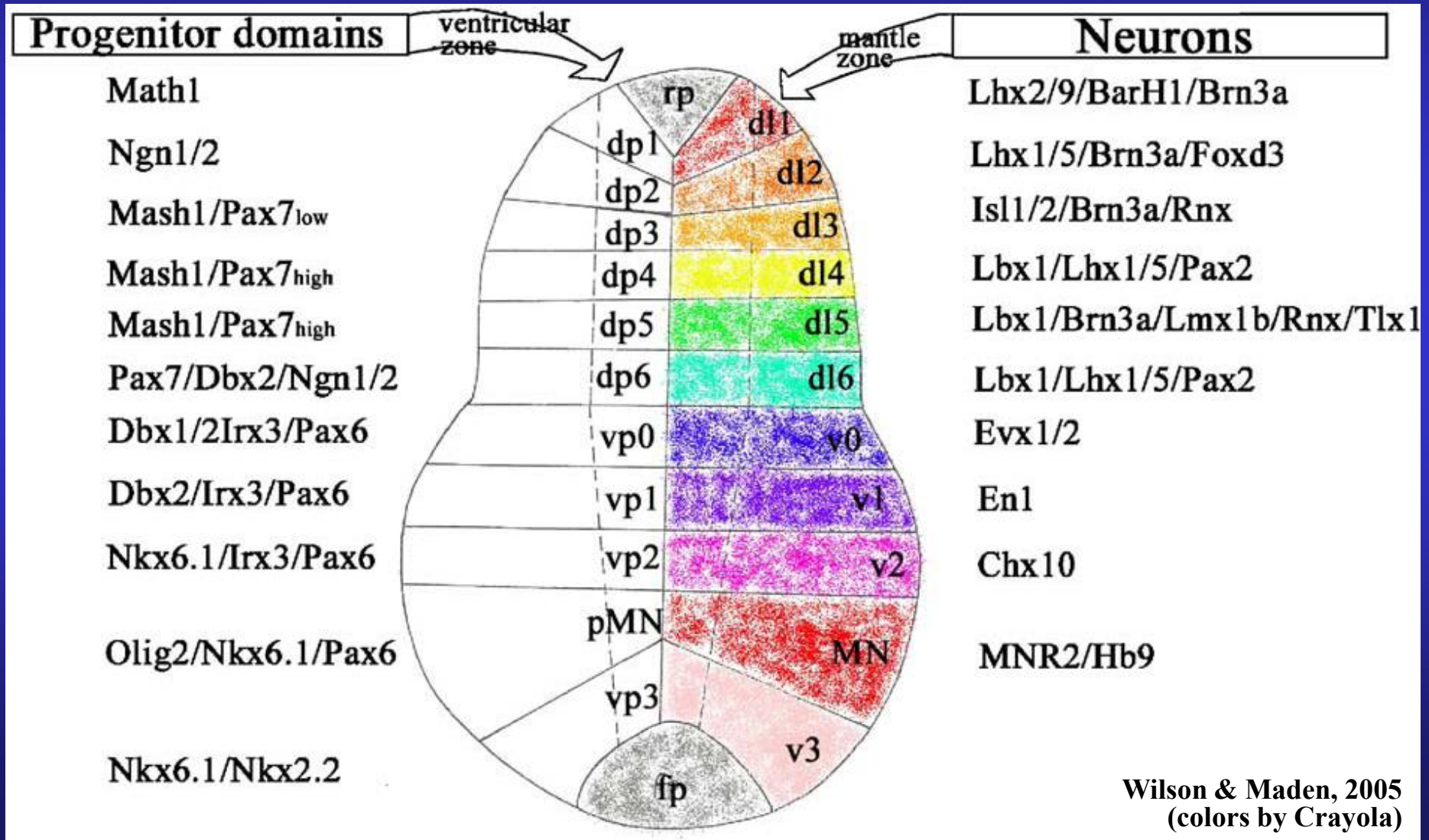
Sonic Hedgehog (Shh)

in

neural tube (spinal cord) patterning

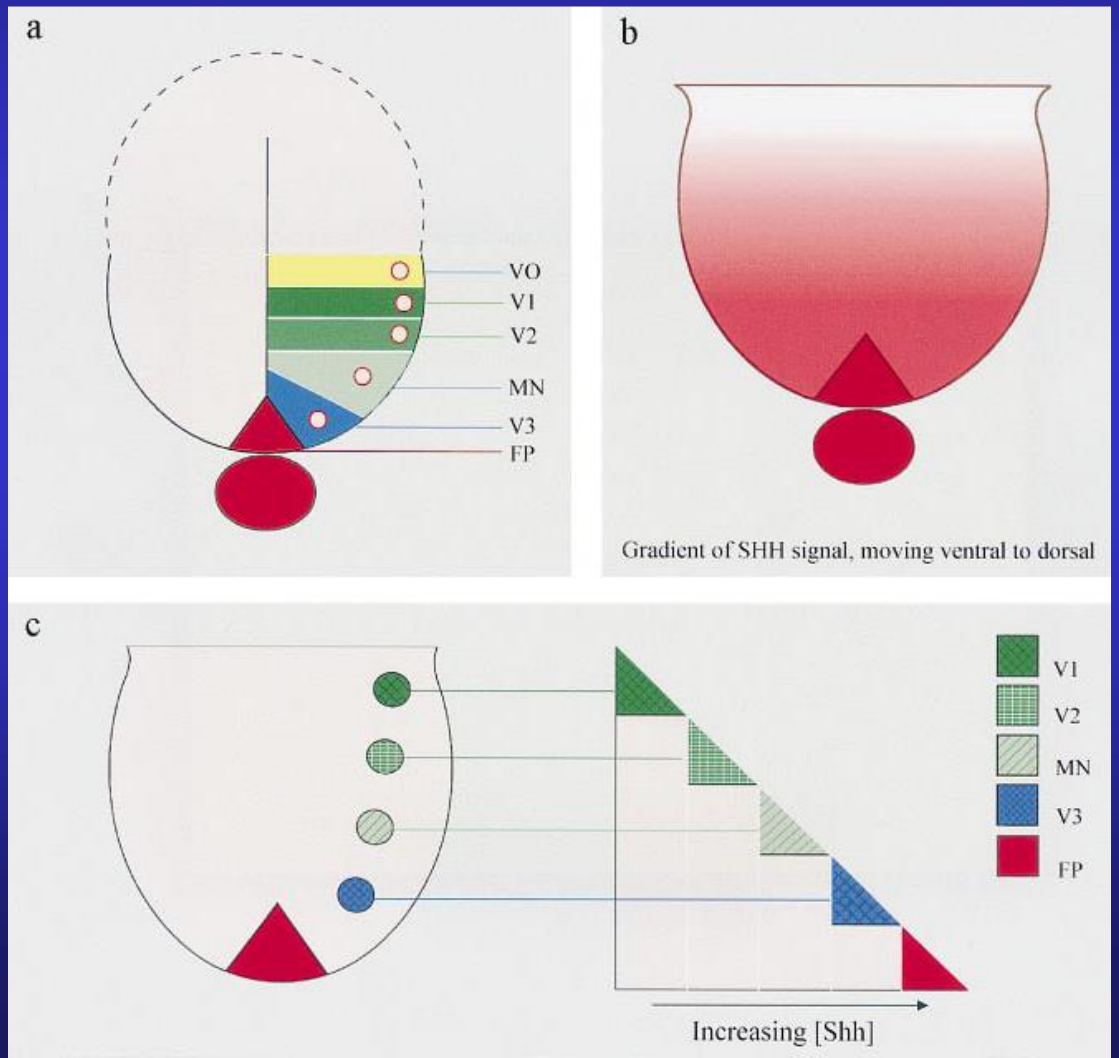


Dorsal-Ventral (D-V) patterning of the neural tube



Wilson & Maden, 2005
(colors by Crayola)

A gradient of Shh emanating from the notochord and floorplate (ventral) patterns the neural tube in the D-V axis



Patten & Placzek 2000

Here's one story in this system that examines two principles:
The role of extracellular proteins that:
(#1) regulate extracellular distribution
and
(#6) act as inhibitors
of the Shh ligand

From the Ph.D. thesis work of Juhee Jeong, with A. McMahon

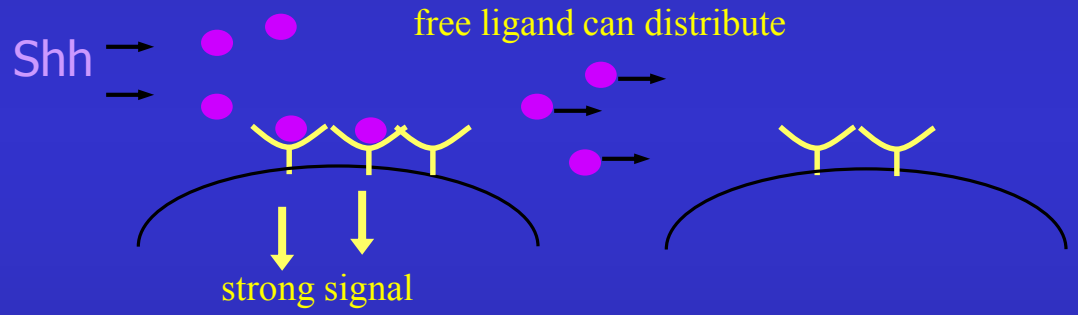
(Jeong & McMahon (2004), Development *132*, 143-154)

(Juhee was a post-doc with John Rubenstein until recently, now faculty at NYU)

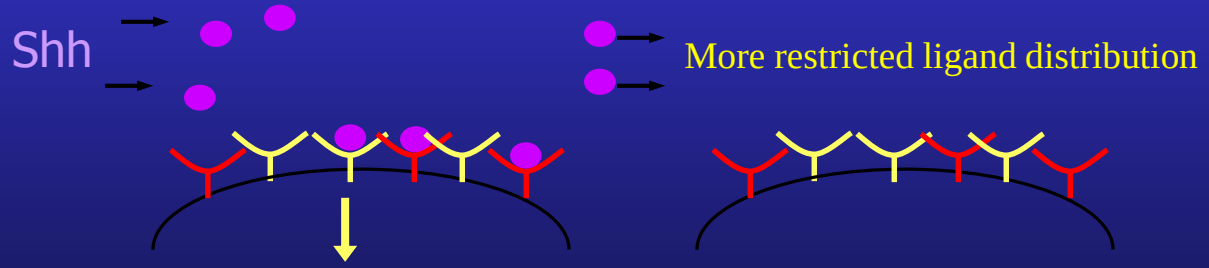
Hypothesis: The Shh signaling gradient is affected by ligand-dependent antagonism (LDA)

Ligand-Dependent Antagonists (LDA): cell surface components that act on the ligand to block its function either by degrading, modifying, or sequestering it.

