



# SENC

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## Sixth Cajal Winter Conference

### Role of glial cells in health and disease

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CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

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# Conference Programme

## **Sunday March 7**

**16:00-20:30 Registration**

**21:00 Dinner**

## **Monday March 8**

**15:15** *F Artigas*. Welcome address

**15:30 Oral communications.** *Chair: A. Araque*

**15:30** J Berenguer de Felipe, S Pons. *miR-22 reduces cerebellar granular neuron precursors proliferation through the down-regulation of the MYCN partner MAX (p.12)*

**16:00** FJ Arenzana, E Medina, C Ortega, D Clemente, F de Castro. *Functional implications of FGF-2 and Anosmin-1 in the proliferation and migration of oligodendrocyte precursor cells from postnatal and adult cerebral cortex (p.13)*

**16:30** N Mecklenburg, S Martínez. *Cerebellar oligodendroglia have extracerebellar origin (p.14)*

**17:00 Plenary lecture**

**Helmut Kettenmann.** *Glial function in neurodegenerative diseases (p.15)*

**18:00 Coffe break**

**18:30 Oral communications.** *Chair: G. Mengod*

**18:30** A Araque. *Endocannabinoids mediate neuron-astrocyte communication (p.16)*

**19:00** H Cabedo. *Schwann cell proliferation versus myelin thickness: a differential role for distinct neuregulin splicing forms (p.17)*

**19:30 Poster session**

**21:00 Dinner**

## **Tuesday March 9**

### **15:30 Oral communications. Chair: F. de Castro**

**15:30** A Di Penta, O Errea, E Moreno, B Fernández, N Escala, P Villoslada. *A new model of neuroinflammation: in vitro cerebellum organotypic cultures challenged with LPS (p.18)*

**16:00** N Gresa, J Serratosa, J Saura, C Solà. *C/EBP $\delta$  inhibition in microglial cells is involved in the anti-inflammatory and neuroprotective effects of the flavonoid chrysin (p.19)*

**16:30** C Sanabra, E Johansson, R Martín, T Vilaró, R Cortés, G Mengod. *Sex-related differences observed in cAMP specific PDE4B splice variants in oligodendrocytes during systemic inflammation (p.20)*

### **17:00 Plenary lecture**

**Marie T. Filbin. *Signaling axonal regeneration in the CNS (p.21)***

### **18:00 Coffee break**

### **18:30 Oral communications. Chair: C. Solà**

**18:30** C Muñoz-Quiles, FF Santos-Benito, MB Llamusi, A Ramón-Cueto. *Repair of chronic spinal cord injuries by adult olfactory bulb ensheathing glia and feasibility for autologous therapy (p.22)*

**19:00** J Selva, SE Martínez, G Egea. *Chronic ethanol exposure decreases RhoA activation in rat astrocytes: increase of the RhoGAP activity (p.23)*

### **19:30 Poster session**

### **21:00 Dinner**

## **Wednesday March 10**

### **16:00 Oral communications. Chair: H. Cabedo**

**16:00** D Clemente, MC Ortega, FJ Arenzana F de Castro. *FGF-2 and Anosmin-1: biomarkers for Multiple Sclerosis (p.24)*

**16:30** R Gorina, M Font-Nieves, L Márquez-Kisinousky, T Santalucia, AM Planas. *TLR4 activation in astrocytes generates a proinflammatory environment through MyD88-dependent and independent pathways not involving IFN- $\beta$ : modulator effects of heparin (p.25)*

### **17:00 Plenary lecture**

**William D. Richardson. *Development and regeneration of myelin (p.26)***

### **18:00 Coffee break**

### **18:30 Oral communications. Chair: R. Gallego**

**18:30** C Sanfeliu, S García-Matas, N de Vera, S Revilla, Y García, L Giménez-Llort, R Cristòfol. *Enhancement of astrocyte functionality as a potential therapeutic tool in Alzheimer's disease (p.28)*

**19:00** P Villoslada, A di Penta A, M Kerschensteiner, C Matute, V Ceña, I Lopez, J Alberch, J Parent, J Serra, O Errea, B Moreno. *Neuroprotective activity of Methylthioadenosine for the treatment of Multiple Sclerosis and other neurological diseases (p.29)*

### **19:30 Poster session and closing**

### **21:00 Dinner**

## Poster presentations

Posters must be mounted on the boards on Sunday 7 or Monday 8 (lobby of Benasque Centre for Science) and will remain on display during the whole conference. Authors should be at their posters at the end of oral sessions.

Poster size is 90 cm x 140 cm (portrait).

P1. LE Maglio, A Araque. *Endocannabinoids mediate neuron-astrocyte communication in somatosensory cortex (p.31)*

P2. A Martinez-Ferre, M Navarro-Garberi, S Martinez. *Diencephalic regionalization is regulated by Wnt signal (p.32)*

P3. C Viéitez, JM Tusell, N Gresa-Arribas, G Dentesano, J Serratosa, C Solà. *CD200R and TREM-2: microglia innate immune markers modulated by pro-inflammatory and anti-inflammatory agents (p.33)*

P4. P Carriba, R Masgrau, L Pardo, E Galea. *Convergence of calcium- and cyclic AMP-dependent signalling into CREB activation in astrocytes (p.34)*

P5. E González-Fernández, MV Sánchez-Gómez, C Matute. *Activation of adenosine receptors induces apoptosis in oligodendrocytes (p.35)*

P6. JA Gomez-Sanchez, M Lopez de Armentia, R Lujan, N Kessaris, WD Richardson, H Cabedo. *Overexpresión of Typelll-b3 neuregulin induces Schwann Cell proliferation, Remak bundle myelination and neurofibromatosis (p.36)*

P7. MC Ortega, R Kozyraki, D Clemente, F de Castro. *Sonic hedgehog and megalin receptor in the biology of oligodendrocyte precursors (p.37)*

P8. M del Caño, MD Ganfornina, D Sanchez. *Protective role of Glial Lazarillo in a model of Spinocerebellar Ataxia (SCA1) in Drosophila (p.38)*

P9. S Alfonso-Loeches, M Pascual-Mora, M Pascual-Lucas, S Fernández-Lizarbe, C Guerri. *Activation of glial cells and innate immunity through the toll-like receptor 4 (tlr4) triggers neuroinflammation and brain damage induced by ethanol (p.39)*

P10. N García-Mateo, D Sanchez, MD Ganfornina. *Is Apolipoprotein D important in myelin clearance by Schwann cells and the recruitment of macrophages after sciatic nerve injury? (p.40)*

P11. R Bajo-Grañeras, D Sanchez, MD Ganfornina. *ApoD, an apolipoprotein expressed by astrocytes, is induced upon oxidative insult via the JNK pathway, and is required for cell survival (p.41)*

P12. M Barceló, A Lewis, F Picatoste, E Claro, G Churchill, R Masgrau. *NAADP plays a role in astrocytic calcium signaling (p.42)*

# **Oral Communications and Plenary Lectures**

**15:30 miR-22 reduces cerebellar granular neuron precursors proliferation through the down-regulation of the MYCN partner MAX**

J Berenguer de Felipe, S Pons, Institute for biomedical research, IIBB-CSIC-IDIBAPS, Barcelona, Spain

