4th Annual Meeting
Mouse Models for Neuroscience Research Network

Center for Genomic Regulation (Barcelona, Spain)
December 1st, 2005

Local Organizing Committee «

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Meeting information «

On December 1, 2005, the fourth Annual Meeting of the Mouse Models for Neuroscience Research Network will be held at the Center for Genomic Regulation (Spain). The Mouse Models of Neuroscience Research Network is part of an initiative to increase scientific research networks in Spain and is supported by the Catalonian Government. The purpose of the program is to facilitate the exchange of information among researchers studying animal models of Neuroscience Research at the molecular, biochemical, pharmacological, anatomical, and functional levels to test hypotheses associated with human neurological disorders. This is achieved by scientific follow-up meetings that facilitate collaborations, ensure the continued availability of scientifically valuable, genetically engineered mice and allow discuss neurobiological and behavioral phenotypic screening tools. The mouse models for neuroscience research should be important tools for functional gene identification as well as providing new and unique altered phenotypes for physiological and behavioral research.

Xarxa Temàtica
Murine Models of CNS Disease
III Pla de Recerca de Catalunya
2000-XT00083
Generalitat de Catalunya

Location «

Salón de actos CMIMA-CSIC
CMIMA / CRG Building
Passeig Marítim, 37-39
Barcelona

Acknowledgements «

The local organizing committee wisth to acknowledge the current sponsors for their generous support to the ***4th Annual Meeting Mouse Models of Neuroscience Research Network***.

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Previous meetings «

Year	*Location*	
2001	Oncology Research Institute - Barcelona, Spain	First Annual Meeting
2002	Center for Genomic Regulation - Barcelona, Spain	Second Annual Meeting
2004	Center for Genomic Regulation - Barcelona, Spain	Third Annual Meeting

Program at a glance «

December 1st, 2005

08:00 – 08:30	Registration
08:30	Presentation and Wellcome
08:30 – 09:30	**Plenary Lecture**: A. Gruart - *Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice*
09:30 – 10:30	**Session I**: *Animal models of neuropsychiatric disorders and their relevance for the development of evidence-based therapy.*
10:30 – 11:00	Coffee Break
11:00 – 12:00	**Invited Seminar**: A. Giraldez - *The roles of miRNAs during vertebrate development*
12:15 – 13:30	**Session II**: *Understanding cellular alterations in neural circuits: animal models for the study of neurodevelopment.*
13:30 – 15:00	Lunch – **Poster Session**
15:00 – 16:00	**Round Table**: *Neuroscience in Spain* J.R. Naranjo, J. Rodríguez, F. Artigas, A. Gruart
16:00 – 17:00	**Session III**: *Use of transgenic animals in the study of signal transduction pathways.*
17:00 – 17:30	Coffee Break
17:30 – 18:30	**Session IV**: *Neuroprotection and neurodegeneration.*
18:30 – 19:30	**Plenary Lecture**: A. Barco - *Role of the CREB activation pathway in the consolidation of synaptic plasticity and memory processes*
20:30	Buses at CRG to go to Razzmatazz
21:00	Buffet + Live Music at Razzmatazz (Sala Lolita)

Social program «

For those **coming to the buffet dinner at Razzmatazz** a **bus will be at CMIMA building at 20:30h** to take you to the party. Please be on time!!!

In case you cannot take the bus find below the address of Razzmatazz:

Razzmatazz
C/ Pamplona 88 passatge interior
Razzmatazz 3 o Sala 3 LOLITA

Take note that **we will offer you a varied cattering with beverages from 21:00 to 23:00h. After this time, the beverages will be at your own charge**.

Program «

08:00 – 08:30 Registration

08:30 Presentation and Wellcome

08:30 – 09:30 **Plenary Lecture**: A. Gruart - *Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice*

09:30 – 10:30 **Session I**: *Animal models of neuropsychiatric disorders and their relevance for the development of evidence-based therapy.*
Chair: F. Berrendero

- F. Berrendero, L. Galeote, B. L. Kieffer, R. Maldonado - *Papel de los receptores opioides mu en los efectos farmacológicos inducidos por la nicotina*
- L. Díaz-Mataix, A. Bortolozzi, M. C. Scorza, M. Toth, P. Celada, F. Artigas - *Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity. Role in atypical antipsychotic action*
- G. Estivill-Torrús, P. Llebrez-Zayas, E. Matas-Rico, E. Gil-Lara, A. Bilbao, C. Pedraza, L. Santín, J. Chun, F. Rodríguez de Fonseca – *The lysophospahatidic acid receptor LPA1: a new regulator for neurogenesis and cognitive processes*

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12:15 – 13:30 **Session II**: *Understanding cellular alterations in neural circuits: animal models for the study of neurodevelopment.*
Chair: M. Morales

- F. J. Tejedor, J. Colonques, J. Ceron, B. Hammerle - *The proliferative centers of the larval optic lobe of drosophila: an experimental model where to study the genetic and molecular bases of neural progenitor cells*
- G. Abello, F. Giráldez, B. Alsina - *Neural versus non-neural regionalization of the otic placode*
- C. Pujades, A. Kamaid, B. Alsina, F. Giraldez **-** *BMP-signaling regulates the generation of hair-cells*
- M. Morales, M. Martínez, M. Calvo, M. Dierssen - *Spine actin dynamics in Dyrk1 transgenic mouse model*

13:30 – 15:00 Lunch – **Poster Session**

15:00 – 16:00 **Round Table**: *Neuroscience in Spain*
J.R. Naranjo, J. Rodríguez, F. Artigas, A. Gruart

16:00 – 17:00 **Session III**: *Use of transgenic animals in the study of signal transduction pathways.*
Chair: J.R. Naranjo

- P. Linares-Clemente, P. García-Junco-Clemente, C.O. Pintado, F. Schmitz, R. Fernández-Chacón - *Imaging synaptic vesicle exo- and endocytosis in cerebellar mossy fiber synaptosomes from SynaptopHluorin transgenic mice*
- R. Gomez-Villafuertes, A. Gutierrez-Adam, B. Pintado, J. Barrio, B. Mellström, J.R. Naranjo - *DREAM regulates Ca2+ homeostasis and viability in cerebellar neurons*
- R. Ruiz, L. Tabares – *Characterization of neutotransmission in neuromuscular degeneration (mmd) mice*

17:00 – 17:30 Coffee Break

17:30 – 18:30 **Session IV**: *Neuroprotection and neurodegeneration.*
Chair: J. Rodríguez

- H. Peluffo, L. Acarin, A. Arís, P. González, A. Villaverde, B. Castellano, B. González - *Neuroprotection from excitotoxic damage by Cu/Zn superoxide dismutase gene delivery to the postnatal rat brain using a modular non-viral protein vector*

- R. Trullas, A. Abad, M. Enguita, N.DeGregorio-Rocasolano - *Over-expression of Neuronal Pentraxin 1 reduces neurite outgrowth before cell death in huan neuroblastoma SH-SY5Y cells*
- J. Alberch, J.M. Canals, J.R. Pineda, D. del Toro, J.F. Torres-Peraza - *Generación de ratones transgénicos con huntingtina mutada y distintos niveles de BDNF para el estudio de la fisiopatología de la enfermedad de Huntington*

18:30 – 19:30 **Plenary Lecture**: A. Barco - *Role of the CREB activation pathway in the consolidation of synaptic plasticity and memory processes*

20:30 Buses to Razzmatazz at CMIMA building

21:00 Buffet + Live Music at Razzmatazz (Sala Lolita)

Instructions for presentations «

Poster Session instructions

Size: Please feel compelled only to use the space necessary to present your work, but limit the size of your poster to **no larger than 120cm (height) x 90cm (wide).**

Please include in your poster: title, authors, institutional affiliation, and sources of support for the work presented.

