

REPORT CATEGORY 1A: Visit by the applicant to another laboratory
Applicant: Alvaro Fernando Nieto Guil.

Host Laboratory: Centro Nacional de Biotecnología-Consejo Superior de Investigación Científica (CNB-CSIC). Spain.
Departamento de Biología Molecular. Laboratory 113.
Principal Investigator: PhD. Marta Nieto.
Stay: 5 months (March-Agust)

Regulation of neuronal polarity establishment by grow factors

The project was developed in CNB-CSIC, with two clear objectives. First improve and implementation of new techniques in the field of neuroscience and methodological transfer to laboratory of Dr.Quiroga in Argentina. Second objective was to deepen the study of growth factors involved in neuronal development "in vivo".

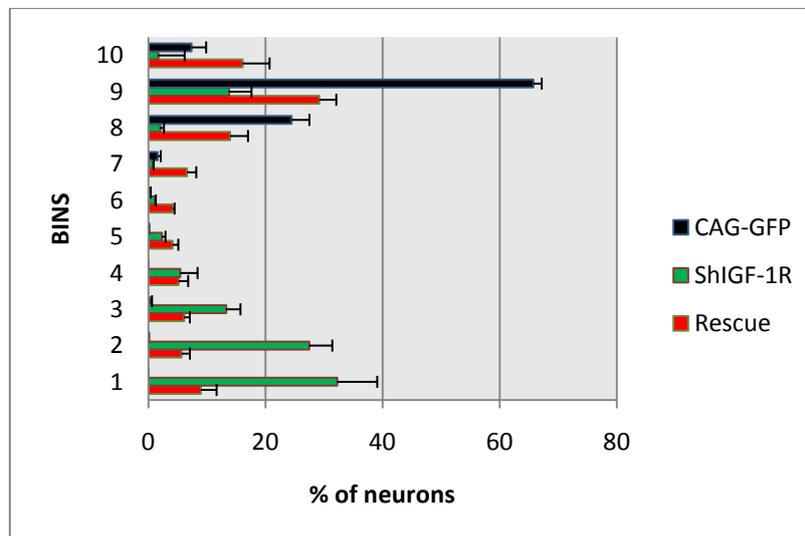
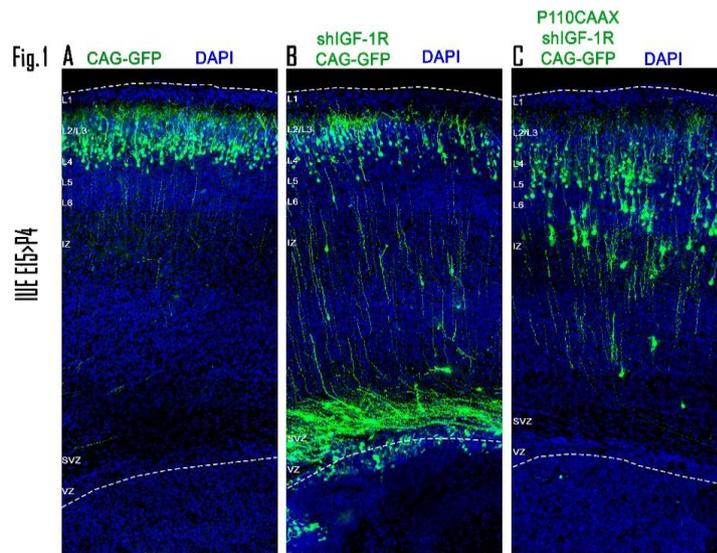
In the first stage, I was instructed in the application of the electroporation technique "*in utero*" (IUE). This methodology allows genetic manipulation of neurons in their natural environment, combining the use of RNA interference technology (shRNA). Using these two tools enables us to analyze the process needed for neurons to develop. By means of this method we can study morphology, location, axon and dendritic development in their ideal environment (in the brain itself). Obtaining new and relevant information in the field.

We used two shRNA designed against two growth factors. We focus on insulin like grow factor receptor type one (IGF-1R), which is widely studied by our group and the other factor under study is TGF- β transforming growth factor β receptor 2 (TGF β -R2), the latter has gained importance in recent years in the field of neuroscience.