MicroRNAs (miRNAs) are short (~22 nucleotides long) non-coding RNA molecules that regulate protein translation through binding consensus sequences normally located on the 3' untranslated regions of their target mRNAs. Although their existence was not revealed until early 1990s, the relevance of miRNAs activity on the regulation of important biological processes as development or cancer, have already been pointed out. A main issue in the cerebellum development, is the control of the cerebellar granule neuron precursor (CGNPs) proliferation. We have previously reported that Sonic hedgehog (Shh) induced proliferation of CGNPs, can be equally reverted by the Bone Morphogenetic Proteins (BMPs) or the direct activation of the Cyclic AMP-dependent Protein Kinase (PKA). Although BMP pathway is not mediated by PKA activation, both induce a similar proliferation stop and neuronal differentiation of CGNPs. Although a correlation between Shh activity and the expression of some miRNAs, as miR-17-92 cluster, miR-125b, miR-324-5p and miR-326 it has already been shown in CGCPs, up to now, no miRNAs have been shown to mediate the anti-proliferative activity developed by BMPs or PKA. To deepen into this issue, we performed a miRNA microarray analysis comparing the expression levels of the miRNA populations expressed in CGNPs either proliferating in the presence of saturating doses of Shh or cultures where neural differentiation was induced either by BMP-2 addition or by PKA activation with the cAMP analog Dibutiryl-cAMP. Interestingly, several miRNA species displayed differential expression levels comparing proliferating versus non proliferating cultures. Noteworthy, miR-19b and 18a, belonging to the miR-17-92 cluster (previously related to Shh activity) were down regulated in both anti-proliferative conditions tested. On the other hand, miR-22 was significantly upregulated in differentiating cells, predicting an anti-proliferative role for this miRNA in cerebellar development. In agreement, transient over-expression of miR-22 in CGCPs reduced Shh induced proliferation. Furthermore, we demonstrated that miR-22 expression significantly reduced the protein levels and the trans-activation activity of Myc Associated Factor X (MAX) one of the best scored among the In silico predicted targets of miR-22. MAX and MYCN both belong to the bHLH transcription factor family and the formation of MAX-MYCN dimers is necessary to produce the active transcription factor. Interestingly, MYCN has been shown to be the main effector of Shh induced proliferation. These results strongly suggest that miR-22 regulation of MAX expression, strongly contributes to the MYCN activity silencing required for cell differentiation. These results together with the suggestive expression patterns shown by miR-22 and MAX in the proliferating CGNPs of the cerebellar external layer (EGL) predicts an important role for miR-22 in the cerebellum development.

**Notes:**

**16:00 Functional implications of FGF-2 and Anosmin-1 in the proliferation and migration of oligodendrocyte precursor cells from postnatal and adult cerebral cortex**

FJ Arenzana, E Medina, C Ortega, D Clemente, F de Castro, Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Paraplégicos, Toledo, Spain

In the mature central nervous system (CNS) of both healthy and sick people, there is a fairly good amount of oligodendrocyte precursor cells (OPCs). Although the physiological relevance of these cells remains to be enlightened, this cell type represents an interesting target to design effective cellular therapies to repair the tissular damage in demyelinating diseases, like multiple sclerosis (MS). In the last years, our group has identified new molecular cues (FGF-2, SHH, Anosmin-1) which are involved in OPC proliferation and migration during development (Bribián et al., 2006; Merchán et al., 2007; Bribián et al., 2008). However, in order to seriously consider OPCs transplants as therapeutic approach in patients with demyelinating diseases, it is mandatory determining the functional properties of mature/adult OPCs. In the present work, we have analysed the role of FGF-2 and Anosmin-1 in the migration of OPCs isolated from cerebral cortex of postnatal (P0, P15) and adult (P60) mice. The cerebral cortex is specially interesting because this CNS region displays a high amount of this cell type.

Our results demonstrate that FGF-2, exogenously added in incubation medium, exerts a chemoattractive effect on OPCs at all stages, while the opposite effect is exerted by Anosmin-1. In both cases, these effects are reverted using a specific inhibitor of FGFR1 (SU5402) and a general inhibitor of FGFRs (SB402451). Regarding proliferation, only FGF-2 increase OPC proliferation, and this mitogenic effect is also via FGFR1. In conclusion, we report that chemoattraction effect of FGF-2/FGFR1/Anosmin-1 system on OPCs is well conserved in forebrain-derived OPCs during all lifespan. In addition, FGF-2 induces mature/adult cortical OPCs to proliferate. Both effects are potentially interesting to understand the pathogeny of demyelinating diseases like MS, as well as to consider these molecules as putative therapeutic targets fo this disease.

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**Notes:**

**16:30 Cerebellar oligodendroglia have extracerebellar origin**

N Mecklenburg, S Martínez, Instituto de Neurociencias, Alicante, Spain

While the origin of neurons and glia in the prosencephalon has been extensively analyzed, the origin of cerebellar glial cells remains sketchily known. It is postulated that the cerebellar neurons and glia originate from the alar rhombomere 1 ventricular epithelium and the rhombic lip. To investigate if specific types of glial cells in the cerebellar cortex have their origin in regions outside of the cerebellar ventricular region, in-ovo transplants were performed using the quail/chick chimeric system. Transplants were performed at chicken developmental stage HH10 and equivalent stage for quail embryos. The chimeric embryos were developed up to HH45 (19 days) to map the localization of donor cells and analyzed their phenotype by immunohistochemistry staining. We found that, cells transplanted from the alar plate of the mesencephalic vesicle along the antero-posterior axis of midbrain, revealed cellular streams migrating from the graft into the host cerebellum, crossing the isthmus border and entering the cerebellum via the velum medullare and folia 1. Mapping the final location of these originally mesencephalic cells showed that they are located in all layers of the cerebellar cortex except the external granular layer. Nevertheless, they were mainly accumulated in the white matter of cerebellar folia, as well as in the superficial levels of internal granular layer and around Purkinje cells, where they are positive for Vimentin, Plp and Olig2. The combinatory analysis of the different grafts allowed us to propose a fate map of chick cerebellar oligodendroglia at neural tube stage.

**Notes:**

## Plenary Lecture

### 17:00 Glial function in neurodegenerative diseases

#### Helmut Kettenmann

Helmut Kettenmann, Max Delbrueck Center for Molecular Medicine (MDC),  
Robert Roessle Str. 10, 13092, Berlin, Germany

Microglial cells are the innate immune cells of the central nervous system. Upon any injury or pathologic insult they transform from the ramified, resting form into activated microglial cells. We have recently found that these cells do not only express receptors typical for immune cells but also receptors for the interaction with brain cells, namely classical neurotransmitter receptors. These receptors are so called 'off-signals' for microglial activation, namely they counteract parameters of activation. Microglial activation is not an all or none process but can considerably vary in the context of the pathology. An interesting example is the interaction of microglial cells with glioma cells. While they have an activated morphology, microglia do not attack tumor cells. In contrast, they promote tumor progression. This is accomplished by the upregulation of MT1-MMP in microglia, an ectoenzyme which activates tumor released proteases.

*Supported by DLR 01GZ0701*

#### Notes:

### **18:30 Endocannabinoids mediate neuron-astrocyte Communications**

A Araque, Instituto Cajal (CSIC), 28002, Madrid, Spain

Accumulating evidence indicate the existence of bidirectional communication between astrocytes and neurons, in which astrocytes exchange information with the synaptic elements, playing active roles in brain physiology.

I will present data demonstrating the existence of neuron-astrocyte signalling mediated by endocannabinoids (ECBs) in both hippocampal and somatosensory cortical slices of mice. In both brain areas, astrocytes express functional type 1 cannabinoid receptors (CB1Rs) that upon activation increase the intracellular calcium through  $G_{q/11}$ - and phospholipase C-dependent release of calcium from internal stores. Depolarization of both hippocampal and layer V pyramidal neurons stimulate the release of ECBs, which activate CB1Rs in adjacent astrocytes, elevating their calcium levels.

In addition, I will present our recent data showing the consequences of the ECB-mediated neuron-astrocyte signalling on synaptic transmission. Endocannabinoids released by hippocampal pyramidal neurons increase the probability of transmitter release at single CA3-CA1 hippocampal synapses. This synaptic potentiation is due to CB1R-induced calcium elevations in astrocytes, which stimulate the release of glutamate that activates presynaptic group I metabotropic glutamate receptors. While endocannabinoids induce  $G_{i/o}$  protein-mediated homosynaptic depression by activation of presynaptic CB1Rs, they lead to heterosynaptic potentiation by activation of CB1Rs in astrocytes. Therefore, astrocytes respond to endocannabinoids that then regulate synaptic transmission, indicating that astrocytes are actively involved in brain physiology.