How to find your location to display your poster: When you register at the meeting, you will receive a conference folder that contains a numbered list of all the poster boards. The number of the abstract corresponds to consecutively numbered poster boards.

Information & Instructions for Speakers

Ideally, we would like your presentation to be in Powerpoint and for you to email your presentation to David Fernández at david.fernandez@crg.es. This will allow us to test your presentation on our laptop, resolve any problems, and allow rapid transition from one talk to the next during the conference.

Alternatively, you can bring your Powerpoint presentation to David Fernández at *Registration Desk* before oral sessions to have your presentation loaded and tested before the session.

Please bring your Powerpoint presentation in at least two of the following media:

- USB 1.0/2.0 memory stick
- CD-Rom
- DVD-Rom

If you choose to bring your own computer to run your presentation, we will only be able to devote a very short time between talks to resolve any incompatibility between your computer and the projector. As a result, we cannot guarantee that your slides will be available for your talk.

We have a PC laptop (Windows 2000) and a MAC laptop.

IMPORTANT NOTE: Microsoft has acknowledged that Powerpoint presentations created on its Windows XP or ME operating systems, and the run on a Windows 2000, **can** have images that are flipped and displayed backwards. Microsoft has indicated that there is no fix for this other than manually correcting the distorted images once the presentation is loaded on the Windows 20000 machine. Also, some animations commands differ between XP and Windows 2000, and as result may not run as intended.

Any question about presentations please contact David Fernández at david.fernandez@crg.es.

Thank you very much again for your time and help. We look forward to your talk and to an enjoyable, fruitul conference in Barcelona.

Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice

A. Gruart
Universidad Pablo de Olavide, Sevilla, Spain.

It is currently assumed that an important functional property of the central nervous system is its capability to transform synaptic activities in order to acquire and to store newly learned information. We have recorded in alert behaving mice the activity-dependent plastic changes taking place at the hippocampal CA3-CA1 synapse during the acquisition, extinction, recall (i.e., retrieval), and reconditioning of an associative task. For this, animals (n = 120 mice) were classically conditioned to evoke eyelid responses, using a trace (CS, tone / US, shock) conditioning paradigm. The tone (2400 Hz, 85 dB) was presented for 20 ms followed 500 ms later by an electrical shock (0.5 ms, 3 × threshold) applied to the trigeminal nerve. A total of four habituation, 10 conditioning and five extinction sessions were carried out. Some animals were summated to two recall sessions or reconditioned for 10 additional sessions. A single pulse presented to Schaffer collateral-commissural pathway, 300 ms after CS presentation, evoked a monosynaptic field EPSP at ipsilateral CA1 pyramidal cells which slope was linearly related to learning evolution. We checked the effects of evoking long-term potentiation (LTP) in experimental animals depending upon the moment when it was triggered across the learning process. For this, LTP was evoked in different groups of animals during conditioning sessions 1-2, 5-6, or 9-10, and during recall and reconditioning sessions, number 1-2 (n = 10 mice per group). LTP evoked by train stimulation of the Schaffer collateral-commissural pathway prevented acquisition, extinction, recall or reconditioning depending upon the moment when it was triggered. The administration of CGP 39551 (a selective NMDA antagonist) prevented LTP induction, the acquisition of an eyelid learned response, and the synaptic changes taking place at the CA3-CA1 synapse across conditioning. According to the present results, we have consistent evidence relating LTP, activity-dependent synaptic plasticity and associative learning in conscious mice.
Supported by Spanish MCYT (BFI2002-00936).

Role of the CREB activation pathway in the consolidation of synaptic plasticity and memory processes

A. Barco
Instituto de Neurociencias de Alicante, Spain

The encoding of new memories in the brain is thought to depend on long-lasting changes in the strength of synaptic connections between neurons, a change that depend, in turn, on transient or permanent alterations in specific patterns of gene expression. The CREB family of transcription factors is one of the core components in the molecular switch that stabilizes long-term forms of synaptic plasticity and converts short- to long-term memory. We are investigating the details of the participation of the CREB family of transcription factors in some of these processes using an multidisciplinary approach based in the generation and characterization of transgenic and knockout mice. We are also interested in exploring the contribution of chromatin remodeling to the perpetuation of synaptic changes and memory stability and particularly in the role of the CREB co-activator, CBP, in these processes.

Session 1: Animal models of neuropsychiatric disorders and their relevance for the development of evidence-based therapy

Chair: F. Berrendero

Papel de los receptores opioides mu en los efectos farmacológicos inducidos por la nicotina

F. Berrendero, L. Galeote, B.L Kieffer, R. Maldonado
Universidad Pompeu Fabra, Barcelona, Spain.

El papel de los receptores opioides mu en diferentes respuestas comportamentales inducidas por la nicotina se ha estudiado mediante la utilización de ratones knockout que carecen de estos receptores. En primer lugar evaluamos los efectos agudos de la nicotina sobre la antinocicepción, mediante los ensayos de la placa caliente y de la inmersión de la cola, y la actividad locomotora en estos animales. El efecto antinociceptivo de la nicotina fue menor en los ratones mutantes mientras que no observamos ninguna diferencia en la respuesta hipolocomotora inducida por esta droga. La tolerancia a los efectos antinociceptivos de la nicotina se desarrolló con mayor rapidez en ausencia de los receptores opioides mu. Además, la actividad funcional de los mismos se incrementó en la médula espinal de ratones wild-type como consecuencia de la administración crónica de la droga. Los efectos reforzantes de la nicotina se evaluaron mediante el paradigma de la preferencia de plaza condicionada. La nicotina produjo claros efectos reforzantes en los animales wild-type pero éstos fueron bloqueados en los ratones mutantes. Finalmente, la expresión somática del síndrome de abstinencia a la nicotina, precipitado en ratones dependientes a la misma mediante la inyección del antagonista nicotínico mecamilamina, resultó también atenuada en los ratones knockout para los receptores opioides mu. Por tanto, los experimentos realizados indican que los receptores opioides mu participan en los efectos antinociceptivos agudos de la nicotina así como en el desarrollo de la tolerancia a este mismo efecto como consecuencia de su administración crónica. Los receptores opioides mu también median los efectos reforzantes de la nicotina y son necesarios para la expresión completa de los signos somáticos de la abstinencia física.