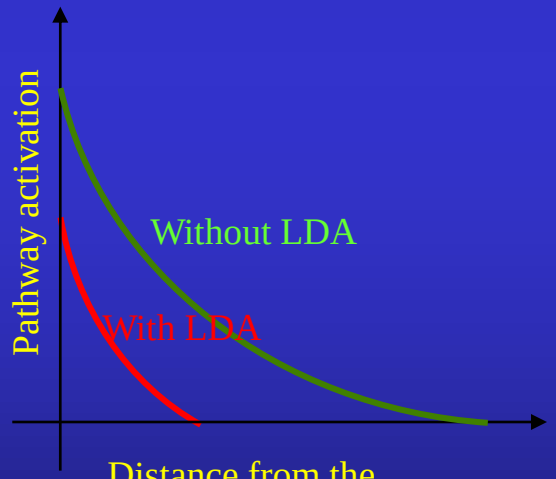
In the absence of LDA:



In the presence of LDA:



Decreased sensitivity of the cell



Hh signaling in *Drosophila*
Complex role of Patched (Ptc)

Ptc: a "receptor" that plays multiple (confusing) roles in Hh signaling

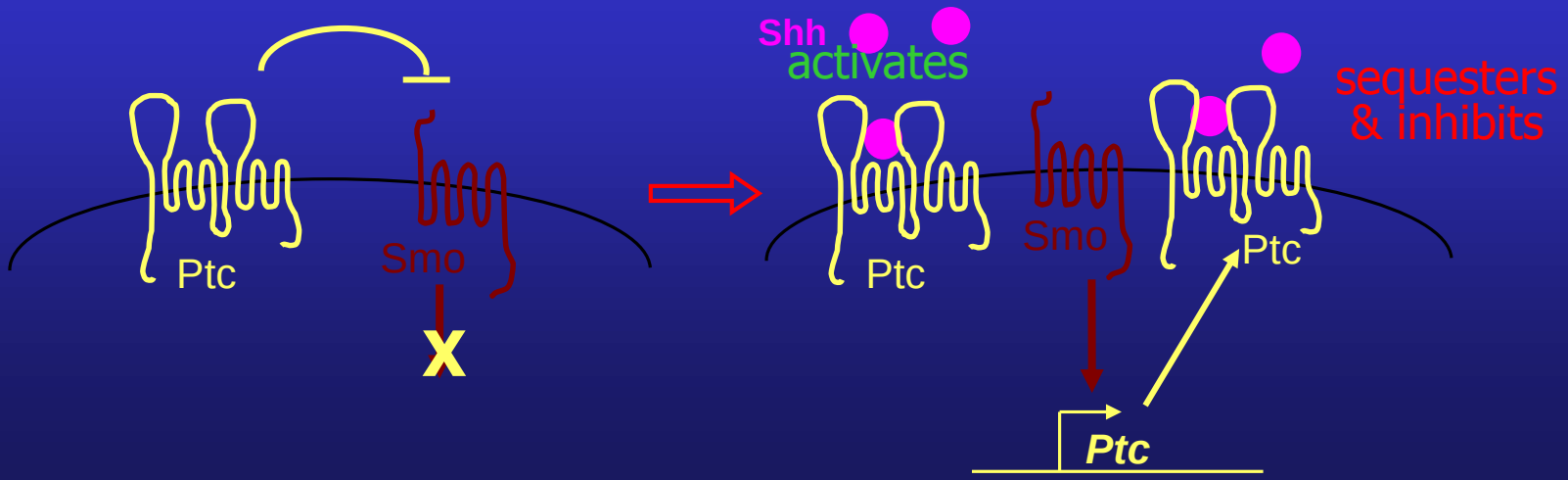
Both a Hh 'receptor' (binds to Hh to activate signaling), and a negative regulator of the Hh pathway

Ligand-independent antagonism (LIA): inhibits **Smo** in the absence of a Hh signal

But Smo becomes *dis-inhibited* upon Hh binding to Ptc
(thus Ptc helps to activate the pathway in the presence of ligand)

Is also a transcriptional target of Hh signaling – i.e. feedback inhibition

Ligand-dependent antagonist: sequesters **Hh** in the presence of a Hh signal



Hh signaling in *Mus musculus*
Complex role of Patched1 (Ptc1)

Ptc1: a "receptor" that plays multiple (confusing) roles in Hh signaling

Both a Hh 'receptor' (binds to Hh to activate signaling), and a negative regulator of the Hh pathway

Ligand-independent antagonism (LIA): inhibits **Smo** in the absence of a Hh signal

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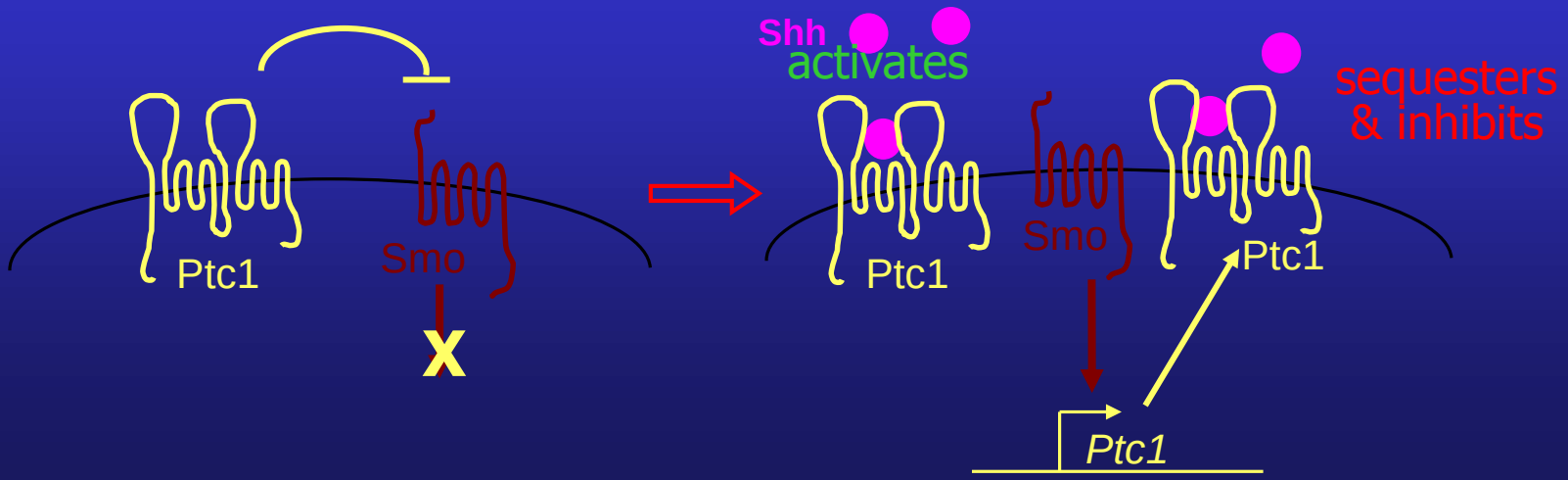
Ligand-dependent antagonist: sequesters **Hh** in the presence of a Hh signal



Hh signaling in *Mus musculus*
Inhibitory role of Hip1

Hip1 (Hedgehog-interacting protein 1) : <u>specific to vertebrates</u>

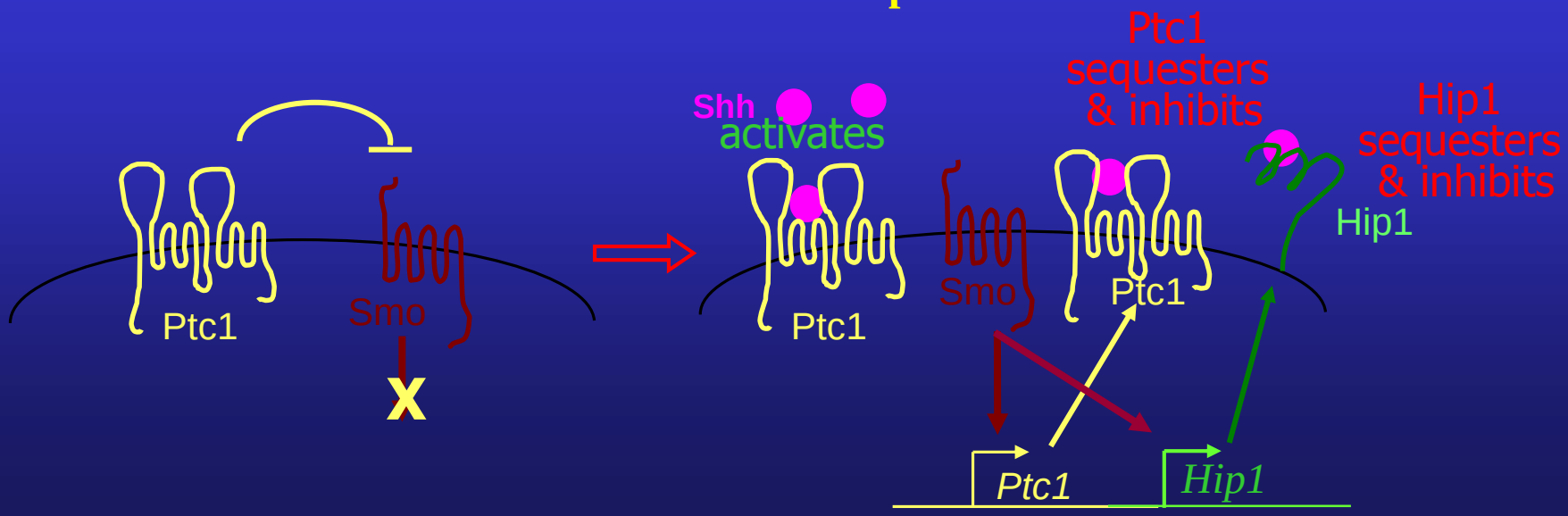
A cell surface protein that binds to all mammalian Hh's

Overexpression/loss-of-function studies: antagonist of Hh signaling

like Ptc1, Hip1 acts to sequester Hh, keeping it from activating the pathway in the cell that expresses it as well as preventing it from traveling to an adjacent cell

Like Ptc1, Hip1 is also a transcriptional target of Hh signaling

Lots and lots and lots of inhibition! – Remember Principle #6!