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#### **Notes:**

**19:00 Schwann cell proliferation versus myelin thickness: a differential role for distinct neuregulin splicing forms.**

H Cabedo, Instituto de Neurociencias de Alicante, Spain

Neuregulins are a group of cell-cell signalling molecules that act as ligands of ErbB receptor tyrosine kinases. NRG1 is a highly spliced gene with more than 15 isoforms, each containing an EGF-like domain that is essential for receptor binding and activation. According to the structure of their N-terminal domains, three main types of splicing products (type I-III) with specific biological roles have been described. It has been shown that type III isoforms (characterized by a cysteine rich domain) are involved in the control of Schwann cell development and PNS myelination. Two different neuregulin type III isoforms have been so far described, type III- $\beta$ 1a and type III- $\beta$ 3. Gain of function studies on the role the type III isoforms in myelination have been exclusively conducted with the type III- $\beta$ 1a splicing form. However, the type III- $\beta$ 3 isoform (also known as SMDF from Sensory and Motor-neuron Derived Factor) is also highly expressed in PNS neurons, suggesting it could have a role in myelin development. To determine the role of this isoform, we have generated transgenic mice that over-express human SMDF under the control of the rat neuron specific enolase promoter. Neuronal over-expression of this neuregulin stimulates Schwann cell proliferation and dramatically enlarges peripheral nerves and ganglia - which come to resemble plexiform neurofibromas- but have no effect on myelin thickness. Our data suggest that, albeit structural similarities between type III $\beta$ 1 and type III $\beta$  3 isoforms, this last neuregulin isoform plays a distinct role in myelination. We propose that subtle sequence differences that drive proteins to lipid raft microdomains (and no plasma membrane localization per se) dictate the differential biological role of neuregulin isoforms.

**Notes:**

**15:30 A new model of neuroinflammation: in vitro cerebellum organotypic cultures challenged with LPS**

A Di Penta, O Errea, E Moreno, B Fernández, N Escala, P Villoslada, Neuroimmunology Group, Department of Neuroscience, Institut Biomedical Research August Pi Sunyer (IDIBAPS), Hospital Clinic, Barcelona, Spain

Multiple sclerosis (MS) is an autoimmune/inflammatory disease of central nervous system (CNS). MS affects more than two million people worldwide and it is known as the main neurological disability among juvenile/adult population. Axonal degeneration, Wallerian degeneration as well as dying-back degeneration, are active phenomena characterized by highly regulated new protein synthesis. The knowledge of the mechanisms implicated in the axonal degeneration is necessary for the formulation of new neuroprotective treatments. Such treatments in MS patients should have a relevant impact in controlling neurodegenerative diseases as well as in preventing chronic dyscapacities.

For this reason, we built a model of neuroinflammation by using organotypic cultures of cerebellum stimulated with LPS for 1 to 96 hours. Such a model mimics endogenous microglial activation, by inducing demyelination and further axonal damage. In particular, we observe by ELISA assay peaks of TNF- $\alpha$  and IL1- $\beta$  release after 3 and 24 hours of LPS treatment respectively. At the same time we also observe an increase of iNOS and GAPDH expression with axonal damage and further demyelination. This suggests oxidative stress as the principal responsible for axonal damage. By morphological analysis after COX-I staining we also observe the formation of multiple axonal spheroid with mitochondria accumulation at end bulbs.

This model allows us to:

1. Establish the dynamic sequence of events for axonal damage, due to neuroinflammation
2. Identify cellular and molecular factors related with axonal damage
3. Analyze the neuroprotective role of different drugs

**Notes:**

**16:00 C/EBP $\delta$  inhibition in microglial cells is involved in the anti-inflammatory and neuroprotective effects of the flavonoid chrysin**

N Gresa<sup>1</sup>, J Serratosa<sup>1</sup>, J Saura<sup>2</sup>, C Solà<sup>1</sup>, <sup>1</sup>Dept. Cerebral Ischemia and Neurodegeneration, Institut d'Investigacions Biomèdiques de Barcelona-CSIC, IDIBAPS. Barcelona, Spain.<sup>2</sup>Unit of Biochemistry, Faculty of Medicine, University of Barcelona, IDIBAPS. Barcelona, Spain

Neuroinflammation, in which reactive glial cells (mainly microglia) are involved, has been suggested to play a key role in the development of neurodegenerative diseases. A significant effort is performed to study new targets and to develop and test anti-inflammatory drugs that could inhibit neuroinflammation. We study the role of CCAAT/enhancer binding protein (C/EBP)  $\beta$  and  $\delta$  transcription factors in neuroinflammation and neurotoxicity induced by reactive microglial cells. C/EBPs could be a therapeutic target against neuroinflammation, their inhibition resulting in neuroprotection.

As a model of neuroinflammation, we induced neuronal death in primary neuronal-microglial cocultures using LPS/IFN $\gamma$  to activate microglia. The lack of specific pharmacological inhibitors of C/EBPs makes it difficult to study their role in neuroinflammation. As several in vitro studies show the anti-inflammatory effect of plant flavonoids in activated macrophages and microglial cells, we determined the anti-inflammatory and neuroprotective effect of the flavonoid chrysin in our model of neuroinflammation, and the involvement of C/EBPs and NF- $\kappa$ B in its mechanism of action. Chrysin pretreatment inhibited the pro-inflammatory phenotype in LPS/IFN $\gamma$ -treated microglial cells and the resulting neurotoxicity in the cocultures. These effects occurred in the presence of a decrease in C/EBP $\delta$  nuclear expression without any effect on C/EBP $\beta$  and p65 nuclear expression. C/EBP $\beta$  and p65 LPS/IFN $\gamma$ -induced DNA binding was neither decreased by chrysin pretreatment.

Our results suggest that the inhibition of C/EBP $\delta$  expression protects from neurotoxicity induced by reactive glial cells, pointing out C/EBP $\delta$  as a possible therapeutic target in the treatment of neurodegenerative processes occurring in the presence of neuroinflammation.

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**Notes:**

**16:30 Sex-related differences observed in cAMP specific PDE4B splice variants in oligodendrocytes during systemic inflammation**

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Intracellular cAMP levels are regulated by a balance between the activities of two types of enzymes, adenylyl cyclase and cyclic nucleotide phosphodiesterase (PDE). Many inflammatory responses involve cAMP and pharmacological manipulation of cAMP levels using specific PDE inhibitors provoke profound anti-inflammatory response.

PDE4 represents a family of cAMP-specific PDEs consisting of four paralog genes (PDE4A-D) each distinguishable by its unique N-terminal sequence. Selective inhibition of PDE4 activity, both *in vitro* and *in vivo*, has several proven anti-inflammatory effects. Furthermore, recent publications indicate that the PDE4B gene is the predominant subtype involved in inflammatory induction by lipopolysaccharide (LPS) in mouse monocytes and macrophages.

Administration of LPS is a well known model of the acute phase response to inflammation. We have analyzed the influence of LPS administration (10mg/kg, i.p.) (as a septic shock model) on the distribution pattern and expression levels of the four PDE4B mRNA splicing variants together with the inflammatory markers, COX-2, IL-1 $\beta$  and TNF- $\alpha$  mRNAs in both male and female mice brain. Characterization of the cell populations involved in the PDE alterations was established by double *in situ* hybridization histochemistry and immunohistochemistry. By semi-quantitative analyses of the autoradiograms we observed that PDE4B2 and PDE4B3 were the only mRNA splice variants that exhibited an altered response to LPS treatment, and this response was furthermore associated with the sex. Whereas in female brain PDE4B2 presented an increase in mRNA levels at both 3 and 24h after LPS treatment, in male brain the expression changed at 3h but remained unaltered at 24h. PDE4B3 mRNA levels showed clear changes in females 24h post-injection, whereas, in male the altered expression was less evident and peaked 8h after treatment.

Knowledge about PDE4B mRNAs expression in mouse brain in both sexes and the alterations provoked by LPS administration might help us to clarify sex-related differences in the susceptibility to autoimmune diseases.