Involvement of 5-HT1A receptors in prefrontal cortex in the modulation of dopaminergic activity. Role in atypical antipsychotic action

L. Díaz-Mataix[1], A. Bortolozzi[1], M. C. Scorza[1], M. Toth[2], P. Celada[1], F. Artigas[1]*
[1]Department of Neurochemistry, IIBB-CSIC (IDIBAPS), 08036 Barcelona, Spain.
[2]Department of Pharmacology, Weill Medical College, Cornell University, New York, N.
*Present address: Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay.

Atypical antipsychotics increase dopamine (DA) release in medial prefrontal cortex (mPFC) an effect possibly involved in the superior effects of atypical vs. classical antipsychotics on cognitive/negative symptoms. We examined the role of 5-HT1A receptors in mPFC on the modulation of dopaminergic activity and the mesocortical DA release in vivo.
The administration of the highly selective 5-HT1A agonist BAY x 3702 (BAY, 10-40 µg/kg i.v.) to anesthetized rats increased the firing rate and burst firing of DA neurons in the ventral tegmental area (VTA) and DA release in VTA and mPFC. These effects were reversed by WAY-100635. The increase in DA neuron activity was prevented by prior cortical transection and the increase in DA release was enhanced by the DA reuptake blocker nomifensine.
The application of BAY in rat and mouse mPFC by reverse dialysis increased the local DA release at low concentration (3 µM) and reduced it at a higher concentration (30 µM). Both effects disappeared in 5-HT1A knockout mice. In the presence of bicuculline, BAY reduced DA release at all concentrations. The application of clozapine and olanzapine (but not haloperidol) in mPFC increased the local DA release in wild-type but not in 5-HT1A knockout mice. Likewise, bicuculline co-perfusion prevented the elevation of DA release induced by the atypical antipsychotics.

These results suggest that 5-HT1A receptors in mPFC enhance the activity of VTA DA neurons and DA release in mPFC. This mechanism appears to be involved in the elevation of DA release produced by the atypical antipsychotics clozapine and olanzapine.

The lysophospahatidic acid receptor LPA1: a new regulator for neurogenesis and cognitive processes

G.Estivill-Torrús[1], P. Llebrez-Zayas[1], E. Matas-Rico[1], E. Gil-Lara[1], A. Bilbao[1], C. Pedraza[2], L. Santín[2], J. Chun[3], F. Rodríguez de Fonseca[1]
[1]Research Foundation IMABIS, Unidad de Investigación, Hospital R.U. Carlos Haya, Malaga, 29010, Spain.
[2]Department of Psychobiology, Faculty of Psychology, University of Malaga, Campus de Teatinos, Malaga, 29071, Spain.
[3]Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037, USA.

The lysophosphatidic acid(LPA) is a bioactive phospholipid acting as intercellular messenger through specific protein G-coupled receptors (LPA1-4). LPA enhances cortical growth by inhibition of neuronal apoptosis when administered exogenously. However, the functional significance of its endogenous signaling remains still unknown. The LPA1 receptor is mainly expressed throughout cortical development in the proliferative regions concomitantly with neurogenesis. Moreover, LPA induces changes in physiological state of hippocampal neurons suggesting a role in cognitive processes.
By use of immunocytochemistry of neural and proliferation markers, ISH for proneural genes, early neuronal precursor cultures and BrdU labeling onto adult mice and embryos lacking the LPA1 receptor we have demonstrated that the LPA1 receptor plays a key role in cortical neurogenesis. The lack of receptor in neuronal precursors triggers a reduction in the proliferative cohort, increase of asymmetric division, shortening of cell cycle and early maturation. As consequence, cortex of adult mice lacking the receptor suffered postnatal neuronal death and shows a reduction in neuronal number for deeper cortical layers. Hippocampus of LPA1 null mice appeared neuronally and morphologically unaltered. However, adult hippocampal neurogenesis is affected with a reduction of the production of new cells even under environmental enrichment condition. Correspondingly LPA1 null mice exhibit difficulties for cognitive tasks. The LPA1 receptor hence comes out as a new cerebral regulator with promising aptitudes to be involved in neurodegenerative disorders requiring neural regeneration or as new target for therapies.
Granted by FIS 01/3032, FIS 02/1643, red CIEN (C03/06, FIS).

Session 2: Understanding cellular alterations in neural circuits: animal models for the study of neurodevelopment

Chair: M. Morales

The proliferative centers of the larval optic lobe of drosophila: an experimental model where to study the genetic and molecular bases of neural progenitor cells

F.J. Tejedor, J. Colonque, J. Ceron, B. Hammerle
Instituto de Neurociencias, CSIC and Universidad Miguel Hernandez, Alicante, Spain.

One of the most relevant problems in developmental Neurobiology is to understand the mechanisms underlying the generation of cellular diversity in the brain. The genetic and molecular analysis of the embryonic nervous system of Drosophila has provided a great deal of information on the processes of segregation of neural progenitor cells and their specification. Despite the extensive conservation of the basic molecular mechanisms of embryonic regionalization between flies and mouse, differences at the level of cellular processes are very clear between the embryonic CNS of Drosophila and vertebrates. For instance, the pattern of division of embryonic neuroblasts (NBs) is very different from that of neuroepithelial progenitors in the vertebrate CNS.
We are using an alternative experimental model where to study the genetic and molecular basis of the proliferation and specification of CNS progenitors: the proliferative centers of the

larval optic lobes of Drosophila from which most cells of the adult Drosophila brain originate. We focus our attention on several aspects of these processes: the pattern of cellular division, the switch from symmetric to asymmetric divisions, the expression/localization of asymmetric cell fate determinants that may be involved in the specification of neural cells, and the genetic analysis of genes which may regulate these processes. This is complemented with gene expression patterns, enhancer-trap reporter expression, and clonal lineage analysis. Our experimental data suggest interesting similarities between Drosophila larval NBs and neuroepithelial progenitors of the vertebrate CNS.
This experimental system is currently applied in our lab to study the role of Minibrain (Mnb) on the regulation of proliferation of neural progenitor cells and to model the possible involvement of the MNB human orthologue in the neuronal deficit of Down syndrome brain. In addition, we use this model system to study two other relevant issues: cell cycle regulation of neural progenitor cells and the genetic evolution of gliogenesis.

Neural versus non-neural regionalization of the otic placode
G. Abello, F. Giráldez, B. Alsina
Universidad Pompeu Fabra, Barcelona, Spain.

