



**UNIVERSITÀ
DEGLI STUDI
DI TRIESTE**



Dipartimento di
Scienze della Vita

COMPARATIVE BRAIN EVOLUTION

Lecture 5

Comparative Neuroembryology (II):
Neuromeres, Longitudinal zones and Brain Regions

Lecture 5:

Comparative Neuroembryology (I): Neuromeres, Longitudinal zones and Brain Regions

1. Neuromeres / Hox genes

2. Longitudinal zones

3. Brain region formation

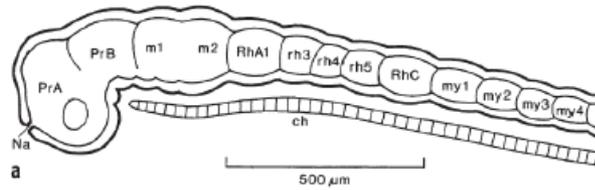
Segmental Development of the Vertebrate Brain: NEUROMERES

neuromeres: serial brain segments

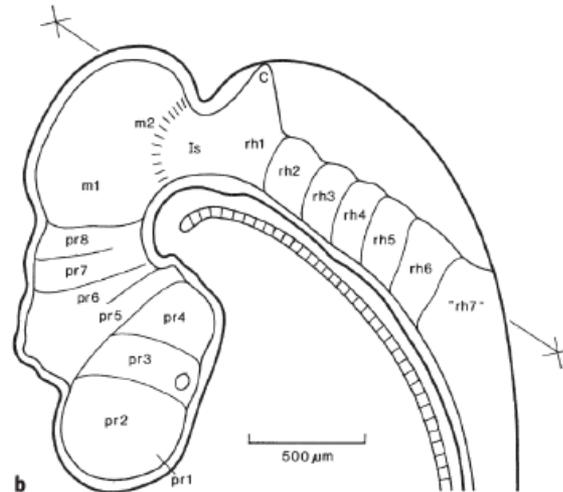
transversely oriented ring-shaped bulges
evident during early development
transitory

Human embryo

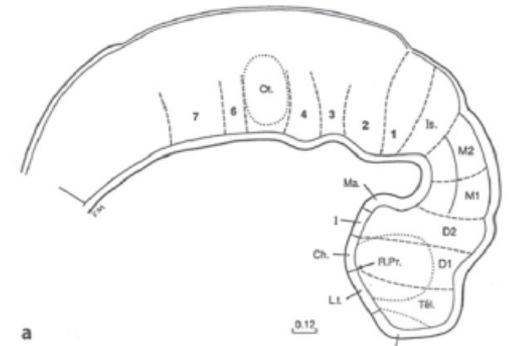
HH-11 (2d)



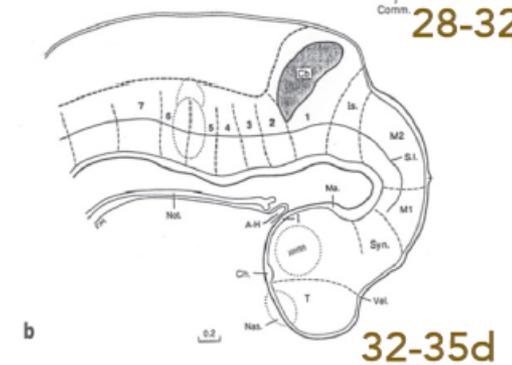
HH-17 (2.5d)



Chick embryo

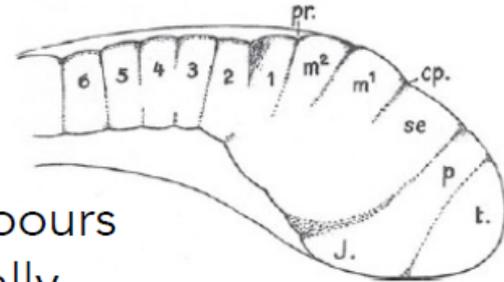


28-32d



32-35d

NEUROMERES



Trout larvae

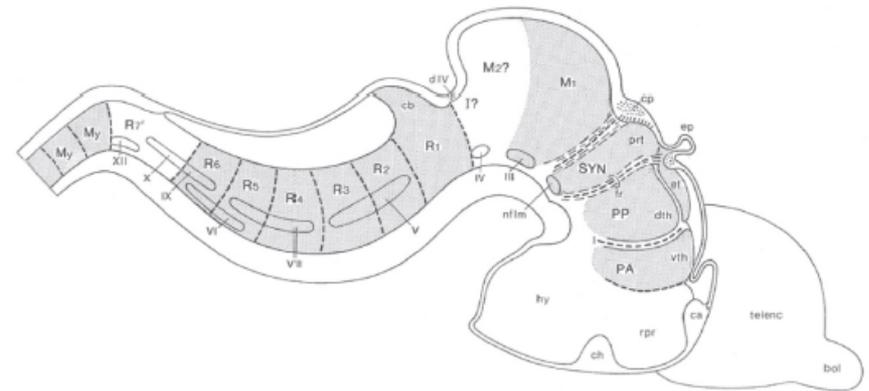
- Each neuromere is **separated** from its neighbours by an external vertical constriction (→internally there's a sharp dorso-ventral ridge)
- Cells from adjoining neuromeres do not extend into another neuromere (?)
- Neuromeres are center of proliferation, early differentiation and migration
 - units of cell lineage restriction (independent developmental fates)
 - Boundaries marked by fibers
 - Fixed relations with peripheral structures

Do Neuromery occur in all parts of CNS?

It "seems" the entire neuraxis shows signs of segmentation during a certain moment in development.

- CNS alternate small and big parts, a model with a fixed number of neuromeres across vertebrates is not possible.

Neuromeres offer repetitive patterns of differentiation.



Neuromeres are **serially homologous**:

- in Cephalocordates, Cyclostomes and Teleosts (spinal cord)
- in all vertebrates: rhombomeres- in rhombencephalon

Neuromeres in the Vertebrate Brain

Mesomeres

m1-m2

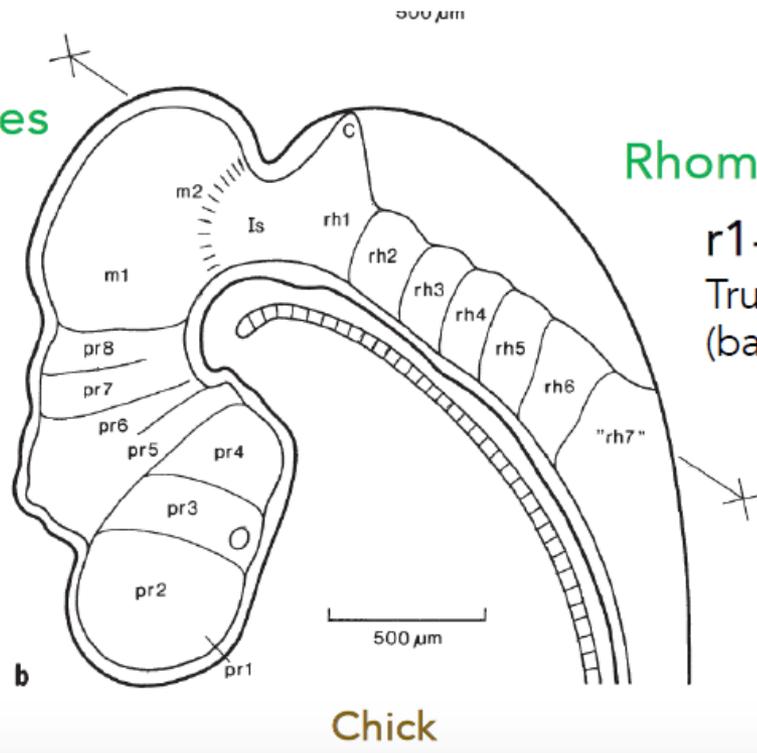
Rhombomeres

r1-r7

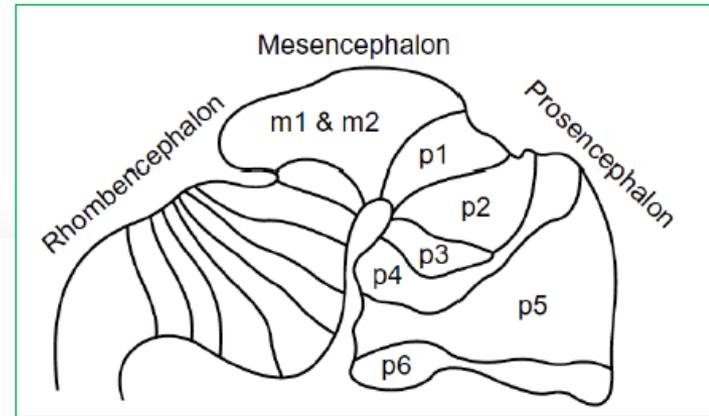
True boundaries
(barriers to migration)

Prosomeres

p1-p6 (or more)



Chick
embryo



1. Prosomeres

- Found in the **diencephalon** and **telencephalon**.
- Give rise to structures like the **thalamus**, **hypothalamus**, and **cerebral cortex**.
- Their segmentation is key to understanding the **evolution of forebrain complexity** across vertebrates.

2. Mesomeres

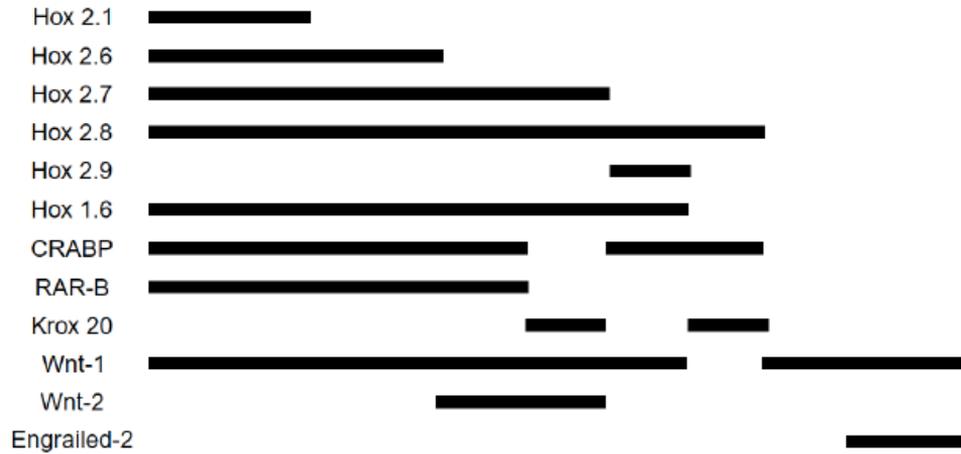
- Located in the **midbrain**.
- Less clearly segmented, but still show regionalization into dorsal (tectum) and ventral (tegmentum) domains.
- Important for **sensory processing** and **motor control**.

3. Rhombomeres

- Segmental units of the **hindbrain**.
- Each rhombomere has a **unique genetic identity**, largely governed by **Hox genes**.
- They guide the development of **cranial nerves** and **brainstem nuclei**.

Genetic Specification of Rhombomeres

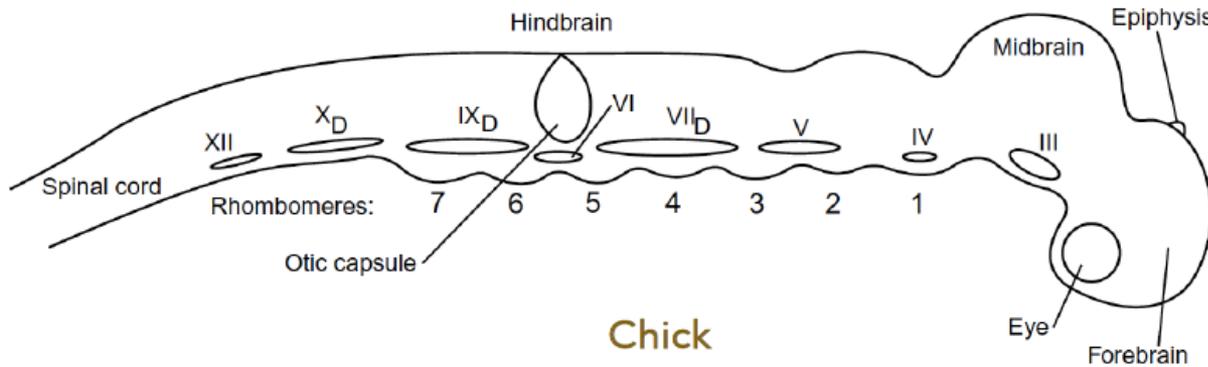
Gene expression patterns



Unique combination in each rhombomere

Mesencephalon:
En-2
Krox20

cranial nerves



Chick embryo

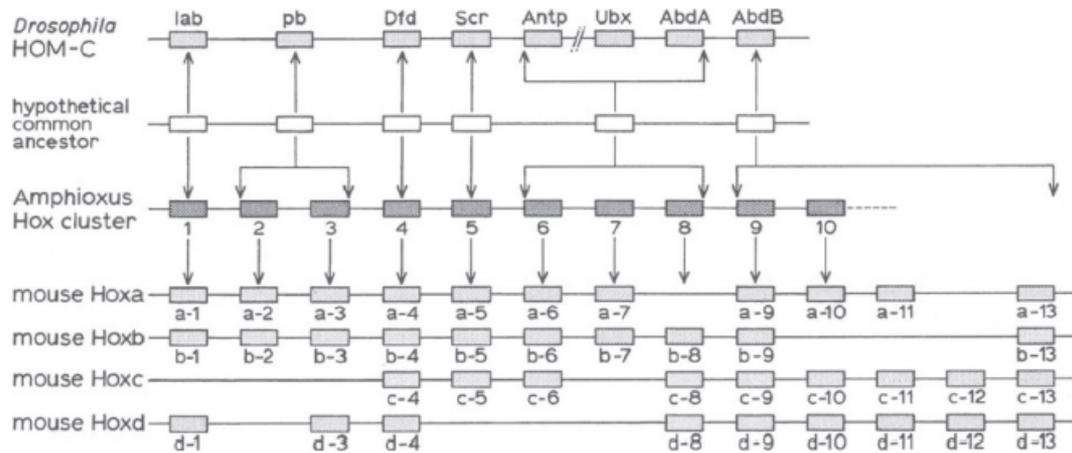
Prosencephalon:
Wnt1-4
Dlx1,2
Nkx2.2

EVO-DEVO

Homeobox genes

- DNA seq 180 bp
- Control spatial patterning in the embryonic CNS
- **Highly conserved**

Comparison of Hox genes help define the fundamental pattern of brain regionalisation in different species.