Question:

How do these two Ligand-Dependent Antagonists affect the gradient of Shh signaling that regulates neural tube patterning in the mouse?

Redundancy of Ptc1 and Hip1 obscures the function of LDA in this system

LDA-specific knock-out of *Ptc1* gives a mild phenotype

Although *Ptc1-/-* (null mutants) die at E8.5 (embryonic day 8.5)

Mt-Ptc1;Ptc1-/- are rescued up to E14.5

(Mt = metallothionine promoter, drives a low level of ubiquitous expression, not responsive to Hh signaling)

Mt-Ptc1 provides low-level expression that is NOT responsive to Shh signaling

Sufficient to inhibit Smo in the absence of a Shh signal

Sufficient to allow for Hh pathway activation in the presence of Shh ligand

BUT **static** low levels do not provide a significant amount of Shh sequestration in the presence of Shh signaling (i.e. no ligand-dependent antagonism)

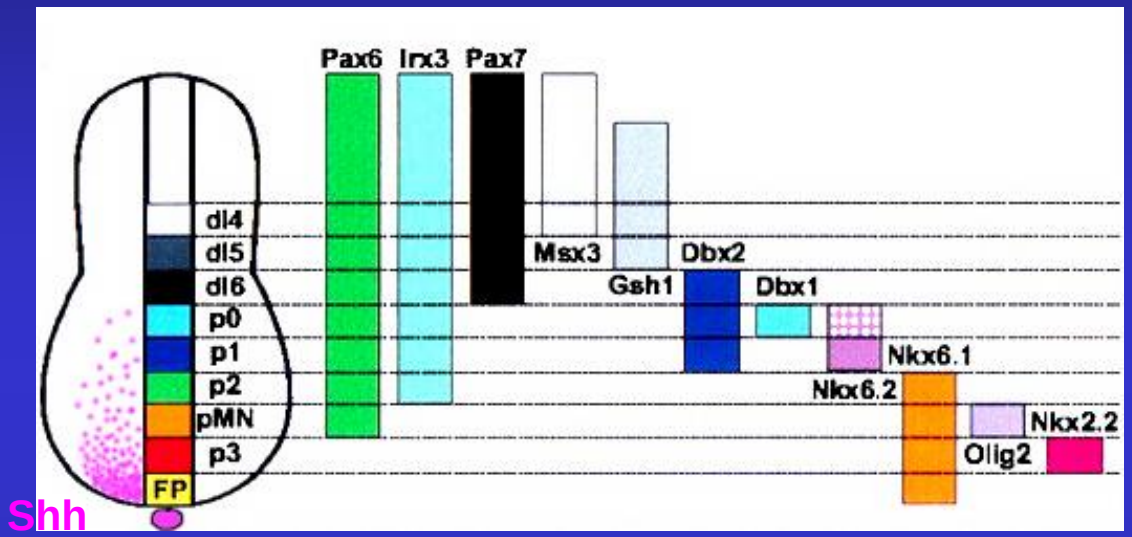
Hip1-/- develop normally up to birth

Hip1-/-;Ptc1-/- double mutants show more severe defects than either *Hip1-/- or Ptc1-/-*

(suggesting redundancy of function between *Hip1* and *Ptc1)*

Complete removal of feedback LDA in

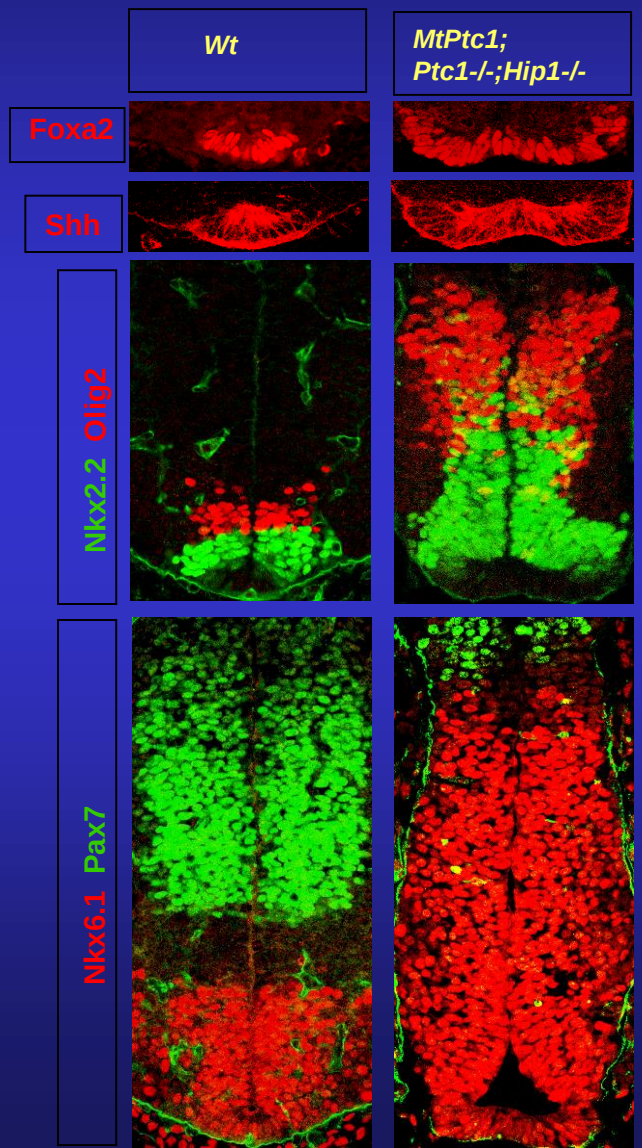
MtPtc1;Ptc1-/-;Hip1-/- mutants provides a model system to examine the Shh gradient in neural tube patterning



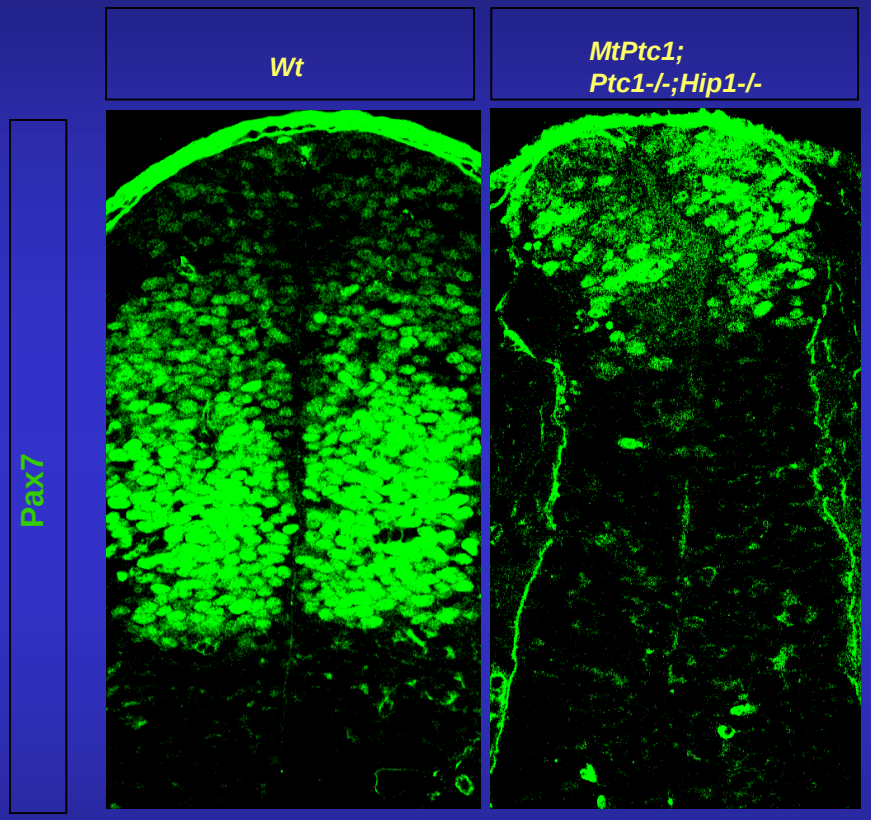
(Persson et al., 2002)

- Shh morphogen gradient along the D-V axis
- Induction or repression of homeodomain proteins with different thresholds
- Refinement of the progenitor domains by cross-repression among homeodomain proteins
- Specification of the neuronal fate based on the “homeodomain protein code”

Expansion of ventral progenitor domains at the expense of intermediate and dorsal domains in *MtPtc1;Ptc1-/-;Hip1-/-*