Most of the sensory input of the vertebrate head is captured from the cranial sensory organs and ganglia that transmit external information to the CNS. Sensory systems share the same developmental origin, are derived from the placodes, ectodermal thickenings that can generate a vast array of specialized cell-types as sensory neurons, supporting cells and/or sensory receptor cells. Only a small region of the otic placode gives rise to neurons. This region that we have called proneural domain is characterized by the expression of proneural genes, FGF10 and Sox3. Complementary to the proneural domain, the so called non-neural territory, expresses Lmx1 and Iroquois1, hairy1 and Serrate1. Our work indicates that anterior-posterior regionalization is a progressive event that is initiated in the ectoderm before otic placode is morphologically visible. At otic cup stage, proneural and non-neural territories presented restricted cell intermingling, as revealed by double DiI and DiO injection experiments. DiI/DiO labelled clones exhibited an antero-postero boundary that was coincident with the FGF0/Hairy1 expression limits. We blocked Notch signalling by the -secretase inhibitor (DAPT) and found that Notch had differentγ functions in the proneural and non-neural domains. Notch regulates cell specification of neuronal cells in the proneural domain by lateral inhibition, but moreover Notch signalling is required for neural/non-neural regionalization by restricting Lmx1 and Irx1 to the posterior compartment. Thus, we propose that two well-defined territories develop at early stages of otic development and Notch signalling pathway is required in both of them.

BMP-signaling regulates the generation of hair-cells
C. Pujades, A. Kamaid, B. Alsina, F. Giraldez
Universidad Pompeu Fabra, Barcelona, Spain.

Bone morphogenetic proteins (BMPs) are diffusible molecules involved in a variety of cellular interactions during development. Bmp4 expression accompanies the development of the ear sensory organs during patterning and specification of sensory cell fates yet, there is no understanding of the role of BMP4 in this process. The present work was aimed at exploring the effects of BMP-signaling on the development of hair-cells. For this purpose, we studied gene expression, cell proliferation and cell death in isolated chick otic vesicles that were grown in vitro in the presence of recombinant BMP4 or the BMP-inhibitor Noggin. Cath1 was used as a marker for hair-cell specification. BMP4 reduced the number of Cath1-cells and, conversely, Noggin increased the size of the sensory patches and the number of Cath1-positive cells. The effect of BMP4 was irreversible and occurred before cell fate specification. Lfng and Fgf10 were expressed in the prosensory domain before Cath1, and their expression was expanded by Noggin. At these stages, modifications of BMP activity did not re-specify non-sensory epithelium of the otic vesicle. The expression of Bmp4 at sensory patches was suppressed by BMP4 and induced by Noggin suggesting an autoregulatory loop. Analysis of BrdU incorporation during 6 and 18 hours indicated that the effects of BMP4 were due to its ability to reduce the number of actively proliferating progenitors and inhibit cell fate specification. BMP4 induced cell death within the prosensory domain of the otic vesicle, along with the expression of Msx1,

but not Msx2. On the contrary, BMP-inhibition with Noggin favored hair-cell specification without changes in the overall cell-proliferation. We propose that about the stage of terminal division, the balance between BMP and BMP-inhibitory signals regulates survival and specification of hair-cell precursors, the final number of sensory hair-cells being limited by excess levels of BMPs.

Spine actin dynamics in Dyrk1 transgenic mouse model

M. Morales[1], M. Martínez[2], M. Calvo[1], M. Dierssen[2]
[1]IDIBAPS-UB, Barcelona, Spain
[2]Center for Genomic Regulation, Barcelona, Spain.

Dendritic spines are the receptive sites of the majority of the glutamatergic synapses in the cortex, being proposed as an important site of neuronal plasticity. In fact, changes in spine number and shape have been documented under a variety of experimental and pathological conditions. An important characteristic of the spine is an enrichment of cytoskeletal actin. Indeed, actin filaments are the major cytoskeletal elements in the spines. How actin in the spine could contributes to synaptic plasticity is unclear, but it probably plays a role in the morphological changes of the spine in response to neural activity, which may reflect the formation of new synapses. Another way the actin cytoskeleton could contribute to synaptic plasticity is by permiting the exocytosis and endocytosis of AMPA-type receptors or mediating local protein trafficking within the dendritic spine. The Drosophila minibrain (mnb/dyrk1A) gene encodes a serine/threonine protein kinase that is required in neuroblast proliferation .The mammalian homologues (DYRK1A: dual-specificity tyrosine-regulated kinase 1) map to human chromosome 21. Experimental evidence supports the involvement of MNB/Dyrk1A in several neurological diseases and cognitive deficits of Down's? syndrome. Furthermore, MNBH/Dyrk1A is over-expressed in Down's? syndrome brains. We have used fluorescence recovery after photobleaching (FRAP) as a method to investigate the actin intracellular dynamics of actin in dendritic spines and its regulation in the Dykr1 transgenic mouse cortical neurons. Embryonic cortical neurons in culture were transfected with a plasmid encoding eGFP-Actin. An individual spine was rapidly photobleached using high-intensity laser illumination. The time course of the fluorescence recovery reflects the rate of actin turnover.
Preliminary Experiments, still in progress, shows that the recovery occurred in two phases; a fast component with a time constant of about 1 s (mostly diffusion) and a slow component with a half time around 40-60 s driven by actin polymerization.

Session 3: Use of transgenic animals in the study of signal transduction pathways

Chair: J.R. Naranjo

Imaging synaptic vesicle exo- and endocytosis in cerebellar mossy fiber synaptosomes from SynaptopHluorin transgenic mice

P. Linares-Clemente[1], P. García-Junco-Clemente[1], C.O. Pintado[2], F. Schmitz[3], R. Fernández-Chacón[1]
[1]Department of Medical Physiology and Biophysics, School of Medicine, University of Seville, Avda. Sánchez-Pizjuán 4, 41009-Seville, Spain.
[2]Center for Animal Production and Experimentation, University of Seville, Espartinas, 41807-Seville, Spain.
[3]Institute for Anatomy and Cell Biology, University of Saarlandes, Homburg, Germany.

Synaptophluorin (Syph) is a pH-sensitive GFP fused to synaptobrevin/VAMP2 that faces the lumen of synaptic vesicles and increases fluorescence of nerve terminals upon exocytosis. In order to image presynaptic activity in different types of synapses, we have generated transgenic mice expressing Syph under the control of the neuronal specific promoter Thy-1. The mice express Syph in a wide variety of neurons all over the brain and the spinal cord. Mice survival and fertility is comparable to control littermates and they do not show any apparent neurological phenotype. One of the transgenic lines (tgSyph-A), displays a characteristic punctate labelling at the cerebellar granular layer. Immunofluorescence analysis of cerebellum with antibodies against GFP and the synaptic vesicle marker SV2, reveals that synaptopHluorin

expression is restricted to subsets of cerebellar mossy fibers. We have then prepared mossy fiber synaptosomes from tgSyph-A mice. As expected, double labeling with antibodies against presynaptic markers and GFP only occurs in a synaptosome subpopulation. We have confirmed the integrity of synaptosomal structure with electron microscopy. Interestingly, the application of depolarizing solutions triggers a significant increase of fluorescence that returns to values close to base line upon restoration of control solution. In addition, those synaptosomes increase their fluorescence upon the application of alkalinizing solutions (ammonium chloride) and supports the notion that the fluorescence increase is due to alkalinization of synaptopHluorin containing vesicles. Those changes on fluorescence levels are consistent with expected changes due to evoked synaptic vesicle exocytosis followed by synaptic vesicle re-acidification after endocytosis. Our data indicate that transgenic mice expressing synaptophluorin bear an important potential to directly investigate presynaptic mechanisms in cerebellar mossy fibers and could be applied to study molecular mechanisms of neurodegeneration in mouse models. Supported by: Human Frontiers Science Program and Spanish Ministry of Education and Science.

