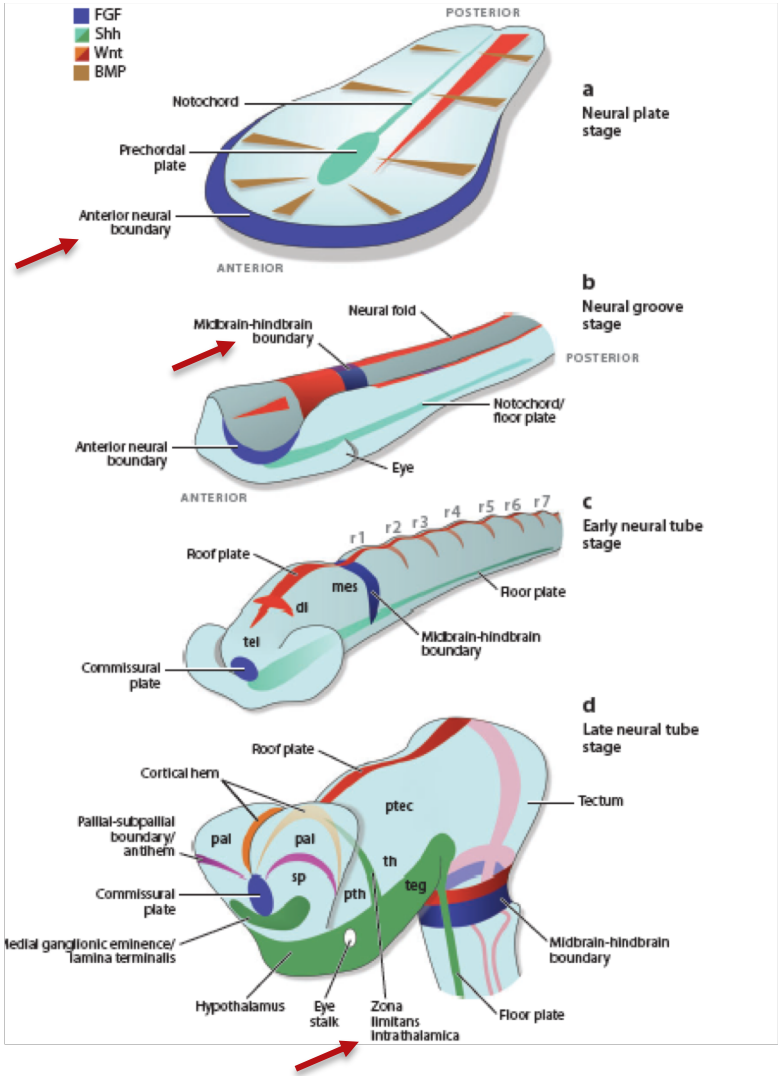
Antero-posterior patterning
&
secondary organizers



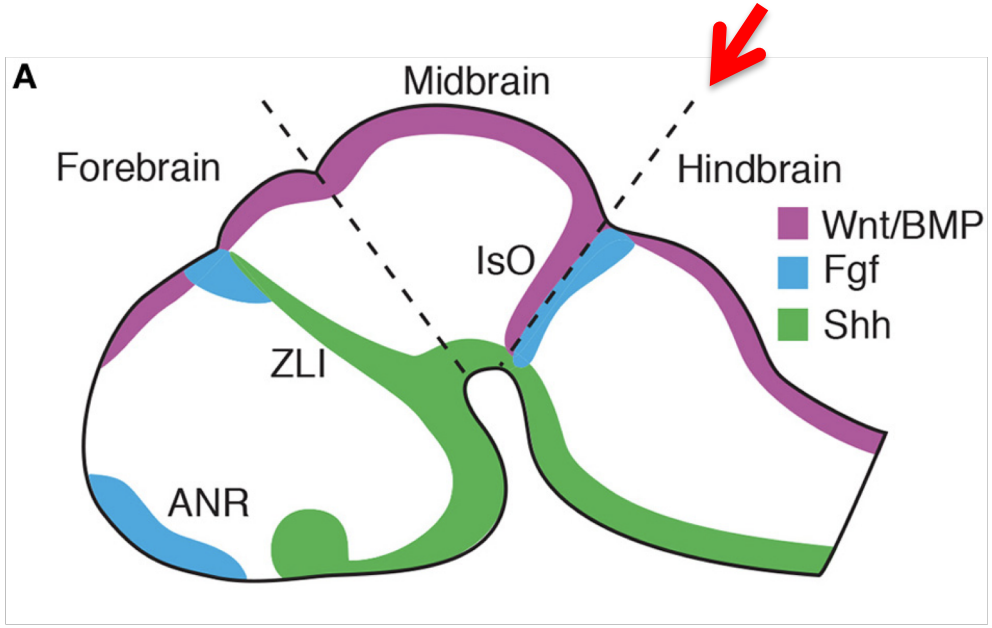
Secondary organizing centers *are established gradually within a given field* ***at the junction between territories*** *that acquire different positional identities*

Three regions in the neural plate and tube have been identified as putative secondary organizers:

ANR
The Anterior Neural Ridge (Boundary)
Commissural Plate

ZLI
Zona limitans intrathalamica

MHB (IsO)
Midbrain-hindbrain boudary

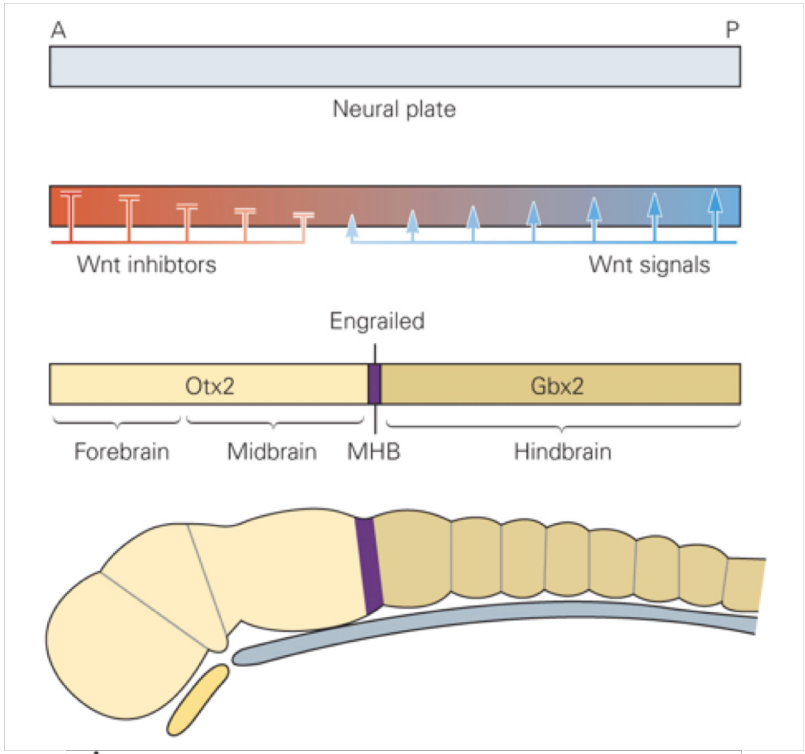


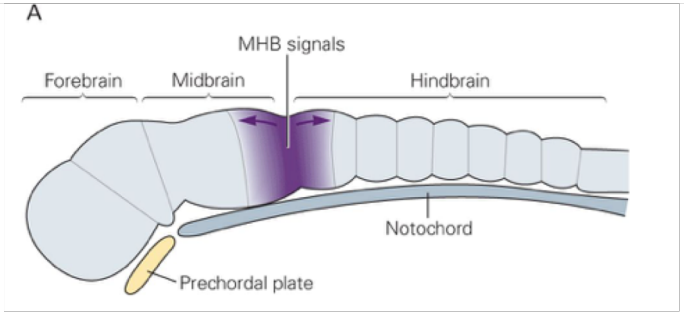
MHB (IsO)

The Midbrain-Hindbrain Boundary or Isthmic organizer

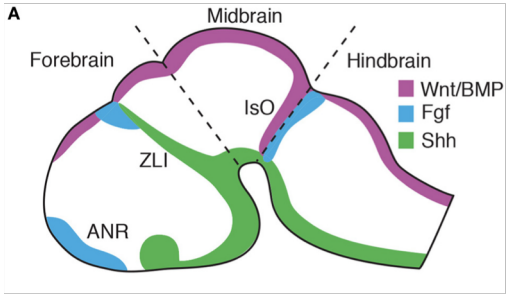
Develops at the mes/met boundary, and co-localizes with a morphological constriction of the neural tube called the **isthmic constriction**

Regulates development of the **mesencephalon** and **metencephalon**





- The **MHB/IsO** emerges after neural tube closure
- Wnt signaling establishes the initial A-P polarity of the neural plate
- The earliest molecular event for the IsO specification is the differential expression in the neural plate of ***Otx2*** in the rostral neuroepithelium and a ***Gbx2*** in the posterior domain
- The MHB expresses **Engrailed** (**En**)
- The MHB is source of **secreted signals** (**Wnt1 and FGFs**) that pattern the midbrain and hindrain and specify neural subtypes



