



**SENC**

Sociedad Española de NeuroCiencia

**Seventh  
Cajal Winter Conference**

**Synaptic mechanisms**

March 20-24, 2011  
Benasque, Huesca (Spain)

 **CSIC**  
Consejo Superior de Investigaciones Científicas

 **cibersam** Centro de Investigación  
Biomédica En Red  
de Salud Mental

# List of Participants

José Aguilera  
Institut de Neurociències  
Universitat Autònoma de Barcelona  
jose.aguilera@uab.cat

Rafael Fernández Chacón  
Fisiología Médica y Biofísica  
Instituto de Biomedicina de Sevilla,  
Hosp. Univ. Virgen del Rocío / CSIC  
/ Universidad de Sevilla  
rfchacon@us.es

Guillermo Álvarez de Toledo  
Physiology and Biophysics  
University of Seville  
gat@us.es

José Javier Ferrero López  
Bioquímica y Biología Molecular IV  
Universidad Complutense de Madrid  
jfferrer@vet.ucm.es

Francesc Artigas  
Neuroquímica y Neurofarmacología  
Instituto de Investigaciones  
Biomédicas de Barcelona (CSIC)  
fapnqi@iibb.csic.es

Roberto Gallego  
Instituto de Neurociencias  
Universidad Miguel Hernández-  
CSIC, Alicante  
roberto.gallego@umh.es

Juan Blasi  
Patology and Experimental  
Therapeutics  
University of Barcelona  
blasi@ub.edu

Angels García Cazorla  
Neurology  
Hospital Sant Joan de Deu,  
Barcelona  
agarcia@hsjdbcn.org

Hugo Cabedo  
Molecular Neurobiology  
Instituto de Neurociencias y  
Fundación Hospital General de  
Alicante  
hugo.cabedo@umh.es

Justo García de Yébenes  
Neurology  
Hospital Ramón y Cajal, Madrid  
jgyebenes@yahoo.com

Núria Casals Farré  
Ciencias básicas  
Universitat Internacional de  
Catalunya, Barcelona  
ncasals@csc.uic.es

Yukiko Goda  
MRC Laboratory for Molecular Cell  
Biology  
University College London, UK  
y.goda@ucl.ac.uk

Graham Collingridge  
MRC Centre for Synaptic Plasticity,  
School of Physiology &  
Pharmacology  
University of Bristol, UK  
gcollingridge@gmail.com

Ana Guadaño Ferraz  
Fisiopatología Endocrina y del  
Sistema Nervioso  
CSIC  
aguadano@iib.uam.es

Reinhard Jahn  
Department of Neurobiology  
Max-Planck-Institute for Biophysical  
Chemistry, Göttingen, Germany  
rjahn@gwdg.de

Cruz Morenilla Palao  
Sensory transduction and  
nociception  
Instituto de Neurociencias de  
Alicante-UMH-CSIC  
cruz@umh.es

Imane Jemal  
Physiology and medical biophysics  
University of Seville  
jemaliman@us.es

Carlos Ortez González  
Neurología pediátrica  
Hospital Sant Joan de Deu-  
Universidad de Barcelona  
ciortez@hsjdbcn.org

Sheila Jordán-Álvarez  
Cellular, Molecular and  
Developmental Neurobiology  
Cajal Institute, Madrid  
sheila@cajal.csic.es

Fuencisla Pilar Cuéllar  
Fisiología y Farmacología  
IBBTEC, Santander  
pilarmf@unican.es

Juan Lerma Gómez  
Direction  
Instituto de Neurociencias de  
Alicante, CSIC-UMH  
jlerma@umh.es

José Jorge Ramírez Franco  
Bioquímica y Biología Molecular IV  
Universidad Complutense de Madrid  
j\_ramirez@vet.ucm.es

María Ángeles Mena  
Neurobiology and Neurology  
Hospital Ramón y Cajal, Madrid  
maria.a.mena@hrc.es

José Rodríguez Álvarez  
Instituto de Neurociencias  
Universidad Autónoma de  
Barcelona  
jose.rodriguez@uab.es

Alfredo Miñano Molina  
Instituto de Neurociencias  
Universidad Autónoma de  
Barcelona  
alfredo.minano@uab.es

Lucía Tabares  
Medical Physiology & Biophysics  
University of Seville  
ltabares@us.es

Julio Morán Andrade  
Instituto de Fisiología Celular  
Universidad Nacional Autónoma de  
México  
jmoran@ifc.unam.mx

Laura Torres  
Physiology and Biophysics  
University of Seville  
latorres@us.es

Ramón Trullas  
Muerte y Proliferación Celular  
IIBB/CSIC  
ramon.trullas@iibb.csic.es

Rebeca Vidal Casado  
Fisiología y Farmacología  
IBBTEC  
vidalr@unican.es

# **Conference Programme**

## **Sunday March 20**

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

**21:00 Dinner**

## **Monday March 21**

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

**15:30 Oral communications.** *Chair: A. Guadaño-Ferraz*

**15:30** J Perucho, MJ Casarejos, A Gomez, RM Solano, J Garcia de Yébenes, MA Mena. *Trehalose protects from aggravation of amyloid pathology induced by isoflurane anesthesia in APP<sub>SWE</sub> mutant mice*

**16:00** AJ Miñano-Molina, J España, E Martín, M Solé, B Barneda-Zahonero, R Fadó, R Trullás, CA Saura, J Rodríguez-Alvarez. *Soluble oligomers of amyloid-beta peptide disrupt membrane trafficking of AMPA receptors contributing to early synapse dysfunction*

**16:30 Plenary lecture**

**Yukiko Goda.** *Regulating synaptic strength across the cleft by N-cadherins*

**17:30 Coffe break**

**18:00 Yukiko Goda.** *Meeting with young neuroscientists*

**18:30 Oral communications.** *Chair: R. Trullas*

**18:30** B Barneda-Zahonero, JM Servitja, AJ Miñano, R Fadó, CA Saura, J Rodríguez-Alvarez. *Synaptic activation mediates neuronal survival by activation of Nurr1*

**19:00** R Vidal, J Pascual-Brazo, E Castro, A Díaz, EM Valdizán, F Pilar-Cuéllar, B Treceño, A Pazos. *A 7 days treatment with the 5-HT<sub>4</sub> receptor agonist RS67333 is required to obtain a complete antidepressant-like response in animal models*

**19:30 Poster session**

**21:00 Dinner**

**Tuesday March 22**

**15:30 Oral communications. Chair: L. Tabares**

**15:30** I Jemal, MA Montes, G Alvarez de Toledo. *Functional organization of synaptic vesicles in hippocampal neurons and their heterogeneity between different synapses*

**16:00** J Lerma, I Aller, RJ Rodrigues. *P2X2/GluN2B: A new ionotropic receptor complex*

**16:30 Plenary lecture**

**Graham Collingridge. *Synaptic Plasticity in the Hippocampus***

**17:30 Coffee break**

**18:00 Graham Collingridge. *Meeting with young neuroscientists***

**18:30 Oral communications. Chair: J. Lerma**

**18:30** P Carrasco, E Gratacós, S Ramirez, J Jacas , M Dierssen, FG Hegardt, N Casals. *CPT1c deficiency impairs feeding behaviour, motor function and memory performance by altering ceramide levels*

**19:00 Round table: *Current challenges in understanding synaptic function***

**20:00 Poster session**

**21:00 Dinner**

**Wednesday March 23**

**15:30 Oral communications.** *Chair: G. Álvarez de Toledo*

**15:30** JL Rozas, L Gómez-Sánchez, P Linares-Clemente, E Vázquez, R Luján, R Fernández-Chacón. *Cysteine string protein-alpha maintains the number of synaptic release sites and dynamin-dependent endocytosis at motor nerve terminals*