DREAM regulates Ca2+ homeostasis and viability in cerebellar neurons

R. Gomez Villafuertes, A. Gutierrez Adam, B. Pintado, J. Barrio, B. Mellström, J.R. Naranjo
CNB-CSIC, Madrid, Spain.

The Na+/Ca2+ exchangers, NCX1, NCX2 and NCX3, are vital for the control of cellular Ca2+ homeostasis. Here we show that a doublet of downstream regulatory element (DRE) sites in the promoter of the NCX3 gene mediates transcriptional repression of NCX3 by the Ca2+-modulated transcriptional repressor DREAM. Overexpression of a DREAM mutant insensitive to Ca2+ (EFmDREAM) in hippocampus and cerebellum of transgenic mice significantly reduced NCX3 mRNA and protein levels without modifying NCX1 and NCX2 expression. Cerebellar granules from EFmDREAM transgenic mice showed increased levels of cytosolic Ca2+ and were more vulnerable to increased Ca2+ influx following partial opening of voltage-gated plasma membrane Ca2+ channels induced by increasing K+ in the culture medium, but survived better in the conditions of reduced Ca2+ influx prevailing in low extracellular K+. Overexpression of NCX3 in EFmDREAM transgenic granules using a lentiviral vector restored the normal survival response to high K+ observed in wild-type granules. Thus, the downregulation of the regulator of Ca2+ homeostasis NCX3 by Ca2+-regulated DREAM is a striking example of the autoregulatory property of the Ca2+ signal in neurons.

Characterization of neutotransmission in neuromuscular degeneration (mmd) mice

R. Ruiz, L. Tabares
Department of Medical Physiology & Biophysics, School of Medicine. University of Seville, Spain.

Neuromuscular degeneration (nmd) mice harbour a spontaneous mutation in the Ighmbp2 gene, a member of a DNA/RNA helicase/ATPase protein family. The nmd mice display a disease phenotype characterized by muscular weakness progressing to severe neurogenic muscle atrophy and paralysis of the extremities. The phenotype of nmd mice is due to a severe degeneration of lower motor neurons, and closely resembles human Spinal Muscular Atrophy with respiratory distress (SMARD; MIM 604320).
We have performed an in vivo electrophysiological characterization of nmd hind limb muscles and have found a decrease in the amplitude of the compound muscular action potentials (CMAPs) at P70, together with a profound depression of the CMAP amplitude with repetitive stimulation, indicative of loss of efficacy in neurotransmission. In addition, in old mutant mice (> P200), the estimation of the number of motor units (MUNE) in the gastrocnemius muscle revealed a severe loss of units and an increase in size of the remaining ones . In contrast, the electrical activity of the diaphragm muscle fibres did not significantly differed from those of control littermates.
To study in more detail the pathophysiological changes taking place in motor neurons in this disease, we have compared synaptic transmission at the neuromuscular junction (NMJ) in nmd and control mice in an in vitro preparation of the levator auris longus muscle. These experiments show that nmd mice have periods of synaptic transmission failure under repetitive

physiological stimulation. The probability of failure occurrence increases linearly with the stimulation frequency. In addition, the size of neuromuscular end-plates, labelled by α-bungarotoxin–rhodamine, were smaller in nmd mice. Taken together our observations show that multiple aspects of neurotransmission in the NMJ are altered in nmd mice. Future work should establish the functional role of the mutated protein, Ighmbp2, in the synapse and the muscle.

Session 4: Neuroprotection and neurodegeneration

Chair: J. Rodríguez

Neuroprotection from excitotoxic damage by Cu/Zn superoxide dismutase gene delivery to the postnatal rat brain using a modular non-viral protein vector

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We have analyzed the neuroprotective efficacy of transient overexpression of the antioxidant enzyme Cu/Zn Superoxide dismutase (SOD) after an excitotoxic injury to the immature rat brain by using a recently constructed modular protein for non-viral gene delivery termed NLSCt. Injection of the NLSCt vector carrying the Cu/Zn SOD transgene 2 hours after intracortical N-methyl-D-aspartate administration showed, after 3 days, improved functional outcome and a reduced lesion volume. In secondary degenerative areas, increased neuronal survival as well as decreased numbers of degenerating neurons and nitrotyrosine immunoreactivity was seen. Interestingly, injection of the NLSCt vector carrying the control GFP transgene also displayed a significant neuroprotective effect, although less pronounced. In conclusion, the transitory expression as well as the appropriate levels of Cu/Zn SOD using the non-viral modular protein NLSCt for gene therapy accounts for the neuroprotective effect, and thus recombinant modular protein vectors can be suitable for in vivo gene therapy.

Over-expression of Neuronal Pentraxin 1 reduces neurite outgrowth before cell death in human neuroblastoma SH-SY5Y cells

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Expression of Neuronal Pentraxin 1 (NP1) is part of the apoptotic cell death program activated in mature cerebellar granule cells by reduction of neuronal activity. NP1 is a glycoprotein homologous to the pentraxins of the acute phase immune response and it is involved in both synaptogenesis and synaptic remodeling. We hypothesized that neuronal pentraxins constitute a genetic sensor that regulates neuronal death or survival depending on synaptic activity. We have now examined the effect of NP1 on neurite outgrowth and cell proliferation in human neuroblastoma SH-SY5Y cells. We found that lentiviral mediated transgene overexpression of NP1 produces a marked reduction in neurite outgrowth. This effect is followed by a significant increase in the number of cells with apoptotic nuclei and with active caspase 3. Silencing NP1 overexpression with lentivirus vector-mediated short hairpin RNA interference (shRNAi) prevents the reduction of neurite outgrowth and rescues cortical neurons from apoptosis evoked by NP1. In addition, different clones of SH-SY5Y cells that permanently overexpress NP1 exhibit a cell proliferation index that is markedly lower that the observed in control neuroblastoma cells. These findings show that NP1 reduces neurite outgrowth, increases both morphological and biochemical signs of apoptosis and reduces cell proliferation, and provide evidence to support the hypothesis that neuronal pentraxins mediate activity-dependent regulation of cell differentiation and survival.
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Generación de ratones transgénicos con huntingtina mutada y distintos niveles de BDNF para el estudio de la fisiopatología de la enfermedad de Huntington

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La neurotrofina BDNF tiene un potente efecto protector sobre las neuronas del núcleo estriado. Por este motivo este factor neurotrófico ha sido propuesto como candidato para ser utilizado en posibles tratamientos neuroprotectores en la enfermedad de Huntington, en la que degeneran principalmente esta población neuronal. Además se ha descrito una correlación directa entre la huntingtina, gen causante de la enfermedad, y la trascripción de BDNF, que está disminuida cuando se produce la expansión de tripletes de CAG en la huntingtina. Para analizar la implicación de esta regulación sobre la fisiopatología de la enfermedad de Huntington hemos cruzado ratones transgénicos con 115 repeticiones de CAGs, R6/1, con ratones heterocigotos de BDNF, generando así líneas de ratones transgénicos para huntingtina mutado con distintos niveles de la neurotrofina. Estos modelos nos han permitido observar que la disminución de BDNF en! dógeno participa en la degeneración de las neuronas encefalinérgicas estriatales, adelanta la aparición de los síntomas y produce una mayor severidad en los trastornos motores.