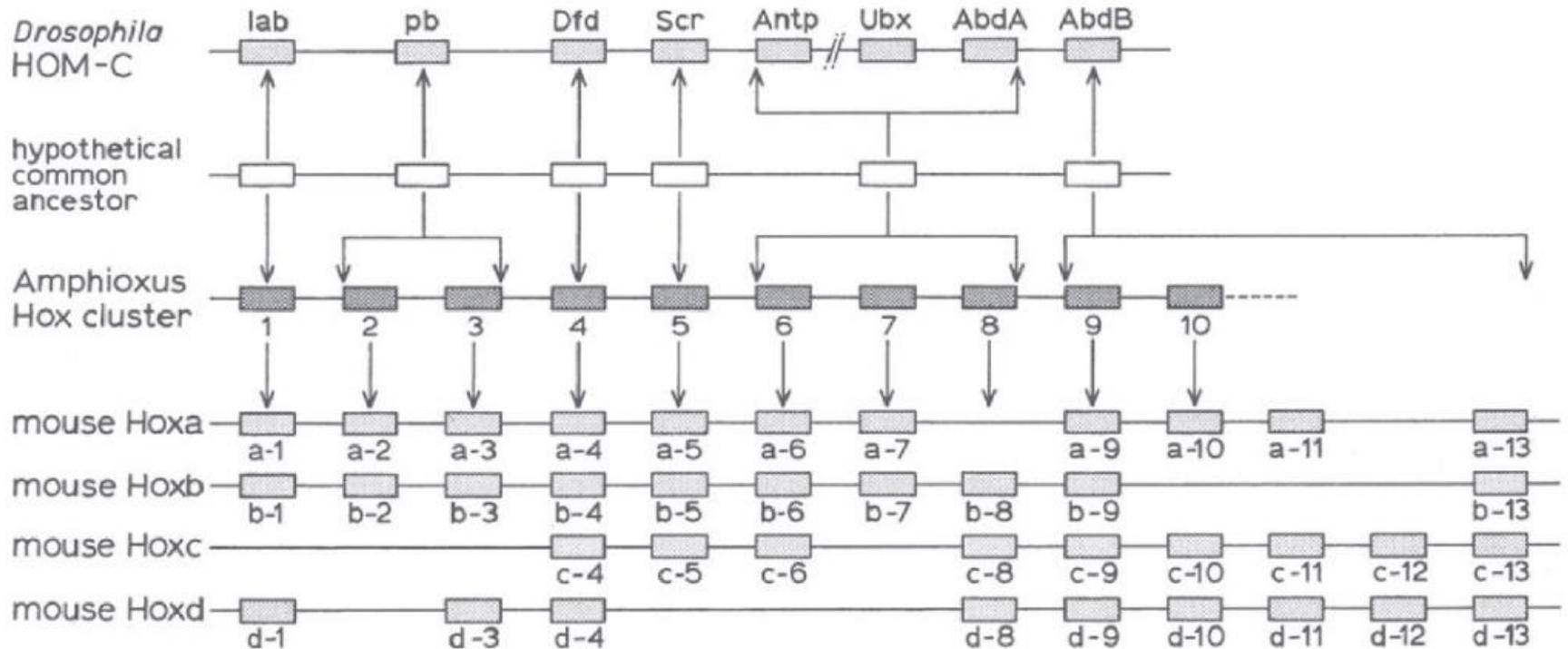


Evolutionary developmental biology

Comparative studies on genomic organisation and diversity of developmental genes → insights into genetic basis for phylogenetic development of the vertebrate brains.

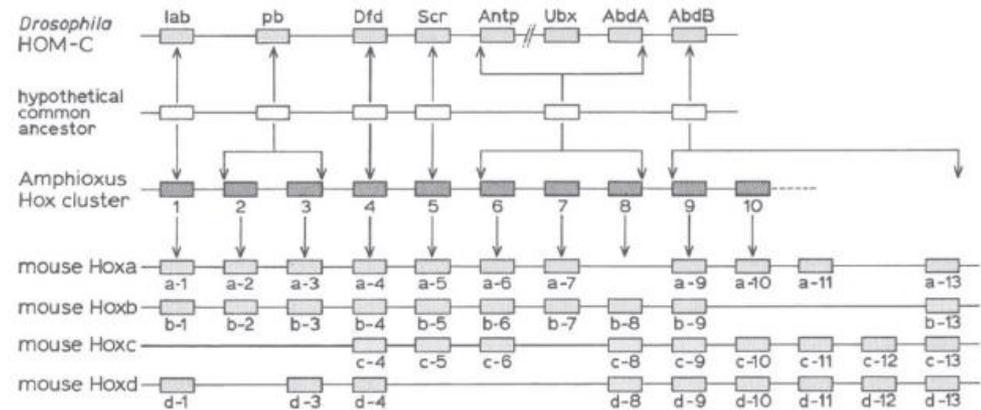
Phylogenesis of the Hox genes

Example: Genome of cephalocordate vs mouse



Single Hox cluster vs multiple Hox clusters

- Increase in number of Hox genes early in chordate evolution



- Hox cluster duplications occurred after the divergence of cephalocordate and vertebrate lineages
- 2 phases of cluster duplication
 - One close to the origin of vertebrates
 - One close to the origin of gnathostomes

Importance of gene duplication in evolution

The great potential of gene duplication in evolution: it leads to increased complexity

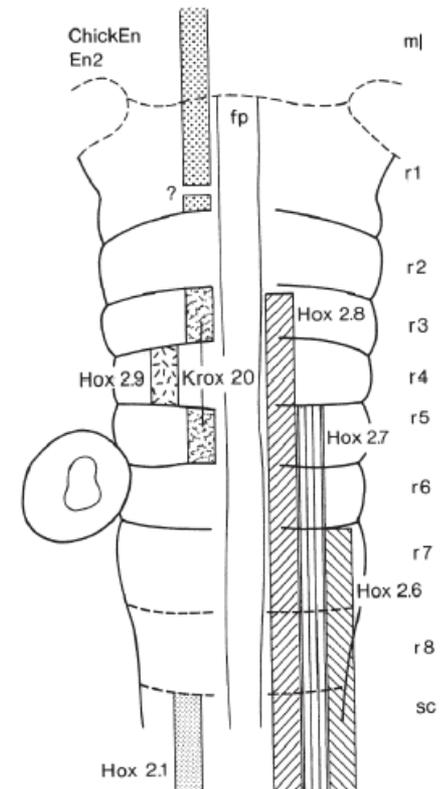
Redundancy, some genes lost, some have new roles and new developmental pathways.

Homeobox-containing genes are very ancient

The first homeobox genes were discovered in *Drosophila* (invertebrates):

HOM genes

In all vertebrates numerous Homeobox-containing genes are found .



Chick
embryo

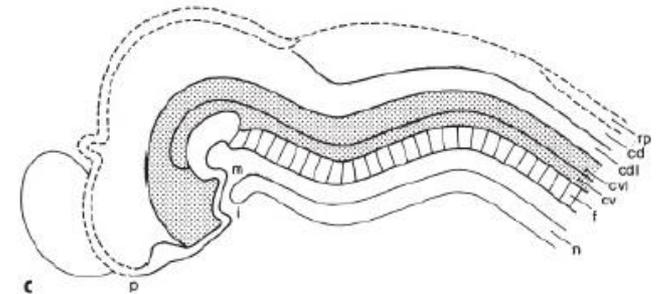
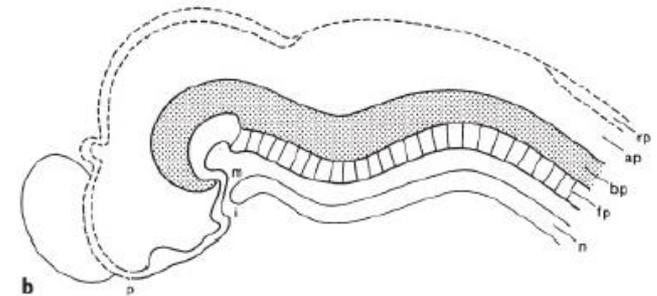
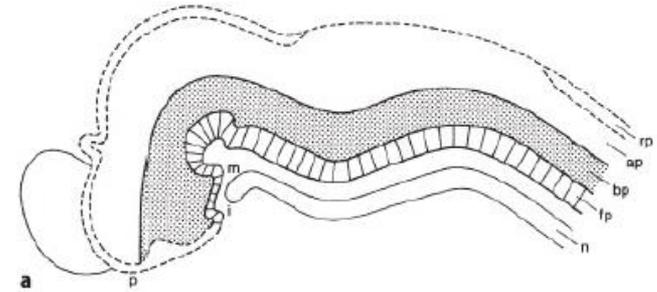
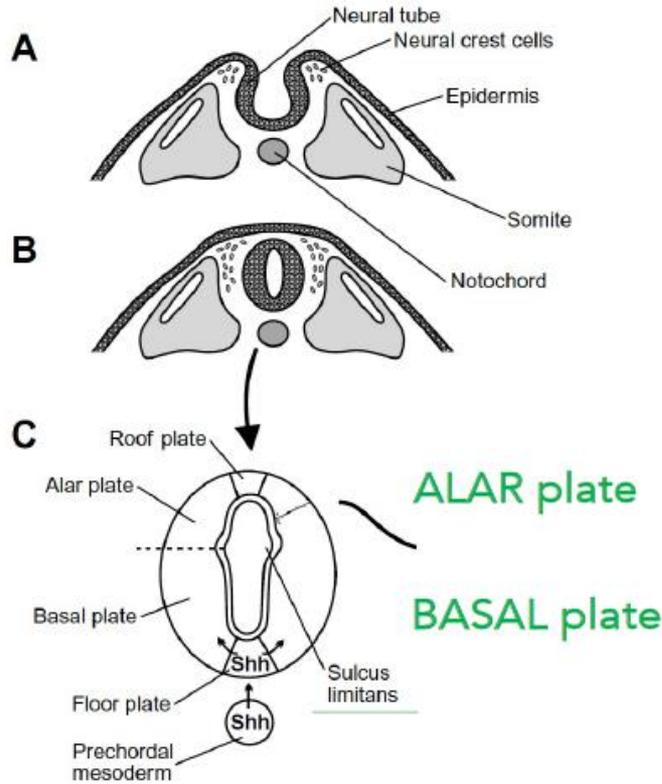
In the developing **central nervous system**, Hox genes:

- Are **expressed in specific domains** along the neural tube.
- Define the **identity of rhombomeres** (segments of the hindbrain).
- Control the **formation of cranial nerves** and **motor neuron pools**.
- Interact with other genes (e.g., **Pax, Otx, Gbx**) to refine brain regionalization.

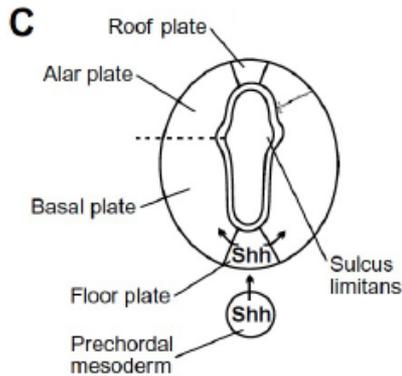
Hox genes help us to:

- Understand how **segmental identity** is genetically encoded.
- Compare **hindbrain organization** across species.
- Explore how **developmental constraints and innovations** shape brain evolution.
- Link **genetic regulation** to **functional anatomy** (e.g., cranial nerve patterning).

