Committee for Aid and Education in Neurochemistry (CAEN)

Report: CATEGORY 1C: Return Home Award

1. Contact details of the applicant:

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2. Supported project:

Effects of poor protein nutrition and stimulating or impoverished environments in the cortex and hippocampus of CJ-1 mice: epigenetic regulation, brain plasticity and behavioral response.

Background

Maternal undernutrition alters brain maturation of the embryo resulting in cognitive and socio-emotional deficits and producing disturbances in learning and memory. These changes extend the postnatal period and continue throughout adulthood [1,2]. Recent studies demonstrate that malnutrition during the prenatal and lactation increases the risk of psychiatric diseases such as depression, personality disorders and schizophrenia [3].

Learning and memory largely depends on the regulation through epigenetic modifications [4]. These epigenetic mechanisms are considered as central elements of brain plasticity, although they are poorly understood [5]. Epigenetic regulation of gene expression allows the integration of environmental and endogenous signals on the genome. The epigenetic machinery is built by molecules that "write" (e.g. enzymes such as methyl- and acetyl- transferases), "read" (bind to marks, e.g. methyl binding proteins) and "erase" (such as demethylases and deacetylases) specific marks modifying gene expression [6]. Recent research demonstrate that exposure to stress during early development has a direct deleterious effect on the quality of physical and mental health [7]. One consequence is increased and long-lasting secretion of glucocorticoids, leading to a deregulation in the homeostatic balance of the hypothalamic-pituitary-adrenal, which is considered a pathogenic factor in disorders of behavior and cognition.

Preliminary data from Dr Canepa laboratory show that poor protein diet produce a delay on morphological and neurological development, anxiety, low level of NGF and BDNF, increased histone H4 acetylation [8]. The general objective of the project is *to study the cellular and molecular basis of cognitive and emotional deficits in mice caused by a low-protein diet during development and lactation*. We will focus in their reversal by restoring a proper neuronal plasticity manipulating gene expression and epigenetic marks. In parallel, we want to study the cell-specific changes produced by malnutrition and the environment.

Hypothesis

Our hypothesis is that the emotional and cognitive deficits resulting from poor protein nutritional status during development and postnatal period are due to, at least in part, an altered regulation of genes involved in learning, memory and modulation of stress response leading to a decrease of brain plasticity. This deregulation of gene expression would be largely mediated by altered epigenetic mechanisms including DNA methylation or histone tail modification. Additionally, these changes in the epigenome can be different between neurons and glia.

Furthermore, we hypothesized that physical stimulation through exposure to an enriched environment would be able to reverse, at least partially, behavioral and cognitive deficiencies of malnutrition; or accentuate them in the case of an aggressive atmosphere or unexciting environment.

Specific aims

In this project we will focus our studies at three different levels: behavioral, cellular and molecular. At behavioral level, we plan **to evaluate the influence of diet alone and/or the environment in memory and learning, anxiety and depression**. At cellular level, we are **going to evaluate changes in apoptotic cell death, neurogenesis and glia phenotype**. **To evaluate brain plasticity by analyzing changes in dendritic spines** (density and morphology) at specific regions of the hippocampus and also in the cortex.

At molecular level, we will **determine the consequences of poor protein nutrition during gestation and postnatal period on levels of general DNA methylation and in specific CpG islands**, such as the promoter of the glucocorticoid receptor and neurotrophins such as BDNF. We plan **to evaluate the effect of**

diet and environment on the expression of methylation and acetylation machinery and the level of molecules involve behavior such as CREB, reelin, PP1 and calcineurin.

Additionally, we will **study the effects of malnutrition and different environments in the cell-type specific levels of methylation and histone tails modification** using immunofluorescence. A more challenging aim is **to set-up a protocol using flow cytometry to separate the different cell types in the brain and evaluate the epigenome in each population**.

Plan of work

During my Master and PhD thesis in Buenos Aires-Argentina, I focused my research in the effects of cytokine expression in microglia activation, inflammation and neuronal survival. Our research demonstrates that the effect of the cytokines is time- and context-dependent producing opposite effects on neuronal survival [9]. During the last decade, the glia function in healthy brain has been intensely studied, demonstrating that glial cells have an important role not only in the normal function of the neurons during development but also the entire life.

