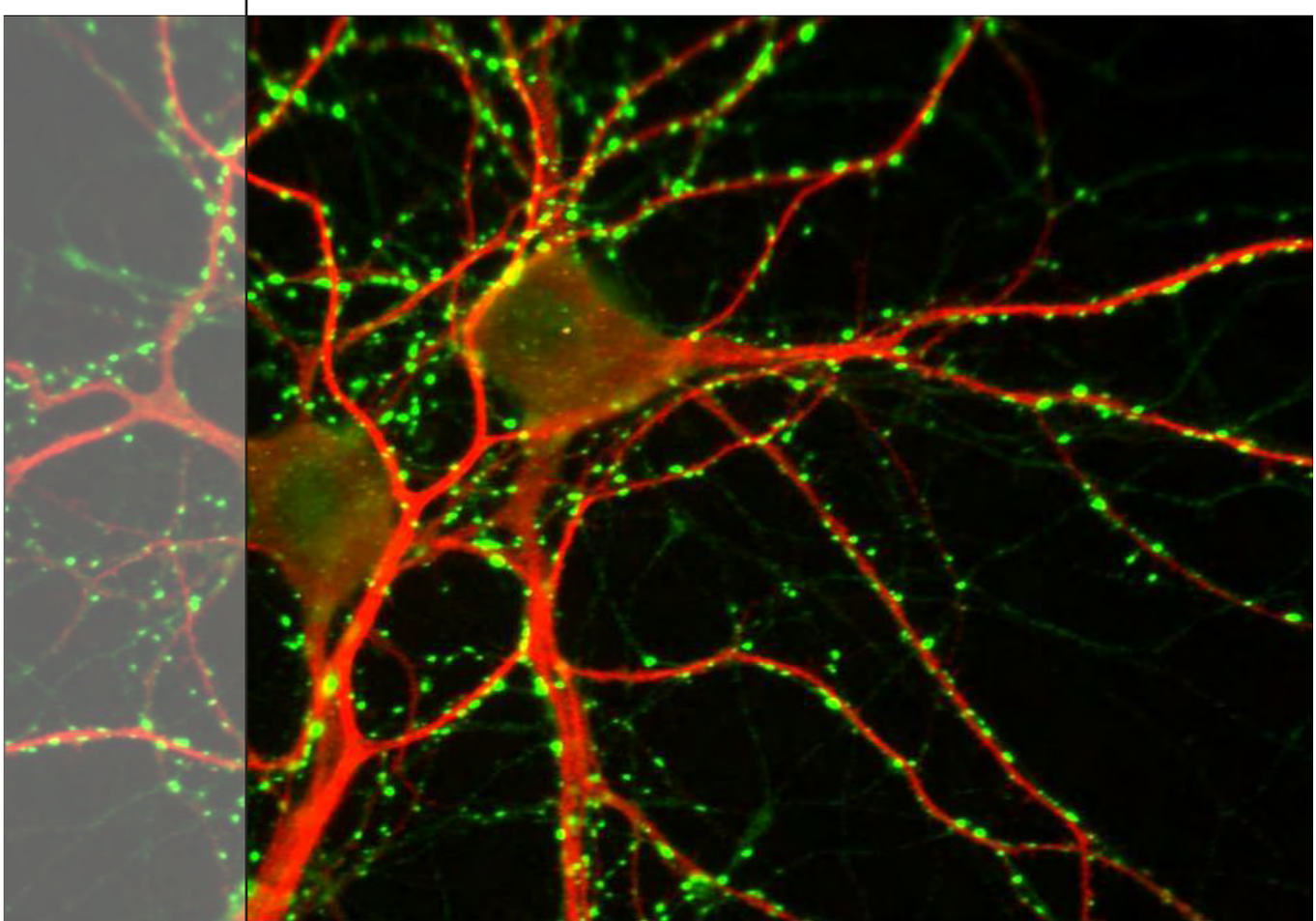
Società Italiana
di Neuroscienze

Università degli Studi
di Milano

Molecular Mechanisms in Neuroscience



5th Meeting
19-20 June 2008

PROGRAMME & ABSTRACTS

Molecular Mechanisms in Neuroscience

5th Meeting

Milan, 19-20 June 2008

Organisers

Fabio Benfenati
Francesco Clementi
Michela Matteoli
Sergio Nasi
Flavia Valtorta

Acknowledgements

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Molecular Mechanisms in Neuroscience
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Programme

THURSDAY, 19 JUNE

8.30-9.00 **On-site Registration**

9.00-9.15 **Welcome to Participants**

9.15-10.00 **Opening Lecture**

Chair: Marina Bentivoglio (SINS President)

Giulio Cossu *(Dibit-HSR, Milan)*
Towards a cell therapy for muscular dystrophy

10.00-12.00 **Symposium 1 - Functional Genomics**

Chairs: Sergio Nasi (CNR, Rome) and
Gian Giacomo Consalez (Dibit-HSR, Milan)

10.00-10.20 **Anna Magnabosco** *(University of Padua)*
Nervous system alterations in *Drosophila melanogaster* sphingolipid metabolic pathway mutants

10.20-10.40 **Alessandra Granata** *(Cancer Research Institute, London UK)*
The dystonia-associated protein torsin-A modulates synaptic vesicle recycling

10.40-11.10 **Giorgio Casari** *(Dibit-HSR, Milan)*
Mutant nicotinic receptors and focal epilepsy, new hypothesis from an *in vivo* study of genetic models

11.10-11.30 **Laura Rusconi** *(University of Insubria, Varese)*
Molecular characterization of CDKL5, a novel kinase involved in Rett Syndrome and infantile spasms

11.30-12.00 **Claudia Bagni** *(Katholieke Universiteit Leuven, Belgium)*
Molecular insights into mental retardation: learning from the Fragile X Syndrome

12.00-15.00 **Poster Session I (Symposia 1 & 2) and Lunch**

14.00-14.30 **Carlo Raviolo** *(Bioscience Division, Millipore SpA)*
Innovations in neural stem cell research

14.30-15.00 **Renato Bertazzo** *(GE Healthcare Europe GmbH)*
Biacore technology in neurosciences

PROGRAMME

15.00-17.00 **Symposium 2 - Development**

Chairs: Lawrence Wrabetz (Dibit-HSR, Milan) and Vania Broccoli (Dibit-HSR, Milan)

15.00-15.30 **Michèle Studer** *(Tigem, Naples)*
The nuclear receptor COUP-TFI in arealization and layer specification in the developing cortex

15.30-15.50 **Giacomo Masserdotti** *(DIBIT-HSR, Milan)*
Cooperation between Notch and BMP signaling in the maintenance of the neural stem cell pool

15.50-16.10 **Laura Cancedda** *(University of California at Berkeley Berkeley, CA)*
Excitatory GABA action is essential for morphological maturation of cortical neurons *in vivo*

16.10-16.30 **Fabrizia Cesca** *(Cancer Research Institute, London, UK)*
Kidins220/ARMS in the neurotrophin pathways: from intracellular trafficking to mouse development

16.30-17.00 **Enrico Tongiorgi** *(University of Trieste)*
BDNF mRNA splice variants as a spatial code to regulate local plasticity of dendrites and spines

17.00-18.00 **Plenary Lecture**

Chair: Daniela Parolaro (University of Insubria, Varese)

Daniele Piomelli *(University of California, Irvine, and IIT, Genoa)*
The endocannabinoid system: from basic mechanisms to therapy

FRIDAY, 20 JUNE

9.00-10.00 **PLENARY LECTURE**

Chair: Enzo Wanke (University of Milan Bicocca)

Egidio D'Angelo *(University of Pavia)*
Plasticity and computation: from neurons to networks

10.00-12.00 **SYMPOSIUM 3 - PLASTICITY**

Chairs: Jacopo Meldolesi (Dibit-HSR, Milan) and Riccardo Fesce (University of Insubria, Varese)

10.00-10.30 **Gian Michele Ratto** *(CNR Neuroscience Institute, Pisa)*
A window with a view: structure and function in the cortex seen through the two-photon microscope

10.30-10.50 **Enrica Maria Petrini** *(CNRS UMR 5091, Bordeaux, France, and IIT, Genoa)*
Endocytic Zones regulate synaptic AMPA receptor abundance and mobility

10.50-11.10 **Silvia Di Angelantonio** *(University La Sapienza, Rome, Istituto Pasteur-Fondazione Cenci Bolognetti, CRiN)*
Activity of adenosine receptors type 1 is required for CX3CL1-mediated modulation of glutamatergic currents in hippocampal neurons

11.10-11.40 **Alexander Dityatev** *(IIT, Genoa)*
Cell adhesion molecules of the immunoglobulin superfamily and hippocampal synaptic plasticity

11.40-12.00 **Tommaso Fellin** *(University of Pennsylvania School of Medicine Philadelphia, USA, and IIT, Genoa)*
Endogenous non neuronal modulators of synaptic transmission control spontaneous cortical activity *in vivo*

12.00-14.00 **POSTER SESSION II (SYMPOSIA 3 & 4) AND LUNCH**

PROGRAMME

14.00-16.00 **Symposium 4 - Degeneration**

Chairs: Flavia Valtorta (Dibit-HSR, Milan) and Fabio Benfenati (IIT and University of Genoa)

14.00-14.30 **Pierluigi Nicotera** *(MRC, University of Leicester)*
Subcellular programmes for neurodegeneration

14.30-14.50 **Paola Rusmini** *(University of Milan)*
Spinal and Bulbar Muscular Atrophy: different approaches to induce ARpolyQ clearance

14.50-15.10 **Laura Gasparini** *(University of Cambridge, Cambridge, UK)*
Axonopathy and accumulation of hyperphosphorylated filamentous tau in retinal ganglion neurons of P301S tau transgenic mice

15.10-15.40 **Massimo Tabaton** *(University of Genoa)*
The cross-talk between the β- and γ-secretase of the β-amyloid precursor protein

15.40-16.00 **Alessia Capotondo** *(DIBIT-HSR, Milan and Vita-Salute University, Milan)*
Dissecting the role of microglia cells in the brain of mice affected by leukodystrophy after hematopoietic stem cells transplantation

16.00-17.00 **Closing Lecture**

Chair: Fabio Benfenati (IIT and University of Genoa)

Giacomo Rizzolatti *(University of Parma)*
The mirror mechanism and its role in cognition

Posters

PS1.01 Ilaria Albieri, Alessia Moiana, Giovanna Calabrese, Dimitris Spiliotopoulos, Elena Cattaneo and G. Giacomo Consalez. **Development of expression cloning approach for the study of neural stem (NS) cell biology.**

PS1.02 Daniela Annibali, Emilia Favuzzi, Bijay Jassal, Sergio Nasi. **Everything you always wanted to know about.... NGF signaling.**

PS1.03 Ruth Adele Antonini, Roberta Benfante, Cecilia Gotti, Francesco Clementi, Diego Fornasari. **The α7 nicotinic acetylcholine receptor subunit duplicate form is downregulated in the monocytic cell line THP-1 on treatment with lipopolysaccaride.**

PS1.04 Roberta Benfante, Helen Lucchetti, Elena Saba, Andrea Raimondi, Simona Di Lascio, Adriano Flora, Francesco Clementi and Diego Fornasari. **Retinoic acid controls PHOX2A expression by means of a dual regulatory mechanism.**

PS1.05 Davide Boido, Pasqualina Farisello, Pietro Baldelli, Fabio Benfenati. **Synapsin knockout mice: an in vitro model of human epilepsy.**

PS1.06 Lucia Caffino, Giorgio Racagni, Marco Andrea Riva and Fabio Fumagalli. **Regulation of Brain-Derived Neurotrophic Factor expression by cocaine in rat brain and its modulation by stress.**

PS1.07 Valeria Capurro, Marta Busnelli, Daniela Braida, Bice Chini, Mariaelvina Sala. **Behavioural characterization of oxytocin mutant mice: implications for autism.**

PS1.08 Doretta Caramaschi, Sietse F. de Boer, Jaap M. Koolhaas. **Serotonin-1A receptors and indiscriminative aggression in genetically selected mouse lines.**

PS1.09 Roberta Castino, Natascia Bellio, David Murphy, Ciro Isidoro**. Akt suppresses autophagy and triggers apoptosis in a model of familial neurohypophyseal diabetes insipidus**.

PS1.10 Giuseppina Catanzaro, Chiara De Simone, Sergio Oddi, Filomena Fezza, Andrea Urbani, Mauro Maccarrone. **Characterization and functional implications of endocannabinoid system in SH-SY5Y cells.**

PS1.11 Alessio Colombo, Cinzia Cagnoli, Ursula Schenk, Maura Francolini, Carolina Frassoni, Giuliana Gelsomino, Lydia Danglot, Thierry Galli, Claudia Verderio, Michela Matteoli. **TI-VAMP-dependent secretory pathway mediates the release of cathepsin B from astrocytes and glioma.**

PS1.12 Mariana Covello, Davide Pozzi, Rosalba D'Alessandro, Anna Fassio, Fabio Benfenati, Pietro Baldelli. **Expression of RE1 silencing transcription factor (REST) in an model of in vitro epilepsy.**

PS1.13 Enrico Defranchi, Mirko Messa, Franco Onofri, Ilaria Monaldi, Marco Milanese, Gianbattista Bonanno, Fabio Benfenati. **Synapsins interact with SH3 domains of proteins involved in synaptic vesicles endocytosis.**

PS1.14 Gabriele Fontana, Cristina Sironi, Vittoria Matafora, Alfonsina D'Amato, Angela Bachi, Daniela Talarico. **Biological activity and regulation of the two isoforms encoded by the *EFA6A* gene in neuronal cells.**

PS1.15 Paola Fragapane, Arianna Rinaldi, Sara Vincenti, Cecilia Mannironi, Francesca De Vito, Alberto Oliverio, Irene Bozzoni, Andrea Mele, Carlo Presutti. **Stress induce alteration in microRNA expression.**

PS1.16 Annalisa Frattini, Silvia De Biasi, Elena Caldana, Arianna Bettiga, Dario Strina, Pietro Poliani, Francesca Rucci, Alesandra Pangrazio, Lucia Susani, Elena Fontana, Laura Vitellaro Zuccarello, Elvira Costantino-Ceccarini. **Neurological impairment in autosomal recessive malignant osteopetrosis due to mutations in OSTM1 and ClCN7 genes.**

PS1.17 Lisa Gasperini, Chiara Piubelli, Serena Becchi, Lucia Carboni. **Proteomic analysis in hypothalamus, hippocampus and pre-frontal/frontal cortex after PACAP administration in rat brain.**

PS1.18 Maila Giannandrea, Niels H Gehring, Fabio Benfenati, Andreas E Kulozik, Flavia Valtorta. **Identification of the molecular basis of epilepsy associated with mutation in human synapsin I.**

PS1.19 M Gravati, G Biella, M Toselli, D Lentini, S Coco, M Parenti, C Gales, A Reversi, M Busnelli, D Losa, B Chini. **Coupling specificity of the human OTR: BRET assays and dual modulation of inward rectifier potassium currents by oxytocin.**

PS1.20 Veronica La Padula, Caterina Giacomini, Angelo Schenone, Massimo Leandri, Laurence Goutebroze, G.Giacomo Consalez, Fabio Benfenati, Anna Corradi. **Peripheral neuropathy in *Ebf2* null mice.**

PS1.21 Loredana Leo, Lisa Gherardini, Virginia Barone, Tommaso Pizzorusso and Giorgio Casari. ***Atp1a2 in vivo* model, developing and phenotyping the knock-in mouse model of Familial Hemiplegic Migraine type 2.**

PS1.22 Anna Linetti, Ilaria Vaccari, Elena Saba, Stefano Gerola, Cristina Montrasio, Francesca Crippa, Pamela Valnegri, Claudia Verderio, Maria Passafaro, Elena Taverna, Paola Carrera and Patrizia Rosa. **Analysis of P/Q-Type Ca2+ channel variants identified in patients with familial hemiplegic migraine.**

PS1.23 Sabrina Lucchiari, Serena Pagliarani, Stefania Corti, Maria Grazia D'Angelo, Francesca Magri, Monica Raimondi, Marinella Carpo, Nereo Bresolin, Giacomo Pietro Comi. **Molecular Characterization of CLCN1, SCN4A, KCNJ2, CACNA1S Genes in Patients with Muscle Channelopathies.**

PS1.24 Alessandra Mallei, Daniele Casale, Roberto Giambelli, Laura Musazzi, Aram El Khoury, Susanne Gruber, Alexander Mathè, Giorgio Racagni, Maurizio Popoli. **Synaptoproteomics of an animal model of depression combining combining genetic vulnerability and early-life stress.**

PS1.25 Cinzia Mallozzi, Marina Ceccarini, Serena Camerini, Gianfranco Macchia, Marco Crescenzi, Tamara C. Petrucci, Anna Maria Michela Di Stasi. **Synaptophysin tyrosine modifications induced by peroxynitrite affect protein-protein interaction and signaling.**

PS1.26 Graziana Modica, Elisa Tinelli, Maria Laura Feltri, Lawrence Wrabetz. **Interaction with BiP chaperone determines the intracellular localization of normal and mutant P0 glycoprotein.**

PS1.27 Luisa Novellino, Fabio Bianco, Cristiana Perrotta, Alessio Colombo, Laura Saglietti, Maura Francolini, Elisabetta Menna, Roberto Furlan, Emilio Clementi, Michela Matteoli, Claudia Verderio. **Vesicle shedding from microglial cells and astrocytes.**

PS1.28 Valeria Padovano, Silvia Massari, Silvia Mazzucchelli, Grazia Pietrini. **Differential regulation of EAAC1 and GLT1 glutamate transporters by calcineurin.**

PS1.29 Rosalba Parenti, Agata Zappalà, Angela Catania, Mario Falchi, Federico Cicirata. **Dynamic expression of Cx47 in brain development and in the cuprizone model of myelin plasticity.**

PS1.30 Laura E. Perlini, Eugenio F. Fornasiero, Flavia Valtorta. **Synapsins and synapse organization: role of site 1 phosphorylation.**

PS1.31 Ilaria Prada, Rosalba D'Alessandro, Andrijana Klajn, Laura Stucchi, Paola Podini, Jacopo Meldolesi. **REST, the transcription repressor of neurosecretion, may govern also gliosecretion.**

PS1.32 Ada Rispoli, Sandra Catania, Elena Cipollini, Giuseppe Pulice, Christina van Houte, Francesca Sparla, Paolo Trost, Rossella Di Giaimo, Marialuisa Melli. **Cystatin B function and Progressive Myoclonus Epilepsy (EPM1).**

PS1.33 Elisabetta Rovelli, Anna Cozzi, Grazia Frizzale, Paolo Santambrogio, Mario Amendola, Alessandro Campanella, Sonia Levi. **Production and characterization of a neuronal cellular model to study neuroferritinopathy.**

PS1.34 F. Rusconi, G. Colombo, E. Fontana, A. Bachi, L. Vitellaro-Zuccarello, R. Zippel, S. De Biasi. **Immunochemical localization of RasGRF1 in the cerebellum and changes in the proteome profile induced by its genetic deletion.**

PS1.35 Mauro Savino, Sara Vincenti, Carlo Presutti, Sergio Nasi. **Neurotrophins and microRNAs: expression profile in PC12 cells upon NGF treatment.**

PS1.36 Manuela Scali, Elisa Brilli, Matthias Köhler, Yuri Bozzi. **Seizures increase importin β1 expression in NG2+ cells in the rat hippocampus.**

PS1.37 Laura Serio, Annamaria Mancini, Andreina Alfieri, Ottavia Barbieri, Simonetta Astigiano, Pasqualina Buono, Francesco Salvatore. **Analysis of human aldolase C mRNA expression in transgenic mice.**

PS1.38 Mario Venza, Maria Caffo, Claudia Torino, Carmelo Genovese, Gerardo Caruso, Concetta Alafaci, Francesco M. Salpietro, Francesco Tomasello. **IL-8 and prostaglandin E synthase-1 expression in low- and high-grade human glioma.**

PS1.39 Annalisa Vicario, Cristina Chiaruttini, Francesca Rapino, Gabriele Baj, Li Zhi, Paolo Braiuca, Lucia Gardossi, Jay Baraban, Enrico Tongiorgi. **Targeting signals in BDNF mRNA coding region direct its trafficking in dendrites.**

POSTERS • Development

PS2.01 Christian Alfano, Luigi Viola, Giulio Srubek Tomassy, Steve Sansom, Gennaro Andolfi, Rick Livesey and Michèle Studer. **Coup-tfI controls projection neuron migration and morphology by negatively regulating Rnd2 expression levels.**

PS2.02 Claudia Asperti, Antonio Totaro, Simona Paris, Marzia De Marni, Ivan de Curtis. **GIT1/Liprin-alpha Complexes in cell motility and early neuronal development.**

PS2.03 Monica Baiula, Santi Spampinato. **Expression of the transcription factor REST at different stages of neuronal differentiation.**

PS2.04 S. Bernascone, J. Erriquez, M. Toscani, A.A. Genazzani, R. Billington, M. Ferraro, C. Distasi. **Adenosine slows the migration of peripheral neurons and glial cells.**

PS2.05 Emanuele Cacci, Maria Antonietta Ajmone-Cat, Tonino Anelli, Stefano Biagioni, Luisa Minghetti. **Effects of activated microglia on neural stem/progenitor cells properties.**

PS2.06 Giovanna Calabrese, Andrea Serio, Ilaria Albieri, Elena Cattaneo and Gian Giacomo Consalez. **Generation of cerebellar GABAergic neurons from NS cells.**

PS2.07 Nadia Canalia, Maria Armentano, Patrizia Aimar, Luca Bonfanti. **Ex-vivo models of Neural Stem Cell niche: antigenic and ultrastructural properties.**

PS2.08 Simona Candiani, Luca Moronti, Roberta Pennati, Fiorenza De Bernardi, Fabio Benfenati, Mario Pestarino. **Evolutionary perspectives of the synapsin gene family in basal chordates.**

PS2.09 Anna Cariboni, Roberto Maggi, Christiana Ruhrberg, John Parnavelas. **Role of VEGF and blood vessels during GnRH-neurons development.**

PS2.10 Daniela Corno, Barbara Cipelletti, Mauro Pala, Letterio Politi, Alessandro Bulfone and Rossella Galli. **Identification of cancer stem cells in a mouse model of medulloblastoma and molecular comparison with normal neural stem cells.**

PS2.11 Valeria Barili, Ruben van Vugt, Giacomo Masserdotti, Aurora Badaloni, Giacomo Consalez, Laura Croci. **EBF transcription factors activate Igf1 gene expression, possibly regulating Purkinje cell survival at birth.**

PS2.12 S. Di Lascio, F. Cargnin, F. Clementi, D. Fornasari, R. Benfante. **Cross-regulation of PHOX2A and PHOX2B transcription factors in the development of the Autonomic Nervous System.**

PS2.13 Francesco Difato, Rajesh Shahapure, Enrico Ferrari, Jummi Laishram, Dan Cojoc, Vincent Torre. **Measurement of the force exerted by neurites during neuronal differentiation.**

PS2.14 Michela Errico, Michele Studer. **The requirement for phox2 genes in the differentiation of ventral-r4 neuron sub-populations.**

PS2.15 J. Erriquez, S. Bernascone, M. Ciarletta, N. Filigheddu, A. Graziani, C. Distasi. **Calcium signals activated by ghrelin and D-Lys3-GHRP-6 in DRG non-neuronal cells.**

PS2.16 Eugenio F. Fornasiero, Dario Bonanomi, Gregorio Valdez, Simon Halegoua, Fabio Benfenati, Andrea Menegon, Flavia Valtorta. **Identification of a developmentally-regulated pathway of membrane retrieval in neuronal growth cones.**

PS2.17 S.G. Giannelli, M. Andreazzoli, P. Rama, V. Broccoli. **Genetic manipulation of murine retinal stem cells.**

PS2.18 Gioacchin Iannolo, Cristina La Rosa, Rita Circo, Lucia Ricci-Vitiani, Concetta Conticello, Ruggero De Maria, Massimo Gulisano. **Distinct Numb isoform modulate NSCs, self-renewal and multipotency versus differentiation.**

PS2.19 Elisa Reisoli, Michela Ori, Stefania De Lucchini, Irma Nardi. **5-HT2B signalling partecipates in retinal and craniofacial morphogenesis during Xenopus laevis development.**

PS2.20 Alessandro Sessa, Chai-An Mao, William H. Klein, Vania Broccoli. **Establishing Eomesodermin role in cortical intermediate progenitor specification and development.**

PS2.21 Giulio Srubek Tomassy, Elvira De Leonibus, Simona Lodato, Denis Jabaudon, Christian Alfano, Andrea Mele, Jeffrey D Macklis, Michele Studer. **Cortical spinal motor neurons require Coup-tf1 for their correct specification to control skilled motor behavior.**

PS2.22 Floriana Volpicelli, Massimiliano Caiazzo, Dario Greco, Luigi Leone, Luca Colucci-D'Amato, Carla Perrone-Capano, Umberto di Porzio. **Nurr1, a transcription factor essential for midbrain dopaminergic neuron development, regulates Bdnf gene expression in vitro.**

PS2.23 Paola Zordan, Laura Croci, Ludovica Piazzoni, Richard Hawkes, G.Giacomo Consalez. **Neurogenins and the specification of cerebellar GABA neurons.**

POSTERS • Plasticity

PS3.01 Massimiliano Aceti, Silvia Middei, Christopher Pittenger, Alison Reynolds and Shane M. O'Mara. **Inducible dominant-negative CREB mutation alters in vivo Long Term Potentiation in mCREB mice.**

PS3.02 Gabriele Baj, Emiliano Leone, Moses V. Chao, Enrico Tongiorgi. **BDNF mRNA splice variants represent a spatial code to regulate the complexity of dendrites and number of spines in specific domains of the dendritic tree.**

PS3.03 Andrea Barberis, Shankar Sachinandanam, Christophe Mulle. **Non-equilibrium activation of GluR6/KA2 kainate receptors determines slow current deactivation kinetics.**

PS3.04 Luca Leonardo Bologna, Michela Chiappalone, Thierry Nieus, Sergio Martinoia. **Constant low frequency stimulation shows changes in responsiveness of in-vitro cortical cultures during development.**

PS3.05 Francesca Calabrese, Raffaella Molteni, Giorgio Racagni, Marco A. Riva. **Stress-induced changes of neuroplastic proteins and modulation by chronic antidepressant treatment.**

PS3.06 Marco Cambiaghi, Rosalia Crupi, Linda Spatz, Hoau-Yan Wang, Mitchell Thorn, Fortunato Battaglia. **Emotional behaviors in autoimmune-prone BAFF transgenic mice.**

PS3.07 Chiara Cerri, Laura Restani, Lamberto Maffei, Matteo Caleo. **Control of cortical binocularity by the corpus callosum.**

PS3.08 Michela Chiappalone, Paolo Massobrio, Mariateresa Tedesco, Fabio Benfenati, Sergio Martinoia. **The study of network dynamics and plasticity in cortical neurons coupled to Micro-Electrode Arrays.**

PS3.09 S. B. Condliffe, C. Grumelli, D. Pozzi, C. Verderio, M. Matteoli. **Voltage-gated calcium channel properties differ in glutamatergic versus GABAergic hippocampal neurons.**

PS3.10 Irene Corradini, Alessia Diletta Zani, Simona Colleoni, Matteo Caleo, Yuri Bozzi, Marco Gobbi, Daniela Braida, Mariaelvina Sala, Claudia Verderio, Michela Matteoli. **SNAP-25 negatively modulates voltage-gated calcium channels and network activity.**

PS3.11 Marco Costanzi, Daniele Saraulli, Clelia Rossi-Arnaud, Vitangelo Barbato, Massimiliano Aceti, Nadia Canu, Vincenzo Cestari. **Recovery from a memory deficit in Ras-GRF1 knockout mice.**

PS3.12 Rosalia Crupi, Marco Cambiaghi, Hoau-Yan Wang, Rene' Hen, Fortunato Battaglia. **rTMS, progenitor cell proliferation and synaptic plasticity.**

PS3.13 Veronica Bianchi, Matteo Vecelio, Pasqualina Farisello, Pietro Baldelli, Fabio Benfenati, Daniela Toniolo, Patrizia D'Adamo. **Cognitive impairments in Gdi1 knockout mice are associated with specific defects in synaptic vesicle pools and short-term synaptic plasticity.**

PS3.14 Federico Esposti, Jacopo Lamanna and Maria Gabriella Signorini. **Long-term effects of AP5 and TTX administration on neuronal networks evaluated on in-vitro MEA cultivations.**

PS3.15 Pasqualina Farisello, Davide Boido, Flavia Valtorta, Pietro Baldelli, Fabio Benfenati. **Study of epileptic activity in hippocampal slices of Synapsins knockout mice.**

PS3.16 Alessandro Faroni, Marinella Ballabio, Bernhard Bettler, Mariapia Colleoni, Giuseppe Lauria, Patrizia Procacci, Valerio Magnaghi. **Altered peripheral myelination in GABA-B1-receptor knockout mice.**

PS3.17 Elisabetta Cesana, Stéphane Dieudonné , Philippe Isope, Lia Forti, Egidio D□Angelo. **The mossy fiber input to Golgi cells in the cerebellum: presence of an NMDA component.**

PS3.18 Giuliana Gelsomino, Elisabetta Menna, Claudia Verderio, Fabio Benfenati and Michela Matteoli. **Functional characterization of presynaptic kainate receptors in primary hippocampal neurons and brain growth cones.**

PS3.19 Carmen Inglese, Damiana Leo, Umberto di Porzio, Carla Perrone-Capano. **The 5-HT receptor 7 is accountable for the ERK-dependent neurite outgrowth in cultured neurons.**

PS3.20 Silvia Leone, Simona Rodighiero, Francesca Gullo, Alida Amadeo, Enzo Wanke. **Neurochemical characterization of cultured neocortical networks.**

PS3.21 Paola Lombardo, Sergio Solinas, Leda Roggeri, Paola Rossi, Egidio D'Angelo. **Granular layer resonance in response to tactile stimulation in vivo.**

PS3.22 Lisa Mapelli, Paola Rossi, Egidio D'Angelo. **Tonic activation of GABAB receptors regulate release probability and the dynamics of synaptic inhibition in the cerebellar glomerulus.**

PS3.23 Cristina Marchetti, Leonardo Restivo, Elisiana Tafi, Maria Angela Rubinacci, Martine Ammassari-Teule, Helene Marie. **Neuronal adaptations, induced by rolipram and imipramine, leading to memory enhancement.**

PS3.24 Emanuele Marconi, Mirko Messa, Claudio Canale, Marco Salerno, Pietro Baldelli, Fabio Benfenati. **Development and plasticity in networks of neurons grown onto micropatterned substrates.**

PS3.25 Paolo Medini, Fabio Benfenati, Bert Sakmann. **Layer- and cell type-specific synaptic inputs and spike outputs of pyramidal neurons along a visual cortical column in vivo.**

PS3.26 Silvia Middei, Christopher Pittenger, Alison Reynolds, Shane M. O'Mara. **Inducible inhibition of CREB phosphorylation impairs memory, prevents learning-induced changes in hippocampal spine density, and blocks in vivo long term potentiation in mCREB mice.**

PS3.27 L. Musazzi, V.S. Barbiero, A. Mallei, S. Zappettini, M. Milanese, R. Giambelli, D. Tardito, G. Bonanno, G. Racagni, M. Popoli. **Effects of stress and antidepressant drugs on presynaptic molecular mechanisms regulating glutamatergic neurotransmission.**

PS3.28 T. Nieus, E. D'Angelo. **A mathematical model of neurotransmission at the input stage of the cerebellum.**

PS3.29 Matteo Pasini, Fabio Fumagalli, Filippo Drago, Giorgio Racagni, Marco A. Riva. **Modulation of intracellular signaling pathways by acute swim stress in rat hippocampus.**

PS3.30 Valentina Pasquale, Paolo Massobrio, Luca L. Bologna, Michela Chiappalone, Sergio Martinoia. **Neuronal avalanches in networks of neurons developing in vitro.**

PS3.31 Francesca Prestori, Daniel Bertrand, Egidio D'Angelo. **Nicotinic stimulation increases glutamatergic transmission in the rat cerebellum.**

PS3.32 Natalia Realini, Daniela Braida, Sandra Guidi, Valeria Capurro, Tiziana Rubino, Renata Bartesaghi and Daniela Parolaro. **Adult cognitive impairment induced by adolescent exposure to THC is associated with altered hippocampal dendritic morphology and synaptic plasticity.**

PS3.33 Laura Restani, Laura Gianfranceschi, Marta Pietrasanta, Lamberto Maffei, Matteo Caleo. **Functional masking of deprived eye responses by callosal input during ocular dominance plasticity.**

PS3.34 Leonardo Restivo, Elisiana Tafi, Martine Ammassari-Teule, H. Marie. **Acute in vivo increase of CREB activity in the hippocampus improves fear memory.**

PS3.35 Milica Cerovic, Paul Baxter, Tiziana Rubino, Daniela Parolaro, Raffaella Tonini. **Bidirectional modulation of corticostriatal synapses after prolonged activation of cannabinoid CB1 receptors.**

PS3.36 Francesca Gullo, Elena Dossi, Antonella Alfieri, Irene Brachini, Andrea Maffezzoli, Silvia Leone, Alida Amadeo, Enzo Wanke. **Functional pharmacology in cultured neocortical networks.**

PS3.37 Letizia Zullo, Michela Chiappalone, Sergio Martinoia, Fabio Benfenati. **The use of Feedback stimulation to reveal intrinsic properties of a neuronal network.**

PS3.38 Anna Linetti, Ilaria Vaccari , Francesca Crippa, Giuliana Roselli, Pamela Valnegri, Maria Passafaro, Carlotta Grumelli, Michela Matteoli, Elena Taverna, Patrizia Rosa. **The role of cholesterol in synapse stability and activity.**

POSTERS ● Degeneration

PS4.01 Valentina Angeloni, Barbara Torsello, Paola Brambilla, Lara Invernizzi, Silvia Bombelli, Roberto A. Perego, Cristina Bianchi. **Expression studies of non receptor tyrosine kinase Arg in SH-SY5Y cells treated with Aβ oligomers.**

PS4.02 V. Annese, M. Di Pentima, G. Minnone, P. Paggi, M.E. De Stefano. **Changes in mRNA and protein levels of metalloproteinase-9 and of members of the plasminogen activator/plasmin enzymatic system in MPTP-induced parkinsonian mice.**

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Abstracts

Oral Communications

(abstracts in chronological order)

Toward the cell therapy for muscular dystrophies

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Mesoangioblasts are recently characterized progenitor cells associated with the vasculature and able to differentiate in different types of solid mesoderm including skeletal muscle. Human adult mesoangioblasts were recently isolated and expanded in vitro from muscle biopsies: they were shown to correspond to a subset of human pericytes and, more importantly to differentiate spontaneously in skeletal muscle with high efficiency.
When both wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of alpha-sarcoglycan null mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype.
Intra-arterial or systemic delivery of wild type, non DLA matched mesoangioblasts in Golden Retriever dystrophic dogs resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration, despite widespread micro-dystrophin expression. These results show efficacy of cell therapy in a large, immune-competent animal and set the rationale for a future clinical trial, using donor cells from an HLA-matched donor under immune suppression. Problems still facing this approach and possible strategies to overcome them will be discussed.

Nervous system alterations in *drosophila melanogaster* sphingolipid metabolic pathway mutants

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The importance of sphingolipids in membrane biology was appreciated early in the twentieth century when several human inborn errors of metabolism were linked to defects in sphingolipid degradation. In fact, this class of lipids is a ubiquitous component of cell membranes and some metabolites: ceramide (Cer), sphingosine (Sph), and sphingosine-1-phosphate (S1P) have important physiological functions, including regulation of cell growth and survival.
Recently the role of sphingolipids in neural cell biology has also become a topic of investigation leading to the recognition that sphingolipids seem to play a key role in neuronal signalling, in particular through their presence in membrane lipid rafts which are involved in numerous cellular processes such as signal transduction, membrane trafficking, cytoskeletal organization, neural adhesion, axon guidance and synaptic transmission.
Homologs of key enzymes belonging to the sphingolipid metabolic pathway have been discovered in *Drosophila melanogaster*, making this an optimal model to study the effects of dysregulation of sphingolipid metabolism on nervous system development, function and integrity.
In particular, our attention focused on *sphingosine-1-phosphate lyase gene (Sply)* null mutants. These animals, in the adult stages, show a profound reduction in flight performance which is probably related to the degeneration of flight muscle fibers and their mitochondria. On the other hand, larvae show locomotor impairments, although their muscles appear morphologically intact. . However, the neuromuscular junctions (NMJ) show significant morphological alterations at the level of the pre-and post synaptic complexes. Moreover, functional imaging studies using the lipophilic strylyl dye FM1-43, highlight anomalous synaptic vesicle recycling, which is confirmed by the electrophysiological analysis of synaptic function. These results suggest that the locomotion defects could be, at least in part, ascribed to the observed NMJ alterations.