Longitudinal zones: alar and basal plate



Longitudinal zones: alar and basal plate



ALAR plate

sensory

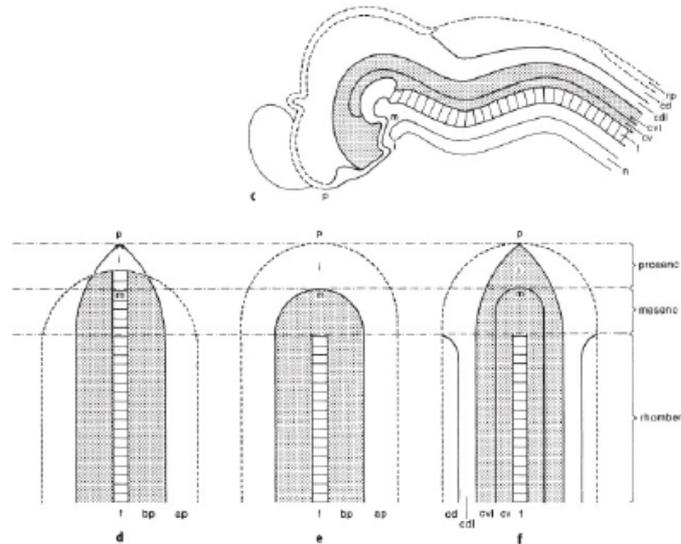
BASAL plate

motor

Sulcus limitans cannot be found rostrally, beyond the isthmus

These four zones can be clearly distinguished in the rhombencephalon, more difficult in the rostral brains.

Longitudinal zones (or columns) are considered both structural and functional entities



Longitudinal zones: alar and basal plate across vertebrates

| | | | |
|-----------------|-------|--|--|
| Rhombencephalon | alar | dorsal area Intermediodorsal | Coincide largely in all vertebrates |
| | basal | Intermedioventral ventral | |
| Mesencephalon | | tectal zone lateral tegmental zone medial tegmental zone | Larger in non-mammals |
| Diencephalon | | Epithalamus Thalamus dorsalis Thalamus ventralis hypothalamus | |
| Telencephalon | | Dorsal pallium Ventral pallium | |

◇ Alar Plate (Dorsal Zone)

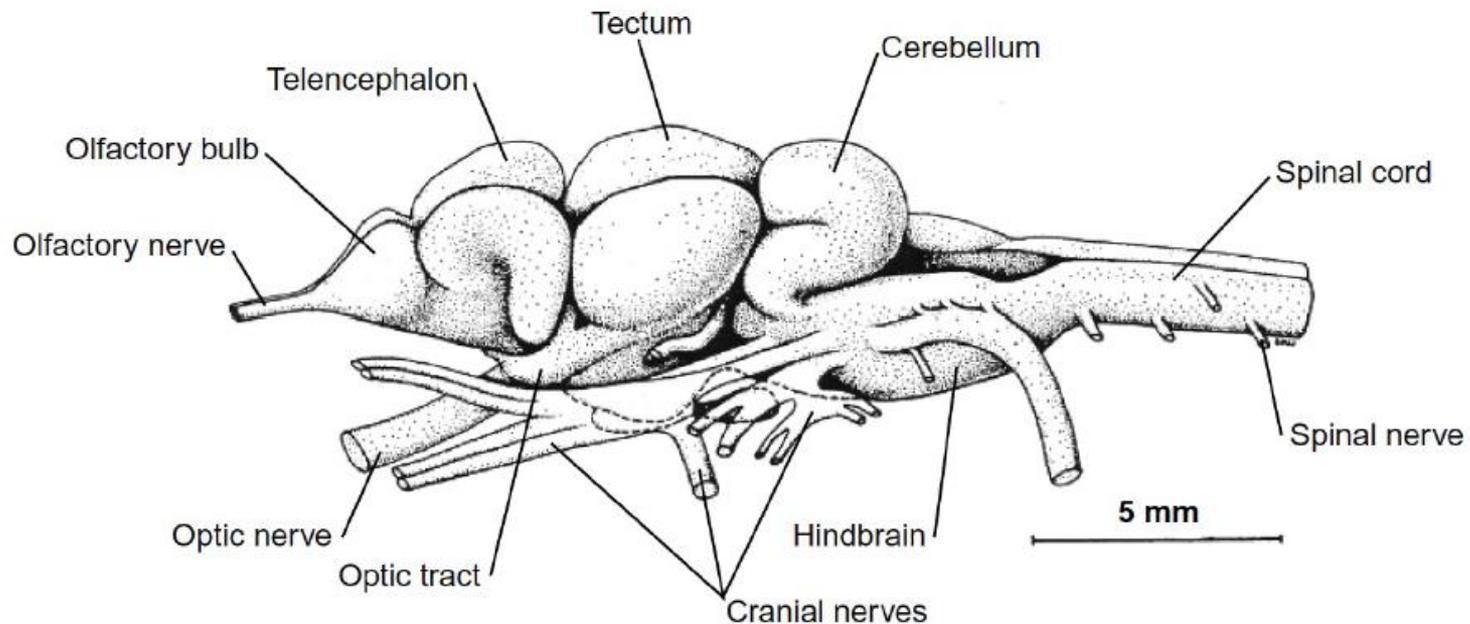
- **Function:** Primarily **sensory**.
- Gives rise to **sensory relay nuclei** in the spinal cord and brainstem.
- In the **forebrain**, it contributes to **higher-order sensory processing centers**, including parts of the **thalamus** and **cerebral cortex**.
- In evolution, the alar plate becomes increasingly complex in species with more advanced sensory systems (e.g., birds, mammals).

◇ Basal Plate (Ventral Zone)

- **Function:** Primarily **motor**.
- Gives rise to **motor neurons** and **motor nuclei** of cranial nerves.
- In the spinal cord, it forms the **ventral horn**, which contains **somatic motor neurons**.
- In the brainstem, it contributes to **motor cranial nerve nuclei** (e.g., oculomotor, facial, hypoglossal).

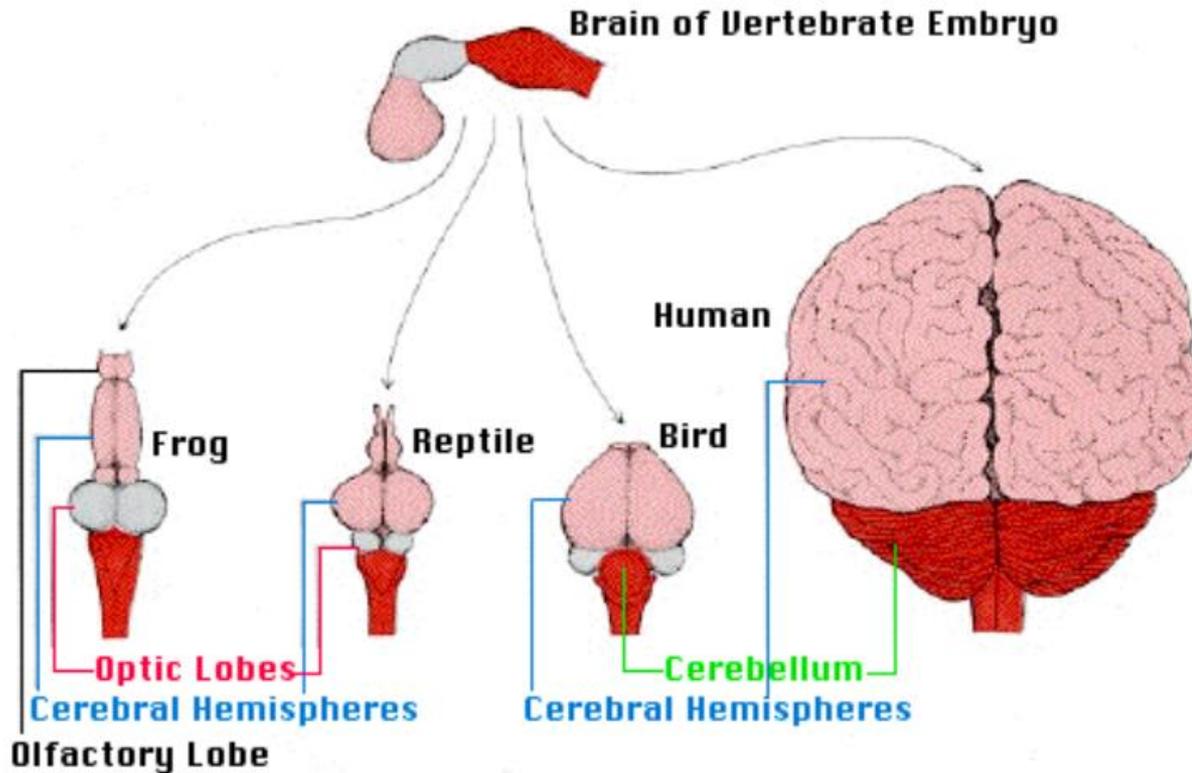
Formation of brain regions across vertebrates

General organization of the brain:



Formation of brain regions across vertebrates

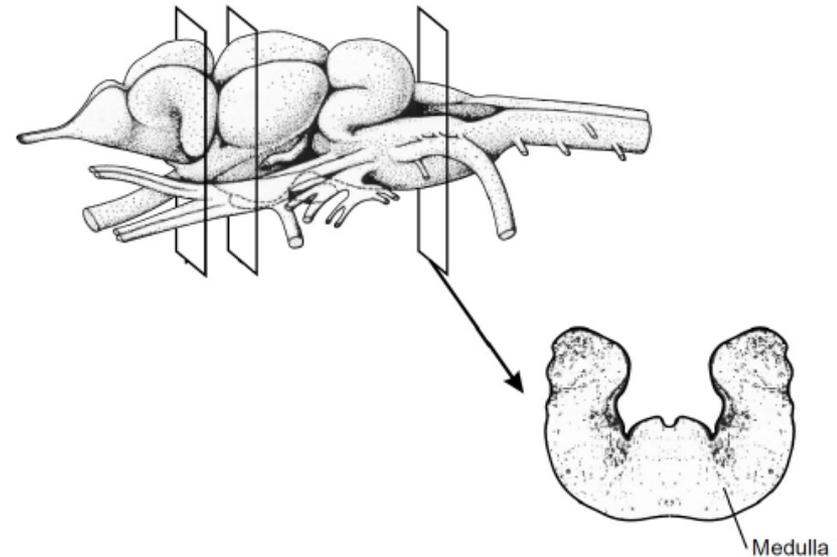
General organization of the brain:



Formation of brain regions across vertebrates

HINDBRAIN (Rhombencephalon)

- Myelencephalon (or medulla oblongata)
- Metencephalon (pons+ cerebellum)



Motor efferents for muscles of jaws, face, lips, cheeks, tongue, throat, eyeball

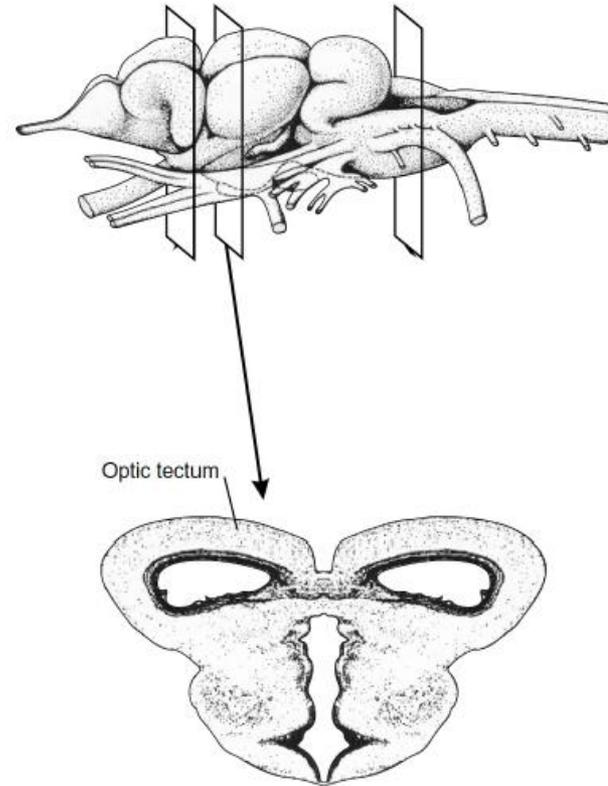
Sensory for “special sense” cranial nerve enter here (e.g. vestibular sense, lateral line system, electric fields in water, infrared sense; touch position pain receptors)

MIDBRAIN (Mesencephalon)

- Tectum (roof of the midbrain)
- Tegmentum (ventral)