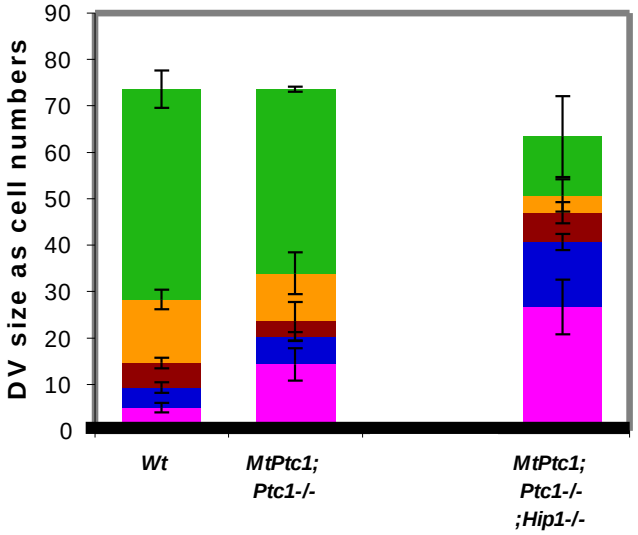


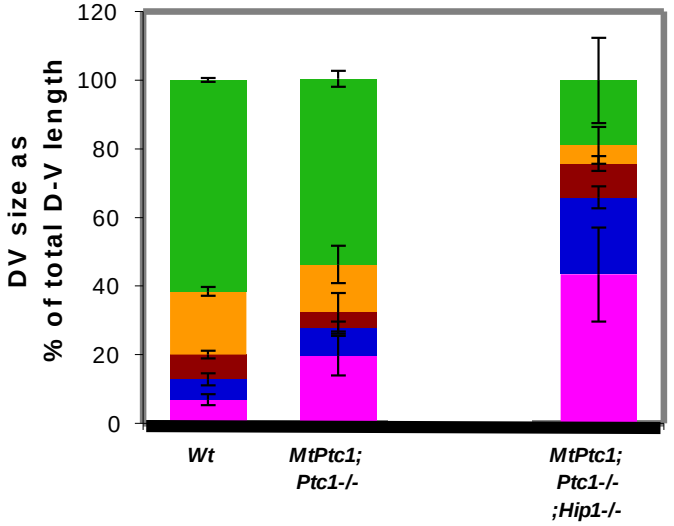
Expansion of ventral domains



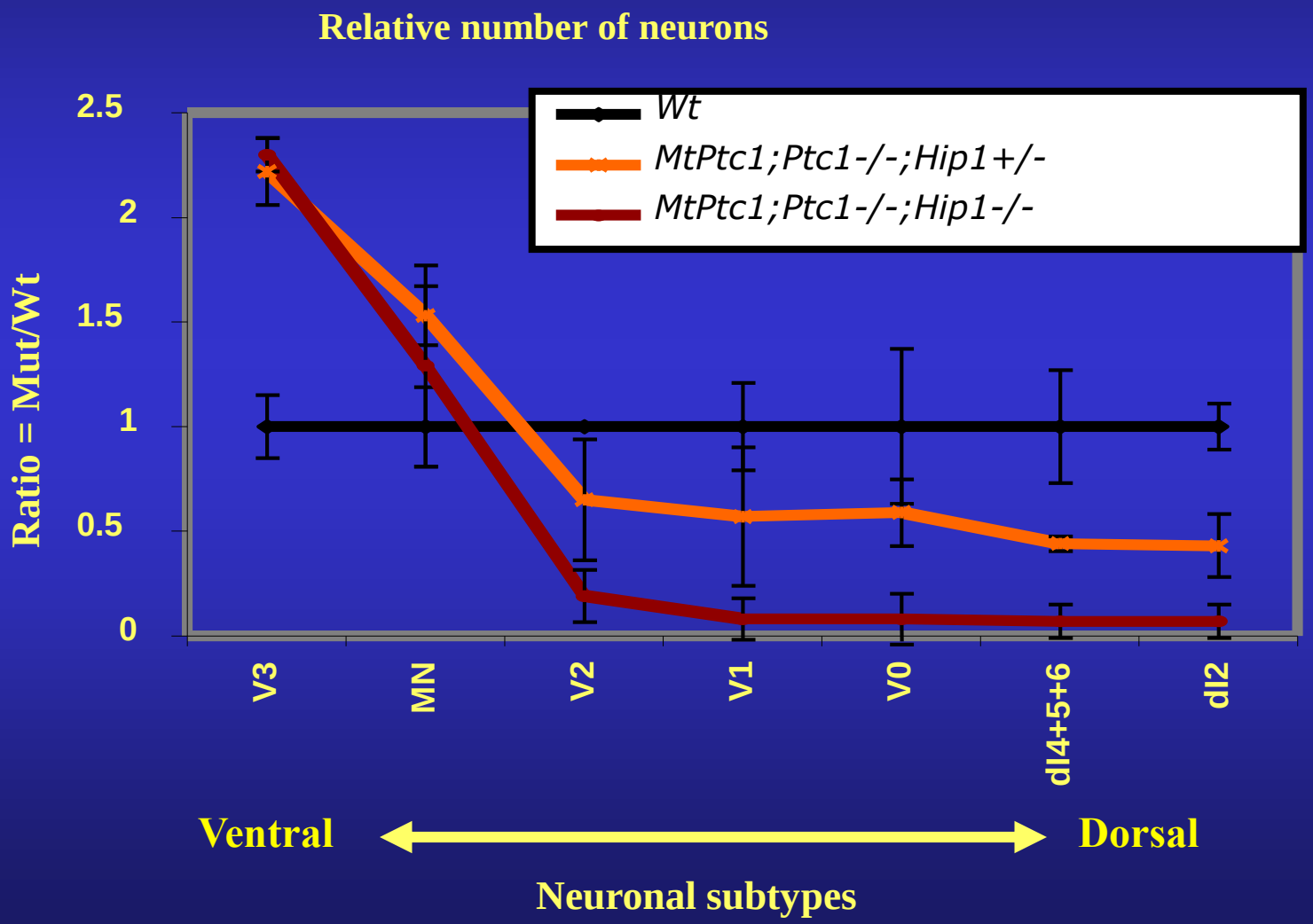
Shrinkage of dorsal domains

Expansion of ventral progenitor domains at the expense of intermediate and dorsal domains in *MtPtc1;Ptc1-/-;Hip1-/-*





Intermediate and dorsal neurons are lost in *MtPtc1;Ptc1-/-;Hip1-/-* spinal cord



Are the Patterning Defects of *MtPtc1;Ptc1-/-;Hip1-/-* neural tube secondary to increased Shh emanating from an expanded floor plate (Shh source)?

Alternate Hypotheses:

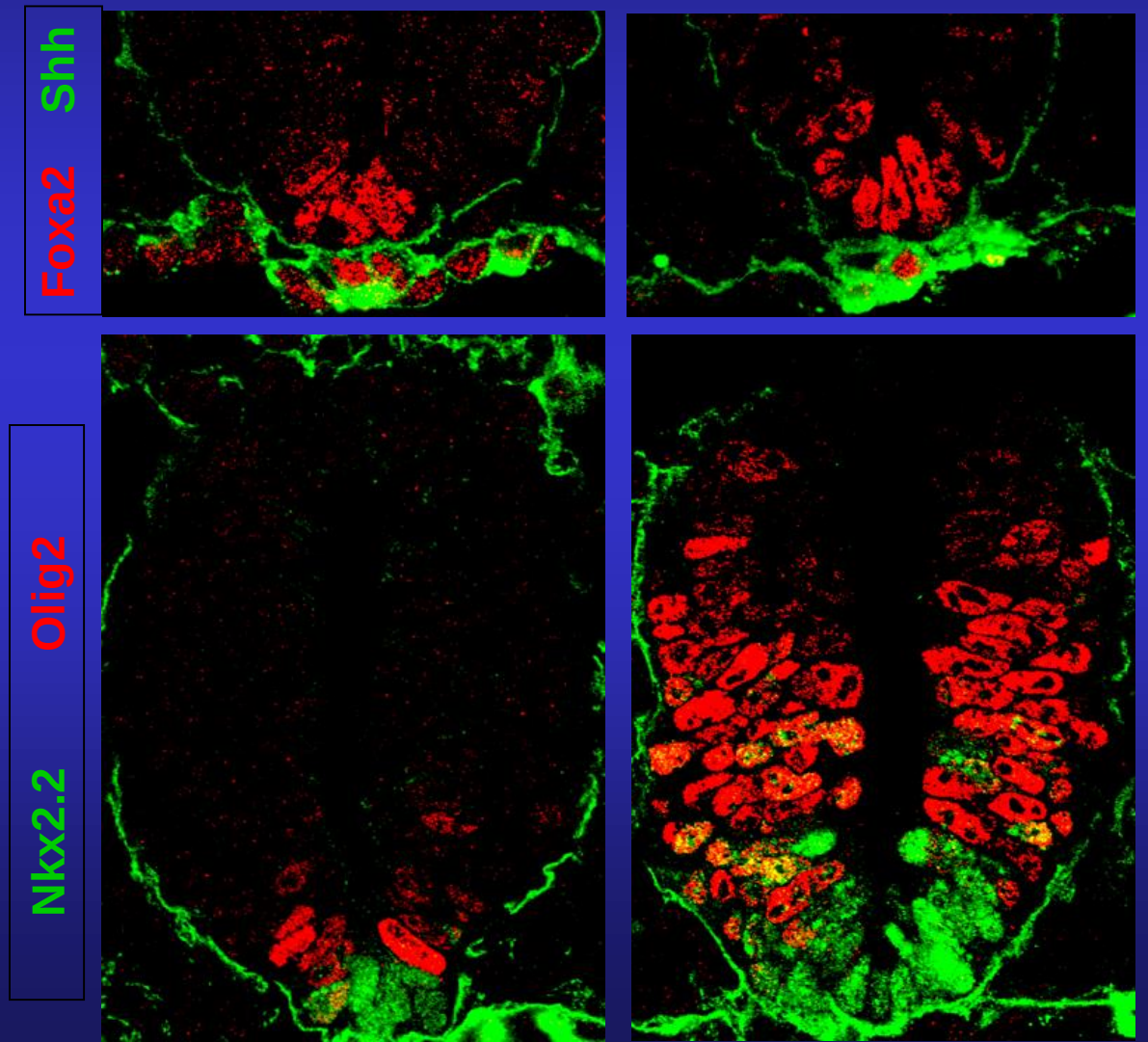
Loss of LDA → increased ligand distribution → expansion of ventral cell types?

Patterning defects of *MtPtc1;Ptc1-/-;Hip1-/-* spinal cord are unlikely to be secondary to an expanded floor plate

Embryonic Day 8.5
(8-9 somites)

At this stage Shh hasn't started to be synthesized in the floorplate, but is only made in the notochord.

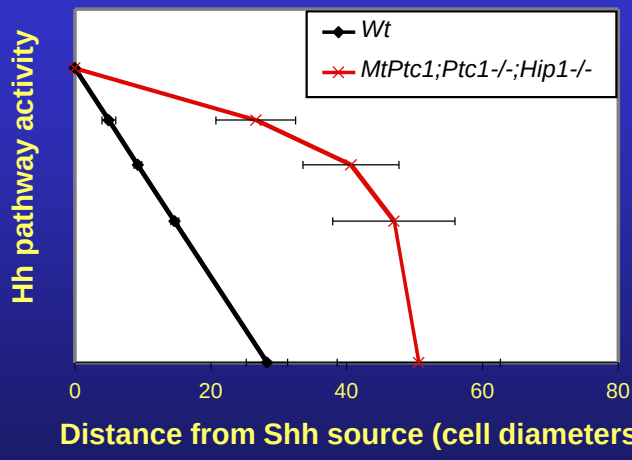
Nonetheless, the ventral neural tube is already expanded, suggesting that Shh made in the notochord is acting at a greater range.



Conclusion (Jeong & McMahon 2004)

Removal of feedback LDA (ligand dependent antagonism) results in severe patterning defects in the spinal cord.