- IsO activity has been first identified in avian embryos - has been found in **all vertebrate species** that have been studied and it is regarded as a **prototypical local organizer** of the embryonic brain

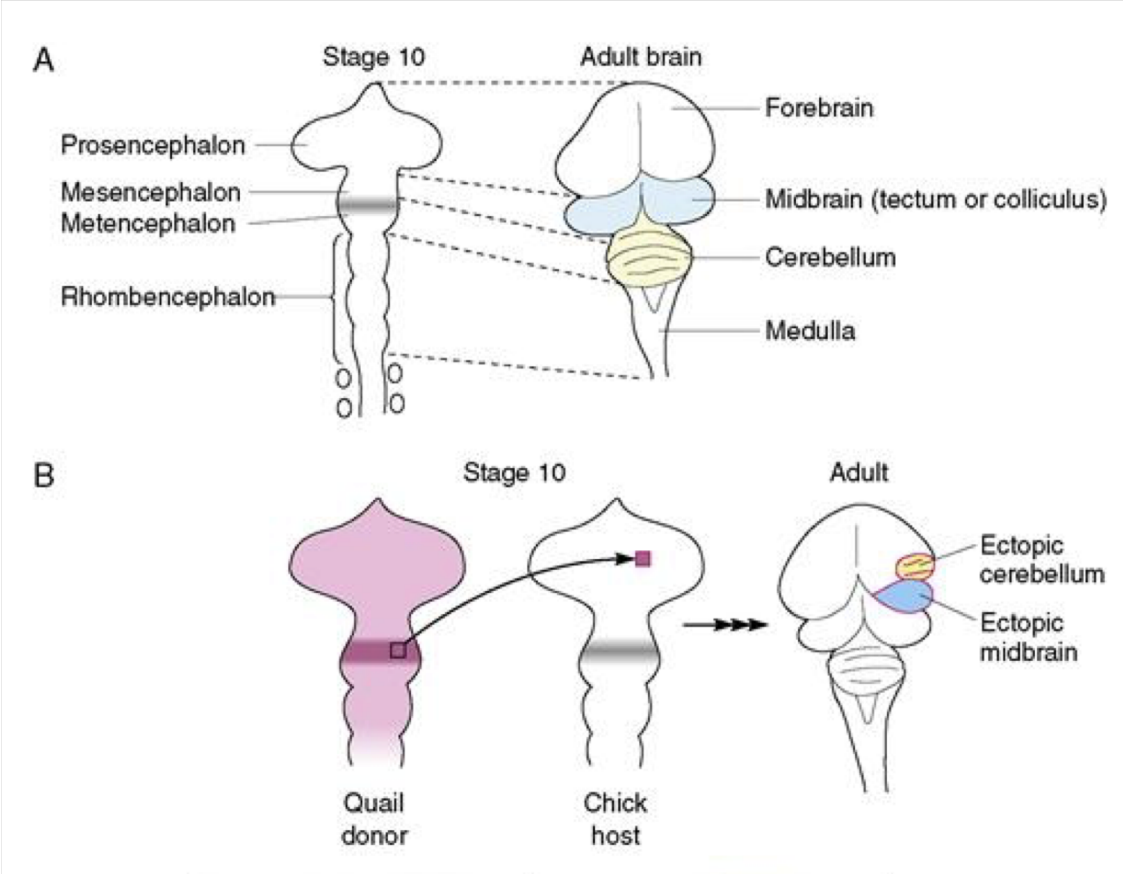
Is the IsO necessary/sufficient for the development of the mes-metencephalic domain?

Which experimental approaches for the functional identification of the IsO?

1. IsO ectopic transplantation

Transplantation of the isthmus to ectopic neural locations → *induction of a mes-metencephalic fate in the host territories that contacted the graft*

→ *Notably the induction of mes-metencephalic tissue was always* ***polarized*** *(*the caudal side of the induced mesencephalon and the rostral side of the induced metencephalon were in contact with the isthmic graft)



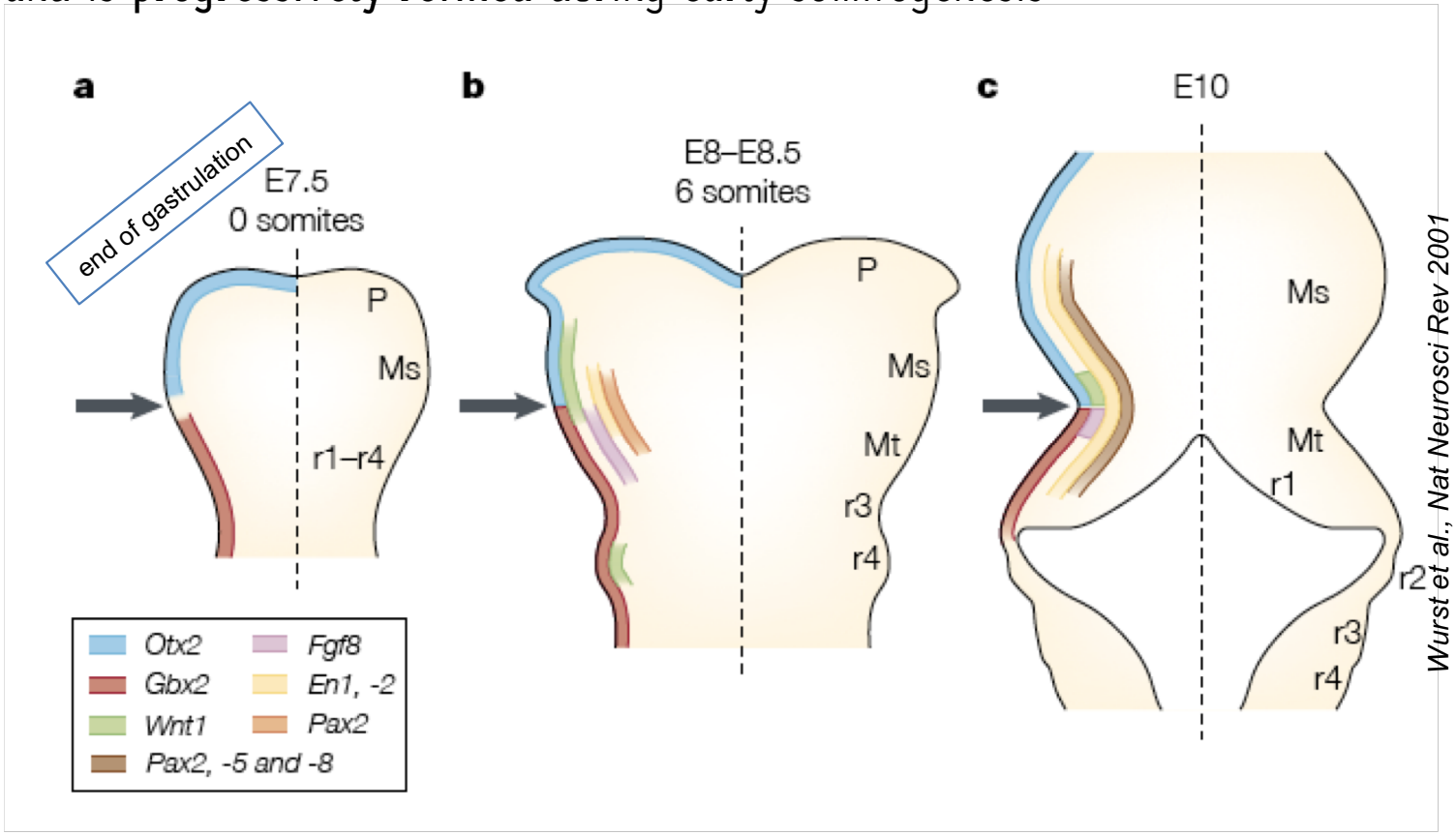
2. IsO removal

Complete removal of the isthmus triggers the loss of the entire mesencephalon and metencephalon

Gene expression at the mid-hindbrain junction (mouse)

the boundary between the midbrain and hindbrain is roughly positioned during late gastrulation and is **progressively refined** during early somitogenesis

The caudal limit of *Otx2* expression and the rostral limit of *Gbx2* mark the mid-hindbrain molecular boundary