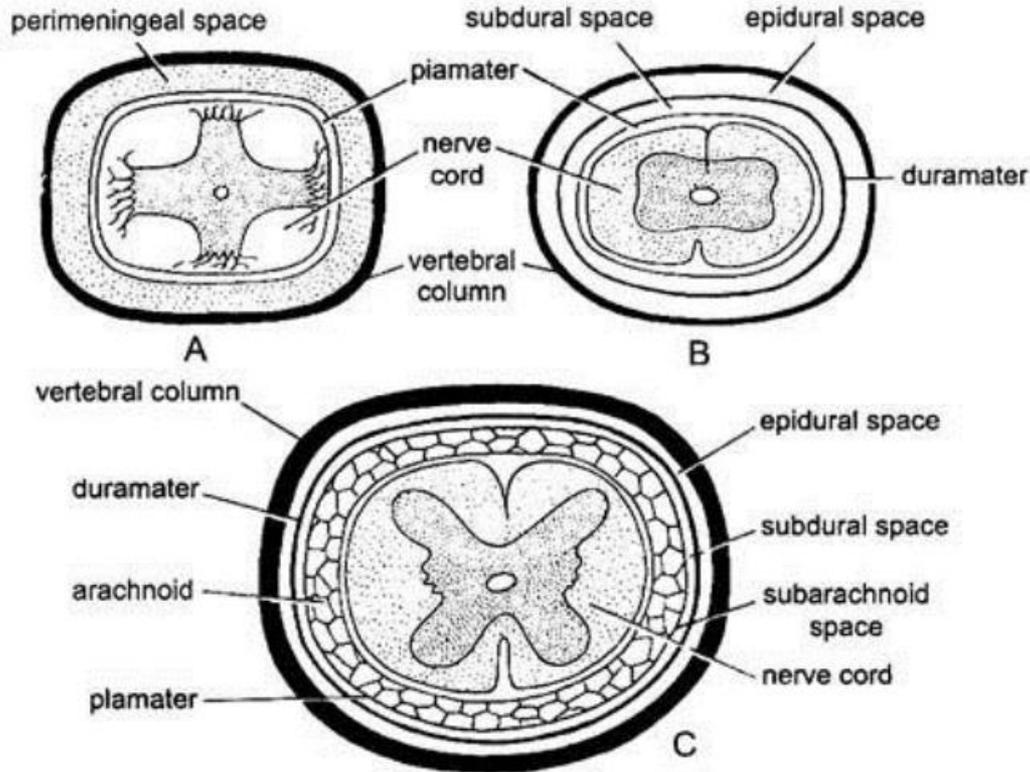
Motor efferents for extraocular muscles (eye movements) and intraocular eye muscles (pupil constr.)

No direct afferent cranial nerve.



The Meninges

fish



Amphibians, Reptiles

2 meninges:

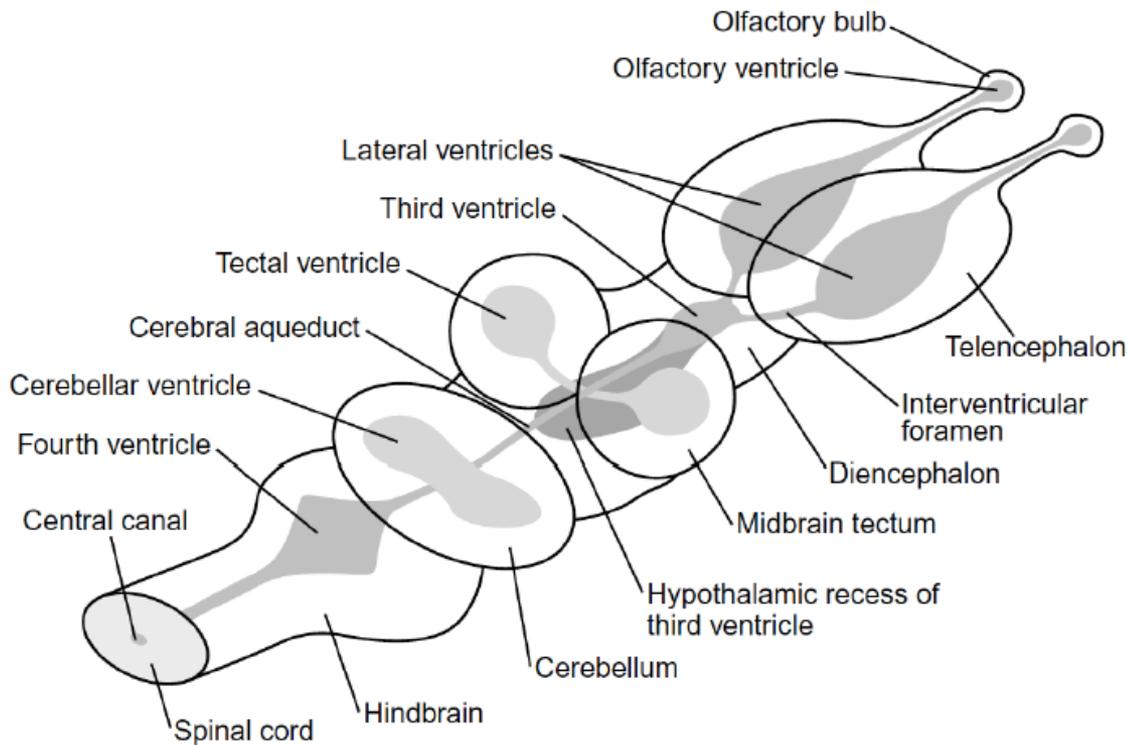
- Dura mater
- Secondary meninx

Mammals & Birds

3 meninges:

- Dura mater
- Arachnoid
- Pia mater

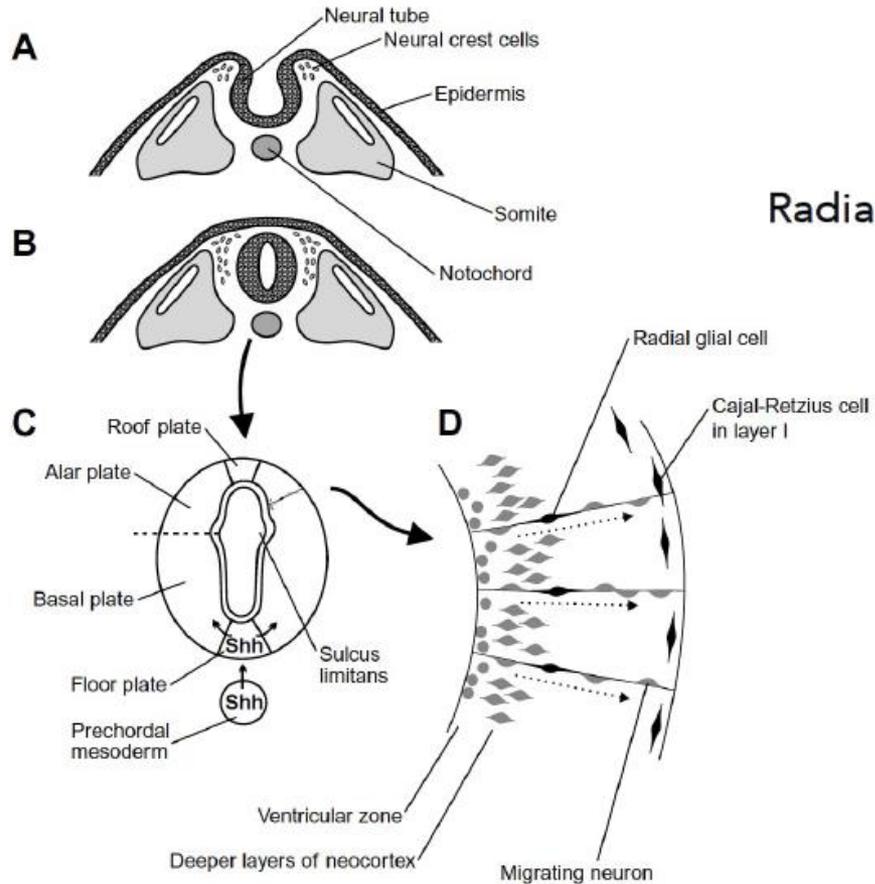
The Ventricular System



Choroid plexus

secretes
cerebrospinal fluid
into ventricles

Neurogenesis and Migration of cells



Radial glial cells

Cajal-Retzius cell

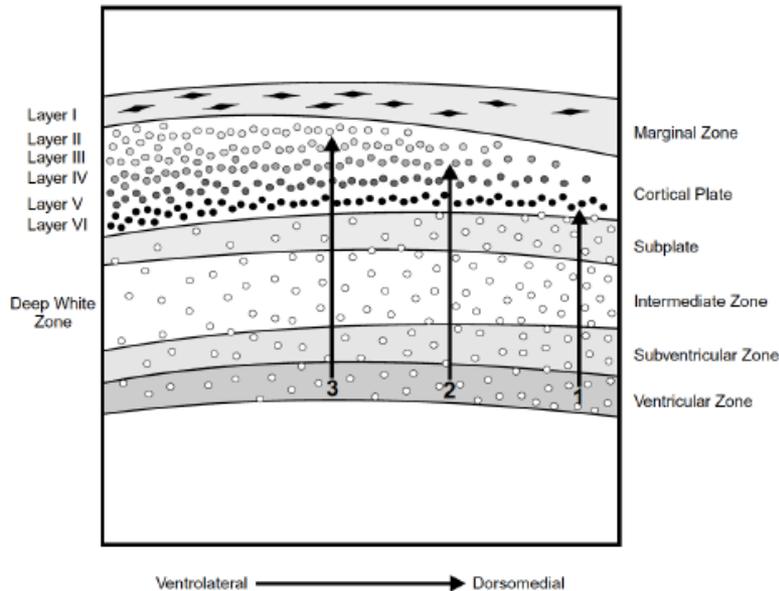
INSIDE-OUT
(for neocortex in mammals)

BMP (dorsally)

Neurogenesis and Migration of cells

Cortices: laminated structures (located more dorsal, alar plate)

Nuclei: scattered cells (loosely or tightly packed, both alar and basal plate)



Neocortex in
MAMMALS
6 layers

Cortex in Reptiles
3 layers

Caudal nuclei before rostral
Ventral nuclei before dorsal

Summary

- Neuromeres (Rhombomeres, Mesomeres, Prosomeres)
- HOX genes and Evo-Devo
- Longitudinal zones
- Brain regions across vertebrates
- Meninges
- Ventricular system



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COMPARATIVE BRAIN EVOLUTION

Lecture 6

Comparative Neuroembryology (III)

Lecture 6:

Comparative Neuroembryology (III): Early Brain Morphogenesis

1. Mechanisms of brain morphogenesis and their evolutionary conservation

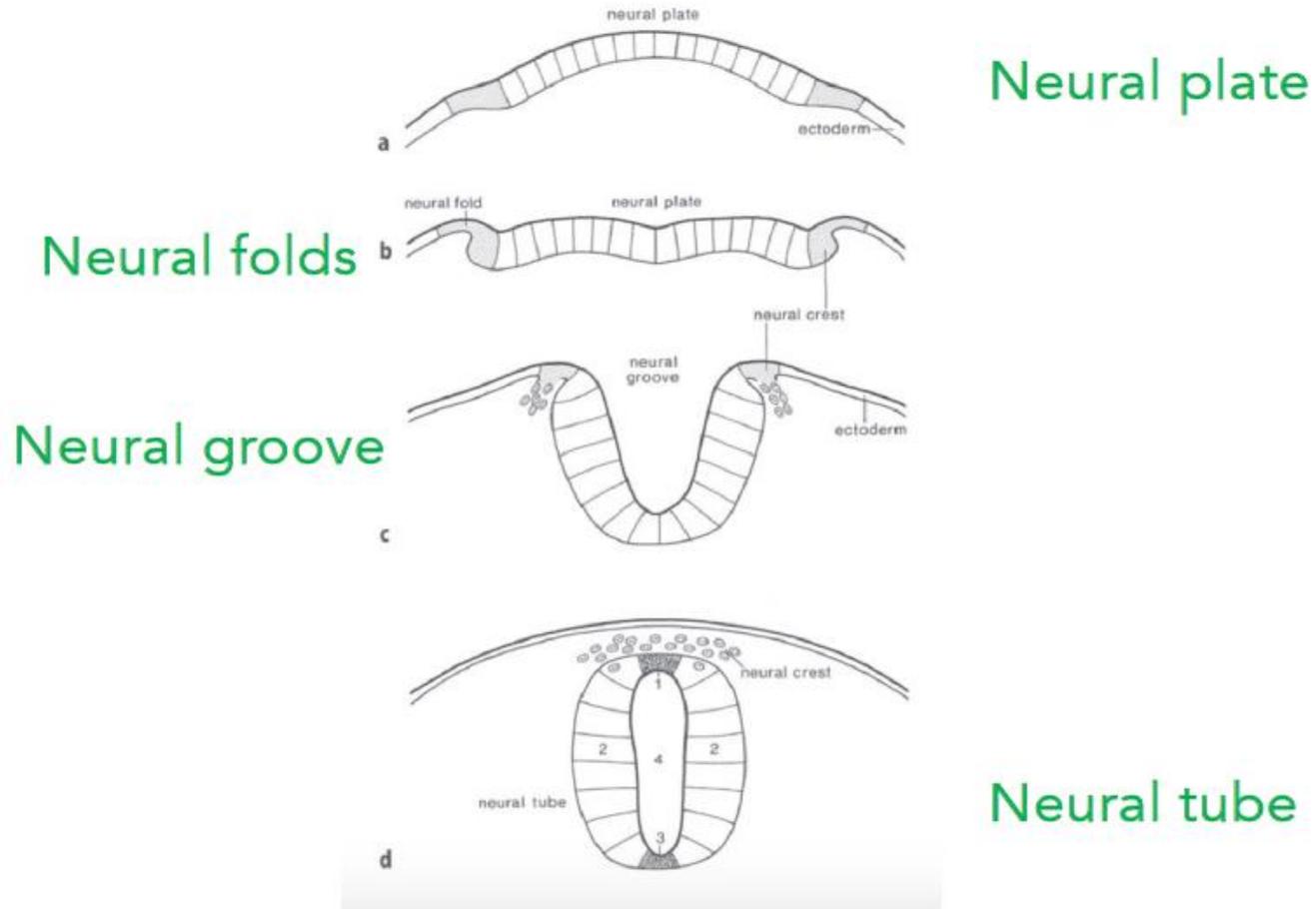
2. Evolution of brain developmental plan: Insights from Agnathans

