NEUROSCIENCE PROGRAM: SECOND YEAR PROJECT PRESENTATION.
MARTES 31 MAYO 2011 SEMINARIO IN

09:00-09:20 Marian Martinez (Director: Victor Borrell) Cellular and molecular mechanisms controlling the expansion and divergence of cortical radial glia.
09:20-09:40 Gabriele Ciceri (Oscar Marin) Single-cell fate mapping in the developing telencephalon.
09:40-10:00 Anna Lucia Conte (Ana Gomis) Characterisation of mechanical mammalian sensory neurons.
10:00-10:20 Rebeca Caires Mugarra (Carlos Belmonte - Elvira de la Peña) Estudio del efecto de sustancias viscoelásticas derivadas del ácido hialurónico en la nocicepción.
10:20-10:40 Luis Baltazar Enoch (Felix Viana) Efecto de los ácidos grasos sobre la actividad del canal TRPA.
10:40-11:00 Géraud Chauvin (Eloisa Herrera) Wiring of functional maps in the visual pathway: a new genetical approach. The role of LMO2 in the retina.

Chairperson: Guillermina Lopez-Bendito

PAUSA 11:00-11:40

11:40-12:00 Aljona Makarova (Ana Carmena) Functional relationships between the PDZ protein Canoe and the Warts/Hippo signaling pathway during asymmetric cell division.
12:00-12:20 Anna Fiorenza (Angel Barco) Role of CREB-regulated microRNAs in neuronal plasticity.
12:20-12:40 Clara Gomis Coloma (Hugo Cabedo) Oncogene-induced senescence as a fail-safe mechanism for the malignant transformation of peripheral nerve tumours: molecular characterization and potentiality as a target for new therapies.
12:40-13:00 Valeria Balmaceda (Javier Saez Valero) Impaired Reelin signaling pathway in Alzheimer's disease.
13:00-13:20 Maria Letizia Campanari (Javier Saez Valero) Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with presenilin-1.
13:20-13:40 Rebeca Corcoles Corcoles (Angela Nieto) Epithelial plasticity in development and cancer.

Chairperson: José Manuel Mingot

Marian Martinez

Cellular and molecular mechanisms controlling the expansion and divergence of cortical radial glia.

In the developing cerebral cortex the fibers of Radial Glia (RG) cells serve as guide and substrate for radially migrating neurons, which travel from their place of birth to their final destination in the cortical plate. Whereas in the lissencephalic mouse RG fibers drive radial migration in strictly parallel trajectories, in gyrencephalic species, like human and ferret, radial migration proceeds following highly divergent trajectories in order for the cerebral cortex to expand in surface area rather than in thickness. Indeed, RG fibers follow highly divergent trajectories in ferret during the formation of cortical folds, which is supported in part by the massive generation of Intermediate Radial Glia Cells (IRGCs). At the molecular level, our preliminary observations show that mice deficient in miRNAs suffer dramatic defects in RG cell biology. Our hypothesis is that the generation of IRGCs and branching of radial fibers are mechanisms to impose divergence on the radial fiber scaffold, thus determining the expansion of the cerebral cortex in surface area. To this aim we will investigate what are the cellular mechanisms involved in the generation of IRGCs from RG cells and in establishing the tangential divergence of the radial fiber scaffold, and the molecular mechanisms controlling RG homeostasis. Our specific aims are:

1. To define the cell lineage of IRGCs in the ferret.
2. To characterize the growth and branching of radial fibers underlying their divergence.
3. To identify molecular mechanisms controlling RG morphology and lineage.

Gabriele Ciceri

Single-cell fate mapping in the developing telencephalon

My PhD project focuses on the clonal analysis of telencephalic GABAergic interneurons. These neurons are born in the ganglionic eminences (GEs), transient structures in the embryonic subpallium, from where they migrate to populate the cortex and other telencephalic regions. Several classes of interneurons exist with different morphologies, neurochemical content and physiological properties. Experimental evidence suggests that interneuron diversity is established early during neurogenesis, but it is still unclear how progenitor cells determine the final properties of each class of interneurons. Our hypothesis is that, like in spinal cord, spatially segregated proliferative regions in the embryonic telencephalon give rise to the different interneuron's subclasses. Indeed, several previous studies (including some from our lab) have demonstrated that regional differences exist in the specification of GEs progenitors. Furthermore, it has been shown that the different subpallial domains contain heterogeneous populations of progenitors. With this in mind, we are trying to develop new technologies to perform single cell fate mapping studies in vivo, combining retroviral lineage-tracing technology with the use of several fluorescent proteins. We have generated different conditional retroviruses each one driving the expression of fluorescent proteins only after Cre recombinase activity. We will inject, at early developmental stages, low titer conditional retroviruses in the lateral ventricle of transgenic mouse embryos expressing Cre under control of specific subpallial promoters to fate-map specific domains. We use ultrasound-guided imaging to accurately control the site and the amount of particles injected. Using this tool, we will be able to label progenies of a small set of progenitor with different colors and to identify lineage restrictions from individual progenitors. Coupling this method with organotypic slices and time-lapse imaging, we will also be able to perform short-term experiments to investigate cell cycle dynamics in GE progenitors as well as the migratory trajectories of neurons derived from the same progenitor. We will then move to adult stages analyzing the distribution, physiology and connectivity of sibling interneurons.

