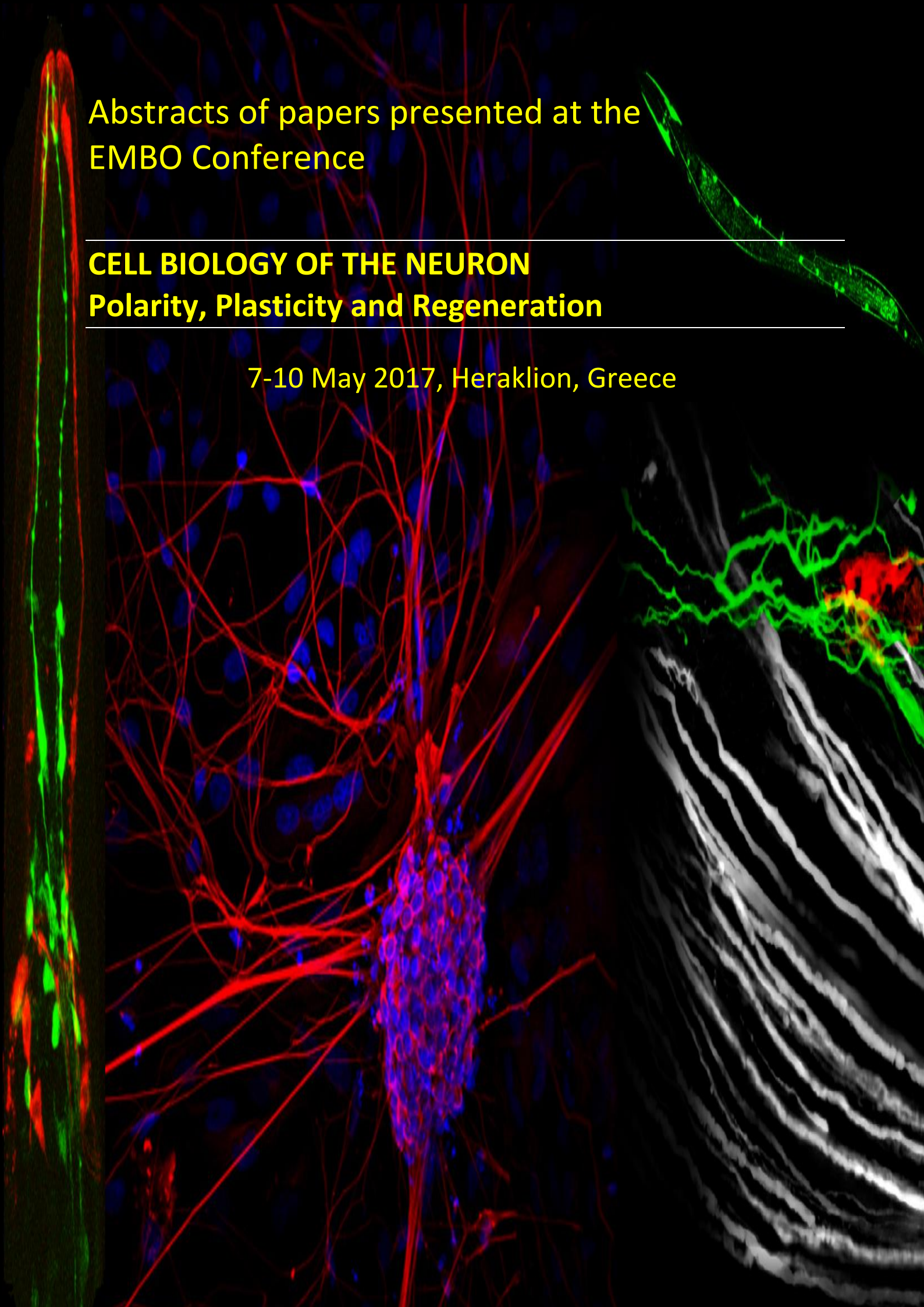
Abstracts of papers presented at the
EMBO Conference

# CELL BIOLOGY OF THE NEURON
## Polarity, Plasticity and Regeneration

7-10 May 2017, Heraklion, Greece



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# Programme

| Sunday, 7 May 2017 | |
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| 11:00-14:00 | **Registration** |
| ***Session 1: Axon and Dendritic Growth***<br>*Chair: Esther Stoeckli [University of Zurich, Department of Molecular Life Sciences, Zurich, Switzerland]* | |
| 14:00-14:10 | Opening Remarks |
| 14:10-14.35 | **Rüdiger KLEIN** [Department of Molecular-Signaling-Development, Max Planck Institute of Neurobiology, Martinsried, Germany]<br>*Understanding contact repulsion: roles of exosomes and phagocytosis* |
| 14:35-15.00 | **Azad BONNI** [Department of Neuroscience, Washington University School of Medicine, St. Louis, USA]<br>*Cell-Intrinsic Regulation of Neuronal Connectivity in the Brain* |
| 15:00-15.25 | **Gaia TAVOSANIS** [German Center for Neurodegenerative Diseases-DZNE, Bonn, Germany]<br>*The Fly Mushroom Body Calyx: a Small Circuit in the Spotlight* |
| 15:25-15.40 | **Sebastian DUPRAZ** [German Center for Neurodegenerative Diseases-DZNE, Bonn, Germany]<br>*RhoA Controls Axon Growth through Myosin II-Actin Mediated Restraint of Microtubule Protrusion* |
| 15.40-16.25 | ***Coffee break*** |
| ***Session 2: Neuronal Polarity***<br>*Chair: Anthony Barnes [Knight Cardiovascular Institute, Oregon Health and Science University, Portland, USA]* | |
| 16:25-16:50 | **Matthew RASBAND** [Baylor College of Medicine, Houston, USA]<br>*The Structure and Function of Axonal Spectrin Cytoskeletons* |
| 16:50-17:15 | **Raman DAS** [Division of Developmental Biology and Medicine, University of Manchester, Manchester, UK]<br>*Cell Behaviour Underlying Neurogenesis in the Developing Spinal Cord* |
| 17:15-17:40 | **Caren NORDEN** [Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]<br>*Deconstructing Neuronal Lamination in the Vertebrate Retina* |
| 17:40-17:55 | **Maya SHELLY** [Department of Neurobiology and Behavior, Stony Brook University, New York, USA]<br>*A Scaffold for cGMP-activity Dictates Dendrite Formation during Neuronal Polarization* |
| 17:55-18:20 | ***Coffee break*** |
| 18:20-19.00 | *Keynote Talk:* **Reinhard JAHN** [Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany]<br>*Molecular Mechanisms Governing Exocytosis of Synaptic Vesicles* |
| 19.00-21.00 | ***Dinner*** |
| 21.00-23.00 | ***Drinks – including speed dating (poster presenters and other participants interact with main/keynote speakers)*** |

| Monday, 8 May 2017 | |
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| ***Session 3: Axon Regeneration***<br>*Chair: Monica Sousa [IBMC/i3S University of Porto, Portugal]* | |
| 09:00-09:25 | **Eran PERLSON** [Department of Physiology and Pharmacology, Tel Aviv University, Sackler Faculty of Medicine, Tel Aviv, Israel]<br>*Location, Location, Location – Amyotrophic Lateral Sclerosis as a Spatiotemporal Mislocalization Disease* |
| 09:25-09:50 | **Yishi JIN** [Division of Biological Sciences, UC San Diego, San Diego, USA]<br>*Novel Pathways of Axon Regeneration Identified from Large-scale Genetic Screen in C. elegans* |
| 09:50-10:15 | **Jerry SILVER** [Department of Neurosciences, Case Western Reserve University School of Medicine, Cleveland, USA]<br>*Overcoming Proteoglycan Barriers to Axon Regeneration and Sprouting* |
| 10:15-10:55 | ***Coffee break*** |
| 10:55-11:20 | **Mike FAINZILBER** [Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel]<br>*The Importins of Axonal Transport in Neuronal Growth and Survival* |
| 11:20-11:35 | **Eric EWAN** [Department of Neuroscience, Washington University in St. Louis, USA]<br>*Role of SCG10 in Axon Regeneration* |
| 11:35-11:50 | **Ulrich HENGST** [Department of Pathology & Cell Biology and The Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, USA]<br>*Control of Axonal mRNA Localization and Translation through an Exclusion Mechanism* |
| 12:00-13:45 | **Lunch Break** |
| ***Session 4: Intracellular Dynamics***<br>*Chair: Peter Schu [Department for Cellular Biochemistry, Georg-August-University Göttingen, Germany]* | |
| 13:45-14:10 | **Jochen GUCK** [Biotechnology Center, TU Dresden, Dresden, Germany]<br>*Mechanosensing of Neurons and Glial Cells in the CNS* |
| 14:10-14:35 | **Volker HAUCKE** [Department of Molecular Pharmacology and Cell Biology, Leibniz-Institute for Molecular Pharmacology, Berlin, Germany]<br>*Retrograde Axonal Transport of Signaling Autophagosomes in Neuronal Complexity and Neurodegeneration* |
| 14:35-15:00 | **Olivier THOUMINE** [Institute Interdisciplinaire de Neuroscience-IINS, CNRS/Université Bordeaux 2, Bordeaux, France]<br>*Role of the Adhesion Molecule Neuroligin-1 in Synaptogenesis Probed by Single Molecule Imaging and Regulation of Tyrosine Phosphorylation* |
| 15:00-15:15 | **Ruud TOONEN** [VU University Amsterdam, The Netherlands]<br>*SNAP-25 Gene Family Members Differentially Support Secretory Vesicle Fusion* |
| 15:15-15:30 | **Meng-meng FU** [Department of Neurobiology, Stanford University School of Medicine, Stanford, USA]<br>*Golgi Outposts Mediate Uniform Microtubule Polarity in Oligodendrocytes* |
| 15:30-15:45 | **Adrian MOORE** [RIKEN Brain Science Institute, Wako-shi, Saitama, Japan]<br>*Important Things have Small Beginnings: Major Branches in the Dendrite Arbor Arise by Stabilization of Single Actin Bundles at the Dendrite Tip* |
| **15:45-18:30** | **Poster Session I: RED SESSION - ODD NUMBERS** |
| 19.00-21.00 | ***Dinner*** |
| 21.00-23.00 | ***Wine and Beer at Poster Session*** |

| Tuesday, 9 May 2017 | |
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| ***Session 5: Synapse***<br>*Chair: Helmut Kessels [Netherlands Institute for Neuroscience, Amsterdam, Netherlands]* | |
| 09:00-09:25 | **Kristian FRANZE** [Department of Physiology, Development and Neuroscience, University of Cambridge, UK]<br>*The Mechanical Control of Neuronal Development and Regeneration* |
| 09:25-09:50 | **Silvio RIZZOLI** [Department of Neuro and Sensory Physiology, University of Göttingen Medical center, Göttingen, Germany]<br>*The Turnover of Mammalian Proteins is Encoded in their Amino Acid and Codon Compositions* |
| 09:50-10:15 | **Michael KREUTZ** [Leibniz Institute for Neurobiology, Magdeburg, Germany]<br>*The EF-hand Ca2+-sensor Caldendrin Directly Transduces Postsynaptic Calcium Signals to Stabilize Branched Actin Filaments in Spines* |
| 10:15-10:30 | **Joris de WIT** [KU Leuven, Department of Neurosciences, VIB Center for Brain & Disease Research, Leuven, Belgium]<br>*A Novel Input-specific Synaptic Organizing Complex Controls Mossy Fiber Synapse Development and Function* |
| 10:30-10:45 | **Katalin SCHLETT** [Neuronal Cell Biology Research Group, Eotvos Lorand Univeristy, Budapest, Hungary]<br>*The Caskin Scaffold Protein Regulates Dendritic Spine Morphology, Learning and Memory* |
| 10:45-11:25 | ***Coffee break*** |
| ***Session 6: Current Hot Topics***<br>*Chair: Britta Eickholt [Charité Cross Over (CCO), Institut für Biochemie, Berlin, Germany ]* | |
| 11:25-11:40 | **Jeroen PASTERKAMP** [Department of Translational Neuroscience, UMC Utrecht, The Netherlands]<br>*A Novel Subtractive Genetics Approach for Dissecting Dopaminergic Pathway Development and Plasticity* |
| 11:40-11:55 | **Konstantinos PALIKARAS** [IMBB-FORTH, Heraklion, Greece]<br>*Mitophagy and Neuronal Homeostasis in C. elegans* |
| 11:55-12:10 | **Peter SOBA** [Center for Molecular Neurobiology, University Medical Campus Hamburg-Eppendorf, Germany]<br>*Maintenance of Scaled Dendritic and Synaptic Growth during Juvenile Development Requires Tao kinase Function* |
| 12:10-13:55 | **Lunch Break** |
| ***Session 7: Neuro-Developmental Processes***<br>*Chair: Oren Schuldiner[Department of Molecular Cell Biology, Weizmann Institute, Rehovot, Israel ]* | |
| 14:00-14:25 | **Jürgen KNOBLICH** [IMBA – Institute of Molecular Biotechnology, Vienna, Austria]<br>*Cerebral Organoids: Modelling Human Brain Development and Tumorigenesis in Stem Cell Derived 3D Culture* |
| 14:25-14:50 | **Melissa ROLLS** [Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, USA]<br>*The Pluses and Minuses of Microtubule Organization in Dendrites* |
| 14:50-15:15 | **Wieland HUTTNER** [Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]<br>*On the Role of Human-Specific Genes, Notably ARHGAP11B, in Neural Stem Cell Amplification and Neocortex Expansion in Development and Evolution* |
| 15:15-15:30 | **Laura F. GUMY** [Division of Cell Biology, Utrecht University, Utrecht, The Netherlands]<br>*MAP2 Defines a Pre-axonal Filtering Zone to Regulate KIF1-Versus KIF5-Dependent Cargo Transport in Sensory Neurons* |
| 15:30-15:45 | **Oren SCHULDINER** [Department of Molecular Cell Biology, Weizmann Institute, Rehovot, Israel]<br>*A Systematic Exploration of Neuronal Remodeling Reveals a Transcription Factor Hierarchy* |
| **15:45-18:30** | **Poster Session II: GREEN SESSION - EVEN NUMBERS** |
| 18:30-19:10 | *Keynote Talk:* **Nobutaka HIROKAWA** [Department of Cell Biology and Anatomy, University of Tokyo, Tokyo, Japan]<br>*Molecular Motors, KIFs and Regulation of Neuronal Function, Morphogenesis, and Brain Wiring* |
| 19.10-21.00 | ***Dinner*** |
| 21.00-23.00 | ***Wine and Beer at Poster Session*** |

| Wednesday, 10 May 2017 | |
|---|---|
| ***Session 8: Synapse and Disease***<br>*Chair: Alexandros Poulopoulos [Department of Pharmacology, University of Maryland School of Medicine, Baltimore, USA]* | |
| 09:00-09:25 | **Corette WIERENGA** [Faculty of Science, Cell Biology, Utrecht University, Utrecht, The Netherlands]<br>*Molecular Pathway Underlying Bouton Stabilization by Semaphorin4D during Inhibitory Synapse Formation* |
| 09:25-09:50 | **Patrik VERSTREKEN** [VIB Center for the Biology of Disease, KU Leuven, Leuven, Belgium]<br>*Fine-Tuning Proteolysis and Exosome Release by Membrane-deforming Heat Shock Chaperones* |
| 09:50-10:05 | **Francesca BARTOLINI** [Pathology & Cell Biology, Columbia University, New York, USA]<br>*Microtubule Hyperstabilization by mDia1 Drives Tau-dependent Synaptotoxicity* |
| 10:05-10:45 | ***Coffee break*** |
| ***Session 9: Neuronal Degeneration*** - *Chair: David Sulzer [Columbia University, New York, USA]* | |
| 10:45-11:00 | **Roman PRASCHBERGER** [Department of Clinical and Experimental Epilepsy, UCL, London, UK]<br>*Elucidating the Pathophysiology of GOSR2-mediated Progressive Myoclonus Epilepsy from Molecule to Neuron* |
| 11:00-11:25 | **Claudia BAGNI** [Department of Fundamental Neuroscience, University of Lausanne, Switzerland]<br>*The disease Associated Protein CYFIP1 Orchestrates Axonal Outgrowth and Brain Wiring* |
| 11:25-11:50 | **David SULZER** [*Columbia University, New York, USA*]<br>*Autoimmune Aspects of Parkinson's Disease* |
| 11:50-12:15 | ***Closing Remarks – End of Conference - Lunch*** |

# ORAL PRESENTATIONS

## *Session 1: Axon and Dendritic Growth*

### Invited Lectures

Understanding Contact Repulsion: Roles of Exosomes and Phagocytosis
**Klein R**

Cell-Intrinsic Regulation of Neuronal Connectivity in the Brain
**Bonni A**

The Fly Mushroom Body Calyx: a Small Circuit in the Spotlight
Baltruscht L, Ranft P, Marchetti G, Bock D and **Tavosanis G**

