Network of European Neuroscience Institutes

2nd International PhD student meeting

22nd of May, 2009

Fodele beach Hotel, Crete, Greece

Program Highlights

Short Student Presentations

Special Lectures

Organizers:

Markella Katidou, Nikos Kourtis, Matthias Rieckher, Kostoula Troulinaki, Manos Vlachos

Institute of Molecular Biology and Biotechnology, FORTH, Crete, Greece

Welcome to Crete and to the 2nd ENInet PhD Symposium!

After the successful first ENInet PhD Symposium last year in Berlin, we, the PhD students at ENI-Crete, took over the task of organizing the second Symposium in Crete.

The main purpose of the symposium is to give the PhD students of the ENInet the opportunity to present their work and interact with other students of the network. We hope that this interaction will lead to fruitful exchange of ideas and experience as well as to potential partnerships and long lasting friendships. In addition to the student presentations we are fortunate to include in our symposium special talks on neuroscience, post-doctoral funding and career prospects.

We would like to thank Prof. Erwin Neher, Prof. Claudina Pousada and Dr. Jan Taplick for accepting our invitation to give special talks during the Symposium. The organization of this Symposium would not have been possible without the generous support of the ENInet steering committee and the help of the ENInet secretary, Doris Harling. In addition, we would like to thank Prof. Nektarios Tavernarakis for the continuous encouragement and guidance during the organization of the Symposium and the IMBB secretary Georgia Houlaki for administrative support.

We kindly welcome you to the second ENInet PhD Symposium 2009 and we wish you a productive meeting.

With best regards,

The organizing committee:

Markella Katidou

Nikos Kourtis

Matthias Rieckher

Kostoula Troulinaki

Manos Vlachos

Institute of Molecular Biology and Biotechnology
Foundation for Research and Technology - Hellas
Heraklion, Crete, Greece

Contents

A. 2nd International PhD Students Meeting Program

Location: "Fodele Beach Hotel", Heraklion, Greece
Date: May 22nd 2009

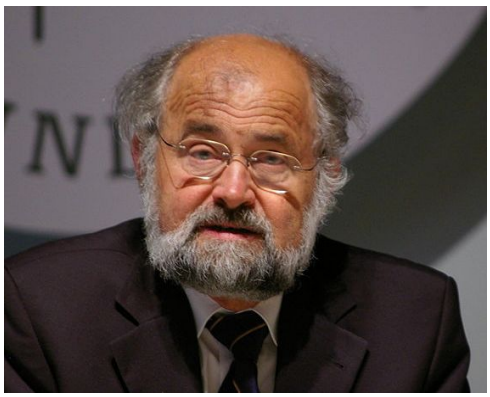
08:45	**Welcome Note**
09:00	**Student talks (session I): Development** **Chair: Markella Katidou**
09:00	Marzena Stefaniuk: Manipulation of *Ttyh1* Gene Expression Influences Cell Morphology in Neuroblastoma and Hippocampal Neurons in vitro
09:10	Mathieu Niquille: Transient Neuronal Populations are Necessary for the Formation of the Corpus Callosum
09:20	Heidi Soellner: Adaptive Plasticity of Neuronal Circuits During Postnatal Mouse Development
09:30	Yasuko Nakamura: PICK1: A Novel Regulator of Dendritic Spine Morphology
09:40	Augusto Escalante: The Transcription Factor Zic2 Determines Axonal Laterality at the Ventral Spinal Cord Midline
09:50	Niklas Senghaas: Boundary Formation and Neuronal Differentiation in the Zebrafish Hindbrain
10:00	Marie Kneib: IL1-receptor Accessory Protein-like 1 (IL1RAPL1) Controls Inhibitory Networks During Cerebellar Development
10:10	**Coffee Break**
10:40	**Round table: Prof. Dr. Erwin Neher**
11:40	**Student talks (session II): Electrophysiology** **Chair: Matthias Rieckher**

11:40	Manuel Molano: Intracellular Information and the Interplay between Activity Fluctuations and Stimulus Encoding in the Barrel Cortex
11:50	Hui Min Tan: M-current Modulation of Gamma Oscillations and Action Potential Phasing in the Hippocampus *in vitro*
12:00	Siobhan Dennis: The Role of SUMOylation in Kainate Receptor Trafficking during Oxygen Glucose Deprivation
12:10	Marta Díaz-Quesada: Diversity of Thalamocortical Short-term Plasticity affects Coding of Natural versus Regular Stimulus Sequences
12:20	Eva Benito: Differential Contribution of Synaptic Plasticity-related Transcription Factors to the Activity-driven Neuronal Transcriptome
12:30	Sabine K. Schmitz: Role of Cyclin-Dependent Kinase 5 Phosphorylation of Munc18 in Synaptic Transmission
12:40	**Lunch Break**
14:00	**Lecture 2: Prof. Dr. Claudina Pousada**
15:00	**Student talks (Session IIIA): Learning and memory** **Chair: Kostoula Troulinaki**
15:00	Cemil Kerimoglu: Formin 2 Regulates Extinction of Learned Fear
15:10	Leiron Ferarrese: Development of a Live Reporter System to Identify CREB-activated Neurons
15:20	Tanja Kuczera: The Anaphase-Promoting Complex in Synaptic Function, Learning and Memory
15:30	**Student talks (Session IIIB): Stem cells** **Chair: Kostoula Troulinaki**
15:30	Oliver Ehm: Examining the Role of the Notch-signalling Pathway in the Regulation of Sox2
15:40	Muhammad Amir Khan: FoxO Transcription Factors in Adult Neurogenesis
15:50	Aida Platero-Luengo: Understanding the Adult Carotid Body Stem Cell Niche
16:00	Isabel Reillo: Evidences for the Widespread Existence of OSVZ in the Developing Cerebral Cortex among Gyrated Mammals

16:10	**Coffee Break**
16:40	**Student talks (Session IV): Cell biology** **Chair: Nikos Kourtis**
16:40	María Hidalgo-Figueroa: GDNF-dependent Mechanisms Required for Catecholaminergic Neuron Survival
16:50	Emilia Horjales-Araujo: Brain Circuitry in the Regulation of Energy Metabolism: Focus on the Link between Starvation and Arousal
17:00	Tony Cijsouw: Role of Synaptotagmin 1 in Large Dense Core Vesicle Dynamics and Release
17:10	Trojanová Johanna: Subcellular Distribution of Presynaptic Chloride-permeable Glycine Receptors
17:20	Henrike Planert: Intrastriatal Synaptic Connectivity of Different Subtypes of the Medium Spiny Projection Neuron in Two Model Systems
17:30	**Lecture 3: Dr. Jan Taplick**
18:30	**Closing remarks**
20:00	**Dinner & Social time**

B. Curriculum vitae of invited speakers

Prof. Dr. Erwin Neher



Personal Data

Date and Place of Birth:	March 20, 1944, in Landsberg/Lech, Germany
Nationality:	German
Marrital Status:	married to Dr. Eva-Maria Neher, five children
Address:	Max-Planck-Institut für biophysikalische Chemie Am Fassberg, D-37077 Göttingen (Germany)

Education

1965	Vordiplom (Physics) Technische Universität, München
1967	M.Sc. (Physics) University of Wisconsin
1970	Ph.D. (Physics) Technische Universität, München

Positions held

1966 - 1967	Graduate student and research assistant in the laboratory of Dr. W.W. Beeman at the University of Wisconsin, Madison, Wisc.
1968 - 1972	Graduate student and post-doc in the laboratory of Dr. H.D. Lux at the Max-Planck-Institut für Psychiatrie, München, Germany
1972 - 1975	Research associate at the Max-Planck-Institut für biophysikalische Chemie, Dept. "Molekularer Systemaufbau", Göttingen, Germany

1975 - 1976	Research Associate as a guest in the laboratory of Dr. Ch.F. Stevens at Yale University, Dept. of Physiology, New Haven, Conn.
1976 - 1982	Research associate at the Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany
1989 - 1990	Fairchild Scholar, California Institute of Technology; Pasadena, USA
1983 -	Director of the Department Membranbiophysik at the Max-Planck- Institut für biophysikalische Chemie, Göttingen, Germany

Scientific awards, honors, memberships

Nobel Prize for Medicine or Physiology together with B. Sakmann (**1991**)
Member of the Order 'Pour le Merite' (**1995**)
Gottfried Wilhelm Leibniz Prize of the Deutsche Forschungsgemeinschaft DFG
Foreign Associate of the National Academy of Sciences USA (**since 1989**)
Fellow of the Göttingen Academy of Sciences (**since 1991**)
Foreign Associate of the Royal Society London (**since1994**)

Honorary degrees

1986	Honorary Professor, University of Göttingen
1988	Dr. h.c., Limburgs Universitair Centrum

Addendum, August 1999

Honorary Degrees

Dr. h.c.