Anna Lucia Conte

Characterisation of mechanical mammalian sensory neurons.

Mechanotransduction, the conversion of mechanical stimulus into a biological response, is detected by mechanoreceptors expressed in sensory neurons but the transduction elements for the mechanical forces are mainly unknown.

The aim of my project is to identify the mechanisms involved in the detection of mechanical stimulus in mammalian trigeminal ganglion neurons, to identify the differences between high and low thresholds neurons, and to associate the expression of mechanosensitive ion channels with these differences.

To this end I am using trigeminal neurons from adult and postnatal (until P5) mice, electrophysiological recordings and calcium imaging techniques (Fura-2). Mechanical stimuli are applied to the surface of the cultured cells using a heat-polished glass pipette driven by a micromanipulator system positioned at an angle of 45º to the surface of the dish.

I have measured the changes in intracellular calcium concentration during the application of mechanical stimulation in neonatal trigeminal neurons. My results reveal a variability on the mechanical threshold of the responses without detecting a clear correlation between the size of the neurons and the mechanical threshold as is indicated by other authors (large-diameter neurons related to low threshold and small neurons related to nociceptive neurons).

I have started the characterisation of the mechanosensitive currents present in neonatal and adult mice based on different inactivation kinetics and intensity of the mechanical stimulus. The objective is to identify the properties of the low and high threshold (nociceptive) mechanical neurons.

Once I have established the properties of the different mechanical neurons I will try to correlate them with the expression of different ionic channels (TRPs) and proteins (Piezo-2) involved in mechanotransduction.

Rebeca Caires Mugarra

Estudio del efecto de sustancias viscoelásticas derivadas del ácido hialurónico en la nocicepción.

La matriz extracelular de los mamíferos está compuesta por diferentes moléculas, siendo el ácido hialurónico (HA), en el sistema nervioso, el componente mayoritario. Estudios previos, de registro electrofisiológico realizados en el laboratorio en el que desarrollo mi tesis, mostraron que la inyección intraarticular de HA de alta viscoelasticidad reduce la respuesta nociceptiva. Estos estudios se realizaron registrando, en animales anestesiados, la actividad eléctrica del nervio safeno, que inerva la articulación de la rodilla en respuesta a estímulos mecánicos nociceptivos. Este efecto ha sido atribuido al comportamiento del HA como un filtro viscoelástico que amortigua la transmisión de fuerzas mecánicas a los canales mecanosensibles de las terminaciones nociceptoras articulares. Durante los procesos inflamatorios articulares (artritis, artrosis) se reducen los niveles de ácido halurónico en el líquido sinovial, lo que explicaría en parte el dolor que acompaña a estos procesos, al disminuir el efecto mecanoprotector del HA. Recientemente, se ha visto que el HA tiene un efecto directo sobre los canales de calcio de las neuronas hipocámpicas modulando su actividad, abriendo así la posibilidad de que los efectos analgésicos del HA en las terminaciones nociceptivas puedan ser, en parte, debidos a una acción directa del HA sobre los canales de membrana implicados en la transducción de los estímulos dolorosos. Entre éstos, destacan los canales de la familia TRP, implicados en la transducción de diversos estímulos físicos y químicos. Las neuronas sensoriales que inervan las articulaciones expresan en su soma los canales TRP, por lo que pueden servir de modelo para analizar el posible papel modulador del HA sobre la actividad de estos canales. El objetivo de este proyecto es estudiar en neuronas sensoriales en cultivo y en líneas celulares que expresen los canales TRP, los efectos de HA mediante registros de calcio intracelular y registro electrofisiológico.