**16:00** R Ruiz, R Cano, WJ Betz, L Tabares. *Active Zones and the Readily Releasable Pool of Synaptic Vesicles at the Neuromuscular Junction of the Mouse*

**16:30 Plenary lecture**

**Reinhard Jahn.** *Mechanism of SNARE-mediated exocytosis of synaptic vesicles*

**17:30 Coffe break**

**18:00 Reinhard Jahn.** *Meeting with young neuroscientists*

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

**18:30** J Blasi, A Muhaisen, J Aleu, A Raptis, L Bahima, L Texidó, M Martín-Satué, J Marsal, C Solsona. *Modulation of endogenous hemichannels by syntaxin in Xenopus oocytes*

**19:00** J Ramírez Franco, D Bartolomé Martín, E Castro, J Sánchez Prieto, M Torres. *Efficient synaptic vesicle recycling after intense exocytosis concomitant with the accumulation of non-releasable endosomes at early developmental stages*

**19:30 Poster session**

**21:00 Dinner**

## Poster presentations

Posters must be mounted on the boards on Sunday 20 or Monday 21 (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. A Montero-Pedrazuela, I Fernández-Lamo, M Alieva, I Pereda-Pérez, C Venero, A Guadaño-Ferraz. *Adult-onset hypothyroidism enhances fear memory and upregulates mineralocorticoid and glucocorticoid receptors in the amygdala*

P2. C Morenilla, M Pertusa, V Meseger, F Viana. *Intracellular signalling and lipid raft segregation of the thermoreceptor TRPM8*

P3. F Pilar-Cuéllar, R Vidal, A Pazos. *Subchronic treatment with the SSRI fluoxetine and the 5-HT<sub>2A/2C</sub> antagonist ketanserine induces an increase in markers related to synaptic plasticity in hippocampus*

P4. RM Solano, MJ Casarejos, A Gomez, J Perucho, J G de Yébenes, MA Mena. *Effects of amyloid (1-42)- $\beta$  peptide on cortical neuron/glia cultures from parkin null mice. Role of autophagy and glutathione homeostasis*

P5. L Torres-Benito, R Ruiz, MA Montes, G Alvarez de Toledo, L Tabares. *Synaptic vesicle expression of VAcHT-pHluorin in septal neurons*

P6. P Linares-Clemente, JL Rozas, P García-Junco-Clemente, JA Martínez-López, ME Vázquez, CO Pintado, R Fernández-Chacón. *Dynamin-dependent endocytosis of freshly exocytosed synaptotagmin at the neuromuscular junction during nerve stimulation*

P7. J Figueiró-Silva, A Gruart, KB Clayton, P Podlesniy, MA Abad, L Benitez, M Enguita, X Gasull, JM Delgado-García, R Trullas. *Neuronal Pentraxin 1 negatively regulates excitatory synapse number*

P8. S Jordán-Álvarez, W Fouquet, SJ Sigrist, A Ferrús, A Acebes. *Presynaptic PI3K promotes the formation of postsynaptic glutamate receptors in the neuromuscular junction of drosophila*

P9. J Ramírez Franco, D Bartolomé Martín, E Castro, J Sánchez Prieto<sup>1</sup>, M Torres. *Developmental regulation of vesicular cycle dynamics in cerebellar granule cells*

# **Oral Communications and Plenary Lectures**

**15:30 Trehalose protects from aggravation of amyloid pathology induced by isoflurane anesthesia in APP<sub>swe</sub> mutant mice**

J Perucho<sup>1</sup>, MJ Casarejos<sup>1</sup>, A Gomez<sup>1</sup>, RM Solano<sup>1</sup>, J Garcia de Yébenes<sup>2</sup>, MA Mena<sup>1</sup>, <sup>1</sup>Department of Neurobiology and <sup>2</sup>Neurology, Hospital Ramón y Cajal, and <sup>1,2</sup>CIBERNED, Madrid, Spain.

**Background:** There is an open controversy about the role of surgery and anesthesia in the pathogenesis of Alzheimer's disease (AD). Clinical studies have shown a high prevalence of these procedures in subjects with AD but the interpretation of these studies is difficult because of the co-existence of multiple variables. Experimental studies in vitro and in vivo have shown that small molecular weight volatile anesthetics enhance amyloidogenesis in vitro and produce behavioral deficits and brain lesions similar to those found in patients with AD.

**Methods:** We examined the effect of co-treatment with trehalose on isoflurane-induced amyloidogenesis in mice. WT and APP<sub>swe</sub> mice, of 11 months of age, were exposed to 1% isoflurane, 3 times, for 1.5 hours each time and sacrificed 24 hours after their last exposure to isoflurane. The right hemi-brain was used for histological analysis and the contra-lateral hemi-brain used for biochemical studies.

**Results:** In this study, we have shown that repetitive exposure to isoflurane in pre-symptomatic mature APP<sub>swe</sub> mice increases apoptosis in hippocampus and cerebral cortex, enhances astrogliosis and the expression of GFAP and that these effects are prevented by co-treatment with trehalose, a disaccharide with known effects as enhancer of autophagy. We have also confirmed that in our model the co-treatment with trehalose increases the expression of autophagic markers as well as the expression of chaperones.

**Conclusions:** Co-treatment with trehalose reduces the levels of  $\beta$  amyloid peptide aggregates, tau plaques and levels of phospho-tau. Our study, therefore, provides new therapeutic avenues that could help to prevent the putative pro-amyloidogenic properties of small volatile anesthetics.

*Supported by FIS 2010/172, Laín Entralgo NDG07/4, CAM 02/02/2006, and CIBERNed 2006/05/059 and 2010.*

**Notes:**

**16:00 Soluble oligomers of amyloid-beta peptide disrupt membrane trafficking of AMPA receptors contributing to early synapse dysfunction**

AJ Miñano-Molina<sup>1,2</sup>, J España<sup>1,2</sup>, E Martín<sup>1,2</sup>, M Solé<sup>1</sup>, B Barneda-Zahonero<sup>1</sup>, R Fadó<sup>1,2</sup>, R Trullas<sup>2,3</sup>, CA Saura<sup>1,2</sup>, J Rodríguez-Alvarez<sup>1,2</sup>, <sup>1</sup>Institut de Neurociències and Departament de Bioquímica i Biologia Molecular Universitat Autònoma de Barcelona, Spain. <sup>2</sup>Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain. <sup>3</sup>Instituto de Investigaciones Biomédicas de Barcelona, CSIC, Barcelona, Spain.

$\beta$ -amyloid ( $A\beta$ ), a peptide generated from the amyloid precursor protein (APP), is widely believed to underlie the pathophysiology of Alzheimer's disease (AD). Emerging evidences suggest that soluble  $A\beta$  oligomers adversely affect synaptic function, leading to cognitive failure associated with AD. The  $A\beta$ -induced synaptic dysfunction has been attributed to the synaptic removal of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors (AMPA). However, it is unclear how  $A\beta$  induces the loss of AMPAR at the synapses. In this study we have examined the effect of  $A\beta$  oligomers on phosphorylation levels of GluR1 serine 845 (ser-845), a residue that plays an important role in the trafficking of AMPARs towards extrasynaptic sites and the subsequent delivery to synapses during synaptic plasticity events. We found that  $A\beta$  oligomers reduce basal levels of ser-845 phosphorylation and surface expression of AMPARs affecting AMPAR subunit composition. Dephosphorylation is related with an increase in calcium influx into neurons through ionotropic glutamate receptors that activate the calcium-dependent phosphatase calcineurin. We demonstrate that calcineurin mediates the reduction in phosphorylation levels of ser-845 GluR1 and subsequent reduction in surface expression of AMPARs. Moreover,  $A\beta$  oligomers block the extrasynaptic delivery of AMPARs induced by chemical synaptic potentiation. In addition, neurons from a genetic mouse model of Alzheimer disease expressed reduced amounts of AMPARs when accumulation of o $A\beta$  and spatial memory deficits start. These findings indicate that  $A\beta$  oligomers could act as a depressor affecting the mechanisms involved in the targeting of AMPARs to the synapses during early stages of the disease.

