Study of the Embryonic Cerebellar Development in the Weaver Condition

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1.Introduction: "Setting the scene"

WHAT WERE THE INITIAL FACTS?

- Cerebellum's development at the prenatal period is complex.
- Cerebellar disorders are involved in pathological situations.
- **Cerebellar Ataxia** is one of these diseases.
- There is a few information about prenatal events and ataxia.
- A deeper study is needed and required for hypothesis formulation.

WHAT ARE WE TRYING TO ACCOMPLISH?

- Study how some proteins are related to cell proliferation.
- Establish how depletion of Purkinje cells (PC) or their neuroblasts affects the proliferative behaviour of granule cells precursors (PGC).
- How prenatal alterations are involved in the weaver's fenotype observed in the cerebellar system.

WHICH ACTIVITIES DID WE CARRY OUT?

- Focus the study at the prenatal period of the homozygous weaver mice.
- Assess specific proteins for their main role in the weaver's condition.

SOME RESULTS ACHIEVED

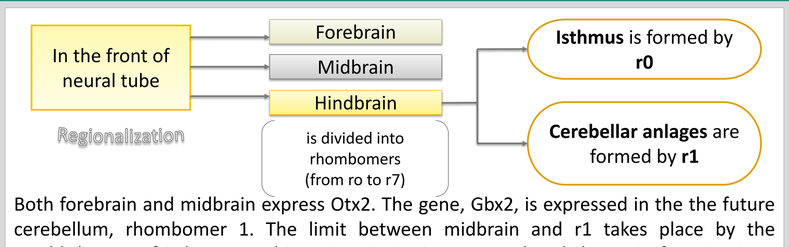
- **Relationship among certain proteins and neuroblasts of PC and granule cells (GC) proliferation might explain adult cerebellar ataxia.**
- Set objectives and formulate the 3 main hypothesis.
- Personal and academic development about ataxia knowledge.
- Principles, methodology, materials and the tutor are key facts to success.

2. What do we know about cerebellar ataxia?

Fig. 1: Artwork about a child with ataxia. The author wants to represent typical impaired walk. (Obtained from Ana Fernandez).

Cerebellar ataxia is a group of neurological disorders showing unbalanced motor movements and a lack of coordination (figure 1). Symtomps are related to oculomotor failures, like kinetic tremor, dysarthria and dysmetria. Futhermore, there are learning deficiencies. In this disorder, it's important to differentiate sporadic ataxias from inherited ataxias. This project is focused on the last ones, and it is supported by a mice' strain that has a pleiotropic mutation. Specially, we refer to a weaver's model.

3. An awesome architectural development



Both forebrain and midbrain express Otx2. The gene, Gbx2, is expressed in the the future cerebellum, rhombomer 1. The limit between midbrain and r1 takes place by the establishment of isthmus. In this region FGF-8 is expressed and the main function is to remove the expression of Otx2 (figure 2).
Isthmus becomes the organising centre of midbrain-hindbrain. Wnt 1 is localised at the back of midbrain. It plays a significant role, which is controlling proliferation and regulating the expression of En1/ 2 and r1.

Fig. 2: Main interactions in an initial stage of the cerebellum's development. The interaction between Gbx2 and FGF-8 has to take place, as well as, Otx2 and FGF-8. (Obtained from Martinez, 2013).

4. Specific origin of different neuron types

In the following stage, isthmus has become narrower and cerebellar anlages have expanded (figure 3). In this point, roof plate (RP) appears between the middle back of hindbrain and cerebellum. RP is really important for the future choroid plexus (Lim1a+).

Later, Atoh1 (=Math1) is expressed (figure 4).

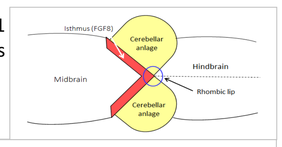
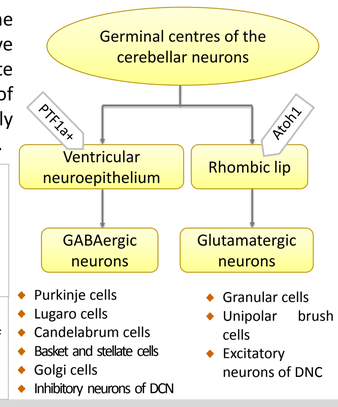


Fig.3: Sketch of the first cerebellum territories. Progressively, isthmus takes a curvature inside the brain. It is the beginning of the isthmus (medial) and cerebellar anlages formation (lateral). (Personal contribution from Lydia Carnicé.)