Wurst et al., Nat Neurosci Rev 2001

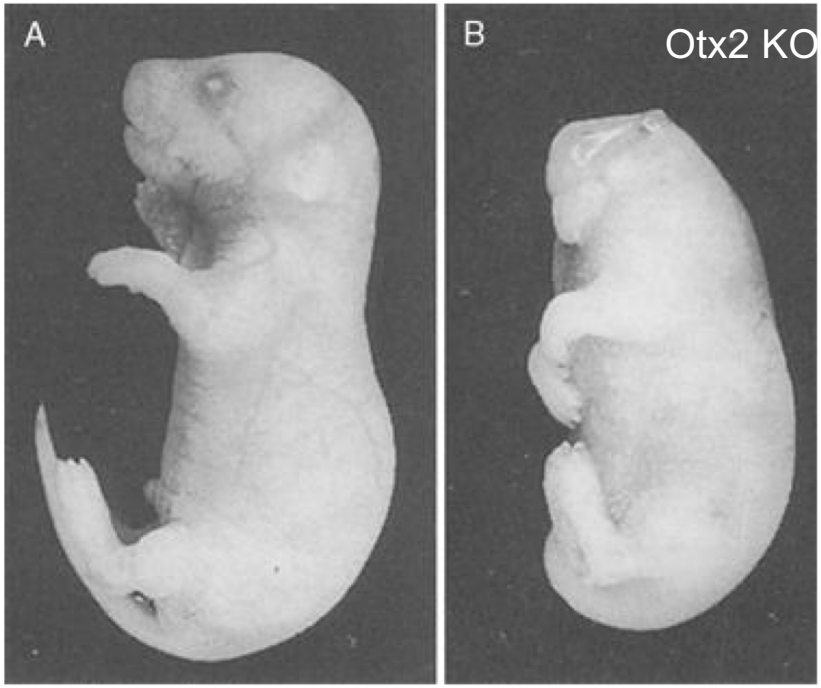
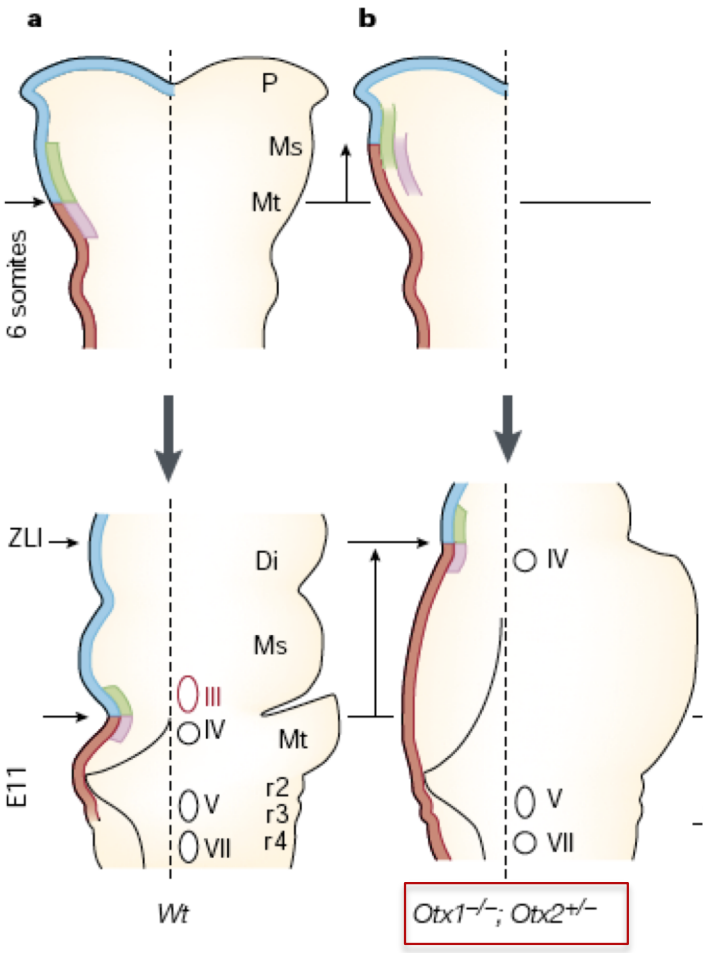


Fig. 2.12 *Otx2* is required for the formation of the mouse head. A dramatic illustration of the importance of the *otx2* gene is the development of the mouse forebrain and rostral head. If the gene is deleted using homologous recombination, embryos without either allele of the gene fail to develop brain regions rostral to rhombomere 3, a condition known as anencephaly. Since many of the bones and muscles of the head are derived from the neural crest, which also fails to form in these animals, the animals lack most of the head in addition to the loss of the brain.From Matsuo et al., 1995

Otx2-/+;Otx1-/- or Otx2-/+;Otx1-/+



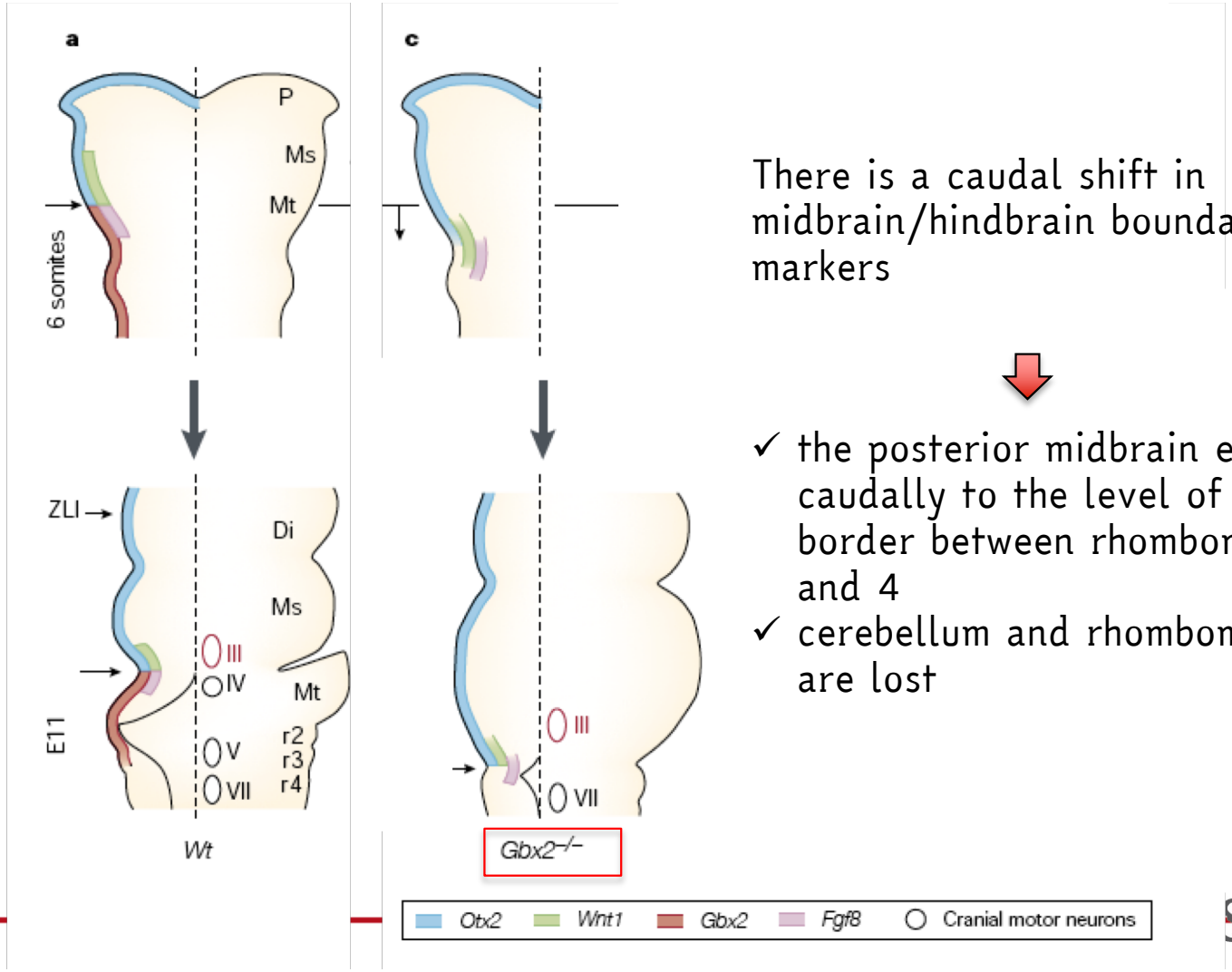
the IsO is shifted anteriorly below a critical threshold of Otx2 function

lack a mesencephalon and extension of metencephalic tissue (giant cerebellum)