Luis Baltazar Enoch

Efecto de los ácidos grasos sobre la actividad del canal TRPA1

Las neuronas sensoriales somáticas y viscerales son funcionalmente heterogéneas y transmiten información tanto nociceptiva como no nociceptiva, la cual incluye tanto estímulos físicos como químicos. Los receptores para la mayoría de estos estímulos son proteínas transmembrana que regulan el flujo de iones a través de la membrana plasmática (canales iónicos). Aquí, la familia de canales iónicos TRP juega un papel primordial, tanto por su amplia distribución en el organismo, así como por sus muy variadas formas de activación e interacción con otras proteínas, lípidos e iones. Dentro de ellas, el efecto de los fosfolípidos de membrana y en particular el papel del PIP2 sobre la actividad de los canales TRP es el mejor estudiado.

Sin embargo, recientemente se ha comenzado a estudiar el efecto que los ácidos grasos tienen sobre la actividad de ciertos canales TRP. Los ácidos grasos se producen durante la digestión de triglicéridos y su papel como moléculas de señalización no es tan claro, por lo que el estudio de éstos comienza a cobrar interés. Estudios realizados en roedores y humanos han mostrado que la administración de ácidos grasos directamente en el duodeno disminuye la ingesta de alimentos, y que probablemente estos efectos sean mediados a través de receptores localizados en neuronas vagales que inervan el intestino.

En experimentos de imagen de calcio con fura-2, hemos observado que la aplicación extracelular de ácido linoleico (50 µM) produce un incremento en los niveles de Ca^{2+} intracelular en células CHO que expresaban de forma inducible el canal TRPA1, mientras que las células CHO control, que no expresan el canal, no responden a dicha aplicación. Igualmente, en neuronas sensoriales del ganglio nodoso se observan incrementos de calcio intracelular en respuesta a la aplicación de ácido linoleico, existiendo además una gran correlación entre las neuronas que responden a ácido linoleico y las que responden a cinamaldehido, un agonista específico del canal TRPA1.

En conclusión, nuestros datos sugieren que el canal TRPA1, un canal iónico activado por frío nocivo y por compuestos irritantes, y que es expresado en una subpoblación de neuronas nociceptivas del ganglio nodoso, tiene un papel primordial en la detección de ácidos grasos.

Géraud Chauvin

Title part 1: **Wiring of functional maps in the visual pathway: a new genetical approach.**

Title part 2: **The role of LMO2 in the retina.**

Our main project aims to shed light on how, during development, functional maps In the early visual pathway, are established, and how Later on, sensory experience can modify them. Using Recent discoveries on molecular cues governing ipsilaterality of retinal ganglion cells such as Zic2 or EphB1, we designed a transgenic mouse where about half RGCs will turn ipsilateral. This new input will induce changes in the visual thalamus as well as in cortical areas, probably priming them for binocular vision. Further implantation of permanent convergent goggles will then permit us to obtain a mouse with a “humanized” visual system: CNS with balanced input from both eyes, and a rostraly shifted ocular focalisation point. Characterisation of this new model should allow us not only to understand the relative contribution of experience, spontaneous activity and genomics in maintaining and refining the organisation of the visual system, but also to probe the limits of plasticity in the mammalian CNS. Our other project is the characterisation and understanding of the role of LMO2 in the retina. LMO2 has been characterised as a cofactor of several zinc finger proteins, which together are able to form different types of transcription complexes which roles has been shown crucial in hematopoietic, but also in certain types of leukaemias. However, if it has been extensively studied in oncology, very few is known about its function in the CNS. Our group has previously show the presence of LMO2 In RGCs Of the embryonic retina, so we’ll use in utero electroportation as a tool for gain/loss of function paradigm in order to unravel LMO2’s function and mechanism of action.

Aljona Makarova

Functional relationships between the PDZ protein Canoe and the Warts/Hippo signaling pathway during asymmetric cell division.

Asymmetric cell division is a universal mechanism for generating cellular diversity and a key process in cancer and stem cell biology. One of the main research lines in our lab is the analysis of this process. Our model system is the fruit fly *Drosophila melanogaster* and, specifically, the stem cells of the central nervous system called neuroblasts (NBs), that divide asymmetrically. Recently, our lab showed a function of the PDZ (PSD-95, Dlg, and ZO-1) domain-containing protein Canoe as a new key regulator of asymmetric NB division. With the aim to further understand the role of Canoe and its interacting partners during this process, different high-throughput screenings have been developed in the lab. Results from a yeast two-hybrid screening identified a protein called Warts as a potential Canoe partner. Warts is a serin/threonin protein kinase, a central factor within the tumor-suppressor signaling pathway known as the Hippo pathway, crucial for regulating organ growth and cell proliferation. Warts has substrates key for cell division and the Warts homolog in vertebrates called Lats negatively regulates Cdc2/CyclinA in a cell-cycle dependent manner. Also, Lats acts as a dynamic component of the mitotic apparatus. Hence, Warts was a good candidate to analyze in depht in relation with Canoe during the process of asymmetric cell division. This was the starting point of my thesis work. The results found so far strongly support functional relationships between Cno and the Warts/Hippo signaling pathway during different events and tissues throughout development.