*Financed by SAF2006-10243/2009-11052. CS is a recipient of a fellowship from the IDIBAPS and EJ from Ministerio de Educación y Ciencia (BES-2007-16588).*

**Notes:**

## Plenary Lecture

### 17:00 Signaling axonal regeneration in the CNS

#### Marie T. Filbin

Marie T. Filbin, Biology Department, Hunter College, 695 Park Ave., New York, NY 10065, USA

A major impediment to axonal regeneration after injury is inhibitors in myelin. Three myelin inhibitors have been identified in, NogoA, MAG and OMgp. One approach to overcome these inhibitors to encourage regeneration is to change the intrinsic state of the axon such that it no longer recognizes these molecules as inhibitory. We have shown that if neuronal cAMP levels are elevated MAG and myelin in general no longer inhibit axonal growth. This cAMP effect is transcription dependent and we have identified 4 very different genes that are up-regulated in response to elevation of cAMP. All 4 of these proteins block inhibition by myelin and they are currently being tested for their ability to promote regeneration in vivo. One of these proteins that is up-regulated is the enzyme Arginase I (Arg I), which is key in synthesis of polyamines. We have shown that the polyamine, putrescine must be converted to spermidine to overcome inhibition and to promote regeneration in vivo. Furthermore, we have shown that spermidine overcomes inhibition by activating the kinase CDK5, though the up-regulation of the CDK5 activator, p35. Up-regulation of p35 is transcription-independent and translation-dependent and requires the spermidine-induced activation of the eukaryotic initiation factor, eIF5a, by hypusination. Another protein that is up-regulated with cAMP is secretory, leukocyte, protease inhibitor (SLPI). SLPI overcomes inhibition by MAG and myelin in a dose-dependent manner. In addition, DRG neurons from animals that received SLPI intrathecally for 24 hours also are not inhibited by MAG and myelin when subsequently cultured. SLPI also promotes optic nerve regeneration when injected intraocularly at the same time as the optic nerve is crushed. Recently we showed that MAG-induces the phosphorylation of Smad2, which is necessary for inhibition. Interestingly, SLPI enters the neuron and accumulates in the nucleus, where it suppresses expression of Smad2. This in turn decreases the amount of Smad2 that is available for phosphorylation by MAG and so blocks inhibition.

*Supported by NINDS NS037060, NS041073 and the NY State SCI initiative.*

#### Notes:

**18:30 Repair of chronic spinal cord injuries by adult olfactory bulb ensheathing glia and feasibility for autologous therapy**

C Muñoz-Quiles<sup>1,2</sup>, FF Santos-Benito<sup>1</sup>, MB Llamusi<sup>1,2</sup>, A Ramón-Cueto<sup>1</sup>  
<sup>1</sup>Laboratory of Neural Regeneration, Institute of Biomedicine at Valencia (CSIC), Spain. <sup>2</sup>Fundación Investigación en Regeneración del Sistema Nervioso, Valencia, Spain

Olfactory Bulb Ensheathing Glia (OB-OEG) promote functional recovery of paraplegic rats and axonal regeneration in their injured spinal cords, after transplantation at acute or subacute stages (up to 45 days). However, the most relevant clinical scenario in humans is the chronic and this, in rodents, occurs beyond the third month. At the chronic stage there are no more cellular and molecular changes at the injury site and, thus, the environmental situation for growing axons is different than that at earlier stages. In addition, chronically injured neurons may present a different intrinsic response to damage. We have evaluated whether adult OB-OEG promote repair of severe chronic spinal cord injuries (SCI) as this has not been previously addressed. Rats with complete SCI, transplanted 4 months after injury, exhibited progressive improvement in voluntary hindlimb movement, not observed in non-transplanted animals. Moreover, the former showed better tissue preservation at the injury site and regeneration of axons from brainstem neurons across and beyond the injury. This histological repair correlated with motor improvement. Functional and histological outcomes did not differ at subacute or chronic stages what is clinically relevant because grafting can be delayed at least 4 months with no decrease in beneficial effects. Thus, autologous transplantation is a feasible approach as there is a time frame for patient stabilization after the accident and before surgery and for obtaining and preparing OB-OEG for grafting. The healing effects of adult OB-OEG on established injuries may offer new therapeutic opportunities for chronic SCI patients.

*Supported by Grants 01/1134; SAF2004-04773; R01 NS054159-01; and Fundación Investigación en Regeneración del Sistema Nervioso.*

**Notes:**

**19:00 Chronic ethanol exposure decreases RhoA activation in rat astrocytes: increase of the RhoGAP activity**

J Selva, SE Martínez, G Egea, Department of Cell Biology, Immunology & Neurosciences, School of Medicine, University of Barcelona, 08036 Barcelona, Spain.

Chronic ethanol exposure alters glucose uptake and glycosylation process in primary cultures of rat astrocytes. These alterations seem to be a direct consequence of dysfunction in the actin cytoskeleton organization and dynamics [1]. Rho GTPases are a well-known family of proteins that govern the organization of the actin cytoskeleton. We have recently reported that rat astrocytes chronically exposed to ethanol show a decrease of activated RhoA (RhoA-GTP), without altering Rac and Cdc42 [2]. Lysophosphatidic acid (LPA) is a strong activator of the RhoA-ROCK signalling pathway that prevents the ethanol-induced alterations in the glucose uptake, in the actin cytoskeleton organization and dynamics [1], and in RhoA-GTP levels. Here we show that the ethanol-induced decrease of RhoA-GTP levels is due to an increase in the RhoGAP activity and that LPA prevents it. Finally, we also provide experimental evidence of the RhoGAP protein that seems to be altered by ethanol exposure in rat astrocytes.

(1) Tomás M, Lázaro-Diéguez F, Durán JM, Marín P, Renau-Piqueras J, Egea G. *J Neurochem*. 2003 Oct; 87(1):220-9.

(2) Martínez SE, Lázaro-Diéguez F, Selva J, Calvo F, Piqueras JR, Crespo P, Claro E, Egea G. *J Neurochem*. 2007 Aug; 102(4):1044-52.

*Supported by grants BFU 2009-07186 and CSD 2006-00012*

**Notes:**

### **16:00 FGF-2 and Anosmin-1: biomarkers for Multiple Sclerosis**

D Clemente, MC Ortega, FJ Arenzana, F de Castro, Grupo de Neurobiología del Desarrollo-GNDe, Hospital Nacional de Parapléjicos, Toledo, Spain.

During development, FGF-2 exerts a mitogenic and chemoattractive effect on the migration of oligodendrocyte precursor cells (OPCs); these are mediated by FGFR1 and down modulated by the extracellular matrix protein Anosmin-1 (Bribián et al., 2006). This protein has a potent role in OPC adhesion during optic nerve development, limiting the migratory capabilities of these cells (Bribián et al., 2008). In demyelinating diseases like multiple sclerosis (MS), a permissive environment that allows migration of OPCs, similar to that during development, should exist to allow an effective remyelination (Dubois-Dalcq et al., 2005). But this occurs only during initial stages in both MS and EAE, when recruited OPCs migrate into the damaged regions to remyelinate (Chang et al., 2002; Wolswijk, 2002). Although there are some reports about the presence of FGF-2 in the cerebrospinal fluid of MS patients (Sarchielli et al., 2008), there are not histological confirmation about this.

In the present study, we have analysed the immunohistochemical distribution of FGF-2, FGFR1 and Anosmin-1 in human samples of MS to elucidate if these molecules are related to the pathogenesis of demyelination and would play significant roles in the remyelination scenario. To complete this, we show that adult cortical astrocytes up-regulate the expression of FGF-2 in pro-inflammatory conditions, and OPCs isolated from adult cerebral cortex respond to FGF-2 and Anosmin-1 as the embryonic OPCs do. Finally, we found a correlation between the levels of FGF-2 in the cerebrospinal fluid (CSF) and the types of histopathological lesions in MS patients, which would be relevant for clinics.