Otx2 Wnt1 Gbx2 Fgf8 Cranial motor neurons

inito

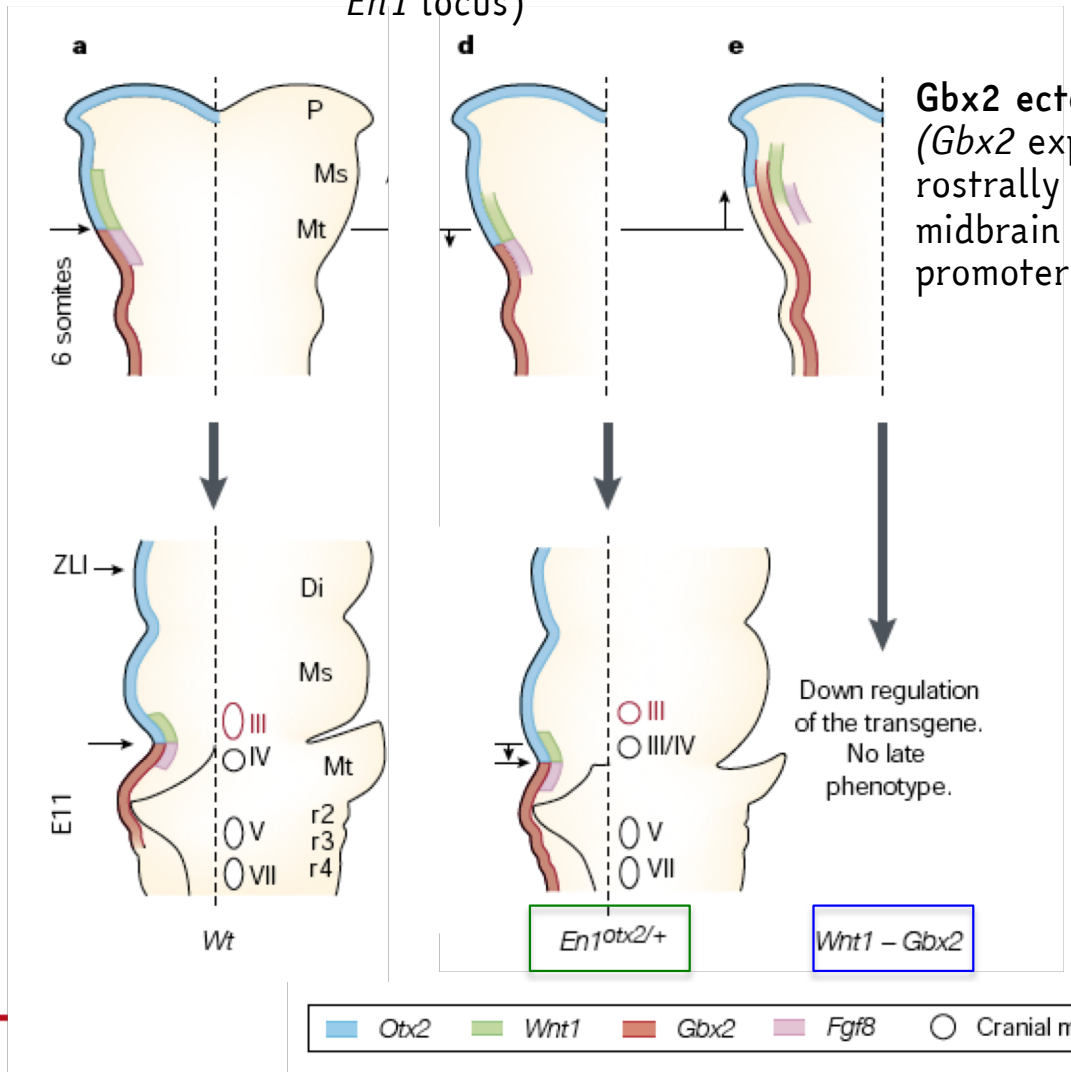
Gbx2-/- mutants:



There is a caudal shift in midbrain/hindbrain boundary markers

- ✓ the posterior midbrain extends caudally to the level of the border between rhombomeres 3 and 4
- ✓ cerebellum and rhombomeres 1-3 are lost

Otx2 ectopic expression
(knock-in *Otx2* gene into the *En1* locus)



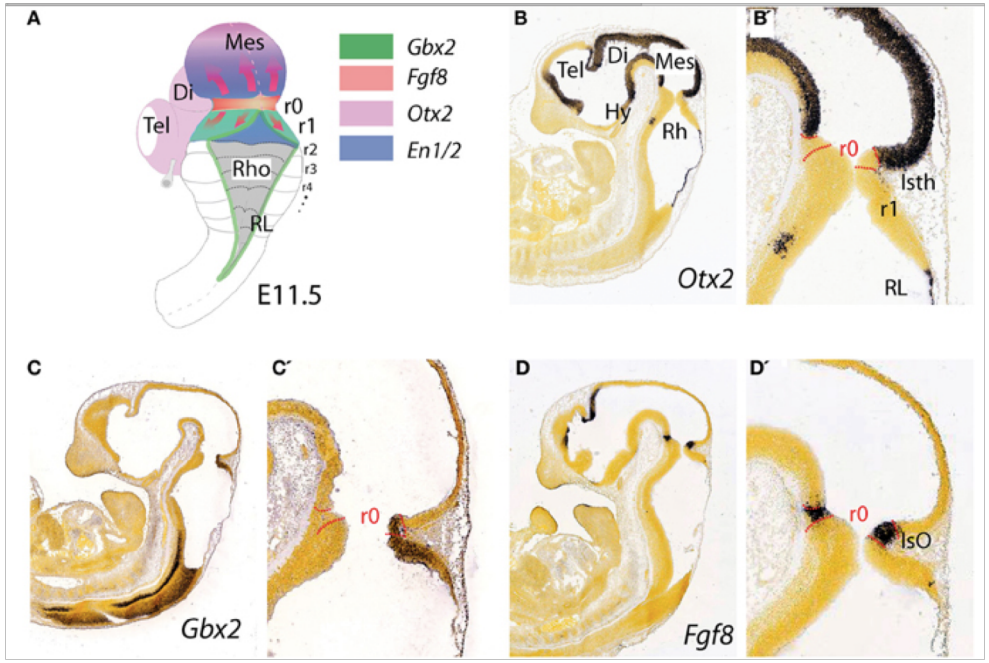
Gbx2 ectopic expression
(*Gbx2* expression driven rostrally into the midbrain by the *Wnt1* promoter)

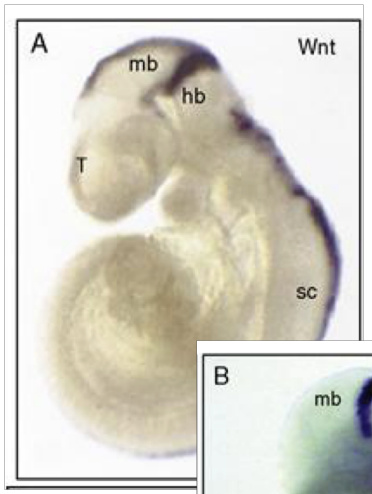
the loss- and gain-of-function experiments converge into a model in which the relative doses of **Otx2** and **Gbx2** proteins control:

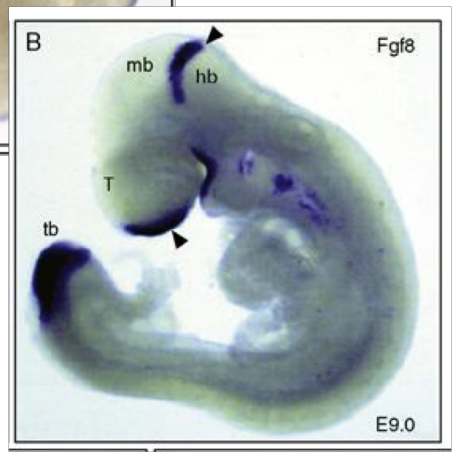
- ✓ the **induction and positioning of the IsO,**
- ✓ the **development of mesencephalic** versus **metencephalic fates** in the normal embryo

Within the mes/met, Otx2 and Gbx2 act antagonistically and are required for positioning and function of the IsO

Molecular characterization of the mid-hindbrain boundary in mice







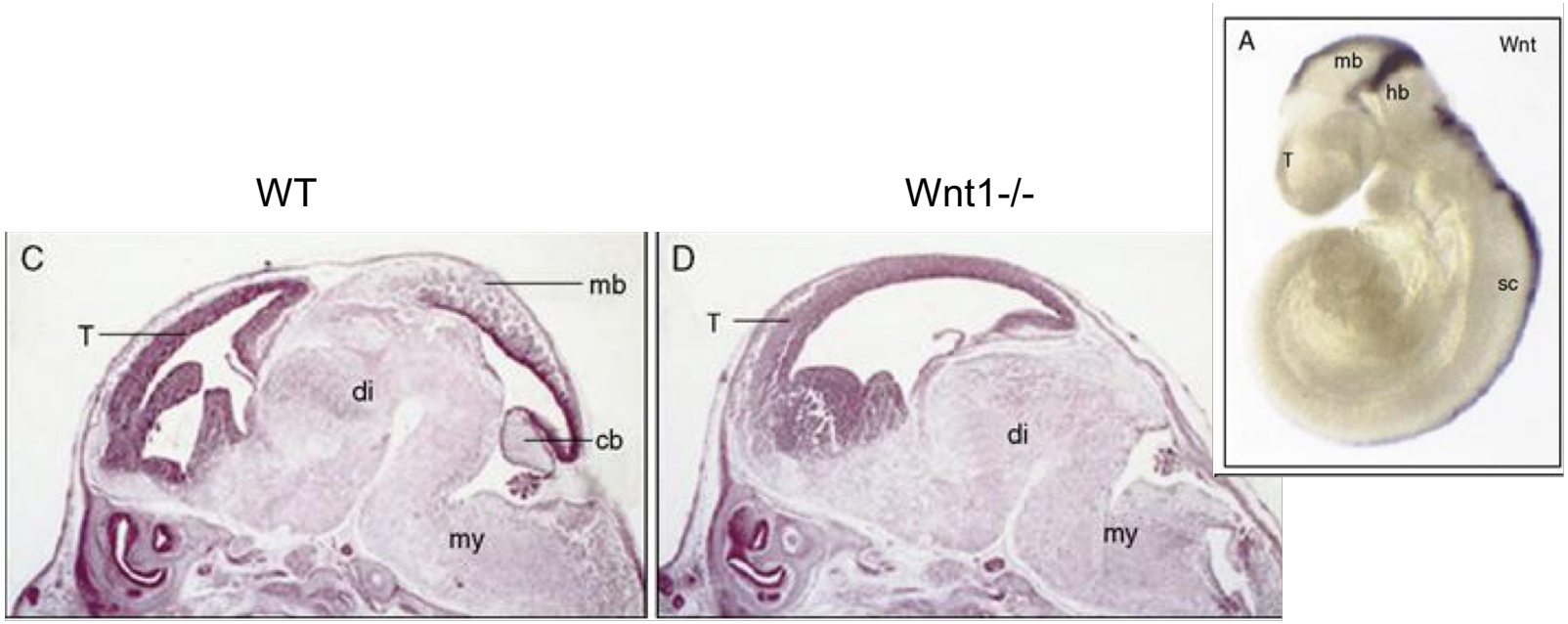
Wnt1 and **Fgf8** are expressed in adjacent bands on either side of the midbrain/hindbrain boundary

Pictures are taken by the **Allen Brain Atlas**....