Los niveles de BDNF endógeno también pueden ser regulados por el polimorfismo del dominio "pro" del BDNF (Val66Met). Utilizando un modelo celular de la enfermedad de Huntington hemos observado que la mutación de la huntingtina afecta mucho más el tráfico intracelular de la forma BDNF Val66Val que en el polimorfismo BDNF Val66Met. Este polimorfismo ha estado asociado a la susceptibilidad a distintas enfermedades neuropsiquiátricas. Nosotros hemos observado que los pacientes con enfermedad de Huntington con 42 a 49 repeticiones de CAG y el polimorfismo de BDNF Val66Met tienen un retraso en la aparición de los síntomas con respecto a los enfermos con BDNF Val66Val. Estos resultados podrían ser explicados por la diferente sensibilidad de la huntigtina mutada en el tráfico intracelular de las distintas formas de BDNF que observamos utilizando el modelo celular.

Todos estos resultados indican que el BDNF es un modulador de los procesos neurodegenerativos activados por la huntingtina mutada y la regulación de sus niveles puede controlar el inicio y severidad de los trastornos motores que se observan en la enfermedad de Huntington.

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1. Role of DYRK1A in neuromotor developmet of cns: implications in down's syndrome

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Motor deficits neuromotor development delay are among the most frequent impairments in Down syndrome (DS), but the neuropathological and molecular bases remain elusive. Here we investigate the motor profile of transgenic mice overexpressing Dyrk1A, TgDyrk1a, a candidate gene hypothesized to cause some of the neurological defects associated with DS. DYRK1A is expressed in the cerebellum and functionally related structures, most brainstem motor nuclei and spinal cord, supporting a role for Dyrk1A in the neural motor pathway involved in neuromotor development during the postnatal period and in controlling motor function in the adult. In previous studies we demonstrated a persistence of immature locomotor patterns, delayed walking activity and retarded general psychomotor development. Here we extended our previous neurodevelopmental screening in TgDyrk1A by using different neurological and behavioral tests. Our results support the notion of a specific motor alteration that impedes the correct performance of coordination/postural adjustment tests. Anxiety-like behavior is detected thus confirming results in adult mice. We also studied the involvement of Dyrk1A in the development of motor cholinergic nuclei in the spinal cord (specially in the ventral horn). Transitory Dyrk1A immunostaining was observed in neurons of reticular formation at PD7 whereas at later stages this pattern was shifted to motoneurons in the facial nucleus. The characterization of this Dyrk1A positive population in the reticular formation demonstrated that GABAergic neurons were mainly coexpressing Dyrk1A. At P14 and in the adult Dyrk1A was observed in cholinegic (ChAT positive) neurons of the facial nucleus. In the ventral horn of the spinal cord expression of Dyrk1A in motoneurons started earlier ion development (PD10) and persisted through adulthood. We propose, that Dyrk1A may play a role in the development of the motor system with functional consequences arising from its overexpression.
Supported by Jerôme Lejeune Foundation.

2. Characterization of the hypothalamic-pituitary-adrenal axis and the dopaminergic system in rats genetically selected in function of anxiety trait

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Stressor-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis is involved in a wide range of stress-related pathologies. In addition, dopaminergic activity is also related to certain aspects of the stress response, including the activation of the HPA axis. In the present work we studied the relationship of the HPA axis and the dopaminergic system to anxiety trait using rats genetically selected in function of its behavior in the elevated plus-maze (EPM): High-anxiety related behaviour/Low-anxiety related behaviour (HAB/LAB) rats. LAB rats spent a high percent of time exploring the open arms of the EPM, whereas HAB rats almost totally avoid such arms, but these two lines also differ in other behavioural traits such as active/passive coping style. It has been reported that HAB rats showed higher levels of prolactin, ACTH and corticosterone than LAB rats after exposure to moderated stressors and are characterized by enhanced vasopressin (AVP) gene expression in the paraventricular nucleus of the hypothalamus (PVN), that may contribute to enhanced anxiety and exacerbated HPA responsiveness to stressors. In the present experiment we show under resting conditions, using in situ hibridization, no differences between these lines in the expression of tyrosine hydroxylase (TH) in dopaminergic areas (substantia nigra, ventral tegmental area and A13). In contrast, HAB rats showed enhanced AVP gene expression in both magnocellular and parvocellular regions of the PVN together with enhanced CRF gene expression in parvocellular PVN and lower levels in central amigdala. This enhanced activity of the HPA axis was

accompanied by normal expression of glucocorticoids and mineralocorticoids receptors in key brain areas. These results strongly indicate a dysregulation of the HPA axis in high anxiety rats.

3. Towards the identification of the anx mutation and of eating behavior pathways in the anx/anx murine model for anorexia nervosa

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Anorexia Nervosa (AN) is a complex disorder where both socio-cultural and genetic factors are supposed to be involved. The mutant anx/anx is a spontaneous murine model for AN, as mutants are characterized by poor appetite, growth failure, and abnormal behavior, such as head weaving and hyperactivity. This model is due to a recessive autosomal mutation, not yet identified, but mapped through linkage analysis close to the Pallidin gene (Pa), located at 122.9 Mb on mouse chromosome 2. Several studies on differential gene expression and on neuropeptide distribution have been performed in order to disclose the etiology of this phenotype, but no additional data on the position of the anx gene has been published. The aim of this study was to identify the gene carrying the anx mutation by combining both linkage and expression microarray analysis. For the linkage analysis, we have genotyped microsatellite markers covering a region of 30 Mb around the Pa gene and over 400 meioses from several experimental crosses of carrier heterozygotes (B6C3Fe-anx A/+a) are being analyzed. Preliminary linkage data reveals that the anx mutation is located close to position 119 Mb (TX119) (lod score 4.5 at a recombination fraction of 0) on mouse chromosome 2. For the microarray study, RNA from cortex and hypothalamus of 3 wild type and three anx/anx mice has been hybridized to 44K oligo microarray Agilent Chips, containing over 40,000 mouse genes. Several pathways are up or down-regulated in this model that would explain some of the phenotypic alterations observed in the anx/anx mice, including the eating regulation. Mutation search using dHPLC should facilitate the identification of mutations in candidate genes contributing to the AN phenotype in the anx model and to define the anx mutation. The data produced should facilitate a better understanding of the mechanisms that underlay feeding and body weight regulation, and the genetic factors involved in the development of eating disorders.