The Dystonia-Associated protein Torsin-A modulates synaptic vesicle recycling

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Dystonia is a common movement disorder, characterised by involuntary muscle contractions, which frequently result in twisting, repetitive movements and abnormal posture. All cases of the autosomal dominant form, early-onset primary dystonia, are caused by a GAG deletion (ΔE) in DYT1 gene, resulting in the loss of a glutamic acid residue in the AAA+ ATPase protein torsinA.
To investigate the function of torsinA, we performed a yeast two-hybrid screening of a brain library and we found that snapin, which binds SNAP-25 and promotes neurotrasmitter release by enhancing the association of the SNARE complex with synaptotagmin, is an interacting partner for both the wt and the mutant ΔE-torsinA. Our results showed that snapin co-localised with ΔE-torsinA in the perinuclear inclusions in transfected human neuroblastoma SH-SY5Y cells and with the endogenous torsinA on secretory granule structures in PC12 cells. In view of these observations, we investigated if torsinA could affect synaptic vesicle recycling by using two independent methods. One is based on monitoring the uptake of the lipophilic dye FM1-43 and the second consists of using an antibody directed against the intravesicular epitope of synaptotagminI. Our analysis showed that wt-torsinA negatively affects synaptic recycling. Conversely, ΔE-torsinA promotes synaptic vesicle endocytosis. In addition, knocking down snapin and/or torsinA using siRNAs technique showed that equally single and double knock-down had a negative effect on the exo-endocytic process. We also observed that the phenotype produced by knocking down torsinA resembles the effect on synaptotagminI membrane exposure caused by ΔE-torsinA. This suggests that ΔE-torsinA could be a loss of function mutant. We are now investigating if torsinA plays a role together with snapin in regulating synaptic trafficking in primary hippocampal neurons.
To date, our findings suggest that torsinA is important for the neuronal uptake of neurotransmitters, such us dopamine, which is responsible of dystonic movements.

Mutant nicotinic receptors and focal epilepsy, new hypothesis from an *in vivo* study of genetic models

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Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE) is a focal form of epilepsy characterized by seizures occurring during non-REM sleep. We have developed an inducible transgenic mouse model carrying the V287L mutation in the β2 subunit of the neuronal nicotinic receptor, which we have shown associated to ADNFLE. Expression of mutant β2 subunit is driven by a nervous system specific tTA transgenic protein and can be silenced by oral doxycycline administration.
Mice expressing mutant receptors display a spontaneous epileptic phenotype, assessed by electroencephalography (EEG), with very frequent interictal spikes and seizures.
Complete silencing of transgene's expression in adult mice is not sufficient to suppress the established epileptic phenotype. On the contrary, silencing of transgene's expression restricted to developmental stages inhibit spreading of epilepsy. We thus suggest that mutant nicotinic receptors are responsible of an abnormal formation of neuronal circuits and/or long-lasting alterations in neuronal networks tuning in developing brain, thus leading to epilepsy.

Molecular characterization of CDKL5, a novel kinase involved in Rett Syndrome and infantile spasms

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Rett Syndrome (RTT) is an X-linked neurological disorder and represents the second cause of mental retardation in females. Mutations in the methyl-CpG binding protein (MeCP2) gene cause the majority of RTT cases. Recently, mutations in the cyclin-dependent kinase-like 5 (CDKL5) gene have been found in some RTT patients with the Hanefeld variant. Pathogenic mutations in CDKL5 were also found in females with early signs of developmental delay and epileptic seizure onset, further reinforcing the importance of this gene in mental retardation and epilepsy.
We are characterizing the role of CDKL5 in the nervous system thereby clarifying the molecular mechanisms involved in disease onset. We have previously shown that CDKL5 and MeCP2 function in a common pathway; in fact, they associate and the kinase is able to mediate the phosphorylation of MeCP2. This suggests that CDKL5 might also play an indirect role in RTT acting as a modifier gene that, by regulating MeCP2 functions, is able to influence disease severity in patients with mutations in MeCP2. Here we will show that both CDKL5 expression and its subcellular localization are highly modulated during embryogenesis and post-natal development. In addition, in adult mouse, CDKL5 protein level and its cytoplasmic/nuclear fraction are tightly regulated in the different brain areas. Moreover, we will present data demonstrating that CDKL5 shuttles between the nucleus and the cytoplasm and that an active nuclear export mechanism is involved in regulating its localization. Our analysis suggests that the C-terminal tail of the kinase is responsible for the cytoplasmic localization. Importantly, we will show that a number of RTT truncating mutations, found in this region, are mislocalized in the nucleus. We believe that this analysis will contribute in drawing a phenotype-genotype correlation in patients with mutations in CDKL5 and in understanding the role of CDKL5 as an MeCP2 modifier gene.

The Fragile X mental retardation protein represses activity-dependent mRNA translation at synapses

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Regulated protein synthesis in neuronal dendrites is crucial for synaptic plasticity and brain development. The Fragile X mental retardation protein (FMRP) represses translation of specific mRNAs in neuronal dendrites; how it exerts this effect, however, is largely unknown. One protein that interacts with FMRP is CYFIP1/Sra1, a component of the WAVE complex involved in actin polymerization. We show that CYFIP1/Sra1 also interacts with the cap-binding translation initiation factor eIF4E through a novel domain that is structurally related to that present in eIF4E-BPs. Reduction of CYFIP1 in neurons leads to an increase of proteins encoded by know FMRP target mRNAs. Neuronal stimulation via BDNF or DHPG, two stimuli that trigger dendritic protein synthesis, causes CYFIP1/Sra1 to dissociate from eIF4E at synapses and lead to the release of the repressed mRNAs. CYFIP1/Sra1 thus mediates the translational repression of FMRP in the brain.

Innovations in neural stem cell research

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ReNCell and ENStem-A cells are two human neural progenitor stem cell lines derived from fetal tissues and from embryonic stem cell lines respectively. Both offer phenotype and genotype stability in addition to a multipotential neural differentiation capacity. Here, we describe the characteristics and the differentiation studies that were performed with these cell lines, which represent a new platform the scientists can use for research and drug discovery applications.

Biacore Technology in Neurosciences

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A quick overview of Surface Plasmon Resonance (SPR) Technology and The Biacore Systems. Examples of applications of Biacore on Neurosciences research field. Different immobilization of ligands strategies and flexibility on the study of interactions. The foundamental role of kinetic and Affinity in the caracterization of biological interactions.

The transcription factor COUP-TFI in cortical lamination and connectivity: a developmental and functional analysis

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The mammalian neocortex is the seat of the highest sensory, motor, and cognitive abilities. Corticogenesis involves the formation of six distinct layers and of functionally specialized areas characterized by specific sets of inputs and outputs. Identifying mechanisms that link area and layer specification is a crucial issue for understanding cortical malformations. We have recently demonstrated that the nuclear receptor COUP-TFI is required to balance cortical patterning into motor and sensory areas (Armentano et al., 2007). Accordingly, in the absence of COUP-TFI, the presumptive somatosensory area acquires motor-like properties as assessed by histological and molecular analyses. We show that this defect might be due to an incorrect specification and migration of upper versus lower layers, which differs from motor to sensory areas. We show that COUP-TFI modulates the sequential specification of lower layers by restricting genes specific for layer V where most of subcerebral projecting neurons reside. Furthermore, COUP-TFI regulates upper layer radial migration by modulating levels of Rnd2, a member of the Rho family of small GTPases, which controls cellular morphology. Tracing experiments indicate abnormal connectivity between the cortex and the spinal cord in post-natal *COUP-TFI* conditional mice. By using a series of behavioral tasks, we have finally demonstrated that COUP-TFI mutant mice are unable to perform skilled reaching movements, suggesting impairment in planning skilled motor activities, normally controlled by the sensorimotor cortex. All together, our data shed new lights into the role for COUP-TFI in several crucial steps of neocortical development and function, and link area specification to cortical layer organization, connectivity and functional behavior.

Armentano M., Chou S. J., Srubek Tomassy G., Leingärtner A., O'Leary D.D.M. and Studer M. COUP-TFI regulates the balance of cortical patterning between frontal/motor and sensory areas. Nature Neuroscience, 2007 10, 2007, 1277-1286.

Cooperation between Notch and BMP signling in the maintenance of the neural stem cell pool

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In cerebellar development, radial glial progenitors located in the ventricular zone (VZ) give rise to different types of neurons. A large body of literature implicates Hes genes in the maintenance of radial glial progenitors. In fact, *Hes5* expression is prominent in the embryonic cerebellar neuroepithelium. ZFP423 is a multi-Zn finger nuclear protein that cooperates with nuclear SMAD1-SMAD4 complexes, and antagonizes EBF proteins, an atypical HLH transcription factor family involved in neuronal differentiation, migration, and survival. Mice carrying targeted deletions of Zfp423 feature a prominent hypoplasia of the cerebellar vermis, a phenotype reminiscent of the Dandy Walker malformation in humans. We found that ZFP423 cooperates dose-dependently with NICD to upregulate *Hes5* and *Blbp* in P19 cells. In C2C12 cells, that do not express the gene, *Hes5* gene transcription is strictly dependent upon the addition of exogenous *Zfp423*. In cells transfected with *Zfp423*, unlike untransfected controls, treatment with exogenous BMP4 further activates NICD-dependent *Hes5* transcription. In keeping with the above findings, ZFP423 knock-down, achieved through specific shRNAs, reduces the level of *Hes5* expression in response to NICD. Finally, in gain-of-function experiments, co-transfection of cells with Ebf genes counteracts the cooperative effect of ZFP423 and NICD on *Hes5* gene regulation. Our results, achieved in cellular systems, suggest that, by cooperating with NICD and nuclear SMAD1-SMAD4, ZFP423 may boost the activity of Notch signaling in maintaining and expanding the pool of cerebellar progenitors, to support the birth of successive waves of glial and / or neuronal progenitors. In vivo studies of Zfp423 mutants are in progress to test this hypothesis

Excitatory GABA action is essential for morphological maturation of cortical neurons *in vivo*

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Amino acid γ-aminobutyric acid (GABA) exerts excitatory actions on embryonic and neonatal cortical neurons, but the *in vivo* function of this GABA excitation is largely unknown. Using *in utero* electroporation, we eliminated the excitatory action of GABA in a subpopulation of rat ventricular neural progenitors by premature expression of the Cl^- transporter KCC_2, as confirmed by the change in the reversal potential of GABA-induced currents. We found that radial migration of cortical neurons derived from the transfected progenitors was unaffected, but their morphological maturation was markedly disrupted. Furthermore, reducing neuronal excitability of cortical neurons *in vivo* by overexpressing an inward-rectifying K^+ channel, which lowered the resting membrane potential, mimicked the effect of premature KCC_2 expression. Thus, membrane depolarization caused by early GABA excitation is critical for morphological maturation of neonatal cortical neurons *in vivo*.

Kidins220/ARMS in the neurotrophin pathways: from intracellular trafficking to mouse development

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Kidins220 (*K*inase *D in*teracting *s*ubstrate of *220* KDa) / ARMS (*A*nkyrin Repeat-*r*ich *M*embrane *S*panning) is a conserved integral membrane protein mainly expressed in brain and neuroendocrine cells. It is a substrate for PKD as well as an intracellular target of the signalling cascades initiated by neurotrophins and ephrins[1,2]. Kidins220/ARMS has been implicated in the process of neuronal differentiation, by mediating the sustained activation of the mitogen-activated protein kinase (MAPK) pathway in response to neurotrophic stimuli[3].
We have shown that the binding of Kidins220/ARMS to the kinesin-1 motor complex is required for the intracellular targeting of Kidins220/ARMS. Furthermore, impairing Kidins220/ARMS trafficking by overexpression of the *k*inesin-1 *i*nteracting *m*otif (KIM) results in an inhibition of MAPK activation and neuronal differentiation in response to NGF[4].
To test the functional role of Kidins220/ARMS in vivo, we have engineered a construct, based on the Cre/LoxP recombination system, which allows the conditional knock out of Kidins220/ARMS in mice. Whilst the Kidins220/ARMS+/- mice are phenotypically normal, the Kidins220/ARMS knockouts die at birth. A preliminary analysis conducted on the brain of mutant embryos has revealed multiple developmental defects. At late stages of development, Kidins220/ARMS -/- embryos display a pronounced enlargement of the brain ventricles. In addition, we identified specific areas of cell death in the thalamic and hippocampal regions. We have further characterised the behaviour of cultured Kidins220/ARMS knockout hippocampal neurons, focusing on their ability to survive and differentiate in response to specific neurotrophic stimuli.

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BDNF mRNA splice variants as a spatial code to regulate local plasticity of dendrites and spines

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Long-lasting changes at the basis of memory storage, require delivery of newly synthesized proteins to the affected synapses. While most of these proteins are generated in the cell body, several key molecules for plasticity can be delivered in the form of silent mRNAs at synapses in extra-somatic compartments where they are locally translated in response to specific stimuli. This "local" mechanism is particularly attractive as it would ensure spatial and temporal specificity to long-term synaptic plasticity. Accordingly, attention has focused on identifying which transcripts are targeted to dendrites and understanding the molecular machinery that regulates this process. One of such mRNAs encodes Brain Derived Neurotrophic Factor (BDNF), a key molecule in neuronal development that plays a critical role in learning and memory and displays abnormal levels in several neuropsychiatric and neurodegenerative disorders.
BDNF is the result of translation of at least 22 transcripts produced by alternative splicing of 9 upstream exons (exon 1-9a), each coding for the 5' untranslated region (5'UTR), spliced to a common downstream exon 9 that encodes the protein and two different 3'UTR sequences (Aid et al. 2007). We have recently shown that different BDNF transcripts are addressed to specific cellular districts (soma, proximal or distal dendrites) following neuronal activation in vivo (Chiaruttini et al. 2007). We therefore investigated the local protein synthesis of different BDNF mRNAs isoforms and the effects on morphology of dendritic arborization in young neurons and on formation of spines in mature neurons in culture. As these mRNA variants showed a specific effect on dendrite architecture in different subcellular districts and are also differentially expressed in response to various stimuli and antidepressant drugs, we propose that they represent a spatial code to regulate BDNF protein expression locally.

The endocannabonoid system and the regulation of pain, anxiety and mood

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The major psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol, affects emotional states in humans and laboratory animals by activating brain CB_1-type cannabinoid receptors. Two primary endogenous ligands of these receptors are anandamide, the amide of arachidonic acid with ethanolamine, and 2-arachidonoylglycerol (2-AG), the ester of arachidonic acid with glycerol. Anandamide and 2-AG are released in select regions of the brain and are deactivated through a two-step process consisting of transport into cells followed by intracellular hydrolysis. Selective pharmacological inhibition of anandamide deactivation – by inhibiting either anandamide transport into cells or its intracellular hydrolysis catalyzed by fatty-acid amide hydrolase (FAAH) – produces analgesic, anxiolytic-like and antidepressant-like effects in rats. These actions are not associated with behavioral responses typical of direct-acting cannabinoid agonists and are accompanied by profound changes in serotonergic adrenergic transmission. On the other hand, selective blockade of intracellular 2-AG hydrolysis – catalyzed by monoacylglycerol lipase – enhances stress-induced analgesia. These findings suggest that anandamide and 2-AG contribute to the regulation of pain and emotion, and that the deactivation of these endocannabinoid lipids might be the target for novel analgesic, anxiolytic and antidepressant drugs.

Plasticity and computation: from neurons to networks

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It is widely accepted that the two fundamental properties of neural circuits, which allow them to elaborate activity patterns needed to control behaviour, are single neuron computations and long-term synaptic plasticity. However, the relationship between these elementary mechanisms and the operations performed by the networks remain speculative in most cases. The cerebellum offers a special opportunity in this sense, since it controls timing of motor operations and can adapt behaviour to new sensory-motor associations. Timing and plasticity can occur already at the input stage, the granular layer, and then be continued through the molecular layer according to a distributed processing scheme. In the past decade, single neuron investigations in vitro and in vivo have highlighted the remarkable timing and plasticity capabilities of the granular layer. In this circuit, Golgi cells can control feed-forward, feed-back as well as lateral inhibition and, by regulating the level of granule cell depolarization, effectively regulate the spatial distribution of LTP and LTD at the mossy fiber – granule cell relay. This, in turn, by exploiting a presynaptic expression mechanism, regulates the dynamics of synaptic activation determining the first spike delay with millisecond precision. This microcircuit sets-up what we call a "time-window matching" mechanism, in which the Golgi cells close the permissive time window for optimal granule cell excitation and long-term synaptic plasticity determines whether spike threshold will be attained within such a window. Thus, the role of synaptic plasticity would be that of fine-tuning pre-wired circuits allowing the granular layer to perform a spatio-temporal transformation of the mossy fiber input to be relayed to Purkinje cells. This concept has wide implications for processing in the entire cerebellar cortex and of the olivo-cerebellar system as a whole.

A window with a view: structure and function in the cortex seen through the two photon microscope

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Two photon microscopy is emerging as a powerful tool to image form and function in the brain in vivo. Although there is a rich and ever-growing literature, two-photon imaging still requires ad hoc solutions specific to each laboratory and to each experimental approach. Neurons and glia can be visualised by bulk loading of membrane permeable indicators or by genetically encoded probes. For example, transgenic mice expressing GFP in selected neuronal population can be used to image the short term dynamics of dendritic spine motility.

The bulk injection of Ca sensitive dye allows to image activity in an intact preparation that, although inherently more complicated than in vitro models, offer the precious opportunity to analyse activity in conditions close to natural. This is very valuable when studying processes depending on intact long range connections that are invariably damaged in vitro. We have devised two models of pharmacologically induced epilepsy in which we can perform simultaneous field recording and calcium imaging: in one model we employ the isolated and perfused brain of the guinea pig, in the other we image calcium changes through a small craniotomy on the occipital cortex. The guinea pig preparation has the great advantage to allow the investigations of structures, such as the entorhinal cortex, that are not accessible in the intact animal. Upon administration of bicuculline both models display an epileptic behaviour with periodic interictal activity followed by high frequency critical activity. Imaging shows that the neuropile signal, largely originating from neurons, mirror the field recordings. In contrast, astrocytes remain silent during periodic bursts and are recruited only after the onset of the critical phase. The analysis of the cross-correlation spectra of the neuropile signal shows that the transition from high frequency to burst like activity is mirrored by the gradual re-emergence of spatial correlation.

Endocytic Zones regulate synaptic AMPA receptor abundance and mobility

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In excitatory neurons, surface AMPA Receptors (AMPAR) are internalized through a clathrin-mediated process. This occurs at endocytic zones (EZs), mostly located adjacent to the postsynaptic density (PSD). Recently it has been shown that dynamin-3 is responsible for this specific EZ localization, since impairment of this protein displaces EZs from the PSD, leads to a loss of synaptic AMPAR and reduces excitatory synaptic transmission.
In the present study we have investigated the influence of EZ positioning on the diffusion and accumulation of AMPARs at individual synapses and EZs. Single particle tracking and high resolution real-time fluorescence microscopy allowed demonstrating that the presence of EZs in the vicinity of the PSD maintains a mobile pool of AMPARs at synapses. During basal activity, displacement of EZs depletes synapses of AMPAR mobile pool. We also demonstrated that chemical stimulation, known to potentiate glutamatergic synaptic transmission, induces mobile synaptic receptor stabilization and AMPAR lateral recruitment to synapses. Displacement of EZs occludes all these effects. Thus we have shown that mobile AMPAR, maintained at synapses by the presence of EZs close to the PSD, are crucial for accumulating and stabilizing AMPAR at synapses during synaptic potentiation. Remarkably, single particle tracking of AMPAR also provided unprecedented evidence for EZs as temporary stabilizing areas for receptors diffusing in the vicinity of the PSD, possibly recruited at synapses to potentiate synaptic transmission.

Activity of adenosine receptors type 1 is required for CX_3CL_1-mediated modulation of glutamatergic currents in hippocampal neurons

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The chemokine fractalkine (CX_3CL1) is constitutively expressed by central neurons, regulating microglial responses including chemotaxis, activation and toxicity. Through the activation of its own specific receptor, CX_3CR1, CX_3CL1 exerts both neuroprotection against glutamate (Glu) toxicity and neuromodulation of the glutamatergic synaptic transmission in hippocampal neurons. Since CX_3CR1 is thought to be primarily expressed on microglial cells, experiments were addressed to understand the pathway of chemokine action on neurons.
Using whole-cell patch clamp recordings, we report that the neuromodulatory effect of CX_3CL1 on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- (AMPA-) type Glu receptor-mediated current (AMPA-current) is absent on cultured hippocampal neurons obtained from $CX_3CR1^{-/-}$ ($CX_3CR1^{GFP/GFP}$) mice. However AMPA-current depression is mimicked by exposing $CX_3CR1^{GFP/GFP}$ neurons to a medium conditioned with CX_3CL1-treated mouse microglial cell line BV2. In addition, we also report that this AMPA-current depression is sensitive to the Adenosine Receptor type 1 (AR1) antagonist DPCPX and that CX_3CL1 induces a significant release of adenosine from microglial BV2 cells, as measured by HPLC analysis.
Furthermore, experiments in cultured hippocampal neurons from $CX_3CR1^{+/+}$ mice and in pyramidal neurons from rat hippocampal slices show that (i) CX_3CL1-induced depression of AMPA-current, is associated with AR1 activity being blocked by DPCPX and (ii) the application of exogenous adenosine similarly induces depression of AMPA-evoked postsynaptic currents, which is again blocked by DPCPX.
We conclude that AR1 activation is necessary for CX_3CL1-induced modulation of glutamatergic currents and adenosine is a candidate mediator of chemokine action on hippocampal neurons.

Cell adhesion molecules of the immunoglobulin superfamily and hippocampal synaptic plasticity

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Two members of the immunoglobulin superfamily of cell adhesion molecules, L1 and the neural cell adhesion molecule NCAM, have been among first cell adhesion molecules suggested to play important roles in synaptic plasticity (Lüthi et al., 1994, Nature 372:777-9). These molecules are also linked to major human brain disorders, including mental retardation, fetal alcohol syndrome and schizophrenia. Analysis of mice deficient in NCAM uncovered that this molecule modulates induction of long-term potentiation (LTP) in the CA3-CA1 and mossy fiber-CA3 synapses in vitro, LTP in entorhinal cortex-dentate gyrus synapses in vivo and several forms of hippocampus-dependent memory. Furthermore, both long-term potentiation and depression in the CA1 region require an unusual carbohydrate, α2,8-linked polysialic acid (PSA), predominantly carried by NCAM. To differentiate between the functions of PSA versus the extracellular domain of the NCAM glycoprotein backbone, we applied NCAM, PSA-NCAM, or PSA to acute slices of the hippocampal CA1 region of NCAM-deficient mice. Remarkably, both PSA and PSA-NCAM, but not NCAM restored normal LTP. Furthermore, contextual and tone memory in NCAM-deficient mice could be partially rescued by injection of PSA-NCAM, but not of NCAM, into the hippocampus, again highlighting the role of PSA. In vitro experiments demonstrate that PSA suppresses activation of NR2B-containing NMDA receptors by low concentrations of glutamate, suggesting that PSA may restrain activity of these receptors extrasynaptically and thus support plasticity of CA1 synapses. In contrast to NCAM-deficient mice, mice conditionally deficient in L1 after cessation of major developmental events have normal CA1 LTP but exhibit interesting impairment in autoassociative spatial memory. Current investigations are targeted to dissect hippocampal synapses in which L1 regulates synaptic plasticity related to this form of memory and to characterize the underlying mechanisms.

Endogenous non neuronal modulators of synaptic transmission control spontaneous cortical activity *in vivo*

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Gliotransmission, the release of molecules from astrocytes, regulates neuronal excitability and synaptic transmission *in situ*. Whether this process affects neuronal network activity *in vivo* is not known. Using a combination of astrocyte-specific molecular genetics, with *in vivo* electrophysiology and pharmacology we determined that gliotransmission modulates slow oscillations, a cortical rhythm characterizing nonREM sleep. Inhibition of gliotransmission by the expression of a dominant negative SNARE domain (dnSNARE) in astrocytes affected slow oscillations, reducing the duration of neuronal depolarizations and causing prolonged hyperpolarizations. These network effects result from the astrocytic modulation of intracortical synaptic transmission at two sites: a hypofunction of postsynaptic NMDA receptors, and by reducing extracellular adenosine, a loss of tonic A1 receptor-mediated inhibition. These results represent the first demonstration that rhythmic brain activity is generated by the coordinated activity of the neuronal and glial networks.

Subcellular programmes for neurodegeneration

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Spinal and Bulbar Muscular Atrophy: different approaches to induce ARpolyQ clearance

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Spinal and Bulbar Muscular Atrophy (SBMA) is a motorneuronal disorder caused by polyglutamine expansion (polyQ) in the androgen receptor (ARpolyQ) protein, which is toxic to neurons. Aggregates are a typical SBMA hallmark; they contain the mutant protein, Ubiquitin and proteasome subunits, suggesting that the Ubiquitin-Proteasome system (UPS) may be involved in their formation. We have already demonstrated that the inactive (ARpolyQ) is localized in the cytoplasm impairing the UPS activity, while Testosterone (T) activation of ARpolyQ leads to protein aggregation into the cytoplasm, without affecting the UPS. This suggests a possibile protective role of these ARpolyQ aggregates in motor neurons. To counteract ARpolyQ toxicity, we have used initially 17-Allylamino-17-demethoxygeldanamycin (17-AAG), an Hsp90 inhibitor, which reduced ARpolyQ toxicity in SBMA transgenic mice. Using immortalized motor neurons (NSC34) expressing ARpolyQ, we have observed that 17-AAG treatment led to faster degradation of mutant AR without affecting the UPS. Using the N-terminal ARpolyQ lacking the binding site to Hsp90 (AR112?HA, courtesy of D. Merry), we found that 17-AAG induced AR112?HA degradation with a similar rate to that previously reported for the full-length ARpolyQ. This suggests that 17-AAG activity does not require the direct interaction between Hsp90 and AR, as it occurs physiologically. Next we evaluated the effect of HspB8 overexpression on UPS functions in presence of ARpolyQ. (proven to be active on other polyQ proteins). (Carra et al., Hum.Mol.Gen. 2005). HspB8 led to a robust reduction of ARpolyQ levels without overloading UPS both in the soluble and in the aggregated forms of ARpolyQ. 17-AAG and HspB8 may represent useful tools to improve ARpolyQ clearance without affecting UPS; it is still unknown whether they act by directing ARpolyQ towards other degradative system, like autophagy. Grants Telethon - Italy (#GGP06063, #GGP07063), University of Milan-FIRST.

Axonopathy and accumulation of hyperphosphorylated filamentous tau in retinal ganglion neurons of P301S tau transgenic mice

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Filamentous tau deposits (*e.g.* neurofibrillary tangles, NFT) are a defining pathological hallmark of tauopathies, which include Alzheimer disease (AD) and familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Altered levels of microtubule-associated protein tau have been detected in the retina and optic nerve of patients with glaucoma, suggesting that pathogenic mechanisms similar to those of tauopathies may underlie retina degeneration. Here, we used the P301S tau transgenic mouse model of tauopathy to investigate whether tau pathology develops in the retina and how it affects the viability of retinal ganglion neurons (RGC). We found that hyperphosphorylated transgenic tau accumulated in RGC of P301S tau retinas. NFT-like lesions first appeared in transgenic RGC at 7 weeks of age and contained tau filaments similar to those found in human NFT. Axonopathy was observed in retina and optic nerve of P301S tau transgenic mice. Axonal swellings were present in proximal, but not distal, optic nerves of P301S and P301SxYFP mice, contained degenerating mitochondria and were immunoreactive for phosphorylated tau. Dystrophic RGC axons accumulating phosphorylated tau were also detected in the retinal nerve fibre layer of 4-week old P301S tau transgenic mice. Axonopathy was not accompanied by overt RGC loss in transgenic retina. In conclusion, axonopathy is dissociated from neuronal loss and precedes NFT formation in the retina of P301S transgenic mice, recapitulating early stages of neurodegeneration. These findings, together with the accessibility of the retina to direct observation and drug delivery, suggest that RGC of the P301S transgenic tau mouse are a good model to investigate tau-driven neurodegeneration. *– LG is recipient of an Alzheimer Research Trust Fellowship – *__current address:__ Dept of Neuroscience and Brain Technologies, The Italian Institute of Technology, Genova, Italy*.

The cross-talk between the β- and the γ-secretase cleavages of the β-amyloid precursor protein

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The endoproteolytic cleavages operated by the γ-secretase and the β-secretase (BACE1) on the β-amyloid precursor protein (APP) result in the production of the β-amyloid (Aβ) species, with two C-terminal variants, at residue 40 or at residue 42. Accumulation in the brain of small, soluble aggregates of Aβ 42 peptides is the central pathogenic event of Alzheimer's disease (AD), and it is produced by different mechanisms. Mutations of presenilin 1 (PS1) gene linked to early-onset familial AD cause relative increased production of Aβ ending at residue 42 (Aβ 42). In sporadic late-onset AD the accumulation of Aβ 42 depends on the presence of apolipoprotein E allele 4, as well as on several age-dependent factors, such as oxidative stress. We discovered that both PS1 mutations and oxidative stress up-regulates the activity of BACE1, activating a positive feedback loop between the γ- and the β-secretase. Mutant PS1 increases BACE1 protein levels, through the overproduction of Aβ 42, the amyloidogenic effect of the altered γ-cleavage on APP. Oxidative stress up-regulates the expression of BACE1, through the γ-cleavage and the opposite signal transmitted by JNK and ERK1/2 pathways. In turn, the increased activity of BACE1 may result in overproduction of N-terminally truncated Aβ species, whose percentage, in the mixture of the Aβ species that compose soluble Aβ, is proportional to the neurotoxicity of Aβ.

Dissecting the role of microglia cells in the brain of mice affected by leukodystrophy after hematopoietic stem cells transplantation

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We previously showed that in the mouse model of Metachromatic Leukodystrophy (MLD) the efficacy of transplantation of gene-corrected Hematopoietic Stem and Progenitor Cells (HSPC) is based on microglia replacement by the myeloid progeny of the HSPC. These gene corrected cells become an effective source of the functional enzyme in the affected Central and Peripheral Nervous System (CNS and PNS), restore the normal microglia scavenger activity and reduce neuroinflammation. However, the actual contribution of donor cells to microgliosis and the importance of the disease burden and of an acute damage to the blood brain barrier in eliciting this phenomenon are still debated. In order to address this issue, we monitored the biodistribution of HSPC at different time points after the transplantation in both irradiated and non-irradiated MLD and WT mice. We followed the short-term fate of these cells using Magnetic Resonance Imaging (MRI), upon magnetic labeling. Their long-term fate was determined by pathology upon transduction with lentiviral vectors encoding GFP. Interestingly, shortly after the transplant we were able to detect labeled cells in the CNS of transplanted mice, preferentially located within areas of adult neurogenesis and of tissue damage in MLD recipients. Moreover, we identified in the c-kit+/sca-1+/Lin- stem cell fraction the population capable of CNS homing. Importantly, in the absence of myeloablation the HSPC, which migrated to the brain shortly after the transplant, survived up to 3-6 months within specific areas, proliferated to minor extent and retained an undifferentiated morphology. However, these cells were not able to provide full and extensive reconstitution of brain microglia, indicating that peripheral engraftment of the donor cells and irradiation are required in order to observe a contribution to microgliosis of the transplanted HSPC. Finally, we are now investigating the role of the environment to define the survival and proliferation of the HSPC in the CNS.

The mirror mechanism in monkeys and humans

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Primates, and humans in particular, are exquisitely social species. Their survival critically depends on their ability to understand what others do and feel. In my talk I will first describe the general properties of a neural mechanism -mirror neuron mechanism- that allows individuals to understand the actions done by others and their emotions. This mechanism produces, in the brain of the viewer, representations of the observed actions and observed emotions in a motor format. Because the observing individuals know the outcome of their motor representations, they are able to achieve, through the mirror mechanism, an immediate, *direct* knowledge of what the others do and feel. In the second part of my talk I will show that, while individual mirror neurons code the "what" of a given motor act (e.g. grasping), their "chained" organization enables the observer to infer the "why" of it (e.g. grasping-for-eating"), that is to read the agent's intention. I will conclude discussing the relationship between mirror mechanism and some aspects of autistic syndrome.

Abstracts

Posters

PS1.01

Development of expression cloning approach for the study of neural stem (NS) cell biology

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During central nervous system development, different types of neurons, and glia are generated from neural progenitors at specific times in a conserved sequential order. Co-operation of extrinsic and intrinsic factors determines changes in gene expression patterns that are the basis for neuronal cell type commitment and differentiation. In our studies of mammalian neurogenesis, we have turned to an in vitro system. Recent publications describe new protocols for the maintenance and propagation of adherent cultures of neural stem (NS) cells (Conti et al., 2005). We are manipulating the genome of NS cells by random integration of dominant enhancer/promoter sequences inserted into Sleeping Beauty transposons. Using this strategy we are generating a gain of function library of genetically modified NS cells. By sequencing and analysing transposon integrations in NS cell clones we have demonstrated the efficiency of our polyA trap system to generate stable genetic modifications in NS cells and to over-express the recruited genes. We will utilize this tool to dissect signaling pathways and regulatory cascades affecting self-renewal, proliferation, survival and fate specification.

PS1.02

Everything you always wanted to know about.... NGF signalling

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Signalling pathways activated upon ligand binding to surface receptors enable cells to sense and respond to changes in their environment. They are crucial for adaptation and cell fate. The neurotrophins NGF, BDNF, NT-3, and NT-4/5 control survival, differentiation, and plasticity of neurons. Their dysfunction is implied in neuro-degeneration, and in mood and anxiety disorders. They signal through a two receptor system: a TRK tyrosine kinase receptor (TRKA, TRKB, TRKC) and p75NTR. Modelling of neurotrophin signalling networks is necessary for integrating experimental knowledge into a coherent picture and to enlighten the underlying mechanisms. Modelling emphasizes the holistic aspects of signalling networks, which disappear if the components are studied in separation. Despite their diversity in function and design, many signalling pathways use the same essential components, which are often highly conserved. We have produced a high quality database of the NGF pathway, which represents the most exhaustive vision of the neurotrophin mode of action up to now. All events of TRKA and p75NTR receptor signalling cascades, from NGF binding to immediate early gene activation, have been annotated on the basis of solid literature data, and none was electronically inferred. The NGF signalling pathway is included into the Reactome knowledgebase, a collaboration among Cold Spring Harbor Laboratory, The European Bioinformatics Institute, and The Gene Ontology Consortium to provide integrated, qualitative views of human biologic processes in a computationally accessible form. The NGF signalling database is expected to be a key tool for future modelling efforts. Once a model has been designed, it will be an invaluable tool for hypothesis testing, simulations of biological response and targeted therapy design.

PS1.03

The α7 nicotinic acetylcholine receptor subunit duplicate form is downregulated in the monocytic cell line THP-1 on treatment with lipopolysaccaride

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The α7 nAChR subunit gene is partially duplicated, and here we investigate its expression in three monocytic cell lines: THP-1, U937 and Mono-Mac-6. Qualitative PCR revealed the presence of the classic α7 gene in these lineages, but we found using Real Time PCR, exclusive expression of α7dup. Both mRNA and protein levels were reduced in THP-1 on LPS challenge, and transcription downregulation was seen to be mediated by a direct mechanism reliant on NF-κB, as its specific inhibitor parthenolide, prevented α7dup transcript reduction. This precise regulation suggests that α7dup may participate specifically in the innate immune system's inflammatory response.

PS1.04

Retinoic acid controls Phox2a expression by means of a dual regulatory mechanism

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The specification of neuronal identity is the result of the interaction between two distinct classes of determining factors: extrinsic factors, including secreted or cell membrane-associated signals in the local environment, and intrinsic factors that often consist of transcription factors cascades. PHOX2A is a homeodomain protein that participates in the network regulating the development of autonomic ganglia. We used an undifferentiated human neuroblastoma cell line to show that retinoic acid, a well-established extrinsic factor that profoundly affects the differentiation of sympathetic neurons at different developmental stages, regulates PHOX2A expression by means of a dual effect: it starts by acting as a positive regulator of gene expression, and later triggers a process, completely evident after 48 hours of treatment, culminating in the selective proteasome-mediated degradation of the PHOX2A protein, whereas the corresponding mRNA remained up-regulated. The persistence of PHOX2A protein, induced by treatment with proteasome inhibitors, resulted in a selective dis-regulation of the trascription of the Dopamine-β-hydroxylase, a well characterized PHOX2A target gene. This suggests that the expression of PHOX2A must be finely regulated during development in order to direct neurons towards the terminal noradrenergic differentiation.

PS1.05

Synapsin knockout mice: an *in vitro* model of human epilepsy

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Epilepsy is a neurological disorder that affects 5% of the world's population. Epilepsy has a strong genetic component; interestingly, mutant mice lacking synapsins, a family of abundant proteins of synaptic vesicles implicated in the regulation of neurotransmitter release and synapse formation, are epileptic. The attacks appear after the third month of age and their severity increases with age. Synapsin KO mice (SynKO) represent valid experimental models of genetic epilepsy in view of the recent identification of several nonsense and missense mutations in the SYN genes in families of patients with partial epilepsy
We used planar Micro-Electrode Array (MEA) recordings to study spontaneous and electrically or chemically evoked epileptiform activities in acute horizontal brain slices obtained from wild type (WT) and SynKO mice.
SynKO slices from adult mice showed a clear epileptiform activity characterized by spontaneous interictal (I-IC) events and ictal (IC) discharges, which were sporadic in WT mice. An electrical stimulation applied at the level of the entorhinal cortex elicited IC and I-IC waveforms only in SynKO slices. The application of the convulsant agent, 4-aminopyridine (4-AP; 200μM) to slices from young mice induced a sustained I-IC activity and rare IC discharges in both WT and SynKO slices. However, I-IC frequency recorded in SynKO was higher than that observed in WT slices. The epileptiform activity of SynKO slices evoked by 4-AP stimulation, studied in slices obtained from old mice, revealed a clear age-related aggravation which paralleled the increase in the severity of the epileptic phenotype observed in vivo. The number of slices which present at least an IC was significantly higher in adult SynKO mice than in WT.
These preliminary results indicate that SynKO mice represent a reliable model of human epilepsy, useful to study how neuronal network hyperexcitabilty due to mutations in SV proteins leads to the development of epileptiform activity.