1993	University of Alicante, Spain
1993	University of Wisconsin, Madison, Wisconsin, USA
1994	Technical University of Munich, FRG
1994	University of Madrid, Spain
1994	Huazhong University of Sciences & Technology, Wuhan, PR China
1995	University of BahÌa Blanca, Argentine

1996	University of Rome, Italy
1999	Hebrew University of Jerusalem, Israel

Awards (mostly together with Bert Sakmann)

1977	Nernst-Haber-Bodenstein, Award of the German Society for Physical Chemistry
1979	Feldberg Award, Feldberg Foundation, London
1982	K.C. Cole Award, Biophysical Society
1982	Harold Lamport Award, New York Academy of Sciences
1983	Spencer Award, Columbia University
1984	Adolf Fick-Preis, Universität Würzburg
1986	Louisa Gross-Horwitz Award, Columbia University
1986	Fidia Research Award Lecture, Fidia Research Foundation
1986	Schunck-Preis, Universität Giessen
1986	Leibniz Award, Deutsche Forschungsgemeinschaft
1989	Gairdner Award,Toronto
1990	Hans Hellmut Vits-Preis, Universität Münster
1990	Bristol-Myers Squibb Research Award, New York
1991	Gerard Prize, American Neuroscience Association

From Les Prix Nobel. The Nobel Prizes 1991, Editor Tore Frängsmyr, [Nobel Foundation],

Editorial activities

1980 - 1987	Journal of Physiology, London
1991 -	Cellular Physiology & Biochemistry
1992 -	Proceedings of the National Academy, Washington D.C.
1993 -	European Journal of Physiology (Pflüger's Archiv)
1996 -	Journal of General Physiology

Information about Prof. Dr. Erwin Neher's career was retrieved from the following websites:
http://nobelprize.org/nobel_prizes/medicine/laureates/1991/neher-autobio.html
http://www.mpibpc.mpg.de/abteilungen/140/erwin_neher/
http://www.mpibpc.mpg.de/cgi-bin/person.cgi?nav=preise&persId=163202&lang=de&inst=biophysikalische_chemie&from=institut

Dr. Jan Taplick

Year of birth:	1969
Nationality:	German
Place of birth:	Leipzig, Germany

Education

1990-1995	Biology studies, University of Leipzig, Germany
1995-1999	Institute of Molecular Biology, Vienna Biocenter, Austria PhD in Biological Sciences: Thesis on "Histone acetylation/deacetylation during growth arrest and proliferation of mammalian cells"

Postdoctoral Training and Employment History

2000-2002	Post-doc, Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel, under the supervision of Prof. Moshe Oren, Research Project: " Involvement of protein acetylation and deacetylation in the regulation of the p53/Mdm2 pathway"
Since 03-2002	Fellowship Programme Manager, European Molecular Biology Organization (EMBO), Heidelberg, Germany
04/2002-12/2002	Assistant Editor, EMBO Reports
Since 01/2008	Deputy Director of EMBO

Fellowship awards

Fellow of the Vienna Biocenter International PhD Programme
Minerva Foundation Postdoctoral Fellowship
EMBO Long-term Fellowship

Prof. Claudina Pousada

Claudina Rodrigues-Pousada is a molecular biologist, and Head of the Laboratory of Genomics and Stress and Full Professor at the Instituto de Tecnologia Química e Biológica (ITQB) in Oeiras, Portugal. She obtained her Ph.D. in Biochemistry at the Institut de Biologie Physico-Chimique, Paris, France, under the supervision of Prof. Donal Hayes. Claudina has been working in the field of signal transduction and gene regulation under stress conditions for more than 25 years.

When she returned to Portugal in 1979, she launched her own laboratory at the Gulbenkian Institute of Science, pioneering the introduction of molecular biology approaches in Portugal. In 2000, she moved to ITQB where she continued to develop her work on stress response.

Claudina Rodrigues-Pousada's work has been published in over 100 papers in leading journals and she has written numerous books chapters. She has been an invited speaker at international scientific meetings and has organized international congresses, in particular the Yeast Conference on Genetics and Molecular Biology in 1995, the 27th FEBS Congress in 2001 and the II International Congress on Stress Response in Biology and Medicine. Apart from these events, she has also organized several workshops, courses and seminars. She has played an important role in introducing molecular biology into the curricula of Portuguese universities.

During her career she has supervised close to 30 PhD and several masters' students. In 1994, Claudina was elected a Member of the European Molecular Biology Organization (EMBO). From 1998 to 2004 she was President of the Portuguese Biochemical Society and served as the Chair of the Executive Committee of the Federation of the European Biochemical Societies (FEBS). In 2005, she was elected chair of the FEBS Working Group on Young Scientists' Careers and she serves as President of the Scientific Committee of the Start-up Company "STAB-GENOMICA". She has served on various national and international scientific committees and regularly serves as a referee for several international journals and funding agencies.

Information about Prof. Claudina Pousada's career was retrieved from the following website:
http://www.set-routes.org/university/profiles/pousada.html

C. Abstracts of oral presentations

Session I: Development

Manipulation of *Ttyh1* Gene Expression Influences Cell Morphology in Neuroblastoma and Hippocampal Neurons In Vitro

M. Stefaniuk and K. Lukasiuk

The Nencki Institute of Experimental Biology, Pasteur 3, Warsaw, Poland

Ttyh1 gene is a member of *Tweety* family of putative large conductance maxi-Cl^- channels. According to our previous data, expression of *Ttyh1* mRNA in the brain is localized in neurons and is increased following *status epilepticus* in the animal model of epileptogenesis. The function of *Ttyh1* has not been elucidated.

Here we aimed at characterization of *Ttyh1* by overexpressing or silencing it in neuroblastoma cell line or in hippocampal neurons *in vitro*.

In hippocampal neurons transfected with plasmid coding Ttyh1-EGFP fused protein, Ttyh1-EGFP was present in dots along the neurites and at the ends of new formed projections. Similar Ttyh1-EGFP localization was observed in neuroblastoma cells. Overexpression of *Ttyh1* in hippocampal neurons *in vitro* induced formation of new, often branched projections as soon as after 24h. Even more intense branching was observed when cells were transfected with *Ttyh1* 7 or 14DIV. *Ttyh1* silencing in hippocampal neurons *in vitro* using siRNA increased the number and length of primary dendrites. In addition, it affected cell morphology causing abnormal pattern of MAP2 distribution.

Since manipulation of Ttyh1 expression influences cell morphology and distribution of cytoskeleton elements, we propose that *Ttyh1* is involved in cytoskeleton functions. It is tempting to suggest, that by influencing the neurite growth and ramification, *Ttyh1* participates in aberrant network formation and by this - the development of epilepsy.