Anna Fiorenza

Role of CREB-regulated microRNAs in neuronal plasticity

The cAMP-responsive element binding protein (CREB) is a transcription factor implicated in the regulation of different forms of neuronal plasticity, learning and memory. Recent work at the Barco's lab, using an unbiased genome-wide screen for transcripts regulated by activity and CREB identified a number of non-coding RNAs, including two microRNAs (miRs), miR-212 and miR-132, that map into an unique intronic locus. MicroRNAs are non-coding short double-stranded RNA molecules involved in post-transcriptional gene expression regulation affecting both mRNA stability and translation.

This project propose to investigate the role of miRs and in particular the CREB-regulated miR132/212 locus in neuronal plasticity with special focus on their role in activity-driven gene expression, regulation of neuronal excitability, long-term potentiation and memory.

To investigate the general role of microRNAs in activity-driven gene expression and adult neuronal plasticity, we will use Dicer knockout mice in two different systems: (1) Hippocampal cultures from $Dicer^{f/f}$ mice will be infected with lentivirus expressing cre recombinase, in order to ablate Dicer activity and, so, microRNAs maturation and function, and investigate the consequences of this ablation in gene expression and neuronal physiology. (2) CaMKIIα-$creERT2/Dicer^{f/f}$ conditional Dicer knockouts will be used for studying the role of microRNA in memory formation and plasticity *in vivo*.

To investigate the specific role of miR-212/132 in activity-driven gene expression, neuronal plasticity and excitability and learning and memory, as well as to identify their targets, we have generated recombinant viruses that either overexpress these miRs in neurons or block their activity by expressing specific inhibitors (miR sponges).

Clara Gomis Coloma

Oncogene-induced senescence as a fail-safe mechanism for the malignant transformation of peripheral nerve tumours: molecular characterization and potentiality as a target for new therapies.

Neurofibromatosis is characterized by the appearance of peripheral nerve tumours (neurofibromas) that although initially benign, can cause serious neurological problems. A significant percentage of these benign tumours progress to form aggressive **M**alignant **P**eripheral **N**erve **S**heath **T**umours (MPNSTs), which respond poorly to chemotherapy, and that require surgical resection and radiotherapy.

The goal of my thesis project is to understand the role of axon-glial signalling in neurofibroma development, and the genetic events that promote neurofibroma progression to malignancy. This will be achieved through the use of disease models developed in genetically modified mice. Previous data from my lab shows that sustained axon-glial signalling *in vivo* (in NSE-SMDF mice) causes early postnatal Schwann cell hyperproliferation and neurofibroma formation. Although the MAPK-pathway remains active in these nerves, cell proliferation halts during adulthood. My preliminary results suggest that these cells enter a state of replicative senescence, a cell cycle control mechanism that prevents malignant tumours developing when proliferation is aberrantly activated by some oncogenes. Thus, one of my objectives is to establish whether replicative senescence is indeed a mechanism of proliferation control in both transgenic mice and human neurofibromas. Interestingly, our data also suggests that disrupting the senescence program (via mutations in the p19Arf/p53-pathway) is sufficient to induce MPNST development. Thus, I also pretend to unveil the role of loss of the senescence program in neurofibroma progression to malignancy. I hope that the results of my project will facilitate the rational design of new therapeutic strategies for a form of cancer with very poor prognosis.

Valeria Balmaceda

Impaired Reelin signaling pathway in Alzheimer's disease.