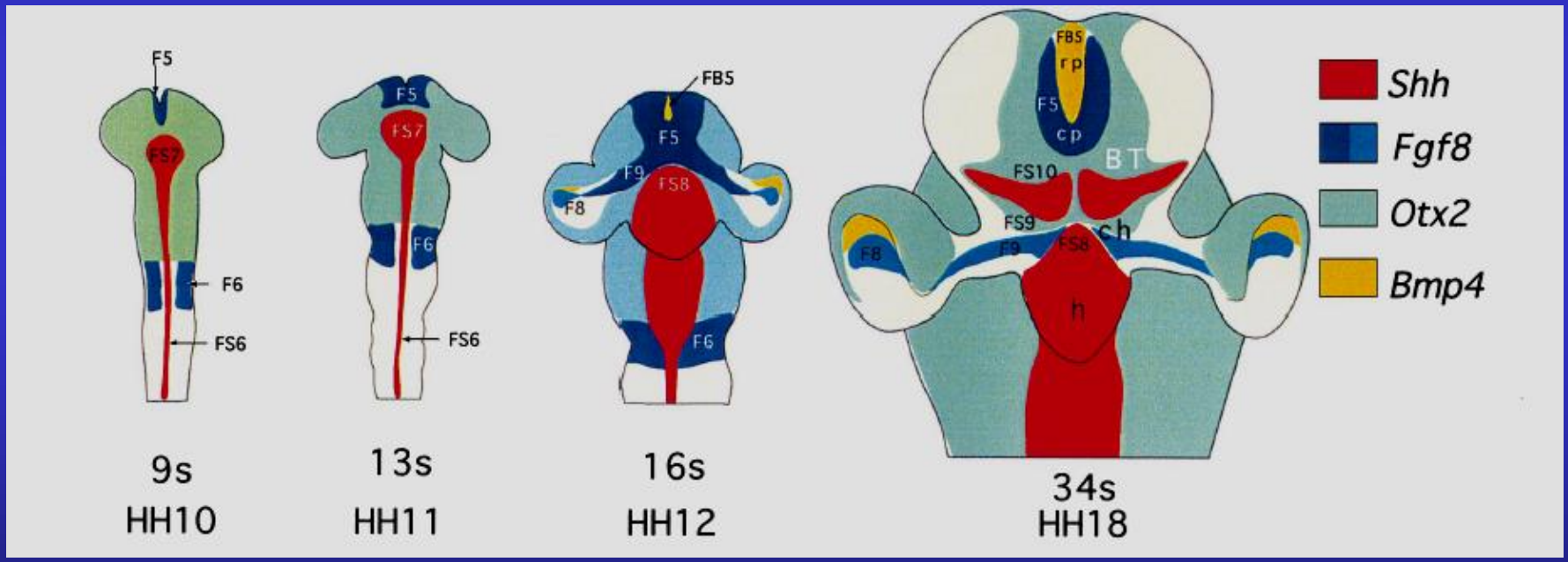
Defects observed are most consistent with the interpretation that during normal development, Ptc1 and Hip1 both make critical contributions to regulating the *intensity* and *spatial range* of Shh signaling.



Finale: “Who cares about the spinal cord, I am only interested in the brain and behavior…”

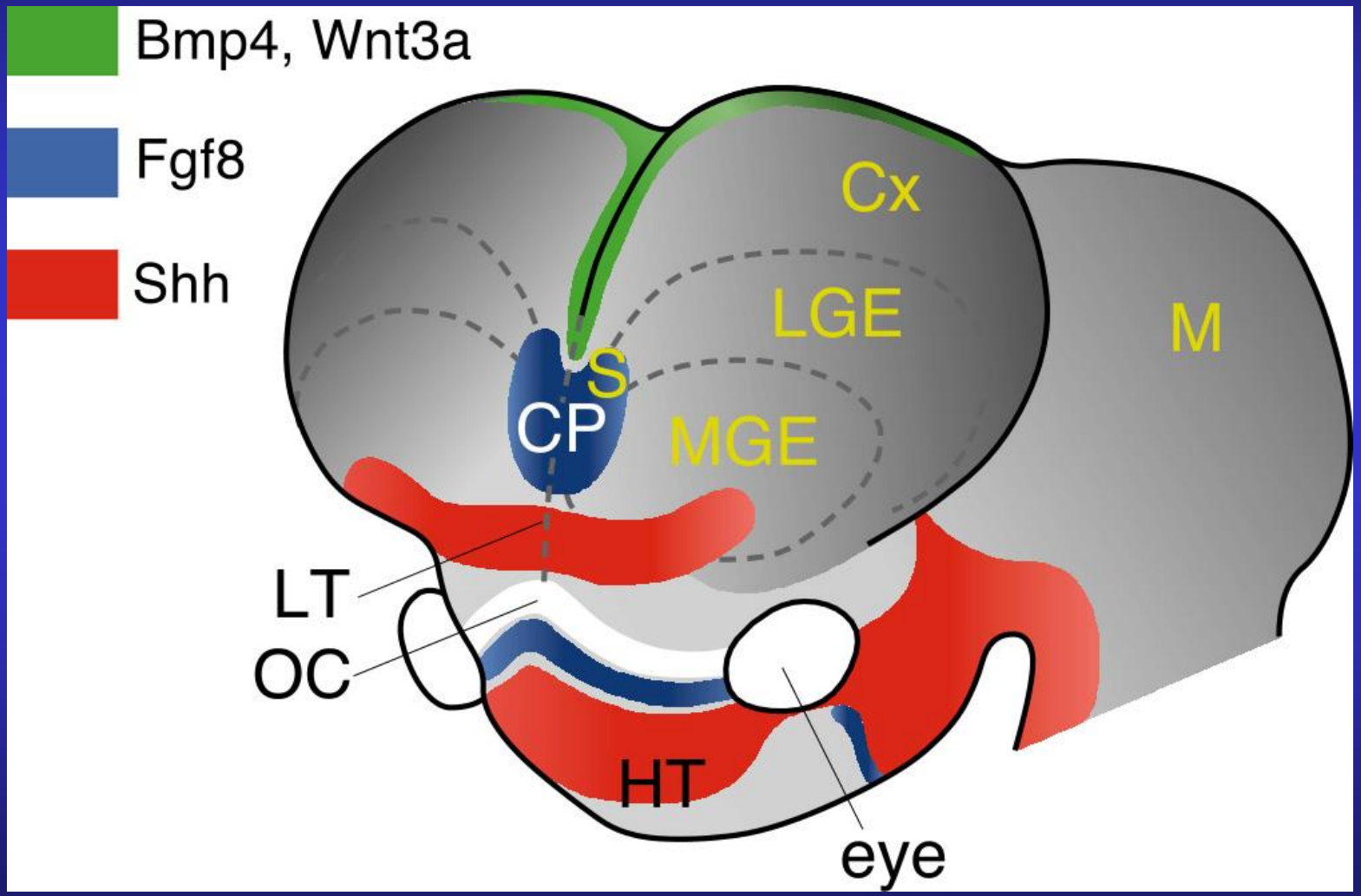
….Fair enough.

Forebrain Patterning Centers
(rostroventral view)