Mechanisms of brain morphogenesis

Mechanisms of Brain Morphogenesis

Benjamin A. Filas, Gang Xu, and Larry A. Taber

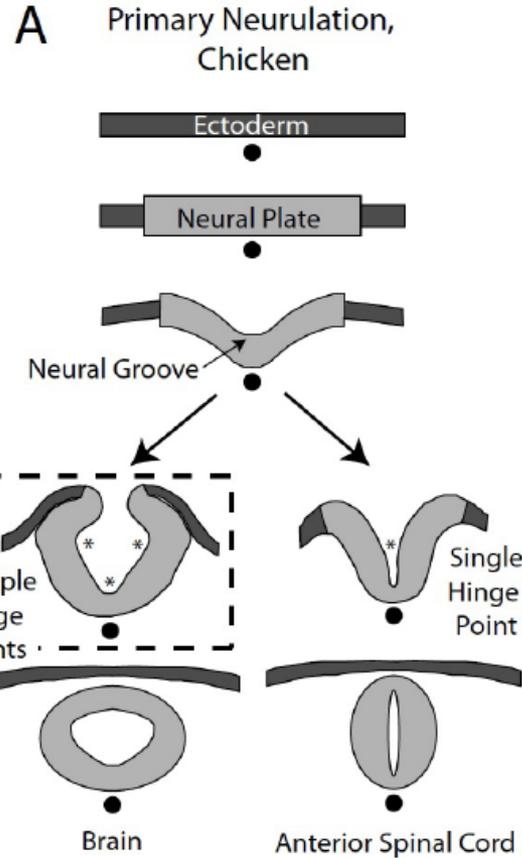
Neurulation



Neurulation

In chicken, mouse, human

Primary neurulation



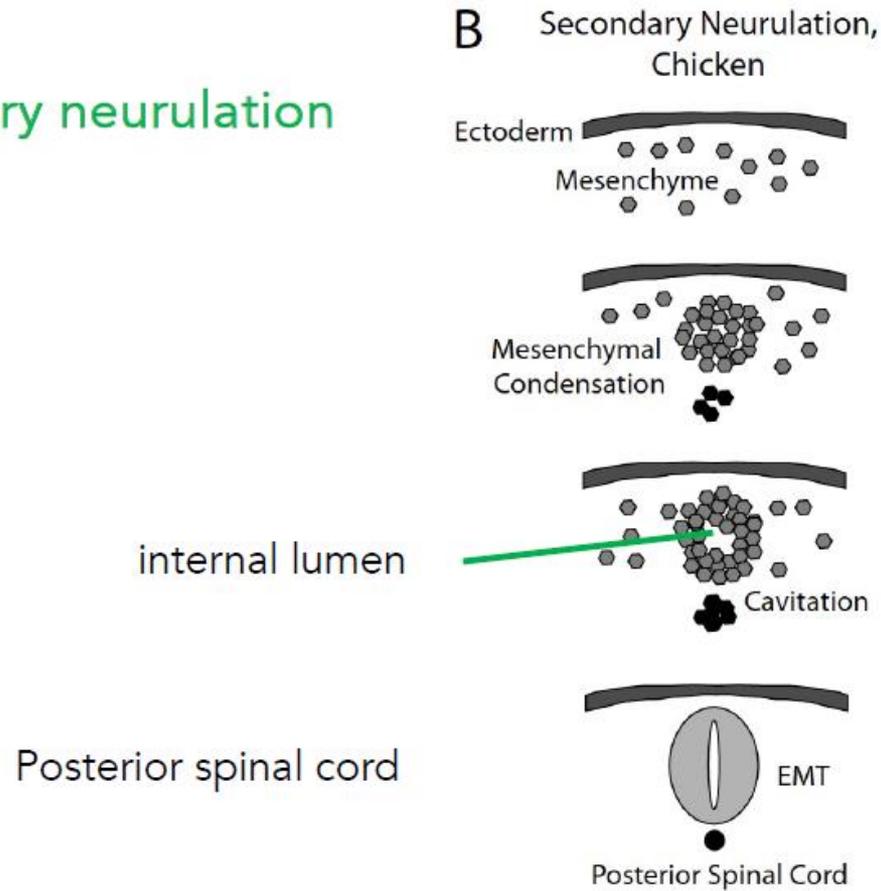
Closure facilitated by 1-3 hinge points

Brain & anterior spinal cord

Neurulation

In chicken, mouse, human

Secondary neurulation



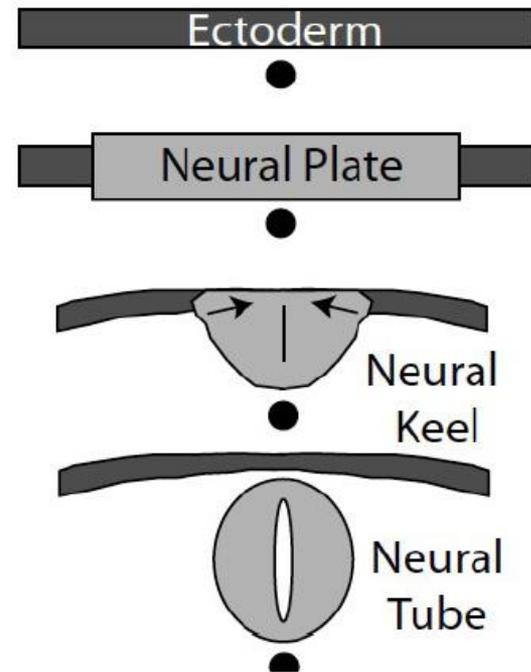
In Zebrafish, Xenopus

Primary and secondary neurulation differences are not so evident.

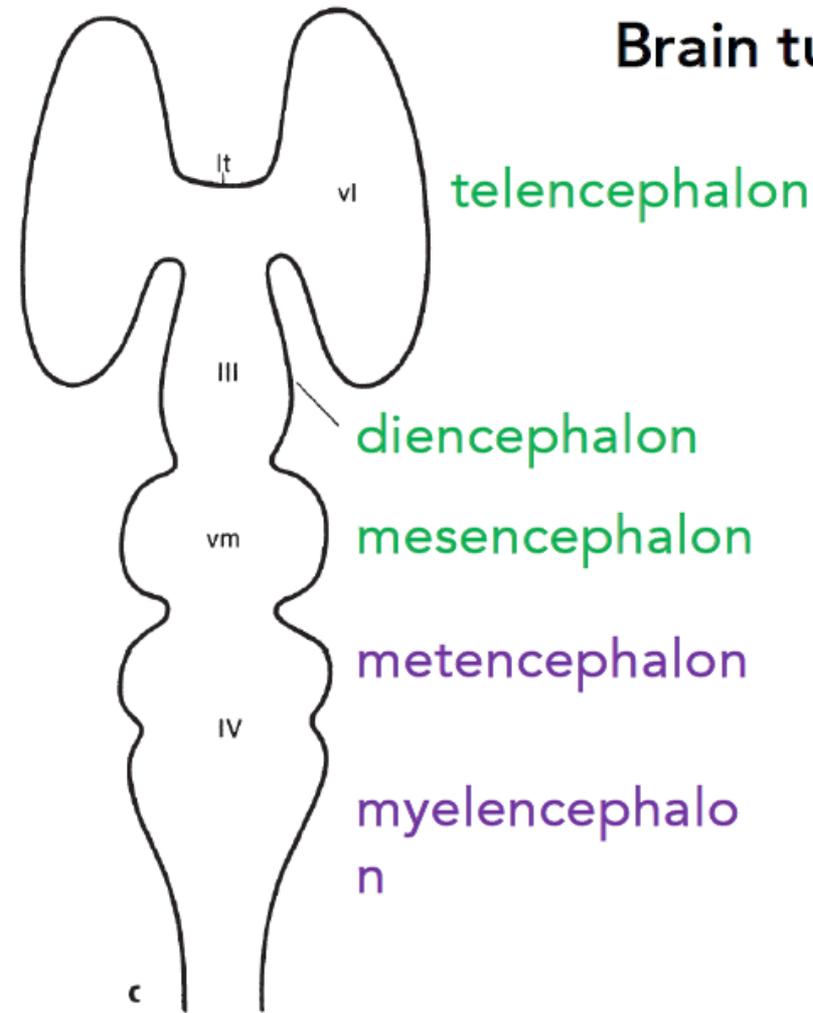
Neural precursors migrate medially
→ **neural keel**

It remodels to form a neural tube.

C Zebrafish Neurulation



Brain tube morphogenesis

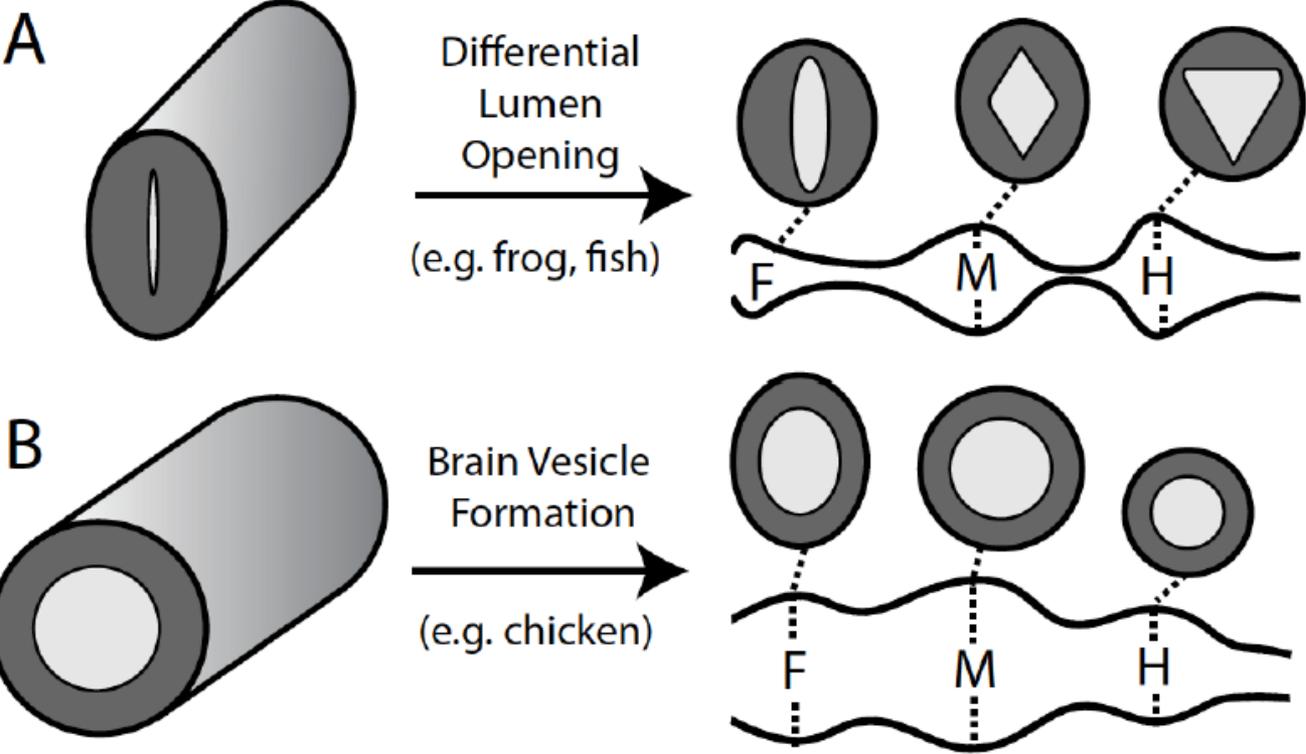


Prosencephalon (forebrain)