Martinez et al., Frontiers in Neuroanatomy, 2013

→ candidate **mediators of IsO activity for** induction and maintenance of polarized mesencephalic and metencephalic fates when transplanted ectopically

Wnt1

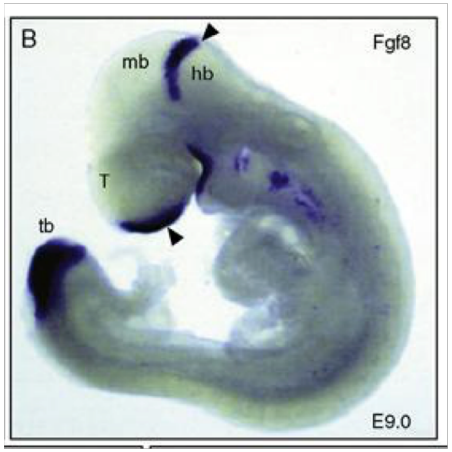


Wnt1-/- mice show dramatic reduction in midbrain/hindbrain structures

and lack of Engrailed1 (En1) expression in the IsO

(En1 KO = phenotype of Wnt1 KO)

FGF8



FGF8 -/- embryos → fail to gastrulate

Fgf8^{neo}/Fgf8^{neo}hypomorphs → survive to birth

a substantial portion of the midbrain as well as isthmus and cerebellum are deleted – similar to KO for wnt1 and en1

...but defects can be due to altereation during gastrulation (Meyers et al., NatureGenetics 1998)

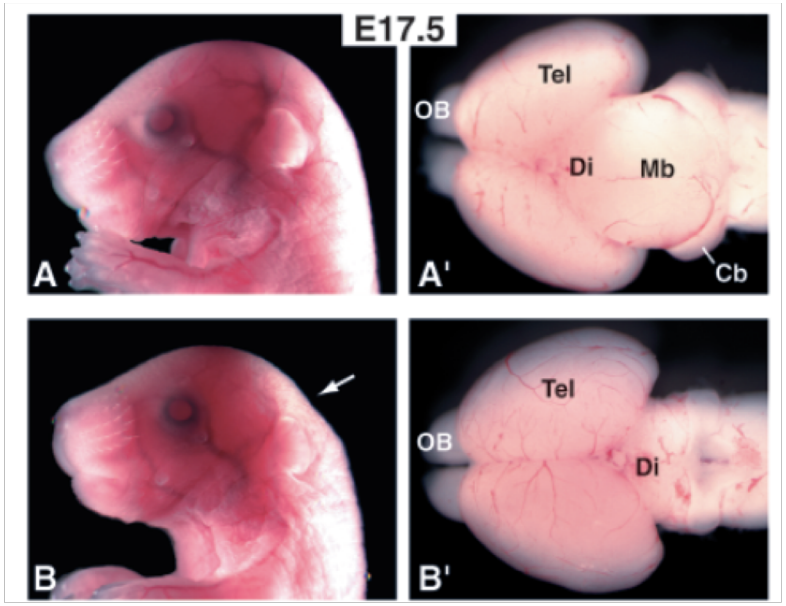
how can be addressed this concern ???

FGF8

Conditional gene inactivation approach (Cre-lox)

FGF8 MHB KO mutants → conditional KO
En1Cre/+,Fgf8flox
(Chi et al., Development 2003)

En1Cre produces sufficient Cre protein throughout the midbrain/ hindbrain boundary region to eliminate *Fgf8* function by 3 somite stage, when *Fgf8* expression normally starts in a subset of *En1*-expressing cells

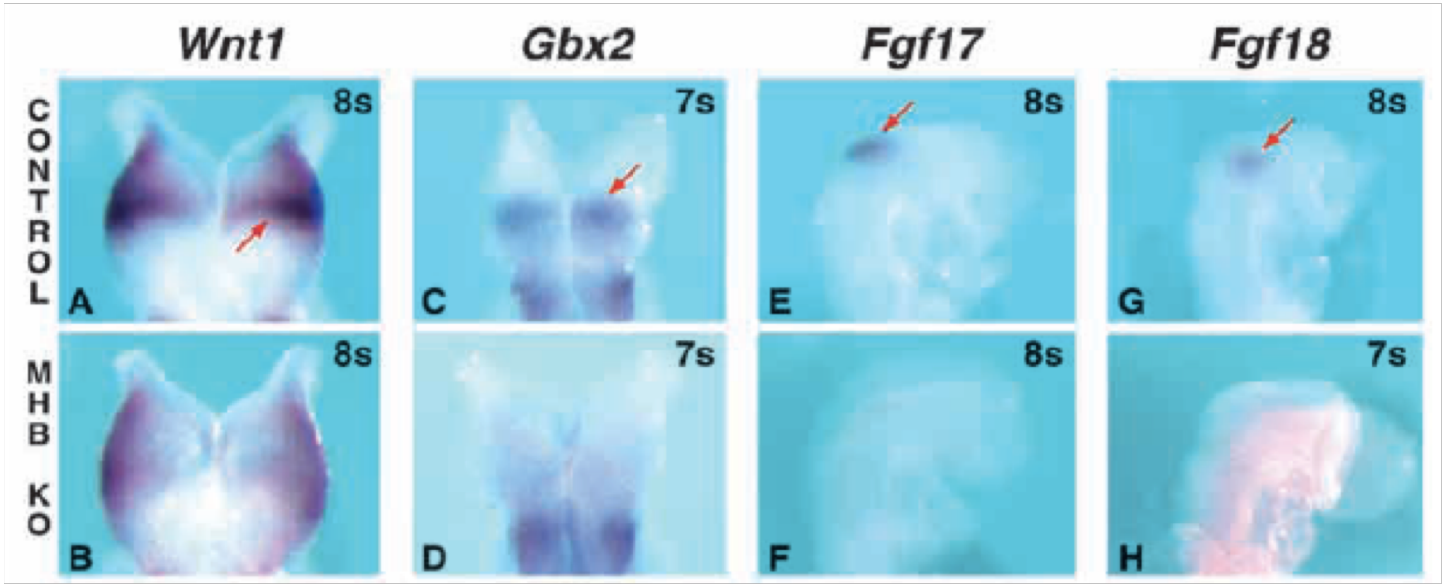


Extensive cell death in the mes/met before E10

Analysis of gene expression in *Fgf8* MHB cKO mutants

→ Ectopic cell death

→ Alteration in gene expression by the loss of *Fgf8* function in MHB

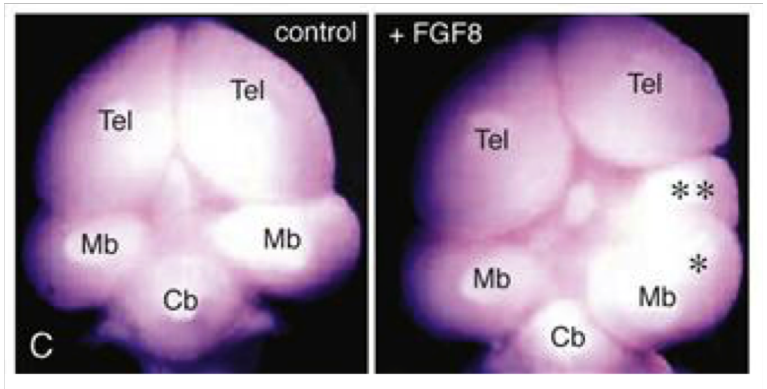


failure to maintain expression of Wnt1 as well as Fgf17, Fgf18, and Gbx2 in the mes/met at early somite stages, and in the absence of the midbrain and cerebellum at E17.5