PS1.06

Regulation of Brain-Derived Neurotrophic Factor expression by cocaine in rat brain and its modulation by stress

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Several lines of evidence show that single or repeated exposure to drugs of abuse produce long-lasting functional and structural changes in brain areas known to mediate drug seeking. Since neurotrophic factors have been shown to regulate synaptic plasticity, they might contribute to the neuroadaptive changes set in motion by drugs of abuse. We investigated the modulation of the neurotrophin brain-derived neurotrophic factor (BDNF) following single or repeated injections with cocaine. In prefrontal cortex, a single dose of cocaine increases BDNF mRNA levels 2 hours after the injection, an effect that persists at least for 24 hours and is paralleled by enhanced expression of mature BDNF protein. Five consecutive injections potentiate this increase 2 hours after the last treatment, with no effect 72 hours later. Conversely, pro and mBDNF protein forms were reduced 2 and 72 hours post-injection in the cytosol. Interestingly, in this fraction of striatum, we found that repeated cocaine injections increased proBDNF levels without altering the mature form of the neurotrophin.
Since it is known that stress is able to modulate behavioral and neurochemical responses to drugs of abuse, we also decided to investigate whether a chronic unpredictable stress could alter the short-term modulation of BDNF expression in prefrontal cortex after a single injection of the psycostimulant. Chronic stress prevented the increased expression of BDNF and its high affinity receptor TrkB after cocaine injection, which was instead evident in unstressed animals.
These results suggest that cocaine differently affects BDNF transcription, translation and protein processing in a region-selective fashion and that stress can interfere with the modulation of BDNF expression in response to the psychostimulant. They also identify a potential molecular target through which stress alters cellular sensitivity to cocaine, and might also be useful in understanding the mechanisms underlying brain vulnerability to stress.

PS1.07

Behavioural characterization of oxytocin mutant mice: implications for autism

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There is a growing interest in the neuropeptide oxytocin (OT) and its receptor (OTR) in the pathogenesis of autism. OT controls the ability to remember individuals previously encountered, a form of social recognition that is essential for the establishment of all complex relationships (Hollander et al., 2003) , suggesting that the OT system is involved in the normal processing of socially relevant cues. The crucial role of OT in regulating the social brain has been recently confirmed in knockout $OTR^{-/-}$ mice, an animal model characterized by marked defects in maternal and social behaviour (Takayanagi et al., 2005).
Aim of the present study was to examine the behavioural phenotype of heterozygous $OTR^{+/-}$ and knockout $OTR^{-/-}$ mice in comparison to wild type $OTR^{+/+}$ mice with particular attention for more specialized behavioural tasks relevant for autism such as the social approach to stranger mouse, the preference for social novelty and reversal of a position habit in an appetitive T-maze task (Crawley, 2007).
Preliminary results indicate that both $OTR^{+/-}$ and knockout $OTR^{-/-}$ mice have a good general health and normal neurological reflexes. However, they are severely impaired in : social recognition and social novelty test, reversal learning in the T-maze task. These findings further confirm that the OTR may play a role in core symptoms characterizing autistic disorders. Furthermore, the deficit found in the heterozygous $OTR^{+/-}$ mice makes these animals an interesting model for the in vivo screening of new pharmacological compounds targeting the OT/OTR system.

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PS1.08

Serotonin-1A receptors and indiscriminative aggression in genetically selected mouse lines

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Aggression has evolved to gain/defend access to resources and mates, and to defend oneself from a threat, and therefore it serves an adaptive purpose when appropriately expressed. In human societies, impulsive violent forms of aggression do not present these context-dependency characteristics and result in a problematic behavior that can be addressed only using specific animal models.
Therefore, we focus on three highly aggressive mouse lines, SAL, TA, and NC900, were established in three independent laboratories by artificially selecting and breeding mice with high trait-aggression levels together from three different mouse strains. Male mice of these three lines, when tested against a docile male cage-intruder, attacked quickly and with high durations and frequency of offensive behaviors. However, when tested against unfamiliar female-intruders or familiar females in a novel cage, most of the SAL males attacked and threatened, while only few TA and NC900 did so and in particular after a period of aggression-reinforcing manipulation. SAL aggression showed a highly repetitive offence pattern with low inhibition and sensitivity to the opponent's submission signals. Differently, inhibitory cues seemed to affect the aggression of TA and NC900.
Since serotonin has been negatively linked with high impulsivity and aggression traits, we investigated the serotonergic system of these mouse lines and their low-aggressive counterparts, LAL, TNA, and NC100. The negative relationship was confirmed in the prefrontal cortex serotonin (5-HT) levels, with SAL mice having the lowest amounts. SAL mice also showed higher 5-HT1A autoreceptor sensitivity to agonists, measured as acute body temperature reduction in response to agonist injection.
We conclude that SAL mice represent a good model for high indiscriminative/uninhibited male trait-aggression and that differential properties of 5-HT1A autoreceptors are linked with serotonin levels in key areas of behavior control.

PS1.09

Akt suppresses autophagy and triggers apoptosis in a model of familial neurohypophyseal diabetes insipidus

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Autosomal dominant familial neurohypophyseal diabetes insipidus (adFNDI) is a progressive, inherited neurodegenerative disorder that presents as polydipsia and polyuria as a consequence of a loss of secretion of vasopressin (VP) from pituitary nerve terminals. VP gene mutations cause adFNDI. Rats expressing an adFNDI VP transgene (Cys67stop) show a neuronal pathology characterized by autophagic structures in the cell body. AdFNDI has thus been added to the list of protein aggregation diseases which are associated with autophagy, a bulk process of lysosomal degradation. In this work we investigated the role of the Akt pro-survival signalling in the regulation of autophagy and of apoptosis linked with Cys67stop. Impairing the autophagy-lysosomal sequestration or the cathepsin D-mediated proteolysis triggered the activation of the intrinsic death pathway of apoptosis in Cys67stop-transfected cells, not in the parental VP-wild type-transfected cells. This was shown by genetic silencing of the lysosomal protease, by the expression of a Vps34 dominant negative, which down-regulates the class III PI3k-dependent signalling needed for autophagic vacuole (AV) formation, and by genetic silencing of Lamp2, a protein indispensable for the fusion of AVs with lysosomes. Ectopic expression of either the wild-type or the Cys67stop proteins did not alter the expression or Akt phosphorylation. Strikingly, the ectopic adenoviral-directed expression of a constitutively active Akt, instead of preserving cell survival, resulted in the suppression of autophagy and precipitated bax-mediated cell death. The present data demonstrate the need for autophagy mediated degradation of Cys67stop peptides that otherwise become toxic, and suggest that, in the presence of mis-folded proteins, the stimulation of the Akt signalling counteracts the beneficial effects of autophagy and precipitates cell death. This can provide an explanation for the late onset and progressive neuronal cell loss observed in hypothalamic magnocellular neurons of adFNDI patients.

PS1.10

Characterization and functional implications of endocannabinoid system in SH-SY5Y cells

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The human neuroblastoma SH-SY5Y cell line is an experimental model for different neurodegenerative disorders. Endocannabinoids represent a new class of lipid mediators which includes amides, esters and ethers of long chain poly-unsatured fatty acids, isolated in many tissues and implicated in different biological actions. The main members of this group are anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol (2-AG). Cannabinoid receptors, their agonists and the enzymes responsible of the synthesis, transport and hydrolysis of AEA, 2-AG and congeners compose the "endocannabinoid system" (ECS). In this study, we report the biochemical, morphological and functional characterization of the main components of the ECS (type 1 and type 2 cannabinoid receptors, CB1R and CB2R; transient receptor potential vanilloid 1, TRPV1; *N*-acyl phosphatidylethanolamine-specific phospholipase D, NAPE-PLD; fatty acid amide hydrolase, FAAH; diacylglycerol lipase, DAGL; and monoacylgliceride lipase, MAGL) in SH-SY5Y cells. We found that these cells possess a full and functional ECS. In addition, we demonstrate that AEA induces, in a dose-dependent manner, apoptosis of SH-SY5Y cells. By proteomic approach, we also show that apoptosis triggered by AEA is paralleled by the modulation of several genes, some of which are specifically implicated in the apoptotic process. In conclusion we show an active ECS in human SH-SY5Y cells, and report an unprecedented proteomic analysis of normal and apoptotic cells induced by the endocannabinoid AEA.

PS1.11

TI-VAMP-dependent secretory pathway mediates the release of cathepsin B from astrocytes and glioma

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Infiltration of malignant brain tumours mainly derives from the local destruction of the extracellular matrix by proteolytic enzymes, such as cathepsins. The mechanism underlying cathepsin's release by glioma is however largely unknown. In the present study we identified a novel vesicular pathway, relying on the v-SNARE TI-VAMP/VAMP7, involved in the storage and release of the cysteine protease cathepsin B in astrocytes and gliomas. Cell fractionation and immunofluorescence experiments revealed that TI-VAMP vesicles are distinct from either synaptobrevin/VAMP-2 positive clear vesicles, releasing glutamate, or secretogranin II-positive large dense core granules, containing ATP. Electron microscopy and biochemical analysis of immunoisolated TI-VAMP positive organelles showed that these vesicles contain pro-cathepsin B, rab 7 and rab 27, are devoid of rab 5 and transferrin receptor, and have a multilamellar appearance, thus are putative secretory lysosomes. Down-regulation of TI-VAMP expression by small RNA-interference decreases pro-cathepsin B release from glioma cell lines. These data indicate a major role for TI-VAMP in cathepsin B secretion and identify the TI-VAMP protein as a novel, potential target for treatments aimed at reducing glioma invasiveness.

PS1.12

Expression of RE1 silencing transcription factor (REST) in an model of in vitro epilepsy

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Repressor Element-1 Silencing Transcription factor (REST) governs the repression of multiple neuronal target genes in non-neuronal cells. In neural progenitors, REST is expressed in the course of embryogenesis to prevent the premature expression of terminal differentiation genes, which takes place only after its down-regulation. In postmitotic neurons of the adult central nervous system, the levels of REST are low but often detectable, reaching the highest levels in the hippocampus. Interestingly, increases of REST expression have been reported in neurons under pathological conditions, such as hypoxia, and during intensive activity. In the present work we studied, by immunocytochemistry and immunoblot, the induction of REST expression in in vitro models of epilepsy triggered by treatment with 4-aminopyridine (4-AP, 100 μM). Six hr treatment of mixed cortical neuronal/astrocytic cultures induced in neurons, with respect to the neurons of untreated cultures, considerable increases of REST levels, whereas treatment for shorter times (90 min) had no effect. Interestingly, these treatments were unable to affect the REST levels of astrocytes. Similar findings were obtained in mouse hippocampal cultures. Neurons stimulated for 3-6 hrs with 4-AP exhibited higher cytoplasmic REST immunoreactivity when compared with untreated neurons. Finally, the data obtained on cultured neurons were confirmed in hippocampal slices obtained from 1 year-old mice where the REST expression levels, detected by immunohistochemistry, were increased in the CA1 region already after 2 hrs of 4-AP treatment. Taken together, our data demonstrate that REST increases during states of intense neuronal network activity, such as epilepsy, and might play a role in the changes of gene expression and the ensuing degenerative events taking place in neurons.

PS1.13

Synapsins interact with SH3 domains of proteins involved in synaptic vesicles endocytosis

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Synapsins (SYNs) are synaptic vesicle (SV)-associated phosphoproteins involved in the formation of presynaptic specializations and in the regulation of neurotransmitter release. Recent studies demonstrated that SYN I migrates to the endocytotic zone of central synapses during neurotransmitter release, suggesting additional functions for these proteins in SV recycling. In order to uncover a possible role in endocytotic processes, we studied their interactions with the Src homology 3 (SH3) domains of molecules involved in SV recycling, namely intersectin and endophilin-1. Both intersectin and endophilin are multidomain dynamin-binding proteins containing five (A-B-C-D-E) and one SH3 domains, respectively. By using Far Western and pull-down assays, we found that SYNs I-II bind with high affinity the A and C SH3 domains of intersectin and the SH3 domain of endophilin-1. The interaction was independent of the phosphorylation state of SYN I at serine residues. The binding to the intersectin A SH3 domain and endophilin SH3 domain decreased the ability of SYN I to stimulate the bundling of actin filaments. Furthermore, these domains show a decreased binding to SV obtained from SYN-KO mice with respect to SV obtained from WT littermates. The functional role of these interactions was assayed by studying the effects of the internalization of intersectin A and endophilin SH3 domains on neurotransmitter release from synaptosomes obtained from WT and SYN-KO mice. Both domains induced a significant decrease in depolarization-evoked neurotransmitter release in WT synaptosomes, while only intersectin A affected the ionomicyn-evoked release. These effects were significantly attenuated in SYN-KO synaptosomes. The specific binding between SYNs to the SH3 domains of proteins involved in SV endocytosis suggests additional functions of the SYNs in the organization of the actin cytoskeleton of the periactive zone and/or in the regulation of the nerve terminal fate of the endocytosed SVs.

PS1.14

Biological activity and regulation of the two isoforms encoded by the *EFA6A* gene in neuronal cells

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The processes of neurite extension and remodeling require a close coordination between the cytoskeleton and the cell membranes. The small GTPase ARF6 plays a central role in regulating membrane traffic and actin dynamics, and its activity has been demonstrated to be involved in neurite elaboration.
EFA6A is a guanine nucleotide exchange factor (GEF) for ARF6. We have shown that two distinct polypeptides encoded by the *EFA6A* gene are expressed in murine neural tissue: a long isoform, EFA6A, and a short isoform, EFA6As. EFA6A encompasses proline-rich regions, a Sec7 domain (mediating GEF activity on ARF6), a PH domain, and a COOH region with coiled-coil motifs. EFA6As lacks the Sec7 domain, and it comprises the PH domain and the COOH-terminal region. The two isoforms have distinct biological activities in primary cortical neurons: EFA6A promotes neurite extension, whereas EFA6As induces dendrite branching.
Expression of the two *EFA6A* isoforms is the result of alternative promoter usage. We have identified the minimal sequence that drives neuron-specific transcription of EFA6As; this region contains multiple consensus sites for Sp transcription factors, that play a role in the regulation of several genes involved in neuronal differentiation and plasticity.
We have explored the role of post-translational modifications in the regulation of the *EFA6A*-encoded polypeptides, and we have shown that the EFA6As protein is phosphorylated in transfected neuronal cells. We have identified the phosphorylated serine residues, and demonstrated that phosphorylation plays a role in dendrite branching induced by EFA6As.
We have shown that EFA6As phosphorylation is reduced by inhibiting the Cdk5 kinase, indicating Cdk5 as the candidate kinase involved in EFA6As phosphorylation. Using phospho-specific antibodies, we have observed that both EFA6A and EFA6As are phosphorylated in murine brain extracts, suggesting that this modification could be involved in regulating their activity *in vivo*.

PS1.15

Stress induce alteration in microRNA expression

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A fascinating area of current research tries to uncover the neural circuits, which underlie specific behaviors, and to determine how these circuits are modified by experience. Human and animals studies have demonstrated that both acute and chronic aversive stimuli can affect neural activity in different brain areas. In particular it has shown that stressful event can induce short-term changes in neural transmission but also long-term changes that Those alterations have been related to impairments in memory but also human diseases such as anxiety and depression. Therefore it is very interesting to determine the molecular mechanism undelying stress induced changes.
Molecular studies in the fruit fly, as well as the analysis of dendritic spine in the development of mouse hippocampus and neurite sprouting from rat cortical neurons, reveal an attractive and conserved mechanism of plasticity involving microRNA (miRNA). These recently identified short RNA molecules operate by regulating local proteins synthesis and probably they maintain synapses in altered state over time. We resolved miRNA expression profiles in different mouse brain regions by an LNA based microarray platform. Different microRNA expression was found in different brain region. Further specific miRNA expression was observed after acute but not repeated stress. The dysregulation of specific miRNA levels will be analyzed with the aim of identifying clusters of miRNAs with common potential targets involved in synaptic plasticity.

PS1.16

Neurological impairment in autosomal recessive malignant osteopetrosis due to mutations in OSTM1 and ClCN7 genes

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Osteopetroses (arOP) are rare bone diseases due to defective bone remodelling. The genes involved in the diseases affect either the functionality of mature osteoclasts (TCIRG1, ClCN7, OSTM1) or the differentiation of the osteoclasts (RANKL).
All forms are characterized by severe bone sclerosis that causes fractures, growth failure anemia, pancytopenia and hepatosplenomegaly due to the reduction of bone marrow cavities. ArOP patients show neurological impairment. These defects affect the children in early life and lead to fatal outcome.
In arOP due to mutations in TCIRG1 the neurological defects (blindness, hydrocephaly) are secondary to bone sclerosis, while in arOP ClCN7 and OSTM1 dependent forms, the neurological impairment (primary retinopathy, severe cortical atrophy) is due to loss of function of these proteins. It has been demonstrated that both proteins co-localize in late endosomes and lysosomes and that ClC-7 and OSTM1 form a molecular complex in which OSTM1 is a β-subunit of ClC-7.
We have demonstrated, that in the ClCN7-dependent form the neurological defects is severe progressive cortical atrophy, while in the OSTM1-dependent form cortical dysplasia, dysmyelination and seizure occur.
We analyzed the nervous system in the spontaneous grey-lethal mouse model that most closely resembles the OSTM1 human arOP. The gl/gl mouse brain is smaller in size and shows loss of demarcation between the white and the grey matter. Histological analysis evidenced hypomyelinated fibres at corpus callosum and cerebellar level, generalized dysmyelinogenesis and presence of gliosis. We confirmed the presence of storage of lipofilic material in the neurons. In addition we found disorganization in pyramidal neurons at cortical level and the presence of high number of GABAergic neurons in the gl/gl brain when compared to wild type control.
Our preliminary data show that the gl/gl neurological involvement appears very complex but clearly suggests that OSTM1 has an important role in the development of the CNS

PS1.17

Proteomic analysis in hypothalamus, hippocampus and pre-frontal/frontal cortex after PACAP administration in rat brain

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The neuropeptide PACAP was discovered due to its ability to increase cAMP intracellular concentration. PACAP effects are mediated through the binding to two receptor classes. Both Type I and Type II binding sites are G protein coupled receptors. PACAP exerts pleiotropic functions, both in the CNS and in peripheral organs, and acts as a hypophysiotropic factor, a neurotrophic and a neuroprotective agent.
In this study, the proteome variations, induced by PACAP treatment, were analyzed in rat hypothalamus, hippocampus and pre-frontal/frontal (PF/F) cortex. To this aim, after central PACAP administration in rats, a proteomic study was performed by 2-D electrophoresis. Maps prepared from PACAP-treated animals were compared to maps obtained from vehicle-treated rats. Proteins showing an altered expression profile after the treatment were revealed by statistic analysis. Subsequently, spots were identified by mass spectrometry.
Identified proteins are correlated to PACAP ability to modulate different molecular processes in CNS. For instance, altered expression levels were observed for proteins correlated to synaptic plasticity: actin in the hypothalamus; stathmin, dynamin, profilin and cofilin in the hippocampus; synapsin in PF/F cortex. Furthermore, a modulation of expression levels of proteins involved in cellular differentiation was revealed: glutathione S-transferase alpha and peroxiredoxin in the hippocampus; nucleoside diphosphate kinase in PF/F cortex. In addition, altered expression levels were detected for proteins involved in neuroprotection, neurodegeneration and apoptosis: ubiquitin carboxyl-terminal hydrolase isozyme L1 and HSP 90-β in the hypothalamus; α-synuclein in the hippocampus; GAPDH and prohibitin in PF/F cortex.
In conclusion, the proteomic analysis, performed in this study, identified some proteins that may be involved in the molecular mechanisms mediating PACAP functions in CNS.

PS1.18

Identification of the molecular basis of epilepsy associated with mutation in human synapsin I

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The synapsins (syn) are a family of synaptic vesicle-associated phosphoproteins implicated in the control of neurotransmitter release and neuronal development. The identification of a nonsense mutation (G1197A) in the SYN1 gene in a family displaying X-linked epilepsy and/or mental retardation points to a role for synI in human epilepsy.
In principle, the presence of a premature stop codon (PTC) could be recognized by the nonsense-mediated mRNA decay (NMD) surveillance mechanism, leading to mRNA degradation and preventing translation of the aberrant protein. To test this hypothesis, we generated a minigene expressing human synI with or without the G1197A mutation. The splicing of the RNAs transcribed from these constructs leads to the exon-junction complex deposition, that is necessary during the NMD process to recognize the presence of the PTC. We measured the human wild type (wt) and nonsense (ns) synI mRNA levels in NMD competent HeLa cells and observed a 90% reduction of the ns synI mRNA with respect to wt synI mRNA. Furthermore, the mRNA transcribed from an intronless full length synI cDNA containing the PTC was not degraded, supporting a role of NMD in the reduction in synI mRNA levels.
To evaluate whether the small percentage of mutated synI which escapes NMD might induce pathologic effects in neurons, we fused it to EYFP to allow its in vivo tracing and expressed it in primary hippocampal neurons derived from either wild type or synI KO mice. The mutant protein lost its selective targeting to synapses and was diffuse throughout the neuron. The mistargeting was not corrected by the presence of endogenous synI. In addition, in neurons expressing mutant synI neuritic extension was impaired. Since synI KO mice display an intense epileptic phenotype, the strong reduction in the expression of synI and the mistargeting of the mutant synI which escapes NMD might explain the epilepsy observed in patients carrying the SYN1 mutation.

PS1.19

Coupling specificity of the human OTR: BRET assays and dual modulation of inward rectifier potassium currents by oxytocin

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It has become apparent that oxytocin (OT) in addition to its role as a circulating hormone, also acts as neuromodulator. OT acts by binding to and activating a GPCR, known to couple to the Gq pathway. However, as already shown for other GPCRs, also the OTR may couple to different G-protein types and effector molecules which in turn could bring to completely different cellular responses. We have explored the coupling specificity of human OTR by applying a BRET assay. The experiments were performed in HEK293 cells co-expressing OTR-Luc, $G\gamma_2$-GFP10 and untagged complementary subunits of the G proteins. We found that the stimulation with OT promotes a significant increase in BRET between OTR-Luc and $G\gamma_2$-GFP2. This increase was significantly potentiated in cells coexpressing $G\alpha_q$, $G\alpha_o$, $G\alpha_{i1}$ and $G\alpha_{i2}$, but not $G\alpha_s$ indicating that $G\alpha_q$, $G\alpha_o$, $G\alpha_{i1}$ and $G\alpha_{i2}$ subunits favoured the interaction between the OTR ang $G\gamma_2$ and therefore suggesting a high level of "promiscuity" for the human OTR. To further explore the coupling specificity of OTR, we took advantage of the well known modulatory action of several GPCRs on IRK family channels and tested the effect of OTR activation on the electrophysiological properties of those ionic conductances, both in GN11 cells, an immortalized GnRH-positive murine cell line exhibiting the features of immature olfactory neurones, and in oligodendrocytes from murine hyppocampal primary cultures. Here we show that OT focal application had a dual action on both GN11 cells and oligodendrocytes by either inhibiting or activating K conductances belonging to the IRK family. The inhibitory action of OT was pertussis toxin (PTX)-insensitive, in line with the view that OTR is a prototypical GPCR coupling to the PTX-insensitive G protein $\Gamma\alpha_{q/11}$. By contrast, the stimulatory action of OT was PTX-sensitive, suggesting that OTR stimulation caused IRK channel activation by coupling to a PTX-sensitive G protein.

PS1.20

Peripheral neuropathy in *Ebf2* null mice

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Ebf2 is a transcription factor largely expressed in the nervous system during development. The phenotypic analysis of the *Ebf2* null mutant demonstrated that, although the *Ebf2* gene is not essential for completion of embryogenesis, the mutant mouse exhibits a combination of neuroendocrine, cerebellar and Peripheral Nervous System (PNS) abnormalities. A preliminary characterization of the PNS phenotype performed postnatally (P30) by electrophysiology on the tail nerves suggests that the sensory system is not altered whereas the motor system is impaired. We found a significant decrease in the amplitude of motor potential of *Ebf2 -/-* tail nerves compared to controls, suggesting a reduction in the number or the calibre of motor axons. Moreover we observed a significant decrease in conduction velocity of mutant motor fibers, suggesting the presence of alterations also in the myelin compartment. In agreement with these results we observed by morphological analysis of sciatic nerves the occurrence of fibers with reduced axonal caliber in *Ebf2 -/-* mice; the expression of *Ebf2* in postnatal motorneurons could account for these defects in motor axons. Moreover, sciatic nerve analysis revealed hypomyelination of several fibers in mutant nerves, the expression of *Ebf2* in Schwann Cells could account for these defects in the myelin sheath. Taken together, these data strongly suggest the occurrence of a motorneuro(no)pathy in *Ebf2 -/-* mice. Whether this phenotype is due to a primary defect in the cell body/axon or in the Schwann Cell is not yet clear.

PS1.21

Atp1a2 *in vivo* model, developing and phenotyping the knock-in mouse model of Familial Hemiplegic Migraine type 2

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Severe attacks of migraine with aura and hemiplegia are the main symptoms of familial hemiplegic migraine (FHM). FHM-2 is an autosomal dominant disease due to mutations of the α2 subunit of Na/K ATPase. We generated and are now characterizing a knock-in mouse model carrying the FHM-2 mutation W887R. Neuroimaging findings have provided evidence that migraine aura arises from cortical spreading depression (CSD), a wave of neuronal depolarization that progresses slowly across the cortex. We have investigated CSD susceptibility in heterozygous knock-in mice by electrophysiological recordings. Mutant mice show reduced threshold and increased velocity of propagation that characterize an increased CSD susceptibility. Homozygous knock-in mice die just after birth. In tissues derived from these animals (E 19.5) the mutant protein, but not its mRNA, was strongly decreased compared to wild type.
The α2 subunit is expressed in neurons during late gestation, while in adult brain it is expressed primarily in astrocytes. This expression pattern suggests a role in the modulation of neuronal activity early in life. To study the cell-specific role of the α2 Na/K ATPase, we are generating a conditional knock-out mouse to obtain selective expression of the Atp1a2 gene in astrocytes or neurons.

PS1.22

Analysis of P/Q-type CA^{2+} channel variants identified in patients with familial hemiplegic migraine

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It has been known for many years that Migraines may have a genetic component and the first gene (CACNA1) involved in a genetic form of inheritable migraine (familial hemiplegic migraine) has been identified on chromosome19.13. This gene codes for the alpha1A subunit of P/Q type calcium channel and we have recently identified new mutations and genetic variants located in the loop II-III of this subunit. This region contains a domain (referred to as synprint: synaptic protein interaction) that is known to bind SNARE proteins (syntaxin 1 and SNAP-25) or synaptotagmin. To understand better the role of these mutations in the activity and physiology of the channel, we are investigating whether they may have a role in channel transport, distribution and interaction with the proteins of exocytic complex. In order to evaluate any effect of these mutation on the transport of alpha1A subunit, cDNAs coding for the human alpha1A (wild type and mutants) were expressed together with the ancillary subunits in different cell types including primary cultured neurons. To follow the expression and distribution of P/Q channels, we have developed different tools, including GFP-tagged forms of the alpha 1A subunit under the control of different promoters, and new antibodies directed against the human isoform of the channel. Expression in HEK cells and cultured hippocampal neurons revealed correct targeting to the membrane of the GFP-tagged form of wild type alpha1A. The analysis of alpha1A variants is under investigation. Preliminary results indicate that point mutations in the synprint region do not largely modify transport and localization of P/Q channel variants in the axon and growth cones. Experiments are now in progress to analyze the interaction of these variants with SNARE proteins.
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PS1.23

Molecular characterization of CLCN1, SCN4A, KCNJ2, CACNA1S genes in patients with muscle channelopathies

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Muscle channelopathies represent a group of neuromuscular disorders caused by functional alteration of ion channels and characterized by genotypic and phenotypic heterogeneity. We analyzed CLCN1, SCN4A, KCNJ2, CACNA1S genes in a cohort of patients whose anamnesis', clinic, and neurophysiologic testing strongly suggested the following diagnoses: Thomsen's and Becker's Congenital Myotonia, Von Eulenburg Congenital Paramyotonia, and Ipokalemic Periodic Paralysis (HOKPP).
Molecular screening of CLC-1 channel led to the identification of seven new mutations (R338X, 1110delC, T533I, V536L, Q812X, G846S, V947E), besides to three mutations previously described (F167L, G190S, M485V). One case of Thomsen's Myotonia and six patients with Becker's Myotonia were observed. Clinical data included: upper limbs myotonia, tongue myotonia, muscular hypertrophy and stiffness; one patient presented with pain not diminishing with exercise (no warm-up), together with a possible neuropathy.
Here we describe two families affected by Congenital Paramyotonia, carrying R1448C and T1313M in SCN4A gene, and showing typical myotonic symptoms cold-worsening.
Furthermore, a 15 years-old proband showed recurrent episodes of ipokalemic periodic paralysis associated with R672H mutation in SCN4A gene inherited in a dominant manner. This study confirms the importance of genetic screening for the confirmation of clinically diagnosed channelopathies.

PS1.24

Synaptoproteomics of an animal model of depression combining combining genetic vulnerability and early-life stress

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Recent studies highlighted the important influence of environmental stress factors on an individual's genetic predisposition to develop mood disorders. Indeed, the experience of stressful events in childhood, such as neglect, abuse or parent loss, was found to increase the risk for the development of depression in adult life. The Flinders Sensitive Line (FSL) rats are a well-validated animal model of depression carrying genetic vulnerability associated to distinct features of pathology and to responsiveness to antidepressant drugs. To reproduce early life stress events the FSL rats and their control, the Flinders Resistant Line (FRL) rats, were subjected to a maternal separation protocol (180 min/day from postnatal days 2 to 14). Treatment with the antidepressant (AD) escitalopram (25 mg/kg/day), was carried out at weeks 11-14 of age. Global analysis of protein expression is a powerful approach to gain insight into the molecular mechanisms underlying vulnerability to psychiatric disorders and the long-term action of drug treatments. Many of the biological targets of AD are localized at synapses; thus to reduce the complexity of the proteome analyzed and to enrich for less abundant proteins, purified nerve terminals (synaptosomes) from prefrontal/frontal cortex and hippocampus of FSL and FRL rats were used. Synaptosomes from 8 rats per group were purified on Percoll gradients and analyzed by two-dimensional polyacrylamide gel electrophoresis.
Protein spots differently regulated in the various comparisons were excised from gels and identified by mass spectrometry analysis, by comparison with SwissProt and NCBI databases. In the various comparisons between groups, proteins related to synaptic function (NSF, CaMKII, Munc-18, Dynamin-1, Clathrin light chain B) and to stress response or oxidative stress and apoptotic pathways (Prohibitin, 14-3-3 protein, Peroxiredoxine 6, Aconitase hydratase, Voltage-dependent anion selective channel protein 1) were identified.

PS1.25

Synaptophysin tyrosine modifications induced by peroxynitrite affect protein-protein interaction and signalling

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The reactive species peroxynitrite (PN) is a potent oxidant that contributes to tissue damage in neurodegenerative disorders. We previously reported that treatment of rat brain synaptosomes with PN induced posttranslational modifications in pre- and post-synaptic proteins resulting in: (i) increase in the SNARE complex formation and exocytosis; (ii) tyrosine nitration and phosphorylation of the synaptic vesicle protein synaptophysin (SYP), and tyrosine nitration of two other pre-synaptic proteins, SNAP25 and Munc18; (iii) upregulation of *c-src* tyrosine kinase activity. The SYP C-terminus characterized by ten copies of a tyrosine-rich pentapeptide repeat [YG(P/Q)QG] mediates the binding with proteins involved in vesicle release and recycling process at nerve terminals. Moreover, SYP C-terminus is a substrate of *c-src*. We showed that SYP could be both phosphorylated and nitrated following PN treatment of synaptosomes. Lower concentrations of oxidant (up to 0.1 mM) preferentially promoted phosphotyrosine formation, whereas higher concentrations (up to 1 mM) resulted in nitrotyrosine formation. In this study, we investigated whether tyrosine residues modifications (nitration and phosphorylation) of SYP affect its interactions. Pull-down assay showed that the phosphorylated form of SYP, but not the nitrated one, binds to *c-src*-SH2 domain and enhances *c-src* kinase activity. Among the nine tyrosines present in the SYP C-terminus, mass spectrometry (MALDI-TOF) revealed that the Tyr250 was nitrated both in GST-SYP nitrated *in vitro* by PN and in SYP purified from 1mM PN-treated synaptosomes. It is noteworthy that a different tyrosine was phosphorylated in SYP isolated from 0.1mM PN-treated synaptosomes in GST-SYP phosphorylated *in vitro*. Our work suggests that modifications of different tyrosine residues induced by PN at the nerve terminals affect SYP function in the mechanisms underlying synaptic plasticity and/or neurotoxicity.

PS1.26

Interaction with BiP chaperone determines the intracellular localization of normal and mutant P0 glycoprotein

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In peripheral nerve, myelin is formed by Schwann cells that enwrap axons in a 1:1 relationship. Myelin protein zero (MPZ) encodes P0, the most abundant glycoprotein synthesized by Schwann cells. Deletion of serine 63 (S63del), in the extracellular domain of P0, causes Charcot-Marie-Tooth 1B (CMT1B) in humans and a similar demyelinating neuropathy in transgenic mice. P0S63del is retained in the endoplasmic reticulum and induces the unfolded protein response (UPR). In S63del mice the UPR is pathogenetic, as ablation of the UPR mediator Chop ameliorates demyelination. S63 is the first amino acid of the β-strand C of the extracellular immunoglobulin-like domain of P0. According to an algorithm described in Bond-Enguldi et al., β-strand C is a putative binding site for the molecular chaperone BiP. We show that the retention of P0S63del depends on the alteration of the hydrophobic/hydrophilic pattern of the β-strand C rather than on the deletion of S63 per se. Furthermore, we show that retention of the mutant protein is likely to depend on binding with BiP. These results suggest that misfolded P0S63del activates UPR by binding BiP and altering its relationship with UPR sensors in the endoplasmic reticulum.

PS1.27

Vesicle shedding from microglial cells and astrocytes

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Microvesicles are particles commonly released from activated haematopoietic, immune and tumor cells, and they are thought to mediate fundamental cellular responses such as homeostasis, immune reactions and intercellular signalling. Presently, very little is known about release and origin of microvesicles in the CNS. In a previous work from our laboratory we demonstrated that microparticles were released by primary cultures of microglial cells upon ATP-mediated activation, and that these vesicles also transported the pro-inflammatory cytokine IL-1beta. In the present study 1) we demonstrate that also primary astrocyte cultures release vesicles upon ATP stimulation, 2) we define the molecular mechanisms underlying formation and shedding of these particles from glial cells, 3) we introduce a protocol for differentiated isolation of microparticles including membrane vesicles and exosomes, 4) and we extend investigation of glial-derived microparticles to *in vivo* systems such as cerebrospinal fluid from rat and human. In particular, we find that binding of ATP to P2X7 receptor induces a signalling cascade in glial cells, involving p38MAPK and acid sphingomyelinase A-SMase as principal key players in the formation and shedding of microvesicles which also contain inflammatory mediators such as IL-1beta. Our very first demonstration of the obligatory role of A-SMase for the release of (IL-1beta containing) particles from glial cells, and the possibility to modulate this pathway through pharmacological treatments open new strategies for the treatment of neuro-inflammatory diseases.

PS1.28

Differential regulation of EAAC1 and GLT1 glutamate transporters by calcineurin

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Glutamate transporters play a fundamental role in maintaining extracellular glutamate below neurotoxic levels. Decreased glutamate transport activity is involved in neurodegenerative diseases such as Alzheimer's, Parkinson's and Amyotrophic lateral sclerosis (ALS). The transport activity and surface expression of neurotransmitter transporters are often dynamically regulated through modulation of their intracellular trafficking. In the Madin-Darby canine kidney (MDCK) cell line, we have shown that PKC activation induces a time-dependent decrease in glutamate transport activity associated with clathrin dependent internalization of the transporter and relocation to recycling endosomes within 20 minutes. Using 32P-metabolic labeling experiments, we showed that PKC activation induces an early transitory peak of phosphorylation of an otherwise dephosphorylated surface transporter (5-10 min), and that accumulation in recycling compartments coincides with dephosphorylation of EAAC1 to its basal level. In addition, we found that PKC-induced decrease in transport activity and the intracellular relocation of EAAC1 also depended on the phosphatase activity of calcineurin (CaN). In contrast, we found that internalization and relocation of the GLT1 astroglial glutamate transporter to EAAC1 containing recycling compartments was dependent on PKC activation but independent of CaN activity. Moreover, an analysis of GLT1 and EAAC1 chimeras revealed that the cytosolic tail of EAAC1 (last 91 cytosolic C-terminal residues), containing sequences for its regulated trafficking, was sufficient to confer CaN dependence to PKC regulated internalization of GLT1. We are currently investigating the possibility that the tail of EAAC1 targets the protein to specific pathways of endocytosis sensitive to CaN activity. The existence of different mechanisms to modulate the trafficking of a specific glutamate transporter may explain the exclusive reduction of GLT1 in ALS.