### Short Talk

RhoA Controls Axon Growth through Myosin II-Actin Mediated Restraint of Microtubule Protrusion
**Dupraz S**, Coles C, Stern S, Brakebusch C and Bradke F

## *Session 2: Neuronal Polarity*

### Invited Lectures

The Structure and Function of Axonal Spectrin Cytoskeletons
**Rasband M**

Cell Behaviour Underlying Neurogenesis in the Developing Spinal Cord
**Das R**

Deconstructing Neuronal Lamination in the Vertebrate Retina
**Norden C**

### Short Talk

A Scaffold for cGMP-activity Dictates Dendrite Formation during Neuronal Polarization
Lee S-I, Cancedda L, Pautot S and **Shelly M**

### Keynote Talk

Molecular Mechanisms Governing Exocytosis of Synaptic Vesicles
**Jahn R**

## *Session 3: Axon Regeneration*

### Invited Lectures

Location, Location, Location – Amyotrophic Lateral Sclerosis as a Spatiotemporal Mislocalization Disease
**Perlson E**

Novel Pathways of Axon Regeneration Identified from Large-scale Genetic Screen in *C. elegans*
**Jin Y**

Overcoming Proteoglycan Barriers to Axon Regeneration and Sprouting
**Silver J**

The Importins of Axonal Transport in Neuronal Growth and Survival
**Fainzilber M**

### Short Talks

Role of SCG10 in Axon Regeneration
**Ewan E** and Cavalli V

Control of Axonal mRNA Localization and Translation through an Exclusion Mechanism
Martínez JC and **Hengst U**

## *Session 4: Intracellular Dynamics*

### Invited Lectures

Mechanosensing of Neurons and Glial Cells in the CNS
**Guck J**

Retrograde Axonal Transport of Signaling Autophagosomes in Neuronal Complexity and Neurodegeneration
Kononenko NL, Claßen GA, Kuijpers M, Puchkov D, Jaworski J and **Haucke V**

Role of the Adhesion Molecule Neuroligin-1 in Synaptogenesis Probed by Single Molecule Imaging and Regulation of Tyrosine Phosphorylation
Chamma I, Letellier, M, Czöndör K, Saphy C, Papasideri I, Tessier B, Sainlos M and **Thoumine O**

### Short Talks

SNAP-25 Gene Family Members Differentially Support Secretory Vesicle Fusion
Arora S, Saarloos I, Kooistra R, Verhage M and **Toonen RF**

Golgi Outposts Mediate Uniform Microtubule Polarity in Oligodendrocytes
**Fu M-m,** Shi R, L C-Y and Barres BA

Important Things have Small Beginnings: Major Branches in the Dendrite Arbor Arise by Stabilization of Single Actin Bundles at the Dendrite Tip
Yoong L-F, Lim H-K, Lackner S, Hong P and **Moore AW**

## *Session 5: Synapse*

### Invited Lectures

The Mechanical Control of Neuronal Development and Regeneration
**Franze K**

The Turnover of Mammalian Proteins is encoded in their Amino Acid and Codon Compositions
Fornasiero EF, Mandad S, Wildhagen H and **Rizzoli SO**

The EF-hand Ca2+-sensor Caldendrin Directly Transduces Postsynaptic Calcium Signals to Stabilize Branched Actin Filaments in Spines
Mikhaylova M, Bär J, Schätzle PE, YuanXiang PA, van Bommel B, Hradsky J, Reddy PP, Loktionov EY, Konietzny A, Raman R, Spilker C, Lopez-Rojas J, Ahsan Raza S, Stork O, Hoogenraad CC and **Kreutz MR**

### Short Talks

A Novel Input-specific Synaptic Organizing Complex Controls Mossy Fiber Synapse Development and Function
Condomitti G, Wierda KD, Rubio SE, Rice HC, Vennekens KM, Savas JN, Gounko NV and **de Wit J**

The Caskin Scaffold Protein Regulates Dendritic Spine Morphology, Learning and Memory
Pusztai S, Fekete A, Bencsik N, Borbély S, Kis V, Pesti S, Buday L and **Schlett K**

## *Session 6: Current Hot Topics*

### Short Talks

A Novel Subtractive Genetics Approach for Dissecting Dopaminergic Pathway Development and Plasticity
Brignani S, Schmidt ERE, Raj DDA, van Battum EY, de Ruiter AA, Schild ES and **Pasterkamp RJ**

Mitophagy and Neuronal Homeostasis in *C. elegans*
**Palikaras K** and Tavernarakis N

Maintenance of Scaled Dendritic and Synaptic Growth during Juvenile Development Requires Tao kinase Function
Hu C, Tenedini F, Saez M, Hoyer N, Calderon de Anda F and **Soba P**

## *Session 7: Neuro-Developmental Pocesses*

### Invited Lectures

Cerebral Organoids: Modelling Human Brain Development and Tumorigenesis in Stem Cell Derived 3D Culture
**Knoblich J**

The Pluses and Minuses of Microtubule Organization in Dendrites
**Rolls M**

On the Role of Human-Specific Genes, Notably ARHGAP11B, in Neural Stem Cell Amplification and Neocortex Expansion in Development and Evolution
**Huttner W**

### Short Talks

MAP2 Defines a Pre-axonal Filtering Zone to Regulate KIF1-Versus KIF5-Dependent Cargo Transport in Sensory Neurons
**Gumy LF**, Katrukha EA, Grigoriev I, Jaarsma D, Kapitein LC, Akhmanova A and Hoogenraad CC

A Systematic Exploration of Neuronal Remodeling Reveals a Transcription Factor Hierarchy
Alyagor I, Berkun V Keren-Shaul, H, Amit I and **Schuldiner O**

### Keynote Talk

Molecular Motors, KIFs and Regulation of Neuronal Function, Morphogenesis, and Brain Wiring
**Hirokawa N**

## *Session 8: Synapse and Disease*

### Invited Lectures

Molecular Pathway Underlying Bouton Stabilization by Semaphorin4D during Inhibitory Synapse Formation
Frias CP, Hu HY, Bresser T, Scheefhals L, van Bergen en Henegouwen PMP, Hoogenraad CC and **Wierenga C**

Fine-Tuning Proteolysis and Exosome Release by Membrane-deforming Heat Shock Chaperones
**Verstreken P**

### Short Talk

Microtubule Hyperstabilization by mDia1 Drives Tau-dependent Synaptotoxicity
Qu X, Yuan FN, Coronal C, Pasini S, Pero ME, Gundersen GG, Shelanski ML and **Bartollini F**

## *Session 9: Neuronal Degeneration*

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Elucidating the Pathophysiology of GOSR2-mediated Progressive Myoclonus Epilepsy from Molecule to Neuron

**Praschberger R**, Low S, Malintan N, Patel N, Houlden H, Krishnakumar S, Kullmann D, Rothman J, Usowicz M, Hodge J and Jepson J

# Invited Lectures

The disease Associated Protein CYFIP1 Orchestrates Axonal Outgrowth and Brain Wiring

Dominguez-Iturza N, Shah D, Li KW, Smit AB, Van Der Linden A, Achsel T and **Bagni C**

Autoimmune Aspects of Parkinson's Disease

**Sulzer D,** Cebrian C, Alcalay R, Gareetti F, Lindestam Arlehamn C, Frazier A and Sette A

# POSTER PRESENTATIONS

1. Investigating the Role of Canonical and Non-canonical Wnts in Axon Outgrowth
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2. Quantitative Map of Proteome Dynamics during Neuronal Differentiation Reveals NCAM1 as Regulator of Dendritic Morphogenesis
   Frese CK, Mikhaylova M, Stucchi R, Gautier V, Liu Q, Mohammed S, Heck AJR, Hoogenraad CC and **Altelaar AFM**

3. The RapGAP SPAR2/SIPA1L2 Provides a Ride-on Service for TrkB Endosomes and Autophagosomes
   **Andres-Alonso M,** Butnaru I, Rafeet-Ammar M, Gomez GM, Yuanxiang PA, Hausrat T, Diaz-Gonzalez S, Sanhueza GA, Schweizer M, Kneussel, M, Spilker C and Kreutz MR

4. The Role of Serotonin Receptors and Extracellular Matrix in Stress-related Disorders
   **Antoniuk S,** Bijata M, Ponimaskin E and Wlodarczyk J

5. Patterns of Spiking Activity of Neuronal Networks in vitro as Memory Traces
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6. Harnessing Electrochemistry for Structural Plasticity Studies - a Glutamate Biosensor
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7. The Role of S-palmitoylation in the Chronic Stress Disorders
   **Bartkowiak-Kaczmarek A,** Zareba-Koziol M, Figiel I, Krzystyniak A and Wlodarczyk J

8. Modeling Microtubule Dynamics in Axonal Growth Cones
   **Beretta CA,** Nedelec F and Engel U

9. Synaptic Remodeling Depends on Signaling Between Serotonin Receptors and the Extracellular Matrix
   **Bijata M,** Labus J, Guseva D, Stawarski, M, Butzlaff M, Dzwonek J, Schneeberg J, Böhm K, Michaluk P, Rusakov DA, Dityatev A, Wilczyński, G, Wlodarczyk J and Ponimaskin E

10. Recycling Endosomes Mediate Local, Golgi-independent Secretory Trafficking in Neuronal Dendrites and Spines
    **Bowen AB** and Kennedy MJ

11. Assessing the place field formation capability of CA1 pyramidal models in Alzheimer's Disease
    **Bozelos P,** Poirazi P

12. Hydrogels Mimicking the Central Nervous System Extracellular Matrix: Applications to 3D Cultures and Neural Regeneration
**Broguiere N**, Zenobi-Wong M and Bradke F

13. Huntingtin-mediated Axonal Transport of APP Determines Synaptic Aβ Accumulation and Memory Alterations in Alzheimer's Disease
**Bruyère J**, Abada Y-S, Fontaine G, Vitet H, Benstaali C, Charlot B, Humbert S, Potier MC, Delatour B and Saudou F

14. Regulation of Dendritic Spine Morphology in Hippocampal Neurons by Copine-6
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15. Vesicular Treadmilling in Axons Defines How Synapses Adapt to High Neuronal Demand
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16. Substrate Elasticity Controls Neurite Initiation through a Bi-stable Switch
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17. Actin Rings: Its Assembly and Physiological Relevance
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18. Profilin-1 is a Key regulator of Actin and Microtubule Dynamics Required for Optimal Axon Growth and Regeneration
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19. Myosin 1b Triggers Axon formation by Controlling Growth Cones and Actin Waves Propagation
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20. AMPK Related Kinase NUAK1 Haploinsufficiency Impairs Cortical Development and Behavior in the Mouse
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21. Local Administration of Lithium Promotes Axonal Regeneration and Functional Motor Recovery after Peripheral Nerve
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22. A Common Conserve Pathway Exists during Inflammation Mediated Neurodegeneration in *Drosophila* Model of Parkinson's Disease
**Dalui S**, Chatterjee S and Bhattacharyya A

23. Nanoscale Alignment of the Periodic Subcortical Cytoskeleton of Axon and Glia at Nodes of Ranvier
**D'Este E**, Kamin D, Balzarotti F, Göttfert F, Velte C, Simons M, and Hell SW

24. Differential Requirement of Sad Kinases for Cortical and Hippocampal Development in Mouse Brain
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25. The AP-2 Complex Has A Specialized Clathrin-Independent Role In Polarity Maintenance In Fungi
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26. Optogenetic Activation of Mechanical Forces to Control Neuronal Polarisation
**Dimitracopoulos A**, Shahapure R and Franze K

27. Neuroepithelial Organization and Polarity Require TorsinA Regulation of the Nuclear Envelope Localized LINC Complex
**Domínguez González B**, Billion K and Goodchild RE

28. The Disease Associated Protein CYFIP1 Orchestrates Axonal Outgrowth and Brain Wiring
**Dominguez-Iturza N,** Shah d, Li KW, Smit AB, Van Der Linden A, Achsel T and Bagni C

29. Actin Cytoskeleton Disruption as a Novel Player in the Pathogenesis of Familial Amyloid Polyneuropathy
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30. Deletion of VTI1a and VTI1b Inhibits Synaptic- and Dense-core Vesicle Fusion due to Missorting of Proteins Required for Regulated Secretion
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31. Microtubule Guidance in Axon Outgrowth
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32. FMR1 Introns Encode MicroRNA's that Provide a Window to Primate Evolution
**Ethell D,** Cave S, Sysko M, Begum A, Hong Y and Irizarry K

33. Excessive Synaptogenesis in Adult Hippocampus of Astrocyte-specific Ephrin-B1 Knockout Mice Impacts Synaptic Functions and Mouse Behaviors
Nguyen AQ, Koeppen J, Garcia M, Hanna S, Obenaus A and **Ethell IM**

34. Synaptic Localization of β-dystroglycan Mediates Homeostatic Plasticity
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35. Principles of Brain Protein Turnover
**Fornasiero EF**, Mandad S, Pena Centeno T, Vidal RO, Wildhagen H, Rammner B, Keihani S, Opazo F, Urban I, Ischebeck T, Kirli K, Rahman R-U, Benito E, Fischer A, Dennerlein S, Rehling P, Feussner I, Bonn S, Urlaub H and Rizzoli SO

36. Cooperative Interactions between 480 kDa Ankyrin-G and EB Proteins Assemble the Axon Initial Segment
**Fréal A**, Fassier C, Le Bras B, Bullier E, De Gois S, Hazan J, Hoogenraad CC and Couraud F

37. The Mitotic Kinase NEK7 Controls Dendritic Morphology in Neurons through Regulation of the Kinesin Eg5
**Freixo F,** Sánchez-Huertas C, Martinez Delgado P, Roig J and Lüders J

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Poobalasingam T, Boddy L and **Gordon-Weeks PR**

39. Long-distance Protein Transport Couples Extrasynaptic NMDAR-activity to Transcriptional Inactivation of CREB in Alzheimer's Disease
**Grochowska KM**, Kaushik R, Gomes GM, Bär J, Bayraktar G, Fusi C, Raman R, Navarro Brugal G, Karpova A and Kreutz MR

40. Aging Diminishes the Regeneration-promoting Effect of Targeting Pten in the Injured CNS
**Hilton BJ**, Geoffroy CG, Zheng B and Tetzlaff W

41. Probing Physiological Relevance of Regenerating Axons after Spinal Cord Injury
Elliott DA, **Husch A** and Bradke F

42. Using Stem Cells to Understand the Underlying Mechanisms of Polygenic Risk of Severe Mental Disorders
**Impellizzeri AAR**, Szabo A, Brincker Fjerdingstad H, Sullivan GJ, Glover JC, Andreassen OA and Djurovic S

43. In Search for Optimal Carbon Nanomaterials Coatings for Efficient Neuronal Regeneration
**Jantas D** and Fraczek-Szczypta A

44. The Role of Ndr1/2 in the Regulation of Neuronal Polarity
**Jin J**, Yang R, Kong E, Hergovich A and Püschel AW

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107. Cell Autonomous Regulation of Neuronal Fatty Acid Synthesis is required for Dendritic Development and Maintenance
**Ziegler AB** and Tavosanis G

# Molecular Mechanisms Governing Exocytosis of Synaptic Vesicles

## Reinhard JAHN

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Neurotransmitter release from presynaptic nerve endings is mediated by Ca2+ -dependent exocytosis of synaptic vesicles. Fusion is carried out by the SNARE proteins synaptobrevin/VAMP, syntaxin 1, and SNAP-25. Upon membrane contact, the vesicular SNARE synaptobrevin forms complexes with the plasma membrane-resident SNAREs SNAP-25 and syntaxin 1. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion ("zipper" hypothesis of SNARE function). The steps of SNARE assembly are controlled both by members of conserved protein families such as the SM- and CATCHR-proteins, and they are tightly controlled by specialist proteins responsible for calcium regulation such as the calcium sensor synaptotagmin and complexins.

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane and that only few SNARE complexes may suffice for effective fusion of bilayers. Furthermore, we have studied intermediate states of the SNARE-dependent fusion pathway. In addition, we have investigated how regulatory proteins such as the SM-protein Munc-18 and the calcium sensor synaptotagmin 1 interact with SNAREs and how they affect their reactivity. Our results lend support to the view that fusion is driven by the energy liberated during SNARE zippering. Moreover, they indicate that there are several energy barriers in the fusion pathway that the SNAREs are well suited to overcome.