FGF8 - GOF

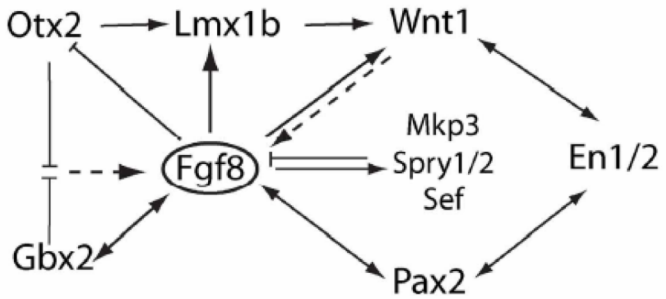
FGF8 normally stimulates cell proliferation in the mes/met and maintains gene expression required for IsO activity

Beads that are soaked with **Fgf8** and implanted into the diencephalon, mesencephalon or rhombencephalon triggered the ectopic expression of mes-metencephalic markers



FGF8 can mimic the effects of grafts of the mes/met boundary region in the chick, in some cases inducing diencephalic tissue to form complete ectopic midbrains as well as cerebellar tissue (these structures were polarized relative to the position of the beads)

FGF8 → mimics organizer activity



H = homebox
TF= transcription factor
M= morphogen
EP= extracellular protein
E = intracellular enzyme
inh= inhibitor of FGF8 signalling

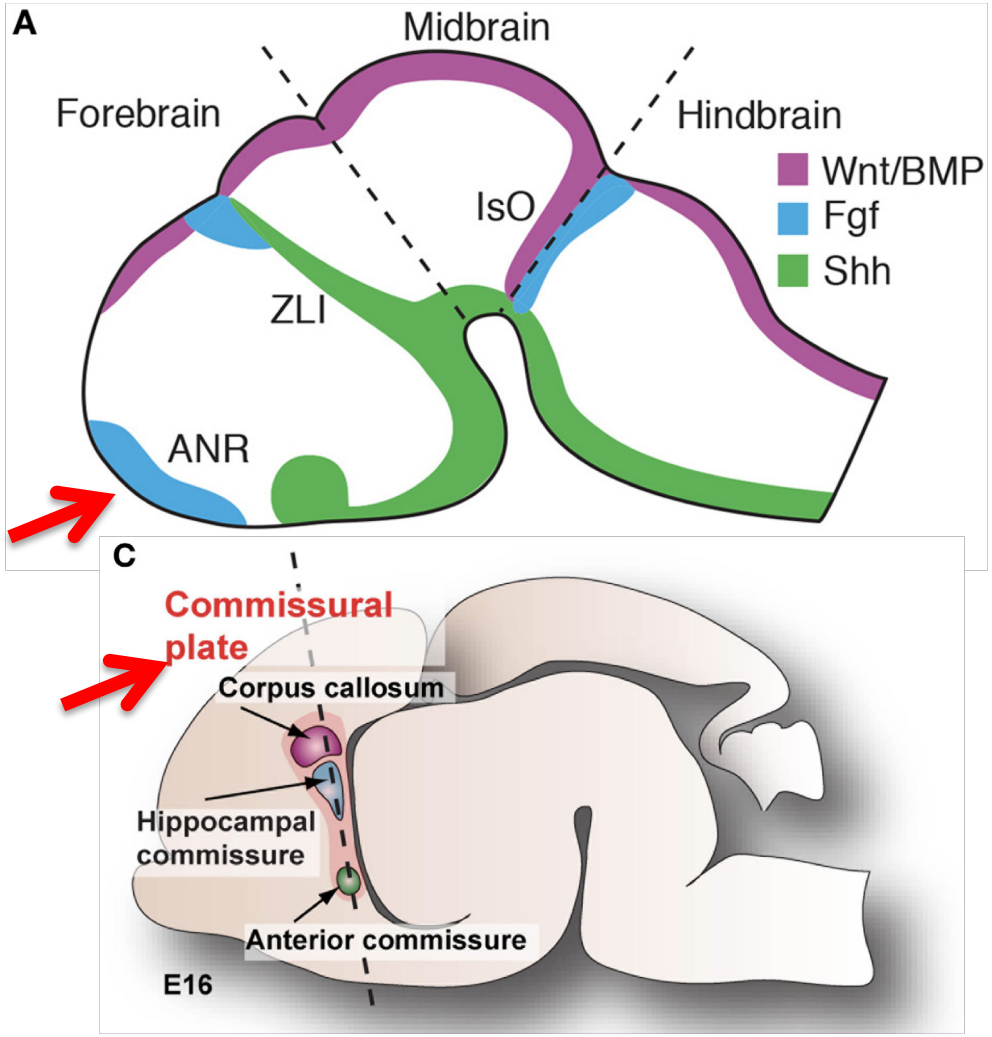
	Otx2	Gbx2	Wnt1	Lmx1b	En1	En2	Pax2	Fgf8	Mkp3	Spry1	Spry2	Sef
	H/TF	H/TF	M/EP	H/TF	H/TF	H/TF	H/TF	M/EP	E/Inh	E/Inh	E/Inh	E/Inh
Di	+++											
	+++											
Mes	+++					+					+	
	+++		+++	+++	++	++			+	++	++	++
Isth		+++		+	+++	+++	+++	+++	+++	+++	+++	+++
R_1		+++			++	++	++		++	++	++	++
R_2												
R_3												

FIGURE 4 | The upper scheme represents the functional interaction (induction/inhibition) of genes that, together with *Fgf8,* are involved in the molecular maintenance of isthmic region at E9.5. The table below summarizes the expression intensity and range of genes along the AP axis of the neural tube focusing on the isthmus. The color code depicts their mRNA expression range from the isthmus toward rostral or caudal regions.

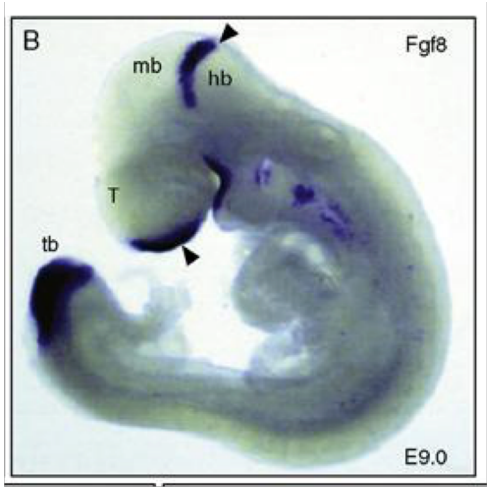
Martinez et al., Frontiers in Neuroanatomy, 2013

ANR

The Anterior Neural Ridge (Boundary) Commissural Plate



Acts as organizer for the forebrain (neocortical patterning)



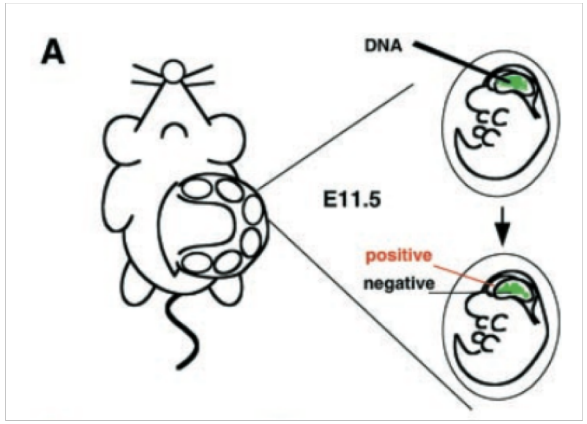
2001; 294 SCIENCE RESEARCH ARTICLES

Neocortex Patterning by the Secreted Signaling Molecule FGF8

Tomomi Fukuchi-Shimogori and Elizabeth A. Grove*

A classic model proposes that the mammalian neocortex is divided into areas early in neurogenesis, but the molecular mechanisms that generate the area map have been elusive. Here we provide evidence that FGF8 regulates development of the map from a source in the anterior telencephalon. Using electroporation-mediated gene transfer in mouse embryos, we show that augmenting the endogenous anterior FGF8 signal shifts area boundaries posteriorly, reducing the signal shifts them anteriorly, and introducing a posterior source of FGF8 elicits partial area duplications, revealed by ectopic somatosensory barrel fields. These findings support a role for FGF signaling in specifying positional identity in the neocortex.