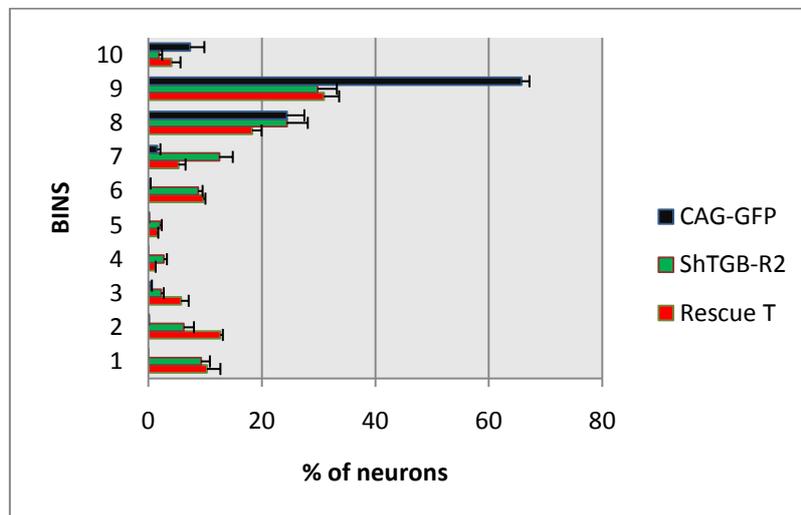
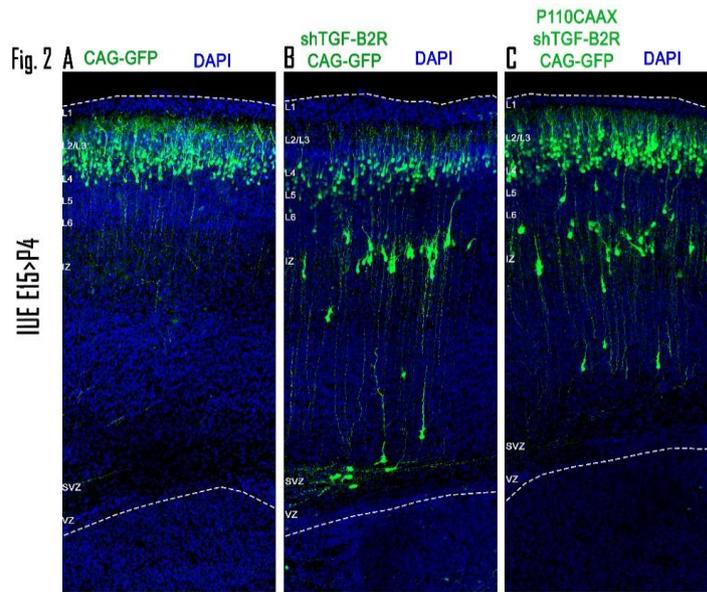
Preliminary Results

Transfection with shIGF-1R, shows a neuronal population are arrested at the base of the ventricle, showing a heterotrophic cell phenotype and are incorrectly oriented. This allows us to understand the need for this ligand for the early stages of development of young neurons. (Fig 1 A and B). These evidence supports our hypothesis that this ligands its necessary for the correct development of the brain.

Previously in the laboratory "in vitro" studies determined the relationship of IGF-1 pathway downstream PI3K (enzyme).This triggers neuronal polarity. For the next experiment we used a catalytic subunit of PI3k called P110, particularly we expressed P110 constitutive active (P110CAAX). With this in mind the experiments be able to restore the cell migration where you can see a large number of affected cells can re-migrate. Again reestablished its development program, these would be the first test of the relationship of this approach in neuronal development "in vivo" (Fig 1 C and quantification).



Furthermore studies with shRNA directed against TGF- β receptor samples a group of cells having an arrest in the intermediate region of the cortex (Fig.2 A and B). The rescue with P110CAAX didn't reverse the phenotype. This creates different scenarios where we wonder whether this phenotype is due to reprogramming of cell identity or alters/ modifies the original cell fate. In this case the rescue with PI3k (Rescue T) not restore the phenotype. (Fig.2 C and quantification). Indicating that both receptors work in different pathways.



Qualitative advantages which means academic exchanges for the development of the doctoral thesis

CAEN 2014 fellowship, allowed me to work with research group that performs this technique routine in his laboratory, I gained procedural expertise and very valuable scientific information it allowed to understand and learn everything about this complex technique. This methodology involves performing surgery (laparotomy) and microinjection; it requires great training and practice. I acquired great training in handling animals, because of the methodology involves performing surgery (laparotomy) and microinjection in the brain it is very difficult. . Thanks to this we are now able to perform this methodology here in Argentina, resulting in a significant scientific improvement to our lab and institute. We were able to identify methodological problems and generate an academic environment in which I was greatly benefited in understanding and “*in vivo*” results in the development of protocols for quantification and image analysis.

Future Projects

In the long term this technique will allow future thesis to assess the complexity of neuronal development in the laboratory. "In vitro" and "in vivo" give us complementary vision of what it's happening during brain development. This experimental relationship will give a qualitative leap succeeding thesis. Also this modern methodology generates competitiveness and new partnerships on both a national and an international setting.



Álvaro Nieto