Transient Neuronal Populations are Necessary for the Formation of the Corpus Callosum

Mathieu Niquille [1*], Sonia Garel [2*], Fanny Mann [3*], Jean-Pierre Hornung [1], Belkacem Otsmane [3], Sebastien Chevalet [1], Carlos Parras [4+], Francois Guillemot [4], Patricia Gaspar [5], Yuchio Yanagawa [6], and Cécile Lebrand [1]

[1] *Department of Cellular Biology and Morphology (DBCM), University of Lausanne, Switzerland*

[2] *Inserm, U784, Ecole Normale Supérieure (ENS), Paris,75005, France.*

[3] *CNRS, UMR 6216, Developmental Biology Institute of Marseille Luminy, Université de la Méditerranée, Marseille, 13009, France.*

[4] *Division of Molecular Neurobiology, National Institute for Medical Research (MRC), Mill Hill, London NW7 1AA, UK.*

[5] *Inserm, U839, Institut du Fer à Moulin, Paris 75005, France*

[6] *Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine and SORST, JST, Maebashi 371-8511, Japan.*

[*] *These authors contributed equally to this work.*

[+] *Present address: Laboratoire de Biologie des Interactions Neurones/Glie INSERM U-711, Hôpital de la Salpetriere, 75651 Paris Cedex 13, France.*

The corpus callosum (CC) is essential for numerous higher cortical functions. While its development has been shown to rely on midline glial cells, CC agenesis is associated with numerous human pathologies, suggesting that a range of developmental defects can result in abnormalities in this structure. Here, we show that two transient populations of midline neurons contribute to the formation of the CC. We report that these two populations of neurons enter the CC midline prior to the arrival of callosal pioneer axons. Using a combination of mutant analysis and in vitro assays, we demonstrate that CC neurons are necessary for normal callosal axon navigation. They exert an attractive activity on callosal axons, in part via Sema3C and its receptor Neuropilin-1. Our study reveals a novel and essential role for these neuronal populations in the pathfinding of a major cerebral commissure and raises new perspectives on pathophysiological mechanisms altering CC formation.

Adaptive Plasticity of Neuronal Circuits During Postnatal Mouse Development

Heidi Soellner [1], José Martínez-Hernández [1], Ben Novitch [2], Andrea B. Huber [1]

[1] *Helmholtz Center Munich, German Research Center for Environmental Health, 85764 Neuherberg, Germany.*
[2] *Department of Neurobiology, David Geffen School of Medicine, UCLA*

Correct wiring of the central nervous system (CNS) during development enables the organism to respond to and interact with its environment. Attractive and repellent guidance cues, e.g. semaphorins, direct outgrowing neurons via the corresponding receptors, e.g. neuropilins, on the axonal surface. Previous studies revealed that absence of neuropilin-semaphorin signaling leads to characteristic miswiring of the spinal motor-sensory circuit. The defects comprise premature ingrowth of sensory and motor axons into the limbs, defasciculation, and altered dorsal-ventral choice of motor neurons at the base of the limb. Surprisingly, some of these mutant mice are viable and thus amenable for postnatal analysis.

We hypothesize that the aforementioned embryonic axon patterning defects are corrected or compensated for during postnatal development.

In order to address this issue, we subject mouse mutants with abolished semaphorin-neuropilin signaling to a three-step-based approach: repetitive behavioural phenotyping at three postnatal timepoints, detailed neuroanatomical analysis, and electrophysiological characterization of relevant neural circuits. Mutant animals showed significant impairments particularly in behavior tests aimed at monitoring motor coordination. Interestingly, mutants segregated into two clearly distinct groups of “wildtype-like performers” and “mutant performers”, and the latter group improved in the execution of the behavioral tests over time. We are currently analyzing the anatomical and functional substrates for this observed improvement in behavior.

Our data suggests that lack of neuropilin-semaphorin signaling during the critical phase of neuronal wiring leads to specific deficits in motor coordination.

PICK1: A Novel Regulator of Dendritic Spine Morphology

Yasuko Nakamura, Jeremy Henley and Jonathan Hanley

Dept.Anatomy, MRC Centre for Synaptic Plasticity, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD

Most excitatory post-synaptic connections in the mammalian brain use dendritic spines. F-actin is found in abundance in spines, and changes in morphology of dendritic spines by the modulation of the actin cytoskeleton are important in synaptic plasticity. PICK1 (protein interacting with C kinase 1) has recently been shown to bind F-actin and the actin-related protein, the Arp2/3 complex, causing an inhibition of Arp2/3-mediated actin polymerization during N-methyl D-aspartate (NMDA)-dependent AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) endocytosis. Here, we have examined the role of PICK1 on dendritic spine morphology through its action in regulating actin polymerization. We have shown that the overexpression of PICK1 decreases spine area and the binding of PICK1 to Arp2/3 which inhibits actin polymerization is required for the decrease in spine size observed.

The Transcription Factor Zic2 Determines Axonal Laterality at the Ventral Spinal Cord Midline

A. Escalante and E. Herrera

Instituto de Neurociencias de Alicante (Consejo Superior de Investigaciones Científicas-Universidad Miguel Hernández, CSIC-UMH). Campus de San Juan, Avda. Ramon y Cajal s/n, Alicante 03550 (Spain)

In bilaterally-symmetric organisms some neural axons cross the midline to project to the other side of the nervous system and some other axons run along their own hemispheric side to project ipsilaterally. This organization allows the brain to integrate sensory information coming from both sides of the body and then elaborate a coordinated response. Thus, during the development of the nervous system there are different classes of axons that at some point of their trajectories have to decide whether or not to cross the midline. In the last decade, the effort of many research groups allowed the identification of a large number of axon guidance molecules that play critical roles in regulating the guidance of both crossing and non-crossing axons at the midline. The LIM homeodomain transcription factors Lhx2,9 have been shown to control the expression of axon guidance molecules required for the crossing of commissural axons at the vertebrate spinal cord. However, transcriptional mechanisms controlling axonal ipsilaterality at the vertebrate ventral spinal cord midline have not being described. Here we show that the transcription factor Zic2, like other members of the Zic family, is expressed in dorsal progenitor during and after neurulation. Later, its expression is restricted to specific subsets of postmitotic neurons located along different dorsal interneuron domains. Conversely to other members of the Zic family, which inhibited cell differentiation when were ectopically expressed at early stages of the spinal cord development, the ectopic expression of Zic2 switches the contralateral trajectory of a group of commisural neurons to an ipsilateral path. These results strongly suggest that the same genetic program that controls ipsilaterality at other midline structures such as the optic chiasm, might also determine axonal laterality in the ventral spinal cord of vertebrates.

Boundary Formation and Neuronal Differentiation in the Zebrafish Hindbrain

Niklas Senghaas, Barbara Solchenberger and Reinhard W. Köster

Institute of Developmental Genetics, Helmholtz Zentrum München, Germany

During development many physical boundaries form within the embryo creating functional and anatomical compartments giving shape to the organism. Some of these boundaries also act as localised signalling centres by expressing specific molecules to distinguish between boundary and non-boundary tissue.

In the vertebrate hindbrain distinct groups of cells form boundaries at the interface of adjacent rhombomeres. These cells mediate neurogenesis of the non-boundary cell population by Wnt and Delta Notch signalling.

We have generated a transgenic zebrafish line expressing a fluorescent reporter under the control of the *lnp* enhancer fragment. This strain specifically marks post mitotic neurons at the interface of the rhombomers. Using this transgenic strain we are addressing where rhombomere boundary cells arise, how they behave during development and which cell type they later represent in the differentiated brain.

In addition, we have isolated the cDNA of *lnp* gene from zebrafish. Sequence analysis revealed that the gene encodes a highly conserved protein with two adjacent transmembrane domains at the N-terminus and a C-terminus with unknown protein structure and function.

In situ hybridization experiments showed that the expression of the gene is highly restricted spatially as well as temporally. The mRNA could only be detected in early stages reaching from 1 cell stage up to long pec. It is expressed in the developing anterior cerebellum close to the midbrain hindbrain boundary (MHB) as well as in iterative stripes running along the rhombomere boundaries. In addition *lnp* expression can be found in the myotome.

Coexpression of fluorescent Lnp fusion proteins in cultured cells together with subcellular markers showed that the Lnp protein is located within structures involved in exo-/endocytosis. In a Tandem Affinity Purification assay followed by mass spectrometry among others we could identify several presynaptic vesicle proteins as possible interaction partners of Lnp.

Based on the data from the expression analysis, localization and the identified candidate interaction partners we suggest that Lnp plays a role in synaptogenesis and vesicle trafficking in early post mitotic neurons in the hindbrain of zebrafish embryos.

Preliminary data from knock down experiments show a reduction of specific axons in the regions of the hindbrain where Lnp is expressed.