In utero microelectroporation

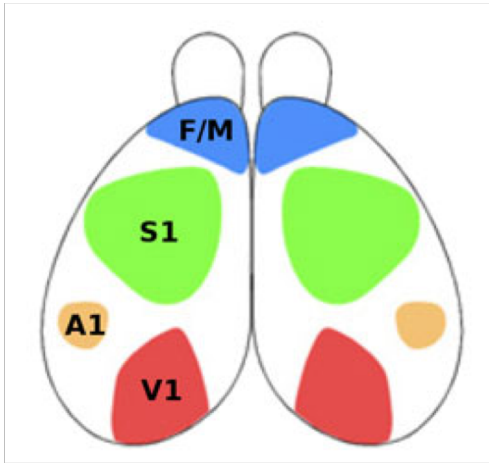


Gene misexpression strategies:

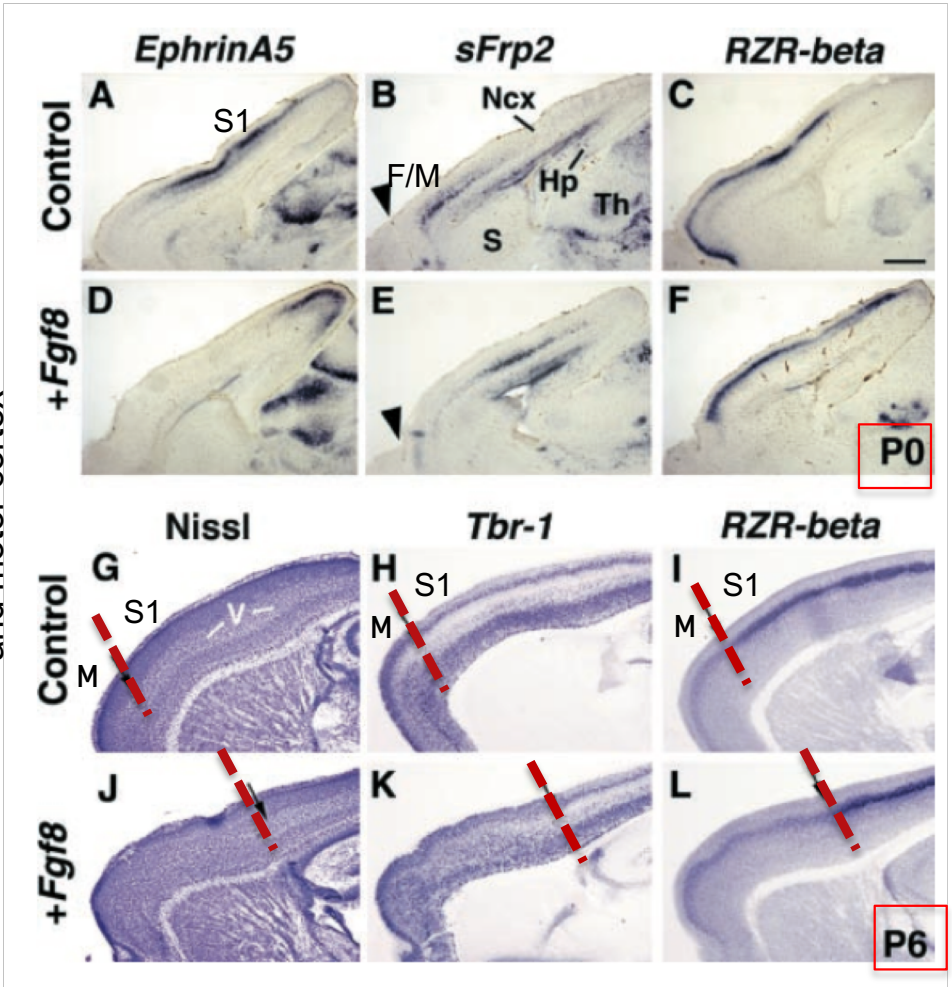
1. augmenting the anterior FGF8 source in the embryonic mouse cerebrum
2. sequestering endogenous FGF8 with a soluble FGF receptor construct
3. introducing a second, posterior source of FGF8

1a. overexpression of FGF8 results in a posterior shift of cortical domains

Embryos were electroporated at embryonic day 11.5 (E11.5) *early in neocortical neurogenesis, before neocortical area identity is determined* - and analyzed postnatally



boundary between somatosensory and motor cortex



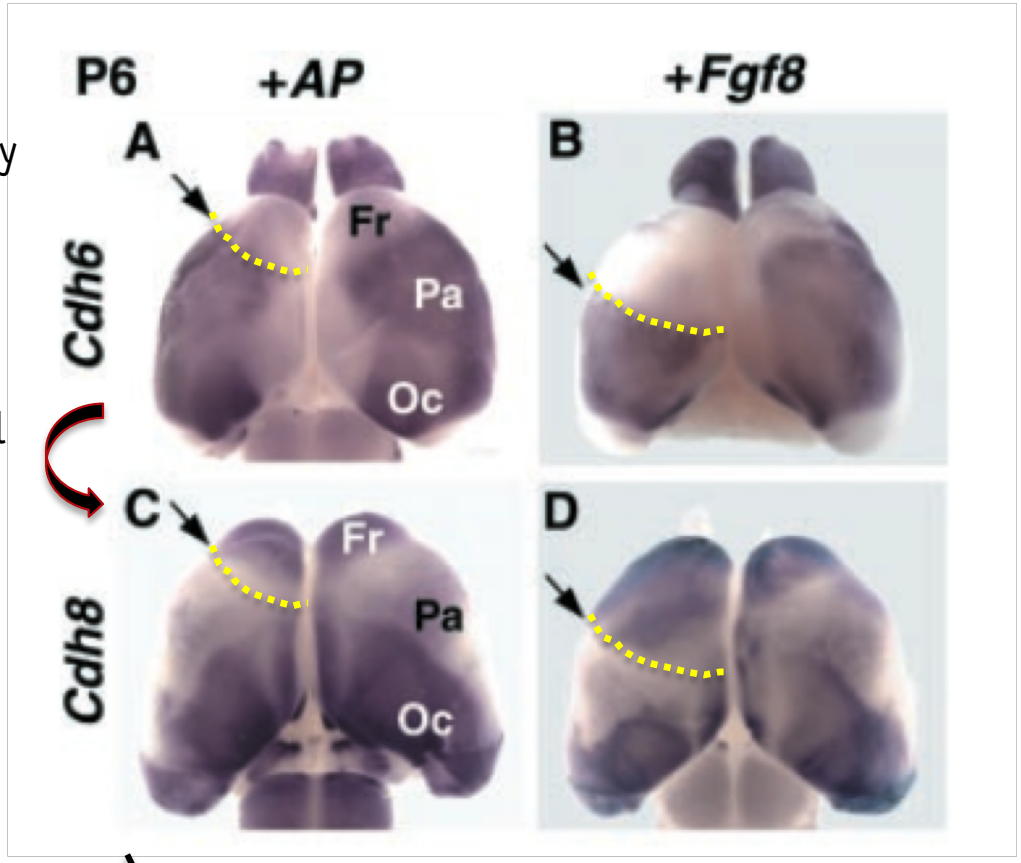
gene expression domains

Cytoarchitectonic boundaries

1b. overexpression of FGF8 results in expansion of anterior neocortical domain with a parallel shrinkage of posterior regions

Fr = frontal/motor
Pa = parietal/somatosensory
Oc = occipital/visual

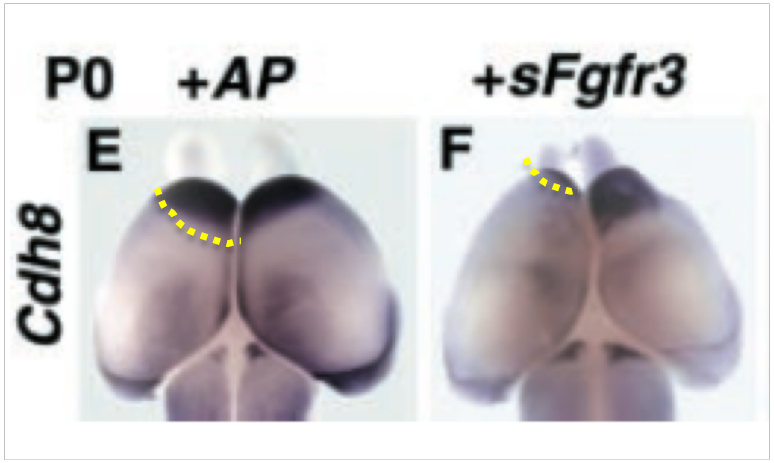
Differential expression



Electroporated hemisphere

2. Reducing endogenous FGF8 signal shifts cortical area boundaries anteriorly

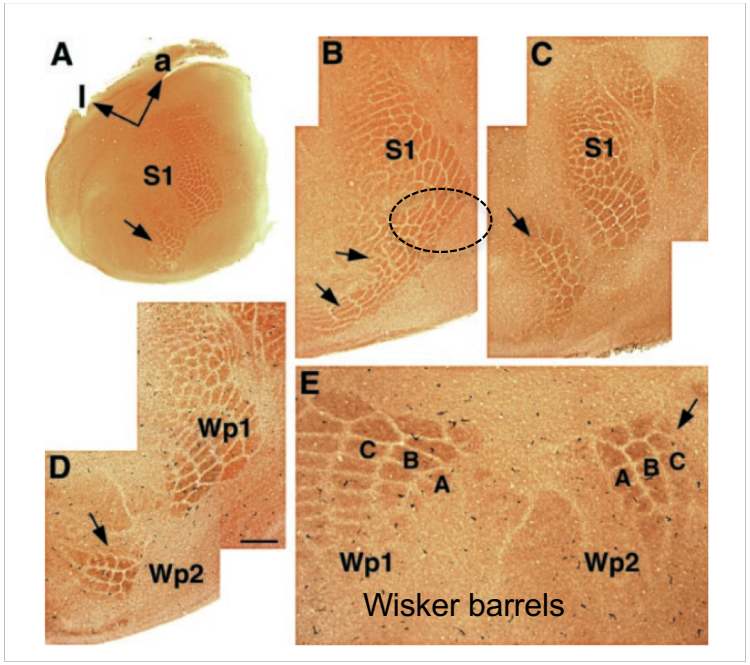
P6
Fr = frontal
Pa = parietal
Oc = occipital