**BACK**
**PROGRAMME**

# Molecular Motors, KIFs and Regulation of Neuronal Function, Morphogenesis, and Brain Wiring

## Nobutaka HIROKAWA

Departments of Cell Biology and Anatomy/ Molecular Structure and Dynamics, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

The intracellular transport is fundamental for neuronal morphogenesis, functioning and survival. To elucidate this mechanism we have identified and characterized kinesin superfamily proteins, KIFs, using molecular cell biology, molecular genetics, biophysics, and structural biology. KIFs transport various cargoes such as mitochondria (KIF1Balpha/KIF5s), synaptic vesicle precursor (KIF1A/KIF1Bbeta), NMDA type (KIF17) and AMPA type (KIF5s) glutamate receptors and mRNAs with a large protein complex (KIF5s) in neurons and other cells along microtubules. Concerning regulation of transport cells use adaptor proteins for recognition of cargos and phosphorylation and hydrolysis of G-proteins for loading and unloading cargoes. Molecular genetics unraveled that KIFs play significant roles not only on basic cellular functions by transporting various important cargoes, but also on fundamental phenomena in life such as memory and learning (KIF17/KIF1A), pain sensation (KIF1A) and development including brain wiring (KIF2A), activity dependent neuronal survival (KIF4), enteric neuronal development (KIF26A), determination of left-right asymmetry (KIF3) and suppression of tumorigenesis (KIF3). Furthermore, our recent studies uncovered deletion of KIFs causes certain diseases such as memory disturbance (KIF17), epilepsy (KIF5A), elevated anxiety (KIF13A), neuropathy (KIF1Bbeta), hydrocephalus (KIF19A), female infertility (KIF19A) and diabetes (KIF12). In this conference I will focus on our recent studies on the mechanisms of activity dependent intracellular transport, regulation of pain sensation and brain wiring and related diseases.

Hirokawa ***JCB*** 94 ;425- ’82 ; Hirokawa et al. ***Cell*** 56: 867- ’89**;** Aizawa et al. ***JCB*** 119 : 1287- ’92 ; Nangaku et al. ***Cell*** 79: 1209- ’94**;** Okada et al. ***Cell*** 81: 769- ’95; Hirokawa ***Science*** 279: 519- ’98;Tanaka et al. ***Cell*** 93:1147- ’98; Nonaka et al. ***Cell*** 95: 829- ’98; Okada and Hirokawa ***Science*** 283: 1152- ’99; Kikkawa et al. ***Cell*** 100: 241- ’00; Setou et al. ***Science*** 288: 1796- ’00; Nakagawa et al. ***Cell*** 103: 569- ’00; Kikkawa et al. ***Nature*** 411: 439- ’01; Zhao et al. ***Cell*** 105: 587- ’01; Setou et al. ***Nature*** 417: 83- ’02; Homma et al. ***Cell*** 114: 229- ’03; Okada et al. ***Nature*** 424: 574- ’03; Ogawa et al. ***Cell*** 116: 591- ’04;Nitta et al. ***Science*** 305:678-’04;Teng et al. ***NCB***. 7:474- ’05; Tanaka et al. ***Nature*** 435: 172- ’05; Okada et al. ***Cell*** 121: 633- ’05; Hirokawa and Takemura ***Nat Rev Neurosci*** 6: 201- ’05; Hirokawa et al. ***Cell*** 125:33- ’06; Midorikawa et al. ***Cell*** 125:371-’06; Guillaud et al. ***NCB***:10 :19-’08 ; Hirokawa and Noda ***Physiol Rev*** 88 :1089- ’08 ; Nitta et. al. ***NSMB*** 15:1067- ’08; Niwa et al. ***NCB*:**10:1269- ’08;Hirokawa et al. ***Nat Rev Mol Cell Biol*** 10:682- ’09; Hirokawa et al. ***Nat Rev Mol Cell Biol*** 10:877- ’09; Zhou et al. ***Cell*** 139:802- ’09; Terada et al. ***EMBO J*** 29:843- ’10; Hirokawa et al. ***Neuron*** 68:610- ’10; Ueno et al. ***Dev Cell*** 20:60- ’11; Yin et al. ***Neuron*** 70:310- ’11; Nakata et al. ***JCB*** 194:245- ’11; Kondo et al. ***Neuron*** 73:743- ‘11; Yajima et al. ***JCB*** 198:315- ‘12; Niwa et al. ***Dev Cell*** 23:1167- ‘12; Nakajima et al. ***Neuron*** 76:945- ‘12; Zhou et al. ***Cell Rep*** 3:509 ‘13; Kanai et al. ***JCB*** 204:395- ‘14; Yang et al. ***Dev Cell*** 31:202- ‘14; Farkhondeh et al. ***J. Neurosci*** 35:5067-’15; Ichinose et al. ***Neuron*** 87;1022-’15; Ogawa et al. ***Cell Rep*** 12;1- ’15; Takei et al. ***J. Neurosci*** 35:15539- ’15; Tanaka et al. ***Neuron*** 90;1215- ’16; (http://cb.m.u-tokyo.ac.jp)

**BACK**
**PROGRAMME**

# Understanding Contact Repulsion: Roles of Exosomes and Phagocytosis

## Ruediger KLEIN

Max-Planck Institute of Neurobiology, Martinsried, Germany

Cells release membranous vesicles known as exosomes that represent a novel mode of intercellular communication. Eph receptor tyrosine kinases and their membrane-tethered ephrin ligands have important functions in neuronal development. Ephrin-Eph signaling requires direct cell contact and is bi-directional: ephrin to Eph signaling is called forward signaling, while Eph to ephrin signaling is called reverse signaling. We observed the release of exosomes containing Ephs and ephrins by different cell types including neurons. Treatment of cells with purified EphB2+ exosomes induces ephrinB1 reverse signaling and causes axon repulsion, suggesting a novel mechanism of Eph/ephrin signaling independent of direct cell contact and the participation of EphB2+ exosomes in neural development.

Cell repulsion induced by ephrin-Eph signaling at cell contact sites is promoted by bi-directional trans-endocytosis (phagocytosis) of clustered Eph/ephrin complexes at cell interfaces. The underlying intracellular signaling pathways are poorly understood. We identified an actin-regulating signaling pathway which allows ephrinB+ cells to trans-endocytose EphB receptors from opposing cells. We further implicate the Rac subfamily of Rho family GTPases and the Rac-specific guanine nucleotide exchange factor Tiam2 as key positive regulators of EphB2 trans-endocytosis. These results indicate the presence of a conserved signaling pathway for EphB trans-endocytosis that removes the physical tether between cells and thereby enables cell repulsion.

I will also touch upon our recent observations that mutant mice with genetic deletions of FLRT1 and FLRT3 adhesion molecules develop macroscopic cortical folds. Cortex folding in these mutants does not require progenitor cell amplification. Instead, absence of FLRT1/3 reduces intercellular adhesion, promotes immature neuron migration and clustering in the cortical plate, thereby leading to sulcus formation in the normally smooth mouse neocortex. These results indicate that regulation of intercellular adhesion of migrating neurons is critical for cerebral cortex folding.

**BACK**
**PROGRAMME**

# Cell-Intrinsic Regulation of Neuronal Connectivity in the Brain

## Azad BONNI

Washington University School of Medicine, St Louis, Missouri

The assembly of neural circuits is critical for the development and function of the brain. Axon and dendrite morphogenesis culminating in synapse formation represent key events that orchestrate the establishment of neuronal connectivity. Using the rodent cerebellar cortex as a model system, we have discovered fundamental epigenetic, transcriptional, and ubiquitin-signaling networks that orchestrate neuronal morphogenesis and connectivity in the mammalian brain. In recent studies, we have identified crucial roles for the nucleosome remodeling and deacetylase (NuRD) complex in the control of presynaptic differentiation and dendrite pruning in the mouse cerebellar cortex. The NuRD complex triggers long-term silencing of developmental genes through alterations of histone tail modifications to promote granule neuron parallel fiber presynaptic differentiation in the mouse cerebellum. By contrast, the NuRD complex dynamically shuts off activity-dependent gene expression via deposition of the histone variant H2A.z at promoters of activity-dependent genes to promote granule neuron dendrite pruning in the mouse cerebellum. These findings suggest that the NuRD complex employs distinct mechanisms to control key aspects of neuronal morphogenesis and connectivity in the brain.

**BACK**
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# The Fly Mushroom Body Calyx: a Small Circuit in the Spotlight

Lothar Baltruschat[1], Philipp Ranft[1], Giovanni Marchetti[1], Davi Bock[2] and **Gaia TAVOSANIS[1]**

[1]German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany; [2]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, United States

In our daily experience we are confronted with a palette of sensory stimuli to which we need to react, for instance by adapting or modifying our behavioral response. We are interested in the cellular mechanisms that underlie the capacity of the nervous system to undergo plastic changes allowing for modulations of behavioral output, including learning.

The insect mushroom body is essential for the formation and retrieval of multiple types of memory, including olfactory associative memories. Olfactory and modulatory inputs converge on mushroom body neurons in the mushroom body calyx forming a dense synaptic meshwork that undergoes experience-dependent structural alterations in several insect species. We characterized anatomical and functional microcircuits within the mushroom body calyx of the adult fruit fly, named microglomeruli (MG). With the availability of a whole brain electron microscopy volume of an adult female fly (Bock et al., unpublished), we reconstructed the complete circuitry of a MG and identified all the neurons that compose it and their local connections. Importantly, we found that they undergo structural changes accompanying the formation of long-term memories. Our data suggest that MGs are autonomous and plastic computational relays.

**BACK**
**PROGRAMME**

# The Structure and Function of Axonal Spectrin Cytoskeletons

## Matthew RASBAND

Spectrins form a submembranous cytoskeleton proposed to confer strength and flexibility to axons and to participate in ion channel clustering at axon initial segments (AIS) and nodes of Ranvier. Neuronal spectrin cytoskeletons consist of diverse β subunits and αII spectrin. Using proteomics, biochemistry, and super-resolution microscopy we show that αII and βIV spectrin interact and form periodic AIS and nodal cytoskeletons, while αII and βII spectrin interact and form a periodic paranodal cytoskeleton. Mice lacking CNS αII spectrin have profound neurological impairments including seizures, disrupted cortical lamination, and neurodegeneration. Mice with αII spectrin-deficient peripheral sensory neurons have intact nociception, but severe ataxia due to preferential degeneration of large diameter axons. We show the density of nodal βIV spectrin is constant among axons, but the density of nodal αII spectrin increases with axon diameter. We propose that nodal αII spectrin may help resist the mechanical forces experienced by axons. Thus, spectrin-dependent cytoskeletons are required for axon function, domain assembly, and axon integrity.

**BACK**
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# Cell Behaviour Underlying Neurogenesis in the Developing Spinal Cord

**Raman M. DAS**

Division of Developmental Biology and Medicine, Faculty of Biology Medicine and Health, University of Manchester, Michael Smith Building, Oxford Road, Manchester M13 9PT, UK

Newborn neurons in the developing vertebrate central nervous system must delaminate from the neuroepithlium, an essential step for generation of normal tissue architecture and formation of functional neuronal circuitry. This involves detachment of an apical process from the ventricular surface followed by migration to the lateral neural tube or to form cortical layers in the brain. However, little is known about the cell behaviour driving this cell state transition. Here, using high-resolution live imaging in chick spinal cord we show that a new form of cell sub-division, named apical abscission, mediates rapid loss of the apical membrane, leading to an acute loss of cell polarity. This process also involves dis-assembly of the sonic-hedgehog transducing primary cilium and retention of the centrosome by the differentiating neuron. Apical abscission takes place in both chick and mouse and requires interdependent actino-myosin constriction and microtubule activity, which generates a focal actin-microtubule tunnel through which the centrosome transits. Centrosome retention is critical for subsequent stages of neuronal differentiation, as a microtubule organising centre during axonogenesis and as the basis for a re-assembled primary cilium. These findings identify new cytoskeletal interactions that orchestrate a key step in the neuronal differentiation programme leading to delamination from the neuroepithelium.

**BACK**
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# Deconstructing Neuronal Lamination in the Vertebrate Retina

## Caren NORDEN

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany

The arrangement of neurons into distinct layers is a conserved feature of the central nervous system giving many areas of the brain its stratified appearance. This layering occurs during development and the precise generation of different neuronal subsets and their correct positioning is crucial for neuronal lamination.

We aim to understand the phenomenon of neuronal lamination in the developing vertebrate retina. Here neurons are arranged in a stereotypic manner that is conserved among species and positioning of neurons into the wrong layer leads to sever defects in retinogenesis and retinal function. We thus aim to decipher cell and tissue biology of neuronal lamination in this highly accessible system. To understand retinal lamination we study translocation modes and division patterns of different neuronal subsets and further investigate how cell-cell and cell-tissue interactions influence lamination outcome. We currently use the zebrafish system as only here can we follow cellular and tissue rearrangements during development in vivo down to the subcellular level.

In my talk, I will focus on the first two neuronal subtypes generated, retinal ganglion cells and cone photoreceptors and share recent findings on their division patterns and translocation dynamics and the underlying mechanisms. In addition, I will discuss how translocation defects can influences retinal lamination events and overall maturation of the retina.

**BACK**
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# Location, Location, Location – Amyotrophic Lateral Sclerosis as a Spatiotemporal Mislocalization Disease

**Eran PERLSON**

Tel Aviv University

Spatiotemporal localization of signals is a fundamental feature impacting cell survival and proper function. The cell needs to respond in an accurate manner in both space and time to both intra and inter cellular environment cues. The regulation of this comprehensive process involves the cytoskeleton and the trafficking machinery, as well as local protein synthesis and ligand-receptor mechanisms. Alterations in such mechanisms can lead to cell dysfunction and disease. Motor neurons that can extend over tens of centimeters are a classic example for the importance of such events. Changes in spatiotemporal localization mechanisms are thought to play a role in motor neuron degeneration that occurs in Amyotrophic Lateral Sclerosis (ALS). In my talk I will discuss such mechanisms that essential for axon/NMJ maintenance.

**BACK**
**PROGRAMME**

# Novel Pathways of Axon Regeneration Identified from Large-scale Genetic Screen in *C. elegans*

**Yishi JIN**

Neurobiology Section, University of California, San Diego, USA

Using single axon injury assay in *C. elegans*, we identified several axon regeneration pathways by systematic genetic screening for 654 selected *C. elegans* genes, based on their orthology to human genes and potential neuronal function or known biochemical role (Chen et al., Neuron 2011). In subsequent studies, we revealed rapid cellular dynamics in response to axon injury, and defined two novel signaling pathways. One involves the conserved microtubule regulator EFA-6, which rapidly redistributes from the cell cortex to microtubule minus ends following axon injury. We showed that this relocalization is critical for its function in repressing axon regrowth (Chen et al., eLife, 2015). Another pathway operates at the level of RNA splicing, dependent on the

CELF family RNA binding proteins. In *C. elegans* the CELF protein UNC-75 is required for axon regeneration. Using CLIP-seq and transcriptomic analyses, we identified a set of genes involved in synaptic transmission as mRNA targets of UNC-75. In particular, UNC-75 regulates alternative splicing of two mRNA isoforms of the SNARE Syntaxin/unc-64. Extending these findings to mammalian axon regeneration, we find that mouse Celf2 expression is upregulated after peripheral nerve injury and that Celf2 mutant mice are defective in axon regeneration. Our data delineate a post-transcriptional regulatory pathway with a conserved role in regenerative axon extension (Chen et al., eLife, 2016). In this meeting, I will describe novel findings through a continued systematic genetic screening for additional 600 *C. elegans* genes.

**BACK**
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# Overcoming Proteoglycan Barriers to Axon Regeneration and Sprouting

## Jerry SILVER

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The glial scar that develops after many forms of CNS trauma as well as the perineuronal net that forms a curtain around synapses throughout the CNS are major obstacles to axon regeneration, sprouting and functional recovery after a variety of traumatic injuries to the brain and spinal cord. The entrapment of axons by the scar and net is due to the production of a family of potently inhibitory extracellular matrix molecules known as the chondroitin sulfate proteoglycans (CSPGs). Because the sugar side chains are known to provide much of the inhibitory properties of CSPGs, their effects can be almost totally removed by enzymatic digestion using local intraparenchymal injections of chondroitinase ABC (ChABC), a bacterial enzyme that degrades chondroitin glycosaminoglycan sugar chains away from their resident core proteins. Many labs have demonstrated that a modest recovery of function can be achieved following acute SCI through application of ChABC. However, the ability to recover function following chronic paralyzing SCI has always been a daunting prospect. Over 50% of human spinal cord injuries cause deficits in respiratory motor function or complete paralysis of inspiratory muscles. We wondered whether combining ChABC and a form of respiratory therapy called Intermittent Hypoxia (IH) might achieve some meaningful amount of restoration of diaphragm function at greatly protracted, chronic stages after cervical hemisection, where endogenous plasticity from the contralateral side well below the level of injury may have been slowly occurring but functionally suppressed by the net. We have examined the potential for restoration of this motor system up to 1.5 years following severe cervical (C2) hemisection where there is no evidence of spontaneous recovery. In spite of complete hemidiaphragm paralysis for up to 18 months, a single injection of chondroitinase ABC into the ipsilateral phrenic motor pool could robustly restore near normal diaphragm function mediated in large part by sprouting of the serotonergic system. Intermittent hypoxia rehabilitation combined with this treatment minimally strengthened and refined the recovered activity to increase the functional effects. The remarkable degree and speed of patterned respiratory motor recovery due solely to the enzyme were completely unforeseen, being both greatly superior to that which occurs acutely and even gaining strength over time. Indeed, our data uniquely establish that increasing plasticity can recover essentially normal respiratory function after a near lifetime of diaphragm paralysis. However, importantly, when driven to excess, the effects of this combination strategy can cause debilitating tonic activity of the ipsilateral hemidiaphragm. Through the controlled regulation of serotonergic sprouting, our treatment strategy triggered a mechanism that ensured robust, patterned respiratory recovery regardless of time post injury. These data give hope that we may functionally improve respiratory related motor system circuitry in the chronically injured human population.