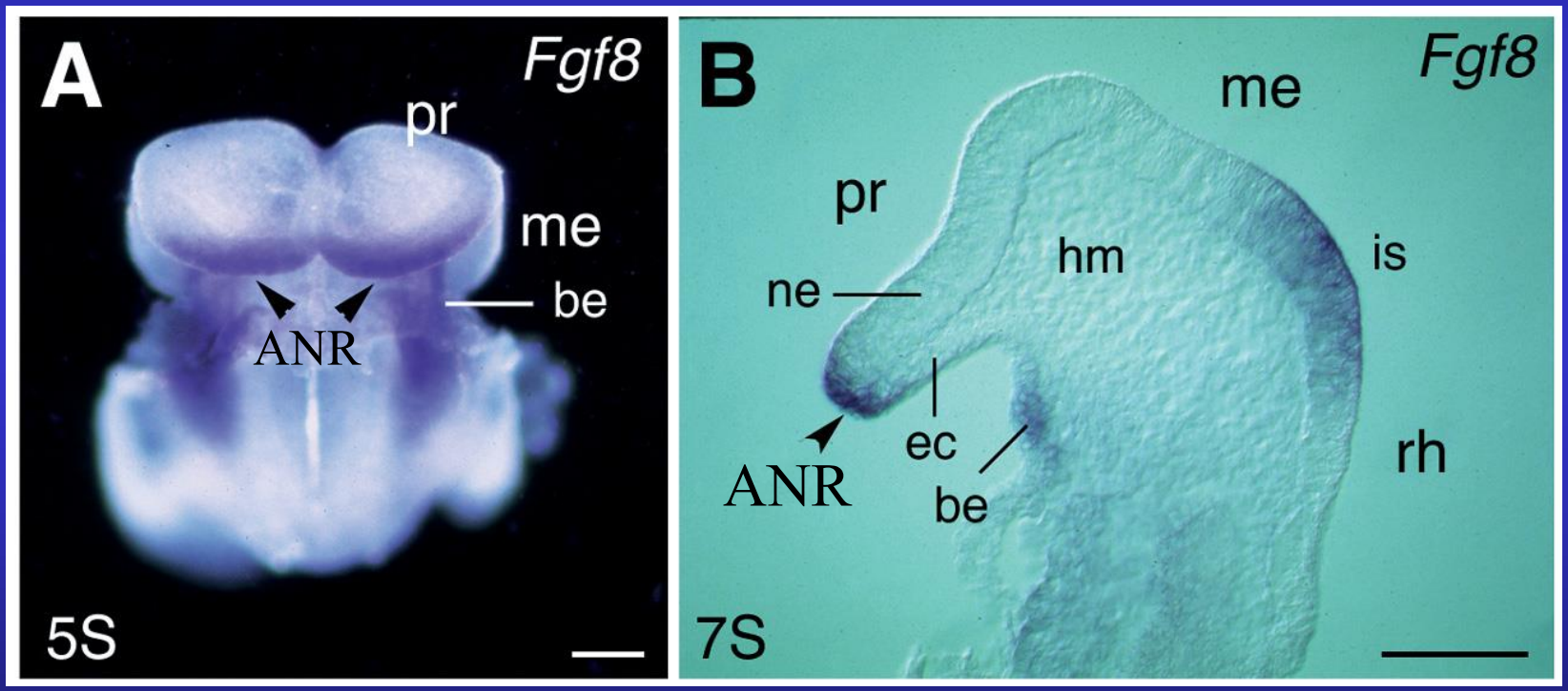


Crossley, Martinez, Ohkubo and Rubenstein, 2001

Patterning Centers in the Forebrain

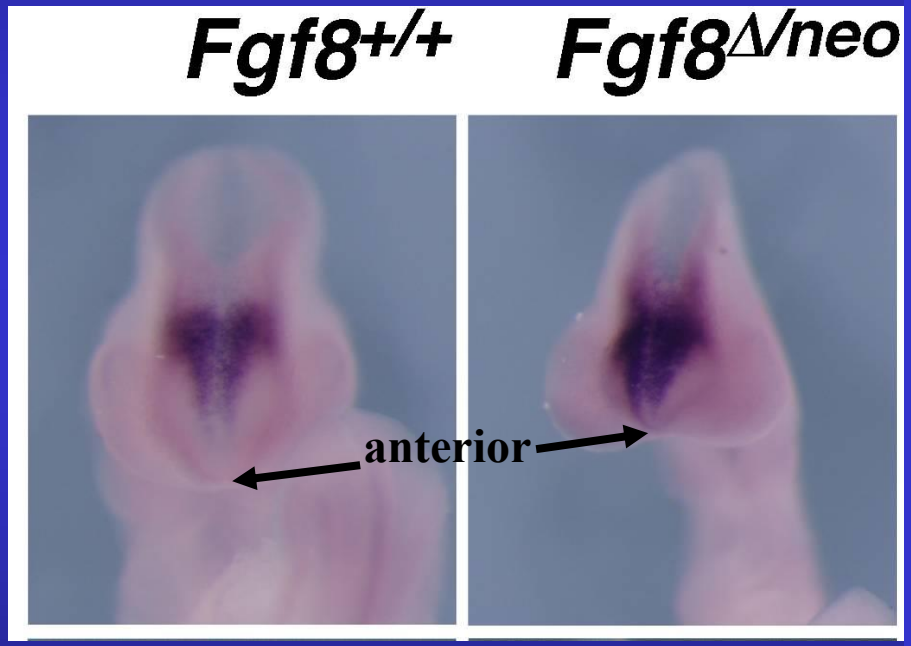


Fgf8 Expression in the Mouse Anterior Neural Ridge (ANR)



Shimamura and Rubenstein, 1997

Wnt8b Expression Expands Rostrally in response to reduced Fgf8 signaling

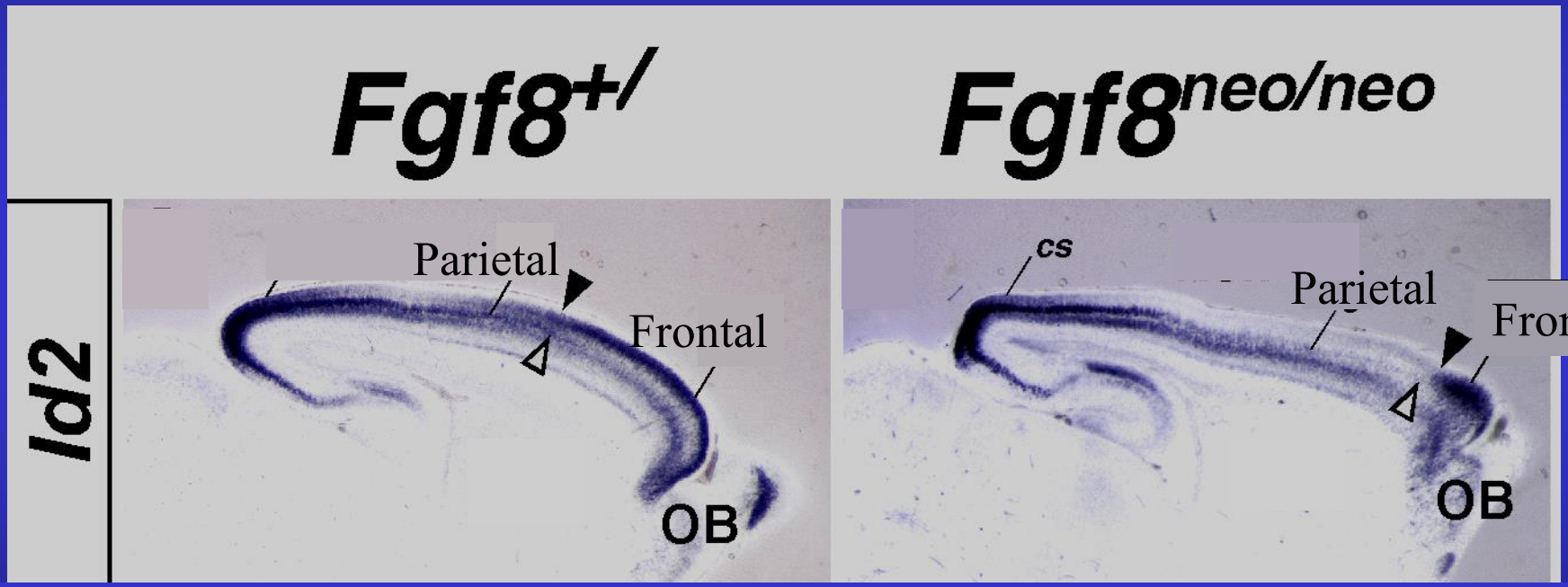


Dynamic/reciprocal interactions between secreted signaling molecules and resulting patterning

Storm et al., 2006

Hypoplasia of the Frontal Neocortex

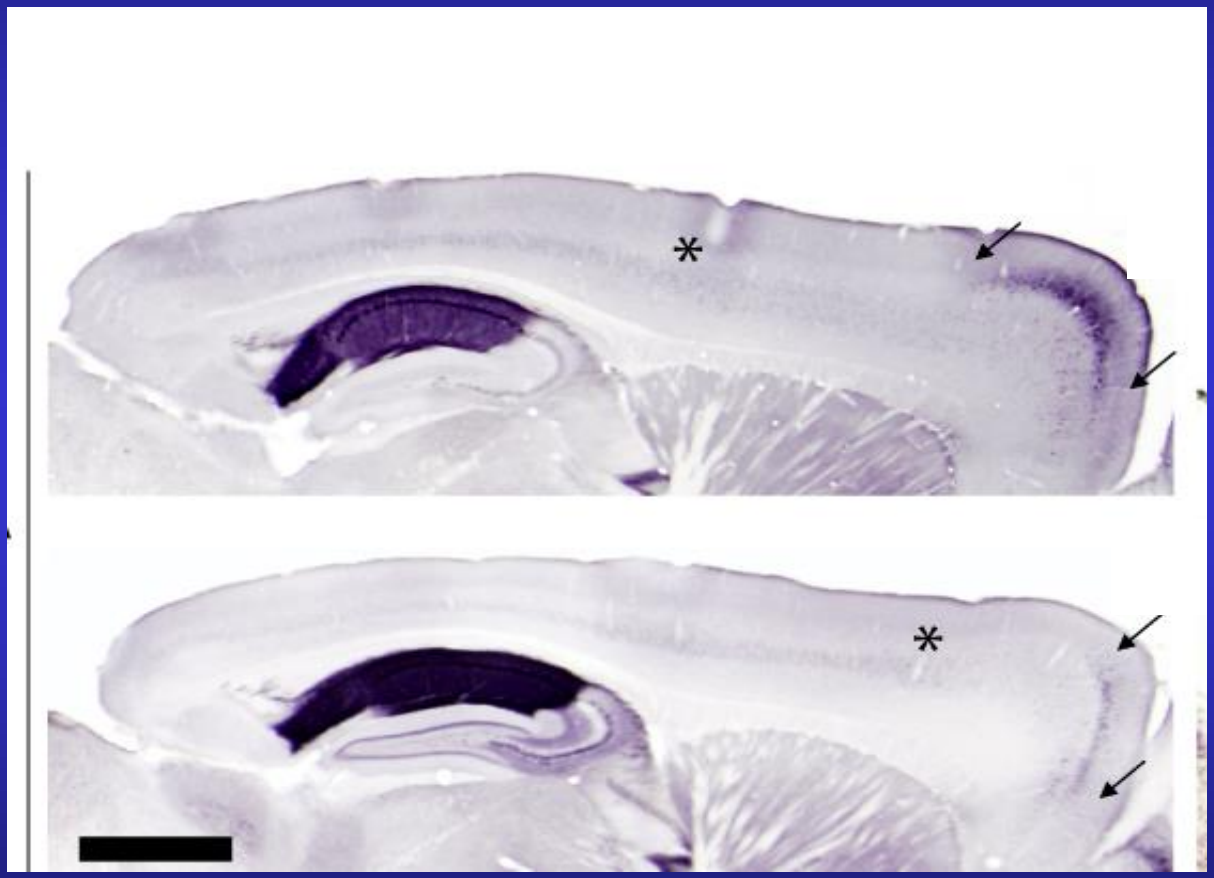
Rostral Expansion of the Parietal Neocortex



E18.5

Garel et al., 2003

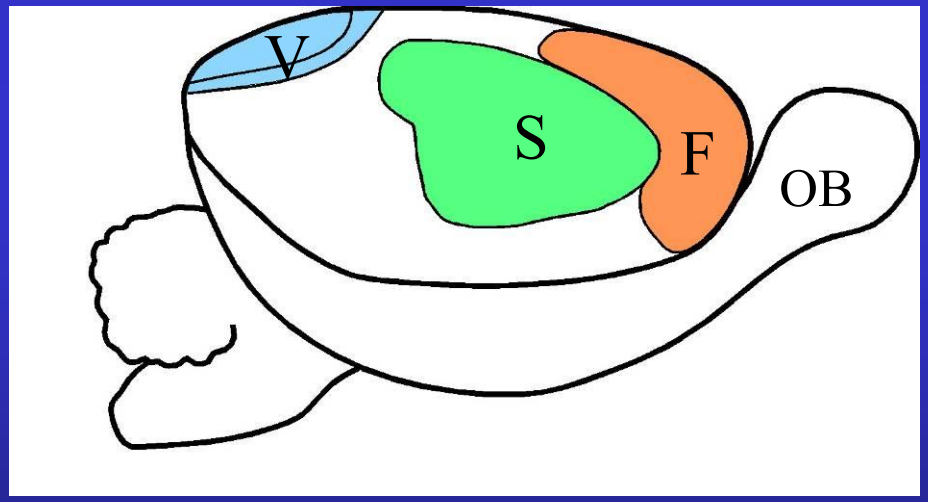
Reduced Frontal Cortex in Adult Fgf17 mutants