4. Prenatal and postnatal characterization of cortical neurons of transgenic mice overexpressing DYRK1A

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Trisomy of chromosome 21 or Down syndrome (DS) is the most common cause of mental retardation. It has been hypothesized that mental retardation is primarily due to deficiencies in neuronal network connectivity in the major cognitive centres in the brain, which secondarily result in impaired information processing. Thus abnormal dendritic trees and reduced dendritic spine numbers in regions involved in cognitive functions, such as the cerebral cortex, could play a crucial role in DS cognitive dysfunction. These structural abnormalities may stem from specific neurodevelopmental or neuroplasticity alterations in the postnatal period. We have analyzed the role of a DS candidate gene, Dyrk1A (the human homologous to Drosophila minibrain) in corticogenesis and in the acquisition of pyramidal cell phenotype, exploring the specific contribution of genetic and microenvironmental factors to the phenotypes. Previous studies in our laboratory showed decreased dendritic branching and reduced spine density in mice overexpressing Dyr1A in a disomic genetic environment, TgDyrk1A mice, and in mice trisomic for a portion of the distal arm of MMU16 homologous to the DS critical region in HSA21, the Ts65Dn mouse, thus suggesting a role for this gene in pyramidal cell structural features. In the postnatal period TgDyrk1A showed altered pyramidal cell phenotype thus

suggesting alterations in the prenatal period. We have thus used primary cultures derived from the cerebral cortex of TgDyrk1A mice to explore the mechanistic aspects that explain the impact of Dyrk1A overexpression on neuronal development. We have determined the Dyrk1A overexpression effects on growth cone morphology, neurite outgrowth and branching. TgDyrk1A cortical neurons presented shorter branches but an increase in the density and number of filopodium. These results indicates an important role of Dyrk1A in the neuritogenesis.
Supported by Jerôme Lejeune Foundation.

5. Characterizing the phenotype of a transgenic mouse model overexpressing β4, α3 and α5 nicotinic acetylcholine receptor subunits.

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Nicotinic acetylcholine receptors (nAChRs), are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. The nAChRs are thought to be (hetero) pentamers composed of homologous subunits. Nicotinic receptors have been related to anxiety responses, mainly those containing beta-4 subunits. The beta-4 subunit is included in a gene cluster, next to the alpha-3 and alpha-5 subunits located on human chromosome 15 in the region 15q24. Previous studies in humans suggested that segmental duplications of this chromosomal region could be involved in panic disorder. To explore the effect of the overexpression of these subunits in anxiety we generated a transgenic mouse model. Two founder mice overexpressing CHRNB4, CHRNA3 and CHRNA5 were produced after microinjection into fertilized B6SJL mouse oocytes, using a construct obtained by digestion of the BAC RP11-335K5 (AC067863). Expression of the transgene was determine by RT-PCR analysis and the distribution pattern of the Chrnb4, Chrna3 and Chrna5 subunits was analyzed by inmunohistochemistry. Transgenic mice were viable and showed no alterations in fertility, perinatal mortality or somatometric parameters. The phenotypic characterization using a battery of tests including sensorimotor and neurological tests showed no differences in sensory or neurological parameters. Activity and anxiety-related behaviour tests, such as the Open Field, Plus-Maze, and Fear Conditioning are in progress.
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6. BACE2 (beta-secretase) overexpression accelerates memory impairment and noradrenergic system dysregulation in mice

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BACE2 (beta-site amyloid precursor protein cleavage enzyme 2) is a beta-secretase that cuts amyloid precursor protein (APP) at the N terminus of amyloid-β protein (Aβ) and is involved in Aβ deposition in brain, a critical feature of Alzheimer's disease (AD) and a frequent observation in Down syndrome (DS) patients after 30 years of age. Several authors have reported the accumulation of both soluble and intracellular Aβ before extracellular Aβ (senile plaques) in DS. Increases in Aβ levels in Down syndrome may reflect the increased expression and protein levels of BACE2 on chromosome 21. The impact of the accumulation of Aβ may have differential effects on development and aging in DS. We have generated transgenic mice overexpressing human BACE2 to study its role in alzheimer's neuropathology in Down syndrome patients. The most consistent neurochemical feature is the lack of sufficient cholinergic input, although there are deficits in other neurotransmitters, including catecholaminergic, serotonergic, and GABAergic activity. Surprisingly, adult TgBACE2 mice show anxiety-like behaviour and an increase in cellularity of NA neurons in LC, together with an increase in the catecholamine synthesising enzyme (tyrosine hydroxylase) levels in the medulla-pons. Recent studies have shown memory impairment in elderly stages that mimics human phenotype of AD pathology. These results demonstrate a disturbance in the noradrenergic and cholinergic system in a murine model of BACE2 overexpression.
Supported by Jerôme Lejeune Foundation.

7. Caracterización de la respuesta hormonal y conductual frente a las situaciones estresantes en un modelo KO para el receptor NK1 de la substancia P

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Las taquiquininas son una familia de neuropéptidos que incluye Sustancia P (SP), neuroquinina A (NKA) y neuroquinina B (NKB) y ejerce su efecto a través de un grupo de receptores (NK1, NK2 y NK3) acoplados a proteínas G. Los efectos biológicos más importantes de estos neuropéptidos están relacionados con el dolor y la respuesta inflamatoria, pero también se les ha relacionado con alteraciones psiquiátricas y con la adicción a drogas. En el presente trabajo se ha investigado el posible papel de SP y su receptor NK1 en la respuesta al estrés, utilizando animales knock-out (KO) para este receptor. En una primera serie de experimentos, no se observaron diferencias entre animales KO y silvestres en la actividad basal del eje hipotalámico-pituitario-adrenal o en su respuesta a estímulos estresantes diversos (ambiente nuevo, inyección de LPS, restraint crónico, social-defeat) Para estudiar si el impacto del estrés crónico podía diferir en ambas líneas, los animales fueron expuestos a estrés crónico por inmovilización (14 días, 2h/día) y se estudió la influencia del genotipo y el estrés crónico sobre la ansiedad (valorada en la prueba de transición luz-oscuridad) o el aprendizaje espacial en el laberinto acuático. El estrés crónico redujo el número de entradas al compartimento iluminado, sin diferencias entre los genotipos. En cambio, en el laberinto acuático no se observaron efectos del estrés pero sí del genotipo: el día del test, los ratones mutantes parecían recordar mejor el lugar donde había estado situada la plataforma durante la fase de adquisición del aprendizaje, a pesar de haber mostrado una menor velocidad de nado y un menor recorrido durante el proceso de aprendizaje. Los resultados no parecen demostrar una participación importante de la SP y el receptor NK1 en los efectos fisiológicos y conductuales del estrés.