*Work supported by grants from MEC (SAF2007-65845), Consejería de Sanidad (ICS06024/00) and Consejería de Educación y Ciencia de Castilla-La Mancha (PAI08-0242-3822), FISCAM (G-2008-C8) and ISCIII (RD07-0060-2007, co-financed by FEDER "Una manera de hacer Europa").*

#### **Notes:**

**16:30 TLR4 activation in astrocytes generates a proinflammatory environment through MyD88-dependent and independent pathways not involving IFN- $\beta$ : modulator effects of heparin**

R Gorina, M Font-Nieves, L Márquez-Kisinousky, T Santalucia, AM Planas, Department of Brain Ischemia and Neurodegeneration, Institut d'Investigacions Biomèdiques de Barcelona (IIBB)-Consejo Superior de Investigaciones Científicas (CSIC), Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain

Brain damage activates the immune system in response to danger signals. Immune responses in the brain are mainly attributed to microglia. However, astrocytes also express toll-like receptors (TLR) and respond to innate immune signals. Here we investigate signalling pathways and released molecules after TLR4 activation in astrocytes, as these responses may have a strong impact in the local environment and surrounding cells. Purified cultures of astrocytes, obtained from wild type rats and mice and from MyD88-deficient mice, were treated with bacterial lipopolysaccharide (LPS). Tools used to interfere with this system include small interference RNA, inhibitory drugs, and heparin; an anticoagulant with antiinflammatory actions. LPS induced early activation of the transcription factor NF $\kappa$ B, through the MyD88 adaptor, and expression of TNF- $\alpha$ , VCAM-1, IL-15, and IL-27. LPS also activated MAPK and induced delayed activation of Jak1/Stat1, which was MyD88-independent but was not mediated by IFN- $\gamma$ . Heparin did not abrogate LPS-induced NF $\kappa$ B activation but attenuated the activation of p38, JNK and Jak1/Stat1 and the expression of negative cytokine regulator SOCS-1 and CXCL10 chemokine (IP-10). Also, heparin blocked LPS-induced ERK1/2 activation and expression of downstream genes, such as Egr-1. Therefore, after TLR4-activation, astrocytes generate a proinflammatory environment, through NF $\kappa$ B, MAPK and Jak1/Stat1, that could promote microglial reactivity, blood-brain barrier dysfunction, leukocyte attraction, adhesion, and infiltration, and could favour certain phenotypes in infiltrating lymphocytes. Heparin modulates this profile by inhibiting MAPK and Jak1/Stat1 signalling, without impairing the transcriptional activity of NF $\kappa$ B.

*Supported by grants from the Ministerio de Educación y Ciencia (SAF2008-04515), Generalitat de Catalunya (CIDEM, RDITSCON07-1-0006) and the European Community (FP7/2007-2013 project, grant agreement n°201024). MF has a PhD grant from MEC.*

**Notes:**

## Plenary Lecture

### 17:00 Development and regeneration of myelin

#### William D Richardson

Richa B Tripathi<sup>1</sup>, Huiliang Li<sup>1</sup>, Konstantina Psachoulia<sup>1</sup>, Laura Clarke<sup>2</sup>, Valeria Burzomato<sup>2</sup>, Kaylene Young<sup>1</sup>, Nicoletta Kessar<sup>1</sup>, David Attwell<sup>2</sup> and William D Richardson<sup>1</sup>, <sup>1</sup>Wolfson Institute for Biomedical Research and Dept of Cell and Developmental Biology. <sup>2</sup>Dept of Neuroscience, Physiology and Pharmacology, University College London, Gower Street, London WC1E 6BT, United Kingdom

Oligodendrocytes (OLs), the myelinating cells of the central nervous system (CNS), develop from migratory glial precursors that are specified in the ventricular zones (VZ) of the embryonic brain and spinal cord. Most oligodendrocyte precursors (OLPs) in the spinal cord develop from the so-called “pMN” progenitor domain in the ventral VZ, which first generates motor neurons before switching to OLP production. We are working to elucidate the mechanisms of neuron-glial fate switching in pMN. For example, we have identified a crucial serine residue in transcription factor OLIG2, the phosphorylation status of which determines the transcriptional co-factors and hence the functional properties of OLIG2. Phosphorylation favours motor neuron generation and de-phosphorylation favours OL lineage specification (Huiliang Li et al. unpublished). It is now important to understand how phosphorylation at this site is regulated.

A minor fraction (~20%) of OL lineage cells in the spinal cord develops from progenitor domains more dorsal than pMN. It is not known whether the dorsally- and ventrally-derived cells are functionally different. To explore this we have generated a reporter mouse *Sox10-lox-GFP-STOP-lox-tdTom*, which expresses GFP constitutively in OLPs and OLs. In the presence of Cre recombinase, GFP is excised and tandem-duplicate Tomato (tdTom) is expressed instead. We crossed this reporter line with *Gsh2-Cre*, which targets Cre to the VZ of the dorsal spinal cord and ventral forebrain, allowing us to visualize ventrally- and dorsally-derived OL lineage cells by intrinsic fluorescence (green or red) and study their physical and electrical properties side-by-side. Preliminary data suggest that OLPs from dorsal and ventral origins have similar electrical and membrane properties but serve different fibre tracts.

A subset of OLPs persists into adulthood in both grey and white matter. We recently showed by fate mapping with *Pdgfra-CreER<sup>T2</sup>* mice that these adult OLPs (“NG2 cells”) continue to generate new myelinating OLs in healthy adult mice up to eight months of age and beyond - though at a steadily decreasing rate that is coupled with a diminishing rate of cell division (self-renewal). We are currently testing whether this extended myelination program is involved in neural plasticity – motor learning and memory, for example. We are also investigating the differentiation potential of NG2 cells following demyelinating damage such as occurs in multiple sclerosis.

*We thank The Wellcome Trust, the Medical Research Council and the European Research Council for support.*

**Notes:**

**18:30 Enhancement of astrocyte functionality as a potential therapeutic tool in Alzheimer's disease**

C Sanfeliu, S García-Matas, N de Vera, S Revilla, Y García, L Giménez-Llort<sup>1</sup>, R Cristòfol, IIBB, CSIC-IDIBAPS, <sup>1</sup>UAB, Barcelona, Spain

Alzheimer's disease (AD) is the most common age-related neurodegenerative disease as, indeed, advanced age is its major risk factor. Amyloid-beta peptide neurotoxicity is the proposed first step in a cascade of deleterious events leading to AD pathology and dementia. Astrocytes provide vital support to neurons and modulate functional synapses. Therefore, the toxic effects of amyloid-beta on astrocytes most probably potentiate the neurodegenerative changes that lead to AD. Conversely, an enhancement of astrocyte functionality would restore brain functions or at least delay neurodegeneration. We studied the effects of amyloid-beta on human astrocyte cultures and in rats in vivo. Conditions mimicking the age-related pro-oxidant environment through buthionine-sulfoximine and ferrous sulfate treatment greatly potentiate amyloid-beta deleterious effects on astrocytes. On the other hand, we have reported that an over-expression of the neurotrophic factor GDNF in hippocampal astrocytes improved the learning and memory of cognitive deficient aged rats in the Morris water maze paradigm (Pertusa et al., *Neurobiol Aging* 29:1366-79, 2008). Now we have over-expressed GDNF in hippocampal astrocytes of 3xTg-AD mice to understand the neuroprotective potential of astrocytes in AD and the preliminary results will be presented. Astrocytes are a candidate cell type for therapeutic interventions against neurodegeneration.

*Supported by grants RD06/0013/1004 from ISCIII, SAF2009-13093-C02-02 from MCINN, 062931 from Fundació La Marató de TV3.*

**Notes:**

**19:00 Neuroprotective activity of Methylthioadenosine for the treatment of Multiple Sclerosis and other neurological diseases**

P Villoslada<sup>1</sup>, A di Penta A<sup>1</sup>, M Kerschensteiner<sup>2</sup>, C Matute<sup>3</sup>, V Ceña<sup>4</sup>, I Lopez<sup>5</sup>, J Alberch<sup>6</sup>, J Parent<sup>7</sup>, J Serra<sup>8</sup>, O Errea<sup>1</sup>, B Moreno<sup>1</sup>, <sup>1</sup>Department of Neuroscience. Institut of Biomedical research August Pi Sunyer (IDIBAPS), Hospital Clinic of Barcelona, Spain; <sup>2</sup>University of Munich, Germany; <sup>3</sup>University of Basque Country, Spain; <sup>4</sup>University of Castilla la Mancha, Spain; <sup>5</sup>CIMA - University of Navarra, Spain; <sup>6</sup>University of Barcelona, Spain; <sup>7</sup>University of Michigan, US; <sup>8</sup>Neuroscience Technologies, Spain

**Background:** Methylthioadenosine (MTA) has anti-oxidant and anti-proliferative properties and was shown to induce cell protection in hepatic cells. We previously demonstrated that exert immunomodulatory effects in the animal model of MS.