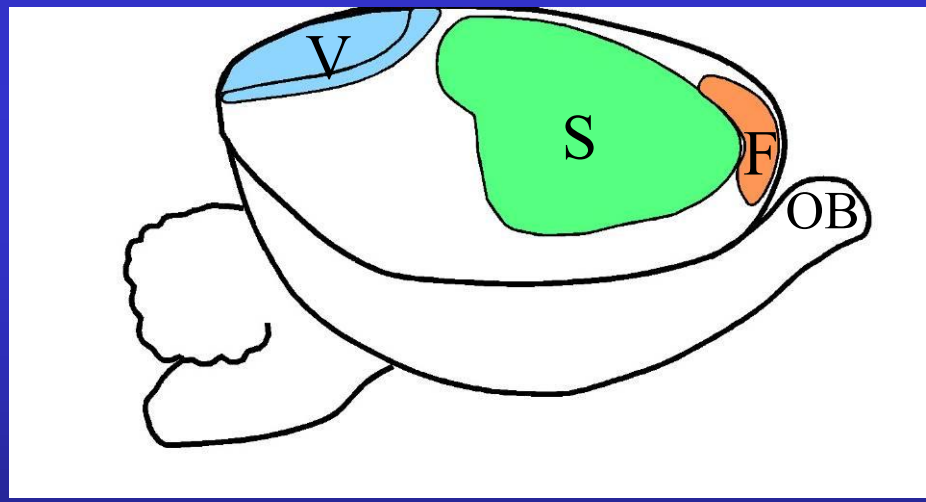
Cholfin and Rubenstein, *2007*

Hypoplasia of the Frontal Cortex Due to Reduced Fgf Signaling

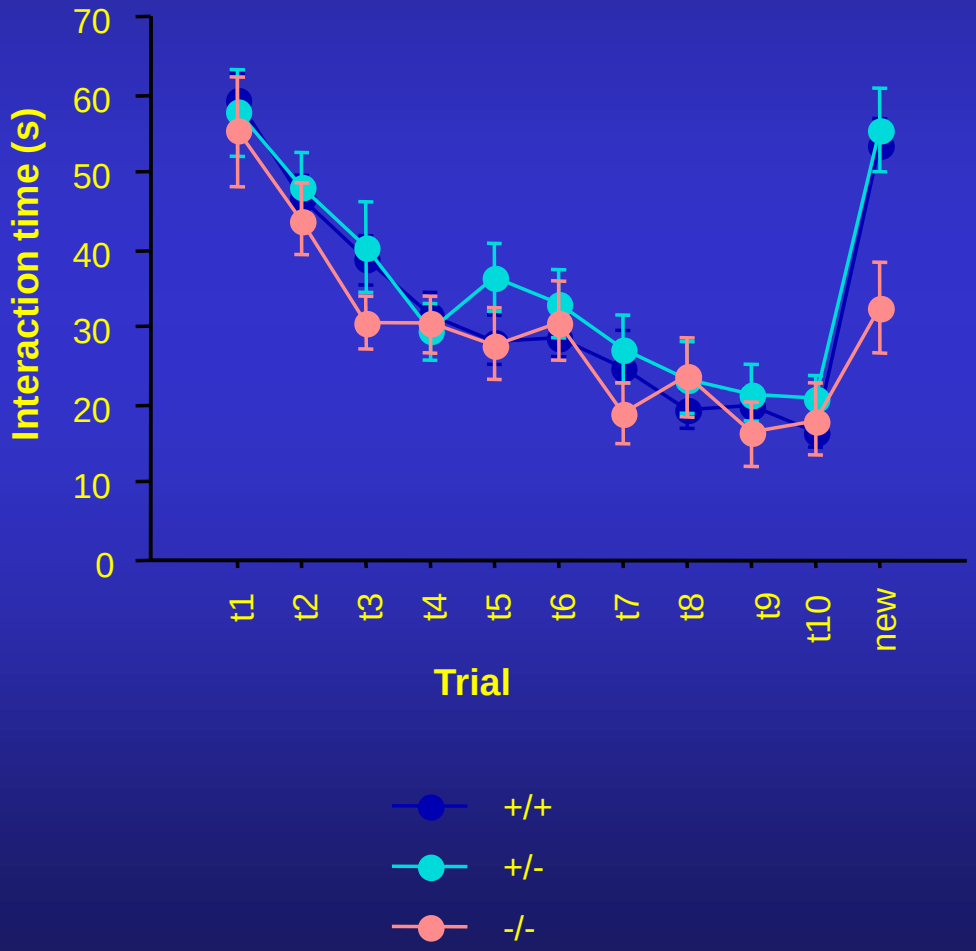
Normal

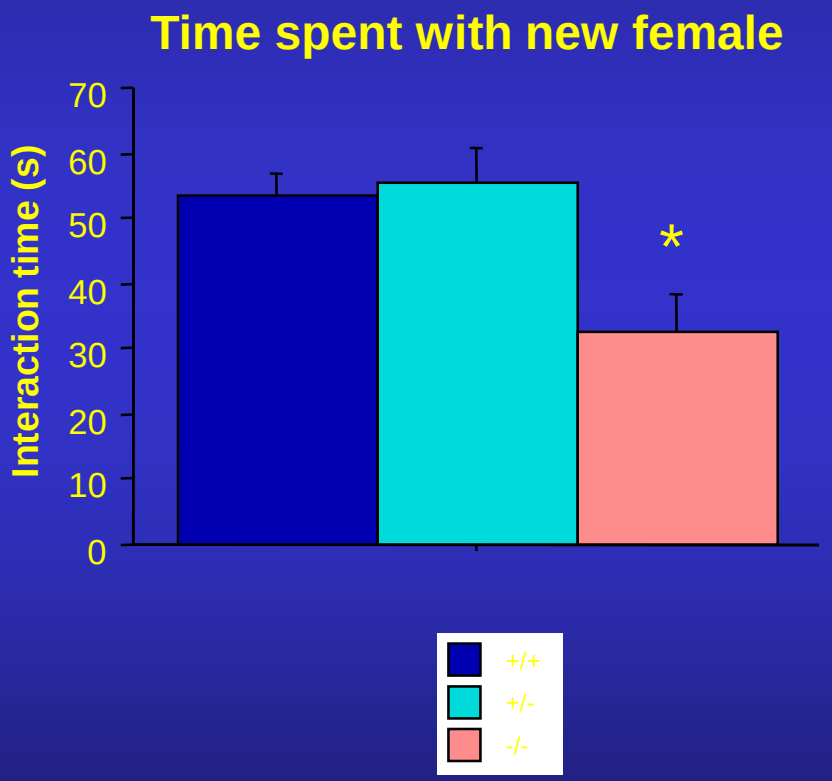


Fgf8$^{neo/neo}$ *or Fgf17*^{KO}



Behavioral Implications:
Social Recognition Test is abnormal in Fgf mutant animals