PS1.29

Dynamic expression of Cx47 in brain development and in the cuprizone model of myelin plasticity

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Connexin47 (Cx47) is a member of the connexin gene family, specifically expressed in oligodendrocytes of adult brain. Previous *in situ* hybridization studies showed Cx47 mRNA in brain regions during postnatal development but no information were available about prenatal life. The present study first analysed the expression of Cx47 protein during pre- and postnatal development of mouse brain and in the cuprizone model of demyelination and remyelination (D/R). The expression of Cx47 was compared to expression profiles of Cx29 and Cx32 by using immunohystochemistry and western blot analisies. The Cx47 was expressed at early embryonic stages and declines during postnatal days towards adulthood. It was distributed along territories populated by developing oligodendrocytes to localize, in adult brain, in a few specific areas. The early expression of Cx47 in respect to Cx32 and Cx29, observed in this study, suggested a specific involvement of the Cx47 in the establishment of primordial contacts of oligodendroglia with other cell-types during brain development. In order to induce D/R, 8 weeks old C57BL/6 mice were provided a powder feed containing 0,2% cuprizone in the diet. The study showed that Cx47 was dynamically expressed at different times of D/R. It showed a persisted up-regulation, in variance to down-regulation of Cx32 and Cx29, which was related to cellular and myelin course occurring during the process. The expression of the Cx47 in the corpus callosum that was the mostly affected area in this experimental model, shifted from oligodendrocytes perykarya (in control animals), to myelin (in early suffering stage), to astrocytes (in the severe injury, when demyelinization occurred) to return to oligodendrocytes (in the recovery process). The dynamic expression profile of Cx47 suggests that it plays an active role in esthablishing an environment supporting remyelination.

PS1.30

Synapsins and synapse organization: role of site 1 phosphorylation

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The synapsins (Syns) are evolutionarily conserved neuron-specific phosphoproteins that localize at the presynaptic level. They are implicated in interactions with synaptic vesicle (SV) proteins, phospholipids and actin playing multiple roles at the synapse. Their biological activity is tightly regulated through phosphorylation.
Syn I is the best-characterized member of the family. It has been shown to control the availability of SV for exocytosis tethering the vesicles to each other and linking them to the actin cytoskeleton, thus regulating neurotransmitter release. It is known from literature that Syn I is involved in synaptogenesis and synaptic plasticity. The residue Ser 9 (site 1), phosphorylated by cAMP-dependent protein kinase and by CaM Kinase I/IV, is involved in precocious stages of neuron development.
We found that the overexpression of the Syn Ia non-phosphorylatable mutant in hippocampal neurons caused a decrease in the number of synapses, while the wild type or the pseudophosphorylated protein overexpression had no effect. Interestingly, any variation of Syn Ia concentration and phosphorylation caused a change in the number of puncta positive for the presynaptic scaffolding protein Bassoon but negative for presynaptic vesicle markers.
Recent data indicate that the formation of synapses involves the delivery of "transport packets" containing several sets of proteins necessary for proper synaptic function. It has been hypothesised that as yet undefined molecules can sense the synaptic microdomain and stop the travelling transport packets leading to the rapid accumulation of vesicles at the sites of synapse formation.
We have not clarified yet the nature of Bassoon positive puncta, however we hypothesize that they could be Bassoon transport packets and that Syn Ia could be involved in their targeting.

PS1.31

REST, the transcription repressor of neurosecretion, may govern also gliosecretion

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REST (RE-1 silencing transcription factor) is a repressor of transcription known to govern the expression of hundreds of neuron-specific genes. Our previous studies in rat PC12 clones, competent and defective of neurosecretion, had shown that the main properties of these cells (and, presumably, of any other neuron/neurosecretory cell) are lost or reacquired depending on their levels of this factor. In fact, stable transfection of full-length constructs in wt PC12 cells, which are very poor of REST induced the decrease expression of numerous genes, encoding proteins of dense core vesicles (DCVs) and of their exocytosis; transfection of a dominant-negative construct combined with trichostatinA (TSA), a blocker of the REST-associated histone deacetylases, induced in the REST-rich defective cells the reappearance of small DCVs, which were exocytized upon stimulation. These results identify REST as the critical factor governing the expression not only of individual genes, but of the whole neurosecretory process via multiple, direct and indirect mechanisms. Neurosecretion resembles in various respects gliosecretion, the process by which astrocytes release glutamate and other products. We wondered therefore whether gliosecretion is also governed by REST. Our preliminary data in astrocyte primary cultures suggest that this might be the case. Transient transfections with the dominant-negative construct combined with the treatment with TSA, induced in astrocytes the appearance of SNAP25 and synaptotagmin1, two key proteins of exocytosis lacking in the non-transfected cells. Molecular, morphological and functional studies are ongoing to clarify in detail the role of the repressor in the expression and properties of the gliosecretory process.

PS1.32

Cystatin B function and Progressive Myoclonus Epilepsy (EPM1)

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Mutations of the cystatin B gene (cstb) are described in the great majority of EPM1 patients. In mammals CSTB is a ubiquitous protein but the disease affects the CNS only. We have recently shown that, in vivo, CSTB has a polymeric structure and interacts with cytoskeletal proteins involved in vesicular transport and that overexpression of CSTB in neuroblastoma tissue culture cells generates toxic cytoplasmic aggregates. Furthermore, in rat cerebellum, CSTB is expressed in the Purkinje cells and radial glia only. Although CSTB, in vitro, shows antiprotease activity, our results suggest the existence of additional role(s) possibly due to its interaction with partner proteins involved in cytoskeletal functions. Under suitable conditions, the CSTB monomer polymerizes in vitro generating amyloid fibers that grow by dimer addition. In an in vitro purified system we show that the native CSTB monomer polymerizes following addition of copper and HSP70 only. Interestingly, these polymers grow by monomer addition, similar to those that we have found in vivo. In contrast, the denatured CSTB monomer does not require copper and polymerizes on addition of HSP70 only, whereas the addition of copper to the native CSTB monomer modifies its structure, possibly destabilizing it. We discuss the effect of the co-transfection of HSP70 and CSTB cDNAs on CSTB aggregate formation in neuroblastoma cells. We are also defining the interaction domains of HSP70/CSTB with each other.

PS1.33

Production and characterization of a neuronal cellular model to study neuroferritinopathy

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Neuroferritinopathies are dominantly inherited movement disorders associated with nucleotide insertions 460InsA (Ln1), 498InsTC (Ln2), 441InsC on the L ferritin gene, that modify the carboxy-terminal residues of the peptide in different extent. More recently a new mutation was identified as a duplication of the 469-484 sequence that replaces the C-terminal 14 amino acid residues with a novel 23 amino acid sequence. The hallmark of these diseases is the presence in the brain of spherical inclusions positive for iron, ferritin and ubiquitin stains. Previous data obtained expressing variants in HeLa cells indicated that iron dyshomeostasis, induced by ferritin alterations, was the principal cause of cellular apoptosis. To better clarify the molecular mechanism of the disease we studied SH-SY5Y neuroblastoma cells transduced with lentivirus and stably expressing Lwt, Ln1 and Ln2 ferritins. The L-variants caused a lower efficiency in ferritin iron incorporation, which is the primary activity of the protein, indicating a dominant negative effect of the mutations. The ferritin lower functionality induced higher amount of the intracellular labile iron pool, which caused the alteration of cellular iron homeostasis parameters. Cytoplasmatic aconitase enzimatyc activity and ROS production were increased in different extent in L-variants respect to the Lwt. Ln2 showed a decrease in proteasome activity of ~ 40% respect to the control and this effect was reverted by treatment with an iron-chelator. The L-ferritin variants expressing cells appeared to be subjected to necrosis, with a reduction of cell viability of about 50-60 % after treatment with ferric ammonium citrate respect to the controls, while apoptosis was no detected. Furthermore, they showed ferritin and iron aggregates, as occur in patient's tissues, indicating that they are good models for the study of pathogenesis of the disease. Also in this cellular model iron appears to be the principal player in neuronal death.

PS1.34

Immunochemical localization of RasGRF1 in the cerebellum and changes in the proteome profile induced by its genetic deletion

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RasGRF1 is a neuron specific Ras exchange factor expressed in the central nervous system. This guanine exchange factor activates the Ras/MAPK pathway in response to calcium and plays a crucial role in synaptic plasticity and memory formation. Mice lacking RasGRF1 fail to become tolerant to most of the behavioral effects induced by chronic cannabinoid exposure and show an altered synaptic plasticity at parallel fiber-Purkinje cell synapses, with an increased basal PKA activity in the cerebellum. To gain information on the role of RasGRF1, we applied morphological and biochemical methods to cerebellar samples from adult wild type and RasGRF1 ko mice. Immunohistochemistry with anti-RasGRF1 antibodies demonstrated that the protein is expressed in cerebellar sections of wild type mice. In particular, immunoreactivity for RasGRF1 was intense in deep cerebellar nuclei and in Golgi cells in granular layer, whereas Purkinje cells and interneurons in molecular layer were only weakly labeled. Double labeling with several neuronal and glial markers confirmed that RasGRF1 immunoreactivity is exclusively neuronal and mostly localized in perikarya and proximal dendritic processes. No staining was observed in control experiments. In RasGRF1 ko mice conventional histological analysis demonstrated a normal laminar organization of cerebellar neurons and immunocytochemistry for neuronal and glial proteins showed a normal staining pattern. Comparative proteomic studies of the cerebellum of RasGRF1 ko mice indicated that the main alterations occur in proteins involved in protein folding and oxidative stress (heat shock proteins and antioxidant enzymes) and in mitochondrial enzymes involved in oxidative metabolism. Collectively these results indicate that deletion of RasGRF1 does not cause major cellular alterations but might alter the cellular responses to oxidative stress and induce mitochondrial dysfunction. Studies have been undertaken to further investigate this aspect.

PS1.35

Neurotrophins and microRNAs: expression profile in PC12 cells upon NGF treatment

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Neurotrophins (NGF, BDNF, NT3, NT4/5) regulate survival, development, and plasticity of neurons. Key aspects of neurotrophin signalling involve control of both transcription and translation of selected messenger RNAs. MicroRNAs (miRNAs), small non-coding RNAs that control translation of target mRNAs, also regulate critical aspects of neuronal development and plasticity. Presumptive binding site for the transcription factor CREB, a major target of neurotrophin signalling, are found in the promoter regions of many miRNAs present in neurons. As a first step towards elucidating miRNA regulation by neurotrophins, we have analysed the miRNA expression in the NGF target cell line PC12. We have employed LNA (locked nucleid acids) miRNA microarray slides, containing capture probes complementary to about 300 mature mammalian miRNAs, to globally analyse miRNA expression upon treatment of PC12 cells with NGF. Data showed that about 30 miRNAs are expressed in PC12 cells, a small number of which appear to be modulated by NGF either positively or negatively. Among the miRNAs that are up regulated, two (mir-23b and mir-29c) are known to be involved in brain development and neuronal differentiation, and two more (mir-207 and mir-212) have CREB binding sites in their potential promoter region. The miRNAs that we have identified so far may represent mediators of neuronal differentiation and plasticity.

PS1.36

Seizures increase importin β1 expression in NG2+ cells in the rat hippocampus

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Importins, also called karyopherins, belong to a large family of proteins involved in cytoplasm-to nucleus transport. Transport machinery generally involves a complex formed by two different importin subtypes (α and β). Both and importins are expressed in the brain, and their expression and localization is regulated by physiological neuronal activity. Little is known about regulation of importin expression in brain pathological conditions. Here we studied the expression of importin β1 (impβ1) in the rat hippocampus following acute and chronic seizures induced by the glutamate agonist kainic acid (KA). Impβ1 protein (but not mRNA) was rapidly up-regulated in pyramidal CA1 neurons following acute KA seizures, as compared to controls. KA-induced impβ1 upregulation was partially prevented by the NMDA receptor blocker MK801. Following chronic seizures, levels of impβ1 mRNA and protein were not up-regulated in pyramidal neurons, whereas an increased number of impβ1-positive cells was detected in the stratum radiatum, as compared to control rats. Quantitative analysis of double-labelling immunohistochemistry experiments revealed a significant increased number of NG2-positive cells expressing impβ1 in chronically treated rats. Conversely, the number of impβ1-positive neurons and astrocytes remained unaltered. These data show a differential regulation of impβ1 expression after acute and chronic seizure activity in the rat hippocampus.

PS1.37

Analysis of human aldolase C mRNA expression in transgenic mice

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Aldolase C is the brain-specific aldolase isoenzyme. We previously demonstrated that construct pAldC2500-LacZ, containing the complete human aldolase C gene promoter region (1200 bp), directs brain-specific LacZ expression in adult transgenic mice (Buono P. et al. FEBS Lett., 2004). Here we report data on human aldolase C mRNA expression in: 1) pAldC2500-LacZ transgenic mice during brain development (E13, E15, P0, P7, P14, P42); 2) adult transgenic mice obtained with pAldC1464-LacZ and pAldC1580-LacZ constructs containing deleted promoter regions of human aldolase C gene.
Preliminary results on pAldC2500-LacZ stabilized transgenic mice line show that: a) AldC-LacZ hybrid mRNA is selectively expressed in CNS during development; b) in brain and cerebellum both endogenous aldolase C and LacZ mRNAs expression level increase from earlier developmental stages to the adult stage where they reach maximal values. The lower expression level of hybrid mRNA, during brain development, suggests that probably some activator/enhancer elements are lacking in the analysed construct.
Preliminary results on pAldC1464-LacZ and pAldC1580-LacZ stabilized transgenic mice lines show that: a) low LacZ mRNA expression is present only in the brain of pAldC1464-LacZ adult transgenic mice; b) high LacZ mRNA expression is present in brain and cerebellum of pAldC1580-LacZ adult transgenic mice; c) both transgenic mice lines show high LacZ mRNA expression in liver, kidney and heart suggesting that these two constructs lack tissue-specificity.

PS1.38

IL-8 and prostaglandin E synthase-$_1$ expression in low- and high-grade human glioma

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Malignant gliomas are highly aggressive tumors of the central nervous system that rely on production of growth factors for tumor progression. In these tumors, IL-8 is up-regulated to promote angiogenesis and proliferation . Although several stimuli, including LPS, PMA, IL-1, PGE2 and TNF-α, have been shown to induce IL-8 production in several cell types, to our knowledge no evidence is available about the intrinsic factors that increase IL-8 expression in glioma. Given that it has been recently reported that prostaglandin E synthase-1 (PGES-1) is constitutively expressed in human glioma cell lines and that PGE2 has a crucial role in the control of growth, survival and angiogenic potential of colorectal and breast tumors, in this report we addressed the expression of IL-8 and PGES-1 in human glioma of different grades of malignancy. The tumors, immediately after surgical resection, were analysed by Real-Time PCR to assess the RNA expression levels of IL-8 and PGES-1. The results obtained showed that the expression of IL-8 is absent in low-grade glioma, and that it markedly increases in a direct positive manner with the grade of tumor malignancy. In accordance, a parallel significant increase in the expression of PGES-1 was observed from low to high grade, although even low-grade glioma showed low levels of this enzyme. These data suggest that IL-8 mRNA may be considered a marker of malignancy, since the IL-8 gene is not expressed in the lower-grade glioma, while it is induced at different levels in relation to the severity of the tumor. Furthermore, we have shown that an high expression of PGES-1 is required to induce IL-8 gene expression. In conclusion, it is conceivable that the aggressive phenotype of glioma may be ascribed to IL-8 and ultimately to an increase of PGE2 production.

PS1.39

Targeting signals in BDNF mRNA coding region direct its trafficking in dendrites

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Targeting of mRNAs into dendrites followed by local protein synthesis is a mechanism that enables neurons to synthesize proteins in close proximity to synapses. For several mRNAs, trafficking in neurites can be modulated by a variety of stimuli including electrical activity and signalling from the neurotrophins BDNF and NT3.However, the signals responsible of this regulated transport are unknown. We have previously shown that endogenous BDNF (Brain Derived Neurotrophic Factor) mRNA is accumulated into the distal dendritic compartment of rat neurons in response to electrical activity,BDNF and NT3 in vitro, and epileptic seizures in vivo. BDNF is encoded by multiple transcripts, produced by alternative splicing of eleven different 5'UTRs, spliced to a common protein coding exon and two different 3'UTRs. In vitro analysis of subcellular localization of chimeric rat and human BDNF-GFP mRNA revealed that the coding region contains a constitutive signal for trafficking in dendrites.Partial deletions of rat CDS demonstrated that selective loss of binding sites for different RBPs severely affects mRNA targeting.Additionally, RNAi experiments showed that the protein Translin is involved in this activity-independent mRNA sorting.Through single nucleotide mutagenesis, in vitro binding studies, analysis of subcellular localization of mutant RNAs and 3D molecular modeling, we could identify the exact recognition site for Translin within the CDS of BDNF and found it to be very simple ($NG(N)_{17}GN$). Moreover, through time lapse analysis we observed that MS2-GFP tagged BDNF CDS mRNA is present in dendrites of living cells in the form of RNA transporting granules.Electrical activity influenced neither the distribution nor the movement of these particles, confirming our previous data.This study provides the first 3D modeling of Translin docking onto a mRNA and strongly suggests that dendritic trafficking of BDNF mRNA is mediated by multiple signals located in different regions of the RNA.

PS2.01

Coup-tfI controls projection neuron migration and morphology by negatively regulating Rnd2 expression levels

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Brain development is a complex and orchestrated process in which many cell populations respond to specific signals and migrate to their final target before being fully functional. During corticogenesis early- and late-generated neurons, which give rise to lower and upper layers respectively, acquire their identity and migrate to their specific targets under the control of several transcriptional regulators. Before settling in their final laminar destination, migrating neurons change their morphology from multipolar-shaped in the subventricular and intermediate zone (SVZ/IZ) to bipolar-shaped before reaching the cortical plate. Such morphological changes require cytoskeleton reorganizations, but little is known about the molecules involved in this process. With the help of a microarray approach between wild-type and COUP-TFI$^{-/-}$ brains, we identified a series of differentially expressed genes1. Among these genes we found cytoskeleton interacting proteins and our attention focused on Rnd2, a member of the Rho family of small GTPases, which is expressed in radially migrating pyramidal neurons and is known to regulate neuronal morphology and/or neurite outgrowth. We demonstrated by quantitative PCR and in situ hybridization that levels of Rnd2 transcripts are doubled in the SVZ/IZ and CP of COUP-TFI$^{-/-}$ embryos. Furthermore, chromatin-immunoprecipitation and promoter array analysis indicated direct binding of COUP-TFI on different sites of the Rnd2 locus. GFP electroporation of COUP-TFI$^{-/-}$ brains in vivo showed an increase in multipolar-shaped neurons in the SVZ/IZ region and impairment in their migration to the cortical plate. With the help of a shRNA vector specific to Rnd2 mRNA, we rescued the neuronal morphological defects and restored cell migration. Thus, appropriate levels of Rnd2 are essential for projection neurons to adopt a correct morphology and migrate into the cortical plate. Taken together, our data demonstrate that COUP-TFI is involved in cytoskeleton reorganization processes and in projection neuron migration and morphology by modulating Rnd2 expression levels.

PS2.02

GIT1/Liprin-alpha Complexes in cell motility and early neuronal development

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GIT1 is a multidomain protein involved in the regulation of adhesion, migration, membrane recycling, and in spine morphogenesis and synapse formation in neuronal cells. GIT1 interacts with the adaptor protein Liprin-alpha. Liprins are scaffolding proteins implicated in synaptogenesis in neurons, and in the adhesion and motility of non-neuronal cells. They interact via SAM domains with the receptor tyrosine phosphatase LAR that regulates adhesion to the extracellular matrix, and active zone assembly at synapses. We have identified a direct interaction between Liprin-alpha and GIT1 that seems to be regulated by the release of an intramolecular inhibitory mechanism. We are characterizing the function of the GIT1/Liprin-alpha complexes during cell motility and neurite extension. Overexpression of Liprin-alpha1 profoundly affects cell morphology by inducing large lamellipodia. Spreading assays on fibronectin showed a 50% increase in the area of Cos7 transfected with Liprin-alpha1, and the relocalization of paxillin and GIT1 to focal complexes at the cell edge. On the other hand, cell adhesion was not evidently affected. The enhancement of cell spreading by Liprin-alpha was prevented by co-expression of a dominant negative mutant of Rac1, and by silencing of GIT1 and LAR expression by siRNA. Moreover, silencing of endogenous Liprin-alpha1, LAR, and GIT1 inhibited basal spreading on fibronectin. Overexpression of Liprin-alpha1 in fibroblasts induced the redistribution of beta1 integrins on the ventral, adherent plasma membrane, without evidently affecting integrin activation. These data support a regulatory role of Liprin-alpha1 in dynamic adhesion and motility. We are currently exploring the molecular mechanisms underlying the effects of Liprin on cell motility and integrin distribution. Furthermore, we are addressing the role of the Liprin-alpha/GIT1 complexes in developing neurons, to analyze the function of this complex in neurite formation and in the establishment and/or maintenance of neuronal polarity.

PS2.03

Expression of the transcription factor REST at different stages of neuronal differentiation

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The repressor element 1-silencing transcription factor (REST) binds to a 21-bp DNA sequence element (RE1) resulting in transcriptional repression of neural-specific genes. The role of REST is complex and tissue-dependent: it declines during neuronal differentiation allowing the expression of the repressed genes while it remains active in non-neuronal cells. In this study we provide evidence that, during early stages of neuronal differentiation induced by IGF-I or PKC activation, REST may repress the expression of several genes not yet required by the differentiation program whereas it declines later. Therefore, the decline of REST is a comparatively later event during maturation of neuroroblasts in vitro and requires specific regulatory pathways. A precocious REST down-regulation, obtained using an antisense strategy, causes a more extended neurite network without preventing overt neurogenesis. Nuclear REST expression is increased in two models of neuronal cells (H19-7 and SH-SY5Y cells) exposed to IGF-I for 2-days while it then declines later in 5-day-treated cells concomitant with a progressive neurite extension. Similarly, the phorbol ester PMA (16nM) is able to increase nuclear REST levels after a 3-day treatment whereas it is down-regulated at later stages. Cell exposure to PKC inhibitors (GF10923X and Gö6976) reverts the effects elicited by PMA alone. REST down-regulation is related to a progressive activity of proteasome: neuroblasts concomitantly exposed to PMA and the proteasome inhibitor MG132 showed a blockade of neuronal differentiation and REST up-regulation. REST decline is clearly related to morphological differentiation and to the expression of growth cone-associated protein 43 (GAP-43), synapsin I and βIII tubulin, three proteins involved in the early stage of neuronal development. Thus transcriptional and post-trasductional events contribute to modulate nuclear REST levels in differentiating neuronal cells in a precise time-frame manner.

PS2.04

Adenosine slows the migration of peripheral neurons and glial cells

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In previous in vitro studies we described the ability of embryonic chick ciliary neurons to migrate in association with glial cells to form cellular aggregates. Changes of the modulus of the velocity of the neuron-glial cell complex were observed in response to treatments that increased or decreased intracellular calcium concentration or the amplitude of spontaneous nonneuronal calcium oscillations. The purpose of this study was to characterize the effects of extracellular application of ATP and adenosine on calcium signalling and their role in the regulation of the rate of migration. Extracellular application of adenosine and ATP directly activated a calcium response in both neurons and glia. Adenosine significantly decreased the modulus of the velocity in isolated glial cells and in neuron-glial cell complexes whereas no differences were observed in the presence of ATP. Experimental manipulations that inhibit the cAMP/PKA signalling pathway reversed the effects of adenosine on cellular complexes and isolated glial cells. Forskolin, which stimulates adenylate cyclase activity mimics the effect of adenosine.
We conclude that the balance between Ca2+ signalling and cAMP signalling modulates the rate of migration of developing peripheral ganglion cells.

PS2.05

Effects of activated microglia on neural stem/progenitor cells properties

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The role played by microglia in the regulation of neurogenesis in pathological conditions is a matter of intense investigation. Activated microglia (MG) have been shown to be directly responsible for the detrimental effects of inflammation on adult neurogenesis. However this view has been challenged by recent studies suggesting that activated microglia are not necessarily neurotoxic or anti neurogenic per se. Microglia activation is in fact a multifaceted phenomenon depending on the nature, intensity and persistence of the activating agent and changes in the microenvironment.
We have recently demonstrated that MG repeatedly challenged with lipopolysaccharide (chronic stimulation) acquire an "atypical" phenotype permissive to the generation of both neurons and astrocytes from neural stem/progenitor cells (NSPC) in vitro, whereas MG receiving single stimulation with lipopolysaccharide (acute stimulation) impaired neuron generation. These different outcomes could derive from the reorientation of microglial functions under chronic stimulation respect to acute stimulation, including the reduced synthesis of pro-inflammatory molecules, such as TNF-α, IL-1α, IL-1β, IL6, NO and the sustained release of immunomodulatory products, such as prostaglandin E2 and IL-10.
We are currently investigating the role of specific soluble factors such as IL-1α on NSPC properties (survival, differentiation, proliferation) exposing the cells directly to the recombinant cytokine or to the MG conditioned medium depleted of IL-1α by means of suitable neutralizing antibody. Our preliminary results suggest that at variance with other pro-inflammatory cytokines IL-1α exert minor adverse effect on NSPC survival and neuronal differentiation.

PS2.06

Generation of cerebellar GABAergic neurons from NS cells

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Malformative and degenerative disorders of the cerebellum are characterized by motor incoordination and mental retardation. They often involve the GABAergic component of the cerebellar cortex, and particularly Purkinje cells (PCs). Although these disorders are currently incurable, the development of robust cell replacement approaches may eventually provide useful tools for their clinical management. In this project, we will manipulate the fate of neural stem (NS) cells to generate specific neuronal progenitor types. Our preliminary results indicate that NS cells grown under proliferative conditions express early positional markers of relevance for cerebellar development, suggesting that NS cells are an ideal tool to generate cerebellar progenitors in vitro. To this end, we will grow cells in a medium containing a cocktail of morphogens to mimic the extracellular environment required for patterning the embryonic cerebellar anlage. After treatment of NS cells with different concentrations of these extrinsic factors and transfection with vectors containing several genes involved in GABAergic fate, we will select cells that express markers of precursors originating from the cerebellar ventricular zone. GABA progenitors obtained in this way will be grafted into wildtype and mutant cerebella in order to assess their ability to migrate, survive and establish proper connections in vivo.

PS2.07

Ex-vivo models of Neural Stem Cell niche: antigenic and ultrastructural properties

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Adult neurogenic sites are specialized microenvironments (niches) regulating neurogenesis within restricted brain regions. In the forebrain subventricular zone (SVZ), continuous neurogenesis is supported by relatively quiescent neural stem cells (NSCs). SVZ stem cells of radial glia origin (B cells) proliferate at low rate, self renew and contribute transit-amplifying progenitors (C cells), that rapidly divide and generate neuroblasts (A cells; Doetsch, Curr Opin Genet Dev, 2003). How the glial population in the stem cell niche goes towards modification during the postnatal period remains to be understood. During the first three weeks after birth radial glia transform into mature parenchymal astrocytes and into SVZ astrocytes (reviewde in Bonfanti & Peretto, Prog Neurobiol, 2006). Complexity can be reduced in many in vitro approaches, yet stem cells change their functional properties which depend on the tissue (niche) environment. In this context, we have developed ex vivo culture models in which tissue explants and organotypic cultures are employed as an alternative way to investigate stem cell niches. Explant and brain slice models offer advantages over other in vitro platforms in that they can replicate many aspects of the in vivo context. SVZ explants preserve cell types of the niche although with changing, experimentally modulable conditions. Slices preserve relationships between the niche and the surrounding regions, also maintaining neuronal activities with intact local synaptic circuitry. Both approaches permit the experimental manipulation of the system. We used mice aged between P0 and P30 to study the main structural/functional changes occurring within the neural stem cell niche during the postnatal period. Finally, we aimed at characterizing the SVZ stem cell niche ultrastructurally, in order to establish to which extent the dynamic niche architecture is maintained.

PS2.08

Evolutionary perspectives of the synapsin gene family in basal chordates

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The synapsins are a family of neuron-specific phosphoproteins that serve multiple roles in neuronal function, such as neurotransmitter release and synaptic plasticity. These molecules play also distinct roles during early stages of neurite outgrowth, axon elongation and synaptogenesis (Ferreira and Rapoport, 2002). In mammals, distinct genes for synapsins I, II and III have been identified, each of which gives rise to different alternatively spliced isoforms. To gain a better understanding of the evolution of synapsin gene family, we cloned and sequenced synapsin genes from two invertebrate chordates, the amphioxus *Branchiostoma lanceolatum* and the ascidian *Ciona intestinalis*, and compared them to known synapsin sequences from mammals and other invertebrates. The amphioxus and Ciona genomes contain a single synapsin gene (*AmphiSyn* and *CiSyn*) that shows a high degree of conservation in exon/introns organization to those found in mammals. Moreover, in these basal chordates some introns of synapsin gene locus encode tissue inhibitor of metalloproteinases (TIMP). In mammals, each of synapsin gene is associated with a specific TIMP: *Syn1-TIMP1*, *Syn2-TIMP4* and *Syn3-TIMP3*, with the only exception of *TIMP2* that is independent from synapsin gene locus. In amphioxus and ascidian genomes we identified two *TIMP* genes that show a different genomic organization: in amphioxus a nested *TIMP* with *synapsin* and an independent *TIMP* were found, whereas in Ciona two nested *TIMP* with *synapsin* gene were present. Such results suggest that the ancestral chordate *Syn-TIMP* locus has undergone duplication events involving only the *TIMP* sequences. The amphioxus and Ciona synapsin protein contains all types of conserved domains (A, C, and E) characteristic of synapsins. Finally, whole mount in situ hybridization experiments reveal some interesting aspects on temporal and spatial expression of synapsin gene during development of amphioxus and Ciona. In both models such gene is expressed in specific regions of the central and peripheral nervous system.

PS2.09

Role of VEGF and blood vessels during GnRH-neurons development

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Gonadotropin-releasing hormone (GnRH) neurons, a small number of neuroendocrine cells scattered in the hypothalamus, play an important role in reproduction. During development, GnRH neurons are born in the olfactory placode and migrate along olfactory nerves in the nasal compartment to gain access into the forebrain and reach the hypothalamus. In humans, defects in their migration result in infertility. The mechanisms involved in the migration of GnRH neurons are under investigation. We have recently described that classical guidance molecules, such neuropilins (NRPs), are expressed by GnRH neurons, and established the importance of NRP2 in their migration in vivo. Using immortalised GnRH-neurons, we found that two distinct NRP ligands regulate their migratory response: the class 3 semaphorins and vascular endothelial growth factor A (VEGF). VEGF is a major regulator of vasculogenesis, interacting with receptor tyrosine kinases (Flt-1/Flk-1) on endothelial cells. Recent evidence indicates that VEGF has additional non vascular-functions. In particular, VEGF can act directly on neurons to produce different effects such survival, axonal elongation and migration. In this study, the interactions between blood vessels and GnRH-system have been tested. Using RT-PCR and enzymatic stainings of VEGF-LacZ reporter mice, we found that VEGF is significantly expressed in the nasal region during development. We also visualised the presence of a network of blood vessels along the migratory path of GnRH neurons. Moreover, isolated mouse embryonic GnRH neurons express specific transcripts for VEGF and Flt-1. Functionally, we found that VEGF exerts pleiotropic effects on immortalised GnRH-neurons, acting on survival, chemomigration and axonal elongation. Taken together, these novel data raise the possibility that GnRH neuronal migration and development are modulated by VEGF signalling, suggesting the existence of a ï¿½cross-talkï¿½ between the vascular and GnRH-neuron systems.

PS2.10

Identification of cancer stem cells in a mouse model of medulloblastoma and molecular comparison with normal neural stem cells

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CSCs have been identified in different brain tumors, including medulloblastomas (MBs), the most common brain tumors in childhood, as tumor founding cells, able to sustain growth and progression of the tumor and to give rise to relapse.
In this study, we focused on the characterization of cancer stem cells (CSCs) in murine spontaneous MBs, derived from mice carrying heterozigous patched mutations. We isolated CSC lines from 2 different MBs (MB CSCs), culturing the specimens under the standard conditions (i.e. in the presence of EGF and FGF2) employed to isolate normal neural stem cells (NSCs). These MB-derived lines display several features that qualify them as CSCs, such as extensive proliferation, self-renewal, multipotency, as well as aberrant differentiation. Moreover, MB CSCs are endowed with tumor initiation ability when injected in mice.
MB is thought to arise from cerebellar neural progenitors that undergo malignant tranformation; in particular, the desmoplastic variant seems to derive from the transformed precursors of the external granule layer (EGL), whereas the classic variant appears to originate from the progenitors of the IV ventricle. To assess whether this presumptive lineage relationship could be revealed by common molecular signatures, we isolated NSCs from the EGL and the IV ventricle, using subventricular zone (SVZ) NSCs as internal control, and subjected them to gene expression profiling together with MB CSCs.
Microarray-based analysis shows that each MB CSC line has a typical molecular signature and, unexpectedly, both MB CSCs appear to share more molecular determinants with SVZ NSCs than with EGL-derived NSCs.
Based on these preliminary findings, by exploiting the relationship existing between the biology of MB CSCs and the physiological cerebellar development, it might be possible to identify still unknown pathogenetic mechanisms and new candidate target useful to develop novel and selective treatments for MBs.

PS2.11

EBF transcription factors activate Igf1 gene expression, possibly regulating Purkinje cell survival at birth

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Ebf genes encode atypical HLH transcription factors (TFs) strikingly conserved in evolution. Originally identified for their roles in the immune system, Ebf genes have been implicated in various aspects of neuronal development. Our group has generated a mouse carrying a null mutation of Ebf2, one of four mammalian members of this TF family. The cerebellum of Ebf2 null mice is reduced in size, and cerebellar foliation is severely altered expecially in the anterior lobes. The Ebf2 -/- cerebellum features prominent Purkinje cell (PC) death at birth. Insulin-like growth factor 1 (IGF1) is an anabolic factor believed to have neuroprotective functions on cerebellar neurons. Although IGF1 is released into the bloodstream by the liver as a hormone, it is also produced locally at many different sites, including the central nervous system, where it acts as a paracrine factor. IGF1 promotes PC survival in vitro and reduces apoptosis in wv/wv granule cell neurons. In Ebf2 -/- PCs, Igf1 expression is downregulated during the first postnatal week, suggesting a role for EBF2 and, possibly, other EBF TFs in Igf1 gene regulation. To address the global role of EBF TFs in Igf1 regulation, we will also take advantage of a new knock-in model that expresses a Cre-inducible shRNA transcript simultaneously targeting Ebf1, Ebf2 and Ebf3 mRNAs. While casting light on the roles of EBF TFs in local Igf1 regulation, our current in vivo studies should also help clarify the role of locally expressed IGF1 in PC survival.

PS2.12

Cross-regulation of PHOX2A and PHOX2B transcription factors in the development of the Autonomic Nervous System

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PHOX2A and PHOX2B are homeodomain transcription factors important for the development of the entire Autonomic Nervous System (both sympathetic, parasympathetic and enteric divisions). PHOX2B is absolutely required at the early stages of development of autonomic ganglia: its expression is triggered by bone morphogenic proteins (BMPs) secreted by the dorsal aorta; it acts as a repressor of negative signals induced by Notch, a BMPs antagonist, and supports MASH1 with which it coordinates the expression of downstream factors as PHOX2A and dHAND, a process necessary for the regulation of the expression of c-RET, TH (tymidine hydroxylase) and DBH (dopamine β hydroxylase) genes which are characteristic of the cathecolaminergic phenotype. During the specification of neuronal identity, control of temporal and spatial expression of PHOX2A and PHOX2B is fundamental, and many studies over the last few years have tried to elucidate the exact molecular mechanisms involved in regulation of their expression. We have demonstrated previously that PHOX2B regulates the transcription of the PHOX2A gene by directly binding and transactivating its promoter. We also characterised the PHOX2B promoter and demonstrated by means of biochemical and functional assays that most of its transcriptional activity is sustained and maintained by an auto-regulatory mechanisms in which PHOX2B binds and transactivates its own promoter.
Chromatin immunoprecipitation (ChIP) assays showed that PHOX2A also participates in the transcriptional complex assembled on the PHOX2B promoter.
The functional significance of this is under further investigation and the results of the various approaches used are the topic of the present poster.