**Objectives:** To assess the neuroprotective effects of MTA in models on MS, Parkinson disease, stroke and Epilepsy

**Methods:** We performed toxicity/excitotoxicity vitro assays with primary neuronal, oligodendrocyte or neuron-astrocytes cultures, optic nerve (ON) and cerebellar organotypic cultures with different doses of MTA. We also assessed the ability of MTA for preventing axonal loss and neuronal death in animal models of MS (EAE in CSF-GFP Tg mice: axonal count and motor evoked potential (MEP)), Stroke (middle cerebral artery occlusion (MCAO) and Transient forebrain ischemia (TFI)), Parkinson disease (MPTP in C57B6 mice) and Epilepsy (Pilocarpine Temporal Lobe Epilepsy (TLE)).

**Results:** In vitro studies revealed that MTA protects neurons against glutamate excitotoxicity. Indeed, MTA prevented excitotoxicity oligodendrocyte loss in vitro and in ON cultures. In cerebellar organotypic cultures stimulated with LPS, MTA reduced myelin loss and decreased axonal transection. In the MS model, reduced axonal loss and recovered MEP. In stroke models, MTA was unable to increase neuronal survival in the MCAO model, but protected partially neurons from death in the TFI. In the MPTP model, high dose MTA (60 mg/kg/day) reduced TH-neuronal loss in the substantia nigra. In the TLE model, MTA decreased NeuN-neuronal loss in hilus, CA3 and CA1 region.

**Conclusions:** MTA is neuroprotective in models of MS and other diseases, deserving further clinical studies for treating brain diseases.

**Notes:**

# Posters

## **P1. Endocannabinoids mediate neuron-astrocyte communication in somatosensory cortex**

LE Maglio, A Araque, Instituto Cajal, CSIC, Madrid, Spain

Although, the functional expression of CB1 receptors (CB1Rs) has been demonstrated in hippocampal astrocytes, their expression in astrocytes from other brain regions remains unknown. We have investigated whether endocannabinoid mediated neuron-astrocyte communication in somatosensory cortex. We recorded the intracellular Ca<sup>2+</sup> levels of astrocytes located in all layers of somatosensory cortex and analyzed the responses to local application of the CB1R agonist WIN 55,212-2.

We have found that:

Cortical astrocytes respond with transient intracellular Ca<sup>2+</sup> elevations to local application of WIN.

The astrocytic intracellular Ca<sup>2+</sup> increases were blocked by the selective antagonist of CB1Rs, AM251 (2  $\mu$ M).

WIN-evoked astrocytic Ca<sup>2+</sup> elevations were abolished by thapsigargin (1  $\mu$ M), that depletes the intracellular calcium stores.

The phospholipase C antagonist U73122 (4  $\mu$ M) prevented the WIN-evoked Ca<sup>2+</sup> elevations in astrocytes.

Ca<sup>2+</sup> elevations evoked by WIN were absent in astrocytes from transgenic mice lacking CB1R expression (CB1R<sup>-/-</sup>).

We have also investigated whether the CB1R-mediated intracellular Ca<sup>2+</sup> signal in astrocytes can be endogenously evoked by endocannabinoids (ECBs) released from layer V pyramidal neuron. We monitored Ca<sup>2+</sup> levels in the astrocytes and depolarized the neuron to stimulate the release of ECBs. We found that neuronal depolarization elevated intracellular Ca<sup>2+</sup> levels in adjacent astrocytes located in layers II/III and V.

These results indicate that cortical astrocytes express functional CB1R, that upon activation increase the intracellular calcium through Gq/11- and phospholipase C-dependent release of calcium from internal stores. Therefore, these results indicated the existence of neuron-astrocyte signalling mediated by endocannabinoids in somatosensory cortical slice of mice.

*We thank A. Zimmer for the generous gift of the CB1R<sup>-/-</sup> mice.*

*Supported by: MICINN (BFU2007-64764) European Union (Health-F2-2007-202167) and Cajal Blue Brain.*

**Notes:**

## **P2. Diencephalic regionalization is regulated by Wnt signal**

A Martinez-Ferre, M Navarro-Garberi, S Martinez, Experimental Embryology Laboratory, Instituto de Neurociencias (UMH-CSIC), 03550, San Juan de Alicante, Spain

Morphogenetic activity of secondary organizers, located in different regions of the developing neural tube, refines the specification of the main domains in the brain primordium. These inductive signals are molecules that belong to four genetic families: Wingless-Int protein family (Wnt), Hedgehog family (Hh), Bone morphogenetic protein family (Bmp) and Fibroblast growth factor family (Fgf). The restricted temporo-spatial expression pattern of these signals seems to be crucial for the regulation of neural tube morphogenesis and cell fate specification.

The zona limitans intrathalamica (ZLI) is a neuroepithelial domain that separates the prethalamus from the thalamus and it is considered as a source of local morphogenetic inductive properties. Genes coding for diffusible morphogens at the ZLI suggest its role as diencephalic secondary organizer. Signaling molecules, such as fibroblast growth factor 8 (fgf8), Sonic hedgehog (Shh), bone morphogenetic proteins (BMPs) 2,4,6 and 7, and Wnts 1,2b,3a,5a,7a and 8b control the expression of regional transcription factors. Thus, some of these signals in the ZLI may represent a source of molecular factors which control proliferation, regionalization and polarity in the diencephalic segments.

Despite the importance of the ZLI, the mechanism of its formation remains poorly understood. Using experimental embryological techniques by introducing microbarriers between the diencephalic basal-alar neuroepithelium and by insertion of inhibitors of the Wnt pathway in the most dorsal alar domain of P2, we studied the molecular mechanisms for generating a permissive territory for Shh expression in the ZLI.

**Notes:**

### **P3. CD200R and TREM-2: microglia innate immune markers modulated by pro-inflammatory and anti-inflammatory agents**

C Viéitez, JM Tusell, N Gresa-Arribas, G Dentesano, J Serratosa, C Solà, Dept. Cerebral Ischemia and Neurodegeneration, Institut d'Investigacions Biomèdiques de Barcelona-CSIC, IDIBAPS, Barcelona, Spain.

Neuroinflammation, in which reactive glial cells (mainly microglia) are involved, has been suggested to play a key role in the development of neurodegenerative diseases. Consequently, a significant effort is performed to study new targets and to develop and test anti-inflammatory drugs that could inhibit neuroinflammation.

Microglia are the principal immune cells of the CNS. In the control brain, microglia are continually surveying their microenvironment, ready to detect changes that could compromise neuronal function. In response to brain tissue injury, these cells develop morphological and functional changes, among them the production of pro-inflammatory mediators with potentially neurotoxic consequences. This reactive/pro-inflammatory phenotype is inhibited in the normal brain through a strict control, in which neuronal cells are believed to play an important role through a series of “off-on” signals. “Off-signals” are present in control conditions and their absence determines the onset of microglial activation. “On-signals” are inducible and their activation determines the onset of a microglial response, either anti- or pro-inflammatory. The mechanism of action of these signals is still unknown.

The objective of the present work is to study the role of CD200R1 (off-signal), and TREM-2 (anti-inflammatory on-signal) in the modulation of the inflammatory response in microglial cells and the resulting neurotoxicity. As a first approach we are determining the response of these proteins to pro-inflammatory and anti-inflammatory stimuli. We believe that the modulation of the signal induced by these molecules may constitute a therapeutic target in the treatment of neurodegenerative diseases occurring in the presence of neuroinflammation.