**BACK**
**PROGRAMME**

# The Importins of Axonal Transport in Neuronal Growth and Survival

## Mike FAINZILBER

Weizmann Institute of Sciences, Department of Biomolecular Sciences, Rehovot, Israel

Dynein-dependent retrograde axonal injury signaling stimulates regenerative responses by the cell body in lesioned peripheral neurons (1). We have previously shown essential roles for importins in dynein-dependent axonal injury signaling. Importin β1 is locally translated in axons, and its binding to importin α constitutively linked to dynein enables retrograde transport of signaling proteins and transcription factors that contain nuclear localization signals (1, 2). My presentation will cover recent insights on the roles of importins and dynein in intrinsic axon scanning mechanisms that control axon growth and neuron length (3, 4). I will further describe unpublished data on new components of this mechanism and how regulation of local translation in axons affects neuronal growth and survival.

1. Rishal & Fainzilber, 2014: Axon-soma communication in neuronal injury. Nature Reviews Neuroscience 15, 32.
2. Perry et al., 2012: Subcellular knockout of importin β1 perturbs axonal retrograde signaling. Neuron 75, 294.
3. Rishal et al., 2012: A motor driven mechanism for cell length sensing. Cell Reports 1, 608.
4. Perry et al., 2016: Nucleolin-mediated RNA localization regulates neuron growth and fibroblast cell size. Cell Reports 16, 1664.

**BACK**
**PROGRAMME**

# Mechanosensing of Neurons and Glial Cells in the CNS

## Jochen GUCK

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It is increasingly being recognized that cells measure and respond to the mechanics of their environment. We are especially interested in the influence of mechanics during CNS development and pathologies. Using quantitative scanning force microscopy we have shown that various neural tissues are very compliant (shear modulus < 1 kPa) and mechanically heterogeneous. We have recreated compliant polyacrylamide (PAA) gel substrate with shear moduli between 0.1 and 30 kPa to match and exceed those of CNS tissue. Various primary neurons and glial cells have been cultured on these gels and their reaction studied. Both primary rat microglia and astrocytes responded to increasing substrate stiffness by changes in morphology and upregulation of inflammatory genes and proteins. Upon implantation of composite hydrogel stripes into rat brains, foreign body reactions were significantly enhanced around their stiff portions *in vivo*. It appears that the mechanical mismatch between a neural implant and native tissue might be at the root of foreign body reactions. Investigations into the molecular mechanisms are underway. Also oligodendrocytes, another type of glial cells, are mechanosensitive as their survival, proliferation, migration, and differentiation capacity *in vitro* depend on the mechanical stiffness of polymer hydrogel substrata. This finding might be linked to the failure of remyelination in chronic demyelinating diseases such as multiple sclerosis. And finally, we have also shown retinal ganglion axon pathfinding in the early embryonic *Xenopus* brain development to be instructed by stiffness gradients. We could even identify a specific molecular mechanism involving piezo1, a stretch-activated ion channel. These results form the basis for further investigations into the mechanobiology of cell function in the CNS. Ultimately, this research could help treating previously incurable neuropathologies such as spinal cord injuries and neurodegenerative disorders.

**BACK**
**PROGRAMME**

# Retrograde Axonal Transport of Signaling Autophagosomes in Neuronal Complexity and Neurodegeneration

Natalia L. Kononenko[1#*], Gala A. Claßen[1#], Marijn Kuijpers[1], Dmytro Puchkov[1], Jacek Jaworski[2], and **Volker HAUCKE**[1]

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Autophagosomes primarily mediate turnover of cytoplasmic proteins or organelles to provide nutrients and eliminate damaged proteins. In neurons, autophagosomes form in distal axons and are trafficked retrogradely to fuse with lysosomes in the soma. Although defective neuronal autophagy is associated with neuronal cell death and neurodegeneration the function of neuronal autophagosomes remains incompletely understood.

In my presentation I will describe our recent studies regarding a novel function of the endocytic clathrin adaptor AP-2 in retrograde axonal transport of autophagosomes that is distinct from its established role in presynaptic vesicle reformation. Our data suggest a mechanism for retrograde axonal transport of BDNF/ TrkB-containing autophagosomes via a non-canonical function of AP-2 and reveal a causative link between autophagy, BDNF/ TrkB signalling, and neurodegeneration.

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**PROGRAMME**

# Role of the Adhesion Molecule Neuroligin-1 in Synaptogenesis Probed by Single Molecule Imaging and Regulation of Tyrosine Phosphorylation

I. Chamma*, M. Letellier*, K. Czöndör, C. Saphy, I. Papasideri, B. Tessier, M. Sainlos, **O. THOUMINE**
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The molecular mechanisms by which early neuronal connections mature into excitatory or inhibitory synapses are still unclear. Here, we examined the role of the adhesion molecule neuroligin-1 (Nlg1) on synapse differentiation in hippocampal neurons, focusing on Nlg1 dynamics, nanoscale organization, and signaling mechanisms.

In the absence of high quality antibodies to neuroligin-1 compatible with live cell imaging, we developed a highly sensitive labeling strategy relying on fluorophore-conjugated monomeric streptavidin to target Nlg1 carrying a short, enzymatically biotinylated tag (Chamma et al., Nature Protocols 2017). We demonstrate efficient and specific labeling of Nlg1 at synapses in both dissociated neurons and and organotypic slices, with reduced steric hindrance and absence of cross-linking compared to multivalent probes (Chamma et al., Nature Comms 2016). We used this method in combination with super-resolution imaging techniques including uPAINT, STED and dSTORM. We reveal that Nlg1 is highly dynamic in the dendritic shaft, and gets diffusionally trapped in trans-synaptic adhesions with axonal neurexin 1β. Those complexes dissolve rapidly upon chemical LTD treatment.

In a second study, we examined the role of Nlg1 phosphorylation in synapse specification, focusing on a unique intracellular tyrosine residue (Y782) located in the gephyrin-binding motif. Expression of two Nlg1 point mutants (Y782A versus Y782F) promoted the assembly of functional excitatory and inhibitory synapses, respectively. The Nlg1Y782F mutant blocked excitatory synapse assembly, as did a Nlg1 mutant lacking the PDZ domain binding motif, suggesting that gephyrin and PSD-95 compete for Nlg1 interaction. These effects seem specific of Nlg1, since the same mutations in Nlg3 did not alter excitatory synapse formation. Strikingly, optogenetic phosphorylation of Nlg1 at residue Y782 using a photoactivatable tyrosine kinase, induced the formation of new dendritic spines (Letellier et al., submitted). Thus, Nlg1 tyrosine phosphorylation is a crucial switch mechanism that may explain the selective role of the Nlg1 isoform in excitatory synapse development.

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PROGRAMME

# The Mechanical Control of Neuronal Development and Regeneration

**Kristian FRANZE**

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During development and pathological processes, cells in the central nervous system (CNS) are highly motile. Despite the fact that cell motion is driven by forces, our current understanding of the mechanical interactions between CNS cells and their environment is very limited. We here investigated the mechanical control of neuronal growth in the developing brain. *In vitro*, growth and migration velocities, directionality, cellular forces as well as neuronal fasciculation and maturation all significantly depended on substrate stiffness. Moreover, when grown on substrates incorporating linear stiffness gradients, axon bundles turned towards soft substrates. *In vivo* atomic force microscopy revealed stiffness gradients in developing brain tissue, which axons followed as well towards soft. Interfering with brain stiffness and mechanosensitive ion channels *in vivo* both led to similar aberrant neuronal growth patterns with reduced fasciculation and pathfinding errors. Importantly, CNS tissue stiffness significantly changed after traumatic injuries. Ultimately, mechanical signals not only directly impacted neuronal growth but also indirectly by regulating neuronal responses to chemical guidance cues, strongly suggesting that neuronal growth is not only controlled by chemical signals – as it is currently assumed – but also by the tissue's local mechanical properties.

**BACK**
**PROGRAMME**

# The Turnover of Mammalian Proteins is encoded in their Amino Acid and Codon Compositions

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We combined here mass spectrometry, deep sequencing, molecular biology, fluorescence imaging and statistics methods to study protein turnover *in vivo*, in the mouse brain. We found that the protein sequences can predict, with a precision close to the accuracy of the wet-lab measurements, four basic parameters that were assumed to be under complex and unpredictable regulation: the protein lifetime, the protein abundance, the abundance of the mRNA transcripts, and the translation rate.

One of the most important factors in the predictions was the nature of the coding sequence of the proteins. Importantly, this observation applied not only to mice, but to human, rat and zebrafish, and, albeit less strictly, to *Drosophila*, *C. elegans*, and plants, as we determined by analyzing ~800 published databases from different organisms. This observation enabled us to fine-tune the lifetime, the abundance, the mRNA abundance, and the translation rate of proteins *in vitro*, by changing the coding sequences, according to the rules observed by studying these parameters in the mouse brain. This result has obvious implications for protein production technologies.

Finally, we have also clarified experimentally the different steps in the mechanism linking the sequence composition to all of the turnover parameters. This mechanism is straightforward, and relates to both protein formation and folding.

These findings define the framework that coordinates protein turnover, at different levels, and propose new ways of understanding protein production, both *in vitro* and *in vivo*.

**BACK**
**PROGRAMME**

# The EF-hand $Ca^{2+}$-sensor Caldendrin Directly Transduces Postsynaptic Calcium Signals to Stabilize Branched Actin Filaments in Spines

Marina Mikhaylova[1,2,3], Julia Bär[1,2], Philipp Schätzle[3], PingAn YuanXiang[2], Bas van Bommel[1], Johannes Hradsky[2], Pasham Parameshwar Reddy[2], Egor Y. Loktionov[4], Anja Konietzny[1], Rajeev Raman[2], Christina Spilker[2], Jeffrey Lopez-Rojas [2], Syed Ahsan Raza[5], Oliver Stork[5], Casper C. Hoogenraad[3], **Michael R. KREUTZ[2,6]**

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Dendritic spines represent the basic cellular unit for memory storage. Remodelling of synaptic strength correlates with changes in the morphological appearance of spines, which reflects an intimate link between functional and structural plasticity. Such changes are based on the unique cytoskeletal organization of dendritic spines comprising of differentially arranged actin filaments. Compartmentalization of calcium-dependent plasticity allows for rapid actin remodelling in dendritic spines. In a recent landmark study Bosch and colleagues (Neuron, 2014) addressed the question of structural and molecular remodelling of dendritic spines following the induction of long-term potentiation (LTP). The authors show that the molecular composition of the synapse changes in four distinct phases. The initial phase of LTP induction is characterized by cofilin-dependent severing of filamentous actin and a massive increase in actin remodelling proteins, which is then followed by a stabilization phase where different F-actin stabilizing and capping proteins gradually increase and only at this later stages reorganization of the postsynaptic density comes into play. However, the molecular machineries translating the initial NMDAR calcium influx into coordinated rearrangement of spinous actin filaments and stabilization of newly formed structures are not well understood. It is in particular unclear how a minimum stable pool of branched actin that is essential for remodelling of F-actin in spines undergoing plasticity is maintained.

In this work, we show that the postsynaptic EF-hand $Ca^{2+}$-sensor caldendrin activates the actin-binding protein cortactin in dendritic spines and thereby stabilizes a synaptic pool of branched F-actin that is essential for the maintenance of long-term potentiation. The CaM-like calcium sensor caldendrin is enriched at synaptic sites. Caldendrin has a unique bipartite structure with a highly basic and proline-rich N-terminus and an EF-hand containing C-terminus. Using a broad methodological approach we show that steep elevations in spinous $[Ca^{2+}]_i$ disrupt an intramolecular interaction of caldendrin that masks a series of PxxP-motifs which then readily associate to the SH3 domain of cortactin. Caldendrin binding keeps cortactin in an active, F-actin-stabilizing conformation, protects a minimal pool of branched F-actin against cofilin-induced severing and primes soluble cortactin for sequential binding to N-WASP/Arp2/3 complex. In agreement with these data, Caldendrin gene knockout or protein knockdown result in a loss of cortactin from activated synapses, higher spinous actin turnover and deficits in spine plasticity, stability and hippocampus dependent learning. In summary, we conclude that caldendrin directly couples elevation of $[Ca^{2+}]_i$ to the stabilization of actin branches in a very early step of temporary gating for F-actin remodelling in dendritic spines.

**BACK**
**PROGRAMME**

# Cerebral Organoids: Modelling Human Brain Development and Tumorigenesis in Stem Cell Derived 3D Culture

**Juergen A. KNOBLICH**

IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Science, Vienna Biocenter, Vienna 1030, Austria

The human brain is unique in size and complexity, but also the source of some of the most devastating human diseases. While many of these disorders have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. With intriguing precision, they recapitulate key brain morphogenetic events like the formation of distinct progenitor and differentiation zones, the generation of neuronal subclasses with distinct layer identities and the establishment of coordinated neuronal activity. We have used patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. More recently, we have developed organoid based models for long distance interneuron migration between distinct areas of the human brain which we are using for modeling neuropsychiatric disorders. Our data describe an *in vitro* approach that recapitulates development of even the most complex organ and can be used to gain insights into disease mechanisms.

**BACK**
**PROGRAMME**

# The Pluses and Minuses of Microtubule Organization in Dendrites

**Melissa ROLLS**

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One of the fundamental distinctions between axons and dendrites is their difference in microtubule polarity. Axons contain exclusively plus-end-out microtubules while dendrites contain a significant proportion of minus-end-out microtubules. This difference in microtubule polarity is one way that different cargoes can be sent from the cell body into axons and dendrites. As microtubules are dynamic, active mechanisms must maintain their organization throughout neuron life. We have been focusing primarily on identifying the ways in which microtubule polarity is controlled in dendrites. Interestingly, dendrite branch points seem to function as hubs for control of microtubule organization. Growing microtubules are steered through branch points by transient linage of a kinesin motor to the plus end. The proteins that mediate this linkage include Apc2 and are concentrated at dendrite branch points. Proteins that regulate generation of new minus ends through nucleation are also concentrated at dendrite branch points. We have uncovered an overlapping set of signaling proteins that is required for localization of both plus and minus end regulators at branch points. This set includes a subset of wnt signaling components making an unexpected link between this pathway and microtubule growth and nucleation.

**BACK**
**PROGRAMME**

# On the Role of Human-Specific Genes, Notably *ARHGAP11B*, in Neural Stem Cell Amplification and Neocortex Expansion in Development and Evolution

**Wieland B. HUTTNER**

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Our group studies neural stem and progenitor cells in the context of the expansion of the neocortex in development and evolution. Two major classes of cortical stem/progenitor cells can be distinguished. First, stem/progenitor cells that reside in the ventricular zone (VZ), i.e. neuroepithelial cells, apical radial glia (aRG) and apical intermediate progenitors, collectively referred to as apical progenitors (APs). Second, stem/progenitor cells that reside in the subventricular zone (SVZ), i.e. basal radial glia (bRG) and basal intermediate progenitors, collectively referred to as basal progenitors (BPs). Neocortex expansion is thought to be linked to an increased abundance and proliferative capacity of BPs.

To gain insight into the genomic changes that underlie neocortex expansion, notably in humans, we have analyzed the transcriptomes of human vs. mouse VZ and SVZ, and of human vs. mouse aRG and bRG. This led to the identification of the human-specific gene *ARHGAP11B* as a major player. Specifically, ARHGAP11B promotes the generation of BPs from aRG and the subsequent BP proliferation, thereby increasing BP abundance. Moreover, ARHGAP11B is able to induce folding of the embryonic mouse neocortex, which normally is smooth. The ability of ARHGAP11B to amplify BPs is based on a single C-to-G base substitution which creates a novel splice donor site, causing a reading frame shift and generating a human-specific 47-amino acid sequence that is thought to be key for BP amplification.