PS2.13

Measurement of the force exerted by neurites during neuronal differentiation

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During neuronal differentiation, neurites are guided towards their final target by molecular cues that are sensed by filopodia and lamellopodia, highly motile structures extruding from the tip of the growing neurite, i.e. the growth cone. Filopodia explore the environment by rapidly moving in all directions, searching for the correct pathway and lamellipodia follow, opening the way to the growing neurite. Cell motility is primarily powered by motor proteins, able to convert chemical energy into cytoskeleton movement and force generation. The kinetics of filopodia and lamellipodia motion has been analyzed but little is known about the force that these neuronal structures exert on their environment. Indeed, the analysis of this force has been limited to theoretical considerations and an experimental analysis has been restricted to samples of isolated filaments.
A quantitative characterization of forces exerted by neurons during neuronal differentiation is necessary for understanding their motility and the precise role of molecular motors. Therefore, we used optical tweezers to measure the force exerted by Rat Dorsal Root Ganglion cells during neuronal differentiation. Results show that force exerted by filopodia is in the order of 1 to 2 pN while lamellipodia being more complex and relatively larger structures exerts force more than 11 pN. Force exerted by Filopodia during its lateral motion and protrusion were also measured.

PS2.14

The requirement for phox2 genes in the differentiation of ventral-r4 neuron sub-populations

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During development the hindbrain is transiently subdivided into eight segmental and distinct regions, named rhombomeres, fundamental for cell lineage restriction.
Facial brachiomotor (FBM) and inner ear efferent (IEE) neurons develop in the ventral rhombomere 4 (r4). The FBM neurons undergo a complex and stereotyped migration and extend their axons dorsally towards the exit point of the VIIth nerve; the IEE neurons project to the inner ear through the stato-acoustic nerve.
We are trying to dissect the roles of the paralogous transcription factors, Phox2a and Phox2b, in ventral r4 neurogenesis, using a knock-in mouse line where Phox2a is expressed in Phox2b territory (Phox2aKI2b).
Loss and gain of function studies have shown that Phox2b is necessary in the ventral r4 to drive progenitor cells to become post-mitotic and to ensure the differentiation of the correct number and type of neurons at the right time and location.
In knock-in embryos, although the proliferating area is expanded, Phox2a is able to promote cell cycle exit, even if, at least early during development, fewer post-mitotic cells are generated compared to the wild-type condition.
FBM neuron generation is reduced in Phox2bKI2a homozygous mice and theyr typical migration is partially impaired. On the other hand, the specification of IEE neurons occurs properly and seems to be improved indicating that Phox2a essentially drives IEE differentiation. Moreover, serotonergic neurons arise ectopically in r4 of Phox2bKI2a homozygous mice showing that Phox2a can’t substitute Phox2b in suppressing serotonergic neuron differentiation.
To understand better the phenotype observed in the knock-in mice, we have performed a microarray analysis and we are characterizing the function of two strongly downregulated neuropeptides, Gap43 and Pcp4 that regulate calcium signalling by binding calmodulin-Ca2+free.

PS2.15

Calcium signals activated by ghrelin and D-Lys3-GHRP-6 in DRG non-neuronal cells

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Ghrelin is a hormone that stimulates growth hormone secretion and regulates energy homeostasis via interaction with its receptor, the growth hormone secretagogue receptor (GHS-R). The effects of this peptide in the peripheral sensory nervous system are at present unknown.
In embryonic chick dorsal root ganglion cells expressing the GHS-R1a, ghrelin induces a cytosolic calcium elevation. In non-neuronal cells, the response was characterized by a transient phase followed by a long lasting rise that was dependent on extracellular calcium concentration. The calcium elevation was dependent on calcium release from thapsigargin-sensitive intracellular stores and on activation of two distinct Ca2+ entry pathways: a receptor activated calcium entry, which was recorded in the presence of the peptide in the external medium and a store operated calcium entry, which activated when ghrelin was removed from the external bath.
In binding studies performed using Fluo-ghrelin, no signals could be detected when cells were incubated with the fluorescent ligand in the presence of a 100-fold excess of unlabeled ghrelin or GHS-R1a antagonist D-Lys3-GHRP-6, indicating that both peptides bind the same receptor. D-Lys3-GHRP-6 failed to antagonize ghrelin-induced calcium signals when applied together the agonist at the same concentration (1 ï M). Surprisingly, application of 1 ï M D-Lys3-GHRP-6 in the extracellular medium activates calcium release from thapsigargin-sensitive intracellular stores and calcium entry via voltage-operated channels or inhibits spontaneous calcium activity in these cells. Finally D-Lys3-GHRP-6, but not ghrelin, promoted apoptosis in DRG cells. These data suggest an important role of the activity of GHS-R1a in developing DRG cells survival.

PS2.16

Identification of a developmentally-regulated pathway of membrane retrieval in neuronal growth cones

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During axon navigation and upon target recognition the growth cone plasma membrane is constantly reconfigured as a result of changes in cytoskeletal and membrane dynamics. The identity and regulation of the membrane pathway(s) participating in remodeling of the growth cone surface remain elusive. Here, we identify a constitutive, high capacity plasma membrane recycling activity in the axonal growth cones which is mediated by a novel bulk endocytic pathway mechanistically related to macropinocytosis. This pathway, involving large compartments distributed at sites of intense actin-based membrane ruffling, requires phosphatidylinositol 3-kinase activity, the small GTPase Rac1 and the pinocytic chaperone Pincher. At early developmental stages, the synaptic vesicle and classical endosomal recycling pathways do not participate in the rapid retrieval of the growth cone plasma membrane. At later stages, during the onset of synaptogenesis, an intrinsic program of maturation leads to downregulation of basal bulk endocytosis and the emergence of depolarization-induced synaptic vesicle exo-endocytosis. We propose that the control of bulk membrane retrieval contributes to the homeostatic regulation of the axonal plasma membrane and growth cone remodeling during axonal outgrowth. In addition, we suggest that the downregulation of bulk endocytosis during synaptogenesis might contribute to the preservation of synaptic vesicle specificity.

PS2.17

Genetic manipulation of murine retinal stem cells

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For long time, mammalian retina has been considered a tissue with low or absent regeneration potential during its entire life. However, recent findings have challenged this view. In fact, the isolation of proliferative cells from the adult ciliary margin and the assessment of retinal endogenous regenerative capability upon damage have revealed unexpected features of the retina. We have isolated mouse retinas from several different stages and looked for lasting cell growth in vitro. We successfully isolated cells from embryonic and postnatal mouse retina, that using serum-free culture conditions can grow stably in vitro. These cells are endowed with all the cardinal properties of stem cells, showing high and stable growth ability in vitro without any signs of senescence or growth-factor independent proliferation. Indeed, cells have been maintained in vitro for more than 6 months up to passage 65. Interestingly, upon mitogens removal all cells differentiated in mature retinal cell types and in particular in Muller glia and photoreceptor progenitors. Interestingly, stem cell derived photoreceptors express a large fraction of molecules of the light cycle, indicating a highly correct and complete differentiation program. However, bipolar, ganglion, amacrine cells were not generally found in the differentiated cultures indicating a biased differentiation potential. This raises the question whether these cells can be induced toward these cell types. In order to answer this question, we genetically manipulated the cell cultures forcing the expression of different master genes of the bipolar, amacrine and ganglion cell fate. In particular, we used Mash1 and NeuroD genes to push bipolar and amacrine cell fate differentiation respectively and Math5 gene to direct ganglion cell formation. Notably, similar results were obtained with cell cultures at early or late in vitro passages. Our results indicate that cultures of retinal stem cells maintain a stable differentiation potential in vitro and may be stimulated to differentiate toward many of the retinal cell types. This strategy aims to obtain pure population of specific retina cell types useful for gene expression profiling and cell transplantation.

PS2.18

Distinct Numb isoform modulate NSCs, self-renewal and multipotency versus differentiation

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Neural stem cells (NSCs) are an attractive tool in neurodegenerative disorders treatment for their potential to generate all mature cells in the Central Nervous System. Unfortunately, our knowledge on NSCs regulation is incomplete.
Numb was originally described in Drosophila as a mutation affecting binary divisions in the sensory organ precursor lineage. Numb is a signaling adapter protein with two protein–protein interaction domains: a Phospho-Tyrosine Binding domain (PTB) and a Proline-Rich Region (PRR) that functions as an SH3-binding domain. There are at least four human Numb isoforms that have been shown to influence in different neural mouse system differentiation vs proliferation. We are investigating on the role of Numb isoforms in NSCs and observed a marked decrease of isoform 2 during differentiation. In particular, the isoform 2 and 4 are present in undifferentiated NSCs, while isoform 2 transcript disappears after differentiation. To explain the role of this switch we overexpressed the isoform 2 in NSCs. The differentiated cells show an increase in neuronal differentiation. Likewise, in the same experiment, we observed a clear reduction of GALC positive cells mediated by isoform 2 overexpression. This scenario is well-matching with a role of Numb in opposition to Notch. In particular, we are testing if different Numb isoforms induce Itch mediated Notch degradation, thus explaining the isoform switch. Preliminary data indicate a different ability of the two isoforms to bind Itch. NSCs overexpressing different Numb isoforms (or domains) are evaluated for replicating activity and differentiating potential. Moreover we are evaluating if a Numb negative regulator, Musashi (MSI1 and MSI2), is able to overrule Numb overexpression effect in NSC differentiative program.
A better knowledge of the mechanisms that regulate human NSCs differentiation may help us to manipulate NSCs for a wide use in the treatment of neurodegenerative diseases.

PS2.19

5-HT2B signalling partecipates in retinal and craniofacial morphogenesis during *Xenopus laevis* development

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Serotonin (5HT) is a neurotransmitter that mediates a wide variety of effects in the central and peripheral adult nervous system. Experimental evidences demonstrated that serotonin even has an important role as growth and differentiating factor for neuronal and non-neuronal cells by controlling proliferation, migration and apoptosis during development.
All the biological actions of 5-HT are mediated by G-coupled receptors and, among these, the 5-HT2B receptor is expressed during CNS, heart and craniofacial development. By using Xenopus laevis as a model system, we demonstrated that 5HT2B receptor loss of function determines a decrease in the proliferation rate of retinoblasts and increases the apoptosis of retinal cells thus resulting in abnormal eye morphology. In order to further investigate 5HT2B role during development, we performed complementary experiments of gene gain of function. The overexpression of 5HT2B, leads to the formation of eyes with irregular form, position and orientation and showing defects in the optic fissure closure and in the pigmented epithelium formation. A detailed molecular analysis pointed out a disorganization of the typical laminar retinal structure and the presence of differentiated neuronal cells in ectopic position. Moreover, as pharmacological treatments with 5HT2 antagonists elicited in mice craniofacial alterations, we are studying the formation of craniofacial muscles and skeletal elements in both overexpressing and depleted 5HT2B embryos. In particular, alteration of 5HT2B expression during embryogenesis results in altered formation of extraocular muscles as well as of the jaw and hyoid cartilages and correlated muscles. We showed that 5HT, via 5HT2B receptor, is among the key extracellular signals that control Xenopus retinal histogenesis and eye morphogenesis. Moreover our results indicate for the first time a direct involvement 5-HT2B receptors in mediating the serotonin action on craniofacial morphogenesis by influencing the formation of skeletal elements and that of the connected muscles.

PS2.20

Establishing Eomesodermin role in cortical intermediate progenitor specification and development

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Eomesodermin (Eomes or Tbr2) is a T-box transcription factor essential for early embriogenesis where it is requested for development of the trophectoderm lineage. However, in the following development, Eomes become expressed in different neuronal structures, and in particular in the cortex where its expression labels a specific population of proliferating basal neuroprogenitors also coined intermediate progenitor cells (IPCs). IPCs have been shown to be transient proliferating cells with specific neurogenic potential, which are formed during radial glia differentiation. These different cell populations may be identified following the expression domains of the transcription factors Pax6 (radial glia), Eomes (IPCs) and Tbr1 (post-mitotic neurons). We are investigating the role played by Eomes in this contest by using both loss and gain of function approaches. Conditional Eomes mutant mice were crossed with Foxg1-cre animals leading to an Eomes specific inactivation in the developing forebrain. In these mutants, IPCs cell formation was strongly impaired leading to severe defects in cerebral cortical layers formation and cell type specification. In order to better investigate the role of Eomes in IPCs determination, we overexpressed the gene in developing cortex by in utero electroporation. After 48hrs, Eomes overexpression elicited drastic changes in the architecture of the developing cortex. In fact, we detected a strong enlargement of the sub-ventricular area coupled with a reduction of the radial glia ventricular layer. These findings were confirmed using specific markers of the different cortical domains. Interestingly, long-term analysis revealed that Eomes overexpression led to a detectable alteration of definitive neuroblast cell fate and relative position in the cortical layers. Altogether, these data identify Eomes as a key molecular determinant of IPCs cells, and alteration of its expression leads to severe changes in cortical development and organization.

PS2.21

Cortical spinal motor neurons require Coup-tf1 for their correct specification to control skilled motor behavior

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During embryogenesis, different regions of the primordial cerebral cortex express unique combinations of transcription factors that control proliferation, cell fate determination and migration. COUP-TFI is an orphan member of the steroid/thyroid hormone receptor superfamily.In the developing mouse cortex COUP-TFI expression pattern is spatially and temporally discrete with a high caudal to low rostral expression gradient. Constitutive null mice for COUP-TFI die at birth and show a complex cortical phenotype and altered thalamocortical connectivity. To better understand the role of COUP-TFI in the specification of cortical cell populations, we have generated a mouse conditional mutant line in which COUP-TFI is selectively inactivated in the cortex. In contrast to the null mice, these animals survive until adulthood. We have previously reported (Armentano et al, Nat Neurosci 2007) that in these mice the patterning of the future areal subdivisions of the adult cortex is strongly affected, resulting in an abnormally enlarged motor cortex at the expense of a reduced and caudally shifted somatosensory area. The rearranged cortical wall retains its normal connections with the thalamus; however, if the abnormally huge motor area is or is not functionally active and properly connected with its subcortical targets (e.g. spinal cord) remain open questions to which is important to answer. In this work we show, through behavioural, hodological (neural connections) and molecular approaches, that COUP-TFI strongly interact with the development of the corticospinal motor system. Corticospinal motor neurons (CSMN) do not differentiate properly and the mutant motor area is not correctly connected with the spinal cord; the cortical functional control of the voluntary movements results lost. These results show that COUP-TFI strongly controls the correct development of the CSMN in the cerebral cortex.

PS2.22

Nurr1, a transcription factor essential for midbrain dopaminergic neuron development, regulates *Bdnf* gene expression *in vitro*

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The transcription factor (TF) Nurr1, a member of the steroid-thyroid nuclear receptor superfamily, is highly expressed in the ventral midbrain during embryonic development and plays an important role in substantia nigra and VTA dopaminergic (mDA) neuron differentiation and maintenance, possibly also during the adult life. *Nurr1* expression is plastic and can be modulated by neuronal activity, including depolarization induced by high potassium. Only a few Nurr1 target genes have been identified and it remains unclear how Nurr1 regulates mDA neuron development and function. To identify novel Nurr1 target genes we have used genome-wide expression profiling in rat midbrain primary cultures, enriched in mDA neurons, following up-regulation of *Nurr1* expression by depolarization. The DNA microarray experiments showed that the brain derived neurotrophic factor gene (*Bdnf*) is amongst the genes upregulated by depolarization. Here we show that *Nurr1* and *Bdnf* expression are both phospholipase C- and protein kinase C-dependent, whilst MAPK independent. In RNA interference experiments the decreased *Nurr1* expression is followed by tyrosine hydroxylase and BDNF mRNA and protein down-regulation, as well as by reduction of the mDA TF *Pitx3* transcripts. Reporter gene assay and chromatin immunoprecipitation experiments performed on midbrain primary cultures using four *Bdnf* promoter constructs show that *Bdnf* is a direct target of Nurr1. Taken together, our findings suggest that Nurr1 controls the development and the function of mDA neurons also via direct regulation of *Bdnf* expression.

PS2.23

Neurogenins and the specification of cerebellar GABA neurons

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All cerebellar cells derive from two germinative neuroepithelia: the ventricular zone (VZ) gives rise to GABAergic neurons, whereas the rhombic lip (RL) produces glutamatergic progenitors. Clonal analyses and studies of mutant animals indicate that GABAergic neurons share a common lineage. As yet, little is known about the cellular/molecular interactions controlling the fate of GABAergic cerebellar neurons. In the cerebellar VZ at key stages of GABAergic neurogenesis, we have detected the expression of three proneural genes, namely Ngn1, Ngn2 and Mash1, that feature incompletely overlapping expression domains. In addition, we have shown that Ngn1 and Ngn2 label large progenitor cell bodies adjacent to a postmitotic domain labeled by Purkinje cell (PC) precursor markers Lhx1 and Lhx5. Finally, we have demonstrated that Ngn1 is expressed in a progenitor domain that is neatly complementary to that labeled by the earliest known GABA interneuron marker Pax2. These observations prompted us to further analyze the roles of Ngn1 and Ngn2 in the genesis of cerebellar GABA neurons and in the choice between different GABA fates / PC subtypes. To this end, we are generating knock−in mice expressing the tamoxifen−inducible Cre recombinase CreERT2 under control of Ngn1 and Ngn2 cis−acting sequences. This approach will allow us to define the critical developmental stages at which different types and subtypes of GABA neurons are generated in the cerebellar VZ, and to clarify the individual and combinatorial roles of Ngns in specifying the diversity of cerebellar GABA neurons.

PS3.01

Inducible dominant-negative CREB mutation alters in vivo Long Term Potentiation in mCREB mice

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The transcription factor cAMP-response element-binding (CREB) is critical for synaptic plasticity. This has been shown insofar by blocking CREB transduction and examining in vitro Long Term Potentiation (LTP) or Long Term Depression (LTD). In this study, we took the opportunity of transgenic mice (Sakai et al., 2002) in which an inducible dominant-negative CREB mutation selectively blocks CREB activation within the cell nucleus thus altering the CREB to function. Different patterns of mCREB expression were found in CA1 or Dentate Gyrus (DG) hippocampal areas in different lines of transgenic mice and mCREB expression was controlled by doxycycline administration. We focused on transgenic mice with DG pattern of mCREB expression to examine whether alterations of CREB function affect spontaneous behaviour in an open field and neural plasticity estimated through measurements of in vivo synaptic transmission and LTP in the Perforant Path-Dentate Gyrus pathway. We report that mutant mice do not differ from controls in terms of spontaneous behaviour and basal synaptic transmission, but are strongly impaired in the induction of long-term potentiation in the dentate gyrus in vivo.
Together, these data suggest that CREB involvement is not required in basal conditions but might be implicated when strong stimulation occurs.

PS3.02

BDNF mRNA splice variants represent a spatial code to regulate the complexity of dendrites and number of spines in specific domains of the dendritic tree

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The neurotrophin Brain Derived Neurotrophic Factor (BDNF) has many different trophic effects and is a key survival factor for neural cells. The rodent bdnf gene generates, through alternative splicing, at least 22 different BDNF transcripts. We have recently shown that exon II and VI BDNF transcripts are localized in dendrites while exons I and IV transcripts are restricted to the cell soma (Chiaruttini et al. 2008) however, the physiological role of these transcripts is still unclear. In this study, we provide evidence that exon II and VI BDNF transcripts can be locally translated dendrites. By selective over-expression or silencing of exon I, II, IV or VI BDNF mRNA isoforms in hippocampal primary neurons at different days in vitro we found that they exert a local effect on cell dendritic arborization and spine number. In particular, we found that BDNF mRNA isoforms located in the cell soma have a prominent effect on primary dendritic arborization and proximal spine development while dendritically targeted isoforms are required to promote complex dendritic arborization and higher number of distal spines. These results strengthen our contention that BDNF mRNA splice variants represent a spatial code to regulate neuronal plasticity locally.

PS3.03

Non-equilibrium activation of GluR6/KA2 kainate receptors determines slow current deactivation kinetics

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Kainate receptors (KARs) are ionotropic glutamate receptors contributing to synaptic currents with a component of small amplitude and slow decay kinetics, a feature likely essential for synaptic integration. In contrast, KARs expressed in heterologous systems mediate current kinetics several-fold faster, for a reason that remains unexplained. As GluR6/KA2 receptors form a major population of KARs in the brain we have studied the biophysical properties of recombinant GluR6/KA2 in HEK293 cells. We have compared the agonist affinity, the desensitization properties and the decay kinetics of GluR6- and GluR6/KA2-currents elicited by different glutamate pulse durations. Strikingly different decay kinetics were found in response to brief (1.5 ms) glutamate applications, whereby GluR6/KA2-currents decayed ~ 10 times-fold slower than those elicited by long (100 ms) glutamate applications. Based on a model simulation, we propose that following brief agonist exposures, GluR6/KA2-currents unmask slow deactivation due to the stabilization of partially bound open states. Interestingly, as the slow GluR6/KA2 current deactivation matched the decay kinetics of KAR-EPSCs, we propose therefore that such GluR6/KA2 gating feature could be responsible for the KAR-EPSCs slow kinetics.

PS3.04

Constant low frequency stimulation shows changes in responsiveness of in-vitro cortical cultures during development

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Background. The electrophysiological activity changes during the in-vitro development of dissociated cortical cells (analysed through MEAs - Multi Electrode Arrays) gained an increasing interest in recent years. Nonetheless, a detailed study on how the responsiveness of the networks changes over time is still missing. Here, we present such a study by analysing how the stimulation promptly influences the spontaneous activity of stimulated cultures during development.
Methods. We monitored the activity of seven cultures of dissociated cortical rat neurons. Recordings were performed up to 28 days-in-vitro (DIVs). The low frequency stimulus (0.2 Hz, biphasic pulse, 1.5V amplitude), was delivered sequentially from eight sites (three minutes per site). The Mean Firing Rate (MFR), Mean Bursting Rate (MBR) and Burst Duration (BD) were observed in the recorded phases of spontaneous activity (30 minutes) preceding and following the stimulus.
Results. During development, all the cultures showed the typical firing increase and onset of bursting activity (Van Pelt et al., 2005). Interestingly, while up to 18 - 21 DIVs the increase in bursting activity after the stimulation phase was negligible (if any), starting from 21 - 25 DIVs the post-stimulus phase showed a significant increment with respect to the MBR and the MFR. Furthermore, it is worth noting that the increment itself grew over time (Fig. 1).
Conclusions. The responsiveness of neuronal networks indicate their capability to modulate in presence of external stimuli and has to be considered as the basis for the plasticity changes the networks undergo during in-vitro as well as in-vivo development. Here, we focused on the way neuronal cultures behave when delivered low frequency stimulation during development, showing a relationship between age of the cultures and responsiveness to external stimuli.
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PS3.05

Stress-induced changes of neuroplastic proteins and modulation by chronic antidepressant treatment

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Although decreased levels of norepinephrine and serotonin may underlie depressive symptoms, compelling evidence now suggests that mood disorders are characterized by reduced neuronal plasticity. In fact, whereas antidepressant drugs rapidly enhance monoamine levels, their therapeutic effects are delayed by several weeks suggesting that adaptive changes may be required for therapeutic activity. Hence, pharmacological intervention may normalize such defects and improve neuronal function through the modulation of proteins and systems important for cellular plasticity and resiliency.
One important system in this context is the neurotrophin brain-derived neurotrophic factor (BDNF), whose expression and function is regulated by pharmacological treatments.
In the present study we investigated whether chronic antidepressant treatment could alter the stress-induced modulation of BDNF (gene and protein expression) and of other molecules belonging to its signaling pathway; we also considered proteins that can regulate the expression of specific BDNF isoforms. The antidepressant used was duloxetine that is a novel serotonin-noradrenaline reuptake inhibitor that differs from other antidepressants by virtue of its balanced potency on both neurotransmitter systems. Moreover, it is known that stress represents an important factor of vulnerability in psychiatric conditions, produces neuropathological changes in the hippocampus and may determine a functional impairment in synaptic activity
In summary, our results consolidate the idea that the neurotrophin BDNF and the related protein may represent a common target of antidepressant treatment. Moreover, we have now provided evidence for a novel degree of modulation, which refers to the possibility that antidepressant drugs might enhance the synaptic pool of the neurotrophin and alter its signaling under challenging conditions, thus supporting the role of these pharmacological agents in the modulation of synaptic function and cellular resiliency.

PS3.06

Emotional behaviors in autoimmune-prone BAFF transgenic mice

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Background: B-cell activating factor of the TNF family (BAFF) is a TNF-like cytokine that is essential for the maturation and survival of peripheral B-cells [1]. BAFF overexpression has been observed in several autoimmune disease such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA) [2], Sjögren's Syndrome (SS) [3] and Multiple Sclerosis (MS) [4]. BAFF transgenic mice develop an autoimmune condition similar to SLE and SS [5]. Neuropsychiatric abnormalities, such as the aforementioned, are often associated with anxiety and mood disorders [6]. **Methods:** This study investigated the effects of BAFF overexpression on depression-like behaviors (Forced-Swim test, Tails Suspension Test) and anxiety-like behaviors (Open Field, Novelty-Suppressed Feeding, Elevated-Plus Maze). We then examined the expression of c-Fos, an established marker for neuronal activation, in response to a mild anxiogenic challenge (15 minutes Elevated-plus-maze exposure). We also quantified the hippocampal progenitor cell proliferation and we elicited Dentate Gyrus (DG) long-term potentiation (LTP) in the presence/absence of GABA blockers. **Results:** Compared to wild type littermates, BAFF transgenic mice showed increased anxiety-like behaviors and increased c-fos expression in cingulate cortex, hypothalamic paraventricular nuclei, DG and basolateral amygdala. They showed decreased progenitor cell proliferation and impaired neurogenesis-dependent and neurogenesis-independent LTP in DG. **Conclusions:** BAFF overexpression alters emotional behaviors, decreases DG neurogenesis and disrupts DG LTP. These results might have implication for treatment of anxiety associated with autoimmune disorders.

PS3.07

Control of cortical binocularity by the corpus callosum

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The corpus callosum is the largest white matter structure in the brain and mediates most of the interhemispheric communication. Visual cortical connections mature by P15 in rodents and are crucial for the developmental maturation of visual cortex during the "critical period" (Caleo et al., 2007). However, other functions of the callosum remain only partly understood. Here we have studied the role of callosal connections in cortical binocularity. To this aim we examined visual responses before and after acute silencing of geniculo-striate pathway. This protocol allows us to isolate visual responses transmitted via the corpus callosum. Cortical binocularity was assessed by measuring the amplitude of the field potential (VEP) evoked by a stimulus grating presented to either eye. We used a unilateral tetrodotoxin (TTX) injection into the geniculate of young rats (age P24-P29) to block thalamic input to visual cortex. As expected, we found in normal rats that responses from binocular region of visual cortex were dominated by inputs from controlateral eye (Contra/Ipsi VEP ratio = 2.4 ± 0,5). Following TTX injection into the geniculate, C/I ratio dropped down to 0.4 ± 0,05 (t-test, p = 0.005). Analysis of VEP amplitudes demonstrated a highly significant decrease of controlateral-eye driven responses (contralateral-eye VEP amplitude before TTX = 326 ± 75 μV, contralateral-eye VEP amplitude after TTX = 68 ± 12 μV; t-test, p = 0.008). There was also a small, non significant reduction of ipsilateral-eye driven responses (ipsilateral- eye VEP amplitude before TTX= 189 ± 52 μV, ipsilateral-eye VEP amplitude after TTX = 160 μV ± 28; t-test, p = 0.4).
We conclude that visual cortex receives a significant input from ipsilateral eye via callosal connections.

PS3.08

The study of network dynamics and plasticity in cortical neurons coupled to Micro-Electrode Arrays

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Investigations on the electrophysiological behavior shown by neural networks are an essential step towards the understanding of brain information processing and computation. Dissociated cortical networks maintained in-vitro, coupled to Micro-Electrode Arrays (MEAs), represent a neurobiological model where the strategies employed by the nervous system to represent and process information can be easily approached and basic physiological mechanisms can be quantitatively characterized. After a few days in culture, neurons connect with each other with functionally active synapses and form a random network that displays spontaneous electrophysiological activity in the form of highly complex temporal patterns, ranging from random spiking to robust and rhythmic bursting behavior. The spontaneous activity changes during the in vitro development (Chiappalone et al., 2006) and can be modulated by chemical and electrical inputs, producing short and/or long-term effects in the in synaptic efficacy and network dynamics.
Here we demonstrate the capability of a cortical network to change its spontaneous activity in response to specific pharmacological stimulation or to change its main properties in neuronal preparations from genetically altered mice. In addition, we will report results on induction of activity changes in cortical networks due to revised protocols of tetanic stimulation (Jimbo et al., 1999; Huang et al., 2004). We found remarkable differences in the electrophysiological activity of the network after the tetanus, suggesting the induction of potentiation at the whole network level. The obtained results demonstrate that large in vitro cortical assemblies can display Long Term Network Potentiation, a mechanism supposed to be involved in learning and memory at cellular level.

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PS3.09

Voltage-gated calcium channel properties differ in glutamatergic versus GABAergic hippocampal neurons

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We have previously demonstrated that GABAergic neurons exhibit increased Ca2+ responsiveness to depolarisation relative to glutamatergic neurons. To determine whether these differences in Ca2+ dynamics represent diverse voltage gated calcium (CaV) channel characteristics, CaV channel properties were recorded from mature neurons in the whole cell voltage clamp configuration. Immediately after recording, cytoplasm was aspirated into the patch pipette for single cell PCR analysis enabling verification of the recorded neurons GABAergic or glutamatergic nature. Inward barium currents (IBa) from IV relations peaked at a potential of 0 mV with a mean of -6.67 ± 0.89 pA/pF in GABAergic neurons. However peak IBa was significantly lower in glutamatergic neurons at -3.91 ± 0.53 pA/pF and shifted to a more hyperpolarised potential of -10 mV. Steady state voltage dependent inactivation curves yielded a V1/2 of -39.1 ± 1.7 mV in GABAergic neurons whereas inactivation curves of glutamatergic neurons were signficantly left shifted with a V1/2 of -21.6 ± 2.6 mV. CaV inactivation rates in glutamatergic neurons were also enhanced relative to GABAergic neurons. In addition, the voltage dependence of activation curve was significantly left shifted in glutamatergic relative to GABAergic neurons. Overall, these results indicate that CaV channel properties differ significantly between GABAergic and glutamatergic neurons. This could be due to either different intrinsic properties of the channels expressed by the two cell populations or the differential expression of modulatory proteins, such as SNAP-25, which is expressed at high levels in glutamatergic but not GABAergic neurons. Further experiments will aim to discriminate between these two possibilities.

PS3.10

SNAP-25 negatively modulates voltage-gated calcium channels and network activity

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SNAP25 is a SNARE protein that participates in synaptic vesicles exocytosis. We previously demonstrated that differential SNAP25 expression in hippocampal neurons regulates intracellular calcium dynamics (Verderio et al, 2004) and that negative modulation of neuronal voltage-gated calcium channels requires SNAP25 activity-dependent phosphorylation (Pozzi et al, 2007). A dysregulation of calcium dynamics due to alterations of SNAP25 expression may thus lead to unbalances of neuronal network activity. In line with these data, alterations of SNAP25 expression have been associated to several neurological diseases, such as schizophrenia (Thompson et al, 1998, 2003; Young et al, 1998; Karson et al, 1999; Fatemi et al, 2001; Mukaetova−Ladinska et al, 2002) and ADHD (Renner et al, 2008).
In this study we aim to investigate whether reduction or lack of SNAP25 may lead to altered network activity in vitro and in vivo and thus to epileptic phenotype. This possibility, previously raised in the coloboma mice model (Zhang et al, 2004), has now been tested using SNAP+/- mice, which display selective reduction of this protein. A significant increase in calcium responsiveness to depolarization was observed in SNAP25+/- and -/- neurons in comparison with wild-type neurons; this result was confirmed in synaptosomes from adult wild-type and SNAP25+/- mice. Behavioural tests on wt and SNAP25+/- mice revealed an increased spontaneous motor activity and a deficit in long term memory in SNAP25+/- mice. In vivo electroencephalographic recordings showed a significant increase in the number of spikes in SNAP25 +/- mice. Moreover, these mice showed a high susceptibility to kainate-induced seizures and a strong sprouting of mossy fibers, which is a marker of spontaneous epileptogenesis in mice after kainate injection.
These data suggest that alterations of SNAP25 expression may contribute to epilepsy, possibly by dysregulating the normal calcium dynamics and thus altering network activity.

PS3.11

Recovery from a memory deficit in Ras-GRF1 knockout mice

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Compelling pieces of evidence exist about the key role exerted by the intracellular pathway Ras/MAPK in the consolidation of emotional memories. An involvement of ERK2 protein in fear memory reconsolidation has been proved in ERK1 knockout (KO) mice, and a line of Ras-GRF1 KO mice – the same used in the present study – showed a severe memory deficit in various behavioral tasks characterized by a critical involvement of the amigdala formation. To explore the way for a possible recovery from the memory impairment, and shed light on its molecular underpinnings, we sub-mitted groups of Ras-GRF1 KO mice and their controls to different experimental procedures in the step-through inhibitory avoidance (IA) task. Remarkably, a full recovery from the deficit was ob-served in KO animals when submitted to a «double training», i.e. a procedure consisting of two shock-delivery sessions administered with an interval of 2 days. Our results suggest a possible re-cruitment of vicarious molecular mechanisms which, depending on the peculiar procedure at issue, can sustain LTM formation in Ras-GRF1 KO mice.

PS3.12

rTMS, progenitor cell proliferation and synaptic plasticity

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Background: To investigate the effects of repetitive transcranial magnetic stimulation (rTMS) on neuronal plasticity in rodents.Long-term potentiation (LTP) phenomena at excitatory synapses are the bases of plasticity and recovery of function after neuronal injuries and degeneration. It has been proposed that rTMS might enhance processes relevant to cognitive and sensorimotor functions. These effects may be promoted through the modulation N-methyl-D-aspartate (NMDA) receptor composition and function, neurogenesis and LTP.
Methods: Following a 5-day rTMS treatment (15 Hz stimulation at 50% stimulator output intensity with a rodent coil), we tested neocortical and hippocampal LTP (Dentate Gyrus-DG, prefrontal cortex), DG stem cell proliferation and NMDAR composition and function in brain from chronic stimulated- and sham-treated mice.
Results: rTMS treatment induced an increase in the amount of LTP all the tested brain areas, an increase in the number of new-generated neurons and an increase of NMDA/glycine-induced NMDA receptor activation without affect on receptor assembly and interaction with PSD-95.
Conclusions: We conclude that chronic rTMS treatment in rodents stimulates proliferation of newly generated neurons in DG and induces an increase of LTP in neurogenic and non- neurogenic areas. Biochemical evidence supports an enhanced NMDAR function that may underlie these plastic changes. These results have important implications for treatment of refractory depression.

PS3.13

Cognitive impairments in Gdi1 knockout mice are associated with specific defects in synaptic vesicle pools and short-term synaptic plasticity

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GDI1 gene encodes for alfaGDI, one of the proteins controlling the activity of small GTPases of the Rab family in intracellular trafficking. Mutations in human GDI1 gene are responsible for X-linked non-specific mental retardation. In mice, the absence of Gdi1 impairs Rab GTPases recycling leading to an accumulation of their membrane-associated form on specific organelles. Moreover, Gdi1 deficient mice (Gdi1KO) showed a specific hippocampus-dependent short-term memory deficit, similar to what is found in XLMR patients. Gdi1KO mice display a defective synaptic vesicle (SV) biogenesis leading to a 50% reduction in the total number of SVs in adult hippocampal synapses. At the active zone SVs appear normal, indicating a defect of SVs reserve pool. In order to evaluate the functional impact of Gdi1 deletion on short-term plasticity at CA1 excitatory synapses of the hippocampus, paired-pulse facilitation (PPF), post-tetanic potentiation (PTP) and synaptic depression were analyzed. Gdi1KO mice show higher PPF, attributable to either a lower release probability or a larger build-up of intraterminal Ca2+. Consistently, a prolonged high-frequency stimulation of Shaffer collateral induces a stronger synaptic facilitation in Gdi1KO mice, which is followed by a depressing phase similar to that observed in wild type animals. The subsequent recovery from depression, obtained by lowering the stimulation frequency, was significantly slowed down in Gdi1KO mice, suggesting an impaired SV recruitment possibly related to the marked reduction in the SV reserve pool. Moreover, we are able to reverse the specific short-term memory deficit of Gdi1KO mice by using a ï¿½spacedï¿½ instead to ï¿½massedï¿½ training protocol in trace fear conditioning and radial maze tasks. The data suggest that lack of Gdi1 alters steps controlling the formation and maintenance of the SV pools possibly through changes in the Rab cycle and that these changes interfere with the efficiency of mental processing.

