Universitat Autònoma de Barcelona

The cerebellum: the paradigm of neurogenesis

Brenda Gavilán Aguilar[1]
[1]Grau en Genètica, Universitat Autònoma de Barcelona, brenda.gavilan@e-campus.uab.cat

1. Introduction to the cerebellum

The cerebellum is an ***ideal paradigm to study neurogenesis*** *because it has a limited number of neuronal types and they are very characterized. Furthermore, all the cells derive from two main germinal centres. The objective of this review is to* ***summarize the development of the cerebellum giving special stress on the genetic factors involved in the determination of the cerebellar territory and in the formation of different cell types.*** *This review is based on experiments performed in* ***mice and rats****, since it is a highly conserved process, the general mechanism can be extrapolated to humans.*

1.1 Localization, function and morphology

Localization: **central nervous system**, specifically in the **hindbrain**, at the inferior-posterior part of the cranial cavity (Fig. 1). Present in all vertebrates, but more evolved in mammals.

Functions: related to equilibrium, fine coordination of posture maintenance and locomotion and behavioural as well as emotional processes.

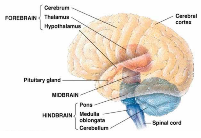

Fig. 1. Schematic representation of the main components of the central nervous system. The cerebellum forms part of the hindbrain and it is connected to the brainstem and to the spinal cord through three peduncles. (©Addison Wesley Longman, Inc.)

Morphology: the mammalian adult cerebellum consists of **two lateral hemispheres** connected to each other through the **vermis** (Fig. 2).

- Sagittal cut in the vermis: 4 primary fissures that divide it into **5 lobes**, that are subdivided into lobules and sublobules. (Fig. 3).
- Section in an hemisphere: **3 lobules** called simplex, ansiform and paramedian.

This structure is called folia, and **foliation** occurs in a stereotypical manner conserved among vertebrates.

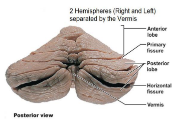

Fig. 2. Posterior view of the mammalian adult cerebellum. (http://antranik.org/central-nervous-system-intro-to-brain-and-ventricles-medulla-oblongata-pons-mid-brain-and-cerebellum/)

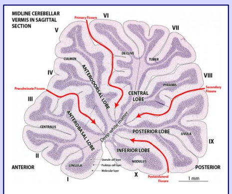

Fig. 3. Sagittal section of the vermis. It is shown the position of the 5 lobes separated by the 4 primary fissures. The lobules are indicated with roman numbers. (http://braindevelopmentmaps.org/taxonomy/term/6/).

This enlarges the surface area allowing a higher number of neurons to be organised into the cortex. Thus, there is an increase in the complexity of the neural circuits, and, consequently, in the processes that the cerebellum is involved in.

1.2 Histological organization in the adult cerebellum

White matter - underneath the cortex: myelinic nervous fibres and glial cell in a tree-shaped structure.

Grey matter - different kinds of GABAergic and glutamatergic neurons and glial cells.

- **Deep cerebellar nuclei (DCN)** – in the white matter: GABAergic interneurons and GABAergic as well as glutamatergic projection neurons.
- **Tri-layered cortex** (Fig. 4) - outer structure:
  - **Molecular layer** - parallel fibres and several GABAergic interneurons.
  - **Purkinje cells monolayer** (GABAergic projection neurons) and Bergmann glia.
  - **Internal granule layer** (IGL) - granule cells and interneurons.

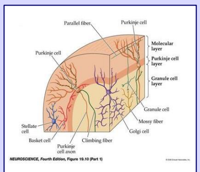

Fig. 4. Distribution of the main cellular types within the adult cerebellar cortex. (http://kin450-neurophysiology.wikispaces.com/Cerebellum)

Neural circuits
Extra-cerebellar inputs: 2 main afferents systems, mossy and climbing fibres, which will converge on Purkinje cells. Moreover, in the three layers we can find inhibitory and excitatory interneurons that interact with each other.
Output: is transmitted by the Purkinje cells which are projected to the cerebellar nuclei.