**Notes:**

## Plenary Lecture

### 16:30 Regulating synaptic strength across the cleft by N-cadherins

**Yukiko Goda**

N Vitureira, M Letellier, I White, Y Goda, MRC Laboratory for Molecular Cell Biology, University College London, UK.

N-cadherin is a  $\text{Ca}^{2+}$ -dependent homophilic adhesion protein that plays an important developmental role in guiding and forming synaptic connections, although it remains expressed at mature excitatory synapses. We have investigated the transsynaptic activity of N-cadherin in regulating synaptic efficacy using FM dyes to monitor vesicle turnover in cultured hippocampal neurons. Interfering with N-cadherin expression in isolated postsynaptic neurons reduces basal release probability at synaptic inputs received by the neuron. Surprisingly, this transsynaptic impairment of neurotransmitter release is accompanied by a significant slowing of vesicle endocytosis. In contrast, in neurons postsynaptically impaired for N-cadherin activity, synapses remain capable of homeostatically upregulating release probability. Our findings reveal that regulation of presynaptic efficacy is molecularly dissociable into two components by the requirement for N-cadherin: one for controlling the level of basal presynaptic strength and the other for adjusting the gain.

*Supported by the MRC and the EU 7th Framework Programme under grant agreement no. HEALTH-F2-2009-241498 ("EUROSPIN" project).*

**Notes:**

**18:30 Synaptic activation mediates neuronal survival by activation of Nurr1**

B Barneda-Zahonero<sup>1,2</sup>, JM Servitja<sup>3</sup>, AJ Miñano<sup>1,2</sup>, R Fadó<sup>1,2</sup>, CA Saura<sup>1,2</sup>, J Rodríguez-Alvarez<sup>1,2</sup>, <sup>1</sup>Institut de Neurociències, UAB, Barcelona, Spain. <sup>2</sup>CIBERNED, Barcelona, Spain. <sup>3</sup>Genomic Programming of Beta-Cells Laboratory, IDIBAPS, Barcelona, Spain.

Cerebellar granule cells (CGCs) differentiate and mature during their migration from the external granular layer (EGL) to the internal granular layer (IGL). Lack of excitatory inputs triggers their apoptotic death. NMDA receptor stimulation promotes the survival of these neurons during that process. It is possible to mimic this process in vitro by culturing CGCs in low KCl concentrations (5mM) in the presence or absence of NMDA. Using this experimental approach, we have obtained whole-genome expression profiles after 3 and 8 hr of NMDA addition to identify genes involved in NMDA-mediated survival of CGCs. Gene ontology analysis of the results identified that most of the regulated genes were included in the anti-apoptotic, development or neurogenesis clusters. One of the identified genes was Nurr1, a member of the orphan nuclear receptor subfamily Nr4a. We show here that Nurr1 is induced by CREB activation in response to NMDA. Silencing Nurr1 by shRNA leads to a decrease in BDNF protein levels and a reduction of NMDA neuroprotective effect. Moreover, we report that Nurr1 increases during cerebellum postnatal development and that Nurr1 and BDNF show a similar expression pattern during development. Thus we conclude that Nurr1 is necessary for NMDA-mediate survival of CGCs.

**Notes:**

**19:00 A 7 days treatment with the 5-HT<sub>4</sub> receptor agonist RS67333 is required to obtain a complete antidepressant-like response in animal models**

R Vidal, J Pascual-Brazo, E Castro, A Díaz, EM Valdizán, F Pilar-Cuéllar, B Treceño, A Pazos, Instituto de Biomedicina y Biotecnología de Cantabria IBBTEC, Santander, Spain. CIBER-SAM, Instituto de Salud Carlos III, Santander, Spain.

In the last years, new drugs are being developed to obtain antidepressant-like effects with a shorter onset of action, such as partial agonists of 5-HT<sub>4</sub> receptors. According to the neurogenic hypothesis of depression, the antidepressant effect could be due to increased proliferation and neurotrophic factors in hippocampus. The aim of this study was to analyze neuroplastic-related changes as BrdU incorporation, BDNF mRNA expression, transcription factors as  $\beta$ -catenin and CREB, all correlated with behavioural tests. The 5-HT<sub>4</sub> receptor partial agonist RS67333 (1.5 mg/kg/day s.c.) was administered for 3 or 7 days to Sprague-Dawley rats. RS67333 was effective in the forced swimming test following 3 and 7 days. However, 7 days were required to obtain antidepressant-like response in the novelty-suppressed feeding and corticosterone-induced anhedonia. BrdU and  $\beta$ -catenin immunolabelling were increased after 3 and 7 days. BDNF mRNA expression was increased in CA3 of the hippocampus (28%,  $p < 0.01$ ) after 3 days, while 7 days increased in CA1 (73%,  $p < 0.01$ ) and DG (53%,  $p < 0.01$ ). Similarly, RS67333 only desensitized the 5-HT<sub>4</sub> receptor-coupled adenylate cyclase after 7 days compared to control ( $E_{max}$  84% vs 135% respectively,  $p < 0.05$ ). CREB (40%,  $p < 0.05$ ), and pCREB (83%,  $p < 0.05$ ) were only significantly increased after 7 days treatment, while pCREB/CREB ratio was increased after both 3 days (93%,  $p < 0.05$ ) and 7 days (84%,  $p < 0.05$ ). These results suggest that 7 days of RS67333 treatment are required to elicit hippocampal neuroplastic changes associated to fully positive behavioural effects, confirming that 5-HT<sub>4</sub> receptor agonism is an encouraging strategy for the development of faster acting antidepressants.

*Supported by Ministerio de Ciencia, Innovación y Tecnología (SAF-07/61862), Fundación Alicia Koplowitz and Fundación de Investigación Médica Mutua Madrileña.*

**Notes:**

**15:30 Functional organization of synaptic vesicles in hippocampal neurons and their heterogeneity between different synapses**

I Jemal, MA Montes, G Alvarez de Toledo, Department of Physiology & Biophysics, School of Medicine, University of Seville, Spain.

There are three functional aspects of synaptic vesicle recycling, the exocytosis, endocytosis and trafficking of vesicles through various pools in nerve terminals. Here we combine two optical probes, Synaptophysin tagged to pHluorin `SypHy` and Styryl dye `FM4-64` for a live imaging of exo-endocytosis of synaptic vesicles. Bafilomycin A1 has been used to examine the partitioning of these probes into recycling and non recycling pools. Neurons in culture were activated by a localized field stimulation using a microelectrode.

We show that a terminal contains about 92 vesicles spreads between the recycling (53 vesicles) and the resting pool (40 vesicles). This implicates a fraction of 54.4% of recycling pool and 45.6% of resting pool. The fast releasable fraction of the recycling pool `readily releasable pool` constitute a 7.4% of the total pool with an average of 9 vesicles per terminal and determine a release probability of 14% for a single vesicle fusion. But these parameters vary a little from neuron to another due to the variation between terminals. From one hand, the total content oscillates between 46 and 262 vesicles, and in the other hand, the repartition between the different pools varies randomly. A fraction of the functional pool determines a synaptic efficacy and it doesn't seem to be related with the spatial location of the terminal. An application of high external calcium does not vary the fraction of the pool but it narrow the range buy a homogenization of the synaptic boutons. Finally, we see that the recycling pool is depleted exponentially suggesting a dynamic equilibrium between pools that allow a compensation

Our assay can be used to investigate the effects of genetic and chemical modulation of the SV cycle. The quantitative parameters that can be extracted with the approaches outlined here should help to elucidate how pools reorganize for potentiation and depression.