Thus Lnp may be a crucial protein for establishing neuronal circuits in the vertebrate hindbrain.

IL1-receptor Accessory Protein-like 1 (IL1RAPL1) Controls Inhibitory Networks During Cerebellar Development

Frédéric Gambino, Marie Kneib, Bernard Poulain, and Yann Humeau

Institut des Neurosciences Cellulaires et Intégratives, UPR3212, Centre National de la Recherche Scientifique, 5 Rue Blaise Pascal, 67084 Strasbourg, France

Abnormalities in the formation and function of cerebellar circuitry potentially contribute to cognitive deficits in humans. In the adult, the activity of the sole output neurons of the cerebellar cortex – the Purkinje cells (PCs) – is shaped by the balance of activity between local excitatory and inhibitory circuits. However, how this balance is established during development remains poorly understood. Here, we investigate the role of IL1RAPL1, a protein linked to mental retardation and autism in the development of mouse cerebellum. Using Il1rapl1-deficient mice, we found that absence of IL1RAPL1 causes a transient disinhibition of deep cerebellar nuclei neurons between post-natal days 10 and 14, although cortico-nuclear synaptic connections are preserved. Upstream, in the cerebellar cortex, we found developmental perturbations in the activity level of molecular layer interneurons, resulting in the premature appearance of giant GABAA-mediated inhibitory post-synaptic currents capable of silencing PCs. We conclude that IL1RAPL1 exerts a key function during cerebellar development by controlling the time course of establishing local excitation/inhibition balance.

Session II: Electrophysiology

Intracellular Information and the Interplay Between Activity Fluctuations and Stimulus Encoding in the Barrel Cortex

Andrea Alenda [1], Manuel Molano [1], Stefano Panzeri [2], Miguel Maravall [1]

[1] *Instituto de Neurociencias de Alicante, UMH-CSIC, 03550 Sant Joan d'Alicante, Spain.*

[2] *Italian Institute of Technology, Robotics, Brain and Cognitive Sciences Department, Via Morego, 30, 16163 Genova, Italy*

Cortical sensory responses underlie stable perception, yet are profoundly variable depending on internal and external context. In the barrel cortex, sensory responses are strongly affected by spontaneous activity fluctuations, a phenomenon that has been addressed mostly by analyzing synaptic responses to discrete stimuli. Responses to complex, extended stimuli have been analyzed only in terms of spiking. Extracting synaptic responses evoked by complex, extended stimuli is a demanding task.

Here we applied a novel information theoretical method to whole-cell recordings in urethane-anesthetized rats, acquired while stimulating whiskers with a complex, prolonged, broad-spectrum waveform. We quantified the stimulus information (reduction in uncertainty) conveyed both by a neuron's membrane potential (i.e., by its integrated synaptic input) and by its spike train output, and thus evaluated contextual effects on single-neuron sensory processing in vivo. We found:

- Significant information is carried in the phase of slow (< 20 Hz) V_m oscillations. Neurons thus have access to the phase information contained in collective activity, previously observed in LFP studies.
- Significant information is also carried in fine-scale, higher-frequency (> 20 Hz) V_m fluctuations (synaptic activity). The information in the two signals is complementary, such that oscillations and fine-scale synaptic activity act as independent information channels.
- Spike timing adds significantly to the information carried in spike counts; so does labeling spikes with the V_m phase at the time of spiking.
- During continuous tactile stimulation, down states are effectively quiescent for information processing, except when a stimulus event facilitates a transition from the down to the up state.

Our approach permits direct evaluation of how response variability affects sensory processing. It bypasses the complications inherent to extracting and comparing synaptic responses, by focusing directly on the functional outcome of synaptic activation. Moreover, the method does not involve any assumption as to the stimulus features that elicit a neuron's response. We expect the approach to be applicable to other experimental designs.

M-current Modulation of Gamma Oscillations and Action Potential Phasing in the Hippocampus *in vitro*

Hui Min Tan and André Fisahn

Department of Neuroscience, Karolinska Institutet

Gamma oscillations are an emergent property of networks of interconnected cortical neurons, and can be induced pharmacologically in the hippocampus *in vitro*. The importance of synaptic connectivity for gamma oscillations has been well studied, but not much is known about the involvement of voltage-gated currents in the generation and modulation of gamma oscillations. In this study we investigate the role of the M-current (I_M) in kainate-induced gamma oscillations using the M-channel blockers linopirdine and XE-991. Application of 10uM linopirdine did not affect the power or peak frequency of gamma oscillations. On the other hand, application of 20uM XE-991 significantly reduced the power of gamma oscillations with no change in peak frequency. Using concomitant recordings of network oscillations and cell-attached or whole-cell recordings, we could show that I_M block by XE-991 resulted in a loss of periodicity (desynchronization) of action potentials relative to the gamma oscillation cycle.

The role of SUMOylation in Kainate Receptor Trafficking during Oxygen Glucose Deprivation

Siobhan Dennis and Dr Jack Mellor

Department of Anatomy, University of Bristol, School of Medical Sciences, Bristol, BS8 1TD

Ischaemic brain damage is largely thought to be caused by excitotoxicity due to over-activation of glutamate receptors. The roles of Ca^{2+} permeable NMDA and AMPA receptors in mediating this excitotoxic damage are well documented but the role of kainate receptors (KARs) is less well known. However, a number of studies have now begun to reveal a role for KARs in mediating ischaemic damage (Lee *et al.,* 2007). Small Ubiquitin like Modifier (SUMO) is greatly upregulated during ischaemia and it has been suggested that this may function as a neuroprotective mechanism (Pei *et al*., 2005; Cimarosti *et al.,* 2008). We discovered that the KAR subunit GluR6a is SUMOylated by SUMO-1 and that this conjugation controls KAR trafficking causing removal of KARs from the post-synaptic membrane (Martin *et al.*, 2007). We hypothesised that neurons may protect themselves from ischaemic damage by increasing SUMOylation of KARs causing their removal from the cell surface and thereby reducing glutamate-mediated excitotoxicity.

To test this hypothesis we exposed hippocampal slices taken from P13-15 rats to 15 minutes of oxygen glucose deprivation (OGD, O_2 and glucose replaced with N_2 and sucrose respectively), an *in vitro* model of ischaemia, and measured synaptic KAR-EPSCs using whole-cell electrophysiological recordings. We also manipulated the degree of SUMOylation within the cell by including SUMO-1, SUMO-2 or the deSUMOylating enzyme SENP-1 in the patch pipette. We initially replicated previous results demonstrating that inclusion of SUMO-1 (4.2 □M) reduced KAR-EPSCs and inclusion of SENP-1 (100 nM) increased KAR-EPSCs (Martin *et al.*, 2007). However, inclusion of SUMO-2 had no effect on the amplitude of KAR-EPSCs. This suggests that SUMO-1 and SUMO-2 play different roles in the regulation of KAR trafficking.

OGD resulted in an average 60.5 ± 10% (n = 8) decrease in the amplitude of KAR-EPSCs (measured 20-25 minutes after OGD). The reduction in KAR-EPSC amplitude suggests internalization of KARs from the membrane since it was not due to changes in pre-synaptic fibre volley. SUMO-1, SUMO-2 and SENP-1 and corresponding inactive control proteins were infused and KAR-EPSC amplitude stabilized before OGD application. OGD caused a 69.4% ± 10% (n = 12) decrease in KAR-EPSC after SUMO-1 infusion that was significantly greater than the decrease induced in interleaved controls using infusion of the inactive version of SUMO-1, SUMO-1□GG (47.05 ± 10%, n = 10, p = 0.047, unpaired t-test). In contrast, OGD after infusion of SUMO-2 caused significantly less reduction in the KAR-EPSC compared to SUMO-2□GG (40.9% ± 10% and 63.4% ± 10% respectively, n = 7, p = 0.048, unpaired t-test). Infusion of

SENP-1 had no effect on the recovery of the KAR-EPSC after OGD compared to the inactive version of SENP-1, SENP-1-C603S ($p = 0.049$, unpaired t-test).