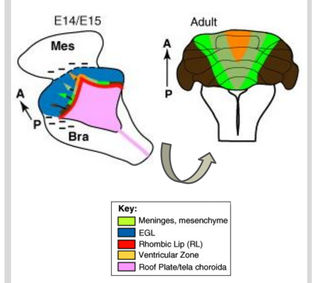


Fig.4: Representation of the main regions of the cerebellum. This picture shows the direction of the GC precursors during the embryonic period (E14/ E15). (Obtained from Chedotal, 2010.)

8. Next steps

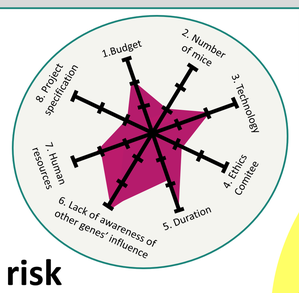
What we can do next?

1. Define a project to prove the hypothesis:
   - Feasibility report and analysis of risk (see an example).
   - Project Plan and activities for the test.
2. Execute and implement the project plan:
   - Do actions to prove and test the hypothesis.
   - The use of other models of hereditary ataxias such as Lurcher, Staggered and Pogo mice.
   - Measure and control of results to reach the object.
   - Validate hypothesis.

or

If the hypothesis are right, the challenge will be accomplished.

If they aren't valid, we will have to reformulate hypothesis.



Analysis of risk to next steps

What we can learn about cerebellar ataxia?

Hypothesis 1

Reduction of Purkinje cells

H1: High levels of E-cadherin are needed to delimit the territory of CP formation at the cerebellum.

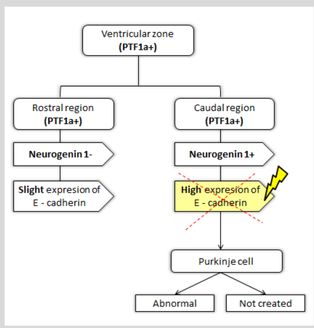


Fig. 10: The first hypothesis is based on the initial stages of development, when CP are created. High expression of E-cadherin is basic to delimit Purkinje cells territory. Any alteration that compromises funcionality of CP could have important consequences. So, it could result as not created or abnormal CP. (Personal contribution from Lydia Carnicé).

Hypothesis 2

Death of descendants

H2: The IGF-2 activity is necessary to strengthen the proliferative activity of SHH.

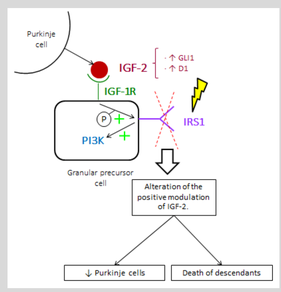


Fig. 11: Factor growing insuline-2 (IGF-2) is produced by CP. This factor is a positive regulator of the SHH pathway. It takes part in this process, improving GLI1 and D1 activities. IGF-2 binds to IGF-1R. Then IRS1 is activated by fosforilation. The last step is the activation of PI3K. If any mutation affects IRS1 structure, its function would be damaged and the number of cells would decrease. (Personal contribution from Lydia Carnicé).

Hypothesis 3

Purkinje cell and Deep Cerebellar Nuclei (DCN) relationship

H3: A correct morfology of CP is required for a precise information transmission to the cerebellum.

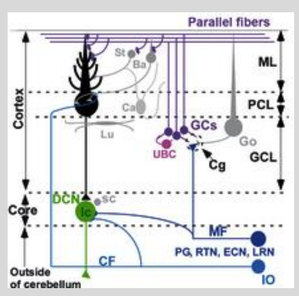


Fig. 12: Cerebellar circuits. CP are innervated by climbing fibers of inferior olive nuclei. Their axon is projected to a specific zone of DCN. The olivo-cortico-nuclear circuit is crucial for the formation of the different longitudinal compartments of cerebellum along medio-lateral axis. If the structure of CP is alterated, it could form an immature axon that coudn't arrive at DCN. The result could be a malformed cerebellum. (Obtained from Hashimoto, 2012).