**Notes:**

### **16:00 P2X2/GluN2B: A new ionotropic receptor complex**

J Lerma, I Aller, RJ Rodrigues, Instituto de Neurociencias de Alicante, CSIC-UMH, San Juan de Alicante, Spain.

In the nervous system, fast signalling is achieved by activation of ligand-gated ion channels. Previous studies have reported a tight interaction and cross-talk between ionotropic receptors, namely between P2XRs and other ionotropic receptors such as nAChRs, 5-HT<sub>3</sub>Rs and GABAARs. These interactions are all characterized by a physical interaction that leads to an activity-dependent cross-inhibition designed to negatively control the excess of function of the other signalling systems by ATP. Since ATP and glutamate are co-released from nerve terminals and P2XRs and NMDARs have an overlapping subcellular localization in the CNS, we decided to study the existence of putative dimerization between P2X and NMDA receptors. P2X and NMDA receptors are coincidence detectors of neuronal function and their interaction would be of particular interest. We found the existence of hybrid P2X<sub>2</sub>/GluN2B receptors, ionotropic receptors composed by subunits from dissimilar receptor families that are able to sense more than one signalling molecule. Indeed, NMDA (300  $\mu$ M) was able to elicit  $[Ca^{2+}]_i$  transients only in HEK293 cells expressing P2X<sub>2</sub> and GluN2B (formerly NR2B) subunits, but not in non-transfected cells or cells solely transfected with GluN2B. Whole-cell patch clamp analysis in combination with a fast perfusion system for agonist application revealed that the activation of this putative P2X<sub>2</sub>/GluN2B receptor requires the co-application of ATP and NMDA. The “hybrid current” was inhibited by APV, PPADS and ifenprodil, but not by MK801. The I/V relationship and Mg<sup>2+</sup>-sensitivity observed for P2X<sub>2</sub>/GluN2BRs were similar to those observed for P2X<sub>2</sub>Rs, but they do not share P2X<sub>2</sub>Rs’ ability to permeate NMDG<sup>+</sup> ions. Co-immunoprecipitation of GluN2B and P2X<sub>2</sub> was also observed in brain tissue, and bimolecular fluorescence complementation studies showed that P2X<sub>2</sub> carries GluN2B to the plasma membrane. Likewise, in P2X<sub>2</sub>-transfected hippocampal neurons the presence of functional P2X<sub>2</sub>/GluN2BRs could also be demonstrated. The present study provides evidence for the formation of a hybrid P2X<sub>2</sub>/GluN2B ionotropic receptor, which may constitute a new concept of functional neurotransmitter receptor.

*Supported by CSD2007-0023 and BFU-2006-007138.*

#### **Notes:**

## Plenary Lecture

### 16:30 Synaptic Plasticity in the Hippocampus

#### Graham Collingridge

Graham Collingridge, MRC Centre for Synaptic Plasticity, School of Physiology & Pharmacology, University of Bristol, UK.

Synaptic plasticity is believed to be important for learning and memory and other cognitive functions. Malfunctioning synaptic plasticity is also widely believed to be a critical component of numerous neurological and psychiatric diseases. We have been interested in understanding some of the molecular mechanisms involved in synaptic plasticity by investigating long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus.

At glutamatergic synapses in the CA1 region of the hippocampus LTP is triggered by the synaptic activation of NMDARs and expressed, in part, by alterations in the number and property of AMPARs expressed at these synapses. The increase in AMPAR number appears to be due to a stabilization of the GluA2-NSF interaction, via the activity of PKMzeta. In contrast, NMDAR-LTD seems to involve mainly internalization of the NSF-sensitive pool of AMPARs, via a process involving alterations in protein phosphorylation.

A key pathway that is emerging as important in NMDAR-LTD is one that involves caspase-3, Akt and GSK-3 $\beta$ . Emerging evidence suggests that this pathway is also involved in the inhibition of LTP by A $\beta$ <sub>1-42</sub>. This lends support to the possibility that the loss of synapses, that is a characteristic feature of Alzheimer's disease, may be due to pathological changes in NMDAR-LTP and NMDAR-LTD.

*Supported by the MRC (UK) and WCU Program (Korea).*

#### Notes:

**18:30 CPT1c deficiency impairs feeding behaviour, motor function and memory performance by altering ceramide levels**

P. Carrasco<sup>1,4</sup>, E. Gratacós<sup>1,4</sup>, S. Ramirez<sup>1,4</sup>, J. Jacas<sup>1</sup>, M. Dierssen<sup>2,5</sup>, F.G. Hegardt<sup>3,4</sup>, N. Casals<sup>1,4</sup>, <sup>1</sup>Departament of Basic Sciences, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya (UIC), Sant Cugat del Vallès, Spain. <sup>2</sup>Centre de Regulació Genòmica (CRG), Parc de Recerca Biomèdica de Barcelona (PRBB), Spain. <sup>3</sup>Departament of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Barcelona, Spain. <sup>4</sup>Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III, Madrid, Spain. <sup>5</sup>CIBER de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.

Carnitine palmitoyltransferase 1 (CPT1) enzymes facilitates the entrance of acyls-CoA inside the mitochondria where they undergo beta-oxidation. Our research group demonstrated that the major isoform in brain, CPT1c, is localized in endoplasmic reticulum of neurons instead of mitochondria, which suggests a complete different role than the two other isoforms. Recently, we have developed CPT1c knock-out (KO) mice in order to elucidate CPT1c function. CPT1c KO mice are viable and fertile and do not present gross brain anatomical alterations. Behaviour experiments demonstrate CPT1c implication in feeding behaviour, motor function and spatial learning, without affecting sensory or autonomous systems. CPT1c KO mice have reduced food intake and reduced peripheral fatty acid oxidation in the fasting state demonstrating the implication of CPT1c in energy homeostasis. On the other hand, behaviour studies reveal that CPT1c deficiency drastically impairs tasks that require strength in the forelimbs, motor coordination and gait. CPT1c KO mice also have impaired spatial learning, as measured in the Morris water maze. Primary hippocampal cultures from CPT1c KO mice present reduced number of mature spines and increased filopodia density suggesting the implication of CPT1c in spine maturation. At the biochemical level, fasted KO mice have increased acyl-CoAs but reduced ceramide and sphingosine levels in some brain regions (hypothalamus, cerebellum and hippocampus). All these results indicate that CPT1c activity, which is increased in situations of low energy availability, plays an important role in neuronal function, probably by regulating the pool of palmitoyl-CoA required for synthesis of sphingolipids.

**Notes:**

**15:30 Cysteine string protein-alpha maintains the number of synaptic release sites and dynamin-dependent endocytosis at motor nerve terminals**

JL Rozas<sup>1</sup>, L Gómez-Sánchez<sup>1</sup>, P Linares-Clemente<sup>1</sup>, E Vázquez<sup>2</sup>, R Luján<sup>3</sup>, R Fernández-Chacón<sup>1</sup>, <sup>1</sup>Instituto de Biomedicina de Sevilla (IBiS), Hosp.Univ. Virgen del Rocío/CSIC/Universidad de Sevilla and Dept. Fisiología Médica y Biofísica and CIBERNED, Sevilla, Spain. <sup>2</sup>Dpto. Química Orgánica, Universidad de Santiago de Compostela, Spain. <sup>3</sup>Dpto. de Ciencias Médicas, Facultad de Medicina and Centro Regional de Investigaciones Biomédicas, Universidad de Castilla-La Mancha, Albacete, Spain.