The results presented suggest that SUMOylation can alter the trafficking of KARs after OGD. However, SUMOylation does not appear to play a major role in the loss of KARs during OGD; as the KAR-EPSC never fully recovered after OGD.

All errors given are standard errors of the mean.

Cimarosti H *et al*. (2008), Neuropharmacology. 54;2:280-289.

Lee Y J *et al*. (2007), J Cereb Blood Flow Metab. 2(5):950-62.

Martin S *et al.* (2007), Nature. 17;447(7142):321-5.

Pei D S *et al*. (2005), J Neurosci Res. 1;82(5):642-9.

Diversity of Thalamocortical Short-term Plasticity Affects Coding of Natural versus Regular Stimulus Sequences

Marta Díaz-Quesada, Miguel Maravall

Instituto de Neurociencias de Alicante, UMH-CSIC, 03550 Sant Joan d'Alicante, Spain

Responses to tactile stimuli appear to depend on the regular or noisy nature of the stimulus: humans can detect the presence of noise in a test stimulus, and regular spiking neurons in the barrel cortex respond more strongly to temporally irregular trains of whisker stimuli than to regular trains with the same average frequency (Lak et al., Cereb Cortex 2008). In this project we aimed to determine whether the short-term dynamics of thalamocortical synapses could be related to this dependence.

We tested short-term depression at thalamocortical synapses using whole cell patch clamp recordings *in vitro* in mouse thalamocortical slices (P15-P19). We stimulated extracellularly using both regular stimulus trains and natural trains derived from spike sequences previously recorded in the VPm thalamic nucleus *in vivo* (Petersen et al., Neuron 2008); the two kinds of stimuli were matched for mean frequency. We repeated experiments varying (1) mean stimulus frequency (5 Hz, 10 Hz), (2) temperature (room and physiological), and (3) extracellular calcium concentration (1-2 mM).

We found that at steady state, there was no clear difference between the mean responses evoked by regular and natural stimulus trains at matched mean frequency. However, upon switching from regular to natural stimuli or vice versa, different synapses behaved remarkably differently. While some synapses had responses that showed significant facilitation upon receiving a stimulus delivered at a novel (higher) instantaneous interval, others depressed or showed no change; still others showed evidence of responding at a preferred interval whether stimulated at natural or regular frequency. These differences can be traced to diversity in the parameters describing the synapses' short-term plasticity.

Much previous work has shown target-dependent plasticity even in single neurons, such that projections to different cell populations display different short-term dynamics. Our results show that the level of diversity in short-term dynamics, even within a comparatively homogeneous population dominated by depression, underlies a range of qualitatively different responses to transitions between regular and noisy stimuli.

Differential Contribution of Synaptic Plasticity-related Transcription Factors to the Activity-driven Neuronal Transcriptome

Eva Benito, Luis M. Valor and Angel Barco*

Instituto de Neurociencias de Alicante, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, Alicante 03550, Spain.

** Corresponding author **abarco@umh.es***

Information processing and memory storage are complex phenomena that require a tightly orchestrated chain of events from the molecular to the systems level. At the molecular level, protein synthesis and de novo gene expression are thought to be necessary for the establishment of long-lasting memories and hence the study of activity-dependent gene expression has been a topic of intense research, leading to the discovery of master genes implicated in learning and memory, such as CREB or SRF. The emergence of high-throughput techniques has allowed a more global approach to the study of the transcriptional programs that participate in the process of information storage. We have used lentiviral vectors to drive the expression of constitutively active versions of the transcription factors CREB, SRF, EGR1 and c-FOS in cultured hippocampal neurons and we have profiled their transcriptomes. Our results show scarce overlapping between the transcription programs controlled by these proteins, suggesting a unique role for each in the event of information processing. We are examining the effect of constitutive activation of the different pathways on electrophysiological properties of cultured neurons, and we are undertaking a bioinformatical approach to gain insight into the regulatory mechanisms for the different transcription factors.

Role of Cyclin-Dependent Kinase 5 Phosphorylation of Munc18 in Synaptic Transmission

Sabine K. Schmitz, Cillian King, Matthijs Verhage and Ruud Toonen

Dept. of Functional Genomics, Center for Neurogenomics and Cognitive Research, Amsterdam, The Netherlands

Cyclin-dependent kinase 5 (Cdk5) and its activators p35 and p39 are mainly found in the nervous system and are thought to be involved in many neuronal processes such as neuronal development, synaptic transmission and neurodegeneration. Here we focus on the role of Cdk5 on synaptic transmission through phosphorylation of the presynaptic protein Munc18-1 using a panel of Munc18-1 mutations: non-phosphorylatable Munc18(T574A) and phospho-mimicking Munc18(T574D). To analyze synaptic transmission and plasticity on a cellular level we use autaptic cultures from munc18-1 null mutant mice that are rescued with either wild-type (WT) Munc18, Munc18(T574A) or Munc18(T574D). First experiments show 4 possible roles for Cdk5 phosphorylation of Munc18 on synaptic transmission: modulation of release probability, readily releasable pool size, neuronal morphology and postsynaptic receptor density.

Session III A: Learning and memory

Formin 2 Regulates Extinction of Learned Fear

Sananbenesi, F., Pinhero, P., Kerimoglu, C., Dettenhoffer, M., Agis-Balbo, R.C., Burkhardt, S., Mulle, C. and Fischer, A.

Laboratory for Aging and Cognitive Diseases, ENI-G, 37077 Goettingen, Germany

The pathogenesis of emotional disorders often involves associative learning that links anxiogenic stimuli to certain life experiences. Among these are phobias and post-traumatic stress disorders which severely affect the life of patients and are an increasing burden to our societies. Treatment of such disorders generally involves the promotion of extinction processes, which are defined as the reduction of an aversively motivated behavior. Therefore understanding the molecular mechanisms underlying extinction may help to develop therapeutic strategies for emotional disorders.

The molecular mechanisms underlying extinction have only begun to be elucidated. Our previous work demonstrated that actin dynamics are essential for fear extinction. Here we provide evidence to Formin2, an actin nucleator, affects hippocampal signaling at the mossy fibre CA3 synapse and is essential for the reduction of learned fear. Formin 2 is highly enriched in the hippocampal mossy fibres and mice deficient for Formin 2 are severely impaired in fear extinction and reversal spatial learning. The molecular mechanisms underlying extinction are currently under investigation.

Development of a Live Reporter System to Identify CREB-activated Neurons

Leiron Ferarrese, Elisiana Tafi, Helene Marie

Laboratory of Molecular Mechanisms of Synaptic Plasticity, ENINET-ROME - EBRI, Via del Fosso di Fiorano 5, 00143, Rome, Italy

Memory is one of the most important and complex brain functions. At a cellular level, it is known to depend on the plasticity in the neurons, especially at the level of the synapses.

One molecular mechanism that has received particular attention is the transcription pathway that is regulated by the transcription factor CREB. It is known that this pathway is activated during synaptic plasticity and during a learning event. Major efforts are underway to characterize the neuronal adaptations resulting from such activation.

Today, there is still no adequate reporter system to recognize live neurons that display recent CREB activity. Such a reporter system would be very useful to link recent neuronal activity-dependent gene expression to structural and functional modifications in adapting synapses.

For this purpose, we are creating an *in vivo* lentiviral mediated reporter system that will allow for live identification of neurons with recent CREB activity. In this system, expression of red fluorescent protein is under the control of CREB-dependent expression. We have in vitro data suggesting that such system is a valid tool in this context.

The final aim of this project is to target this reporter to different brain areas in rodents and identify CREB-activated neurons participating to memory dependent behavioral tasks for further live electrophysiological analysis.

The Anaphase-Promoting Complex in Synaptic Function, Learning and Memory

Tanja Kuczera, Farahnaz Sananbenesi, Roman Stilling and Andre Fischer

European Neuroscience Institute Göttingen, Grisebachstr. 5, 37077 Göttingen, Germany

The Anaphase-Promoting Complex (APC) is a multisubunit ubiquitin ligase that targets proteins for degradation by the 26S proteasome. The Anapc2 subunit is essential for APC activity. So far the function of APC has been mainly linked to cell cycle progression but most recent findings also indicate a role of APC in synaptic function.