**BACK**
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# Molecular Pathway Underlying Bouton Stabilization by Semaphorin4D during Inhibitory Synapse Formation

Cátia P. Frias, Hai Yin Hu, Tom Bresser, Lisa Scheefhals, Paul M.P. van Bergen en Henegouwen, Casper C. Hoogenraad and **Corette J. WIERENGA**

Division of Cell Biology, Faculty of Science, Utrecht University, The Netherlands

Changes in inhibitory connections are essential for experience-dependent circuit adaptations, and inhibitory defects may underlie neurodevelopmental diseases such as autism. Inhibitory axons and their presynaptic boutons can undergo rapid changes, but the molecular mechanisms underlying these dynamics and their role in inhibitory synapse formation are currently unclear. By monitoring inhibitory axons over time in organotypic hippocampal slices, we show that stabilization of presynaptic boutons is the first step in inhibitory synapse formation and that this process is regulated by the guidance protein Semaphorin4D (Sema4D). Sema4D signaling induces inhibitory bouton stabilization within tens of minutes without affecting bouton disassembly. We show that this signaling pathway requires ongoing neuronal activity, and involves activation of receptor tyrosine kinase MET and actin remodeling. Our data indicate that actin plays an important role during synapse formation and demonstrate a novel link between MET, a known autism risk factor, and inhibitory presynaptic dynamics.

**BACK**
**PROGRAMME**

# Fine-Tuning Proteolysis and Exosome Release by Membrane-deforming Heat Shock Chaperones

## Patric VERSTREKEN

VIB Center for the Biology of Disease, KU Leuven, Belgium

Chaperones safeguard proteome integrity by balancing protein (un)folding with proteolysis. We recently identified several chaperones in a screen for brain-enriched proteins that remodel membranes, revealing an intriguing link bewteen membrane dynamics and protein quality control. We focused on a presynaptically enriched chaperone, Hsc70, and demonstrated that it deforms the endosomal membrane to drive intraluminal vesicles formation into multivesicular bodies (MVBs). This Hsc70 activity is responsible for turning over a particular set of presynaptic proteins, leaving a rejuvenated protein pool and promoting neurotransmission.

We are now pursuing how another membrane deforming chaperone, Hsp90, affects synaptic organelles, protein levels and function. Our data suggest that Hsp90 and Hsc70 inversely regulate the balance between protein degradation and extracellular release via exosomes. This work reveals unique strategies used by chaperones to coordinate synaptic proteostasis and membrane dynamics.

**BACK**
**PROGRAMME**

# The disease Associated Protein CYFIP1 Orchestrates Axonal Outgrowth and Brain Wiring

Nuria Domínguez-Iturza[1,2], Disha Shah[3], Ka Wan Li[4], August B. Smit[4], Annemie Van Der Linden[3], Tilmann Achsel[1,2] and **Claudia BAGNI[1,2,5,6,*]**

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Autism (ASD) and schizophrenia (SCZ) are neurodevelopmental disorders characterized by defects in brain connectivity. Copy-number variants of the *CYFIP1* gene in humans have been linked to neurodevelopmental disorders, such as ASD and SCZ. CYFIP1 has a dual role, regulating actin polymerization and protein synthesis. Here, we show that *Cyfip1* heterozygous mice have decreased functional brain connectivity and exhibit a slow-growth phenotype of the callosal axons during development. While the axons eventually reach their correct target areas, defects in the callosal structure persist into adulthood. The axonal proteome revealed an imbalanced production of proteins involved in actin cytoskeleton remodelling and axonal transport that is likely causative of the delayed axonal growth. The observed delay in axonal positioning and the decreased brain connectivity caused by the *Cyfip1* haploinsufficiency might explain its genetic association to psychiatric disorders.

**BACK**
**PROGRAMME**

# Autoimmune Aspects of Parkinson's Disease

**David SULZER**[*1], Carolina Cebrian[1], Roy Alcalay[1], Francesca Garretti[1] (Columbia University), Cecilia Lindestam Arlehamn[2], April Frazier[2], Alessandro Sette[*2]

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While adult human neurons are not typically antigen presenting cells, many substantia nigra dopamine neurons express MHC-I. The death of these neurons caus the motor disorders of Parkinson's, and in mouse substantia nigra neurons, the appropriate combination of neuronally presented antigen and T cell causes cell death. In blood of ~40% of Parkinson's patients and few age matched controls, CD4+ and CD8+ T cells are present that respond to two regions in alpha-synuclein, a protein misprocessed in the disorder. As degradation of alpha-synuclein and other proteins by lysosomes changes with disease and age, it is possible that autoimmune response to neoepitopes play roles in neurodegenerative and other aging related disorders.

**BACK**
**PROGRAMME**

# RhoA Controls Axon Growth through Myosin II-Actin Mediated Restraint of Microtubule Protrusion

**Sebastián Dupraz**[1*], Charlotte Coles[1], Sina Stern[1], Cord Brakebusch[2] and Frank Bradke[1]

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Axon growth is a key step during neuronal polarization and the basis to achieve brain connectivity. The Rho-GTPase RhoA is thought to orchestrate this process. However, the physiological role of RhoA is still unclear. Here, we demonstrate *in vitro* and *in vivo* that genetic ablation of RhoA enhances axon growth but leaves the neuronal polarization program intact. Biochemical analysis followed by pharmacological and molecular manipulations showed that RhoA restrains axon growth by activating myosin II-mediated changes in the actin cytoskeleton, which in turn prevents microtubules to protrude into the leading edge of the growth cone. Thus, our data depict a physiological function of RhoA modulating axon growth during neuronal development.

**BACK**
**PROGRAMME**

# A Scaffold for cGMP-activity Dictates Dendrite Formation during Neuronal Polarization

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Studies over the last decade have established axon formation as the initiating event in neuronal polarization. These studies assumed that dendrite formation is a passive process that follows axon formation by default. We previously demonstrated that axon formation is initiated by a cyclic AMP (cAMP)-driven phosphorylation cascade. We here show that subsequent to axon establishment, dendrite formation is also a promotable process, driven by cyclic GMP (cGMP)-activity. We identify a cGMP-scaffold that recruits the cGMP-synthesizing enzyme soluble guanylate cyclase (sGC) to upregulate cGMP-levels, thereby instructing dendrite formation in cultured hippocampal neurons and in the embryonic brain. The cGMP-scaffold further recruits Plexin co-receptors for Semaphorin3A, a key extracellular regulator of neuronal polarity in the embryonic brain, which mediates a rise in cGMP. Our results support an active, stepwise process for both axon and dendrite formation in neuronal polarization, that might be driven by extracellular cues *in vivo*.

**BACK**
**PROGRAMME**

# Role of SCG10 in Axon Regeneration

**Eric Ewan** and Valeria Cavalli

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Spinal cord injury (SCI) damages long projecting axons leading to loss of sensory and motor function. Spinal neurons fail to activate a pro-regenerative program and consequently do not regenerate after injury. In contrast, injured peripheral neurons activate a pro-regenerative program leading to mostly successful axon regeneration and functional recovery. We previously discovered that after peripheral (sciatic) nerve injury microtubule deacetylation at the injury site facilitates axon regeneration. However, after thoracic SCI, we observe that microtubule deacetylation is impaired near the injury site, leading to increased level of acetylated microtubules. Therefore, we hypothesize that alterations in microtubule dynamics may fail after SCI, contributing to the failure of spinal axon regeneration after injury. SCG10 is a microtubule destabilizing factor that previously was linked to enhanced neurite outgrowth capacity *in vitro*. We found that SCG10 is enriched in the growth cone of regenerating peripheral sensory (sciatic) and motor (ventral root) axons after nerve injury. In contrast, ascending spinal sensory axons do not express SCG10 after SCI. Interestingly, if spinal axons are put into a pro-growth state (induced by a prior injury to the sciatic nerve, known as a preconditioning injury), SCG10 accumulation occurs specifically in the tips of injured conditioned spinal axons. This suggests the possibility that SCG10 accumulation contributes to successful spinal axon regeneration. We are currently testing this hypothesis via viral infection of DRG neurons *in vivo* using AAV8 viral vectors to modulate SCG10 levels. These experiments will reveal whether SCG10 contributes to spinal axon regeneration observed with the preconditioning injury model, as well as whether enhanced SCG10 expression alone can stimulate axon regeneration after SCI. We are also determining whether any pro-regenerative effects associated with SCG10 are related to microtubule dynamics, particularly deacetylation in the growth cone of regenerating axons.

**BACK**
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# Control of Axonal mRNA Localization and Translation through an Exclusion Mechanism

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Compartmentalized control of gene expression is crucial for the establishment and maintenance of polarity in neurons. The transcriptomes present in axons, dendrites and soma are distinct in their composition and complexity, and local protein synthesis in the neuronal periphery has been recognized as crucial for essentially all aspects of neuronal development, function and regeneration. In the case of axons, the localized transcriptomes and associated translatomes are highly dynamic; for example, the localization of individual mRNAs to axons and their local translation are regulated during neurodevelopment, and both injury and neurodegenerative signals trigger the rapid axonal recruitment and translation of unique sets of mRNAs. Currently, axonal localization is understood to result exclusively from the active transport of mRNAs. This transport is mediated through RNA-binding proteins that recognize and bind to sequences in the transcripts. Despite intense efforts, only a couple of these sequence elements have been identified, such as the beta-actin zipcode.

Here, we used a motif discovery algorithm to identify novel sequence elements that would be informative for axonal localization of mRNAs. Instead of identifying sequence elements that were overrepresented in axonal versus total transcriptomes (indicating an instructive role in axonal transport), we discovered a sequence element that was highly underrepresented in axonal transcriptomes, suggesting that its presence in mRNAs is incompatible with axonal localization. We identified this sequence as a recognition site for two mammalian members of the PUF family, Pumilio (Pum) 1 and 2. These two paralogs are believed to be functionally interchangeable, but we found that while Pum1 is found throughout the neurons, including axons and growth cones, Pum2 expression is restricted to the cell body. We hypothesized that Pum2 binding to mRNAs might be a mechanism to retain transcripts in the somato-dendritic compartment. We found that insertion of a Pum-binding element in the beta-actin mRNAs is sufficient to its prevent axonal localization, and knockdown of Pum2 increased the axonal expression of endogenous mRNAs containing the Pum-binding element. shRNA-mediated knockdown of Pum2 in the developing mouse cortex significantly reduced axonal growth and branching in transfected neurons, and in vitro, greatly reduced the regeneration of injured axons.

Together, our data identify Pum2-mediated retention of mRNAs in the cell body as a novel mechanism to shape the axonal transcriptome. Control of mRNA localization by Pum2 is required for axonal development and regeneration.

BACK
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# SNAP-25 Gene Family Members Differentially Support Secretory Vesicle Fusion

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Neuronal dense-core vesicles (DCVs) transport and secrete neuropeptides necessary for development, plasticity and survival, but little is known about their fusion mechanism. We show that *Snap-25* null mutant (SNAP-25 KO) neurons, previously shown to degenerate after 4 days in vitro (DIV), contain fewer DCVs and have a reduced DCV fusion probability in surviving neurons at DIV14. At DIV3, before degeneration, SNAP-25 KO neurons show normal DCV fusion, but one day later fusion is significantly reduced. To test if other SNAP homologs support DCV fusion, we expressed SNAP-23, -29 or -47 in SNAP-25 KO neurons. SNAP-23 and -29 rescued viability and supported DCV fusion in SNAP-25 KO neurons, but SNAP-23 more efficiently. SNAP-23 also rescued synaptic vesicle (SV) fusion while SNAP-29 did not. SNAP-47 failed to rescue viability and did not support DCV or SV fusion. These data demonstrate a developmental switch, in hippocampal neurons between DIV3-4, where DCV fusion becomes SNAP-25 dependent. Furthermore, SNAP-25 homologs support DCV and SV fusion neuronal viability to a variable extent, SNAP-23 most effectively, SNAP-29 less so and SNAP-47 ineffective.

**BACK**
**PROGRAMME**

# Golgi Outposts Mediate Uniform Microtubule Polarity in Oligodendrocytes

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Oligodendrocytes are specialized glial cells in the central nervous system that produce myelin, the fatty layers of insulation that wrap around axons to facilitate efficient action potential conduction. Unlike Schwann cells in the peripheral nervous system that ensheath a single axonal segment, one oligodendrocyte can ensheath multiple axonal segments and consequently extends multiple processes. These microtubule-rich processes are elaborate and highly branched, yet it is unclear how they are organized and how they form. We now show using live-cell imaging that microtubules in oligodendrocytes have uniform polarity, with growing plus-ends directed away from the cell body. Interestingly, though polarity is consistent throughout oligodendrocyte differentiation, speeds of polymerization vary at different developmental time points. In addition, oligodendrocytes contain Golgi outposts, which act as a source of acentrosomal microtubule nucleation at sites that are far from the cell body. In order to screen for candidate Golgi outpost interactors, we used our lab's RNA-Seq database to identify microtubule-associated proteins with high expression in oligodendrocytes. We identify TPPP (tubulin polymerization promoting protein), which selectively localizes to Golgi outposts but not to Golgi bodies in the cell body. Knockdown of TPPP results in aberrantly mixed microtubule polarity, which leads to disorganization of oligodendrocyte branching structures. We are currently identifying additional putative Golgi outpost specific proteins using mass spectrometry of TPPP-associated proteins and evaluating myelin structure and behavior phenotypes of TPPP knockout mice. Together, our data demonstrate that microtubule organization is highly regulated both temporally and spatially and crucial for oligodendrocyte development.

**BACK**
**PROGRAMME**

# Important Things have Small Beginnings: Major Branches in the Dendrite Arbor Arise by Stabilization of Single Actin Bundles at the Dendrite Tip

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Dendrite arbor branching patterns determine the number, distribution and integration of neuron inputs; neuron firing properties; and ultimately the activity of a neuron within a circuit. Not all dendrite branches are equal. As mature dendrite arbor pattern is the compound outcome of a series of branching events, specific branches created early in dendrite outgrowth delineate the arbor into its distinct main subtrees and underlie its targeting into correct innervation fields. Nevertheless, while extensive analyses have revealed key processes that pattern terminal dendrites and spines, mechanisms constructing the critical major branches within the tree remain fundamentally unknown. In part, this is because in order to identify these mechanisms requires the association of individual subcellular molecular events occurring early in outgrowth with later mature arbor-wide pattern—an integrated approach spanning different spatiotemporal levels. Here, we reveal how highly localized stabilization of single F-actin bundles at the growing dendrite tip is the key precipitating event that generates major branches. By a genetic screen utilizing *in vivo* imaging coupled with automated dendrite feature detection and quantification, we identify the atypical myosin (MyoVI) as a principal player in this process. We show that underlying major branch formation is a transient local upregulation of anterograde-directed microtubule nucleation at the dendrite tip, a process spatially separable from continuous background dendrite microtubule nucleation. MyoVI drives localized stabilization of single F-actin bundles at the tip and these bundles in turn capture and target anterograde-directed microtubule polymerization events into discrete filopodia, driving tip-splitting for major branch creation. Moreover, differential use of MyoVI generates the diverse arbor complexities of different neuron types. Our findings establish how early individual cell biological events feed-forward to subdivide the mature dendrite arbor and create diversity in neuron form and function, thus defining critical neuronal features underlying circuit wiring and computation.

**BACK**
**PROGRAMME**

# A Novel Input-specific Synaptic Organizing Complex Controls Mossy Fiber Synapse Development and Function

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The function of neural circuits depends on precise patterns of connectivity and the properties of synaptic transmission at specific synapses. The hippocampal mossy fiber (MF) synapse, which connects dentate gyrus granule cells with CA3 pyramidal neurons, is a major information processing pathway and has unique structural and functional properties. The molecular mechanisms regulating MF synapse development and its specific structural-functional properties are largely unknown. Recently, we identified the presynaptic heparan sulfate proteoglycan Glypican 4 (GPC4) as a novel synaptic organizing protein with a key role in excitatory synapse development. GPC4 is strongly enriched in the presynaptic compartment of MF synapses, but its role in MF synapse development or function is currently unknown.