Cysteine String Protein-alpha (CSP-alpha) is a synaptic vesicle protein that prevents neurodegeneration of presynaptic terminals. CSP-alpha knock-out mice suffer from a lethal neurological phenotype that is evident after the second postnatal week. We have found striking changes in the synaptic vesicle cycle using electrophysiology and imaging at the neuromuscular junction (NMJ). Exocytosis at the synaptic terminals is decreased in CSP-alpha KO mice at P16-20. Nerve terminals fail to sustain prolonged release. At these synapses, the SNARE protein SNAP25, is dramatically decreased. That observation could explain a lower number of transmitter release sites observed in mutant synapses. In addition, we have studied the synaptic vesicle cycle in mutant and wild-type synapses. To monitor synaptic exo- and endocytosis in vivo we have generated transgenic mice that express in neurons synaptopHluorin (SpH) but lack CSP-alpha. Combining imaging and electrophysiology, we have uncovered a reduction in the size of the recycling synaptic vesicle pool and a defect in endocytosis in mutant synapses. Dynamin-dependent endocytosis taking place during the stimulus is particularly reduced in the absence of CSP-alpha. Such a defect could also explain the reduced size of the recycling pool during prolonged stimuli. Our results reveal that CSP-alpha function on the synaptic vesicle is broader than initially envisioned being CSP-alpha required for the long term maintenance of synaptic exo- and endocytosis.

*Supported by MICINN BFU2007-66008, P07-CVI-02854, ISCIII, Fondo Europeo de Desarrollo Regional (FEDER).*

**Notes:**

**16:00 Active Zones and the Readily Releasable Pool of Synaptic Vesicles at the Neuromuscular Junction of the Mouse**

R Ruiz<sup>1</sup>, R Cano<sup>1</sup>, WJ Betz<sup>2</sup>, L Tabares<sup>1</sup>, <sup>1</sup>Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, Spain. <sup>2</sup>Department of Physiology and Biophysics, University of Colorado School of Medicine, Aurora, Colorado, EEUU.

The fusion of synaptic vesicles with the plasma membrane is a highly regulated process that takes place at specific locations of the presynaptic membrane called Active Zones (AZs). The relationships between AZs, quantal release, and vesicle replenishment are not well understood in a mature synapse. We have measured the number, distribution, and other properties of AZs in mouse motor nerve terminals and combined these observations with electrophysiological estimates of the size of the readily releasable pool (RRP) of synaptic vesicles. On average, we counted 850 AZs per terminal. Assuming two primary docked vesicles per AZ, we predict a total of 1700 vesicles optimally positioned for exocytosis. Electrophysiological estimates of the size of the RRP gave an average value of 1730 quanta during 100 Hz stimulation, in satisfying agreement with the morphology. The RRP size estimated from the model is frequency dependent, more than doubling as stimulation frequency increases from 1 to 100 Hz, suggesting that, at low frequencies, many AZs are apparently silent. The model involves depletion of the RRP during a train following an exponential time course, leaving vacant release sites that are reoccupied by new, or recycled, vesicles, and is compatible with the positional priming model.

*Supported by grant BFU2007-61171 from the Spanish Ministry of Education and Science Grants (MEC.). R.C. has a Training Grant BES2008-005122 from MEC.*

**Notes:**

## Plenary Lecture

### 16:30 Mechanism of SNARE-mediated exocytosis of synaptic vesicles

#### Reinhard Jahn

Reinhard Jahn, Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany.

Neurotransmitter release from presynaptic nerve endings is mediated by  $\text{Ca}^{2+}$  - dependent exocytosis of synaptic vesicles. Exocytotic membrane fusion is mediated by the SNARE proteins synaptobrevin/VAMP, syntaxin 1, and SNAP-25. Upon membrane contact, the vesicular SNARE synaptobrevin forms complexes with the plasma membrane-resident SNAREs SNAP-25 and syntaxin 1. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ("zipper" hypothesis of SNARE function). Furthermore, the final steps of SNARE assembly are controlled by several additional proteins including the calcium sensor synaptotagmin, complexin, and the SM protein Munc-18. In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane (1) and that – at least in vitro – a single SNARE complex suffices to bring about effective fusion of bilayers (2), a conclusion that very recently has been corroborated by independent evidence from other laboratories (3). In addition, our data also shed some new light on the mechanism of the calcium sensor synaptotagmin, which is presently controversially discussed in the field. Furthermore, we have extended our studies on the composition of synaptic vesicles using quantitative methods, resulting in a refined molecular model of this trafficking organelle (4).

(1) Stein, A., Weber, G., Wahl, M.C., Jahn, R.(2009) Helical extension of the neuronal SNARE complex into the membrane. *Nature* 460, 525-528

(2) Van den Bogaart, G., Holt, M.G., Bunt, G., Riedel, D., Wouters, F.S., Jahn, R.(2010) *Nat Struct Mol Biol* 17, 358-365

(3) Mohrmann, R., de Wit, H., Verhage, M., Neher, E., Sorensen, J.B. (2010) *Science* 330, 502-505

(4) Takamori, S., Holt, M., Stenius, K., Lemke, E.A., Grønborg, M., Riedel, D., Urlaub, H., Schenck, S., Brügger, B., Ringler, P., Müller, S.A., Rammner, B., Gräter, F., Hub, J.S., De Groot, B.L., Mieskes, G., Moriyama, Y., Klingauf, J., Grubmüller, H., Heuser, J., Wieland, F., Jahn, R.(2006) Molecular anatomy of a trafficking organelle. *Cell* 127, 831-846

**Notes:**

**18:30 Modulation of endogenous hemichannels by syntaxin in *Xenopus* oocytes**

J Blasi<sup>1</sup>, A Muhaisen<sup>2</sup>, J Aleu<sup>2</sup>, A Raptis<sup>1</sup>, L Bahima<sup>1</sup>, L Texidó<sup>1</sup>, M Martín-Satué<sup>1</sup>, J Marsal<sup>1</sup>, C Solsona<sup>1</sup>, <sup>1</sup>Laboratori de Neurobiologia Cel·lular i Molecular, Departament de Biologia Cel·lular i Anatomia Patològica. Campus de Bellvitge, Universitat de Barcelona, IDIBELL-HUBc, Barcelona, Spain. <sup>2</sup>Developmental neurobiology and neural regeneration (A.M.) and Neuroscience Technologies SLP (J.A.), Parc Científic de Barcelona, Spain.

SNARE proteins are key elements of the molecular machinery for fusion of cellular membrane compartments. Syntaxin 1A is one of the components of the SNARE protein complex (together with SNAP-25 and synaptobrevin/VAMP1-2) that mediate the fusion of secretory vesicles with the plasma membrane. In addition to its direct interaction with a series of proteins involved in the mechanism of exocytosis, syntaxin 1A also interacts with a number of membrane proteins which are related to ion flow or transport of substances across plasma membrane. Here we show the effect of syntaxin 1A on the ionic flow through hemichannels, the hexameric connexin structures that form gap junctions.

Using two electrode voltage-clamp we recorded endogenous hemichannel currents in *Xenopus* oocytes by lowering the extracellular calcium concentration. The activated currents were sensitive to gap junction blocking agents such as heptanol, octanol and flufenamic acid.

In syntaxin 1A-expressing oocytes the amplitude of the current activated by low extracellular calcium concentration was reduced. In contrast, the amplitude of the current was enhanced in oocytes injected with the light chain of botulinium neurotoxin C1, a neurotoxin that cleaves syntaxin 1.