PS3.14

Long-term effects of AP5 and TTX administration on neuronal networks evaluated on in-vitro MEA cultivations

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We present results concerning the long-term effects of TTX and AP5 on the spontaneous activity of 10 div neuronal networks on multi-electro arrays (MEA). With "long-term" we mean the effects 30 minutes after the drug wash-out, i.e. after a considerable activity recovery. We evaluated these effects studying the intra-burst activity with a 0.1ms resolution. We adopted two research methods: i) an high-resolution cross-correlation burst analysis (introduced by ourselves) and ii) a topological burst analysis.
The first method, used for the analysis of correlations among bursts (before drug administration and in the post-recovery period), estimates network correlations and synchronizations. Then we analyze burst topologies (second method) distinguishing among bursts that involve more or less than 25% of MEA channels (Global or Local burst respectively).
Results demonstrate that both TTX and AP5 raise the burst number respect to the pre-drug period but with different features. AP5 raises the burst mean cross-correlation value (+10%), i.e. the mean correlation among bursts, especially in local bursts, and scatters CCF values (mean CCF standard deviation increasing). TTX decreases the mean CCF value (-7,1%) and faintly reduces cross-correlation values standard deviation, compacting CCF values. Moreover, AP5 slightly increases the number of local bursts and marginally modifies the global/local ratio but TTX shocks the network dynamic dramatically increasing the global burst number and reducing local bursts.
Results obtained with our innovative method show that AP5 is unable to twist the activity in an after-pruning network but can desynchronize sub-networks. TTX, on the other side, effects on the whole network synchronization. The notable activity increment in the post-TTX tract (+66%), together with the Global activity explosion and the mean burst cross-correlation value decrement, suggests the possibility of a long-term detrimental effect on inhibitory mechanisms.

PS3.15

Study of epileptic activity in hippocampal slices of Synapsins knockout mice

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Synapsins I/II/III (Syns) are abundant proteins of synaptic vesicles (SV) implicated in the regulation of neurotransmitter release and synapse formation. Syns mutant mice show spontaneous and sensory stimuli-evoked epileptic seizures and a non-sense mutation in the SYN1 gene was recently found to be associated with familial X-linked epilepsy. We used patch-clamp and field potentials recordings to study neuronal network activity and properties of synaptic transmission in acute hippocampal slices of triple knockout (TKO) mice.
Field excitatory postsynaptic potential (fEPSP) recordings from CA1 stratum radiatum showed higher paired-pulse facilitation in TKO mice. The fEPSPs recorded in response to a stimulus train of 20 sec @ 20 Hz was characterized by an initial facilitation, followed by a depressing phase. Both phases were significantly enhanced in TKO mice, showing an impairment in glutamatergic neurotransmission. Patch-clamp recordings revealed a lower input resistance (Rin) and a depolarized membrane potential in mutant CA1 pyramidal neurons. The treatment of slices with the convulsant 4-aminopyridine (4AP; 200 ï M) increased neuronal Rin, neuronal excitability and frequency of spontaneous excitatory postsynaptic currents. 4AP induced also epileptiform discharges: inter-ictal (I-IC), ictal (IC) and long-lasting hyperpolarized events. The frequency of evoked I-IC events was higher in TKO mice.
These results indicate that TKO mice represent an interesting model of human epilepsy useful to study how altered synaptic transmission due to mutations in SV proteins increases neuronal excitability leading to development of epilepsy.

PS3.16

Altered peripheral myelination in GABA-B1-receptor knockout mice

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γ- aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the nervous system, which interacts with ionotropic and metabotropic GABA receptors. Emerging evidences indicate that these receptors play significant physiological roles also in the peripheral nervous system (PNS). In particular, γ-aminobutyric acid type B (GABA-B) receptors are implicated in Schwann cell proliferation and differentiation. An approach to elucidate that biochemical, morphological and functional parameters of peripheral nerve fibers depend on GABA-B receptors is the study of GABA-B1-deficient mice, which are devoid of functional GABA-B receptors. We have observed that GABA-B1-deficient mice exhibit morphological and molecular changes in peripheral myelin, including an increase in the number of irregular fibers and in the expression levels of the myelin proteins PMP22 and P0. Moreover, the number of small myelinated fibers and small neurons of the lumbar dorsal root ganglia is higher in GABA-B1-deficient than in wild type mice. Furthermore, we have shown that GABA-B1-deficient mice show alterations in gait and nociceptive sensitivity. Aδ-sensitive fibers seem to be increased in these mice. In conclusion, our findings implicate GABA-B receptors in the PNS myelination process and raise the possibility that PNS alterations contribute to the sensory phenotype of GABA-B1-deficient mice.

PS3.17

The mossy fiber input to Golgi cells in the cerebellum: presence of an NMDA component

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Golgi cells (GoCs) are the main inhibitory interneurons in the cerebellar granular layer. They are thought to be activated by mossy fibers (Mf), but this synaptic connection has not been investigated. We made whole-cell voltage-clamp recordings from GoCs in rat parasagittal cerebellar slices (P17-23; 32°C). Excitatory synaptic currents (EPSCs) evoked by white matter extracellular stimulation had a multimodal latency distribution, in which two populations were distinguished: i) short-latency EPSCs, with relatively invariant latency (1.4 ms; CV 0.11), presumably originated from direct Mf-GoC inputs. In fact our arrangement of the stimulation electrode ruled out direct activation of the granule cell (GrC)-GoC pathway. Accordingly, 2-photon Ca^{2+} imaging revealed fast (within 2 ms from stimulus) activation of basolateral GoC dendrites. ii) "Long-latency" EPSCs, with broader latency distribution (median 4.4 ms; CV 0.24), were enhanced by the GABAaR blocker SR95531 and inhibited by the NMDAR blocker D-APV, consistent with pharmacological sensitivity of GrC firing (D'Angelo et al., 1995), and presumably arose from the disynaptic Mf-GrC-GoC pathway. Mf-GoC EPSCs evoked at low frequency and threshold intensity (-70 mV), were fast, low-amplitude (-30/-70 pA) events, SR95531-, strychnine- and D-APV-insensitive, abolished by NBQX, and with a paired-pulse ratio (at 100 Hz) around 1. At -70 mV in a Mg^{2+}-free solution, a slowly rising and decaying EPSC component, abolished by D-APV, was revealed. When stepping at different potentials (–60 to +60 mV) in normal saline in the presence of non-NMDA GluR and inhibitory input blockers, the I-V plot of the EPSC peak showed the negative slope conductance typical of NMDA currents (reversal around 0 mV). The average current at +60 mV (27 pA) was abolished by D-APV. In conclusion, we show the existence of an NMDA-mediated current at the Mf-GoC input, which might underlie synaptic plasticity phenomena. *Supported by EC grant SENSOPAC and INGENIO project of ESF and Regione Lombardia, Italy.*

PS3.18

Functional characterization of presynaptic kainate receptors in primary hippocampal neurons and brain growth cones

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Excitatory glutamatergic transmission involves a variety of different receptor types, classically distinct in ionotropic and metabotropic but recently has been shown that AMPA and Kainate receptors have also metabotropic functions. In the past years we have demonstrated that AMPA receptors are expressed at the presynapse where induce a local activation of MAPK and a phosphorylation of synapsin I. In order to address whether endogenously released glutamate can also act on presynaptic kainate receptors, we have investigated the expression of various KAR subunits in Growth Cone Particles and in synaptosomes. Both synaptosomes and GCPs were enriched of GluR6,7 and KA2 subunits of KAR. To investigate whether the expression of KAR subunits in the GC is paralleled by the insertion of functional KARs on the plasma membrane, 5±7 DIV old neurons were loaded with Fura-2 applied in the presence of TTX. Exogenous application of the agonist induced a [Ca2+]i transient, thus indicating that KAR subunits are assembled in functional receptors . We are currently investigating whether second messenger pathways are coupled to presynaptic KAR in growth cones, by looking at the occurrence of post-translational modifications of selected presynaptic substrates, upon exposure of growth cones to kainate. In a first set of experiments we focused on synapsin I, which is the major presynaptic substrate for signalling pathways involving PKA, MAPK and CaMK. Cultured hippocampal neurons at 5-7 DIV were stimulated with kainate or forskolin, fixed and immunostained with anti-synapsin I antibody. Similarly to what already reported for the PKA activator forskolin, treatment with kainate induced a dispersion of synapsin I immunoreactivity in axonal growth cones, which was prevented by the specific PKA inhibitor H89. All together, these data provide support to the possibility that glutamate may act on AMPA and/or kainate presynaptic receptors, possibly affecting distribution of presynaptic proteins and synaptic vesicle recycling.

PS3.19

The 5-HT receptor 7 is accountable for the ERK-dependent neurite outgrowth in cultured neurons

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The 5-HT receptor 7 (Htr7) is the most recently identified member of the family of G-protein-coupled 5-HT receptors. This receptor has a widespread distribution in the brain and is involved in thermoregulation, circadian rhythm, mood regulation, sleep, learning and memory. Several antipsychotics and antidepressants have high affinity for the Htr7, suggesting that it is a potential target for the treatment of neuropsychiatric disorders. We have recently shown that the expression of the Htr7 is strongly and persistently upregulated in the striatum of rats chronically treated with the psychostimulant methylphenidate (Adriani et al., 2006, Neuropsychopharmacology, 31: 1946-1956). We are studying the signalling pathway activated by the Htr7 in striatal primary cultures obtained from E16 rat embryos. Virtually all cells in our cultures expressed the neuronal Tuj1 marker, as shown by co-expression with the nuclear marker DAPI, thus indicating that most cultured cells were neurons. Treatment of these cultures with 8-OH-DPAT, a Htr7 agonist, induced ERK phosphorylation and a significant increase of the neurite length, evaluated by Tuj1 immunostaining, when compared to untreated cultures. These effects were blocked by application of SB269970, an Htr7 selective antagonist, or by application of the MEK inhibitor U0126. These results show that stimulation of the Htr7 regulates ERK-dependent neurite outgrowth in striatal neurons in vitro, suggesting a crucial role of this receptor in controlling neuronal morphology and plastic changes of striatal circuits.

PS3.20

Neurochemical characterization of cultured neocortical networks

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The isolation and in vitro maintenance of neurons allows the examination of their cellular function so that culture systems are widely used for analysis of developmental and pathogenic mechanisms. Networks of cultured cortical neurons, spontaneously assembled from dissociated cells, are characterized by spontaneous rhythmic activity that resembles observations in brain areas in vivo. Our aim was the morphological and neurochemical characterization of primary murine cortical cultures which were studied with MEA (micro-electrode-array) technology that allows recording of in vitro neural spiking activity. By means of immunocytochemical approaches for light and confocal microscopy and using neuronal, glial and synaptic markers a preliminary identification of the different cell populations and of their morphological features was performed. This analysis was then combined with some markers of the GABAergic system to understand the relationships among the different components of the neural network. At 7-8 days in vitro our culture showed a majority of MAP2 (microtubule associated protein 2) positive (+) neurons with long processes; the neuronal component was confirmed by NeuN (neuronal nuclear antigen) immunoreactivity, but a small glial population was also identified with the specific marker GFAP (gliofibrillar acid protein). Some synaptic vesicular markers, the protein synaptophysin and the glutamate transporters (VGLUT) showed a considerable number of synaptic terminals, some of them clearly excitatory (VGLUT+). Preliminary experiments indicated the presence of GABAergic neurons and the diffuse expression of α1 and α4 GABA A receptor subunits both in cell bodies and in growing processes. On these basis this culture system may prove to be a promising tool to better understand the electrophysiological and pharmacological properties of cortical networks and to correlate some pathological alterations with dysfunctions of glutamatergic and/or GABAergic circuits.

PS3.21

Granular layer resonance in response to tactile stimulation *in vivo*

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Local field potentials (LFP) were recorded from the granular layer of Crus IIa in 20-26 days-old urethane anesthetised Wistar rats. The recording electrode was positioned into the granular layer at 300-500 micro-meters from the cerebellar surface. The evoked responses were elicited by tactile stimulation of the whisker pad by delivering 30 ms pulses at 30 psi through a plastic pipette tip. The LFP was composed of 3 waves called T, C and D corresponding to the trigeminal and cortical responses reported in previous studies (Morissette and Bower 1996; Roggeri et al., submitted). In order to obtain precise measurements of changes in response to different stimulation patterns we considered only the T wave, as it establishes a direct link (via the trigeminal nucleus) between the sensory input and the cerebellum. The amplitude of the T component was sensitive to the frequency of stimulus repetition. The size of the T wave decreased non-monotonically from 1 Hz to 16 Hz and showed an enhanced response between 6 Hz and 9 Hz (10.4±7.5%, n=7; $p<0.05$, unpaired t-test) suggesting a resonant behaviour of the granular layer. Interestingly, the resonant frequency coincides with that of granule cells and Golgi cells responses to current injection revealed in vitro (D'Angelo et al., 2001; Solinas et al., 2008). The relationship between circuit resonance and single cell and synaptic properties is currently being investigated using a detailed computational model of the granular layer.

PS3.22

Tonic activation of $GABA_B$ receptors regulate release probability and the dynamics of synaptic inhibition in the cerebellar glomerulus

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The quantal properties of synaptic transmission are fundamental for determining the dynamic and plastic behaviours of central synapses. Here we report an investigation of inhibitory synaptic transmission between Golgi (GoC) and granule cells (GrC) in the cerebellar cortex. We performed whole-cell patch-clamp recordings on GrCs in slices measuring synaptic currents evoked by GoC axon stimulation (eIPSCs), spontaneous events (sIPSCs, due to GoC autorhythmic activity) and miniature currents. Our results show that transmission at this synapse is quantal in nature, consisting of multiquantal sIPSCs and eIPSCs. eIPSCs from putative single GoC-GrC connections have been studied by applying the binomial theory and multiple probability fluctuation analysis (MPFA). Increasing the Ca^{2+}/Mg^{2+} ratio in the extracellular solution determines a raise in the probability of release (p) and an increase in eIPSC mean amplitude while reduces the coefficient of variation (CV) and the failure rate. CV was used to estimate the quantal parameters p, n (number of release sites) and q (quantal size). With a physiological Ca^{2+}/Mg^{2+} ratio we obtained p=0.33±0.03 (n=3), n=5.76±0.53 (n=3) and q=8.47±0.94 pA (n=9). p, n and q obtained with MPFA were consistent with those obtained with the CV method. Brief high frequency trains at either low or physiological Ca^{2+}/Mg^{2+} ratio, revealed the coexistence of short-term facilitation and depression. Application of the $GABA_B$R blocker CGP55845 modified eIPSC trains by increasing the first eIPSC and accelerating the rate of decrease of subsequent eIPSCs, suggesting that $GABA_B$Rs, activated by ambient GABA, operate a tonic inhibition of release through a p reduction. This modulation did not affect the total charge transfer. These results suggest that the GoC-GrC synaptic connection, as a part of the cerebellar glomerulus, is endowed with a presynaptic $GABA_B$ receptor-dependent mechanism controlling the sharpness of the onset of inhibition.

PS3.23

Neuronal adaptations, induced by rolipram and imipramine, leading to memory enhancement

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We treated rats with a combination of cAMP modulators, namely rolipram and imipramine, to study the neuronal adaptations through which these drugs improve memory formation.
To address this issue, we injected rats intra-peritoneally for 4 days with rolipram+imipramine (RI), or placebo solution, at P19-P25, after which either behavioral or electrophysiological tests were carried out.
First, we assessed the memory improvement potential of this treatment through the hippocampus-dependent contextual fear conditioning task and found that memory of the shock to context association was significantly increased in RI (vs placebo) chronically (but not acutely) treated rats. To explore the physiological mechanisms through which memory formation was increased, we performed whole cell patch clamp recordings from hippocampal CA1 pyramidal cells after chronic RI treatment. We observed differences in AMPA and NMDA receptor transmission. We found that, in RI chronically treated rats (but not acutely treated rats), the AMPA/NMDA ratio was decreased while no difference was observed in the paired-pulse ratio, suggesting specific post-synaptic, but not pre-synaptic, alterations. We observed an increase in spontaneous AMPA miniEPSC amplitude, but not frequency, while a minimal stimulation protocol revealed no significant changes in the number of silent synapses. Furthermore, long-term potentiation (LTP) and spine density were also significantly increased in RI chronically treated rats.
Collectively, these results indicate that hippocampus-dependent memory enhancement correlate with specific CA1 neuron adaptations that could form the molecular substrate for increased memory formation.

PS3.24

Development and plasticity in networks of neurons grown onto micropatterned substrates

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Various molecules, during development of central nervous system, act on axons as guidance factors activating dynamic rearrangements of actin cytoskeleton and promoting the formation of plastic neuronal networks. We generated artificial neuronal networks onto substrates patterned with different adhesion and guidance molecules by using micro-contact printing (microCP) in order to characterize molecular and functional aspects of synaptic transmission and plasticity. We used poly-dimethylsiloxane stamps to pattern poly-D-lysine in various geometries with the aim to optimize adhesion conditions for neurons on substrates. By using immunofluorescence we evaluated different specific markers for structure (actin, microtubules) and synaptic function (phosphorylation, synaptic vesicle proteins and neurotransporters) to assess how different patterning geometries can influence neuronal growth and network development. Moreover structural organization of the network was analyzed by atomic force microscopy. The functional parameters of synaptic transmission of micropatterned neuronal networks was assessed by patch-clamp techniques. Since the drying step in conventional microCP could induce protein denaturation and loss of function, we applied an indirect microCP by which we patterned onto the surface an intermediate drying resistant molecule (protein A) that binds with high affinity and specificity the guidance cues, expressed as recombinant proteins (i.e. L1-Fc, NCAM-Fc). Finally we found that glutathione and streptavidin could be used as bridging molecules because of their ability to resist to drying step and bind with high affinity Glutathione-S-Transferase and biotin respectively. This approach make possible to pattern more than one factor on the same surface by combining different bridging molecules, allowing to study the simultaneous effects of multiple patterned molecules on the structural and functional differentiation of the neuronal network.

PS3.25

Layer- and cell type-specific synaptic inputs and spike outputs of pyramidal neurons along a visual cortical column *in vivo*

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The cortical column is the building module of the primary visual cortex in all mammals tested so far. *In vitro* studies provided a detailed knowledge of the physiology of synaptic connections of neocortical circuits within a visual cortical column. Nevertheless, we still lack a detailed knowledge of how sub- and supra-threshold spontaneous activity varies along a visual cortical column *in vivo*, and how the synaptic inputs driven by an optimal visual stimulus is converted to a spike output by the morphologically distinct pyramidal neurons that populate different cortical layers. This knowledge is crucially lacking for experimental rodents, widely used models to study visual cortical development and plasticity.
To address this issue, we performed intrinsic signal imaging (ISI)-targeted *in vivo* patch clamp recordings of pyramidal neurons in the binocular primary visual cortex of pigmented rats followed by neuronal reconstructions.
Subthreshold and suprathreshold activity were highly layer and cell type-specific. When tested with optimal stimuli, sub- and supra-threshold responsiveness vary with the laminar position and, within a layer, with the morphological identity of recorded neurons. Moreover, sub- to supra-threshold conversion of the RF size varied according the anatomical identity of the neuron. Finally, the relative distribution of simple and complex cells varied along a column. Interestingly, we found that within layer 5, the main output lamina of the neocortex, the two morphologically distinct populations of pyramids that project to different subcortical targets (slender- and thick tufted), have different spontaneous activity, visual responsiveness, orientation selectivity and receptive field structure. Thus, representation of a standard visual stimulus is highly layer- and cell type specific along a cortical column in vivo. This approach clarifies how a cortical column of visual cortex functions in the intact brain.

PS3.26

Inducible inhibition of CREB phosphorylation impairs memory, prevents learning-induced changes in hippocampal spine density, and blocks *in vivo* long term potentiation in mCREB mice

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CREB is implicated in memory formation, likely through its effect on long term synaptic plasticity mechanisms. Here we investigate the possibility that CREB controls large-scale plasticity phenomena and interferes with both the activity (LTP) and the connectivity (spine density) of brains circuits critically involved in contextual memory formation. To investigate these phenomena we measured in vivo long term potentiation and dendritic spine density elicited by contextual fear conditioning (CFC) in conditional mCREB mutant mice and their controls. We found that mutant mice were impaired performance in the CFC task. In addition, although basal dendritic spine density in CA1 was unaffected by the conditional CREB deletion in this region, these mice did not show the learning-dependent increase in CA1 spine density found in the control mice. Furthermore, induction of long-term potentiation in the CA1-Subiculum pathway in vivo was also altered in mCREB mice, although basal synaptic transmission was not. Together, our data suggest that CREB is recruited for large-scale changes in the activity and connectivity of brain circuits mediating formation of contextual fear memory.

PS3.27

Effects of stress and antidepressant drugs on presynaptic molecular mechanisms regulating glutamatergic neurotransmission

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We have recently demonstrated that depolarization-evoked release of glutamate was markedly reduced in the hippocampus of rats chronically treated with different antidepressant drugs (AD). The functional changes in glutamatergic neurotransmission were accounted for by alterations in protein-protein interactions regulating neurotransmitter release and function of the presynaptic SNARE protein complex, mediating neurotransmission release. Stress is a key factor in the pathogenesis of mood disorders. Since we found that AD reduced glutamate release, we hypothesized that AD could also reduce glutamate release induced by stress. Therefore, aim of this study was to analyze the effects of stress and chronic AD on depolarization-evoked release of glutamate and on selected presynaptic molecular mechanisms involved in the regulation of glutamatergic neurotransmission. Rats were treated with fluoxetine or desipramine (10 mg/kg) for 2 weeks and then subjected to acute unpredictable Footshock Stress (FS). Pre-frontal/frontal cortices (P/FC) were dissected and synaptosomes and synaptic membranes were purified. The FS paradigm induced, in P/FC, a marked increase of depolarization-evoked release of glutamate, which is prevented by chronic AD treatment. Moreover, while FS induced an increase of αCaM kinase II phosphorylation, we did not found major changes in presynaptic interactions involved in the regulation of glutamate release. Therefore, we hypothesized that the reduced glutamate release could be due to downstream mechanisms. We found that FS increased 100 kDa SNARE complex accumulation in presynaptic membranes, while AD did not prevent this increase. In summary, glutamate release induced by FS is related to accumulation of 7S SNARE complexes at the presynaptic membranes. Since chronic AD prevented the increase of glutamate release induced by FS, we speculate that, in AD-treated animals, accumulated SNARE complex are not primed for fusion.

PS3.28

A mathematical model of neurotransmission at the input stage of the cerebellum

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The granule cell (GC) of the cerebellum has some peculiar properties compared to other cells of vertebrates brain. The GC is characterised by a small soma (diam=6um) and by few excitatory and inhibitory inputs (2 to 6). Despite this simplicity, excitatory and inhibitory GC synaptic inputs are enclosed into a structure, called cerebellar glomerulus, enwrapped by a glial sheet. A major consequence of such synaptic organization is that (1) neurotransmitter diffusion causes cross-talk between nearby sites protracting synaptic responses and (2) excitatory and inhibitory input cross regulates each other by activating presynaptic receptors. Excitatory (EPSCs) and inhibitory synaptic currents (IPSCs) also display short-time plasticity in the form of depression and facilitation, which are strongly affected by neurotransmission diffusion and by presynaptic regulation. LTP or LTD induction at mossy fibre to GC regulates short-term plasticity by increasing or decreasing release probability, respectively. The IPSC dynamics also undergo regulatory effects by the activation of presynaptic GABAB receptors causing a decrease of the release probability.
Based on the numerous experimental investigations carried out at the GC input stage we developed a mathematical model of neurotransmitter release, diffusion and binding to postsynaptic receptors. Parameterization of the models was achieved by fitting the whole EPSCs and IPSCs traces such that the entire dynamics of the synaptic event could be described. The models point to the critical role played by release probability and neurotransmitter diffusion for regulating excitatory and inhibitory transmission.

PS3.29

Modulation of intracellular signaling pathways by acute swim stress in rat hippocampus

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Increasing evidence implicates stress as an important factor of vulnerability to mental illnesses. Hence, we decided to investigate the rapid changes produced by a mild acute stress in order to understand mechanisms set in motion by the cell as an attempt to "cope" with the challenging condition. We therefore investigated the intracellular signaling changes brought about by a 5 minutes forced swim stress in rats: Among the proteins involved in stress-related plasticity, we examined the expression of the neurotrophin BDNF, expression and phosphorylation of the MAP kinase ERK1/2, which mediates BDNF signals, the glutamate-related proteins α calcium/calmodulin-dependent protein kinase II (αCaMKII) and NMDA receptor subtypes NR1 and NR2B.
We found that the levels of the mature form of BDNF (mBDNF) were increased in P2 and S2 fractions in rats exposed to stress for 2 days, with no changes in BDNF propeptide levels (proBDNF). The analysis of the MAP kinase pathway revealed that acute swim stress enhances ERK1 activation on day 2, in P1 and S2 compartments of hippocampus.
We then evaluated the effects of stress on proteins of the glutamatergic synapse. Acute swim stress reduced the activation of αCaMKII in all subcellular fractions, mainly 5 minutes post-stress on both days in the P1 and P2 fractions. In the S2 compartment, αCaMKII activation is reduced on both days. Differently from αCaMKII, the activation of NR-1 subunit is increased, with no changes in NR-2B activation.
Taken as a whole, our results indicate that acute swim stress is able to modulate the response of important signaling pathways whose regulation is involved in plastic processes related to psychiatric disorders. Our experimental paradigm might be useful to investigate if and how single vs. repeated pharmacological treatment with psychotropic drugs (antidepressants or antipsychotics) will be able to modulate stress-related signaling, and whether drug modulation of stress-induced effects might be of therapeutic value.

PS3.30

Neuronal avalanches in networks of neurons developing *in vitro*

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Periods of synchronized bursting activity are usually generated by different in vitro models for the study of neural dynamics (acute and cultured brain slices, cultures of dissociated neurons): in 2003, Beggs and Plenz (Beggs & Plenz, 2003) demonstrated that within these synchronous epochs there exists a more sophisticated embedded form of dynamics, called *neuronal avalanche*.
In this work, we analyzed the electrophysiological activity produced by networks of dissociated cortical neurons cultured onto Micro-Electrode Arrays (MEAs) by considering the approach proposed by Beggs & Plenz, (2003) and we asked whether and how neuronal avalanches are intrinsic to the network formation and stabilization.
Therefore, we considered recordings of spontaneous activity during development, as well as a large-scale network computational model, developed to interpret the experimental results.
We observed different distributions of avalanche sizes and durations, i.e. sub-critical, critical or super-critical, depending on both the age and the development of cultures. These behaviors correlate with the level of synchronization among bursts and the ratio between bursting and random spiking activity. In particular, criticality was found in correspondence to medium synchronization among bursts and poor random spiking activity. We confirmed these results through the application of specific drugs affecting the balance between coherence and variability in the network's activity (i.e. acetylcholine and bicuculline). These hypotheses were also confirmed by the computational model, in which we mimicked both the spontaneous activity and the effect of these substances on the culture and we found the same results as in the experiments.

References

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PS3.31

Nicotinic stimulation increases glutamatergic transmission in the rat cerebellum

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Neuromodulatory systems of the brain have been suggested to profoundly impact on neurotransmission and long-term synaptic plasticity, the cellular correlate for learning and memory. The cerebellum, that is involved in procedural memory, receives abundant cholinergic innervation and shows a dense nicotinic acetylcholine receptor (nAchRs) expression. However, the functional effects of nAchRs in the cerebellum are still largely unknown. To address this issue we have performed voltage-clamp recordings in whole-cell configuration in the granular layer of acute slices obtained from the cerebellar vermis of 18/22-day old rats (32 °C). A brief application (100 sec) of nicotine (1 microM) significantly enhanced glutamatergic synaptic transmission (19.4±5.4% EPSC amplitude increase, n=56; $p<0.001$). The effect was transient (<60 sec), indicating nAchR desensitization. Nicotinic AchRs can be located in the presynaptic terminals thereby enhancing release of various neurotransmitters in tissue culture, synaptosomes and brain slices. We have therefore investigated whether a similar mechanism could operate in the cerebellum. Nicotine application altered the relationship between two EPSCs in a pair, so that the paired-pulse ratio (PPR) decreased by 36.8±5.7% (n=8; $p<0.005$). Moreover, a high Ca^{2+} buffer concentration in the intracellular solution (10 mM BAPTA) was still accompanied by a significant PPR change during nicotine application (-39.3±11.5%, n=6; $p<0.05$) supporting its presynaptic origin. Interestingly, a further effect of nicotine application was to lower LTP threshold. A single 100 ms / 100 Hz burst, that would normally evoke long-term depression (Maffei et al, 2003; Gall et al, 2005), induced LTP when applied in the presence of nicotine. These results suggest that cholinergic stimulation mediated by nAchRs potently regulates information transmission along the mossy fibre pathway of the cerebellum.

PS3.32

Adult cognitive impairment induced by adolescent exposure to THC is associated with altered hippocampal dendritic morphology and synaptic plasticity

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Marijuana and hashish are the illicit drugs most frequently used by human adolescents. Given the continued neurodevelopment throughout adolescence, adolescents may be more vulnerable than adults to certain neural consequences of heavy marijuana use, for example cognitive behavior. The current study therefore aimed to assess whether an experimental model of adolescent chronic exposure to D^9-THC, may induce lasting effects on learning and memory in male rats.
Adolescent male rats have been treated with THC or its vehicle from 35 to 45 postnatal days (PND). Once animals reached the adult age (75 PND) the following tests have been performed: the 8 arm radial maze task to investigate specific aspects of spatial memory and the passive avoidance test to evaluate emotional learning. No alteration was found in emotional memory, but in the 8 arm radial maze THC-pre-treated animals exhibited a worse performance in comparison with vehicle group, suggesting a deficit in spatial memory.
To correlate memory impairment to altered neuroplasticity, level of proteins involved in synaptic plasticity was investigated in the hippocampus and prefrontal cortex.
A significant decrease in pre- and post- synaptic proteins expression (VAMP2, PSD95) and in the astroglial marker GFAP was found selectively in the hippocampus of pretreated rats, indicating a possible alteration in neuroplasticity.
Finally, to parallel these changes to alteration in dendritic morphology, Golgi-Cox staining was performed in the dentate gyrus of the hippocampus. Male pretreated rats had a significantly lower total dendritic length and number than vehicle group. Spine density analysis showed that THC also induced a decrease in this parameter.
The biochemical results suggest that THC pretreated rats may establish less synaptic contacts and/or less efficient synaptic connections throughout the hippocampus and this could represent the molecular underpinnings of the cognitive deficit induced by THC treatment in adolescence.

PS3.33

Functional masking of deprived eye responses by callosal input during ocular dominance plasticity

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Brain circuits are shaped by experience during "critical periods" early in life. Monocular deprivation (MD) is a well-known paradigm of experience-dependent plasticity, in which cortical neurons exhibit a change in ocular dominance (OD) towards the open eye. The mechanisms of OD plasticity are only partly understood.
Here we demonstrate a crucial involvement of callosal connections in the synaptic modifications occurring during MD. Rats at the peak of the critical period were deprived for 7 days, resulting in the expected OD shift towards the open eye. Acute microinjection of the activity blocker muscimol into the visual cortex contralateral to the recording site restored binocularity of cortical cells. Blockade of interhemispheric communication selectively enhanced deprived eye responses with no effect on open eye-driven activity. We conclude that callosal inputs play a key role in functional weakening of less active connections during brain plasticity.

PS3.34

Acute in vivo increase of CREB activity in the hippocampus improves fear memory

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Memory formation requires long-lasting plasticity and persistent neuronal-network modifications achieved through activity dependent-transcription. The transcription factor cAMP response element binding protein (CREB) is induced by activity-dependent phosporylation. Resulting CREB-dependent transcription promotes both long-lasting forms of hippocampal synaptic plasticity and stable encoding of memory traces. Whether an acute increase of CREB-dependent transcription in hippocampal neurons is sufficient to promote an improvement of hippocampal-dependent memory formation remains to be determined. Here we show that an acute increase in CREB activity, by local in vivo expression of a constitutively active form of CREB (CREBCA) in hippocampal neurons using viral-mediated gene transfer (Sindbis), results in a significant improvement of memory performance in contextual fear conditioning. In vivo infection of adult mice CA1 or dentate gyrus (DG) principal excitatory neurons with viruses encoding CREBCA or GFP (Control) was achieved by stereotaxic surgery. One day later, mice were trained in the contextual fear conditioning task with a single mild footshock (0.3mA). Twenty-four hours after the completion of the training session, mice were subjected to a retrieval test in the conditioning chamber. Mice from CREBCA groups displayed higher levels of freezing as compared to mice from GFP groups indicating that the increase in CREB activity in CA1 or DG neurons improves memory performance in a hippocampal dependent task. These results were obtained in absence of pain- or anxiety-related effects of CREBCA infection. In addition, high acute in vivo expression of a dominant negative form of CREB (CREBDN) in DG does not prevent memory formation suggesting that CREB-dependent transcription is sufficient to improve memory but might not be necessary for memory formation in this hippocampal dependent task.

PS3.35

Bidirectional modulation of corticostriatal synapses after prolonged activation of cannabinoid CB1 receptors

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The dorsal striatum regulates motor output and appears to be involved in memory and drug habits.
High-frequency stimulation of excitatory cortical inputs to the striatum induces either a long-lasting increase (HFS-LTP) or decrease (HFS-LTD) of synaptic transmission. Both forms of long-term synaptic plasticity are dependent on glutamate and dopamine release in the striatum.
CB1 receptor agonists, such as Δ9-tetrahydrocannabinol (THC), the main psychoactive component of marijuana, inhibit the release of glutamate from corticostriatal synapses. Additionally, endocannabinoid signalling is required for HFS-LTD.
Chronic CB1R activation results in tolerance to cannabinoid-induced locomotor effects. We therefore investigated whether repeated THC administration differentially modulates LTD and LTP at corticostriatal synapses.
Mice exposed to a pharmacological protocol inducing THC tolerance (10 mg/Kg, 2 daily s.c. injections, 4.5 d treatment) were tested for changes in long-term synaptic plasticity in the dorsolateral striatum. In vehicle-treated mice, high-frequency stimulation of corticostriatal synapses triggered a form of endocannabinoid mediated LTD that, conversely, was not observed after chronic exposure to THC. This effect was prevented by the in-vivo pre-treatment with the cannabinoid antagonist SR141716A (3 mg/Kg).
Glutamatergic synapses in the dorsolateral striatum are also capable of expressing an NMDA- and dopamine-dependent form of LTP, after removal of Mg2+ from the bathing medium. Under these experimental conditions, the HFS protocol induced an LTP of comparable magnitude in both vehicle and THC-treated mice. However, after the induction of LTP by HFS, low-frequency stimulation (2Hz, 10 min) depotentiated the synaptic strength to pre-LTP levels in control mice, but it was unable to restore baseline synaptic responsiveness in tolerant mice.
These results provide the first evidence that chronic THC exposure blocks LTD and the reversal of LTP in the dorsolateral striatum. This suggests that bidirectional changes at corticostriatal synapses may be relevant for the development and maintenance of cannabinoid addictive processes.

PS3.36

Functional pharmacology in cultured neocortical networks

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The isolation and in vitro maintenance of neurons allows the examination of their cellular function so that culture systems are widely used for analysis of developmental and pathogenic mechanisms. Networks of cultured cortical neurons, spontaneously assembled from dissociated cells, are characterized by spontaneous rhythmic activity that resembles observations in brain areas in vivo. Our aim was the physiological characterization of primary murine cortical cultures which were studied with MEA (microelectrode-array) technology that allows recording of in vitro neural spiking activity. We applied various types of blockers of voltage-dependent ion channels, neurotransporters and antiepileptic drugs to test the presence of synaptic transmission, of intrinsic pacemaker activity and observe the putative efficacy of therapeutic drugs. We will show that it is possible to obtain dose-response curves of various drugs affecting the network activity. For example we tested four compounds: 1) tetrodotoxin (TTX, a toxin that is specific for many isoforms of the sodium voltage-gated channels), 2) ZD7288, a specific blocker of the CNS pacemaker ion channels, 3) SKF89976A (SKF) known as a specific blocker of the transporter (GAT1) for the inhibitory neurotransmitter GABA, and 4) carbamazepine (CBZ), one of the most common antiepileptic drugs. It is known from the literature that these drugs have the following range of IC50 : TTX, 3-10 nM for CNS channels; SKF, 100-300 nM; ZD7288, 20-30 µM; CBZ, 20-300 µM. Our results will show that the MEA-derived data show an optimal correlation with those reported, thus confirming the functional quality of the technique. The results about the functionality of GAT1 protein are peculiar of this technique because all the other putative methods are not easily implementable functionally. On these basis our culture system may prove to be a promising tool to better understand the electrophysiological and pharmacological properties of cortical networks.

PS3.37

The use of Feedback stimulation to reveal intrinsic properties of a neuronal network

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Coupling in vitro cultures with Micro Electrode Arrays (MEAs) represent the first attempt at feeding a reduced nervous system with electrical stimuli determined by its own spontaneous spiking activity. This basic idea is behind our choice to develop and use a feedback stimulation (FB) protocol. Network dynamics of *in-vitro* and *in vivo* cortical assemblies can be modulated through electrical stimulation at various frequencies and through FB which seems to be less traumatic for the cells.
By using FB we wanted to study the plasticity effect of a stimulus sequence expressing the network spontaneous spike timing. First step focused on the development of a recording/stimulating system in an artificial open-loop configuration. The occurrence time of extracellular action potentials was detected and then fed back (as an external stimulation) into the same network. We analyzed the effect of FB stimulation on the network spike timing.
Cortical cells from 18 days embryonic WT mice were cultured and plated on MEAs; spontaneous activity at 18-22 days *in-vitro* was analyzed. The electrode channels showing a good cellular activity were chosen as stimulating channels. We develop a MatLab Spike detection algorithm to extract the spike timing sequence and assign a single biphasic (+/-) pulse (750mV and 0.25ms per phase) to each spike detected. Preliminary results show a general increment in the network responsiveness after the stimulus. We observed an increase in the network burst frequency and a stronger synchronization throughout the culture. Increasing the stimulation amplitude the tendency towards synchronization was enhanced as well as the network burst frequency.
The use of this simple bi-directional interface allow us to explore simple form of artificially input-output behaviour. Next step is to develop a closed loop hybrid-biorobotic system and study its plastic properties.