Using an in vivo knockdown strategy, we find that GPC4 controls the morphological maturation of MF presynaptic terminals. To dissect how GPC4 exerts this function, we performed a proteomic screen to identify novel GPC4 binding partners. We identify a largely uncharacterized orphan G-protein coupled receptor (GPCR) as potential GPC4 interactor. Biochemical analysis demonstrates that GPC4 binds to this GPCR in a heparan sulfate-dependent manner, while immunohistochemical studies indicate a postsynaptic localization of this receptor specifically at the MF synapse. Further, we find that GPCR expression on the surface of non-neuronal cells stimulates presynaptic differentiation in co-cultured neurons in a GPC4-dependent manner. In knockout mice lacking the orphan GPCR, basal transmission and the characteristic presynaptic facilitation of MF synapses are strongly impaired. Ultrastructural analysis reveals impairments in MF presynaptic bouton size, active zone, and postsynaptic density morphogenesis. Neighboring CA3-CA3 synaptic inputs on the same dendrite however are functionally and ultrastructurally normal in these knockout mice. Taken together, our results identify a novel, input-specific synaptic organizing complex required for MF synapse development and function.

**BACK**
**PROGRAMME**

# The Caskin Scaffold Protein Regulates Dendritic Spine Morphology, Learning and Memory

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Caskin (CASK interacting protein), a scaffold protein possessing ankyrin repeats, SH3 and tandem SAM domains and a proline-rich C-terminal region, is expressed in the brain in high amounts and is located at synapses. While CASK mutations have been implicated in neurological diseases, little is known about how the two Caskin isoforms, Caskin1 and 2 influence synaptic functions.

We have investigated the effects of Caskin1 on dendritic spine morphology. Confocal and electron microscopy of cultured neurons revealed that overexpressed Caskin1 was present predominantly in the somatodendritic region of neurons and was enriched especially in dendritic spine heads, colocalizing with the postsynaptic scaffold protein PSD95 and Shank2. Immunoprecipitation further confirmed that Shank2 and Caskin1 localized within the same complex. Importantly, overexpression of Caskin1 increased the amount of more mature, mushroom-shaped dendritic spines in the expense of filamentous spines of cultivated neurons. We also examined the spine morphology of Caskin1,2 double knock-out (Caskin$^{dKO}$) mice and their heterozygous littermate controls in the hippocampus CA1 str. radiatum by electron microscopy. Both the area and the length of the postsynaptic density (PSD) were significantly decreased in Caskin$^{dKO}$ mice, indicating a lack of proper organization of the postsynaptic dendritic spines.

LTP formation in hippocampal slices was impaired in Caskin$^{dKO}$ animals while basal synaptic activity seemed to be normal. Behavioural tests showed that the lack of Caskin did not influence general locomotor activity but Caskin$^{dKO}$ mice had deficits in memory formation, including novelty recognition and spatial memory. Taken together, our results prove a previously unnoticed postsynaptic role of Caskin scaffold proteins, regulating dendritic spine morphology and learning abilities possibly via interacting with members of the PSD.

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**PROGRAMME**

# A Novel Subtractive Genetics Approach for Dissecting Dopaminergic Pathway Development and Plasticity

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The midbrain dopamine system is involved in the control of cognitive and motor behavior. Midbrain dopamine neurons (mDA) are grossly divided into two anatomically and functionally distinct subpopulations: substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) neurons. SNc neurons make precise connections with dorsal striatum (nigrostriatal projections), while VTA neurons target ventral striatum and cortex (mesocorticolimbic projections). Both pathways collectively run in the medial forebrain bundle (MFB) towards the forebrain. To distinguish between different subsets of dopaminergic projections *in vivo*, and to identify subset-specific developmental programs, we designed BAC transgenic mice called *Pitx3-ITC* mice. The subtractive genetic strategy we have developed relies on the expression of different fluorescent proteins in different subsets of mDA neurons in a single mouse. *Pitx3-ITC* mice display labeling of SNc neurons and selective visualization of nigrostriatal projections in the MFB and in striatum, from early embryonic development onwards. Combination of *Pitx3-ITC* mice with 3D-imaging of solvent cleared organs (3DISCO) technology and light sheet imaging allows for 3D analysis of neuronal migration and axonal/dendritic development of SNc neurons. Interestingly, *Pitx3-ITC* mice show that nigrostriatal axons are not dispersed throughout the MFB, but rather accumulate in the dorsal MFB. This pre-target sorting of nigrostriatal axons may be important for the correct innervation of the dorsal striatum. Further, *PitX3-ITC* mice have begun to reveal the molecular mechanisms that are important for mDA neuron migration. In conclusion, the *Pitx3-ITC* genetic strategy offers the unique possibility of differentially labeling dopaminergic subsets and visualizing their projections, thereby comprising a unique tool for unveiling the molecular mechanisms underlying previously unexplored aspects of dopaminergic system development, such as the pre-target sorting of nigrostriatal axons in the MFB, and plasticity in health and disease.

**BACK**
**PROGRAMME**

# Mitophagy and Neuronal Homeostasis in *C. elegans*

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Mitochondria are essential for energy production and have vital roles in calcium signalling and storage, metabolite synthesis and apoptosis, among others, in eukaryotic cells. Neuronal cells are particularly dependent on proper mitochondrial function. Thus, maintenance of neuronal homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the elimination of damaged or superfluous mitochondria. Mitochondrial impairment has been implicated in several age-related neurodegenerative diseases. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the effects of mitophagy deficiency in neuronal physiology. To address this question, we developed two composite, *in vivo* imaging approaches to monitor mitophagy in neurons. Neuronal mitophagy is induced in response to oxidative stress. Mitochondrial dysfunction leads to transportation of axonal mitochondria towards the neuronal cell body, in calcium- and an AMPK-dependent manner. Autophagy deficiency increases mitochondrial number in neurons of age-matched nematodes and abolishes mitochondrial axonal transport upon stress. Additionally, impairment of mitophagy results in enhanced cell death in *C. elegans* models of neurodegeneration. Our results indicate that mitophagy contributes to preserve mitochondrial homeostasis and neuronal health.

**BACK**
**PROGRAMME**

# Maintenance of Scaled Dendritic and Synaptic Growth during Juvenile Development Requires Tao kinase Function

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During juvenile growth of an organism maintenance and appropriate scaling growth of dendrites and synapses is critical to ensure network function. However, to date we have little information how specific growth and connectivity is maintained and regulated at the molecular level.

We have established a quantitative model to study these questions using the Drosophila nociceptive network, which is fully functional throughout larval development and undergoes substantial growth and plasticity to maintain behavioral output. In this system, sensory dendrites of nociceptive neurons retain body wall coverage by scaling growth and also add circuit specific synapses in the central nervous system enabling appropriate responses to noxious stimuli during organismal growth.

We have identified the conserved Tao kinase as a critical regulator of both dendritic and synaptic growth. We show that Tao function regulates developmental scaling growth via its kinase activity by altering both microtubule and actin dynamics. Thereby, it promotes appropriate body wall coverage of sensory dendrites and synaptic output. Surprisingly, we found that Tao function is also required for circuit specific addition of synapses between connected neurons as deregulation of Tao function results in loss of specific and concomitant addition of ectopic synapses. Our results show that Tao kinase is a critical regulator of circuit integrity and maintenance during juvenile development.

**BACK**
**PROGRAMME**

# MAP2 Defines a Pre-axonal Filtering Zone to Regulate KIF1- Versus KIF5-Dependent Cargo Transport in Sensory Neurons

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Polarized cargo transport is essential for neuronal function. However, the minimal basic components required for selective cargo sorting and distribution in neurons remain elusive. We found that in peripheral sensory neurons the axon initial segment is largely absent and that microtubule associated protein 2 (MAP2) defines the cargo filtering zone in the proximal axon. Here, MAP2 directs axonal cargo entry by coordinating the activities of molecular motors. We show that distinct kinesin motors differentially regulate cargo velocity: kinesin-3 drives fast axonal cargo trafficking, while kinesin-1 slows down axonal cargo transport. MAP2 inhibits 'slow' kinesin-1 motor activity and allows kinesin-3 to drive robust cargo transport from the soma into the axon. In the distal axon, the inhibitory action of MAP2 fades out, leading to regained kinesin-1 activity and vesicle distribution. We propose that selective axonal cargo trafficking depends on the MAP2-defined pre-axonal filtering zone and the ability of cargos to switch between distinct kinesin motor activities.

**BACK**
**PROGRAMME**

# A Systematic Exploration of Neuronal Remodeling Reveals a Transcription Factor Hierarchy

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Developmental neuronal remodeling is essential for sculpting the mature nervous systems of both vertebrates and invertebrates during development. Remodeling often involves the elimination of existing connections followed by the creation or stabilization of proper, functional connections. Our knowledge of the mechanisms that regulate remodeling are far from being complete. We believe that understanding the mechanisms of developmental remodeling should shed light on the mechanisms that regulate axon degeneration and growth during development, injury and disease. In addition, defects in neuronal remodeling have been linked to human diseases such as schizophrenia and autism.

The *Drosophila* mushroom body (MB), a central nervous system (CNS) structure involved in learning and memory, is comprised of three types of neurons (γ, α'/β', and α/β), sequentially born from four identical neuroblasts. MB γ neurons undergo stereotypic remodeling such that their larval connections undergo pruning during early metamorphosis and later regrow to adult specific lobes. The genetic power of *Drosophila*, and especially the ability to perform detailed mosaic analyses, together with the stereotypy of the MB development make this a unique system to study the mechanisms of remodeling.

Previous work, from our lab and others, has demonstrated that both pruning and regrowth of MB γ neurons are regulated by nuclear receptor complexes, suggesting that the entire process is tightly regulated on the transcription level. We therefore used state of the art RNA-seq technology to uncover the transcriptional landscape of MB γ neurons at unprecedented developmental resolution. We sequenced MB γ neurons every three hours during the first thirty hours of metamorphosis and uncovered clustered expression of genes and pathways. Focusing first on DNA binding proteins, we identified ten, out of them seven unknown, transcription factors and DNA binding proteins that are required for various aspects of neuronal remodeling. By resequencing MB neurons that are mutant in specific transcription factors, we constructed a temporal sequence of transcription factor hierarchy controlling MB remodeling. Finally, by looking at the response of the different developmental clusters to the mutations in these transcription factors, we also uncovered defined functional gene groups and pathways that are regulated by the transcription factor network in a concerted fashion.

Our work has revealed the dynamic transcriptional landscape of an identifiable neuron type at unprecedented developmental resolution. Our dataset is a developmental atlas of MB γ neuron transcriptome. Mutant analysis has revealed the hierarchy and temporal sequence of different developmental programs regulating neuronal remodeling.

**BACK**
**PROGRAMME**

# Microtubule Hyperstabilization by mDia1 Drives Tau-dependent Synaptotoxicity

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Oligomeric Amyloid β1-42 (Aβ) plays a crucial synaptotoxic role in Alzheimer's disease (AD), and hyperphosphorylated tau facilitates Aβ toxicity. The link between Aβ and tau, however, remains a topic of debate. We hypothesized that a primary activity of Aβ is to activate pathways that alter microtubule behavior and/or tubulin post-translational modifications associated with microtubule longevity, and that these changes trigger a cellular stress response that leads to tau hyperphosphorylation in an attempt to restore normal microtubule stability. In this study we show that Aβ acutely hyperstabilizes axonal and dendritic microtubules in hippocampal neurons (21DIV) by reducing microtubule plus end dynamics. Silencing or acute inhibition of the formin mDia1 suppresses this activity and corrects the synaptotoxicity and deficits of axonal transport induced by Aβ. We explored the mechanism of rescue and found that microtubule hyperstabilization promotes tau hyperphosphorylation and tau-dependent loss of dendritic spines through regulation of PP1 activity. Together, these results uncover a novel role for mDia1 in Aβ-mediated synaptotoxicity and demonstrate that inhibition of microtubule dynamics is a driving factor for the induction of tau-mediated neuronal damage.

**BACK**
**PROGRAMME**

# Elucidating the Pathophysiology of GOSR2-mediated Progressive Myoclonus Epilepsy from Molecule to Neuron

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Mutations in the Golgi SNARE protein GOSR2 cause progressive myoclonus epilepsy (PME). Patients with this form of PME typically present with ataxia around age three, followed by cortical myoclonus and generalized tonic-clonic seizures. However, despite GOSR2's known role in mediating ER to Golgi and intra-Golgi membrane fusion, it is still unclear how GOSR2 mutations result in a selective neuronal phenotype with the clinical hallmarks of severe ataxia and hyperexcitability.

We are utilising an array of complementary approaches to elucidate the disease mechanism of GOSR2-PME. As components of this strategy, SNARE dysfunction is being experimentally assessed in liposome fusion assays; mutant GOSR2 localisation and Golgi trafficking investigated in patient fibroblasts; and the impact of the disease mutations upon nervous system development and function studied in a novel in vivo model of GOSR2-PME.

Liposome fusion studies revealed reduced membrane fusion rates due to the pathogenic GOSR2-PME mutations. In addition, we found in patient fibroblasts that greatly reduced amounts of mutant GOSR2 localises to the Golgi apparatus. These findings imply that anterograde cargo transport is impaired due to the formation of fewer fusogenic cis-Golgi SNARE complexes. The seeming paradox of how a global trafficking defect might eventually cause a selective neurological disorder is being investigated in a *Drosophila* model of this disease - the first in vivo GOSR2-PME model to date. Given the critical importance of Golgi trafficking in dendrite development, we hypothesized that dendritic growth might be impaired. Indeed, we found profoundly shortened dendritic arbors with a total length reduction of up to two thirds in normally highly elaborate larval sensory neurons. This finding may explain one key feature of this disorder – ataxia – since cerebellar Purkinje cells with their highly elaborate dendritic trees would likely be most profoundly affected by such a dendritic growth deficit. In addition, we found that motoneuron synapses in our disease models exhibit gross morphological abnormalities and synaptic retraction, combined with hyperactivity.

From our experiments a mechanistic framework emerges which understands GOSR2-PME as a neurodevelopmental disorder with dendritic arborisation defects and morphologically abnormal, hyperactive excitatory synapses. Together, these multifaceted defects may simultaneously cause ataxia and shift circuit excitation-inhibition balance towards hyperexcitation. Besides having provided a multi-level insight into GOSR2-PME's pathophysiology, this study reiterates the importance of the early secretory pathway in dendritic development and suggests a critical role of the Golgi apparatus in presynaptic development and function.

**BACK**
**PROGRAMME**

**Poster - 001**

# Investigating the Role of Canonical and Non-canonical Wnts in Axon Outgrowth

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Axon outgrowth occurs during development of the central nervous system (CNS), and is imperative for proper function of neurons. Axon outgrowth is regulated by extracellular cues including Wnts. Wnts are cysteine rich lipid-modified glycoproteins that act as morphogens and modulate various neuronal processes during development. Canonical Wnts, such as Wnt3a, signal through -catenin pathway and induce gene transcription during cell proliferation and renewal, whereas, non-canonical Wnts, such as Wnt5a, signal in a -catenin independent manner through components of the planar cell polarity (PCP) pathway and modulate the cytoskeleton. Non-canonical Wnts have been shown to be involved in axon guidance, neurite outgrowth and dendritic arborisation, processes that also require components of PCP pathway. However, a precise role of Wnt-PCP signaling in axon induction and outgrowth remains poorly understood. Thus, in this study, the role of Wnts in the morphogenesis of primary mouse embryonic cortical neurons was investigated. Initial studies showed that treatment of primary cultured embryonic mouse cortical neurons (E15-16) from CD1 mice with IWP2 and LGK974 inhibited axon formation. IWP2 and LGK974 inhibit porcupine, an acyl transferase required for Wnt secretion, and thus block secretion of all Wnts. Addition of Wnt5a-conditioned media (CM), a source of Wnt5a, as well as purified Wnt5a to primary cortical neurons promoted an increase in axon length by 1.5-2 fold. In contrast, addition of Wnt3a-conditioned media, a source of canonical Wnt3a, to neurons had no effect on axon length. Wnt3a was confirmed to be active as tested using a Dvl-phosphorylation assay in breast cancer cells. The role of PCP component Prickle (Pk) was also investigated. Knockdown of Pk1 and Pk2 in primary cortical neurons using siRNAs inhibited axon formation. Altogether, the results indicate that endogenous Wnts are required for axon outgrowth and that non-canonical Wnt5a can enhance axon outgrowth. Further investigation is required to understand the mechanism of Wnt-PCP signaling in axon induction/outgrowth. Overall, this will increase our understanding of the effect of Wnts on axon induction/outgrowth, and may uncover means to overcome defects and injury in the CNS.