They expressed a soluble form of the FGF receptor (sFgfr3) close to the anterior FGF source to sequester endogenous FGF8

the left frontal *Cdh8*-high domain is unchanged by *AP* (E), reduced by *sFgfr3* (F)

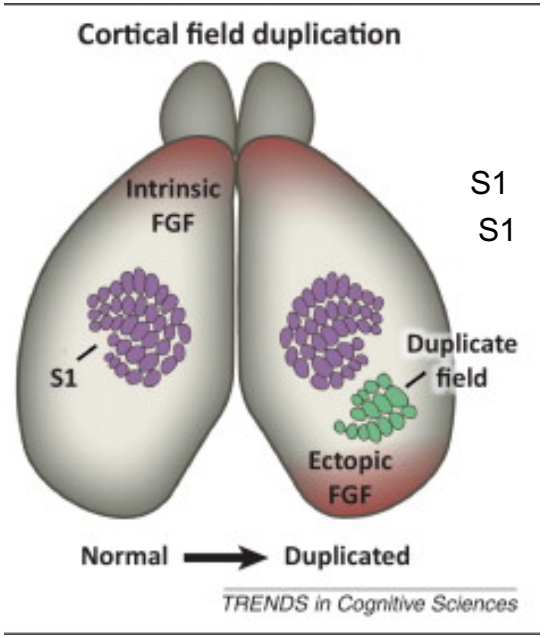
3. A posterior source of Fgf8 generates ectopic S1 barrels: FGF8 specifies positional identity



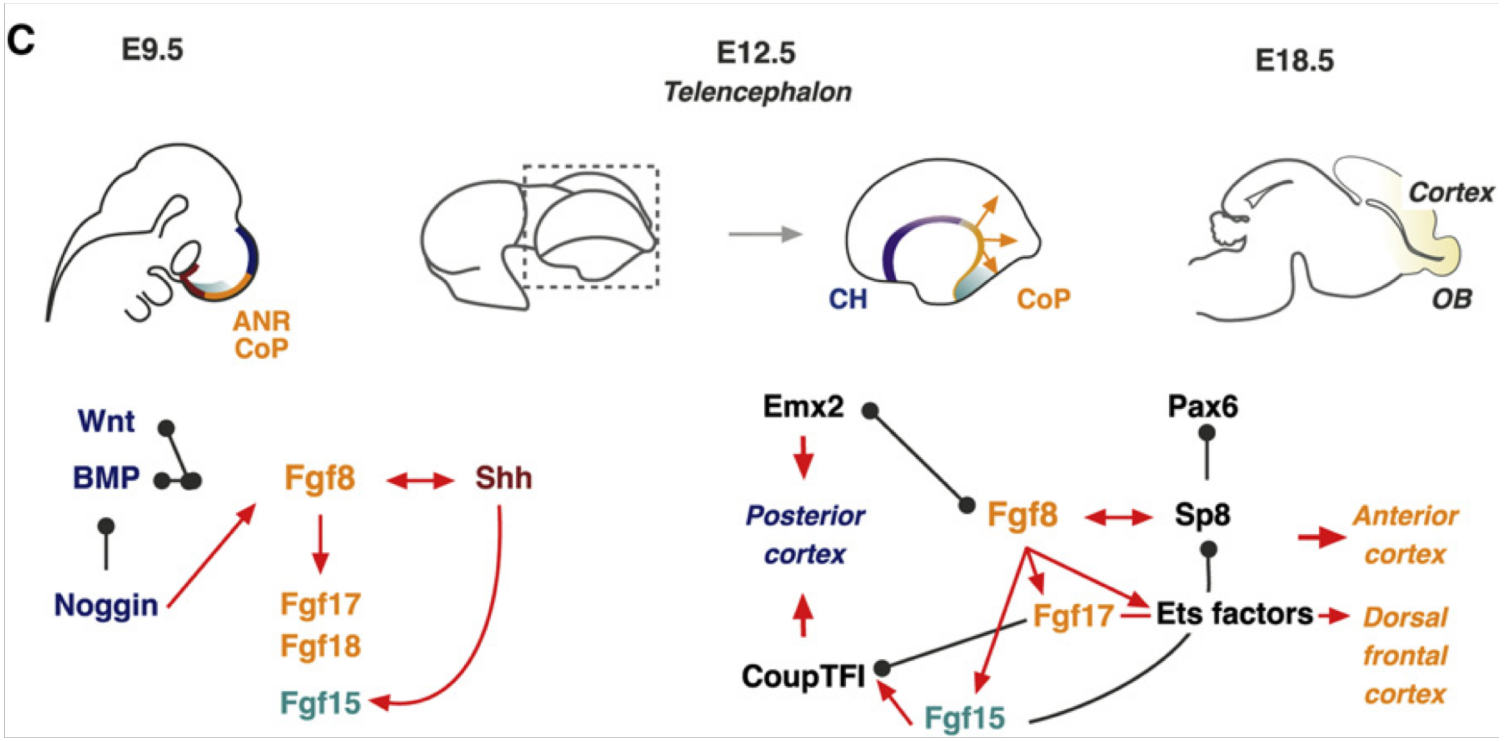
Fukuchi-Shimogori and Grove Science 2001

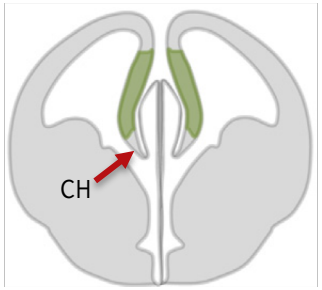
In rodent S1, an array of barrels reflects the pattern of whiskers on the animal's snout, each barrel is innervated by thalamocortical axons carrying sensory information from a single whisker

Mirror-image duplication



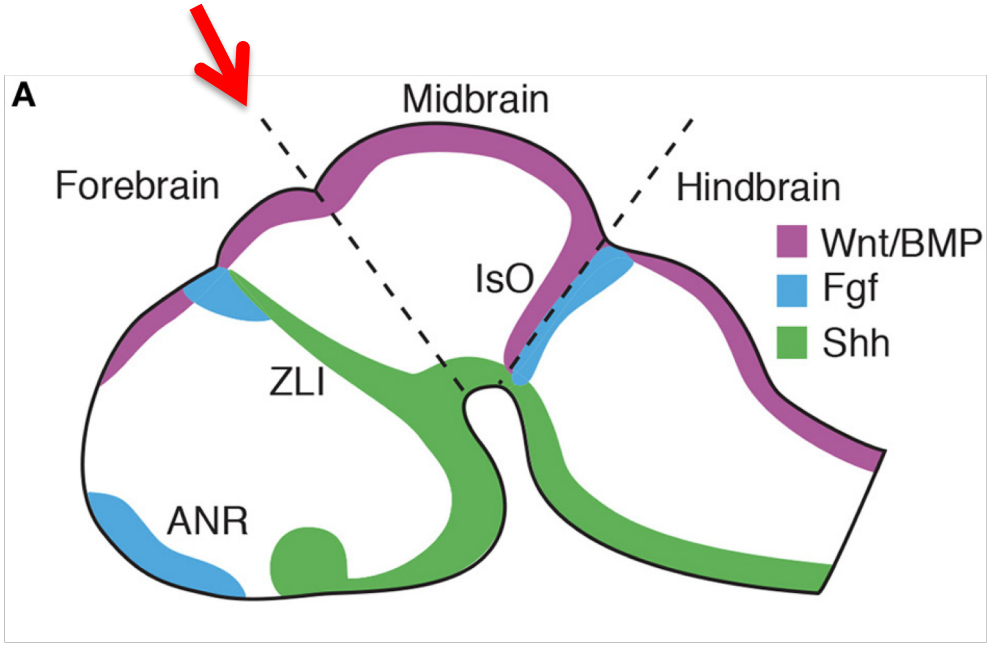
FGF8 as a classic diffusible morphogen in neocortex: it *forms a diffusion gradient along the entire anterior to posterior (A/P) axis of the neocortical primordium, and acts directly to impart positional identity, both close to the FGF8 source, and at a distance. (Toyoda et al., Development 2010)*





CH = cortical hem
ANR = anterior neural ridge
CoP = commissural plate

FGF8 interacts with Wnt/Bmp from the cortical hem (CH) and Shh from the ventral neural tube, and induces ''anterior'' transcription factors (e.g., Sp8, Pax6, Ets) and represses ''posterior'' factors (Emx2, CoupTF1). FGF17 patterns a subdomain of the anterior cortex, while FGF15 counteracts FGF8/17 activities



ZLI

Zona limitans intrathalamica

The ZLI is a narrow transverse region between prosomer 2 and 3

ZLI secretes signaling molecules that generate the patterning of the **diencephalon**

The ZLI is the only structure in the alar plate that expresses signal molecules of the Hedgehog family (Shh)