*Supported by grant PI081396 (Instituto Carlos III of the Ministerio de Ciencia e Innovación of Spain).*

**Notes:**

#### **P4. Convergence of calcium-and cyclic AMP-dependent signalling into CREB activation in astrocytes**

P Carriba<sup>1,2</sup>, R Masgrau<sup>1,2</sup>, L Pardo<sup>1,2</sup>, E Galea<sup>1,2,3</sup>, <sup>1</sup>Institut de Neurociències. <sup>2</sup>Departament de Bioquímica, Universitat Autònoma de Barcelona. <sup>3</sup>Institut Català de Recerca i Estudis Avançats (ICREA), Spain

Although much evidence implicates CREB in synaptic plasticity and learning, most of the studies have focused exclusively on neurons with little consideration for astrocytes, despite evidence that these cells are key regulators of synaptic plasticity. The current project of the laboratory focuses on CREB in astrocytes with three general questions: 1. How is CREB activated?; 2. What are the consequences of CREB activation, including genes and functions?; and 3. Is CREB-dependent transcription (CREB-dt) altered in pathological states? In dealing with question # 1 we report that the calcium-elevating gliotransmitters ATP and noradrenaline stimulated CREB-dt in primary cortical astrocyte cultures, as judged by CRE-luciferase assays. CREB activation was completely blocked by BAPTA, a chelator of intracellular calcium, and by H89, a protein kinase A inhibitor, indicating obligatory roles of calcium and cAMP-dependent signaling in the activation of CREB-dt by ATP and noradrenaline. Also, CREB-dt was partially inhibited by ERK1/2 inhibitor U0126, indicating a facilitatory, rather than obligatory role of ERK1/2. The canonical view holds that CREB is regulated by the coactivator CBP that binds to pCREB, and by the family of calcium/calcieneurin-dependent coactivators TORCs. ATP and noradrenaline caused pCREB formation (western blots) with no effect of BAPTA, suggesting that pCREB and calcium triggered CREB-dt independently. Overexpression of DN-TORC2, but not DN-TORC1, inhibited the ATP and noradrenaline-elicited CREB-dt. Unexpectedly, the calcineurin inhibitors CsA and FSK506 did not inhibit CREB-dt, revealing that TORC2 can be activated in astrocytes by a mechanism other than calcineurin. Ongoing studies are aimed at dissecting signaling pathways.

#### **Notes:**

## **P5. Activation of adenosine receptors induces apoptosis in oligodendrocytes**

E González-Fernández, MV Sánchez-Gómez, C Matute, Department of Neurosciences, University of Basque Country, Leioa, Spain

Adenosine is a potent biological mediator which acts on four G-protein coupled receptors: A<sub>1</sub> and A<sub>3</sub>, primarily coupled to G<sub>i</sub> family G proteins, and A<sub>2A</sub> and A<sub>2B</sub>, mostly coupled to G<sub>s</sub> proteins. This neuromodulator can be released from most cells, including neurons and glia, but its concentration increases dramatically during cellular stress and brain injury. Moreover, recent studies have shown that adenosine receptors activation is involved in myelination, as well as in apoptosis and neurodegenerative diseases. In this study we have found that rat oligodendrocytes *in vitro* express all four subtypes of adenosine receptors, transporters ENT1 and ENT2 and the enzymes adenosine deaminase and adenosine kinase. Viability assays demonstrated that overstimulation of adenosine receptors reduces oligodendrocyte viability in a dose dependent manner. In turn, we observed that adenosine induces apoptotic cell death through both the intrinsic and extrinsic pathway, depending on the concentration of the agonist. Adenosine also causes a high increase in ROS levels which suggests an alteration in mitochondrial function. In addition, selective activation of adenosine receptors showed that the A<sub>3</sub> receptors are primarily responsible for adenosine toxicity to oligodendrocytes. Specifically, A<sub>3</sub> receptor activation triggers oxidative stress, mitochondrial membrane depolarization and oligodendrocyte death via intrinsic apoptosis pathway. Moreover, selective stimulation of A<sub>3</sub> receptor induces a decrease in the levels of MAP kinases phosphorylation ERK1/2 and Akt.

Together, these findings indicate that oligodendrocytes are endowed with the molecular machinery to process adenosine signals, and that those signals can be deleterious to oligodendrocytes and contribute to disease onset and/or progression.

*Supported by grant SAF2007-62380 from MCINN, CIBERNED and Gobierno Vasco.*

**Notes:**

## **P6. Overexpression of Type III-β3 neuregulin induces Schwann Cell proliferation, Remak bundle myelination and neurofibromatosis.**

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Type III neuregulins exposed on axon surfaces control myelination of the peripheral nervous system. Two different neuregulin type III isoforms have been so far described, type III-β1a and type III-β3. It has been shown, that threshold levels of type III β1a neuregulin dictate not only the myelination fate of axons but also myelin thickness. However, no *in vivo* data exists on the role of the type III-β3 splicing form. To study the biological role of this isoform, we have produced transgenic mice overexpressing it in neurons (using the NSE promoter). Neuronal overexpression of this isoform in mice stimulates Schwann cell proliferation and dramatically enlarges peripheral nerves (sciatic and vagus) and ganglia but have no effect on myelin thickness. The nerves display neurofibroma-like properties, such as abundant collagen fibrils and abundant dissociated Schwann cells, and in some cases develop big tumors. Moreover, the organization of Remak bundles is dramatically altered; the small-caliber axons of each bundle are no longer segregated from one another within the cytoplasm of a nonmyelinating Schwann cell but instead are close packed and the whole bundle wrapped as a single unit, frequently by a compact myelin sheath. We also found that transgene alters profoundly the developmental expression pattern of transcription factors controlling Schwann cell differentiation and myelination (Krox-20, Sox-10 and Oct-6). Because Schwann cell hyperproliferation and Remak bundle degeneration are early hallmarks of type I neurofibromatosis, we suggest that sustained activation of the neuregulin pathway in Remak bundles can contribute to neurofibroma development.

### **Notes:**

## **P7. Sonic hedgehog and megalin receptor in the biology of oligodendrocyte precursors**

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Low-density lipoprotein receptor-related proteins (LRPs) are cell-surface receptors with diverse biological functions during development. Megalin (also known as LRP2 or gp330) is a multiligand receptor involved in the development of the CNS and the transport of molecules across the blood brain barrier (Willnow *et al.*, 1996; Zlocovic *et al.*, 2008). Among its several ligands, the morphogen Sonic hedgehog (Shh) is one of the most studied. Indeed, the knock-out for megalin shows similar phenotype than the one for Shh, which suggests close related actions of both factors during development (McCarthy *et al.*, 2002).

Previous work of our laboratory demonstrated that Shh promotes the migration and proliferation of the oligodendrocyte precursor cells (OPCs) during development (Merchán *et al.*, 2006). In the present work, we analyse the putative role of megalin receptor, which is exclusively present in astrocytic cells, in Shh-mediated migration and proliferation of the OPCs. We detect spatio-temporal parallelisms between the expression of megalin and the Shh-related proteins Ptc-1 and Gli1, which can be explained in terms of the colonization of the optic nerve by the two different OPC subpopulations (plp-dm20<sup>+</sup> and PDGFR $\alpha$ <sup>+</sup>). On the other hand, we show that the blockade of megalin impedes OPC proliferation and interferes on Shh chemoattractive effect. In both cases, our data points out to a necessary Shh internalization by astrocytes via megalin receptor and its subsequent release by the same cell type previous to exert its effect on OPCs.

In addition of during development, Shh is also important in demyelinating diseases, like multiple sclerosis (Wang *et al.*, 2008). The role of megalin in this disease has been studied through the analysis of the distribution of this receptor and its cellular characterization in mice spinal cords of Experimental Autoimmune Encephalomyelitis (EAE), the animal model of multiple sclerosis. The expression of megalin is highest during the relapsing phase of EAE, since it is up-regulated in hypertrophied astrocytes in the periplaque and in infiltrated macrophages within the demyelinated area. Our data shows that megalin and Shh are present in those situations where the mobilisation of endogenous OPCs is necessary: e.g. during development and in the phase of demyelinating diseases in which remyelination still occurs.