2. Cerebellar development

Inheritance of key factors in each specific lineage + Precise pattern of **environmental cues** following specific spatial-temporal schedule = **Cerebellar neurogenesis**

2.1. Determination of the cerebellar territory

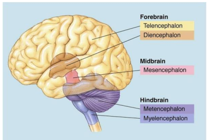

Fig. 5. Adult central nervous system that will be formed from the vesicles of the neural tube. The cerebellum, together with the pons, belonging to the metencephalon and this to the hindbrain. (http://mikeclaffey.com/psyc2/images/organization-fore-hind-brain.jpg).

The different parts of the brain (Fig. 5) arise from the anterior part of the **neural tube.** A combination of different **transcription factors will pattern the five brain vesicles** that will form the different organs.

During the early development (embryonic day E8.5 and E9.5) of the neural tube the hindbrain is transiently fragmented into segments called **rhombomeres** whose borders align with the expression boundaries of certain **Hox genes.**

The cerebellar territory arises from the **rhombomere 1** between the domains of *Otx2* and *Hoxa2*. Anterior to this territory, there is the mesencephalon that will differentiate into the tectum. The **isthmus** is a constriction between them that is considered an **organizing centre** for both. The position of it will be determined by the expression domain of ***Fgf8***, and this depends on the cross-regulating mechanism described in Fig. 6.

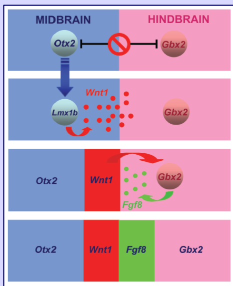

Fig. 6. Cross-regulating mechanism that determines the cerebellar territory. *Otx2* is expressed in the midbrain and *Gbx2* in the hindbrain, they repress each other creating two separated territories. *Lmx1b* is induced by *Otx2* in the midbrain, where Lmx1b represses *Fgf8* cell-autonomously, but triggers the expression of *Wnt-1*. Secreted Wnt-1 stimulates the expression of *Fgf8* in neighbouring cells. As Gbx2 represses Lmx1b, the expression of *Fgf8* is only found in the border between *Otx2* and *Gbx2*. There, we find narrow rings that express *Wnt-1* or *Fgf8* that determine the rostral and caudal part of the isthmus respectively. Moreover, Gbx2 promotes the development of the cerebellum in the metencephalon (hindbrain) through the suppression of *Otx2*. (B. Carletti et F. Rossi, 2008).

Fgf8 **presents two splicing isoforms** with different strength in the signal that they transduce.

- **Fgf8b** is 33 base pairs longer that **Fgf8a** (phe32 allows stronger binding to receptor).
  - **Fgf8b** strong signal present: cerebellar development.
  - **Fgf8a** weaker signal present: tectum development.

Also, *Otx2* raises the threshold of Fgf8 activity required for developing cerebellar trait.

Other genes involved (Fig 7):

En1/2 and Pax2/5
- Redundant functions creating a **positive feedback loop with *Fgf8*** allowing the maintenance of each other's expression in the boundary between mesencephalon and the rhombomere 1.

Grg4
- **Antagonize with the isthmus activity** repressing *Fgf8*, *En2* and *Pax5*. It also participates in the precise positioning of the isthmus repressing *Pax2*, which is essential for the induction of Fgf8.

Ras-ERK signalling pathway
- Fgf8 signal is transduced by Ras-ERK signalling pathway, **phosphorylating Irx2**, an activator of the cerebellar development in the rostral hindbrain (Fig 7).

Sprouty genes
- **Regulate negatively Ras-ERK** in the midbrain maintaining the appropriate level of Fgf8 signalling necessary for normal growth and patterning.

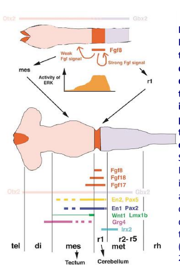

Fig. 7. Localization of the expression domains of the different genes that participate in the precise positioning of the isthmic constriction. Strong signal Fgf8 activates, in the required amount for cerebellar development, the ERK activity. (T. Sato et al. 2004).