The aim of the project is to characterize the function of the APC during learning and memory. To this end we generated mice in which *Anapc2* is specifically deleted in principal neurons of the forebrain. Locomotor function and basal anxiety was normal in *Anapc2* deficient mice. However we detected a number of interesting phenotypes in male and female mice lacking *Anapc2*. For example working and long term memory consolidation is enhanced. The underlying molecular mechanisms are currently under investigation. Together with the fact that certain subunits of the APC are deregulated in post-mortem tissue of Alzheimer's disease patients our preliminary data suggest that APC might be involved in the pathogenesis of Alzheimer's disease.

Session III B: Stem cells

Examining the Role of the Notch-signalling Pathway in the Regulation of Sox2

O. Ehm [1], C. Göritz [2], L. Espinosa [3], B. Kempkes [4], E. Kremmer [5], A. Bigas [3], J. Frisen [2], D. C. Lie [1]

[1] *Helmholtz Center Munich, Munich, Germany, Institute for Developmental Genetics Research Group Adult Neurogenesis and Neural Stem Cells*

[2] *Department of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institute, SE-171 77 Stockholm, Sweden*

[3] *Institut d'Investigacio´ Biome` dica de Bellvitge Gran Via km 2.7, Hospitalet and Institut Municipal d'Investigacions Me` diques-Hospital del Mar, Dr. Aiguader, 88, 08003 Barcelona, Spain*

[4] *Helmholtz Center Munich, Munich, Germany, Institute of Clinical Molecular Biology*

[5] *Helmholtz Center Munich, Munich, Germany, Institute of Molecular Immunology*

During adulthood neural stem cells continuously give rise to new neurons in the dentate gyrus of the hippocampus. The maintenance and differentiation of neural stem cells have to be tightly balanced in order to sustain hippocampal neurogenesis throughout lifetime. The Notch-signalling pathway has been implicated in stem cell maintenance in several stem cell systems. In the present study, we addressed the question whether Notch-signalling is involved in adult hippocampal stem cell maintenance.

Conditional knockout of the transcription factor RBPJk, a down-stream mediator of Notch-signalling, in hippocampal stem cells, lead to a significant reduction in the number of radial glia like stem cells. This resulted in increased proliferation and differentiation of SGZ stem cells into immature neurons at an early time-point after the induction of the knockout. At a later time-point, neurogenesis was largely diminished.

Previous studies have indicated that the transcription factor Sox2 is necessary for the maintenance of hippocampal stem cells during adulthood. We examined whether Sox2 is involved in Notch-signalling mediated stem cell maintenance. Immunohistochemical analysis, using reporter animals for the Notch signalling pathway, revealed that Notch-signalling is active in many of the Sox2-positive cells in the SGZ. Conditional knockout of RBPJk lead to a significant decrease in the number of Sox2-positive cells in the SGZ, suggesting that Notch-signalling is regulating Sox2 expression.

Subsequent *in vitro* analysis in isolated hippocampal stem cells could confirm the effect of Notch-signalling on Sox2 expression. Preliminary ChIP analysis revealed the presence of RBPJk and the intracellular domain of the Notch receptor on the Sox2 promoter, indicating that Sox2 is a direct target of the Notch-signalling pathway in adult hippocampal stem cells. Taken together these data suggest that Notch signalling plays an essential role for adult neural stem cell maintenance through the regulation of Sox2.

FoxO Transcription Factors in Adult Neurogenesis

Muhammad Amir Khan, Tobias Schwarz, Chichung Lie

Helmholtz Zentrum München, Institute of Developmental Genetics, Adult Neurogenesis Group

FoxO transcription factors are involved in many aspects of cellular functions, such as metabolism, transformation, cell cycle arrest, differentiation, cell death and protection from stress stimuli. There are currently four members in this group; FoxO1, FoxO3, FoxO4 and FoxO6. FoxO transcription factors can bind to the same target sequence and can show overlapping functions. Recent studies have indicated a role for FoxO transcription factors in adult stem cell regulation. For example, FoxO1-/-, FoxO3-/-, FoxO4-/- triple conditional knock out mice revealed a significant decrease in the long-term hematopoietic stem cell population.

The adult hippocampal dentate gyrus is one out of two regions of the adult mammalian brain where neural stem cells generate new functional neurons throughout adulthood. We investigated the expression of FoxO proteins in the adult neurogenic areas in the brain. Interestingly, we found the activity of FoxO proteins in Sox2 and GFAP positive cells in the subgranular zone of the hippocampal dentate gyrus. Sox2 and GFAP positive cells represent the putative stem cells in the subgranular zone, arguing that FoxO proteins can play a role in the maintenance of quiescence in these cell types. We have also detected the activity of FoxO proteins in the immature neurons in the dentate gyrus. FoxO proteins are involved in controlling the differentiation of many cell types arguing a similar role in the new born immature neurons in the dentate gyrus. Moreover, we have found that the activity of FoxO proteins is differentially regulated in proliferating and differentiating neural stem cells in vitro. At present we are generating a GLAST creERT2::FoxO1/3/4 conditional knockout line to investigate the role of FoxO proteins in adult neural stem cell maintenance. Finally, we have also begun to use Cre-recombinase encoding retroviruses to investigate the role of FoxO in immature neurons.

Understanding the Adult Carotid Body Stem Cell Niche

Aida Platero-Luengo, Rocío Durán, José López-Barneo, and Ricardo Pardal

Instituto de Biomedicina de Sevilla - IBiS, Hospital Universitario, Virgen del Rocío, Universidad de Sevilla, Sevilla 41013, Spain

Recent observations in our lab identified the mammalian carotid body (CB) as a niche of neurogenesis with a recognizable physiological function in adult life. This organ is responsible for the detection of hypoxemia and is able to adapt to a persistent stimulus by increasing the number of neuronal, type I cells. We have previously shown that this neurogenesis depends on the proliferation and differentiation of a subpopulation of glia-like stem cells or type II cells. However, the specific mechanisms by which CB stem cells (CBSCs) are activated in hypoxia are unknown. We hypothesize that blood vessels and CB neuronal cells might have an important role on instructing the stem cells to proliferate and differentiate. To elucidate this mechanism we are using different methodological approaches: 1.- In vitro cultures to test the effect of different niche factors on the proliferation and differentiation of CBSCs; 2.- PCRs to study the expression of specific receptors and signalling factors in the different CB cell types separated by flow cytometry; 3.- Electron microscopy to study the cytoarquitecture of the CB niche, and immunochemistry approaches to reveal the expression of specific proliferation and differentiation markers in vivo. Understanding the physiology of CBSCs is crucial not only to learn about adult neurogenic niches but also to improve the use of these cells for therapeutics and to better understand the pathophysiology of the organ.

Evidences for the Widespread Existence of OSVZ in the Developing Cerebral Cortex Among Gyrated Mammals

Isabel Reillo, M. Ángel García-Cabezas and Víctor Borrell

Neurogenesis and Cortical expansion, Neuroscience Institute of Alicante; Av. Ramón y Cajal s/n, 03550 San Juan de Alicante

The cerebral cortex of higher mammals undergoes massive surface area expansion and folding during development, which is thought to be responsible for the evolutionary expansion in cognitive performance. During cortical development, progenitor cells are located in the ventricular (VZ) and subventricular (SVZ) zones. This basic organization seen in rodents is further expanded in primates, where subventricular progenitors are found subdivided in an inner subventricular zone (ISVZ) and outer subventricular zone (OSVZ). The existence of an OSVZ is thought unique to primates and responsible for the outstanding expansion and folding of their cerebral cortex. We have examined the organization and structure of the cortical progenitor layers in other species with a convoluted cerebral cortex. We find that in ferret, cat and sheep numerous cortical progenitors are located outside of the cell-dense SVZ, in a layer so far identified as IZ. These progenitors were largely absent in non-gyrated species like mouse and guinea-pig. “IZ” progenitors express a combination of transcription factors, and are organized in specific sublaminae, typical of the human OSVZ. Our findings provide strong evidence that the OSVZ is widely present in the developing cerebral cortex of most gyrated species, which supports the notion that progenitors in this layer play a central role in cortical expansion and folding during development and evolution.