Reelin is an extracellular glycoprotein that modulates synaptic function and plasticity in the mature brain, thereby favouring memory formation. Increasing evidences support a link between Reelin and components of its signaling pathway with the main hallmark of Alzheimer's disease (AD) [1-3]. Moreover, our group showed increased Reelin expression in affected brain areas of AD patients, and also an altered glycosilation pattern of Reelin[4]. Recently, we demonstrated that in vitro treatment with β-amyloid peptide, Aβ42, led to increased Reelin levels and altered Reelin glycosylation pattern[5]. Reelin exerts its biological function by binding to lipoprotein receptors, the very-low-density lipoprotein receptor (VLDLR) and apolipoprotein E receptor 2 (ApoER2)[6-10]. Interestingly, presenilin 1 (PS1) the catalytic component of the γ-secretase protease complex that process β-amyolid precursor protein (APP), can play a role in the intramembranous cleavage of other transmembrane proteins such as ApoER2[11,12].

The aims of this research project are two. First, we plan to evaluate the effects of the impaired processing of PS1 on ApoER2 and Reelin. Interestingly, we have detected a significant increase in both ApoEr2 and Reelin in the brain of a PS1-KO mice model. These results lead us to investigate if PS1-mediated ApoER2 processing is affecting Reelin levels. The genetic modulation of ApoER2 and pharmacological modulation of PS1 will be useful tools. Second, we plan to analyze whether changes in the glycosylation of Reelin impairs its physiological function. In this point, we are interested in analyzing how altered Reelin glycoforms, triggered by Aβ42, can affect its signaling pathway and ultimately the regulation of tau phosphorylation.

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Maria Letizia Campanari

Revisiting the role of acetylcholinesterase in Alzheimer's disease: cross-talk with presenilin-1.

Acetylcholinesterase (AChE) is an enzyme that degrades the neurotransmitter acetylcholine. In mammals, AChE is encoded by a single ACHE gene. The diversity in the transcribed products arises from alternative mRNA splicing [1], which allows the production of two distinct AChE variants within the brain: the major T (tail) variant encoding amphiphilic monomers, dimers and tetramers, as well as hetero-oligomers via the interaction with the non-catalytic proline-rich membrane anchor (PRiMA). During the progression of Alzheimer's disease (AD), the distribution of brain AChE molecular forms changes with prevalence in the lighter monomers, whereas the major cholinergic tetramers decrease. The significance of these changes is intriguing and still unexplored. Similarly, the early increase in AChE levels around β-amyloid plaques and its physiopathological consequences remain to be deciphered. Finally, recent studies from our group [6] demonstrate an interaction between AChE and Presenilin 1 (PS1), the catalytic subunit of the gamma-secretase complex which processes the amyloid precursor protein (APP).
In this project, analyzing human samples, as well cellular systems modulated by genetic over-expression and silencing and pharmacological treatments (with Aβ peptides and AChE inhibitors), we should resolve changes in AChE expression and specific AChE/PS1 interaction. Our methodological approach include determination of AChE activity (enzymatic levels), western blot analysis (protein levels of the different AChE variants, PS1 levels), sucrose density gradients (AChE molecular forms analysis), and QRT-PCR analysis (transcripts levels). We will study the specific interaction between the different AChE variants and PS1 and define the subcellular level and catalytic/non-catalitic character of AChE in the modulation of PS1 levels.

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Rebeca Corcoles Corcoles

Epithelial plasticity in development and cancer.

The epithelial to mesenchymal transition (EMT) converts adherent and polarized epithelial cells into mesenchymal cells with migratory and invasive properties. The first EMT in the embryo defines the territories that will become neural or mesendodermal. Later on, rounds of EMT and the reverse process, mesenchymal to epithelial transition (MET) are crucial for the formation of many organs and tissues. Reactivation of the EMT programme in the adult promotes tumour progression and organ fibrosis, and recent findings indicate that the EMT can also confer stem cell properties.
The main inducers of the EMT are transcription factors of the Snail, Zeb and Twist families. Our lab has greatly contributed to the field by describing and characterizing the first EMT inducers (Snail factors) and by showing additional functions associated with them, including the regulation of cell proliferation and survival. We have also identified Prx1, another transcription factor that can trigger EMT in embryos and in cancer cells (unpublished). One question that emerges is why the organism needs so many EMT inducers and whether there are differences in the EMT triggered by each of them. Therefore, the aim of this project is to characterize the cells that have undergone EMT triggered by each individual factor or by a combination of them, as different developing tissues and human tumors usually express several inducers. We will pay particular attention to cell behavior in 3D cultures and in vivo and will examine parameters related to cell movement, proliferation, survival and stemness. As the reverse process, MET, is important for cell differentiation and metastasis formation, we will examine whether these factors need to be downregulated for the colonization of migratory embryonic or malignant tumour cells to form organs or metastasis, respectively, with obvious implications in morphogenesis and cancer progression.