At E9.5 the ***closure of the neural tube*** *is complete with the exception of certain areas like the boundary between the mesencephalon and the metencephalon, the rhombic lip.*

2.2. Germinal centres

The next phase of the development consists in the formation of **two germinal compartments**, the ventricular zone (VZ) and the rhombic lip (RL), this begins between E9 and E11. It is followed by the **specification of the different classes of the cerebellar cells**. At this stage, the cerebellar anlage is formed by two symmetric bulges placed dorsolateral that will eventually fuse in the primitive cerebellar plate. The inner and outer germinal layers of this cerebellar plate constitute the VZ and the rostral RL.

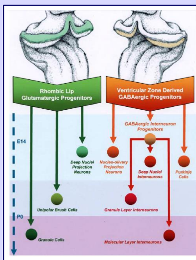

Fig. 8. Origin and approximate time formation of the main neuronal types of the cerebellum classified according to its origin. Depending on if the neuroepithelium where the progenitor comes from is VZ or the RL, the neuron will have GABAergic or glutamatergic neurotransmitter phenotype respectively . (K. Letto et al. 2006).

The generation of the neuronal types is well **compartmentalized** (Fig. 8). However, at birth these neuroepithelia disappear and the dividing precursors migrate to the white matter or along the pial surface.

There is also a **restriction in time**, the neurons are generated following a precise time schedule (Fig. 9).

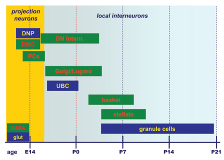

Fig 9. Schedule that the different neuronal types follow in the cerebellar development. Projection neurons arise first, whereas the local interneurons are generated during late embryonic development and early postnatal. *Green: GABAergic. Blue: glutamatergic. DNP: deep nuclear projection neurons. DNO: nucleo-olivary projection neurons. PCs: Purkinje cells. DN intern: deep nuclei interneurons. UBC: unipolar brush cells.* (B. Carletti et F. Rossi, 2008).

2.2.1. Neuronal populations from the ventricular zone

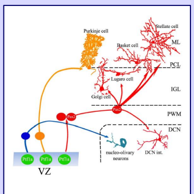

Fig. 10. Different pools of *Ptf1-a*-expressing cells and the final localization of the GABAergic neurons that originate. There is a correlation between interneurons birthdate and their placement, so in the cortical layers the earliest-born are locate deeper whereas the later-born settle in more superficial positions. (K. Leto et al. 2012).

The **GABAergic** progenitors are characterized by the expression of ***Ptf1-a*** which is needed to avoid the default granule cell development program. The progenitors are organized in **microdomains** (Fig. 10) distinguished by different expression profiles. (Ex: progenitor cells expressing *Neurogenin 1* and *2* → projection neurons).

Projection neurons
- Are born between E10.5 and E12.5.
- **Nucleo-olivary projection neurons** (DNO) and **Purkinje cells** (PCs).
- Acquisition of mature phenotype through **cell-autonomous mechanisms**.

Inhibitory interneurons
- Are originated from a single population of ***Pax2***-expressing since E13 cells.
- The types of inhibitory interneurons with different expression markers are: **basket, stellate, Golgi, Lugaro** and **candelabrum** in the cortex and the **deep nuclear interneurons**.
- These cells delaminate into the prospective white matter and divide up to the postnatal (P) development. They maintain full potentialities until P15 when they will maturate because of **environmental cues**. This experience-dependent refinement of local circuit has a critical role in the cortical plasticity.

We can also find **astrocytes** and **oligodendrocytes** precursor cells.
- Oligodendrocytes and GABAergic interneuron precursors express ***Ascl1*** → but they are not related.
- Common precursor between GABAergic interneurons and astrocytes, both expressing ***Gfap***. *Ascl1* who determines the fate choice enhancing the generation of interneurons.

2.2.2. Cerebellar neurons originated in the rhombic lip

RL is located **between the IV ventricle and the roof plate**, in an **opening of the neural tube**. Its progenitors express ***Math-1***, and will give rise to **glutamatergic** neurons. This factor is expressed since E9.5 and provides essential information for this lineage. It is dynamically regulated by the **antagonistic interaction** between ***Notch1*** signalling in the cerebellar primordium and **BMPs** secreted by the roof plate.