The co-expression of syntaxin 1A and Munc18a at different molar ratios revealed a modulatory effect of Munc18a on syntaxin 1A activity. Moreover, a direct correlation between the amplitude of the ion flux through hemichannels and the ratio of Munc18a:syntaxin 1A injected was observed.

Taken together, these results demonstrate a modulatory effect of syntaxin on the ionic current supported by hemichannels.

*Supported by grant SAF 2008/00732.*

**Notes:**

**19:00 Efficient synaptic vesicle recycling after intense exocytosis concomitant with the accumulation of non-releasable endosomes at early developmental stages**

J Ramírez Franco<sup>\*1</sup>; D Bartolomé Martín<sup>\*1</sup>; E Castro<sup>2</sup>; J Sánchez Prieto<sup>1</sup>; M Torres<sup>1</sup> <sup>1</sup>Department of biochemistry and molecular biology IV, School of veterinary, UCM, Madrid, Spain. <sup>2</sup>Department of biochemistry, molecular biology and physiology, Faculty of health sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain.

\*These authors contributed equally to this work.

After exocytosis of neurotransmitter-containing synaptic vesicles, endocytosis is fundamental to maintain synaptic transmission. As the different endocytotic mechanisms differ in their efficiency to generate releasable synaptic vesicles, using FM1-43 dye to track vesicle recycling should produce a variety of unloading behaviors if these endocytotic pathways coexist within a nerve terminal. FM1-43 loss identifies two types of synaptic boutons in cultured cerebellar granule cells that underwent weak and strong loss of the dye, respectively. Since decreasing the extent of exocytosis dramatically increases the proportion of synaptic boutons that undergo strong FM1-43 depletion, as well as dramatically reducing the number of endosome-like structures, we conclude that efficient synaptic vesicle recycling occurs concomitant with the formation of non-releasable endosomes in both types of synaptic boutons, although to a different extent. Furthermore, cell culture maturation increased, while chronic blockage of synaptic activity decreased the proportion synaptic boutons that undergo extensive dye loss. Moreover, electron microscopy techniques revealed the accumulation of these endosomes under high stimulation conditions, while they were present to a lesser extent when cells were faced to milder stimuli. This pathway has been demonstrated to have a role in non-mammalian cells and in chromaffin cells, but remains to be established if it is being put into play in neurons during synaptic transmission.

*Supported by grants from the Spanish 'Ministerio de Educación y Ciencia' (BFU2006-01012) and (BFU2009-07092) to MT, (BFU2007-64154 and BFU2010-16947) to JS-P, and the 'Instituto de Salud Carlos III' RD06/0026, the 'Comunidad de Madrid' (S-BIO-0170/2006) and the UCM-CAM (CCG07-UCM/SAL-2150) to J S-P and MT.*

**Notes:**

# Posters

## **P1. Adult-onset hypothyroidism enhances fear memory and upregulates mineralocorticoid and glucocorticoid receptors in the amygdala**

A Montero-Pedrazuela<sup>1</sup>, I Fernández-Lamo<sup>1</sup>, M Alieva<sup>1</sup>, I Pereda-Pérez<sup>2</sup>, C Venero<sup>2</sup>, A Guadaño-Ferraz<sup>1</sup>, <sup>1</sup>Department of Nervous System and Endocrine Pathophysiology, Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain. <sup>2</sup>Department of Psychobiology, Universidad Nacional de Educación a Distancia, Madrid, Spain.

Hypothyroidism is the most frequent hormonal alteration in adult life with a wide range of disease severity including learning and memory impairments and emotional disorders. However, the deleterious effects of hypothyroidism on the adult brain are poorly understood and underestimated.

Here we have explored the consequences of adult TH deficiency on emotional learning and memory by using a classical Pavlovian fear conditioning paradigm in adult hypothyroid and euthyroid rats.

Our results demonstrate that adult-onset hypothyroidism does not affect learning acquisition but potentiates fear memory consolidation, delays memory extinction and exacerbates spontaneous recovery of fear memory. These pathological effects were associated with an enhancement of corticosterone release after auditory fear conditioning training and with an increase in glucocorticoid and mineralocorticoid receptors expression in the lateral and basolateral nuclei of the amygdala.

Our findings are the first to demonstrate fear memory potentiation in adult-onset hypothyroidism. Inappropriate regulation of fear can lead to psychological alterations as post-traumatic stress disorders. This study suggests that adult-onset hypothyroidism induces an increased susceptibility to emotional memories and suggest an enhanced corticosteroid signaling in the amygdala as one of the possible pathological mechanisms.

*Supported by grant BFU2007-62979 (A.G.-F) from the Ministry of Science, Spain and Innovation.*

**Notes:**

## **P2. Intracellular signalling and lipid raft segregation of the thermoreceptor**

### **TRPM8**

C Morenilla, M Pertusa, V Meseger, F Viana, Instituto de Neurociencias-UMH-CSIC, San Juan de Alicante, Spain.

TRPM8 is a member of the Transient Receptor Potential (TRP) channel family expressed in small sensory neurons and is activated by cold, cooling substances such menthol and icilin and voltage. How TRPM8 is temperature activated is still poorly understood. Several mechanisms have been proposed: they include changes in membrane tension due to temperature-dependent lipid bilayer rearrangements. In light of the heterogeneous organization of biological membranes into specialized subdomains, with special biochemical and biophysical properties, we asked whether TRPM8 shows a preferential localization that may explain its gating and signalling properties. Here, we show that TRPM8 channel is partially localized in detergent-resistant membranes, called lipid-rafts, in both heterologous expression systems and in sensory neurons. This localization is in part directed by the N-glycosylation at its residue N934 because mutant TRPM8-N934Q is efficiently expressed and transported to the cell surface but displaced from raft microdomains.

The localization of TRPM8 in lipid rafts (cell signalling platforms) suggests this channel could activate intracellular signalling pathways to regulate gene expression in cold sensory neurons. To explore this hypothesis we performed preliminary experiments showing that TRPM8 activation by both menthol and cooling results in phosphorylation and activation of ERK1/2 and the cAMP response element binding protein CREB (a transcription factor). These results suggest that indeed TRPM8 activation can drive a change in gene transcription program and therefore modulate the sensory neuron physiology.

*Supported by funds from the Spanish Ministry of Education and Science projects BFU2007-61855 to F.V. and from the Generalitat Valenciana project GV/2007/113 to Cruz Morenilla and CONSOLIDER-INGENIO 2010 CSD2007-00023.*

### **Notes:**

### **P3. Subchronic treatment with the ssri fluoxetine and the 5-HT<sub>2A/2C</sub> antagonist ketanserin induces an increase in markers related to synaptic plasticity in hippocampus**

F Pilar-Cuéllar, R Vidal, A Pazos, Departamento de Fisiología y Farmacología, CIBER-SAM, Instituto de Salud Calos III, Cantabria, Spain. Instituto de Biomedicina y Biotecnología de Cantabria IBBTEC (Universidad de Cantabria-CSIC-IDICAN), Cantabria, Spain.