At the Iberoamerican Glia Network Symposium during the I Congress IBRO-LARC of Neurosciences for Latin-American, Caribbean and Iberian peninsula (Brazil, 2008), I contacted Dr Laia Acarin, who was conducting a project focused on the study of glial cells and inflammatory processes in postnatal development and hypoxic/ischemic injury, in which later I participate as Postdoc with a Marie Curie Fellowship. We found that during postnatal development there is a tiny control of inhibitory/modulatory immune receptors in microglia which reduce the inflammatory response during developmental cell death [10]. These regulatory receptors are also upregulated during postnatal Hypoxia/ischemia (Chertoff et al, manuscript in preparation). This project gave me the great opportunity to work abroad in an excellent environment of research at the Autonomous University of Barcelona, develop a short stay in Paris and participate in several international meetings, congresses and courses. Thanks to ISN and IBRO support, I have the pleasure to participate in the Organization Committee of the "ISN Advanced School In New Approaches In Glial Cell Research" and in the Young Investigator Training Programme Committee, respectively, organizing several activities around the FENS Forum 2012 in Barcelona, which opened a new word of possibilities to contribute in the growing of other scientists around the word, which I am planning to continue in near future.

In the present project we plan to evaluate the effects of diet and environment in behavior, brain plasticity and epigenome. Our experimental design includes 6 groups of CF-1 mice: a *normal nourished mums* (23% protein) and low-protein malnourished mums (8% of protein from one week before mating to end of lactation). After weaning, the litters of each nutritional state will be subjected to one out of *three different environments: enriched* (more social interaction, bigger space and toys), *normal and poor* (small cages and less social interaction). The animals will be evaluated during lactation and at 1 week and 5 weeks after weaning.

The research plan requires the following type of experimental techniques:

- Western blot and biochemical / immunohistochemical analysis of "readers", "erasers" and "writers" of epigenetic machinery on mice brain, including sample preparation and microscope analysis, in which I have an extensive experience in this techniques, obtained during my PhD and Postdoc.
- DNA methylation analysis in brain extracts, by using *Cytosine-extension assay* (for global or CpG islands DNA methylation) or *Bisulphite conversion and amplification* (for specific CpG islands such as promoter of Glucocorticoid Receptor and BDNF) in which I count with the help and training of the members of Dr Canepa laboratory.
- Flow cytometry analysis of brain populations to evaluate cell specific changes in epigenome. I have experience on the use of this technique in cell culture, however the use of this technique in brain samples will be a challenge that I hope it moves to the cutting-edge research avenues the science in Argentina.
- Behavioral testing for development, memory, learning, emotional deficits. I am familiar with several of the test and I will count with the group support for the ones that I do not used before.

Expected outcomes

At the beginning of 2014, I plan to return to my home country where I will be part of the projects held by Dr Eduardo Canepa, in the laboratory of Molecular Biology at the University of Buenos Aires-Argentina to share with them knowledge and experience that I acquired during my ten years in the neuroscience field. Dr Canepa Group, mainly focus on cell cycle and DNA repair, has recently started a new project in neuroscience field, which involves one of the hottest topics from a very original point of view as very little is known about cell specific epigenome changes and molecular mechanisms involve in the context of protein malnutrition and environment. The focus on behavior joined at evaluation of cellular and molecular levels will give the project a great possibility of success and a wide understanding of the consequences of the diet and environment in daily life. My experience on glia biology, neurodegeneration and neuroinflammation during development and adult life will furnish different point of view to his group.

As I mentioned before, I plan to work in the laboratory of Dr Canepa, from which I will gave the opportunity to apply my background on glia research to one of the hottest topics. I will have the complete

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support of Dr Canepa to develop my own ideas and also his financial support. However, Argentina is a country with a great scientific tradition by chronically ridden by socio-economic crisis and lack of the proper scientific funding in addition to a double cost of scientific material due to import and taxes cost. The support given by ISN will be used to buy consumable material for the experiments but also the basic equipment that will give me the bases for setting up own laboratory in the near future. As I mentioned before, in the past ISN generously sponsored my training at different levels and the financial support of the Return grant will proven critical for the successful establishment of my independent research in my home country.

References

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3. Detailed budget of how the funds were spent:

My salary was paid by the National Research Council (CONICET), I applied to the "group formation grant" given by the National Agency for the Promotion of Science and Technology (ANPCyT). The following budget includes equipment, services of pre-existent equipment and consumables.

Animals + housing costs	998.12
Calibration of micropipettes (x4 different sizes) - Service of -80ºC Freezer - Equipment for behavioral test (Barnes Maze)	1463.85
Primary and secundary antibodies for IHC and WB	1982.94
Reactive for Molecular biology (primers, polymerase, retrotranscriptase, etc)	1672.57
Materials for tissue cutting and mounting for IHC (blades, glass slides, coverslips, mounting media, etc) and consumables (microtubes and tips)	590.23
Chemicals for general solutions (running and transfer buffers, Methanol, PBS, SDS, paraphormaldehide, etc)	438.42
TOTAL COST	7146.13

The invoices are in Argentine Pesos and the change was calculated in American Dollar in accordance with the National Bank reference on the payment day.