The fate-restricted cerebellar precursors leave the RL in **three migration waves**:

- From E10.5 to E12.5 **glutamatergic projection deep nuclear neurons** spread rostrally in direction to the subpial position to the nuclear transitory zone. While they are migrating, they express *Pax6*, *Tbr1* and *Tbr2*.
- From E14 to E21 progenitors of **unipolar brush cells** (UBC) and **glutamatergic interneurons of the granular layer** migrate either rostrally or dorsally. UBC transiently go to the white matter before their final homing in the internal granular layer. They also express *Tbr2*.
- From late embryogenesis to early postnatal life **granule cell progenitors migrate tangencially** along the cerebellar surface and form the external granular layer **(EGL)** (Fig. 11). They express *RU49*, *Zic1* and *Zic3*. In E13 the progenitors are already committed to granule cell fate, but they need these and other extrinsic signals from the EGL to maturate.

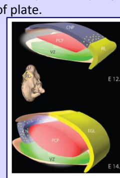

Fig. 11. Migration model of the main cerebellar neuronal types. Formation of the EGL. (D. Morales et M. E. Hatten, 2006).

2.3. External granular layer

The EGL is generated between E15 and P15 by the **granule cell precursors** coming from the RL and covers the entire cerebellar surface. It has two sections, the outer half (**proliferative**) and the inner (**pre-migratory**) .

Purkinje cells stimulate the proliferation of granule cells secreting **sonic hedgehog** (Shh) that has a crucial role in embryonic and adult brain patterning (Fig 12). The **targets of this pathway** are cell cycle regulators and SHH pathway factors.

Granule cell precursors, experiment a proliferation peak at P5-P8 and thereafter declines and stops at P15. This is because when they reach the inner most part of the EGL (the premigratory) the **response to Shh is switched off** thanks to specific **glycoproteins** (laminin in outer and vitronectin in the inner) or **accumulation of cell cycle inhibitors in the inner EGL.** Post-mitotic granule cells migrate to the internal granular layer (**IGL**) apposed the Bergmann glia, whose maturation is induced by Shh as well.

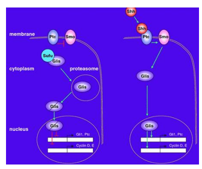

Fig. 12. Shh pathway signalling. *Left: in absence of Shh.* PTC inhibits SMO which is not translocated to the primary cilia and Gli3 is constitutively cleaved and converted to its repressor form. *Right: when Shh is present.* Shh binds to PTC, SMO is not inhibited and it is accumulated in the cilia. cAMP production stops and Gli3 is not cleaved and it becomes a transcriptional activator together with Gli2 and Gli1.(C. Vaillant et D. Monard, 2009).

Remarks:
- **Gli3R** (Shh pathway) is needed for the *Fgf8* expression in the isthmus, so it has an **integrative role** between such crucial pathways in cerebellar development.
- **Foliation patterns** observed in the cerebellum and the **great growth** experimented are caused by the granule cell proliferation and it is controlled by the amount of Shh signalling.
- The disposition of the **fissures** is also genetically determined but the responsible pathway is not known yet.

2.4. Overall view (Fig. 13)

1. Determination of the **isthmic constriction** in the neural tube (IsO in the picture) at the rhombomere 1.
2. Formation of the two main **germinal neuroepithelia** (VZ and RL) indicating the migration direction of their precursors.
3. Formation of the **EGL and the *Shh* signalling** coming from the PCs that permits the growth and the foliation.
4. In the last picture, the **granule cells have reached the IGL** and we can observe the final shape of the cerebellum.

So, all in all, it is a very coordinated and precisely regulated process that has some interesting characteristics that makes them a paradigm for neurogenesis that helps us to understand better such a complicated process. However, there are still a lot of questions that need to be solved to understand the process completely.

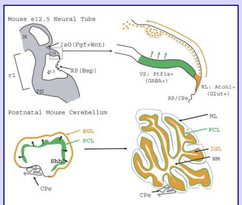

Fig. 13. Schematic view of the overall of the cerebellum development. (Atoh1 = Math-1). (K.A. Aldinger et al. 2009).

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