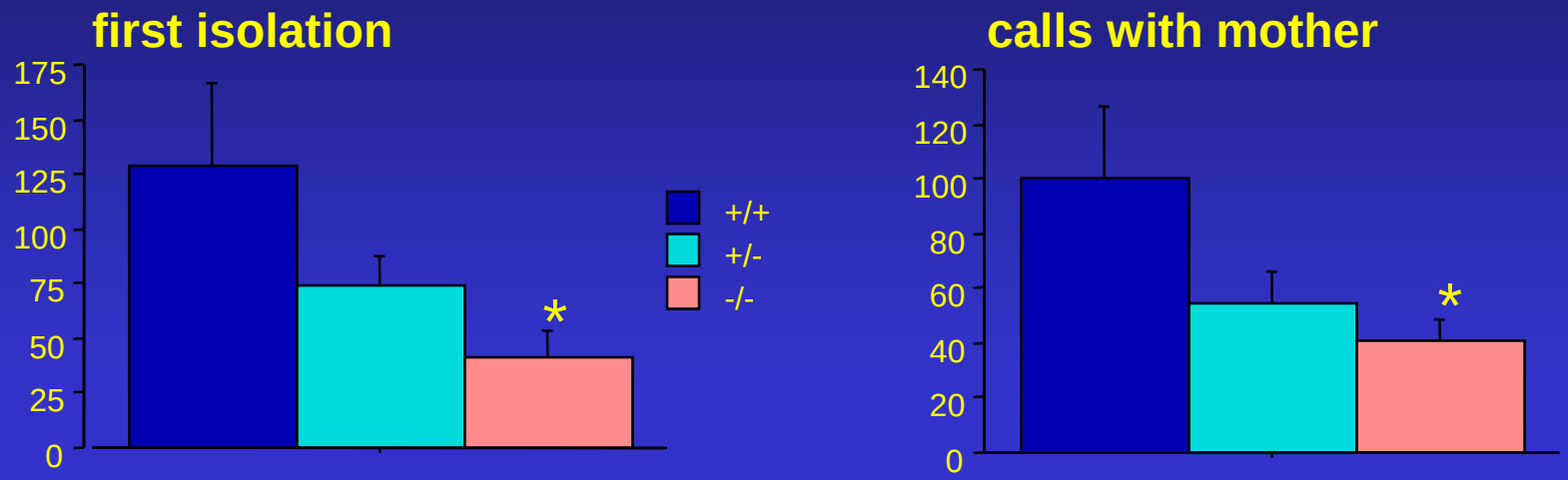




*: p < .05 vs. +/+ and +/-

Scearce-Levie et al, 2007

Behavioral Implications:
Ultrasonic Vocalization in Fgf mutant Pups

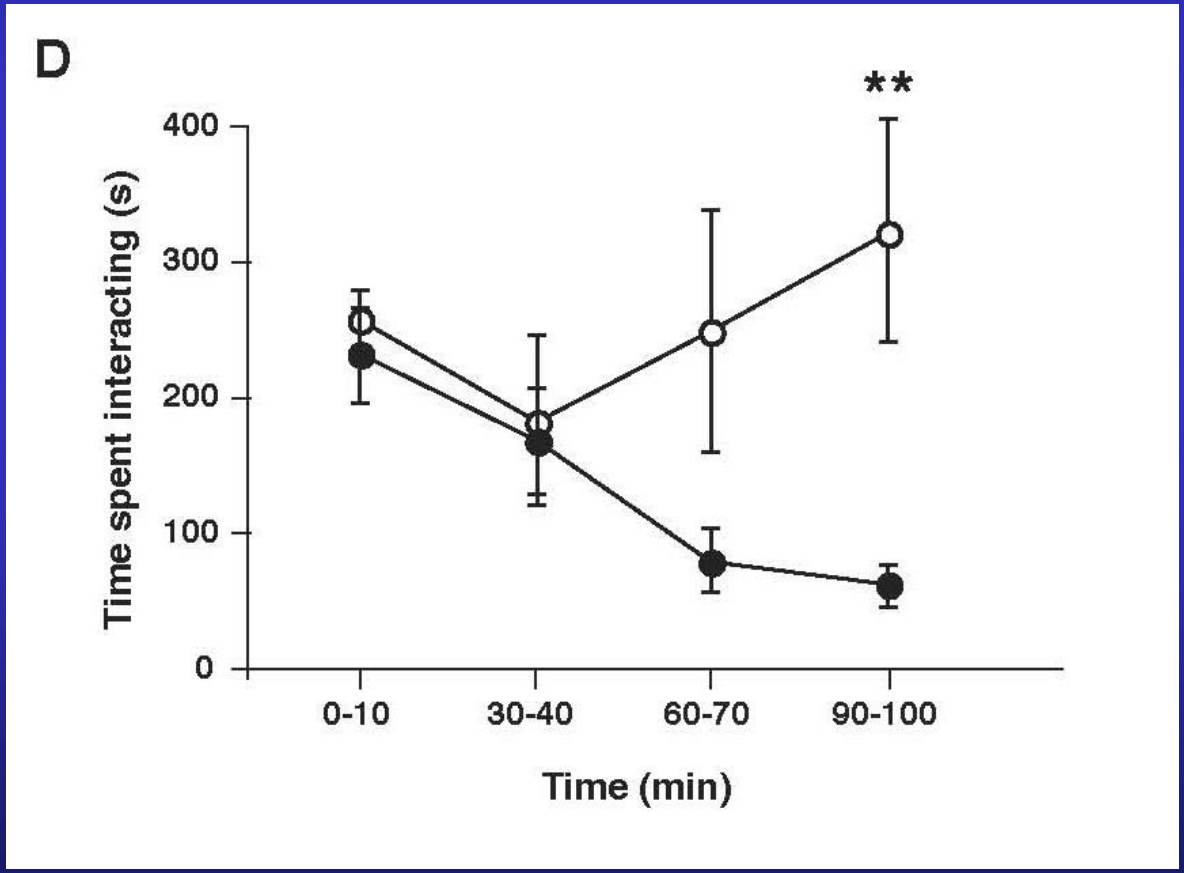


Fgf mutant pups “talk” (call) less to their mothers…

Scearce-Levie et al, 2007

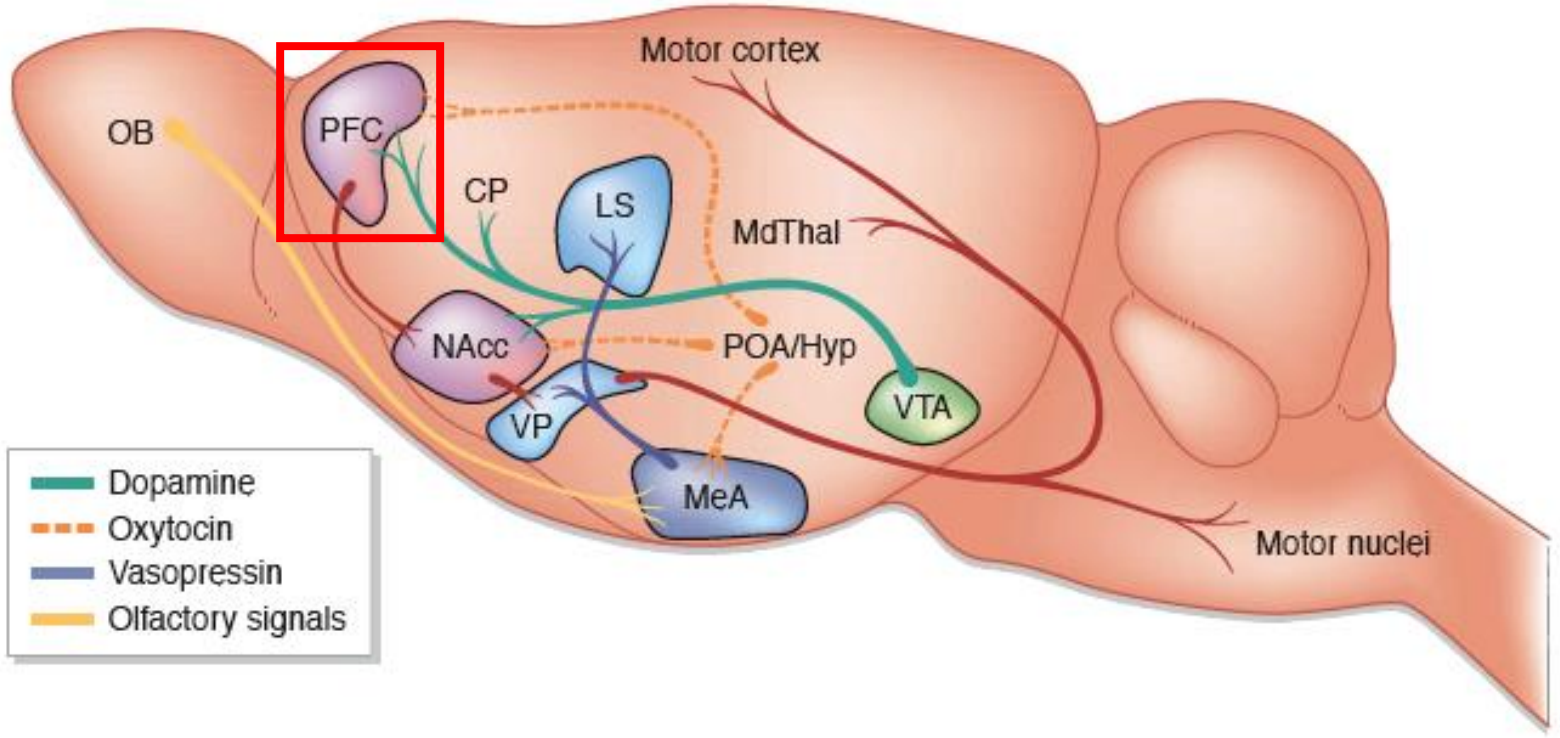
Behavioral Implications:

FGF17 Mutants (black circles) Spend Less Time Socially Interacting Than Wild Type Litter Mates (white circles)

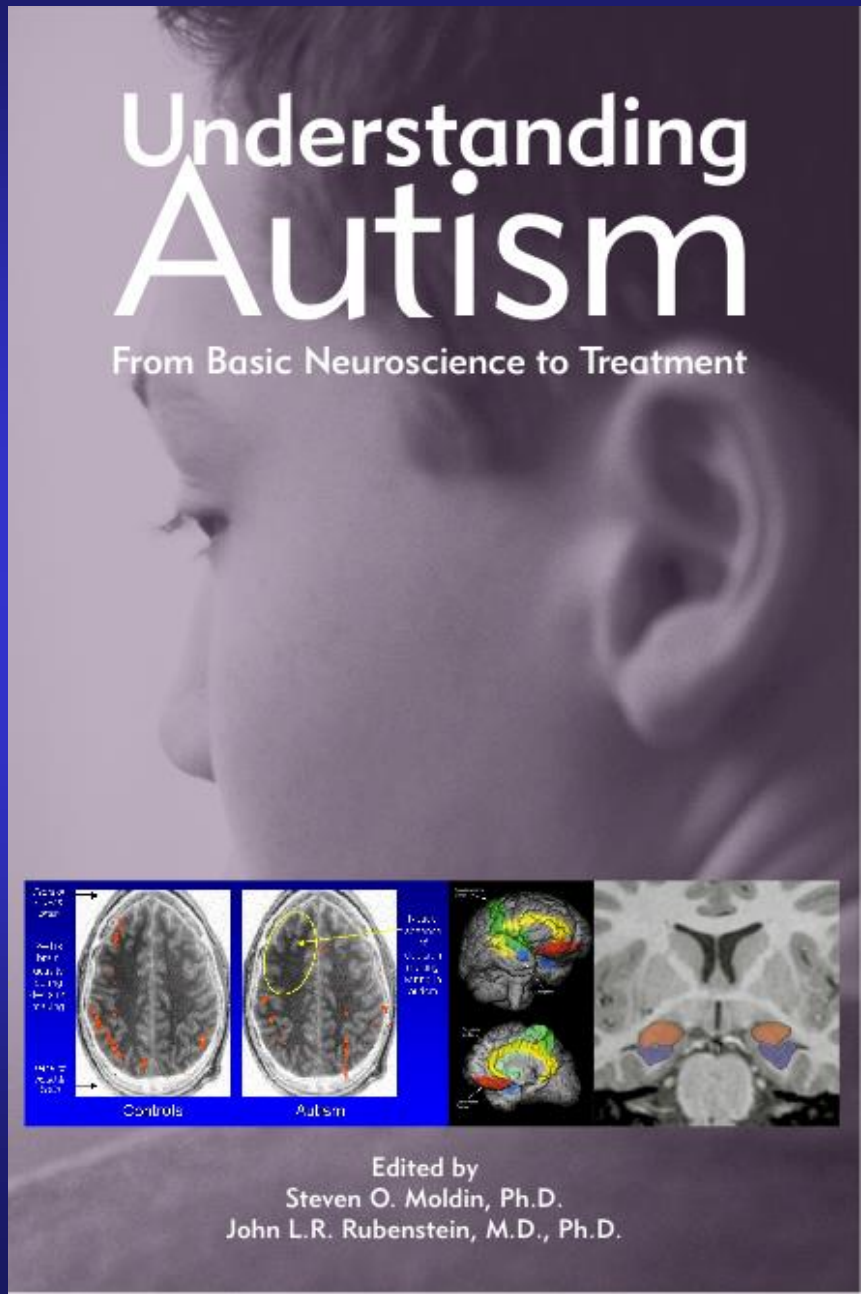


Lennart Mucke, Eric Roberson, Kim Scearce-Levie, Jeremy Cholfin, John Rubenstein

Circuitry Implicated in Social Interactions in Rodents: from Young and Wang



A major justification for John Rubenstein's work on secreted signaling centers and for my lab's work on neurodevelopmental signaling is its potential relevance to psychiatric disorders such as autism



And Bipolar Disorder (Cheyette lab recent press)

Cheyette Lab: Recent press about our work:

SCIENCE AAAS (online news)

New clues to how lithium soothes the bipolar brain may shed light on other mental illnesses

http://www.sciencemag.org/news/2016/10/new-clues-how-lithium-soothes-bipolar-brain-may-shed-light-other-mental-illnesses/

A dog and his ligand…