Session IV: Cell biology

GDNF-dependent Mechanisms Required for Catecholaminergic Neuron Survival

María Hidalgo-Figueroa [1,3], Sonia Bonilla [1], Lin Gao [1], José López-Barneo [1,2,3], and Alberto Pascual [1,2,3]

[1] *Instituto de Biomedicina de Sevilla, Hospital Universitario Virgen del Rocío/ CSIC/ Universidad de Sevilla. Sevilla, Spain*

[2] *Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Spain*

[3] *European Neuroscience Institute (ENI). Seville, Spain*

GDNF is a potent neurotrophic factor that protects catecholaminergic neurons from toxic damage and induces fiber outgrowth. However, the actual role of GDNF in the normal adult brain is unknown, despite GDNF-based therapies are considered promising for neurodegenerative disorders. We have generated a conditional GDNF-null mouse to suppress GDNF expression in adulthood. After *GDNF* ablation animals showed a progressive hypokinesia and a selective decrease of brain tyrosine hydroxylase mRNA, accompanied of pronounced catecholaminergic cell death, affecting most notoriously the locus coeruleus, the ventral tegmental area (VTA) and the substantia nigra (SN) (1). Associated with these neuronal lost, a striatal lost of dopaminergic synapses in GDNF depleted animals was observed. These data demonstrate that GDNF is indispensable for adult catecholaminergic neuron survival.

In order to dissect the brain regions relevant for the maintenance of the different brain areas affected by the GDNF depletion, we are characterizing which neuron subtypes express GDNF at different stages during development. We will use a knock-in mouse that expresses the ß-galactosidase enzyme under the control of GDNF promoter and will colocalize its enzymatic activity with several neuronal populations by using different neuronal markers. This work will allow for using more restricted CRE lines, and mapping which neuronal subtype is producing the GDNF required by SN, VTA and LC.

To study how this factor regulates neuronal survival is necessary to understand the transcriptional and translational program regulated by GDNF. To this end, we are isolating synaptosomes from Striatum and performing differential proteomics analysis. In parallel, we are extracting RNA from SN and VTA to characterize changes in the RNA profile of these cells after GDNF depletion.

In global we expect that this experimental approach will help in deciphering the mechanisms controlled by GDNF and required for neuronal survival.

1. Pascual A., Hidalgo-Figueroa, M. et al., Nature Neuroscience, 2008.

Brain Circuitry in the Regulation of Energy Metabolism: Focus on the Link between Starvation and Arousal

Emilia Horjales-Araujo and Christian Broberger

Department of Neuroscience, Karolinska Institutet. Retzius v. 8,171 77 Stockholm, Sweden

All motivated behaviours, including food intake, require arousal. When energy stores ebb, we enter a higher state of alert to facilitate the search and ingestion of food. However, the pathways whereby starvation signals elicit wakefulness are not well known. Here, we present data suggesting that a large, but poorly explored cell population in the lateral hypothalamus that express thyrotropin-releasing hormone (TRH), may act as such a link. These cells receive input from the brain's metabolic sensor, the hypothalamic arcuate nucleus, and in turn project to key arousal regions such as the cerebral cortex. Moreover, we demonstrate that TRH can shift network activity in higher brain regions from sleep-like network rhythms to the firing patterns typical of wakefulness. These data suggest a mechanism and functional context for TRH-induced arousal previously shown in experimental animals and humans.

Role of Synaptotagmin 1 in Large Dense Core Vesicle Dynamics and Release

Tony Cijsouw, Matthijs Verhage, and Ruud F.G. Toonen

Department of Functional Genomics, Center for Neurogenomics and Cognitive Research, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands

Secretory vesicles, like synaptic vesicles and large dense core vesicles (LDCVs), in neurons and neuro-endocrine cells go through a number of stages before being released. After translocation of the vesicle to the target membrane, the vesicle docks, sequentially primes and fuses with the membrane after sensing a Ca^{2+} stimulus. The SNARE protein complex (SNAP-25, Syntaxin 1, Synaptobrevin) is essential in this process. The major calcium sensor triggering fusion is Synaptotagmin 1 (Syt1). Electrophysiological data shows Syt1 to be important in the burst phase of release, while the sustained release is unaffected. Recent data from *in vitro* liposome fusion assays suggests an additional vesicle clamping function for Syt1. To understand Syt1 function, it is important to study all steps of secretion (*i.e.* docking, priming, and fusion) in a spatially and time resolved manner. Therefore, we investigated the role of Syt1 in the dynamical behavior of LDCVs in the secretory pathway of Chromaffin cells using Total Internal Reflection Fluorescence (TIRF) microscopy. TIRF enables us to monitor fluorescently labeled vesicles close to the membrane with high spatial and time resolution. Initial results showed that in Syt1 -/- cells LDCVs are located further away from the membrane compared to Syt1 +/+ cells. This suggests that, in addition to its calcium-sensing role, Syt1 acts as a docking/clamping factor in SNARE mediated exocytosis of LDCVs.

Subcellular Distribution of Presynaptic Chloride-permeable Glycine Receptors

Trojanová J. [1], Hrušková B. [1], Kulik Á. [2] and Tureček R. [1]

[1] *Institute of Experimental Medicine, Prague, Academy of Sciences of the Czech Republic*
[2] *Institute of Anatomy I and Cellular Biology, University of Freiburg, Germany*

Glycine is a major inhibitory transmitter in the mammalian brainstem and spinal cord. Its action is exerted through glycine receptors (GlyRs). GlyRs are pentameric structures composed of α(1–4) and β subunits. Activation of GlyRs leads to an anionic conductance and, depending on the intracellular chloride concentration ($[Cl^-]_i$), either inhibits or excites neurons. Previously, we have identified GlyRs in the calyx of Held, a giant nerve terminal in the medial nucleus of the trapezoid body. Due to a high $[Cl^-]_i$, activation of the receptors depolarizes the terminal, induces calcium entry and enhances glutamate release from the calyx. It is not known, however, whether Ca^{2+} directly facilitates the glutamate release or whether it acts indirectly, e.g. by Ca^{2+}-dependent recruitment of synaptic vesicles from a reserve pool.

The aim of this study was to reveal the subcellular distribution of GlyRs on the calyx of Held and to determine the spatial relationship between GlyR and the glutamate release zones. We expected that a tight co-localization of GlyRs and the active zones would favor the direct way of the modulation.

We have found that both the distribution and composition of presynaptic GlyRs differed from what has been known for postsynaptic GlyRs. The postsynaptic receptors form $\alpha_1\beta$ heteromers clustered by the β subunit associated protein, gephyrin. Presynaptic α_1 containing GlyRs lacked the β subunit and were diffusely distributed in the calyceal membrane. The quantitative distribution of GlyRs was then studied in serial sections obtained from 6 different calyces of Held. The results from 3D reconstructed terminals indicated that GlyRs were preferentially located on the non-synaptic face of the presynaptic membrane. Furthermore, the receptors were concentrated in cellular compartments containing one or more of the glutamate release zones.

The results are consistent with the indirect modulation hypothesis. The data support the view that presynaptic GlyRs are tuned to sense low concentrations of glycine delivered by spillover.

Intrastriatal Synaptic Connectivity of Different Subtypes of the Medium Spiny Projection Neuron in Two Model Systems

H. Planert, S. Grillner, B. Robertson, G. Silberberg

Karolinska Institutet, Stockholm, Sweden

Striatonigral and striatopallidal medium spiny projection neurons differentially express D1 and D2 receptors and neuropeptides, and are thought to have opposite implications in the regulation of behavior. To investigate the electrophysiological characteristics of MSN subtypes, as well as their connectivity within the striatum and interactions with glutamatergic and dopaminergic afferents is therefore crucial to better understand striatal function. Recently, MSN subpopulations have been investigated using BAC D1 and BAC D2 mice, in which cells that express the receptor are labeled with EGFP (1, 2, 3, 4, 5, 6, 7). We describe intrinsic and connectivity profiles of MSNs in two model systems: with respect to their projection target by retrogradely labeling striatonigral neurons in rats, as well as in relation to receptor expression using BAC mice.