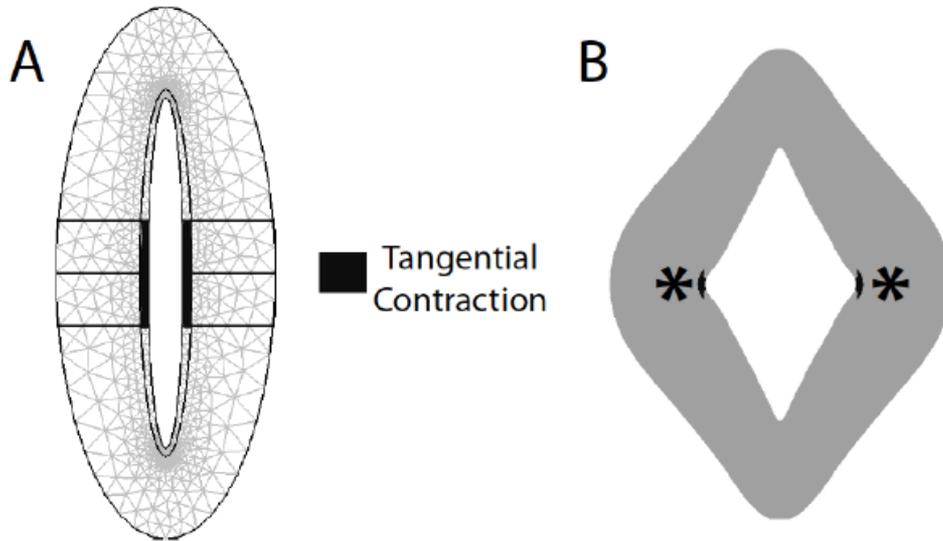
Mesencephalon (midbrain)

Rhombencephalon (hindbrain)

Lumen opening

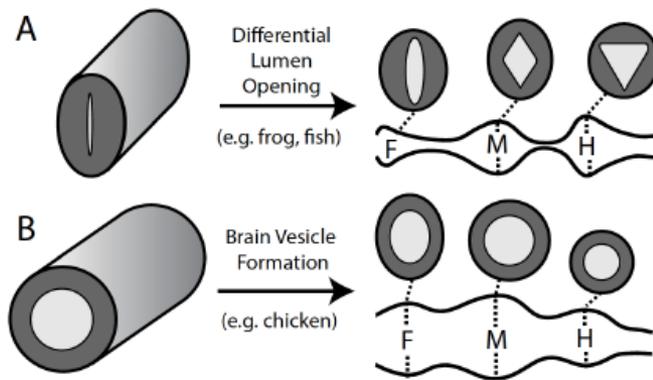


Which mechanisms regulate lumen opening?



- Early active contraction (inhibiting myosin by blebbistatin exposure prevents this process)
- Later relaxation to facilitate expansion (in response to fluid pressure in the lumen)

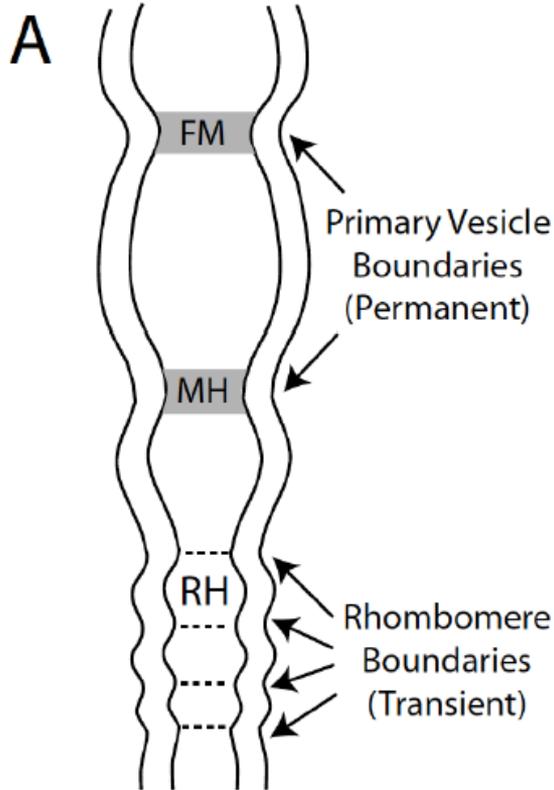
Brain vesicle formation



Differential lumen opening set the initial pattern for brain vesicles.

Actomyosin can remodel the tube into its 3D structure.

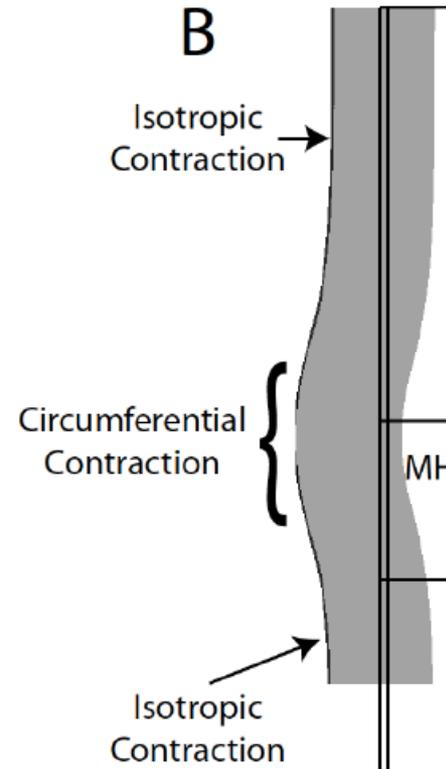
Brain vesicle formation



Primary brain vesicles separated by the permanent **fore-midbrain (FM)** and **mid-hindbrain (MH)** boundaries.

Rhombomeres (RH) are transient, sequential bulges in the early hindbrain.

- Extrinsic forces
they come from surrounding tissues
and shape brain tube
- Intrinsic forces in the developing neural
tube



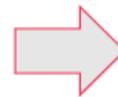
Rhombomere formation

Rhombomeres: periodic bulges in the hindbrain, regions of cell lineage restriction and differential gene expression

In **chicken embryo**

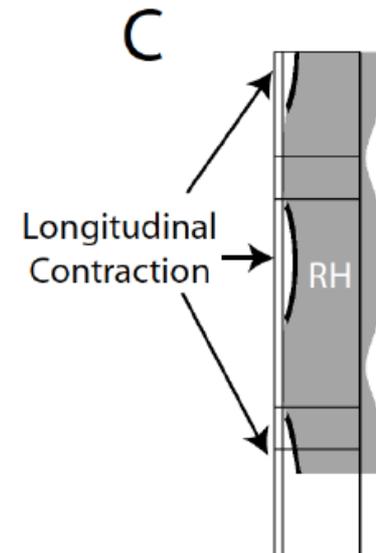
Cell proliferation rates, apical F-actin concentration, and extracellular space are increased in boundary regions

Extracell. space or apical contraction
(at the boundaries)



Rhombomere
formation

- 1) pattern of axonal migration
- 2) Cell differentiation
- 3) Gene expression

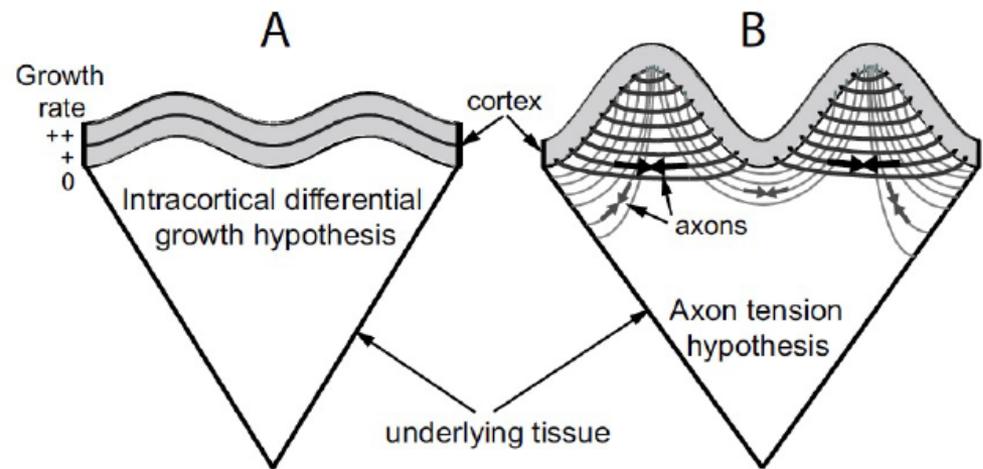


Cortical folding: different hypothesis

In large mammals,

Folding of the cortex begins after neuronal migration and proliferation.

- Primary folding patterns
- Secondary folds

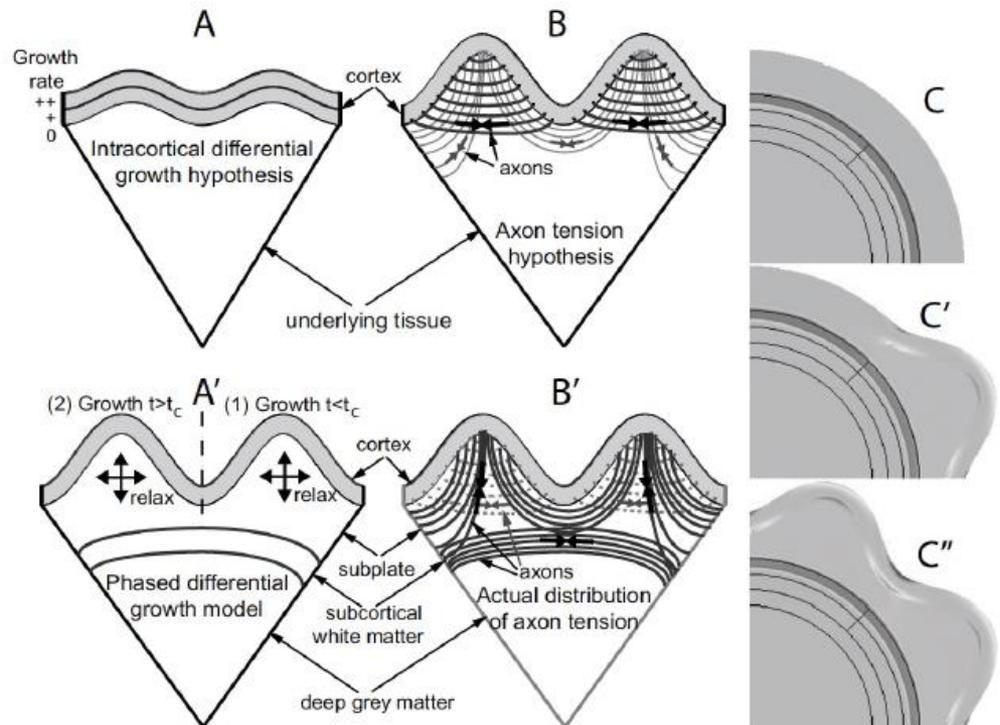


Cortical folding: different hypothesis

Different hypothesis

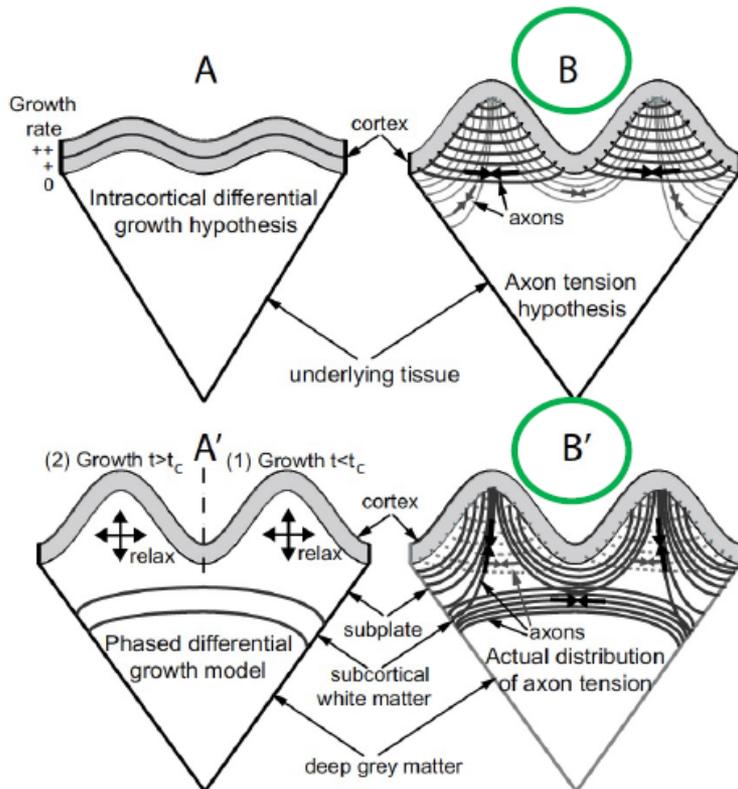
1. Brain grows faster than the skull
(compressive forces on the brain, causing folding)

BUT exp show the brain can fold without external constraints



Cortical folding: different hypothesis

Phased differential growth as a mechanisms for cortical folding



3. Axonal tension hypothesis

Tissue dissection from folding **ferret brain**

Axonal tension is significant but the principal directions of this tension are different from those predicted by axon tension hypothesis, so it cannot play a mechanistic role during folding.