8. Understanding Psychopathology of Panic Disorder: Consequences of NTRK3 Overexpression on Fear Circuit

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It has been suggested that neurotrophic factors may participate in the pathophysiology of anxiety disorders. The neurotrophin type 3-receptor (NTRK3) is expressed in the locus coeruleus (LC) and its ligand, NT-3, has a role as a survival factor for noradrenergic neurons. Inappropriate hyper-responsiveness of the NA system is observed in panic disorder (PD) patients and during panic attacks. The main symptom of persons with anxiety/panic disorders is their inability to correctly identify the fear-related information that may depend on altered responsiveness of fear circuits. In a first series of experiments we performed a detailed neuromorphometrical analysis of amygdala and hippocampus by means of stereological techniques. We showed a tendency to an increased cellular density in all hippocampal subregions in TgNTRK3 and a significant increase in the density and number of cells of the basolateral amygdala, and a tendency to an increase in its volume. To analyze the possible functional consequences of these structural modifications, we used neurobehavioral paradigm like Fear Conditioning (FC). We showed an increased sensitivity to context and non-context FC in TgNTRK3 24 hours after training and 1 week after test. We propose that the hypersensitive fear system in PD depends on hypertrophy of key regions of the fear circuit, as the basolateral amygdala that significantly disrupts the formation of emotional memories. This increase in emotional memory efficacy may depend on an increased release of norepinephrine from LC evoked by the foot-shock during the training phase of FC. These results provide evidence for the involvement of NTRK3 in anxiety leading to a neurotrophic hypothesis for the pathogenesis of PD. This work was supported by SAF2001-1231, SAF2004-02808 and DURSI (Generalitat de Catalunya).

9. Papel del TNFα en la muerte apoptótica provocada por la deprivación de oxígeno y glucosa (OGD)

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Para estudiar los mecanismos relacionados con el factor de necrosis tumoral alfa (TNF-α), implicados en la muerte apoptótica generada por isquemia, hemos utilizado el modelo de deprivación de oxígeno y glucosa (OGD) en cultivos mixtos de corteza cerebral en los que habíamos descrito que un 50% de la muerte es apoptótica (Malagelada et al., 2005). En el presente trabajo observamos que en cultivos sometidos a OGD presentan una activación de la caspasa 8 desde el primer momento de la reperfusión. Cuando se utiliza un inhibidor específico de la caspasa 8 se observa una disminución de células con la caspasa 3 activa y también se detecta una reducción significativa de la muerte neuronal inducida por la OGD. Para determinar si la activación de la caspasa 8 en la OGD venía mediada por el TNF-α a través de su receptor de tipo 1 (TNFR1) se utilizaron cultivos de corteza cerebral de ratones knock out para este receptor. En estos cultivos de knock out de TNFR1 no se observa la activación de la caspasa 8 tras la OGD, tampoco se observa un aumento de células con la caspasa 3 activa. Estos datos indican que la activación de la caspasa 8 en la OGD viene mediada por el TNF-α a través del TNFR1, y que esta activación es necesaria para una posterior proteólisis de la caspasa 3.

10. Modulación de la expresión génica por calcipresina 1 en cerebelo de ratón en desarrollo

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La calcipresina 1 está codificada por DSCR1, un gen del cromosoma 21 humano que se sobrexpresa en cerebro de pacientes con síndrome de Down (SD). La calcipresina 1 es un modulador de la actividad calcineurina, la serina-treonina fosfatasa más abundante del cerebro que participa en la regulación de la transcripción, el crecimiento axonal y la proliferación y diferenciación neuronal. Los niveles de expresión de Dscr1 mostrados por hibridación in situ son elevados durante el desarrollo del SNC del ratón, hecho que sugirió su participación en neurogénesis. Utilizando un anticuerpo específico frente a calcipresina 1 hemos observado abundante expresión de la proteína en distintas regiones del cerebro del ratón en desarrollo, incluida el cerebelo. En esta última región, se ha analizado el efecto de la pérdida de función de calcipresina 1 en el transcriptoma. Para ello se comparó, mediante microarrays, los perfiles de expresión génica de cerebelos de ratones Dscr1-/- con los de controles de camada Dscr1+/+. Se realizaron réplicas biológicas usando un chip de oligonucleótidos de 22K (Agilent). El análisis final comprendió la cuantificación de la señal (Gene Pix 6.0, Axon) y la normalización de la expresión génica utilizando el paquete analítico R-Limma (Lowess). Los resultados clasificados según un valor estadístico (B≥97%) mostraron un grupo de genes diferencialmente regulado en el ratón knockout, con un valor absoluto de fold-change superior a 1,2. Estos resultados sugieren que la sobrexpresión de Dscr1 podría contribuir a algunas de las alteraciones observadas en el desarrollo del cerebelo en modelos murinos de SD.

11. La inactivación del receptor Dopaminérgico D1 bloquea el aprendizaje espacial y la potenciación sináptica duradera (L-LTP)

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La dopamina es un neurotransmisor que se une a cinco tipos de receptores dopaminérgicos, divididos farmacológicamente en dos grandes familias, la familia D1 (D1 y D5) y la D2 (D2, D3 y D4). La dopamina juega un papel importante en procesos de aprendizaje y memoria, sin embargo, se desconoce el papel que juega cada uno de estos receptores en los distintos aspectos del aprendizaje y la memoria, así como los mecanismos moleculares que subyacen al proceso cognitivo. Para estudiar el papel del receptor dopaminérgico D1 en la memoria y en el aprendizaje espacial, utilizamos el laberinto acuático de Morris y la potenciación sináptica

duradera (LTP) sobre rodajas de hipocampo en animales genéticamente modificados para la inactivación del receptor D1 (D1-KO) en comparación con animales salvajes de la misma camada (WT).
Los animales D1-KO presentan déficit en el aprendizaje espacial en el laberinto acuático de Morris que no aparece en los ratones salvajes. En la región CA1 del hipocampo, los D1-KO no muestran alteraciones en la transmisión sináptica basal ni en el fenómeno de facilitación sináptica inducida mediante pares de pulsos homosinápticos. Sin embargo, la magnitud de las fases temprana y tardía de la LTP en los animales D1-KO es inferior a la obtenida en los individuos WT.
Nuestros resultados indican que el receptor D1 es crítico para el aprendizaje y la consolidación de la memoria espacial y para la plasticidad neural involucrada en el mantenimiento de la actividad sináptica a largo plazo.

12. PET technology for the study of CNS: first steps at IAT-PRBB

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Positron emission tomography (PET) offers different possibilities for studying the central nervous system (CNS). This technique requires the isotopic labelling of a molecule that will act as a tracer. After intravenous administration, the radiotracer will distribute selectively through the body according to the location of its biological targets. This selective accumulation makes it possible the acquisition of images, allowing visualizing the biological process of interest. 18-F, 15-O, 13-N and 11-C are the positron emitter radioisotopes most commonly used for PET labelling. Among them, 11-C has proven to be ideal since 12-C is present in almost every bio-molecule and both isotopes can be exchanged without modifying the biochemical properties of the molecule that will serve as radiotracer. However, the short half-life of these radioisotopes makes their use difficult as they need to be produced and linked to the tracer very quickly, and in close proximity to the place where the resulting radiotracer will be used. Institut d'Alta Technologia (IAT) counts with infrastructure for the production and use of a wide range of positron emitter radiotracers in both humans and small animals. In this work, we present real cases of different applications of the PET tracers suitable for the study of CNS in rodents already tested at IAT: 18F-Fluodeoxiglucose (FDG) as an indirect measure of cerebral activity, 13N-NH3 as a tracer for blood flow, and 11C-PK11195 as a tracer for the expression of peripheral benzodiazepine receptors.

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