A neurotrophic hypothesis has been recently proposed to explain the molecular effects of antidepressant drugs. Chronic treatment with antidepressants increases hippocampal proliferation and the expression of neurotrophic factors as brain derived neurotrophic factor (BDNF). It has been suggested that drugs with a dual profile neurotransmitter transporter inhibitor/5-HT<sub>2A</sub> antagonist could enhance antidepressant responses. We have studied the effects of a 7 days treatment with the serotonin reuptake inhibitor (SSRI) fluoxetine (5 mg/kg/day; i.p.), ketanserin (0.1 mg/kg/day; i.p.) and fluoxetine+ketanserin (association group), on BDNF and TrkB mRNA expression, cell proliferation,  $\beta$ -catenin, N-cadherin and BDNF protein levels in hippocampus, and 5-HT<sub>1A</sub> receptor functionality. Forced swimming test (FST) was carried out to confirm the antidepressant response, and the coadministration group showed a reduction in the immobility time compared to vehicle ( $p < 0.05$ ). BDNF mRNA expression was increased in the association group in CA3 ( $47 \pm 10\%$ , vs vehicle;  $p < 0.05$ ) and DG ( $79 \pm 14\%$ , vs vehicle;  $p < 0.001$ ) of hippocampus, with no changes in TrkB mRNA and BDNF protein.  $\beta$ -catenin protein level was increased in association group in total homogenate ( $33 \pm 7\%$ , vs vehicle;  $p < 0.001$ ) and membrane ( $34 \pm 10\%$ , vs vehicle;  $p < 0.01$ ) fractions of the hippocampus, together with N-cadherin in total homogenate ( $37 \pm 10\%$ , vs vehicle;  $p < 0.01$ ) and membrane fraction ( $76 \pm 13\%$ , vs vehicle;  $p < 0.001$ ) of the fluoxetine+ketanserin group. BrdU incorporation and 5-HT<sub>1A</sub> receptor functionality were unchanged. These results suggest the involvement of 5-HT<sub>2A</sub> antagonism in the antidepressant-related neuroplastic effects is related to the role of  $\beta$ -catenin associated to N-cadherin in cell-cell adhesion, instead to its role in the Wnt/ $\beta$ -catenin pathway associated to proliferation.

*Supported by Fundación Alicia Koplowitz, Fundación de Investigación Médica Mutua Madrileña and Ministerio de Educación y Ciencia (SAF-2007/61862).*

**Notes:**

#### **P4. Effects of amyloid (1-42)- $\beta$ peptide on cortical neuron/glia cultures from parkin null mice. Role of autophagy and glutathione homeostasis**

RM Solano<sup>1</sup>, MJ Casarejos<sup>1</sup>, A Gomez<sup>1</sup>, J Perucho<sup>1</sup>, J G de Yébenes<sup>2</sup>, MA Mena<sup>1</sup>, <sup>1</sup>Departments of Neurobiology, and <sup>2</sup>Neurology, Hospital “Ramón y Cajal”, Madrid, Spain and CIBERned <sup>(1,2)</sup>.

**Objective:** To investigate the effects of oligomeric amyloid (1-42)- $\beta$  peptides (A $\beta$ 1-42) on the survival, cell types and signalling pathways in cortical neuron/glia cultures from WT and PK-KO mice.

**Background:** The production and aggregation of amyloid  $\beta$  peptides (A $\beta$ ) has been linked to the development and progression of Alzheimer’s disease. A $\beta$ 1-42 is increased in patients affected by AD, both in familial and sporadic cases. Parkin mutations impair ubiquitin-proteasome pathways and produce familial Parkinson’s disease (PD) in humans. Parkin may play a role in the processing of A $\beta$ 1-42.

**Methods:** *Transgenic animals* were obtained from 129SV/C57BL6 wild type (+/+) or parkin null (-/-) mice (Itier et al. 2003). Neuronal and glial cortical primary cultures were derived from littermate -/- and +/+ embryos obtained from homozygous colonies previously generated by heterozygous parkin +/- intercross. The genotype was confirmed by PCR analysis of tail tissue and by WB analysis of parkin in the cultures (Casarejos et al. 2005; Solano et al. 2008). *Neuronal and glial cortical primary cultures* were obtained at 16 days of gestation and grown in Neurobasal medium supplemented with B27, during 7 days.

**Results:** The treatment of mixed neuron/glia cultures with A $\beta$ 1-42, 4.4  $\mu$ M, for 48 h induced significant cell death by necrosis (trypan blue) and apoptosis (TUNEL). These effects were both more pronounced in WT than in PK-KO neurons. In WT cultures the treatment with A $\beta$ 1-42 reduced both the total number of cells as well as the number of astrocytes, expressed as the area of GFAP+ cells. However, the treatment with A $\beta$ 1-42 increased more the percentage of microglia in PK-KO than in WT cultures. The temporal profile of activation of ERK 1/2 and AKT proteins showed a rapid increase of phospho-proteins with a maximum increase of activation 10 min after treatment with A $\beta$ 1-42 in PK-KO glial cells. Pre-treatment with an inhibitor of GSH synthesis and the inhibition of autophagy, reverted the increased resistance to A $\beta$  of the PK-KO cultures.

**Conclusion:** Neuron/glia PK-KO cultures are more resistant to A $\beta$ -induced toxicity than WT cultures.

*Supported by FIS 2010/172, Laín Entralgo NDG07/4, CAM 02/02/2006, and CIBERned 2006/05/059 and 2010.*

**Notes:**

## **P5. Synaptic vesicle expression of VAcHT-pHluorin in septal neurons**

L Torres-Benito, R Ruiz, MA Montes, G Alvarez de Toledo, L Tabares, Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, Spain.

Fusion of pHluorin (a pH-sensitive green fluorescent protein) with distinct synaptic vesicle proteins, as synaptobrevin, synaptophysin, synaptotagmin, or the vesicular glutamate transporter (VGLUT), allows real time monitoring of exo-endocytosis. We have used a new fusion protein formed by the acetylcholine vesicular transporter and pHluorin (VAcHT-pHluorin) to monitor neurotransmission in cholinergic septal neurons.

Septal neurons in primary culture (DIV12) from P0-P1 rats were transfected with the VAcHT-pHluorin construction under the CMV promoter. At DIV20-22 the level of expression and the subcellular localization of the protein were checked by immunofluorescence confocal microscopy (VAcHT was detected with anti-GFP antibodies). Transfected neurons showed fluorescent punctata over neurites and somas. These fluorescence spots colocalized well with the synaptic vesicle protein SV2.

In live imaging experiments, VAcHT-pHluorin transfected neurons responded to electrical stimulation and high potassium solution (70 mM) with punctuate fluorescence transients, indicative of the functionality of the recombinant protein. These results show that this new construction could be of interest for studying the dynamic of exo-endocytosis and the effect of the over-expression of the VAcHT on neurotransmitter release.

### **Notes:**

**P6. Dynamin-dependent endocytosis of freshly exocytosed synaptophysin at the neuromuscular junction during nerve stimulation**

P Linares-Clemente<sup>1</sup>, JL. Rozas<sup>1</sup>, P García-Junco-Clemente<sup>1</sup>, JA Martínez-López<sup>1</sup>, M E Vázquez<sup>2</sup>, CO Pintado<sup>3</sup>, R Fernández-Chacón<sup>1</sup>, <sup>1</sup>Instituto de Biomedicina de Sevilla, IBiS, Hosp.Univ. Virgen del Rocío/CSIC/Universidad de Sevilla and Dept. Fisiología Médica y Biofísica, and CIBERNED, Seville, Spain. <sup>2</sup>Dpto. Química Orgánica y Centro Singular de Investigación en Química Biológica y Materiales Moleculares, Universidad de Santiago de Compostela, Spain. <sup>3</sup>Centro Producción y Experimentación Animal, Universidad de Sevilla, Spain.