Evolution of brain developmental plan: Insights from Agnathans



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Review

Evolution of the brain developmental plan: Insights from agnathans

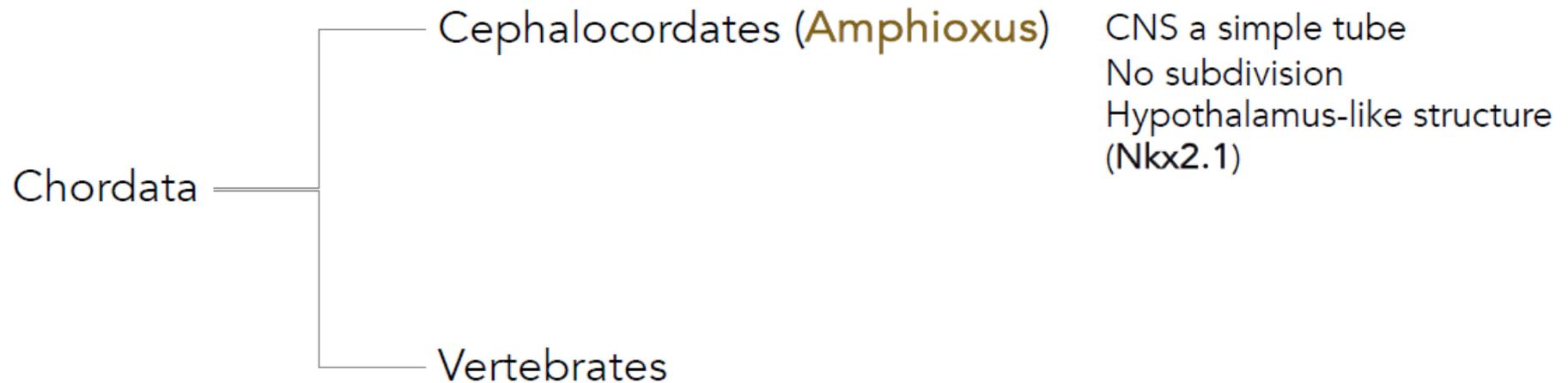
Yasunori Murakami^{a,*}, Katsuhisa Uchida^b, Filippo M. Rijli^a, Shigeru Kuratani^b

^a*Institut de Génétique et de Biologie Moléculaire et Cellulaire, UMR 7104, CNRS/INSERM/ULP, Illkirch Cedex, CU de Strasbourg, France*

^b*Evolutionary Morphology Research Team, Center for Developmental Biology (CDB), RIKEN, Kobe, Japan*

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Evolution of brain developmental plan: Insights from Agnathans

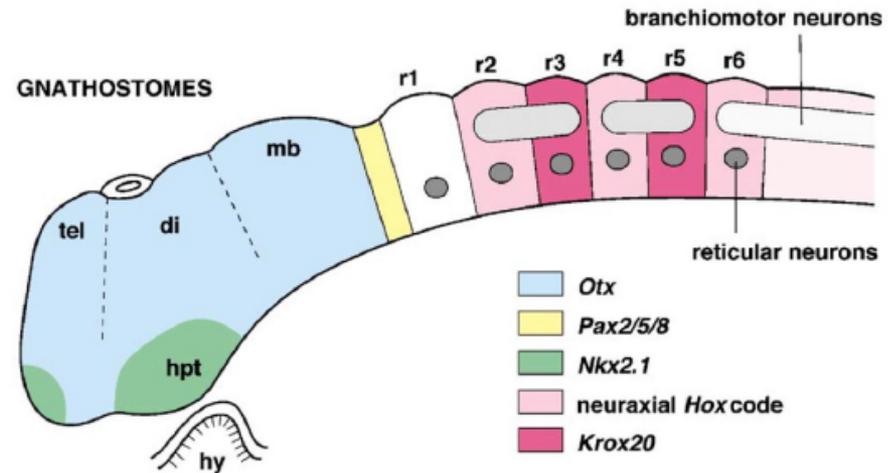


Neuromeres, regulatory genes and evolution of brain development plan

rhombomeres

Developed as compartments inhibiting cell movements into adjacent segments.

In vertebrates: Hox and Krox20



Neuromeres, regulatory genes and evolution of brain development plan

Amphioxus neural tube

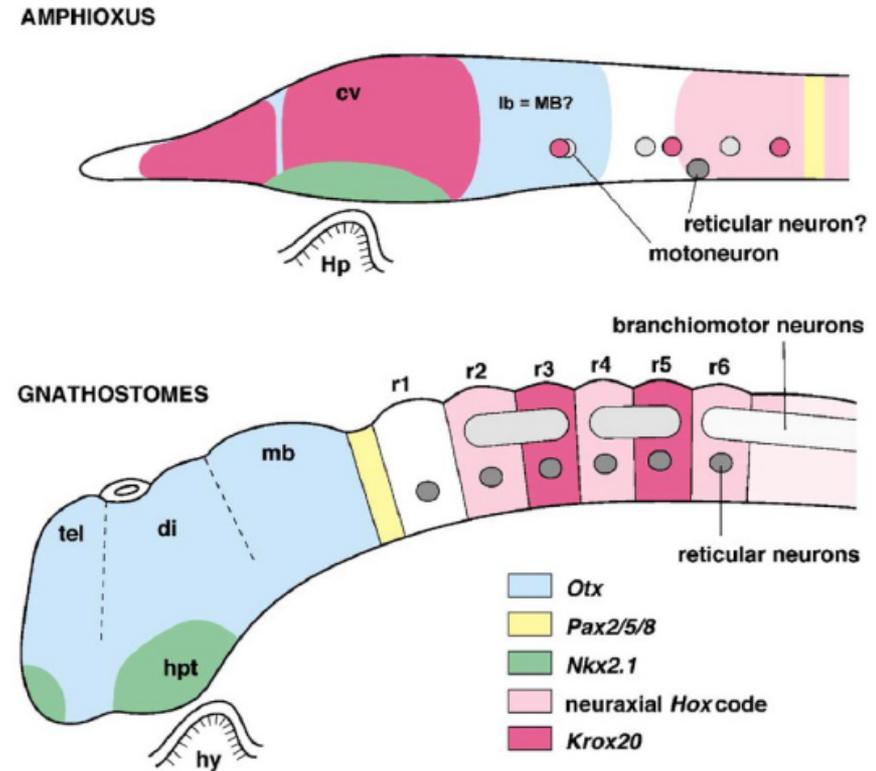
has domains that can reflect homology to the vertebrate brain.

SIMILARITIES

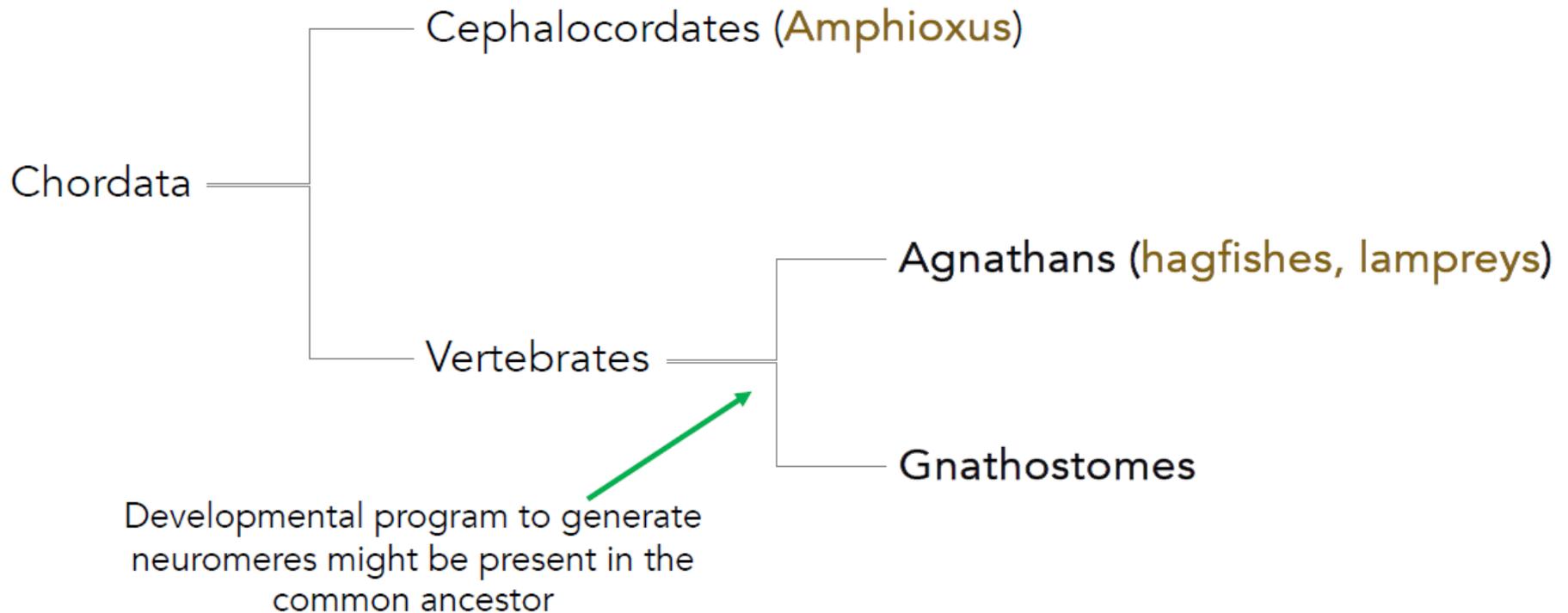
- Vertebrate hindbrain-like domain (Hox)
- Islet gene is iterated as in vertebrates

DIFFERENCES

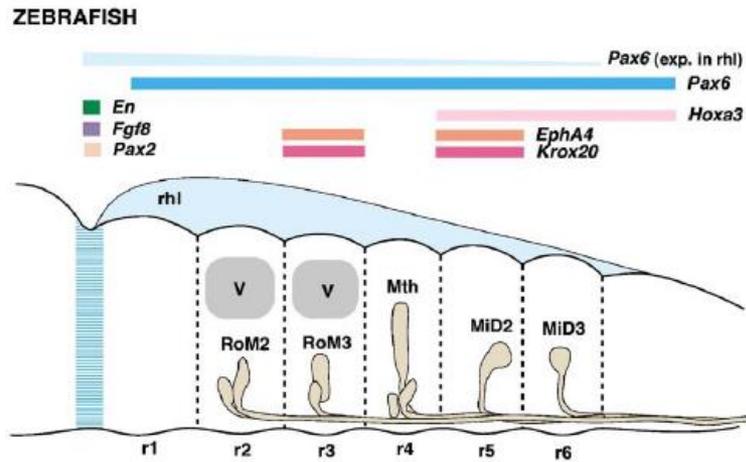
- AmphiKrox20 not striped expression pattern & part. Overlaps w/ AmphiHox
- Truly segmented domains not present



Comparison of Gnathostomes and Lamprey (Agnathans)



Comparison of Gnathostomes and Lamprey (Agnathans)



Example 1. Mauthner neuron (reticular neurons)

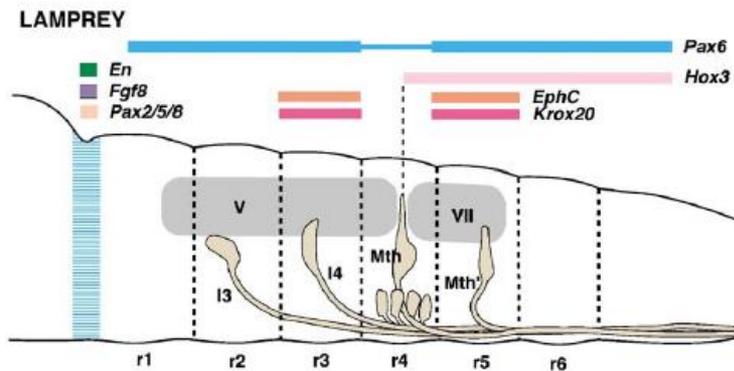
Function: escape response (stimulate contralateral motor activity)

Reticular neuron axons into spinal cord

They develop in association with rhombomeres in both
Mauthner neurons develop always in r4 in both.

Example 2. Krox20 & Eph expression

Expressed in r3 and r5 in lamprey, similar to gnathostomes.