Reformulate hypothesis

5. Homozigous weaver mice

Weaver is a semi-dominant mutation based on a unique based pair substitution of a serine to a glicine at the residue 156. This change affects a gene that codifies for a G-protein-activated inward-rectifying potassium channel (Girk 2). The importance of this event is the lost of the potassium selectivity. Consequently, there is an influx of Na^+ and Ca^{2+} ions. Many neurons are affected, such as dopaminergic neurons (pars compacta), PC and neurons of DCN. Most neurons' death is at the medial zone (vermis). In the postnatal period there are many relevant histological characteristics (figures 5, 6, 7 and 8). Weaver's death takes place in early stages, specially at the moment of weaning. Moreover, males are sterile and females can have offsprings

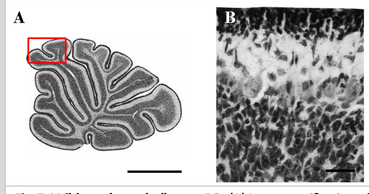


Fig. 5: Wild type's cerebellum at P8 . (A) Low magnification of the sagittal section cerebellar vermis from a wild-type mice. Scale bar: 1mm. (B) High magnification at the level of the culmen. Scale bar: 50µm. (From Cytology and Histology, Biosciencies – Hystotheque).

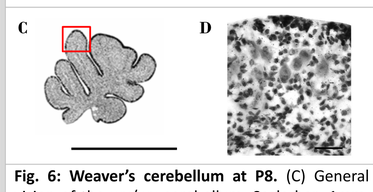


Fig. 6: Weaver's cerebellum at P8. (C) General vision of the wv/wv cerebellum. Scale bar: 1mm. (D) Enlargement of figure C (red square). Scale bar: 50µm The disorganitzation of cortex layers is obvious. In these histhological slides, the critical aim is to appreciate the drastic changes in both situations. (From Cytology and Histology, Biosciencies – Hystotheque).

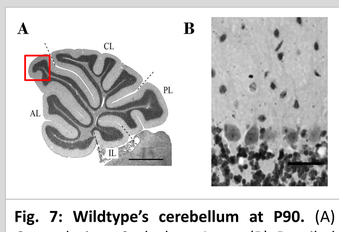


Fig. 7: Wildtype's cerebellum at P90. (A) General view. Scale bar: 1mm. (B) Detailed observation of figure A. All cortex layers are well-defined. Scale bar: 50µm. (From Cytology and Histology, Biosciencies – Hystotheque).

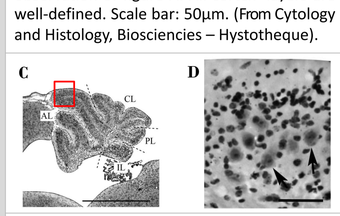


Fig. 8: Weaver's cerebellum at P90. (A) General view. Scale bar: 1mm. (B) Enlargement of figure C. Arrows are shown CP. Scale bar: 50µm. (From Cytology and Histology, Biosciencies – Hystotheque).

7. Analyse and formulate (Activities to achieve objectives)

For all objectives: Understanding all actual Knowledge, procedures & studies related to cerebellar ataxia

1 Some genes that are implicated during prenatal development in the cerebellum. A cause-effect between E-cadherin and CP is pretended (figure 10).

ACTIONS

1.1 Know the expression of different genes that participated.
1.2 Study of the territories that genes delimit.
1.3 Analyse the expression of E-cadherin territory.
1.4 Relate different types of neuron to their territory.
1.5 Quantify CP at the territory that express E-cadherin.

Then, first hypothesis has been formulated.

2 Setting consequences of an alteration in IRS1, considering PGC as the main affected cells, due its implication in SHH pathway (figure 11).

ACTIONS

2.1 Study of IGF-2 pathway as a positive modulator of precursor granular cells (PGC).
2.2 As SHH is present in many pathways, one of them is chosen which will be the most positive regulator of SHH (IGF-2).
2.3 Show that an alteration in IRS1 implies lost of interaction with SHH, and this fact is the main cause for the reduction of PGC.

Then, second hypothesis has been formulated.

3 Abnormal CP can influence cerebellum circuits and information transmission (figure 12).

ACTIONS

3.1 Set information transmission circuit . As we are focused on information that should arrive to cerebellum, it has been considered climbing afferents fibres, so they send information to cerebellum through the axon of the PC.
3.2 Determine if in normal conditions, information arrives to cerebellum. Study of the foliated morphology of cerebellum.
3.3 Once it's set that information didn't arrive in normal conditions to cerebellum:
3.3.1 State that the circuit has a fail because CP are deformed.
3.3.2 State that there is a low number of circuits due to the fact that CP are in low number.