Fluorescent latex beads were injected into the substantia nigra pars reticulata of 12 to 13 day old rats. On postnatal days 13 to 19, simultaneous whole-cell recordings were obtained from multiple neighboring retrogradely labeled (striatonigral), and nonlabeled neurons in the acute slice. Similar experiments were conducted in BAC D1 mice (postnatal days 21 to 30) and the results of the two systems were compared. Membrane properties as well as synaptic interconnectivity of the different MSN subtypes were tested with step and ramp current protocols and by stimulating neurons individually.

Retrogradely labeled as well as EGFP positive neurons exhibited characteristic MSN properties such as inward rectification and ramp response to step current injection. Our results also suggest a lower excitability of striatonigral MSNs, as had been concluded for D1 receptor expressing neurons (2, 5). Synaptic connections included synapses between labeled and nonlabeled MSNs, and from fast-spiking interneuron onto labeled and nonlabeled neuron populations. In BAC D1 mice, we describe differences in connection probabilities in relation to EGFP and therefore D1 receptor expression.

1. Heintz, Nat Rev Neurosci 2: 861-870 (2001).
2. Kreitzer & Malenka, Nature 445: 643-647 (2007).
3. Day et al., Nat. Neurosci 9(2): 251-257 (2006).
4. Shen et al., Nat Neurosci 10(11): 1458-1466 (2007).
5. Cepeda et al., Eur J Neurosci 27: 671-682 (2008).
6. Ade et al., J Neurosci 28(5): 1185-1197 (2008).
7. Taverna et al., J Neurosci 28(21): 5504-5512 (2008).

D. List of participants

Name of Participant	*Principal investigator*	*Institute*	*Email*
Hui Min Tan	André Fisahn	*The European Neuroscience Institute at Karolinska Institutet, Stockholm*	Hui.Tan@ki.se
Emilia Horjales-Araujo	Christian Broberger	*The European Neuroscience Institute at Karolinska Institutet, Stockholm*	emilia.horjales@ki.se
Marta Díaz-Quesada	Miguel Maravall	*The Instituto de Neurociencias de Alicante*	mdiaz@umh.es
Siobhan Dennis	Jack Mellor	*Neuroscience at Bristol*	anxsd@bristol.ac.uk
Marie Kneib	Yann Humeau	*The European Neuroscience Institute at Strasbourg*	mkneib@gmail.com
Manuel Molano	Miguel Maravall	*The Instituto de Neurociencias de Alicante*	mmolano0@hotmail.com
Eva Benito	Angel Barco	*The Instituto de Neurociencias de Alicante*	ebenito@umh.es
Mathieu Niquille	Cécile Lebrand	*Department of Cellular Biology and Morphology (DBCM), University of Lausanne, Switzerland*	mathieu.niquille@unil.ch
Augusto Escalante	Eloisa Herrera	*The Instituto de Neurociencias de Alicante*	aescalante@umh.es
Yasuko Nakamura	Jonathan Hanley	*Neuroscience at Bristol*	Y.Nakamura@bristol.ac.uk
Sabine Schmitz	Ruud Toonen	*Center for Neurogenomics and Cognitive Research Amsterdam*	sabine.schmitz@cncr.vu.nl
Niklas Senghaas	Reinhard W. Köster	*Institute of Developmental Genetics, Helmholtz Center Munich*	niklas.senghaas@gsf.de
María Hidalgo Figueroa	José López-Barneo	*The Neuroscience Institute at Seville*	mahifi@hotmail.com
Aida Platero-Luengo	Ricardo Pardal	*The Neuroscience Institute at Seville*	aidplalue@alum.us.es
Henrike Planert	Gilad Silberberg	*The European Neuroscience Institute at Karolinska Institutet, Stockholm*	Henrike.Planert@ki.se
Muhammad Amir Khan	Chichung Lie	*Institute of Developmental Genetics, Helmholtz Center Munich*	amir.khan@helmholtz-muenchen.de
Isabel Reillo	Víctor Borrell	*The Instituto de Neurociencias de Alicante*	ireillo@umh.es
Tony Cijsouw	Ruud Toonen	*Center for Neurogenomics and Cognitive Research Amsterdam*	tony.cijsouw@cncr.vu.nl

Johanna Trojanová	Rostislav Turecek	*The Institute of Experimental Medicine ASCR in Prague*	trojanova@biomed.cas.cz
Kerimoglu Cemil	Andre Fischer	*The Neuroscience Institute at Göttingen*	Cemil.Kerimoglu@mpi-mail.mpg.de
Oliver Ehm	Chichung Lie	*Institute of Developmental Genetics, Helmholtz Center Munich*	oliver.ehm@helmholtz-muenchen.de
Heidi Soellner	Andrea Brösamle Huber	*Institute of Developmental Genetics, Helmholtz Center Munich*	heidi.soellner@helmholtz-muenchen.de
Tanja Kuczera	Andre Fischer	*The Neuroscience Institute at Göttingen*	Tanja.Kuczera@biologie.uni-goettingen.de
Leiron Ferarrese	Helene Marie	*The European Research Institute Rome*	l.ferrarese@ebri.it
Marzena Stefaniuk	Katarzyna Lukasiuk	*The Nencki Institute at Warsaw*	mstefani@nencki.gov.pl

Welcome to Crete!

Crete is the largest island in Greece and the fifth largest in the Mediterranean with a diverse area of more than 8,000 square kilometres. It is an island with an exquisite 1,000 kilometer-long coastline dotted with numerous coves, bays and peninsulas, which afford a multitude of soft, sandy beaches along the beautifully blue Mediterranean Sea. Crete was considered to be the birthplace of Zeus and the natural resting place of gods. It is also the home of Minoan civilization with important archaeological finds at Knossos, Phaistos and Gortys. Today, Crete is one of Europe's most popular holiday destinations.

Crete consists of four prefectures: Chania, Rethimno, Heraklion and Lasithi. Heraklion is the major city and capital of Crete, located in the middle of the island. It is the fourth largest city in Greece with around 155,000 residents. Heraklion is an industrialised city with a busy harbour and a very busy airport known as “Nikos Kazantzakis” International Airport, in honour of the most famous Cretan writer worldwide.

Knossos is the site of the most important and best known Minoan palace complex in Crete. It is located some 5 km south of Heraklion. According to tradition, Knossos was the seat of the legendary Cretan king Minos. The Palace is also connected with further legends, such as the myth of the Labyrinth and the Minotaur, as well as the story of Daidalos and Ikaros. Excavation has revealed that the site was continuously inhabited from the Neolithic period (7000-3000 B.C.) until Roman times.

The Archaeological Museum Of Heraklion holds the remains of the 3000-year old Minoan civilization, which grew around the nearby legendary palace of Knossos (of Minotaur fame), as well as Byzantine churches and a well-preserved Venetian wall and fortress from the 15th century. Popular highlights include the Phaestos disc, classic Hellenic and Roman sculptures, frescoes, jewellery, wall-paintings and pottery.

Heraklion holds also the Natural History Museum of Crete. It has wonderful displays and good descriptive details about the flora and fauna, the wildlife, of Crete.

Another great place that is very close to the city (~15Km east) is the new Aquarium ("CretAquarium" or "Thalassocosmos"). A 5000 square metre structure, it is both a research centre (housing the Institute of Marine Biology & Genetics and the Institute of Oceanography) and a fun, impressive aquarium with 32 tanks (representing interesting underwater Cretan sea landscapes) and 50 viewing points. 2500 organisms of 200 Mediterranean marine species, from hunter sharks to lobsters, to colourful jellyfish.

Finally, Heraklion hosts the School of Sciences and Engineering and that of Health Sciences, which belong to the University of Crete. It is also the seat of the Foundation of Research and Technology - Hellas (ITE-FORTH), one of the largest research centres in the country, and the Technological Educational Institute of Crete. There are 8 University Schools and 11 Technical Schools (TEI) in total.

Καλώς Ήλθατε στην Κρήτη!