BACK

**Poster - 002**

# Quantitative Map of Proteome Dynamics during Neuronal Differentiation Reveals NCAM1 as Regulator of Dendritic Morphogenesis

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Neuronal differentiation is a multistep process that shapes and re-shapes neurons by progressing through several typical stages, including axon outgrowth, dendritogenesis and synapse formation. To systematically profile proteome dynamics throughout differentiation we took cultured hippocampal neurons at different developmental stages and monitored changes in protein abundance using a combination of stable isotope labeling and high-resolution tandem mass spectrometry (LC-MS/MS). We used our systematic and in-depth proteome analysis of hippocampal neurons in culture to established a quantitative map of neuron-specific proteome dynamics during developmental stages 2/3, 4 and 5. Our data set comprises 6,753 protein identifications of which more than 4,300 were quantified over all time points, covering crucial neuronal developmental processes including axon outgrowth, dendrite formation and synaptogenesis. About one third of the proteins reveal substantial changes in protein expression throughout the neuronal differentiation, clearly highlighting the extensive reprograming of the proteome. To highlight the strength of our resource, we focused on neural cell adhesion molecule 1 (NCAM1) as a regulator for dendritic outgrowth during neuronal development. The transmembrane isoform of NCAM1, NCAM180, is strongly upregulated during dendrite outgrowth, highly enriched in dendritic growth cones and interacts with a large variety of actin binding proteins. Inducing actin polymerization rescues the NCAM1 knockdown phenotype, suggesting that NCAM180 stimulates dendritic arbor development by promoting actin filament growth at the dendritic growth cone. Based on the obtained data on NCAM1, we feel that our quantitative map of neuronal proteome dynamics is a rich resource for further analyses of the role of other identified proteins in neurodevelopmental processes.

**BACK**

**Poster - 003**

# The RapGAP SPAR2/SIPA1L2 Provides a Ride-on Service for TrkB Endosomes and Autophagosomes

**Maria Andres-Alonso**[1,2], Ioana Butnaru[2], Mohamed Rafeet-Ammar[2], Guilherme M. Gomes[2], PingAn Yuanxiang[2], Torben Hausrat[3], Silvia Diaz-Gonzalez[1], Gustave Acunha Sanhueza[2], Michaela Schweizer[4], Matthias Kneussel[3], Christina Spilker[2], Michael R. Kreutz[1,2]

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Brain-derived neurotrophic factor (BDNF) binds and activates TrkB receptors located on axonal terminals and thereby initiates retrograde signaling. TrkB signaling endosomes contribute to several aspects of neuronal function and play an important role in plasticity of mossy fibre (MF) synapses. However, mechanisms of TrkB endocytosis and retrograde transport are not well understood. The Trk receptors and their ligands have been shown to be internalized as a complex in a clathrin-dependent manner to specialized vesicular compartments and sorted to diverse pathways leading to several physiological responses. The sustained ERK signalling by TrkB is mediated directly by binding of signalling adaptors, and the stimulation of Rap-1 based signalling pathways. In line with these findings, long-term signalling by TrkA has been hypothesized to be specifically mediated through endosomes associated with Rap1, which have been shown to be long-lived endosomes that persist from the nerve terminal to the cell soma.

The SPAR/SIPA1L family comprises three membres of neuronal RapGAPs with a unique domain organization and differential expression in neurons. All three members are serine-rich proteins and they share a PDZ domain and a RapGAP domain for the small GTPases Rap 1 and 2 (Spilker et al., 2010). SPAR2/SIPA1L2 is highly abundant in granule cells of the dentate gyrus and cerebellum. Here, we show that the protein binds directly to TrkB, via a minimal 12 aa domain in the TrkB juxtamembrane region, and links TrkB to a dynein motor via a direct interaction with snapin. Interestingly, SPAR2/SIPA1L2 concomittantly associates with Light chain3 and autophagosomes and TrkB endosomes / autophagosomes can traffick together in a SPAR2/SIPA1L2 dependent manner. This ride on service seems to impact on the retrograde transport of TrkB signaling endosomes. SPAR2/SIPA1L2 knock-out mice show among others structural defects in the MF projection, impaired BDNF-dependent MF LTP, increased surface expression of TrkB and deficits in pattern separation which requires MF plasticity. Application of a Tat-peptide encompassing the binding region for TrkB in SPAR2/SIPAL2 induces a similar phenotype in vivo and in vitro in wild-type mice like those observed in knock-out mice. Collectively the data suggest that SPAR2/SIPA1L2 can regulate BDNF-induced Erk activation and mossy fibre plasticity via a direct interaction with TrkB and that RapGAP activity is involved in termination of TrkB activity and fusion with autophagosomes.

BACK

**Poster - 004**

# The Role of Serotonin Receptors and Extracellular Matrix in Stress-related Disorders

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Neuroplasticity has been a subject of a great interest due to its pivotal role in the central nervous system. Structural alterations of neuronal and synaptic networks integration are also critically involved in the neuropsychiatric disorders, in particular depression. Based on the clinical observations on the efficacy of antidepressants targeting serotonergic system, it has been suggested that serotonin and its receptors play an important role in modulation of pathological plasticity.

It is known that matrix metalloproteinase (MMP-9) is one of the key biomarkers of depression and its polymorphism is involved in pathogenesis of bipolar disorder. However, underlying molecular mechanisms and possible interplay between serotonergic system and extracellular matrix remain poorly understood.

Previously, we have shown *in vitro* that stimulation of serotonin receptor 5-$HT_7$ contributes to the activation and/or release of MMP-9 to the extracellular space, which in turn triggers intracellular signals that induces the elongation of dendritic spines. In this study we investigated possible role of 5-$HT_7$R/MMP-9 signaling pathway in stress-related disorders. Using the mouse model, we have shown that activation of 5-$HT_7$ receptors caused increase of MMP-9 activity in the prefrontal cortex and hippocampus and resulted in depressive-like phenotype. Thus, the obtained results may indicate involvement of the cross-talk between 5-$HT_7$R and MMP-9 in the pathogenesis of depression *in vivo*.

BACK

**Poster - 005**

# Patterns of Spiking Activity of Neuronal Networks *in vitro* as Memory Traces

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Neuronal cultures *in vitro* plated on the multi-electrode arrays are very promising as an experimental model to study basic principles of learning. But it is still an open question if patterns of spontaneous activity in neuronal cultures can be interpreted as memory traces and if these traces can be modified in a learning-like manner.

In our work we tested hypothesis that population bursts of activity in neural networks *in vitro* represents memory traces that can be potentially reconfigured by learning in the closing loop stimulation protocol. We studied experimentally *in vitro* development of spontaneous bursting activity in neuronal cultures as well as how this activity changes after open or closed loop stimulation. Results demonstrate that bursting activity of neural networks *in vitro* self-organize into a few number of stereotypic patterns which remain stable over many days. External electrical stimulation increases a number of simultaneously present activity patterns with majority of bursts still classified as belonging to the dominant cluster.

Application of cluster analysis to the spontaneous population bursts along the neuronal network development for two cultures demonstrates that for the both cases more than 50% of bursts belong to only one dominant cluster. Almost all of remaining population bursts falls into two equally sized clusters covering 40% of bursts in total. This confirms previous findings that self-organization in the process of neuronal culture maturation leads to emergence of a limited number of dynamical modes with every represented by its own attractor.

Dominant population bursts and activity that emerge after stimulation have significantly different patterns of activations. As all patterns were present before the stimulation and stimulation affected only their frequencies it can be suggested that learning is implemented mostly via switching between existing attractors of activity.

BACK

**Poster - 006**

# Harnessing Electrochemistry for Structural Plasticity Studies - A Glutamate Biosensor

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Dendritic spines structure plays a special role in synaptic plasticity in the brain. The new, recently described morphological form of dendritic spines is spine head protrusion (SHP) which is the protrusion on the top of the head of spines. Although the function of SHPs is still unknown, researchers believe that their presence is associated with the processes of remodeling of dendritic spines structure and creating new synaptic connections. We hypothesize that formation of SHPs occurs as a result of increased activity of neuronal networks triggered by induction of chemical long-term potentiation. Increase in neuronal activity leads to increase in neurotransmitter release to extracellular matrix and also leads to an increase in proteolysis of extracellular matrix compounds. The studies have already shown (Richards et al., 2005) that exogenous application of neurotransmitter glutamate to medium of organotypic hippocampal culture induces the formation of SHPs. On this basis, it is hypothesized that the formation of SHPs occurs as a result of the neurotransmitter release to the extracellular matrix. Due to the fact that exogenous iontophoretic application of neurotransmitter is an invasive method and does not constitute direct evidence of the formation of SHPs we would like to verify this theory using electrochemical measurements. To this end, we will measure hydrogen peroxide from a modified glutamate biosensor, which is proportional to the endogenous concentration of glutamate after chemically induced stimulation in dissociated hippocampal culture. In our project, we would like to determine the genesis of SHPs formation in dissociated neuronal culture using developed glutamate biosensor. We believe that SHPs formation plays an important role in phenomenon of synaptic plasticity, underlying learning and memory processes.

BACK

**Poster - 007**

# The Role of S-palmitoylation in the Chronic Stress Disorders

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One of the environmental factors that can lead to development of anxiety and major depression disorders (MDD) is chronic stress. Chronic stress-related emotional and cognitive impairments are associated with various changes in the specific brain regions and alterations in synaptic proteins activity. Precise regulation of structural and functional synaptic integrity is critical for neuronal network connectivity and proper brain functions.

Post-translational modifications (PTMs) that occur on a protein are one of the best described mechanisms of synaptic proteins regulation. Protein S-palmitoylation (S-PALM) is a lipid modification described by the attachment of palmitate (C16) to cysteine thiol via thioester bond. As it was shown in the most recent study, palmitate reversibly modifies numerous classes of neuronal proteins, including synaptic scaffolding proteins, neurotransmitter receptors and secreted signalling molecules. Until now, most studies were focused on the physiological role of S-PALM, with limited reference to the role of S-PALM in pathogenesis of neuropsychiatric disorders, including MDD. One class of identified S-PALM proteins - serotonin receptors (5-hydroxytriptamine or 5-HT receptors) - might link S-PALM with MDD.

In order to understand the role of S-PALM in psychopathological changes we applied animal model of chronic restraint stress with complex behavioural and biochemical evaluations of stress effects. Wild-type C57BL/6J mice were subjected to the procedure of immobilization followed by force swimming test (FST), dark-light box test, sucrose preference test and measurement of serum levels of corticosterone. Each of the above-mentioned tests measures different component of depression, namely: decreased mobility, anxiety, anhedonia and the level of stress hormones during and at the end of the experiment respectively.

In the following steps of our study we identified 1385 S-PALM peptides assigned to 451 proteins among postsynaptic density proteins. In the group of 451 identified S-PALM PSD proteins, 3 were present only in control mice and 60 were exclusively present in mice exposed to chronic restraint stress. This was achieved using mass spectrometry (MS) - based Acyl-Biotin Exchange method.

Aberrant modifications of important postsynaptic density proteins may contribute to chronic stress disorders development by affecting the functions of these proteins.

**BACK**

**Poster - 008**

# Modeling Microtubule Dynamics in Axonal Growth Cones

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The nervous system consists of neurons forming polarized cell protrusions called axons. At the growth cone of an axon, actin filaments establish a dynamic network that leads the formation of lamellipodia and filopodia. While actin polymerization provides the driving force necessary for axon elongation, plus-end microtubule (MT) dynamics have a crucial role in axon navigation. These two processes are regulated by extracellular guidance cues that locally affect cytoskeleton dynamics influencing axon targeting. However, how the balance between plus-end MT dynamics and actin polymerization influence axon targeting remains poorly understood.

We investigate the role of microtubule and MT-actin interactions in growth cones of primary spinal cord neuronal culture of *Xenopus laevis*. Indeed, we combine live imaging of MTs with different tracking techniques to quantify parameters of dynamic instability, such as polymerization, de-polymerization and the transition between those two states. To understand how these parameters influence MT behaviors, we build a mathematical model of growth cone MT dynamics using the *Cytosim* software (Nedelec et al., 2007). In our model, we incorporate MT dynamics within realistic growth cone morphologies and include a simplified actin network to simulate MT-actin interactions and retrograde flow. Simulations of growth cone MT dynamics in growth cone shapes obtained from image data suggest that growth cone architecture has a strong influence on MT distribution and length. For instance, to obtain the typical splayed arrays of MTs, MT-actin interactions are fundamental. To validate the simulation results and to further investigate growth cone dynamics, we experimentally increase plus-end MT-acting binding and image neurons in which MTs are stabilized with taxol.

Combining microscopy, image analysis and modeling, we established a steady-state model of growth cone MT dynamics. In simulations of growth cones with symmetric (straight growth) and asymmetric (turning) architectures, MTs distribution is similar to what we measure in cultivated neurons. Indeed, MT distribution depends on the MT intrinsic properties and interaction with the actin network. To further explore MT dynamics per-se and MT-actin interactions, we will modify the simulation environment to have different parameters of dynamic instability on one side of the growth cone. Intriguing, this approach will also help us to understand the role of MT dynamics during growth cones turning response.

BACK

**Poster - 009**

# Synaptic Remodeling Depends on Signaling Between Serotonin Receptors and the Extracellular Matrix

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The rewiring of synaptic circuitry pertinent to memory formation in the brain has often been associated with morphological changes in dendritic spines and extracellular matrix (ECM) remodeling. Here, we linked these processes by uncovering the signaling pathway involving the serotonin 5-HT7 receptors (5 HT7R,) the matrix metalloproteinase-9 (MMP-9), the hyaluronan receptor CD44, and the small GTPase Cdc42. We find that 5-HT7R stimulation increases local MMP 9 activity triggering dendritic spines remodeling, synaptic pruning and impairment of long-term potentiation (LTP). The underlying molecular machinery involves 5-HT7R-mediated activation of MMP-9, which leads to CD44 cleavage followed by Cdc42 activation. Pharmacological/genetic suppression of this pathway rescues the 5-HT7R-induced synaptic changes and the deficit in LTP. Our results thus reveal causal interactions in a previously unknown molecular mechanism regulating neuronal plasticity.

BACK

**Poster - 010**

# Recycling Endosomes Mediate Local, Golgi-independent Secretory Trafficking in Neuronal Dendrites and Spines

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Long-term storage of memories in the central nervous system depends on the local dendritic synthesis and membrane trafficking of new synaptic proteins such as AMPA-type glutamate receptors (AMPAR). While traditional cell biology dictates that newly synthesized integral-membrane proteins require processing and sorting by the Golgi apparatus (GA) for trafficking, the GA is notably absent from most neuronal dendrites. Consequently, whether secretory cargoes are locally trafficked in dendrites, and if so, the identity and spatial organization of the organelles responsible for trafficking them remain unclear. We have utilized an inducible-ER release system in combination with live-cell fluorescence microscopy to define the dendritic organelles involved in trafficking new AMPA receptors. We found that upon exiting the dendritic endoplasmic reticulum (ER), AMPARs initially undergo spatially restricted entry into nearby ER-Golgi intermediate compartment (ERGIC). AMPARs are subsequently forward trafficked through the recycling endosome (RE) network which is often found in close proximity to dendritic ERGIC. This pathway is critical for biosynthetic protein trafficking, including to individual synaptic sites via spine-resident REs, as disrupting RE function drastically impairs the surface delivery of newly-released AMPARs. Surprisingly, RE-mediated surface delivery of AMPARs still occurred in the absence of normal GA function, indicating that locally translated proteins may be directly trafficked through this pathway without requiring processing by the somatic GA. Thus, in addition to its canonical role in recycling membrane proteins, the RE network also participates in the local, GA-independent trafficking of new synaptic proteins. Ultimately, this pathway could deliver locally synthesized proteins to support translation-dependent forms of neural plasticity.

**BACK**

**Poster - 011**

# Assessing the place field formation capability of CA1 pyramidal models in Alzheimer's Disease

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A variety of critical computational tasks is thought to be affected in senile neurodegenerative diseases, in ways not thoroughly explored. Remarkably, in Alzheimer's disease (AD), spatial navigation skills exhibit a gradual deterioration. Thereupon, it is no coincidence that CA1 hippocampal subfield shows particular vulnerability to neurodegenerative triggers. While the dynamic interplay between various structural aberrations -and, specifically, the dendritic morphology and/or synaptic rearrangements- is consistently reported in the literature, a systematic examination of the causative parameter space is still missing. In order to understand when and why the initial compensational adjustments to disease are gradually overtaken by functional losses, there is a need for a novel approach that could offer clear insights.

In this study, we set out to explore the robustness of the computational capacity of a CA1 pyramidal neuron. In order to assess this, we studied the place field formation capability in a computational single-cell model in which structural alterations were induced. Specifically, we used an elaborated version of the biophysically and morphologically realistic CA1 model, published by Poirazi et al., 2003, that employs a structural flexibility scheme. This scheme allows the appropriate redistribution of the active properties along the dendrites of any morphological 3D reconstruction. Next, by using a computational tool named REMOD (previously developed in our lab), we produced genealogies of manipulated morphologies from ancestor ones, thus, mimicking the dendritic complexity shrinkage effect, ramifying throughout AD's progressive pathology. Consequently, we tested the same place field formation protocol across all the morphological genealogies, while applying various synaptic arrangements and exploring the parameter space of reported ion conductance ranges. Finally, we mapped the recorded electrophysiological responses on a simulated exploration space and yielded the corresponding place field maps.