PS3.38

The role of cholesterol in synapse stability and activity

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In previous studies we demonstrated that P/Q type calcium channels (Cav2.1) and SNARE proteins colocalize in membrane subdomains enriched in cholesterol isolated from synaptosomes. In this study we investigate whether lipid microdomains in hippocampal neurons may play a role in presynapse stability and function. Firstly, we demonstrate the presence at synaptic boutons of aliquots of SNARE proteins and Cav2.1 channels in cholesterol-enriched domains.To analyze the role of lipid microdomains in presynapses, hippocampal cultures were treated with fumonisin B1 (inhibitor of sphingolipid synthesis), and mevastatin or squalestatin, two drugs know to affect the synthesis of cholesterol by inhibiting HMG-CoA reductase or squalene synthase. In untreated neurons, immunoreactivity for Cav2.1 and the synaptic vesicle marker synaptobrevin2 appears as small puncta along the dendritic branches. In fumonisin, mevastatin or squalestatin treated cultures, the density of puncta immunolabeling for the presynaptic proteins was reduced, and the size of the remaining puncta appears to be increased. To analyze whether the morphological modifications observed with the drugs were associated with alteration of neurotransmitter release, the exo-endocytic recycling of synaptic vesicles was monitored in two ways: i) selective uptake in synaptic vesicles of the fluorescent styryl dye FM1-43 and ii) internalization of an antibody directed against the luminal epitope of the synaptic vesicle protein synaptotagmin. The results demonstrated that whereas fumonisin and mevastatin do not inhibit the activity dependent uptake of FM1-43 or synaptotagmin antibodies, squalestatin induces a significant reduction of synaptic vesicle exo-endocytosis. Altogether these data suggest that lipid microdomains play an important role in stability of presynapses, and that cholesterol depletion may impair the synaptic vesicle cycle.

PS4.01

Expression studies of non receptor tyrosine kinase Arg in SH-SY5Y cells treated with Aβ oligomers

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Aggregated β-amyloid (Aβ), the main component of senile plaques in the brain of Alzheimer patients, may damage neurons in vivo and induces degeneration patterns in SH-SY5Y neuroblastoma cells. Since the non receptor tyrosine kinase Arg, through cytoskeletal interactions, has a role in neurulation and in dendritic spine morphogenesis and stability, the aim of our study is to evaluate the putative involvement of the different 5'- and 3'- Arg isoforms in neurodegenerative pathways induced in SH-SY5Y cells by Aβ treatment.
We treated undifferentiated SH-SY5Y cells with low concentration (0,1 μM) of Aβ(1-42) oligomers, to reproduce a condition which may occur in the early stage of Alzheimer disease, and ATRA (All Trans Retinoic Acid)-differentiated SH-SY5Y cells with higher concentration (2,5 μM) of oligomers, to reproduce the Aβ neurotoxic effect.
In undifferentiated SH-SY5Y cells 0,1 μM Aβ oligomers treatment induces a quick formation of neuritic processes evidenced by a significant increase of neurites/cell after 24 hours, and a significant elongation of neuritic length after 48 hours. The expression of total Arg transcript and protein, evaluated by Real Time PCR and western blot, was increased in Aβ treated cells at any time analyzed and a switch in the quantitative expression of the two 3'- isoform Arg transcripts was also evidenced .
An increase of Arg expression has been evidenced also in SH-SY5Y cells differentiated by 6 day treatment with 20 μM ATRA and in these cells the studies on cellular and molecular effects induced by 2,5 μM Aβ treatment are ongoing.
The demonstration of Arg involvement in neurotrophic and/or neurodegenerative pathways induced by different concentrations of Aβ aggregates might be important to clarify the pathogenetic mechanisms of neurodegeneration in Alzheimer disease and might be useful for new therapeutic approaches.

PS4.02

Changes in mRNA and protein levels of metalloproteinase-9 and of members of the plasminogen activator/plasmin enzymatic system in MPTP-induced parkinsonian mice

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Despite a well-described pathological phenotype, the molecular pathways leading to nigrostriatal neuron degeneration in Parkinson's disease remain to be fully understood. A role for metalloproteinases (MMPs) and plasminogen activator (PA) /plasmin systems in neurodegenerative diseases has been proposed. However, their involvement in PD pathogenesis is still seldom, or none, explored. Here we report on changes in mRNA and protein levels of MMP-9 and of some components of the PA/plasmin system: plasminogen, tissue-type PA (tPA), annexin II and LRP (tPA-plasminogen receptors) and PAI-1 (PA inhibitor-1), in the substantia nigra pars compacta (SNpc) of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropiridine (MPTP)-injected mice, an animal model of parkinsonism. C57/Bl6 mice receive acute administration of 80 mg/Kg of MPTP and are killed 1, 24, 48 and 72h, 1 and 2wk after last MPTP injection. Control animals receive saline administration. We observe a statistically significant decrease, respect to control, in MMP-9 mRNA level after 1h, followed by a significant increase at 24h. After this date, mRNA levels remain higher than control. Protein levels of both pro- and active MMP-9 increase significantly at 24h, similarly to mRNA, decrease near control by 72h and further increase at 1-2wk. mRNA levels of PA/plasmin system components change in accord to one another: all mRNAs increase 1 h after last MPTP injection (being significant only for annexin II and LRP) and then decrease to control levels, or significantly lower, at 24-48h. Preliminary data on tPA protein level, however, show a significant decrease by 48h. Our results suggest an involvement of MMP-9 in two events characterizing MPTP-induced parkinsonism: early SNpc neuronal degeneration (24-48h), which associates with an inflammatory response, and late axonal regeneration of survived neurons. Interpretation of the data on the PA/plasmin pathway require further experiments to evaluate the enzymatic activities.
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PS4.03

Alpha-synuclein and its A30P mutant form regulate actin cytoskeleton dynamics

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Alpha-synuclein (Syn), a neuronal protein highly enriched in the pre-synaptic compartment, is the major component of Lewy bodies and its mutated forms (A30P, A53T, E46K) are related to familial Parkinson's disease. Many physiological roles have been proposed for Syn, including the regulation of synaptic vesicle pools and of neurotransmitter release. The actin cytoskeleton regulates many aspects of synaptic function: a direct/indirect dysregulation of its organization may be responsible for the synaptic loss and neurotransmitter release impairment that cause neurodegeneration.
Using concentrations of the proteins similar to those found in the brain, we show that Syn and A30P modulate actin dynamics *in vitro* and in cellular systems. Using an *in vitro* fluorometric assay, we observe that Syn slows down actin polymerization, possibly by monomer sequestration, while A30P fastens it.
In neuroblastoma cells (N2a), we show that the delivery of A30P by streptolysin-O, as well as its transient or stable transfection, causes a change in cell morphology with disruption of actin stress fibers. In stably expressing epithelial clones (MDCK), we follow the reconstitution of actin cytoskeleton after latrunculin-A-induced depolymerization. We find that Syn slows down the re-formation of actin filaments, while A30P causes its chaotic polymerization, with the transient assembly of discrete structures trapping A30P and few other actin binding proteins, such as the cadherin/catenin complex. We also show that the expression of the two Syns can determine an alteration of cytoskeleton-based processes, such as cell migration and membrane turnover. Syn expressing MDCK cells migrate faster, while A30P expression inhibits cell migration. In both N2a and MDCK clones, A30P disrupts regulated exo/endocytosis.
Elucidating Syns interaction with cytoskeletal actin may contribute to the understanding of their role in neuronal physiology as well as in neurodegeneration.

PS4.04

Dopamine precipitates apoptosis of APPswe-overexpressing neuroblastoma cells following its endocytosis and processing to Aβ

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Alzheimer's disease (AD) is a late-onset neurological disorder with progressive loss of memory and cognitive abilities as a result of excessive neurodegeneration. One of the best characterized feature of AD is the accumulation of extracellular aggregates of beta amyloid (Aβ) peptides and it knows that APP plays a crucial role in the pathogenesis of Alzheimer disease. Beta-amyloid peptide (Aβ) , derives from alterated processing of APP, and mutations of the APP gene are responsible for rare cases of familial AD. One of these mutation is the double mutation (K670N e M671L) called Swedish mutation. Heterozygous mice overexpressing APP-swe develop diffuse plaques in the hippocampus and cortex and are therefore a good in vivo model for AD study.
In this study we used neuroblastoma cells N2Awt and N2A-APPswe. Neuroblastoma cells N2A, stably over-expressing APP-swe show an higher sensitivity to Dopamina (DA) treatment than N2A-wt cells. We investigated on the cytotoxic mechanism of action of DA in N2A-APP. Dopamine treatment induces in these cells translocation and processing of APP followed by beta-amyloid secretion. Upon DA stimulus APP is rapidly translocated into endosomal-lysosomal compartments. We show that the silencing of Dynamin, a GTP-ase involved in endocytic vesicles recruitment, prevents APP processing and apoptotic cell death in N2A-APP suggesting the central role of endocytosis in cytotoxic DA-dependent processing of APP.
We also investigated on the specific role of Cathepsin D and autophagic pathway in APP processing and cell death.

PS4.05

Modulation of beta-secretase expression and activity in primary neurons and astrocytes

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BACE1 (β-site APP cleaving enzyme 1) is the main β-secretase of the brain and the key enzyme in the production of the neurotoxic amyloid-β peptides that accumulate in the brain of Alzheimer's disease (AD) patients. Since amyloid-β accumulation is considered the major cause of neuronal damage and death in AD, changes in BACE1 expression and/or activity may play a critical role in the progression of the disease.
The recent evidence of an increase in BACE1 protein level and β-secretase activity in some AD patients, has prompted a number of studies which report changes in BACE1 expression or β-secretase activity both in animal models and cell cultures.
We investigated the mechanisms that control β-secretase expression in rat primary cultures of astrocytes and neurons. The main questions we addressed were: i) the possible contribution of astrocytes in the production of amyloid-β, with particular regard to their state of activation; ii) the definition of the conditions in which BACE1 expression is modulated in neurons.
Regarding the first aspect, we observed a reduced β-secretase activity in astrocytes activated with cytokines. Interestingly, the β-secretase activity in astrocytes was due to the expression of BACE2 (a BACE1 homologue), and a decrease in BACE2 levels was monitored after treatment with cytokines.
With respect to neurons, we showed that various experimental treatments that had been reported to modulate BACE1 expression and/or activity in neuronal cell lines are not effective in primary neurons. However, we found a stimulus able to downregulate BACE1 protein levels in our experimental model.

PS4.06

Identification of an APP partner with a bionformatic approach and experimental validation

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Alzheimer disease (AD) is a neurodegenerative disorder characterized by a progressive and irreversible decline of cognitive function. Altered proteolytic processing of the Alzheimer amyloid precursor protein (APP) is well established as one of the major pathogenetic mechanism underlying AD. However, despite extensive efforts, the normal functions of this protein and their relationships with the abnormal processing remain still elusive. To investigate these issues, we have used a conserved coexpression-based bioinformatic approach, which in theory is capable to reveal the possible functions and the potential interactors of any protein. In good agreement with the literature, this analysis has revealed that APP may play a crucial role in biological processes related to cell-matrix interaction. The top scoring hit of our bioinformatic screening was the heat shock protein Hsp47, a collagen-binding protein that assists the molecular maturation of procollagen. Therefore, we are experimentally addressing the hypothesis that HSP47 is a functional partner of APP. To this aim we are conducting expression and functional studies. Although previous studies have shown that HSP47 is expressed at very low levels in CNS, we have found that Hsp47 is present in primary cultures of hippocampal neurons and astrocytes. Moreover, Hsp47 is upregulated in mouse hippocampus with after kainate-induced seizures, with a kinetic similar to the 770 APP isoform. On the other hand, we have found that HSP47 is capable to counteract the effects on cell migration induced by APP overexpression. In conclusion these results indicate that Hsp47 may functionally interact with APP and its pathway.

PS4.07

Transplantation of neuronal/glial restricted precursors and mesenchymal stromal cells following spinal cord injury in adult mice

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Spinal cord injury (SCI) often results in permanent neurological deficits below the injury site, a pathological state of functional damage to local neurons and axons fibres. In recent years cell transplantation seems a promising approach in spinal cord regeneration to minimize tissue damage.
We reproduced an experimental model of spinal cord compression in the mouse. We transplanted into the SCI lesion cavity mesenchymal stromal cells (MSCs), obtained from bone marrow of EGFP-expressing mice. Another group of mice received grafts of neuronal (NRP)- and glial (GRP)-restricted precursors, obtained from the neural tube of E12 EGFP embryos. Injured mice without graft served as controls. Spinal cords were examined 12-19-26 days post injury: we have investigated the survival and the effects of these cells on cellular environment, glial scar formation and microglial activation, compared with control mice.
Injected MSCs and NRPs/GRPs survived well and integrated into the host spinal cord tissue: whereas MSCs invaded especially the damaged area, NRPs/GRPs tended to surround it. In some cases, NRPs/GRPs expressed glial and neuronal markers.
Preliminary data about the study of lesion volume suggested that controls display a larger glial scar than grafted animals. Sensory and motor tests demonstrated that transplantation of MSCs and NRPs/GRPs promoted some improvement in treated animals, compared with controls.
These results suggest the therapeutic potential of such cells, since both can survive for a long time, differentiate, integrate in the host injured spinal cord and promote functional recovery after SCI.
Support from Girotondo Onlus.

PS4.08

Effects of beta amyloid-metal complexes on SHSY5Y neuroblastoma cells: gene expression profile analysis

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The essential features of Alzheimer's disease are the presence of extracellular plaques constituted mainly of beta-amyloid peptide deposits and neurofibrillary tangles in the brain. Although the mechanism leading to the development of the pathology is still an object of debate there are many evidences which link the altered metabolism of metal ions with the progression of the disease.
In this connection, we investigated, performing a microarray analysis of 35130 genes, the gene expression of SH-SY5Y neuroblastoma cells treated with amyloid-metal complexes and with the metals alone (iron, zinc, copper, aluminium).
This comparative study showed that the up-regulated genes after cells treatment with Abeta-Fe were associated with the regulation of enzyme activity (APH1A), intracellular transport (CCS), metabolism (LOX). In presence of Aβ-Zn the microarray analysis revealed selective up-regulation in level of transcripts encoded by genes associated with the intracellular signalling cascade (ESR2) and metal ion homeostasis (FTL, TF). The modulated genes of the Aβ-Cu showed the over-expression of genes implicated in cell communication (APPBP1), nuclear division (NOLC1), protein metabolism (MRPS21). These results demonstrated that different Aβ-metal complexes produced a different pattern of gene expression. However, the most important finding was obtained in the presence of Aβ-Al that produced the over-expression of genes directly related to Alzheimer's disease (AD) such as Amyloid-like protein 1 and 2 precursor and Microtubule-associated protein tau (MAPT). Based on these data, Aβ-Al complex seemed to be strongly involved in the etiopathogenesis of Alzheimer's disease.

PS4.09

Wild-type and mutant presenilin-2 decrease the calcium content of intracellular stores: beyond the leak channel

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Fibroblasts from FAD patients carrying mutations in either PS1 (M146L, P117L) or PS2 (M239I, T122R) show a reduced instead of an exaggerated calcium release from intracellular stores (Zatti et al., 2004 & 2006; Giacomello et al. 2005). These and other PS mutants (PS1-A246E, PS1-D385A, PS1-H163R, PS2-N141I, PS2-D366A), as well as the wild type (wt) form of these proteins, share the same feature when transiently over-expressed in different cell lines and rat primary neuronal cultures. Such effect is more evident for PS2 variants which dramatically reduce the store calcium content and also cause lower steady-state calcium concentrations at the level of the endoplasmic reticulum (ER) and the Golgi apparatus, as demonstrated by organelle-targeted aequorins (Zatti et al., 2006).
We here show that in HeLa and SH-SY5Y cell lines as well as in MEFs (either wt or devoid of endogenous PSs) transient expression of wt or mutant PS2 reduces the store calcium content by both increasing the ER calcium leakage and reducing the ER calcium uptake by SERCA pumps. The full-length (FL) but not the N- and C- terminal dimer of the protein is directly involved in store calcium handling, while its action seems independent on the ryanodine receptor or the ribosomal translocon complex. It is still under investigation whether the FL protein contributes to the leakage pathway by functioning as a channel per se (Tu et al., 2006) or by increasing the leakage of IP3 receptors.
Giacomello et al., Neurobiology of Disease 18, 638-648, 2005.
Tu et al. Cell 126, 981-993, 2006.
Zatti et al., Neurobiology of Disease 15, 269-278, 2004.
Zatti et al., Cell Calcium 39, 539-550, 2006.

PS4.10

Mitochondrial ferritin influences cellular iron availability and limits ROS formation

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Mitochondrial ferritin (FtMt) is a nuclear-encoded iron-sequestering protein that specifically localizes in mitochondria with incompletely characterized functions. Studies on mice revealed a tight tissue-specific expression pattern, preferentially in cells characterized by high-energy consumption. Previous cellular studies showed that FtMt expression affected iron homeostasis and rescued the phenotype of the Friedreichï¿½s ataxia (FRDA) yeast model. To clarify the physiological role of FtMt in humans we tested its properties on HeLa cells and on skin fibroblasts from FRDA patients. After FtMt expression, cells resulted protected against short-term oxidative stress, promoted by H2O2 or Antimicyn A insults, and against long-term oxidative stress, occurring in enhanced mitochondrial respiratory metabolism or in FRDA fibroblasts. Depending on cell types and treatments, FtMt expression reduced Reactive Oxygen Species (ROS) development, from 30% to total inhibition, retained ATP production and mitochondrial iron-sulphur cluster enzymes activities, with a consequent positive effect on cell viability. A 25% reduction of cytosolic and mitochondrial labile iron pools were observed during accomplishment of FtMt cytoprotection, particularly occurring in glycolytic growth where FtMt was slowly degraded. Differently, in enhanced respiratory condition, FtMt was faster degraded and determined iron redistribution among cellular compartments, which promoted mitochondrial enzymes activities increase without affecting cytosolic iron status. These results accounted for the expression pattern observed in mouse tissues and indicated that the regulation of mitochondrial iron availability and ROS limitation are primary functions of FtMt. These properties suggested that FtMt could be useful in pathological conditions characterized by defective respiration, such as FRDA.

PS4.11

Microtubule dynamics imbalance in MPTP model of Parkinson's disease

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Dysfunction of microtubule system is emerging as a novel contributing factor in Parkinson's disease (PD). Recent data indicate that microtubules differently interact with mutated proteins in PD and that the dysfunction of microtubule is involved in the mechanism of action of model drugs in PD. However, the role of tubulin in pathogenic events remains elusive.
We reported earlier that MPP+, the toxic metabolite of the parkinsonism producing neurotoxin MPTP, binds specifically to tubulin and affects microtubule dynamics by acting as a destabilising factor in vitro. Here we investigated whether alteration in MT dynamics could underline neuronal death occurring in cultured cells and in mice treated with the neurotoxin. By FRAP (fluorescence recovery after photobleaching) experiments of YFP-tubulin in NGF-differentiated PC12 cells we found that MPP+ induces a significant reduction of tubulin mobility at the neuronal tip and along the neurite. By time lapse imaging of cells expressing the MT-associated protein EB3-GFP, we show a significant reduction of MT dynamics following exposure to MPP+. Finally, we have analysed post-translational modifications of tubulin known to be associated with differently dynamic MTs and show an enrichment in stable MTs in the corpus striatum of MPTP-treated mice at early time points. These results provide the first evidence that MT dynamics is specifically targeted during neurodegeneration induced by MPTP both in cultured cells and in mice. Since dynamics is crucial in MT biological functions, we hypothesise that the altered dynamic behaviour of microtubules could represent a novel pathogenic pathway triggering neuronal cell death in PD.

PS4.12

Effects of nandrolone administration on the neuromuscular junctions in diaphragm of G93A mice, animal model of familial Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a late onset neurodegenerative disease characterized by selective loss of upper and lower motor neurons; this loss induces weakness of skeletal muscles, paralysis and death by respiratory failure within 5 years from diagnosis.
In ALS, motoneurons degeneration proceeds as a "dying back" process, starting with disaggregation of presynaptic terminal at the neuromuscular junctions (NMJs) and progressing toward the cell soma in the spinal cord.
Approximately 2% of all ALS cases are due to mutations in the gene encoding for Cu, Zn Superoxide dismutase (SOD1); in the first part of this work we focused on the analysis of the integrity of the NMJs and the evaluation of the percentage of innervated NMJs in diaphragm muscles in C57BL6 mice transgenic for the ALS-linked hSOD1-G93A mutation, that are examined at the onset of disease.
We observed that, already at this early stage of the disease, G93A mice showed a marked decrease in the number of intact diaphragm NMJs compared to their WT littermates. Moreover, in both WT and G93A mice, the administration of the anabolic agent nandrolone induces an increase in the percentage of innervated NMJs, as compared to untreated mice. From these results we conclude that nandrolone administration can partially protect NMJs from early denervation in diaphragm muscles of G93A mice. We then investigated how nandrolone adminstration can mediate the preservation of NMJs integrity. We looked at several features that are known to be involved in ALS associated degeneration, namely mitochondrial alteration and swelling and synaptic vesicle density in nerve endings. Our results showed that, at the onset of the disease, these structures are only slightly altered and nandrolone has only minor effect on them.
In diaphragms of G93A mice extensive axonal sprouting can be observed, suggesting that compensatory reinnervation (a process that has been always observed at later stage of disease) started as early as P85-90.

PS4.13

Early microglial activation and beta-amyloid perivascular deposition in a mouse model of hypertension

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Genetic Alzheimer's disease (AD) accounts for only few AD cases and is almost exclusively associated to increased production of the β-amyloid peptides (Aβ) in the brain. Instead, the majority of patients is affected with the AD sporadic form and typically has an altered Aβ clearance from the brain. The identification of factors that influence the onset and progression of sporadic AD is a key step toward understanding its mechanism(s) and developing successful therapies. Increasing epidemiological studies describe a strong association between AD and cardiovascular risk factors, particularly with hypertension. Besides its well known peripheral outcomes, hypertension exerts detrimental effects on the cerebral circulation, favouring chronic brain hypoperfusion, a condition associated to impaired energy substrates delivery to brain tissue, probably resulting in alterations typical of AD. However, a clear demonstration of a pathophysiological link between cardiovascular risk factors and AD aetiology is still missing. To deepen our knowledge of the mechanisms involved in brain response to hypertension and their possible role in Aβ deposition in the brain, a key step in AD pathogenesis, we studied a mouse model in which permanent transverse aorta coartaction (TAC), results in a persistent increase in blood pressure and, in the long term, in chronic hypoperfusion of both cerebral hemispheres. We evaluated, at different time points after TAC, the extent of microglial activation in relation to the Aβ deposition, evident four weeks after hemodynamic disruption. Microglia morphological changes were observable already one day after TAC, indicating their clear-cut reaction, preceding Aβ deposition. In addition, we analyzed the levels of cytokine mRNAs and lipid mediators, as molecules linked to inflammatory response and oxidative stress. They were altered in a time and brain region specific fashion, possibly in relation to hemodynamic changes and/or hypometabolism.

PS4.14

Hyperhomocysteinemia and Alzheimer's Disease: alteration of methylation metabolism, oxidative stress and amyloid production in TgCRND8 mice fed with B vitamins deficient diet

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The mechanism underlying Alzheimer's disease (AD) is still an area of significant controversy. There are widely confirmed reports of an association between hyperhomocysteinemia, oxidative stress and AD. Glutathione (GSH), the most abundant mammalian antioxidant, is synthesized from Hcy via the transsulfuration pathway. Deficiencies of folate, vitamin B12 and B6, cofactors of enzymes involved in Hcy metabolism, could lead to decreased S-adenosylmethionine/S-adenosylhomocysteine ratio (SAM/SAH, methylation potential, MP). Nitric oxide (NO) is involved in physiological functions and pathological processes leading to tissue damage due to its free radical nature. The production of NO is related to Hcy metabolism through the regulation of ADMA (asymmetric dymethyl arginine) levels. It has already been shown that gene methylation is involved in β-amyloid production through the regulation of PS1 and BACE expression. Herein, we studied the relationship between Hcy, NO, brain and liver SAM/SAH and GSH/GSSG ratio and β-amyloid in TgCRND8 mice fed with B vitamins deficient diet. TgCRND8 mice, that have an accelerated amyloid accumulation, together with 129Sv mice were fed with a deficient diet (without folate, vitamin B12 and B6) or with a control diet. They were thereafter sacrified to analyse Hcy, NO, SAM/SAH, GSH/GSSG in plasma and tissues and β-amyloid accumulation. The deficient diet lead to a marked hyperhomocysteinemia, to brain amyloid plaque deposition, to a decrease of SAM/SAH ratio in brain and liver and to an increase of GSH/GSSG ratio in TgCRND8 brain probably due to the attempt to compensate for the increased oxidative damage resulting from β-amyloid. There is also a brain NO decrease in diet B in TgCRND8 mice, while there are no significant differences in plasma. Experiments with SAM administration are actually in progress to clarify the relationship between alteration of MP and redox homeostasis caused by hyperhomocysteinemia in AD.

PS4.15

Alzheimer's disease related VGF-peptide/s in the mammalian nervous system

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A 4.8 kDa fragment of the VGF neuro-endocrine precursor protein was found to be significantly reduced in cerebro-spinal fluid of patients affected by Alzheimer's disease. VGF mRNA is selectively expressed in certain neurons and endocrine cells, and promptly responds to neurotrophins, or to neural/electrical activity. The VGF precursor shows >10 potential cleavage sites, and is indeed processed to low MW peptides. We raised antibodies to C- and N-termini of the above 4.8 kDa peptide (human VGF408-17 and 373-82, respectively), to be used these in parallel with antibodies to human and rat VGF precursor C-terminus. Samples of bovine hypothalamus (n=6) and rat brain (n=6) were paraformaldehyde-fixed for immunohistochemistry, and water-extracted for ELISA. VGF 408-17 immunoreactivity was confined to hypothalamus (suprachiasmatic nucleus, periventricular region and median eminence), thalamic areas and cortical perikarya, as opposed to the widespread distribution of peptides at VGF precursor C-terminus (entire hypothalamus and most brain areas). Staining was abolished in absorption controls (up to 30 pmol/ml homologous peptide). With the VGF373-82 antisera, initial experiments on hypothalamus showed a localization profile comparable to VGF408-17 peptides. Tissue concentrations of VGF373-82 and VGF408-17 peptides (ELISA: ID50= 0.2 and 0.002 pmol/well, respectively) were lower in rat and bovine hypothalamus (~5-14 pmol/g, either species), compared with VGF C-terminus peptides (100 and 600 pmol/g, rat and bovine, respectively), in agreement with the restricted distribution shown in immunohystochemistry. Hence, the Alzheimer's d. related peptides are found in cortical neuronal perikarya, as in discrete neuronal systems in hypothalamus and thalamic areas. Experimental studies are warranted, to address the regulation of such peptide/s' its cleavage from the VGF precursor, as well as their relevant alterations in connection with neuronal damage in neurodegenerative diseases.

PS4.16

***In vitro* astrocyte activation: a cellular model to investigate the role of glia cells in neurodegenerative diseases**

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Astrocytes are the most abundant glial cells in the brain and are considered key players in many physiological and pathological processes observed in the central nervous system. Moreover, they can enter in an ï¿½activated stateï¿½, characterized by increased proliferation, morphological alterations and modulation of gene expression, playing either detrimental or protective role in the progression of many neurodegenerative diseases. In particular the interplay between astrocytes and microglial cells is thought to control the activation process via secretion of biologically active molecules, such as pro-inflammatory cytokines. A detailed in vitro characterization of the process of activation was performed in rat cortical astrocytes by studying the different phenotypes that are induced upon administration of various stimuli (e.g. IL1beta, TNFalpha and INFgamma). Moreover, we investigated the effect of lypopolysaccharide on the expression of markers of activation (i.e. the release of IL6, PGE2, and NO, and the induction of iNOS and COX2) in astrocytic cultures with various extent of microglial contamination. We also evaluated the effects of amyloid beta on astrocitic preparation, in light of the presence of reactive astrocytes around the senile plaques in Alzheimerï¿½s disease brains. Preliminary evidences suggest that amyloid beta can contribute to astrocyte activation when acting concomitantly with proinflammatory cytokines. This effect will be further investigated in light of the possible interactions of astrocytes with other cell types (i.e. microglia) and activating stimuli.

PS4.17

Beta-amyloid and homocysteine plasma levels and DNA methylation in Alzheimer disease and in Down syndrome

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by the abnormal accumulation of amyloid beta peptide (Abeta), from amyloid precursor protein (APP), in the brain. Down Syndrome (DS) is the most common genetic disorder characterized by the presence of an extra copy of chromosome 21. DS subjects show sings of progressive cognitive decline earlier than healthy population, and most of them develop Alzheimer's type dementia at the age of 50-55 years; this is probably due to the triplication of APP gene, located on chromosome 21. The aim of this study was to evaluate sAPPα and Abeta 1-42 plasma levels in AD and DS in order to identify a possible peripheral marker of the pathology. Moreover we analysed homocysteine (hcy) plasma levels and total DNA methylation levels to evaluate a possible role of hcy in influencing gene expression by modification in methylation status.
Demographic and clinical information were taken, after informed consent, from 40 AD patients, 21 DS subjects and 71 age-matched controls (both for AD and DS group). sAPPα and Abeta 1-42 plasma levels were evaluated by commercially available ELISA kits. Hcy levels were determined by RP HPLC method. Total DNA methylation status was determined using HpaII restriction enzyme followed by [H^3]dCTP incorporation. Statistical analyses were performed using GraphPad Software.
We demonstrated that both AD and DS had higher levels of sAPPα and Abeta 1-42 than controls, indicating that DS subjects are a good model for the study of early stages of dementia. Moreover AD showed higher levels of hcy with respect to controls, thus confirming hcy association with dementia. Nevertheless no difference in total DNA methylation status and no correlation between hcy and DNA methylation were found in AD and DS. The evaluation of specific methylation levels in genes involved in AD, as BACE and PS1, will be necessary to suggest a possible link between Abeta/hcy and regulation of gene expression.

PS4.18

Transplanted neural stem cell-derived motor neurons improve SMARD1 disease phenotype

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OBJECTIVE: We explored the therapeutic potential of neural stem cell-derived motor neurons into a Spinal Muscular Atrophy with Respiratory Distress type 1 (SMARD1) model (nmd mice).
SMARD1 is an autosomal recessive form of infantile motor neuron disease due to mutation in the IGHMBP2 gene. Cell replacement therapy could be a possible therapy for motoneuron diseases including SMARD1.
METHODS: We isolated a neural stem cell poluation (NSCs) from murine spinal cord of a transgenic mouse expressing GFP only in motor neurons (HB9-GFP) by immunomagnetical positive selection for the stem cell marker LewisX (Le+). These cells were expanded as adherent monolayer and differentiated into motor neurons with a cocktail of growth factors including sonic hedgehog (Shh) and retinoic acid (RA). Differentiated motor neurons were purified by immunomagnetic selection for p75NTR antigen and grafted into spinal cord in nmd mice, an animal model of SMARD1. We assessed the long-term survival of transplanted motor neurons examining their integration with host tissues by immunohistochemistry, and confocal microscopy. Transplanted mice were evaluated for their motor performance using an accelerating rotarod device and survival was recorded.
RESULTS: In vitro, Le+ NSCs derived motor neurons were positive for Hb9-GFP as well as for neuronal and motor neuronal markers. They extended long axons and formed neuromuscular junctions with skeletal myotubes. After transplantation of purified MNs in nmd spinal cord, numerous Hb-9-GFP+ MNs were readily identified in the host anterior horns. These cells showed colocalization with neuronal and motor neuronal markers (choline acetyltransferase). Furthermore, donor cell-derived motor axons were observed within the ventral roots of transplanted animals. Treates nmd mice presented an improved phenotype with delayed disease progression and increased life span.
CONCLUSIONS: Our data demonstrate that transplantation of purified motor neurons has a positive effect on the SMARD1 disease phenotype and open new possibilities for the development of cell therapy in patients with SMARD1 and other motor neuron disease.

PS4.19

Chronic nicotinic drug treatments affect nicotinic and glutamate receptor number and localization and lead to neuroprotection against glutamate toxicity

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Nicotine is an alkaloid contained in tobacco leaves that selectively binds neuronal acetylcholine receptors (nAChRs), a heterogeneous class of cationic channels with high calcium permeability, which can play an important role in calcium-dependent events such as neurotransmitter release, regulation of second messanger cascades, cell survival and apoptosis. Preliminary experiments performed in our laboratory, show that chronic exposure of primary cortical neurons to nicotine and nicotinic drugs, not only leads to change in number, subunit composition and function of nAChR subtypes, but also changes the levels of ionotropic (AMPA and NMDA) and metabotropic glutamate receptors (mGluR5). In particular, we found that chronic nicotinic drug exposure can change the localization of these receptors in particular cell domains (from the cell surface to intracellular membranes) and/or their recruitment in membrane lipid microdomains (or rafts). Lipid rafts are membrane subdomains enriched in sphingolipids and cholesterol that form platforms that recruit signalling complexes, neurotransmitter receptors, ion channels and other synaptic proteins. It has been previously shown that nicotine can be neuroprotective in various neuronal models, but the nAChR subtype(s) as well as the intracellular pathways involved are different depending on cell model and neurotoxic agent.
In this study we analysed in primary cortical neurons, the neuroprotective effect of nicotine and nicotinic antagonists on glutamate-induced neurotoxicity. We found that chronic treatments with nicotine and homomeric alpha7 receptor specific antagonists were neuroprotective whereas dihydro-beta-erythroidine, a heteromeric receptor specific antagonist, was not. Moreover, disruption of lipid rafts leads to loss of the neuroprotective effect of nicotine and studies are in progress to identify whether this is due to change in the membrane localization and/or signalling of nAChR subtypes.

PS4.20

Effects of Abeta administration on neprilysin expression in fibroblast cell lines from AD patients and controls

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Neprilysin (NEP) is a type II transmembrane protein bound to the cell membrane, where it normally degrades beta-amyloid (Abeta) intracellularly and on cell surface. Cerebral NEP levels have been reported to decrease with age and in Alzheimer's disease (AD), possibly contributing to disease pathogenesis by compromising Abeta catabolism. Neprilysin was shown in various peripheral tissues and since 1987 it was reported that NEP was present in human fibroblasts as glycosylated protein of about 94 kDa.
We decided to use human fibroblasts from AD patients and controls to evaluate the effect of Abeta administration on NEP expression and activity. Fibroblasts were obtained from a skin biopsy and maintained in DMEM at 37°C in 5% CO2 atmosphere. Abeta 1-42 peptide was aged for 5 days and then administered to cell cultures for 48 hours. Fibroblasts were recovered and frozen at -20°C until use. NEP expression was detected using specific antibodies both by FACS and by western blotting. We confirmed the presence of NEP in its glycosylated form (about 90kDa) both in patients and in controls; pre-incubation of cell extracts with PNGase F induced a decrease in protein molecular weight (MW 80kD). After Abeta 1-42 administration, the signal revealed by immunoblotting appeared to be divided in two bands, the first one at the correct MW, the second one slightly lower. We hypothesized that Abeta 1-42 could influence NEP maturation process favoring the production of different isoforms. The evaluation of NEP activity in treated and untreated cells may evidence a correlation between the presence of a lower protein form and a possible difference in enzyme activity. These results could be useful in providing a peripheral model to study the role of NEP in Abeta catabolism and the possible negative effects of the peptide on NEP function. This could support the role of this enzyme in AD pathogenesis suggesting new therapeutic tools for such a multifactorial disease.

PS4.21

The role of metabotropic glutamate receptors 7 in Parkinson's disease

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In Parkinson's disease (PD), the loss of dopamine neurons leads to glutamatergic hyperactive pathways in the basal ganglia. Metabotropic glutamate receptors (mGluRs) play a critical role in the modulation of synaptic transmission and are therapeutically promising targets for the treatment of Parkinson's disease. Among the mGluR family, mGluR7 is the most widely expressed in the basal ganglia, but its role in this pathology is not known. Recently, AMN082 emerged as the first highly selective and potent positive allosteric agonist of mGluR7. The present study aimed at testing AMN082 action in several rodent models of PD. AMN082 oral administration was tested in 6-OHDA models of early or late stages of PD after 6-OHDA infusion either bilaterally in the striatum or unilaterally in the substantia nigra pars compacta. AMN082 (5 and 10 mg/kg) alleviated the akinetic deficits measured by increased of mean delayed responses and reaction times (RTs) to release a lever at a cue onset while 20 mg/kg had no effecting in 6-OHDA lesioned rats. In addition low doses of AMN082 reduced apomorphine-induced contralateral circling in unilateral 6-OHDA lesioned rats and reversed haloperidol-induced catalepsy. To evaluate the specificity of AMN082 action on mGluR7, mice with deletion of mGluR7 ($mGluR7^{-/-}$) and controls ($mGluR7^{+/+}$) were tested in the catalepsy test. $mGluR7^{-/-}$ mice were less sensitive to the effects of haloperidol than control mice and AMN082 reduced the latency to step down the rod of $mGluR7^{+/+}$ but not of $mGluR7^{-/-}$ mice. These results suggest a critical role of mGluR7 in the regulation of motor functions and a potential role of AMN082 at low concentrations for PD treatment.