Synaptic endocytosis is essential at nerve terminals to maintain neurotransmitter release by exocytosis. Here, at the neuromuscular junction (NMJ) of synaptophysin (spH) transgenic mice, we have used imaging to study exo- and endocytosis simultaneously occurring during nerve stimulation. We describe two sequential endocytosis components. During the early component of endocytosis spH-molecules freshly exocytosed are internalized. That component is sensitive to the blocking of GTPase activity of dynamin with dynasore and resistant to myristyl trimethyl ammonium bromide (MiTMAB), a competitive agent that inhibits dynamin by blocking dynamin binding to phospholipid membranes. During the late component of endocytosis, takes place the internalization of the spH molecules that pre-exist at the plasma membrane before stimulation starts. This component is blocked by impairing dynamin binding to phospholipid membranes with MiTMAB. The mechanism responsible for early endocytosis is tightly coupled to exocytosis activation and late endocytosis might arise upon saturation of the early component. Our study shows the co-existence of two sequential synaptic endocytosis mechanisms: one that internalizes vesicular components immediately after they are released and another that internalizes vesicular components pre-existing at the plasma membrane surface.

*Supported by HFSP, MICINN BFU2007-66008, JA P07-CVI-02854, ISCIII and Fondo Europeo de Desarrollo Regional (FEDER).*

**Notes:**

## **P7. Neuronal Pentraxin 1 negatively regulates excitatory synapse number**

J Figueiró-Silva<sup>1</sup>, A Gruart<sup>4</sup>, KB Clayton<sup>2</sup>, P Podlesniy<sup>1</sup>, MA Abad<sup>2</sup>, L Benitez<sup>1</sup>, M Enguita<sup>1</sup>, X Gasull<sup>3</sup>, JM Delgado-García<sup>4</sup>, R Trullas<sup>1,2</sup>. <sup>1</sup>Neurobiology Unit, Institut d'Investigacions Biomèdiques de Barcelona, Consejo Superior de Investigaciones Científicas, IDIBAPS, Barcelona, Spain. <sup>2</sup>Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III. <sup>3</sup>Laboratory of Neurophysiology, School of Medicine, University of Barcelona, Spain. <sup>4</sup>División de Neurociencias, Universidad Pablo de Olavide, Seville, Spain.

The number of synaptic contacts between neurons changes during development and in response to variations in neuronal activity. Such dynamic regulation of synapse number requires the presence of mechanisms that constraint those changes to within physiological limits. However, the molecular composition of those mechanisms is not well characterized. In addition to synapse number, neuronal activity also controls neuronal survival. Thus, low neuronal activity triggers the mitochondrial gene-expression dependent program of apoptotic cell death in mature neurons. Recent evidence indicates that pro-apoptotic proteins of this intrinsic program of cell death, besides playing a role in controlling cell death, also regulate synapse formation and elimination. Neuronal Pentraxin 1 (NP1), a glycoprotein predominantly expressed in the nervous system, is part of the gene-expression dependent intrinsic program of apoptotic cell death evoked by reduction of neuronal activity. NP1 is associated with dystrophic neurites in Alzheimer's disease-affected brains and we found that NP1 acts as a negative regulator of excitatory synapses, indicating that, besides cell death, NP1 also modulates synaptic function.

*Supported by grants SAF2008-03514 (to R.T), BFU2008-00899 (to J.M.D.-G.), BFU2008-03390/BMC (to A.G.), BFU2005-01572 and FIS 08/0014 (to X.G.); by the European Community grant FP7/201714 (to A.G) and by the Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED).*

### **Notes:**

## **P8. Presynaptic PI3K promotes the formation of postsynaptic glutamate receptors in the neuromuscular junction of drosophila**

S Jordán-Álvarez<sup>1</sup>, W Fouquet<sup>2</sup>, SJ Sigris<sup>2</sup>, A Ferrús<sup>1</sup>, A Acebes<sup>1</sup>.<sup>1</sup>Cajal Institute, Madrid, Spain. <sup>2</sup>Neurowissenschaftliches Forschungszentrum, Berlin, Germany.

The phosphoinositide 3 kinase (PI3K) is involved in pathways for cell survival and gene expression which can lead to human diseases like cancer, diabetes or Alzheimer. In *Drosophila*, up- or down-regulation of PI3K, AKT and GSK3, acting within a novel tyrosine kinase receptor signaling pathway, control the formation and maintenance of synapses. The new synapses are functional and elicit changes in larval and adult's locomotor behavior. Interestingly, PI3K activation is able to induce new synapses in aged neurons. This synaptogenic pathway appears functionally conserved in mammals.

To detect possible postsynaptic changes due to PI3K presynaptic increase, we analyzed changes in the number of ionotropic glutamate receptors, located in the postsynaptic densities (PSDs) of the larval neuromuscular junction. The *in vivo* formation of glutamatergic synapses has been well characterized. Here, we analyzed the formation and growth of GluRs using immunohistochemistry and FRAP (Fluorescence Recovery After Photobleaching) in animals overexpressing PI3K. The results show an increase in the number of postsynaptic densities directly related to the higher number of synapses produced by PI3K. In addition, the FRAP analysis indicates that the kinetics of assembly of GluRs in experimental animals is similar to the controls. Using the Gal4-repressor (Gal80<sup>ts</sup>) for a temporal activation of PI3K, we determined that new synapses are formed within 20-30 h approx. Taken together, the data indicate that the PI3K dependent synaptogenesis causes an increment of PSDs, without changing the dynamics of glutamate receptors assembly.

*Supported by MCINN grants BFU2006-10180 and BFU2009-12410. S.J.A. was funded by fellowship BES-2007-16597.*

**Notes:**

## **P9. Developmental regulation of vesicular cycle dynamics in cerebellar granule cells**

J Ramírez Franco<sup>1</sup>; D Bartolomé Martín<sup>1</sup>; E Castro<sup>2</sup>; J Sánchez Prieto<sup>1</sup>; M Torres<sup>1</sup>, <sup>1</sup>Department of biochemistry and molecular biology IV, School of veterinary, UCM, Madrid, Spain. <sup>2</sup>Department of biochemistry, molecular biology and physiology, Faculty of health sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain.

Synaptic development involves acquisition of full competence for exocytosis and endocytosis of synaptic vesicles. Styryl dye FM1-43 has been used to study the exo-endocytotic cycle of synaptic vesicles at nerve terminals in cultures. We have used this approach to analyse synapse development at cultured rat cerebellar granule cells (CGCs). Functional nerve terminals have been established according to quality parameters that rely on: coefficient of variation and slope of the baseline and the extent of the distaining. Development of the culture involves an increase in the % of synaptic boutons that reach the quality parameters. Maturation does not significantly affect the initial fluorescence level, but changes the kinetics and extent of the distaining process. Group 1 boutons distain more than 60% of the dye with a rapid kinetics, while group 2 boutons distain only 40% of the dye with slower kinetics. Maturation increases the proportion of group 1 nerve terminals in the culture. Synaptic activity blockade with the Na<sup>+</sup>-channel blocker tetrodotoxin produces a delay in maturation, decreasing the percentage of group 1 boutons. CGCs need a sustained depolarization state of their membrane potential to mature. It is thought that this situation would be equivalent to a permanent synaptic activity state. At least three endocytic ways have been described at central synapses. During strong stimulation, mechanisms of bulk endocytosis are implemented, these mechanisms show a strong calcium dependence and are slower than clathrin mediated endocytosis, moreover these mechanisms involve the retrieval of a large membrane moiety and intermediate steps between the retrieval and subsequent exocytosis, which explains a higher fluorescence levels at group 2 boutons after loading process, the lower degree of dye releasing and the slower exocytic kinetic at this boutons. Therefore, maturation of cultured CGCs involves a transition between different endocytic ways that predominates at different stages.

*Supported by grants from the Spanish 'Ministerio de Educación y Ciencia' (BFU2006-01012) and (BFU2009-07092) to MT, (BFU2007-64154 and BFU2010-16947) to JS-P, and the 'Instituto de Salud Carlos III' RD06/0026, the 'Comunidad de Madrid' (S-BIO-0170/2006) and the UCM-CAM (CCG07-UCM/SAL-2150) to J S-P and MT.*

### **Notes:**