Evolution of the coupling of segmentation, neuronal specification and Hox expression in the hindbrain

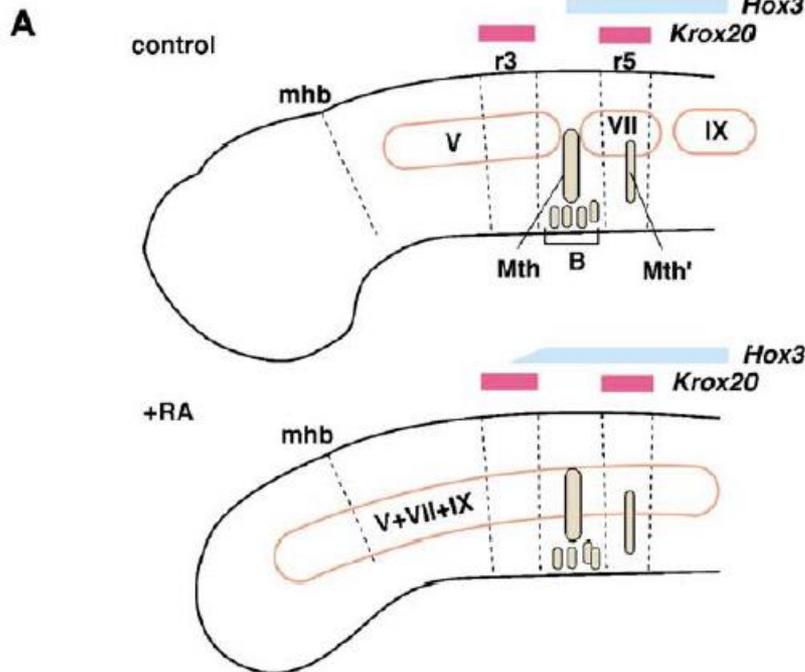
Gnathostomes

1. Motor nuclei & cranial nerves are generated in association w/ rhombomeres
2. Transition trigeminal –facial motor neurons at r3-r4 border
3. Similar to lamprey

Lamprey

1. Cranial motor nerve roots innervate individual branchial arches
BUT
They are not in register w/ rhombomere boundaries
2. It occurs at middle r4 (LjHox3)
3. RA exogenous admin. to lamprey embryo induces rostral shift of LjHox3 & posteriorization of branchiomotor neuron identity

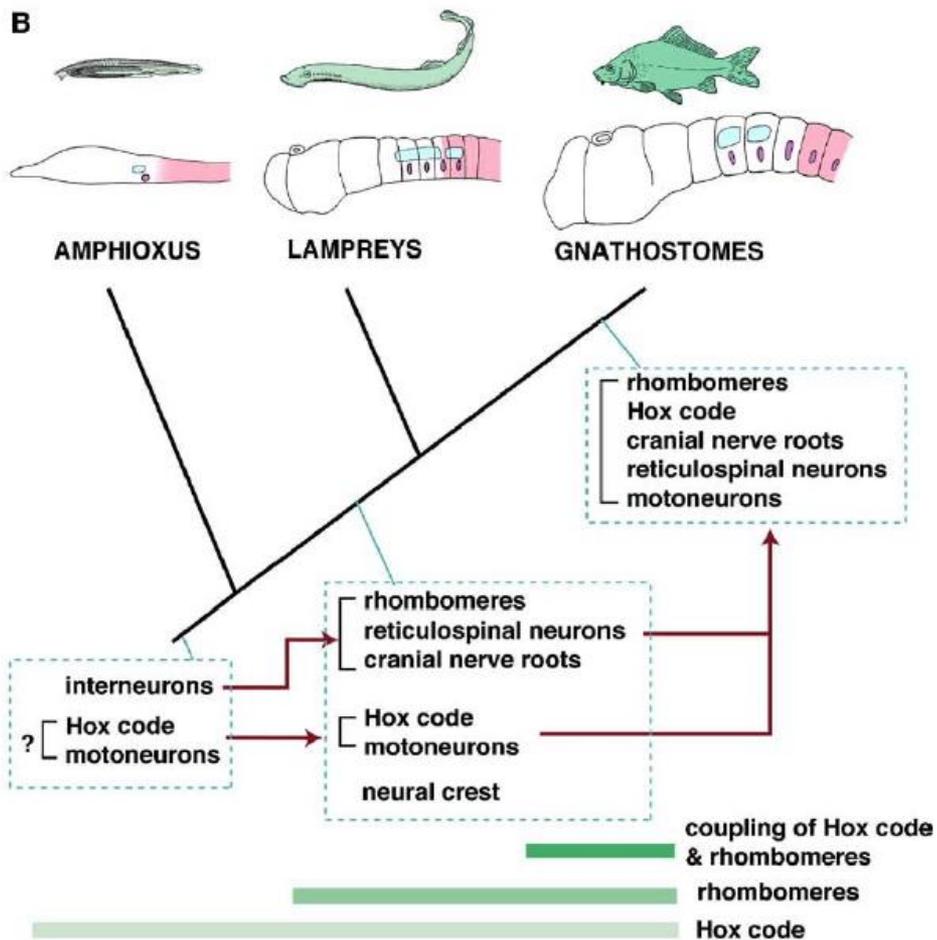
Evolution of the coupling of segmentation, neuronal specification and Hox expression in the hindbrain



Lamprey

3. RA exogenous admin. to lamprey embryo induces rostral shift of LjHox3 & posteriorization of branchiomotor neuron identity, Similar to: **Gnathostomes**

In lamprey, variations of Hox-dependent branchiomotor neuron identity, along A/P axis do NOT seem to be constrained by hindbrain segmentation (similar to gnathostome spinal cord, where Hox genes are required for motoneuron positional specification, despite the absence of neuromeres),



Lamprey:

2 independent programs in the hindbrain

Gnathostomes:

Only one combined program

Hox is ancestral (present in Amphioxus already)

Evolution of the cerebellum

Gnathostomes: Midbrain-hindbrain boundary (MHB)
Fgf8 patterns cerebellum

Midbrain-hindbrain boundary (MHB)
LjFgf8/17 expression

Lamprey:

It does NOT have Purkinjee cells and cerebellar nuclei or cerebellar & pre-cerebellar systems
That requires **Pax6** (lampreys does not express Pax6 in the rhombic lip)

Cerebellum might have appeared in Gnathostomes thanks to Pax6 in the rhombic lip.

Evolution of the telencephalon

Telencephalon is the most recent brain structure.

Amphioxus: no telenc.

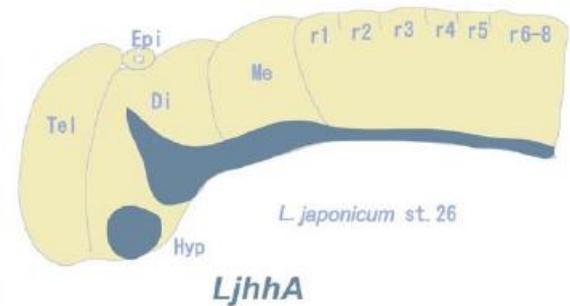
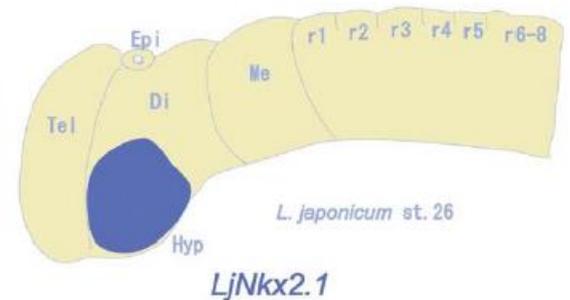
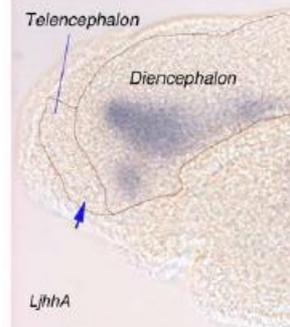
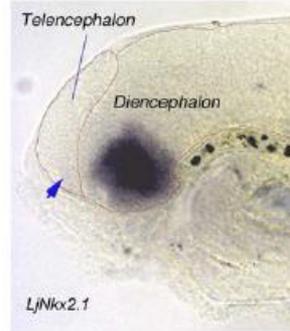
Lamprey: have overt telencephalon
express Pax6, Emx

Similar pallial structure but cortex and
hippocampus poorly evident;

Small dorsal thalamus

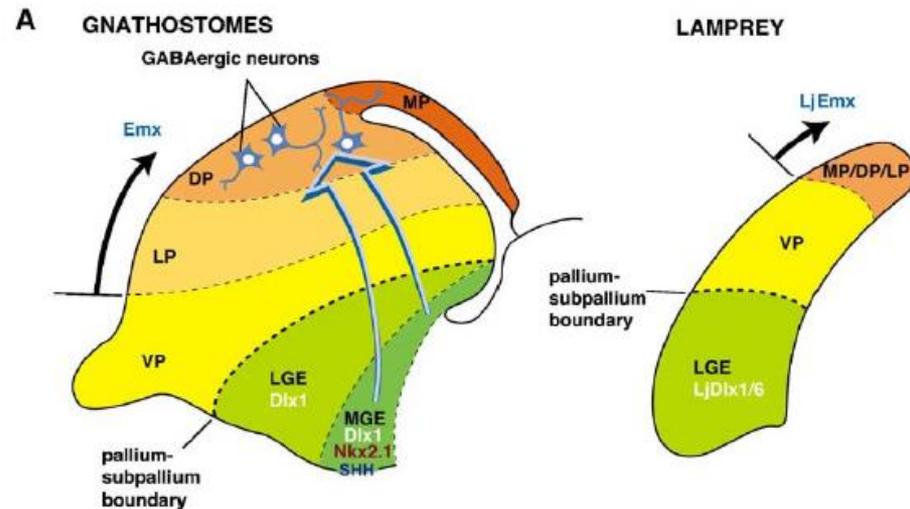
Ventral thalamus express LjDlx1/6 and
not Nkx2.1.

B

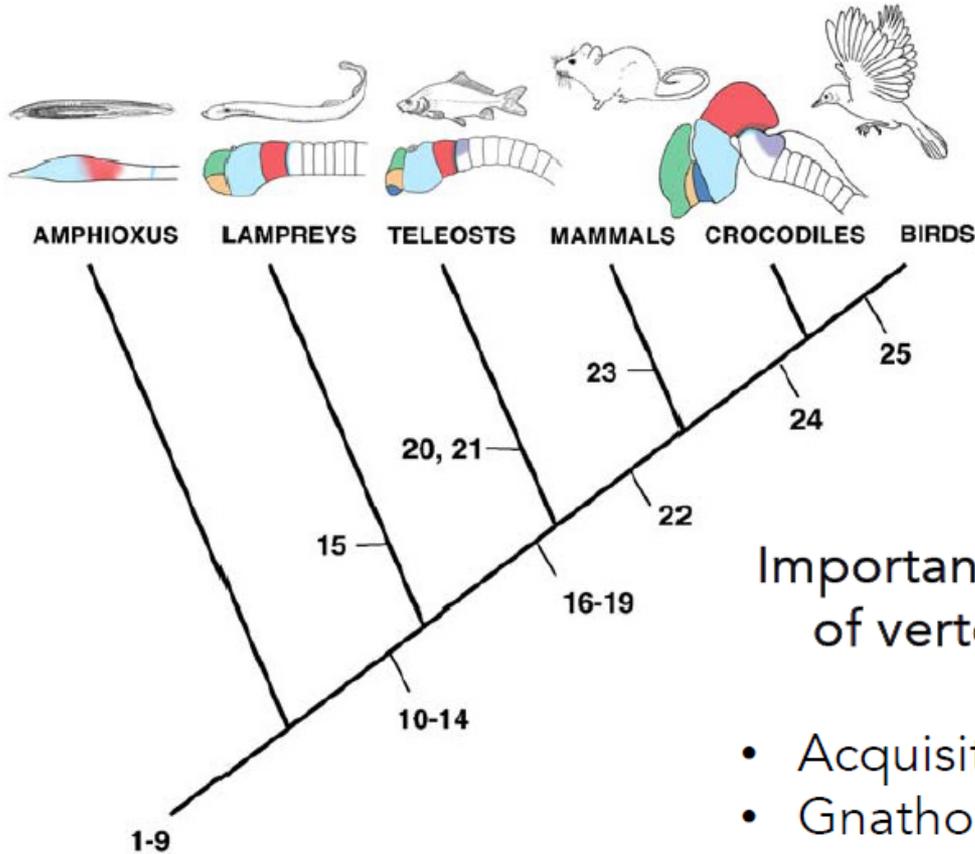


Evolution of the telencephalon

Lamprey: have overt telencephalon
express Pax6, Emx
Similar pallial structure but cortex and
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Small dorsal thalamus
Ventral thalamus express LjDlx1/6 and
not Nkx2.1.



Conclusion



Important changes acquired around the split of vertebrates from ancestral chordates:

- Acquisition of neuromeres
- Gnathostome-like cerebellum & telencephalon





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