PS4.22

Protective effect of cholesterol depletion in rat brain tissue anoxia

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Anoxic condition in the Central Nervous System induces a loss of explicit memory, properly in hippocampal region, due to oxidative stress and excitotoxic mechanisms. Learning and memory processes are sustained by long term synaptic plasticity (LTP) that in CA1-CA3 hippocampal subfield is NMDA-dependent. The NMDA receptors are also responsible for the excitotoxic damage, during an ischemic insult, referred to as post ischemic LTP, consistent in a NMDA receptors prolonged activation by large amount of glutamate concentration. There are some evidences from literature that these receptors are inserted in the post-synaptic cleft in lipid microdomains or "lipid rafts", rich in cholesterol and sphingomyelin. In the present work we studied the protective effect of cholesterol depletion in rat hippocampal slices exposed to anoxia by using Godukhin's protocol. Three episodes of five minutes anoxia were applied both in absence and in presence in the perfusion medium of Methyl-beta-Cyclodextrin (MbCD) to deplete cholesterol from the lipid rafts destabilizing receptor's protein structures. We investigated CA1 population spike (PSs) in stratum pyramidale of hippocampal region. Adult male Wistar rats (6-9 weeks old) were used: hippocampal slices (450 microm) were prepared and incubated in an interface tissue chamber, with a medium (95%O2/5%CO2) at 33.5 â,'C(pH= 7.4). We observed in anoxic condition (95% N2 / 5% CO2- Oxygen Glucose Deprivation), the disappearance of PSs just after the first anoxic episode and a post-ischemic LTP insurgence 60 min after the end of the three episodes of anoxia. In MbCD treated slices, on the contrary, we observed only a 20% of PS reduction and no post-ischemic LTP. These results suggest a possible role of MbCD in preventing anoxia damage and indicate that manipulation of the lipid components of plasma membrane rafts in neurons may provide a new approach to the treatment of ischemic disorders.

PS4.23

A new approach to study pre-mRNA splicing alterations in a cellular model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) represents the third most common neurodegenerative cause of adult death, after Alzheimer's disease and Parkinson's disease. ALS is a late-onset progressive neurodegenerative illness characterized by the selective loss of motoneurons and progressive muscle atrophy. The process of motor neuron degeneration is complex and involves multifactorial influences. Several alterations have been proposed as concurring to its pathogenesis, among them oxidative stress, mitochondrial dysfunction, impaired axonal transport, excitotoxicity and protein misfolding. On the other hand, it is known that alternative pre-mRNA splicing has an important role in the control of gene expression. Humans employ tissue-specific and developmental stage-specific alternative splicing to generate a large variety of proteins in a specific cell at a specific developmental stage. Neurological disorders are not the exceptions that can escape from aberrations of the splicing machinery and defects in the splicing of individual mRNAs have also been claimed to participate in the pathogenesis of ALS. Consequently, it was hypothesized that aberrant alternative mRNA processing, induced by oxidative stress, may contribute to the development and progression of ALS. With the aim to investigate this possibility we treated SH-SY5Y cells, a human neuroblastoma cell line, with a mitochondrial complex 1 inhibitor able to evoke an oxidative stress condition in the cell, and using the microarray technology, we analyzed the genes that are affected by modified alternative mRNA splicing. In a preliminary analysis, we found significant changes in three out of six alternatively spliced mRNAs of our "in vitro" model. In addition, we investigated the effects of the treatment at proteomic level, particular attention was focused on proteins involved in the selection of mRNA splicing sites; we observed modifications on some of them following the pharmacological challenge. The integration analysis of high-throughput genomics data and the proteomics study represent a powerful resource to understand the molecular mechanisms underlying ALS, this fundamental step is essential for the design of novel therapeutic strategies.

PS4.24

Altered levels of NGF and its receptors in the superior cervical ganglion and peripheral targets of mdx mice

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The superior cervical ganglion (SCG) of mdx mice, an animal model for Duchenne muscular dystrophy, has 36% fewer neurons than the wild-type (WT). Neuronal loss occurs between 5 (P5) and 10 (P10) postnatal days and affects neurons innervating muscular targets (i.e. iris), but not those innervating non-muscular targets (i.e. submandibular gland). However, surviving neurons, irrespective of the type of their peripheral target (muscular and non-muscular), show reduced axonal defasciculation and terminal sprouting, expression of a neuronal dystrophic phenotype. Accordingly, we hypothesized that, in mdx mice, insufficient provision of neurotrophic factors by dystrophic muscular targets, combined with alterations in the assembly/organization of axonal cytoskeletal proteins, could be responsible for SCG neuron loss. We investigated the levels of NGF in SCG target organs (submandibular gland and iris) of P5, P10 and adult WT and mdx mice by Western immunoblot and Elisa. No changes in the level of total NGF was observed between WT and mdx mouse iris. Differently, pro-NGF forms, which are known to be responsible for apoptotic signaling, were more expressed in P5 and P10 mdx mouse iris, respect to the WT. This also combines with a significant decrease, in the SCG of newborn mdx mice, in the protein level for both TrkA and its phosphorylated form (pTrkA), which is the receptor activated by NGF binding. The level of the pan-neurotrophin receptor p75NTR was also significantly lower in P10 and adult mdx mouse SCG, respect to the WT. In conclusion, we hypothesize that a decrease in the neurotrophic survival signaling and a consequent increase in the apoptotic-promoting signaling within the ganglion, along with intrinsic neuronal cytoskeletal alterations and dystrophy-related muscular damages, may be responsible for selective neuronal loss in mdx mouse SCG.

PS4.25

Mechanisms of iron-mediated toxicity in primary cultures of neurons and astrocytes

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Iron is essential for many physiological processes but, when in excess, it can be toxic for cells. In particular, it can promote the formation of Reactive Oxygen Species (ROS) and this can play an important role in the pathogenesis of many neurodegenerative disorders. In order to investigate the effects of iron mediated-toxicity, we set up the conditions to control acute iron overload and to evaluate the ensuing status of intracellular oxidation, with specific attention to ROS production. Experiments were performed in rat primary cultures of neurons and astrocytes. Rapid iron entry into the cells (monitored by the quenching of FURA-2) impaired the reducing potential of the cells and promoted ROS production (monitored by DCFDA), ultimately leading to cell death. Depletion of intracellular glutathione further increased ROS levels and speeded up cell death.
Finally, we found that the condition of astrocyte activation, a change in phenotype induced by proinflammatory cytokines, was able to protect cultured astrocytes from the effects of acute iron overload. We are currently investigating at the molecular level the differences between resting and "activated" astrocytes in terms of their capability to detoxify H_2O_2, iron and ROS.

PS4.26

A potential role for neural stem cells (NSCs) in the etiopathogenesis of Tuberous Sclerosis Complex (TSC)

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Tuberous sclerosis complex (TSC) severely involves the central nervous system, where tubers, i.e. focal lesions characterized by the loss of the normal cortical cytoarchitecture and by the presence of abnormally enlarged neurons and glial cells, can be frequently observed. The atypical cells found in tubers express the same markers normally detected in neural progenitors located in the embryonic ventricular zone (VZ) and in the adult subventricular zone (SVZ), suggesting a pathogenic lineage relationship between VZ/SVZ progenitors and the aberrant cells within the tuber itself. To corroborate this hypothesis, we are characterizing NSCs from animal models of TSC, both *in vivo* and *in vitro*. Due to the embryonic lethality associated with TSC1 complete inactivation, we are taking advantage of a conditional mouse model for TSC1 crossed with promoter-specific Cre (Emx1-Cre) mice to selectively target the mutation to radial glia cells and highly undifferentiated cortical progenitors. We are currently analyzing the effect of TSC1 loss of function in different neurogenic areas *in vivo*, by staining sections of control and mutant mice (Emx1-Cre^{+}/$TSC1^{c/c}$) for NSC-specific markers. At the same time, we are investigating the role of TSC1 in NSCs, isolated *ex vivo* from the neurogenic zones of control and mutant mice at different time points, by using the NeuroSphere Assay (NSA). Similarly, we have also generated NSCs from $TSC^{c/c}$ mice and are subjecting them to *in vitro* TSC1 inactivation by a Cre deleter lentivirus. In both cases, the effect of TSC1 inactivation in NSCs will be evaluated by analyzing the cardinal features of NSCs, i.e. self-renewal and multipotency. All these studies will help us to dissect the temporal and spatial windows in which TSC1 could affect VZ/SVZ progenitors development and to investigate whether its alteration could have a causative role in the formation of the aberrant cells found in tubers.

PS4.27

JNK activation and autophagy following peripheral sciatic nerve lesion in adult mouse

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Neuropathic pain is caused by nervous system lesion or inflammation: symptoms may include allodynia and hyperalgesia. Useful animal models to study neuropathic pain consist in the sciatic nerve ligation (SNL) and axotomy. Axonal injury elicits changes in macromolecule synthesis in the corresponding DRGs cell bodies and activates several signalling pathways that may lead to both cell death and regeneration. In this study we analyze two aspects related to neuronal cell death following SNL: 1) the involvement of MAPK cascades in peripheral nociceptive sensitization and maintenance of chronic pain and 2) autophagic activity.
C57BL6 and JNK2 ko mice underwent SNL or sciatic nerve crush. After perfusion (3, 24, 48h and 5 days from surgery), ipsi- and contralateral L4, L5 DRGs were dissected; untouched and sham operated animals were used as controls. We performed IHC for p-c-jun, cleaved-caspase 3, and ATF-3. Neuronal degeneration was evaluated by Fluoro Jade B (FJ). Gomori's method was used to identify autophagic vacuoles. JNK and p-JNK expressions were analyzed by WB.
Our results showed no neuronal degeneration in the DRGs 12 h after SNL and a weak increase at 24h in ipsilateral ones. An intense p-c-jun and cleaved caspase 3 staining was detected in ipsilateral DRG neurons 24h after SNL whereas a faint labelling was seen in the contralateral ones. WB analysis of operated and sham-operated JNK2 ko DRGs showed a similar different JNK activation between ipsi- and contralateral ganglia with a decrease of 54 kDa vs 46 kDa isoforms. The numbers of Gomori positive neurons estimated by Stereo-Investigator revealed that at 24h (40.5%) and 5d (44%) from surgery neuronal positivity increased in injured L5 DRGs vs untouched animals (28.50%).
Increased c-Jun immunoreactivity and autophagy upregulation could represent either signs of neuronal degeneration or attempts to promote cell regeneration.
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PS4.28

Zinc pre-exposure enhances NMDA excitotoxicity in primary neuronal cultures from a transgenic ALS model mice

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Amyiotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the selective degeneration of motoneurons in spinal cord, brainstem and cerebral cortex. There are approximately 10% reported familial cases of this disease (FALS). Of these, 10%-20% are linked to inherited mutations in superoxide dismutase 1 gene (Cu-Zn SOD-1). FALS pathology is mimicked in transgenic G93A mice overexpressing a mutant human SOD-1 (hSOD-1) gene containing a glycine93→alanine (G93A) substitution. Glutamate excitotoxicity is implicated in the neurodegeneration in FALS disease; zinc, in brain, is present in synaptic vesicles of glutamatergic neurons and is released into the synapse with neuromodulatory effects at several receptors. In brain, glia may provide substrates for neuronal metabolism and remove glutamate from extracellular space.
Our purpose is to investigate the effect of Zn on N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors in both primary pure neuronal cortical E15 cultures and P0 neuronal/glia co-coltures, the interaction of neurons and glia under excitotoxic conditions and their possible role in ALS pathology. We have tested in both culture conditions the toxicity exerted by NMDA alone and the Zn+NMDA, and we have seen effects of a Zn pre-exposure followed by NMDA injury. We found that zinc enhances NMDA toxicity in G93A genotype and the presence of glial cells seems to protect from neuronal death. Moreover, superoxide anion (O2•-) production appears implicated NMDA excitotoxicity. We found higher superoxide anion (O2•-) levels during NMDA/Zn toxic injury in pure neuronal cortical E15 cultures compared to P0 neuronal/glia co-coltures. In addiction, in G93A genotype, Zn pre-exposure followed by NMDA injury resulted in a longer presence of high superoxide anion (O2•-) concentrations in the cytosol. Thus, corfirming the increased sensitivity to the glutammate mediated excitotoxicity of the neurons bearing the mutant hSOD-1.

PS4.29

NGF controls the amyloidogenic pathway in target neurons

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The impairment in neurotrophin supply such as NGF and BDNF in some brain areas like hippocampus and cortex is among the causes of the onset and progression of Alzheimer Disease (AD).
Here we report that in NGF-differentiated PC12 cells and in hippocampal and cortical neurons the interruption of NGF signaling induces the activation of the amyloidogenic pathway with consequent intra and extracellular accumulation of amyloid Aβ peptides, which are the most toxic fragments directly implicated in the development of AD. These peptides are partially released and largely accumulates intracellularly as aggregates which are soluble only with strong detergent treatments, generally employed to dissolve senile plaques. The pool of released Aβ peptides induces an increase of APP and PS1 protein levels and appears to cause apoptotic death also in healthy neurons by creating a feedforward toxic loop. These events are prevented by beta and gamma secretase inhibitors, by antibodies directed against Aβ peptides, or by partial silencing of APP mRNA, by siRNA. The same cultured neurons deprived of serum die but APP and PS1 over expression does not occur, Aβ production is undetectable and cell death is not inhibited by anti Aβ antibodies.
These results demonstrate that the interruption of NGF or BDNF supply to target neurons induces overproduction of Aβ, intra and extracellular accumulation, and spreading of its toxic action to healthy neurons. These events are not a simple consequence of an apoptotic trigger due to withdrawal of a trophic signal and further point out a possible link between these events and the onset of Alzheimer disease.

PS4.30

Analysis of the stress transducer, PERK, in sciatic nerves of the CMT 1B neuropathy mouse

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Charcot-Marie-Tooth 1 disease is one of the most common inherited neuropathies, affecting 1/2500 individuals. This pathology is characterized by the loss of myelin sheath integrity in the Peripheral Nervous System (PNS) resulting in slowed nerve conduction velocity, hind limbs muscular atrophy, and postural abnormalities. This neuropathy is caused by mutations in a wide range of genes; one of them is Myelin Protein Zero (MPZ), which is expressed by Schwann cells, the myelin forming glia of the PNS. MPZ encodes the most abundantly expressed protein of peripheral myelin and is required to tightly compact the structure of myelin allowing saltatory conduction. When the MPZS63del, a mutation causing CMT1B in humans, is expressed in mouse together with wild type alleles, it produces a demyelinating neuropathy that mimics the corresponding human disease. S63del is correctly expressed and translated but does not reach the myelin sheath being retained in the ER-Golgi complex. The accumulation of unfolded proteins into ER lumen is generally followed by induction of the Unfolded Protein Response (UPR), an adaptive mechanism aimed to relieve ER stress. S63del accumulation triggers a canonical, dose dependent, UPR with increased phosphorylation of eIF2alpha, ATF6 cleavage, IRE-1 induced XBP-1 splicing and CHOP induction. Genetic ablation of CHOP restores motor, electrophysiological and morphological abnormalities in S63del mice suggesting that the UPR is pathogenetic and maladaptive. Since CHOP is downstream of the PERK and eIF2alpha, we wanted to study the effects of PERK ablation in normal and S63del mice. For this purpose we bred S63del mice with PERK heterozygous null animals. Here we will present the preliminary behavioural, morphological and biochemical data from these mice to address whether PERK is maladaptive in S63del nerves.

PS4.31

Early electrophysiological changes in the excitability of neonatal rat hypoglossal motoneurons caused by free oxygen radicals

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease whose main feature is the progressive and selective loss of motoneurons in the spinal cord and brainstem. Despite extensive research, the mechanisms of neuronal injury are complex and incompletely understood: among them, oxidative stress and excitotoxicity are suggested to play a pivotal role. In the 25 % of cases, ALS has predominant bulbar-onset as it involves early, severe degeneration of brainstem motor nuclei. Amongst brainstem motor nuclei, the nucleus hypoglossus, responsible for tongue muscle innervation, is one of the most vulnerable to this disease. It is, however, unclear how hypoglossal motoneurons respond to oxidative stress in functional terms. The present study sought to investigate the early events associated with acute oxidative stress by using, as a model, a slice preparation of the neonatal rat brainstem. Hypoglossal motoneurons were recorded with whole-cell patch clamping during application of H_2O_2 as a donor of free oxygen radicals. In voltage clamp configuration (holding potential = -70 mV), bath application of 1 mM H_2O_2 (20 min) reduced the frequency and peak amplitude of spontaneous synaptic events, indicating decreased network excitability. The same effect was observed in the majority of motoneurons when the concentration of H_2O_2 was halved, and persisted when fast glutamatergic synaptic transmission was pharmacologically blocked by 50 μM APV and 20 μM CNQX. The voltage-activated persistent inward current evoked by depolarizing ramps was reduced. Under current clamp, 1 mM H_2O_2 increased the spike threshold and the firing frequency induced by current pulses, an effect resistant to APV and CNQX. All these effects were poorly reversible on washout. These data suggest that even a relatively short oxidative stress damaged the motor output of the nucleus hypoglossus through a combination of changes in network and motoneuron excitability.

PS4.32

Transcriptional regulation by Atrophin in development and neurodegeneration

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Atrophin (*Atro*) is the only Drosophila homolog of human *Atrophin-1*, the DRPLA disease gene. DRPLA (Dentatorubralpallidoluysian Atrophy) is a polyglutamine disease, caused by an expansion (over 48 repeats) of a polyQ tract in Atrophin-1, and characterized by brain specific neurons degeneration, together with psychiatric and motor symptoms. Atrophins are ubiquitous transcriptional cofactors involved in neuronal development and survival. Drosophila Atrophin itself contains two polyQ tracts, Q11 and Q14, of 11 and 14 glutamines respectively. To find what is the Atro function in gene expression regulation, what are the genes whose expression is regulated by Atro, and possibly misregulated by DRPLA-modeling Atro mutations, we are coupling gene expression with chromatin profiling in flies overexpressing wild-type or polyQ-expanded Atro. We have generated transgenic flies carrying wild-type Atro and two DRPLA fly models carrying polyQ-expanded Atro forms, which show retinal neurodegeneration. Taking advantage of the TARGET (Temporal And Regional Gene Expression Targeting) system, we have induced the transgenes expression in the adult retina, performing microarrays based genome-wide expression profiling at time points preceding neurodegeneration and we are carrying on data mining and validation. We are also generating a cell culture model based on Drosophila neurons inducibly overexpressing wt and polyQ-expanded Atro forms to verify their effects on gene expression in neuronal cells, by quantitative RT-PCR.
Finally we have generated an in vivo system based on flies which inducibly express Atro fused with Dam (DNA adenine-methyltransferase), to perform genome-wide chromatin analysis by the DamID (Dam Identification) method. This technique will allow us to find out DNA-protein interaction sites by methylation profiling and to identify the Atro direct transcriptional targets.

PS4.33

Title: Ceftriaxone treatment improves phenotype in a murine model of spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a genetic devastating motoneuron disease leading to infant death. No effective therapy is currently available for SMA and treatment is usually supportive. Thus, the identification of new therapeutic strategies is of critical importance. Beta-lactam antibiotics such as ceftriaxone may offer neuroprotection in motoneuron disease by increasing glutamate transporter expression. Since they are currently being evaluated in clinical studies in patients with amyotrophic lateral sclerosis, we evaluated their effect, in particular of ceftriaxone, in a SMA mouse model.
We injected daily intraperitoneally ceftriaxone in SMA mice from postnatal day 5 to death and we recorded phenotype modification and survival of treated mice versus untreated.
Treated animals present an ameliorated neuromuscular phenotype and an increased survival that correlate with protection of motoneurons and neuromuscular units. Ceftraxione effects is linked to increased expression of sodium-dependent glutamate transporter 1 (GLT1) in the spinal cord. This study provides the evidence of a potential therapeutic effect of this drug in SMA phenotype.

PS4.34

Transplantation of neural stem cells derived from murine embryonic (mES) ameliorates spinal muscular atrophy phenotype

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OBJECTIVE: To explore the potential of stem cells to treat Spinal Muscular Atrophy (SMA).
SMA is a devastating genetic motoneuron disease without effective treatment. A potential therapy for SMA could be stem cell transplantation both trough neuron replacement and/or neuroprotective effect.
METHODS: We generated a homogeneous neural stem cell population (NSCs) from a murine Embryonic Stem (mES) cell line expressing the green fluorescent protein (GFP).
We induced motoneuron differentiation exposing ES-NSC cells to a growth factors cocktail ("priming") followed by Retinoic Acid and sonic hedgehog. The phenotype was evaluated by morphology and immunocytochemistry. As next step, we transplanted the ES-NSCs, after "priming", intratechally in SMA (Smn–/–SMN2+/+SMN 7+/+) neonate mice to evaluate their ability to 1) migrate toward the parenchyma 2) incorporate functionally within the host environment 3) ameliorate the disease phenotype. The fate of transplanted cells was monitored by immunohistochemistry and confocal analysis. Neuromuscular evaluation (hand grip and open field tests) was performed and survival was recorded.
RESULTS: ES-NSCs cells grow as adherent monolayers, are self-renewing and co-express neural stem marker (nestin, SOX1 and LewisX). ES-NSCs can be induced towards a motoneuronal fate as demonstrated by the positivity for HB9, Islet1 and Choline acetyl transferase (ChAT) and formation of neuromuscular junctions in coculture with myotubes. In vivo, the intratechally transplanted NSCs are able to migrate across the meninges, integrate in the parenchyma and robustly differentiate into neuron and motor neurons with axon projecting into anterior roots. In several behavioral tests, transplanted mice exhibited significant functional improvement. Furthermore the survival was significantly extended in comparison to vehicle treated SMA mice (19 vs 13 days, $p < 0.001$).
CONCLUSIONS: Our data provide the first evidence that NSC derived from ES may exert a significant therapeutic effect on the SMA phenotype opening the path for the development of a combined cellular and molecular therapy for this disease.

PS4.35

Voltage gated sodium channels in an ALS mouse model: a semi-quantitative mRNA assay

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting the motor neuron (MN) system, leading the affected individuals to paralysis and death. In 90-95% of instances there is no apparent genetic linkage (sporadic ALS), but in the 5-10% of cases the disease is inherited in a dominant manner (familial ALS) and is not clinically distinguishable from the sporadic form. Mutations in the abundant, ubiquitary expressed cytoplasmic enzyme Cu/Zn superoxide dismutase (SOD1) account for 20% of familial ALS and transgenic mice expressing such mutations develop a fatal human-like disease. A common feature of both sporadic and familial ALS is the altered excitability of the cortical and spinal motorneurons, as shown in electrophysiological studies carried out in a transgenic mouse model of the disease (SOD1-G93A).
The present study's purpose is to determine if the voltage gated sodium channel isoform Nav 1.6, fundamental for the maturation and activity of spinal MN, and the best known accessory subunits, Beta 1, 2 and 3, show an altered expression in the spinal cord of SOD1-G93A mice, as a possible cause for the abnormal excitability of the MN system. A semi-quantitative assay has been performed at the RNA level, using both traditional and real time RT-PCR, in RNA samples derived from total spinal cord and from laser microdissected neurons from lumbar sections.

PS4.36

Fat, a tumor suppressor involved in retinal degeneration

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Fat is a cell adhesion molecule belonging to the cadherin family and conserved across species. It is considered to be a tumor suppressor that plays a role in cell proliferation and organ size since lethal mutations display hyperplastic overgrowth of cells during development. How Fat regulates these mechanisms is not fully established but it has been shown recently to act through the Hippo tumor suppressor Pathway. One of the first molecules shown to interact with Fat is Atrophin, the unique Drosophila homolog of human Atrophins. The protein is linked to a Human Neurodegenerative Disease, DRPLA, caused by a poly-Q expansion within the *atrophin* gene. Fat and Atrophin control similar processes during development of neuronal tissues and display strong genetic interactions. Our analysis shows that *fat* mutant clones, also in the fly eye, display age dependent abnormalities in the morphology of the photoreceptors, the neuronal cells that constitute retinal tissue. The severity of the phenotype increases with the age. These defects suggest a role of Fat in neuronal homeostasis and are enriched when removing one copy of *atrophin* in the same background for *fat*. Since Fat is involved in regulating the Hippo tumor suppressor Pathway, we also analyzed the phenotype of the retina in fly eyes mutants for some other activators of this pathway. Preliminary data suggest a genetic link between the Hippo Pathway and *fat* retinal degeneration. In conclusion we argue that Fat, alone or in cooperation with Atrophin, is involved in retinal neurodegeneration and that it could act to prevent retinal neurons death also with the help of other players.

PS4.37

Pheripheral markers of vascular damage in Alzheimer's disease and Mild Cognitive Impairment

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Background: although Alzheimer Disease (AD) is mainly considered a neuronal disease, many evidences point out a vascular involvement in its etiology. Homocysteine (Hcy) may cause arterial and venous thrombosis, but the mechanism by which it induces vascular damage is largely unknown. Tissue Factor Pathway Inhibitor (TFPI) is the only known regulator in the normal coagulation pathway and the increased expression of TFPI is postulated to play an important role in the control of thrombogenesis. Vascular endothelial cells, the main site of TFPI synthesis, react to elevated Hcy administration in-vitro, and TFPI antigen and activity have been found increased in pathologies in which mild-Hyperhomocysteinemia (mHHcy) is well known to play a key-role. Thus, TFPI may represent an endothelial cell damage marker.
Aim: to investigate whether mHHcy is a risk factor for AD, and whether it is associated with alteration of endothelial function, we have evaluated: Hcy, as risk factor for vascular and cerebrovascular disorders; TFPI, as inhibitor of the extrinsic coagulation cascade, regulating both pro-coagulant and pro-inflammatory processes; TXB2, as marker of platelet activation.
Patients and methods: 72 consecutive AD patients and 32 MCI subjects were enrolled. Neuropsychological evaluation, neuroradiological imaging and laboratory examinations were performed to rule out other causes of dementia. 53 healthy subjects (ctrl), age and sex matched, were also investigated. Hcy, TFPI and TXB2 were evaluated using HPLC and ELISA assay respectively.
Results: TFPI and Hcy levels were found increased in AD ($p<0.001$) and MCI subjects ($p<0.01$).
Plasma TBX2 appeared to be high only in AD ($p<0.05$) and the same parameter was positively correlated with TFPI levels ($P<0.0001$; $r=0.71$). Moreover, only in mHHcy AD subgroup, Hcy was significantly correlated with TFPI increased levels ($P<0.001$; $r=0.56$).
Conclusions: our data give further rationale to confirm the vascular involvement in AD pathogenesis and underline the importance of monitoring Hcy and coagulation factor levels as markers of vascular damage in this pathology.

PS4.38

Vesicular monoamine transporter (VMAT2) mRNA levels are reduced in platelets from patients with Parkinson's disease

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Despite advances in neuroimaging, the diagnosis of idiopathic Parkinson's disease (PD) remains mainly clinical. The identification of biological markers for an early diagnosis is of great interest to start a neuroprotective therapy aimed at slowing, blocking or reversing the disease progression. Vesicular monoamine transporter (VMAT2) represents the main protein responsible for sequestration of cytoplasmic dopamine (DA) into synaptic vesicles for storage and subsequent release. Thus, VMAT2 impairment can regulate intra- and extracellular DA levels, influencing oxidative stress and neuronal death. Since *in vivo* imaging studies have demonstrated that VMAT2 is reduced in PD patients beyond what could be explained by a loss of nigrostriatal neurons and that this reduction might be a pathogenic feature of PD, as an exploratory study we assessed VMAT2 mRNA levels in platelets from sporadic PD patients to identify a possible peripheral biomarker of disease. After informed consent, 20 PD patients, including a subgroup of 7 *de novo* patients, and 20 age- and sex-matched controls were recruited from the Dept of Neurology, San Gerardo Hospital, Monza, and the Parkinson Disease Centre, Milano. Motor (UPDRS III and V) and cognitive (MMSE) functions were evaluated. 10 ml of venous blood were taken to isolate platelets. VMAT2 mRNA levels were quantified by RT-qPCR using beta-actin as endogenous control. A 30% reduction ($p<0.05$) of VMAT2 mRNA was demonstrated in PD patients vs. controls. No difference was found between VMAT2 mRNA levels in *de novo* vs. treated PD patients. No correlation was observed between VMAT2 mRNA levels and demographic or clinical characteristics. The reduction of VMAT2 mRNA in platelets from PD patients, which reflects the condition described in the brain, suggests the possible use of this parameter as a valid biomarker for PD. Further studies in a greater number of cases and assessment of VMAT2 protein levels and function are needed to confirm our data.

PS4.39

The trieste-autoimmune brain atlas (taba) project: anti-neural antibodies characterization in ataxic patients

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Ataxia is a disorder of the motor coordination of familiar, traumatic or sporadic origin. It has been hypothesised that autoimmunity may contribute to the causes of ataxia of non-genetic origin and in particular that association with other autoimmune diseases such as celiac disease (CD) may enhance the probability to develop cerebellar ataxia. Through the project Trieste-Autoimmune Brain Atlas (TABA) we have developed a novel test to quantify anti-neural antibodies in serum or liquor based on semi-quantitative immunohistochemical analysis of rat brain sections. The cut off has been established on the staining reactivity of more than 100 healthy blood donors. The test specificity is 95%, sensitivity 84%, predictive negative value (PNV) 96% and positive predictive value (PPV) 82%. Our comprehensive data base, organized as a digital brain atlas, contains the staining pattern produced by more than 750 sera on 14 brain regions and allows detecting in principle any type of anti-neural reactivity even from the rarest disease (www.units.it/taba). Using this methodology, we have investigated the anti-neural reactivity in the serum of 10 patients with ataxia associated with CD, also called gluten ataxia (GA), 10 patients affected by idiopathic ataxia, 10 with genetic ataxia and 10 healthy blood donors, by immunohistochemistry on rat brain sections and western-blot on human medulloblastoma and larynx carcinoma cell-line lysates. Furthermore, we selected two phage antibody libraries from patients with gluten ataxia on rat brain lysate to isolate specific antibodies against neuronal antigens. We found 11 sera with anti-neural reactivity, 5 were from GA patients, 5 from idiopathic and 1 from genetic ataxia. Furthermore we isolated from the phage library, two antineural antibodies cross-reacting with TG6 a member of transglutaminases family, widely expressed in the brain. These results support the possibility of an autoimmune ethiology of ataxia especially if associated with CD.

PS4.40

Sulforaphane protects and rescues SH-SY5Y cells against 6-Hydroxydopamine-induced toxicity

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Parkinson’s disease (PD) is a neurodegenerative disorder with a selective loss of dopaminergic neurons in the substantia nigra. Evidence suggests that oxidative stress is one of the major factors responsible for the dysfunction or death of dopaminergic neurons. Isothiocyanates (ITCs), present in cruciferous vegetables, are known as cancer chemopreventive agents and strong inducers of phase II detoxification enzymes. Among the various ITCs, sulforaphane (SUL) has recently gained attention as a potential neuroprotective agent. In this study, we investigated the neuroprotective potential of SUL in a neuronal cell model of PD. In particular, we demonstrated that treatment of human dopaminergic SH-SY5Y cells with SUL prevents the oxidative stress and neuronal death induced by 6-hydroxidopamine (6-OHDA), a specific neurotoxin. In parallel, we found a potent indirect antioxidant activity of SUL on neuronal cells that could be ascribed to increased GSH levels and phase II enzyme activities, such as glutathione-S-transferase, glutathione reductase and NADPH-quinone. Interestingly, SUL also showed an ability to rescue the neuronal death induced by 6-OHDA through the activation of neuronal survival pathways such as PI3K/AKT and MEK/ERK. Taken together, these findings suggest that SUL may have a positive impact on PD to retard or reverse the accelerated rate of neuronal degeneration.

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PS4.41

Mechanisms involved in the reversal phytoestrogen genistein-evocated nociceptive hypersensitivity in a mouse neuropathy model induced by chronic constriction injury

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The great interest in soy isoflavones therapeutic use as an alternative to the endogenous estrogens is not only restricted to hormonal pathologies, but it extends to inflammatory, neurodegenerative diseases and pain. The present work investigates the effect of the isoflavone genistein, a nutriaceutical largely present in soybean, on neuropathic pain. Genistein binds the estrogen receptors (ER) with higher affinity for ERß that is highly expressed in neuronal and immune cells. Neuropathy was induced in C57BL/6J male mice by sciatic nerve chronic constriction injury. Genistein, administered subcutaneously (s.c.) starting from third day after nerve lesion, time- and dose-dependently reversed pain hypersensitivity. Genistein-induced relief of hyperalgesia and allodynia could be due to the activation of classical nuclear receptors, its antioxidant, antiinflammatory and immunomodulating effects. We showed that a specific ERß antagonist prevented both antiallodynic and antihyperalgesic action of phytoestrogen, while a specific ERa antagonist was ineffective, thus suggesting the involvement of ERß in genistein effect. The non-selective ER antagonist, ICI 182,780, reversed only the genistein antiallodynic effect. The antioxidant effects are also involved, since the same genistein dose that relieved the neuropathic pain also reversed ROS and malondialdehyde increases in injured paw tissues, and further increased the specific activity of antioxidant enzymes, such as glutathione-related enzymes and catalase. Both specific ERß antagonist and non selective ER antagonist didn't reverse the genistein antioxidant activity. Immunomodulatory and anti-inflammatory activities could be also responsible of phytoestrogen-induced neuropathic pain relief, since the peripheral and central NF-kB, nitric oxide system and pro-inflammatory cytokine over-activation was reversed by genistein. There are in progress studies of ERß involvement in the phytoestrogen-induced reversal of NO system increase. Collectively these results suggest that genistein could ameliorate painful neuropathy by multiple mechanisms.

PS4.42

Immunolocalization of alpha-synuclein in the rat spinal cord by two novel monoclonal antibodies

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Our study provides the first immunohistochemical evidence of the presence and distribution patterns in the rat spinal cord of α-Synuclein, a soluble acidic protein well known to be richly expressed in the central nervous system and to be closely related with the pathogenesis of some neurodegenerative diseases including Parkinson and Alzheimer diseases. We used two novel homemade monoclonal antibodies (2E3 and 3D5) recognizing two different epitopes of α-Synuclein (1). Both antibodies were able to localize α-Synuclein within the nerve terminals, whereas only 3D5 was able to also localize it within the nucleus of the neurons. α-Synuclein-immunoreactive nervous elements were widely recognized along the entire extent of rat spinal cord and in almost all the laminae of gray matter. However, they appeared particularly concentrated within the laminae I, II, VII and X and more scattered in the others. Double immunostaining procedures allowed us to demonstrate the co-localization of α-Synuclein with synaptophysin in the nerve presynaptic terminals, with neuropeptide Y in lamina I, II, IX and X, and its close relationships with tyrosine hydroxylase immunoreactive neurons in lamina VII and X. Interestingly, the α-Synuclein immunoreactive neurons showed poor content of calbindin 28kD. Our findings could help understanding some early clinical symptoms of the neurodegenerative diseases, such as protopathic pain and disautomonic disorders and indicate the spinal cord as a probable start point of them, according to the "ascending theory" of Parkinson disease (2).
(1) Yu et al. Neuroscience, 145: 539-555, 2007
(2) Braak et al. Acta Neuropathol. , 113 (4) : 421-429, 2007.

PS4.43

Molecular effects of beta-amyloid and glutamate in human fibroblasts. Correlation between ERK1/2 and MMSE in Alzheimer's disease patients

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Amyloid-beta (Abeta) affects cellular functions leading to neuro-degeneration and memory impairment via critical signal transduction processes, including stress-activated kinases and ERK pathways. Activation of JNK and p38 has been shown in degenerating neurons from Alzheimer's disease (AD) patients. Moreover, astroglial activation of ERK has been observed in early stages of the disease.
We used fibroblasts (fb) obtained from AD patients and age-matched controls, to investigate these pathways and bio-molecular modifications associated to various stages of disease or induced by beta-amyloid and glutamate (glu) treatments. In these peripheral cells from AD patients, abnormal APP processing and modifications of glutamatergic system were previously demonstrated.
By western blot and phospho-elisa assay we observed phosphorylated p38 and JNK in fb from AD patients and in control fb following glu and Abeta treatments. We also showed ERK deactivation in fb from mild AD patients and ERK phosphorylation in cells from moderate/severe patients. A significant inverse correlation between phosphorylated-ERK status and MMSE was demonstrated. In treated control fb, as in mild AD patients, a decrease of ERK activation was shown.
To better investigate Abeta and glu involvement in alterations of ERK pathway, we tested the MEK/ERK inhibitor effect. By preliminary data, we hypothesized that Abeta and glu may display similar effects on ERK down-regulation but acting through different steps. Glu might activate the classic MEK/ERK cascade, while Abeta action may be MEK-independent and it might involve the CaMKII pathway. Moreover, our results showed that fb respond to these treatments, suggesting the presence of potential specific receptors, NMDA-like. The comparison of ERK activation pathway observed in AD and control fb, after glu and Abeta treatments, may elucidate some molecular mechanisms involved in early or late stages of the pathology.

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