Then, third hypothesis has been formulated.

6. Goals, objectives, measurements & how we know that we achieve it

Main goal: To contribute in new sources of knowledge on cerebellar ataxia , considering the embryonic period in the cerebellar developement a key element in this process .

First Objective

Find the possible relationship between abnormal E-cadherin and the consequent hindrance of CP formation.

Relevant information could be added by the analysis of the elements that control how the distribution of the different cellular types at the beginning of the cerebellar development must be. The boundaries between genes in prenatal life become really important.

Second Objective

Setting consequences of an alteration in insuline receptor substrate (IRS1), considering PGC as the main damaged cells, due to its implication in SHH pathway.

Hedgehog family is an important target that require special attention.

SHH is secreted by CP at the direction of granular external layer. SHH binds to his receptor, Patched 1 (PTC), activating another receptor called Smoothened (SMO). Then the translocation of SMO takes place in the primary cilia of PGC (figure 9). At the end, GLI family (1, 2 and 3) is activated and many genes are regulated because they are implicated in the cellular cycle.

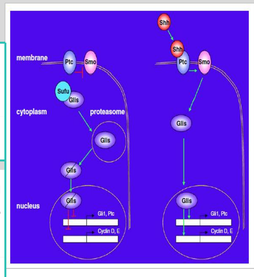


Fig. 9: Regulation of Sonic Hedgehog (SHH). At the left side, all the events that take place when SHH is absent are shown. At the right side, SHH is present and contributes to the expression of some transcription factors, such as GLI family. The activity of SHH is crucial for the proliferation, differentation and migration of precursor granular cells (PGC). (Obtained from Vaillant, 2009).

Third Objective

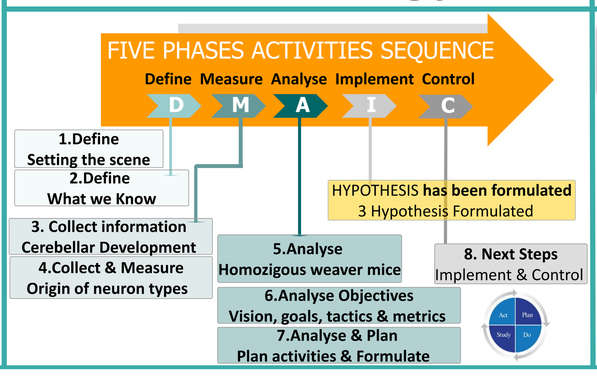
Show that aberrant CP can affect cerebellum circuits and information transmission.

Cerebellum has two types of afferent inpunts, mossy and climbing fibers.

It has been confirmed that information arriving to cerebellum (so in quantity as in quality) is directly conditioned by errors in CP transmission. Moreover, the alteration of CP structure is part of the transmission circuit.

Methods & Materials

Methodology



Reference Materials

To verify all the hypothesis, immunohistochemistry will be used. This technique is based on the use of specific antigens in tissues (figure 13).

1. Paraffin-embedded or frozen tissue .
2. Apply the primary antibody.
3. Apply enzyme-conjugated secondary antibody.
4. Fluorescence microscope visualisation.
5. Digitalisation image.

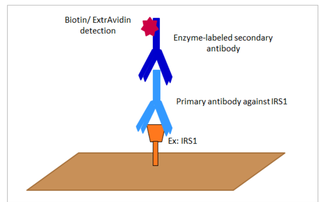


Fig. 13: Representation of an indirect immunohistochemistry. One example of the second hypotesis is shown. The target protein is IRS1. (Personal contribution from Lydia Carnicé.)

Principles of success

BEHAVIOURS

- Commitment with the program.
- Be creative when looking for answers to apply tactical approaches.
- Research of tools, materials or methods that help you achieve the success.

TACTICS

- Materials are necessary to analyse, formulate and conduct objectives.
- Methodology is a key to understand the current situation.
- State a consistent sequence of all the phases in order to reach the objectives.

ADVICES

- Use materials, reviews, public knowledge...
- Never stop, continue with next steps (feasibility/ risk study, project to prove, implement...).

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