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### **Notes:**

## **P8. Protective role of Glial Lazarillo in a model of Spinocerebellar Ataxia (SCA1) in Drosophila**

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Glial Lazarillo (GLaz) is a Lipocalin expressed by glia in Drosophila. The expression of its human homologue (Apolipoprotein D) increases with aging and in many neurodegenerative diseases. We have shown that Lazarillo protects against oxidative stress and plays a role in axonal regeneration after injury. Nevertheless its molecular mechanism of action is unknown.

We are using Spinocerebellar Ataxia type I (SCA1) as a model of neurodegenerative disease to test the GLaz mechanism of action. By driving the expression of polyglutaminated human Ataxin1 (ATXN1-82Q) to the fly photoreceptors, the retina degenerates and allows us to observe its pathogenic effects *in vivo*.

GLaz alleviates the degeneration caused by ATXN1-82Q. The eye external morphology is restored and the retinal tissue maintains its structure when GLaz is over-expressed in photoreceptors. Likewise the absence of GLaz exacerbates neurodegeneration.

We are now testing more physiological models where expression of ATXN1-82Q and GLaz is directed to glial cells. Behavior and stress resistance studies will allow us to test the effects of GLaz over-expression. As a second approach, we are generating a model of late motoneuron degeneration, so that we can check if GLaz is also beneficial when co-expressed with ATXN1-82Q in the cell type sensitive to the polyglutaminated Ataxin1 in patients.

Finally, we are generating flies where GLaz levels of expression are controlled in its native cell type (the glial cells) while ATXN1-82Q is expressed and causing degeneration in motoneurons, thereby achieving the closest model to the real pathological situation.

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**Notes:**

## **P9. Activation of glial cells and innate immunity through the toll-like receptor 4 (TLR4) triggers neuroinflammation and brain damage induced by ethanol**

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Glial cells and TLRs play critical role in brain injury. Activation of TLRs signaling can trigger stimulation of glial cells, production of inflammatory mediators, neuroinflammatory damage and neurodegeneration. Alcohol abuse induces brain damage and sometimes neurodegeneration. Although the mechanisms involved in these effects are unknown, we demonstrated that chronic ethanol consumption increases cytokines and inflammatory mediators in brain, activating signaling pathways associated with neuroinflammation, triggering cell damage. We have further shown that ethanol is able to activate TLR4 signaling in astrocytes and macrophages, suggesting that activation of TLR4 response by ethanol could be an important mechanism of ethanol-induced neuroinflammation. The present study aims to establish the potential role of TLR4 in ethanol-induced microglial activation and brain damage. By using primary culture of microglial and astroglial cells from *wild-type* (TLR4<sup>+/+</sup>) and *TLR4-deficient* (TLR4<sup>-/-</sup>) mice, we observed that TLR4 is critical for ethanol-induced inflammatory signaling in these glial cells, since knockdown of TLR4 by siRNA or cells from TLR4<sup>-/-</sup> mice, abolished the activation of MAPKs and NFκB pathways and the production of inflammatory mediators. *In vivo* studies also show that while chronic ethanol intake by TLR4<sup>+/+</sup> mice up-regulates CD11b (microglial marker) and GFAP (astrocyte marker), it increases caspase-3 activity, iNOS, COX-2 and several cytokines (IL-1β, TNF-α, IL-6) in cerebral cortex, the TLR4 deficiency protects against ethanol-induced glial activation, induction of inflammatory mediators and apoptosis. These findings not only provide the first evidence for the critical role of TLR4 response in ethanol-induced neuroinflammation, but they also provide new insight into the mechanisms participating in ethanol-induced brain injury and possible neurodegeneration.

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**Notes:**

**P10. Is Apolipoprotein D important in myelin clearance by Schwann cells and the recruitment of macrophages after sciatic nerve injury?**

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Glial cells express Apolipoprotein D (ApoD) during development, adulthood and aging of the vertebrate nervous system. We have shown that ApoD plays an important role in the events that follow sciatic nerve injury.

ApoD is secreted by Schwann Cells. These cells play a key role in both the clearance of myelin debris after crush injury, and the recruitment of macrophages to the injured site. Using ApoD knock-out and transgenic mice we have demonstrated that the lack of ApoD delays myelin clearance, and increases the number of macrophages in the injured site, all of which contributes to a considerable delay in axonal regeneration. We use this experimental paradigm to understand the molecular mechanisms underlying the function of this glial protein in the PNS. We propose the following working hypotheses: (1) *ApoD helps in presenting myelin components to Schwann Cells and macrophages*, (2) *ApoD works as an extracellular signalling molecule modulating the recruitment of macrophages to the damaged site (directly or indirectly by regulating the activity of gene networks)* and (3) *ApoD modulates the lipid composition of myelin to make it prone to phagocytosis*. To contrast these hypotheses we are using different combinations of macrophages and Schwann cells co-cultures from ApoD-KO and *wt* mice. We are assaying the phagocytosis of labelled myelin by flow Cytometry and immunocytochemistry. This approach should lead us to distinguish whether ApoD exerts direct effects on phagocytosis and/or on myelin composition, or alternatively acts through the regulation of signalling between Schwann cells and macrophages.

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**Notes:**

**P11. ApoD, an apolipoprotein expressed by astrocytes, is induced upon oxidative insult via the JNK pathway, and is required for cell survival**

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The study of glial derived factors induced by injury and degeneration is important to understand the response of the nervous system to these deteriorating conditions. In this work we focus on Apolipoprotein D (ApoD), a Lipocalin expressed by astrocytes and oligodendrocytes in the CNS. ApoD expression is strongly induced upon CNS aging, injury or neurodegeneration (e.g. Alzheimer or Multiple Sclerosis).

We have previously demonstrated that ApoD has a protective function in animal models (mouse and fruit fly) against the oxidative stress produced by paraquat (PQ), a redox-cycling herbicide widely used to induce neurotoxicity.

This work has two main objectives: (I) to understand how ApoD expression is controlled in the context of the glial response to oxidative insult, and (II) to ascertain whether ApoD has a protective role for glial cells.

We use glial cell cultures (murine primary cortical astrocytes derived from wild-type or ApoD-KO mice, and human cell lines) subjected to PQ treatment as our experimental model. We have investigated whether the stress activated MAPK pathways (signaling through ERK, p38 or JNK) control ApoD expression in astrocytes. We have also tested how ApoD influences glial cell reactivity, viability and cell death upon increased oxidative stress.

The major findings of this study are: (I) ApoD expression is controlled by the JNK pathway when astrocytes are treated by PQ. (II) ApoD-KO cortical astrocytes have a decreased viability and are less resistant to PQ, indicating that ApoD contributes to the self-protection of glial cells upon damage.

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**Notes:**

## **P12. NAADP plays a role in astrocytic calcium signaling**

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Elaborated intracellular Ca<sup>2+</sup> signals and the occurrence of Ca<sup>2+</sup> waves spread from astrocyte to astrocyte provide these cells with a specific form of excitability. The control of such excitability is a key element in the exchange of information between astrocytes and other cell types in the brain and in the astrocyte regulation of synaptic transmission. Nicotinic Acid-Adenine Dinucleotide Phosphate (NAADP) is a new second messenger that release Ca<sup>2+</sup> from lysosome-related acidic organelles in a wide variety of cells. NAADP induced Ca<sup>2+</sup> responses can be amplified by Ca<sup>2+</sup> induced release Ca<sup>2+</sup> from the endoplasmatic reticulum (ER) and by extracellular calcium entry. Here we made use of NAADP-AM, a membrane-permeable analogue of NAADP to show that intracellular NAADP is capable to mobilize Ca<sup>2+</sup> from lysosomes in cortical astrocytes. Furthermore, we demonstrated that disruption of NAADP and lysosomal signalling with Ned-19 (100 µM), a new antagonist of the NAADP receptors, Bafilomycin (2 µM), an inhibitor of the V-ATPase, and GPN (50 µM), a disruptor of lysosomal-related intracellular vesicles, reduce the magnitude of Ca<sup>2+</sup> responses initiated by extracellular application of ATP (100 µM) but not Bradykinin (1 µM). Interestingly, NAADP participates in the propagation of mechanically induced calcium waves, a process in which release of endogenous ATP has been previously shown to be a key element.

### **Notes:**