4. Obtained results

The work performed during this year allowed me to present a Poster at the XXIX Annual Meeting and SAN - ISN Small Conference and course "New mechanisms of neuro-glial interaction : Their contribution to nervous system development and repair"; Septembre 29-October 3, 2014 Huerta Grande , Córdoba-Argentina.

The **abstract** was as follow:

Maternal undernutrition alters brain maturation of the embryo resulting in cognitive and socio-emotional deficits and producing disturbances in learning and memory. These changes extend the postnatal period and continue throughout adulthood, mainly regulated through epigenetic modifications, although they are poorly understood.

Our aim is to study molecular basis of deficits in mice caused by a low-protein diet (LP) during development and lactation. We focus our study on the consequences of poor protein nutrition on morphological and neurological development and methylation machinery.
The experimental design includes 2 groups of CF-1 mice: a normal nourished mums (23% protein) and low-protein malnourished mums (8% of protein) from 5 days before mating to end of lactation. We monitor weight and the morphological and neurological development in both sexes littermates. We evaluate the changes on mRNA expression of glucocorticoid receptor (GR) and methylation machinery members at P21.
LP reduced the weight of the littermates without affecting the litter size. We also observed a delay on development parameters such as outer ear detachment, opening of auditory pavilion and sound tactil and visual reflex. Preliminar data showed increased expression of GR and MeCP2 on female LP hippocampus. We also evaluate if DNA methylation is affected by LP.
In summary, maternal undernutrition affects brain development, the stress response and methylation machinery.

Work in progress

We observed that hipoproteic diet produce a delay on physical and neurodevelopment in both sexes, showing sliht differences between females and males. However, at weaning all reflexes studied seems normal. The hipoproteic mice were smaller than controls till weaning.
Moreover, we collected samples for immunohistochemistry from females and males at postnatal day 21 (P21) and after 5 weeks in normal, enriched or poor environment; the analysis for changes on glial cells and neurons in our paradigm is under progress. In addition, samples for molecular studies of epigenetic modulators from hippocampus, hypothalamus, cortex and cerebellum were collected at 3 and 8 weeks (after 5 weeks on different environments). Preliminar data showed an increase on glucocorticoid receptor and MeCP2 in female hippocampus at P21. The analysis of other molecules and regions are under progress.
Additionally, I submitted the solicited manuscript to Journal of neurochemistry entitled, "Protein malnutrition and brain development". The manuscript has been assigned the manuscript number JNC-2014-0782 and it is under review.
We expect to complete this analysis and present the second part of our work at the ISN meeting 2015 in Cairns-Australia. Furthermore, the most remarcable observations will be part of a manuscript which will be submited to a high level scientific journal.

I attach some pictures from the Laboratory of neuroepigenetics and the SAN meeting and SAN -ISN course, 2014

Laboratory of Neuroepigenetics -

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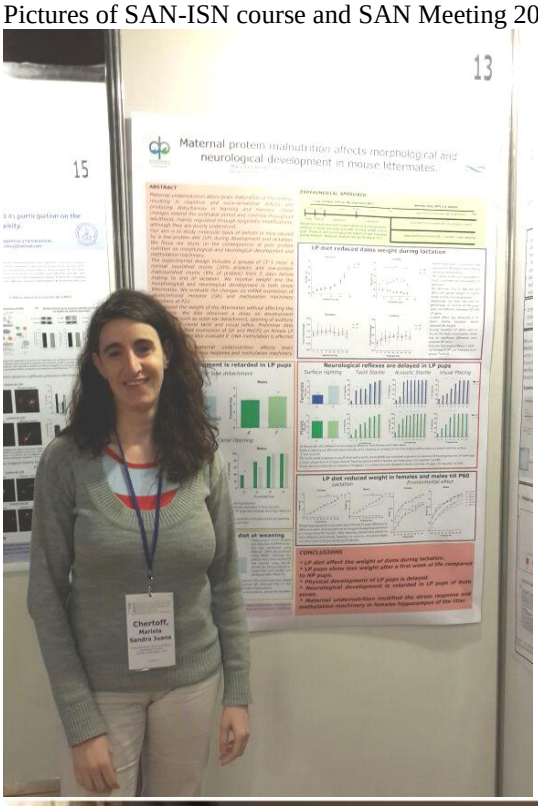
Pictures of SAN-ISN course and SAN Meeting 2014.



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