Preliminary results suggest that the apical tree shrinkage can lead to spatially expanding and noisier place fields, presumably due to the higher cellular excitability, manifested through both higher firing rates and increased bursting. This could offer a viable explanation for the spatial navigation impairment, pinpointed down to a specific and well-characterized neural computational task. Further exploitation of the proposed manipulation scheme could retrieve valuable, and yet unsuspected, qualitative and quantitative results, hence aiding the development of targeted interventions for the prevention of AD's cognitive decline.

**BACK**

**Poster - 012**

# Hydrogels Mimicking the Central Nervous System Extracellular Matrix: Applications to 3D Cultures and Neural Regeneration

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The extracellular matrix of the brain is based largely on a backbone of hyaluronan (HA), grafted with chondroitin sulfate proteoglycan brushes (CSPGs) and cross-linked by tenascins. HA is therefore the backbone polymer of choice to construct brain extracellular matrix mimetic hydrogels. A number of chemical processes have been used to cross-link HA into hydrogels, but none of them could simultaneously provide fast gelation, full compatibility with cell encapsulation, tissue adhesion, and *in situ* gel formation possibilities.

In order to overcome these issues, we designed an HA derivative cross-linkable into hydrogels with the enzymatic activity of a mammalian transglutaminase, coined HA-TG. The specificity of the enzyme to its recognition sites enabled cell encapsulation without loss of viability, and in the absence of any reactive chemicals that could compromise cell behavior. The gelling speed was dependent on the enzyme concentration, and gelling speed of ~30 s could be obtained, which made these gels not only convenient for in vitro cultures, but also appropriate for in situ gelling during a surgical procedure. Additionally, the cross-reactivity to some proteins present in a lesion (including at least fibrin, the natural substrate of the transglutaminase) enabled adhesion, which proved important to prevent cyst formation and obtain good integration with surrounding tissues. Finally, the gels are based on GMP grade components already in clinical use, which would facilitate clinical translation.

The gels were effective in supporting 3D neuron cultures in vitro. In particular, dissociated E17 rat cortical neurons could be encapsulated with high viability, fast neurite extension, standard polarization and maturation as seen by immunocytochemistry, and with rapidly-forming and long lasting synchronous spiking activity. Dorsal root ganglia from E10 chick embryos could also be grown in the gels, and nerve growth factor (NGF) could be immobilized in the gels with retention of activity. Finally, the gelation, integration, neurite outgrowth, and *in vivo* stability were studied in a rat spinal cord injury model, with a T8 lateral hemi-section. Preliminary results showed the gels were able to support both neurite outgrowth and fibroblast infiltration. The gels slowly degraded over a period ranging from a few days to a few weeks depending on the HA-TG concentration. No adverse reaction such as macroscopic encapsulation or cavitation was observed. Ongoing experiments aim to further characterize and optimize the *in vivo* efficacy of the material.

In summary, HA-TG gels present an array of physical and biochemical properties that make them an ideal platform for a variety of neural tissue regeneration applications, from cell encapsulation with high viability to growth factor immobilization and physical support of neurite outgrowth. Further investigation is needed in order to determine if they can provide improvements after spinal cord injury.

BACK

**Poster - 013**

# Huntingtin-mediated Axonal Transport of APP Determines Synaptic Aβ Accumulation and Memory Alterations in Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the abnormal trafficking of the amyloid precursor protein (APP), precursor of Aβ peptides whose levels are crucial for AD. However, little is known about how APP is transported within neurons. It is thus of crucial importance to understand the mechanisms that regulate anterograde and retrograde APP transport within axons and dendrites and whether transport impact on Aβ production.

Our laboratory along with other groups has identified huntingtin (HTT) -the protein that when mutated causes Huntington's disease- as a key regulator of the transport of trophic factors such as Brain derived neurotrophic factor but also of other cargos such as receptors, endosomes and APP. HTT not only facilitates transport in axons and dendrites by promoting the efficiency of molecular motors but can also regulate directionality through its phosphorylation at serine S421.

Here, we investigated whether HTT phosphorylation at S421 affects APP axonal transport and accumulation of Aβ at the synapse. We used primary neurons from newly engineered $Hdh^{S421A}$ and $Hdh^{S421D}$ mice in which this particular site mimics absence (S421A) or constitutive phosphorylation (S421D), and microfluidic devices that reconstitute cortico-cortical connections coupled to spinning-disk confocal videomicroscopy.

We found that absence of phosphorylation at S421 regulates APP axonal transport and Aβ peptide accumulation at the synapse. By modifying the genetic status of the cultures in the pre or post compartments, we found that axonal transport rather that dendritic transport of APP determines the accumulation of Aβ at synapse. To test whether modifying anterograde transport of APP impacts on disease in vivo, we crossed $Hdh^{S421A}$ with APPswe/PS1L166P mice. In vivo, absence of HTT S421 phosphorylation restores synaptic morphology and ameliorates memory alterations in the AD model.

In conclusion, anterograde transport of APP is a crucial component for Aβ peptide accumulation at the synapse and implies HTT protein as a potential modifier of disease progression.

BACK

**Poster - 014**

# Regulation of Dendritic Spine Morphology in Hippocampal Neurons by Copine-6

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Dendritic spines compartmentalize information in the brain, and their morphological characteristics are thought to underly synaptic plasticity. In this study we identified copine-6 as a novel modulator of dendritic spine morphology.

We found that BDNF -a molecule essential for long-term potentiation of synaptic strength- upregulated and recruited copine-6 to dendritic spines in hippocampal neurons. There, copine-6 promotes recycling of activated TrkB receptors back to the plasma membrane surface. This, in turn, modulates BDNF-TrkB signaling via Erk which is necessary for BDNF-induced increases in mushroom spines in hippocampal neurons. Overexpression of copine-6 increased mushroom spine number and decreased filopodia number, while copine-6 knockdown had the opposite effect and dramatically increased the number of filopodia, which lacked PSD95.

Manipulation of post-synaptic copine-6 levels affected mEPSC kinetics and evoked synaptic vesicle recycling in contacting boutons, and post-synaptic knockdown of copine-6 reduced hippocampal LTP and increased LTD. Thus, copine-6 regulates BDNF-dependent changes in dendritic spine morphology to promote synaptic plasticity.

BACK

**Poster - 015**

# Vesicular Treadmilling in Axons Defines How Synapses Adapt to High Neuronal Demand

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In the classical model of axonal transport, secretory and synaptic vesicles travel anterogradely from cell body to distal synapses where they fuse to membranes to deliver their cargoes. Conversely, retrograde vesicles represent endocytic vesicles that travel back to the soma for signaling, recycling or degradation. This classical model therefore predicts that blocking access to the axon (from the soma or from synapses) should dramatically affect the global flow of vesicles. However, we found that blocking vesicular secretion in the soma or blocking endocytosis at the synapse has very limited effect on axonal trafficking. Based on these observations, we hypothesized that most anterograde and retrograde vesicles form a single bidirectional pool that circle back and forth along the axon, thus following a vesicular treadmilling mode of transport.

Using high-resolution videomicroscopy of photoconvertible cargoes and microfluidic devices that isolate cell bodies, axons and synapses, we found that 70% of photoconverted vesicles are transported back and forth with surprisingly regular phases that correlate with axonal length. We further found that moving vesicles that arrive at axon extremities (axon initiation segment and tip) pause for minutes before reversing in the opposite direction. We next compared the kinetics of treadmilling vesicles in axons from neurons with low activity and no synapse versus high activity and functional synapses. In low-activity axons we observed that only 5% of treadmilling vesicles exit at each turn compared to 50% exit in high-activity axons, suggesting a regulation by synaptic activity. Based on these results, we developed a mathematical model with either classical or treadmill parameters. We found that only treadmill trafficking allows fast refilling of the synaptic pool for efficient release upon high neuronal activity. We are now testing this hypothesis using photoconvertible cargoes and repeated, sustained neuronal stimulation.

In conclusion we propose a novel model for axonal transport of secretory and synaptic vesicles that we named vesicular treadmilling. This transport is dynamically regulated by the refill capacity of the synaptic pool and allows rapid and efficient adaptation to high neuronal demand.

BACK

**Poster - 016**

# Substrate Elasticity Controls Neurite Initiation through a Bi-stable Switch

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Neurite initiation is the first step in neuronal development and occurs spontaneously in soft tissue environments. While mechanisms regulating morphology of migratory cells on rigid substrates in cell culture are widely known, how soft environments modulate neurite initiation remains elusive. Using mathematical modeling, pharmacologic inhibition, and genetic approaches, we revealed that paxillin-dependent endocytosis and adhesion can form a component of a bi-stable switch to control neurite initiation in a substrate stiffness-dependent manner. On soft substrates, most paxillin binds to endocytic factors and facilitates vesicle invagination, elevating neuritogenic Rac1 activity and the expression of genes encoding the endocytic machinery. By contrast, on rigid substrates cells develop extensive adhesions, increase RhoA activity and sequester paxillin from the endocytic machinery, delaying neurite initiation. Our results highlight paxillin as a core molecule in substrate modulus-controlled morphogenesis and define a mechanism whereby neuronal and migratory cells respond differentially to environments exhibiting varying mechanical properties.

**BACK**

**Poster - 017**

# Actin Rings: Its Assembly and Physiological Relevance

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Neurons are highly polarized cells that depend on cytoskeleton organization to establish their shape and function. Advances on super resolution microscopy allowed the identification of a membrane periodic skeleton composed by actin rings interconnected by spectrin tetramers. However, the assembly and function of this structure remains largely elusive. Recent findings support the relevance of additional components of the membrane periodic skeleton apart from actin and spectrin. In this respect, our previous data suggest that alpha-adducin is essential to control actin ring diameter. We are currently dissecting, by STED nanoscopy, how actin rings are nucleated, elongated and organized using specific inhibitors and knocking down relevant actin-binding proteins. Additionally, we are modeling the contribution of the membrane periodic skeleton to the electrophysiological properties of axons.

We expect that our findings open new perspectives on the study of the physiological roles of axonal actin.

BACK

**Poster - 018**

# Profilin-1 is a Key regulator of Actin and Microtubule Dynamics Required for Optimal Axon Growth and Regeneration

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Although actin is well recognized as a key player in axon growth, how different actin-binding proteins control its dynamics is not fully understood. Here we investigated the role of profilin-1 (Pfn1) in axon growth and regeneration. Profilins provide the pool of competent ATP-actin monomers to be added to F- actin ends to support their polymerization. In vitro, Pfn1 knockdown severely impaired actin retrograde flow, microtubule growth speed, and axon formation and growth. In vivo, mice with an inducible neuronal deletion of Pfn1 had decreased axon regeneration. In a model with increased regeneration capacity, Pfn1 activity was increased in the growth cone of regenerating axons. In line with these findings, overexpression of constitutively active Pfn1 strongly enhanced actin and MT dynamics, and axon growth. In summary, we show that Pfn1 is a determinant of axon growth and regeneration acting as a key regulator of both actin and MT dynamics.

**BACK**

**Poster - 019**

# Myosin 1b Triggers Axon formation by Controlling Growth Cones and Actin Waves Propagation

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Despite notable advances over the past decades, understanding how axons are formed and neuronal polarity is established remains a major challenging question. Several myosins 1 that are single head molecular motors have been identified in neurons but their function is still unclear. By using live cell imaging, high resolutive microscopy, gene silencing and gene overexpression we demonstrate that one of these myosins, myosin1b triggers the formation of axon and is required for the establishment of polarity of mouse embryonic hippocampal and cortical neurons in primary culture. We observed that myosin1b is associated with the axons. Depletion of myosin1b or inhibition of its motor activity delays the stage of development of the neurons and inhibits the formation of the axon. As the other myosins 1, myosin1b is associated with membrane via the binding of a PH motif to phosphoinositides. A single mutation in this motif inhibits myosin1b ability to form axon. Thus, myosin1b motor activity and its binding to plasma membrane via Pip2 are both required to form axons.

The growth cones and the actin waves that propagate along the neurites are two important players for axon formation and they share a similar organization of their actin network. We show that myosin1b regulates the propagation of actin waves, and controls actin dynamic and the stability of the filopodia both in the growth cones and actin waves. This function required the integrity of myosin1b motor activity as well as its ability to bind phosphoinisitides. Furthermore manipulation of myosin1b expression affect the distribution of microtubules in the growth cones and the distribution of Kif5 a microtubule associated motor involved in the transport of cargo along microtubules for the specification of the axon.

Together our data demonstrate that myosin 1b controls the formation of axons and the establishment of neuronal polarity by regulating actin dynamics and filopodia both in the growth cones and actin waves and indirectly microtubules dynamics and microtubules dependent membrane traffic.

**BACK**

**Poster - 020**

# AMPK Related Kinase NUAK1 Haploinsufficiency Impairs Cortical Development and Behavior in the Mouse

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The molecular mechanisms underlying axon branching and circuit formation in the developing brain are still poorly understood. Impairment of these processes can lead to sociallydevastating neurodevelopmental defects such as Autism Spectrum Disorders (ASD), schizophrenia or mental retardation. We recently identified a novel signaling pathway involving two kinases LKB1 (STK11) and NUAK1 (ARK5) and controlling terminal axon branching in the mouse cortex through the capture of mitochondria at nascent presynaptic sites (Courchet et al. Cell 2013). Recently, systematic genomic studies in humans identified de novo loss of function mutations associated with sporadic forms of ASD in several kinases downstream of LKB1, including NUAK1. Yet the relevance of these mutations to ASD phenotypes and the functional or behavioral consequences of disrupting the LKB1/NUAK1 pathway remain largely unknown. We now present evidence that the inactivation of one allele of the NUAK1 gene results in a complex phenotype including growth retardation, lateral ventricles enlargement and thinning of the cortex. Furthermore we observed a gene dosage effect of NUAK1 expression on mitochondria trafficking and terminal branching in cortical neurons. We adopted a genereplacement strategy to further characterize in vivo some of the mutations identified in NUAK1 linked to ASD. Finally, we observed that NUAK1 heterozygosity disrupts the behavior of mice and results in a decreased sensory gating and longterm memory formation. Overall, our results indicate that NUAK1 is haploinsufficient for the development of cortical connectivity and support the hypothesis that mutations in NUAK1 could participate to the etiology of neurodevelopmental disorders.

**BACK**

**Poster - 021**

# Local Administration of Lithium Promotes Axonal Regeneration and Functional Motor Recovery after Peripheral Nerve

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Peripheral nerve injuries may result in loss of motor function, sensory function, or both. Such injuries may occur by trauma or acute compression. Demyelination can occur as a result of neuronal or Schwann cell injury and is usually accompanied or followed by axonal degeneration. Recovery may eventually occur, but it is slow and frequently incomplete. Current researches have extensively focused on the new approaches for the treatment of peripheral nerve injuries. To date, few therapeutic treatments are available. They are mostly based on anti-inflammatory agents.

Lithium is an enzymatic inhibitor of GSK3β, and mimics the Wnt/β-catenin signaling pathway. This drug is widely used as a long-term mood stabilizer in the treatment of bipolar and depressive disorders Lithium has demonstrated neuroprotective qualities and therefore shows great potential therapeutic benefit for some neurodegenerative diseases. In the present research we demonstrate the effect of topical lithium administration on peripheral nerve regeneration and recovery of motor function following peripheral nerve injury in rats.

Forty-eight adult male Sprague Dawley weighing 250-300 g were used. 10 mm sciatic nerve defect was bridged using a silicone conduit filled with at three different dosages lithium dilution. Animals were randomized into one control group and five experimental groups (n=8/group) administered the following (1) sham; control with no treatment (2)Nerve Autograft; (3)Only silicone conduit; (4)silicone conduit+1,5mEq lithium; (4)silicone conduit+2,5mEq lithium; (5)silicone conduit+5mEq lithium; (5)silicone conduit+15mEq lithium. At 12 weeks, sciatic nerve samples were harvested for evaluation of behavioral testing, sciatic nerve functional study, gastrocnemius muscle mass, and histomorphometric analysis. Our data showed high doses of lithium (5mEq and 15mEq) showed better performance in promoting nerve regeneration and functional recovery than low dose of lithium (2,5mEq). Topical lithium administration is capable of promoting nerve regeneration after nerve injuries, highlighting the therapeutic values of lithium as a neuroprotective drug for peripheral nerve injury treatments.

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Keywords: Peripheral nevre injury, Lithium, Regeneration, Functional Recovery

BACK

**Poster - 022**

# A Common Conserve Pathway Exists during Inflammation Mediated Neurodegeneration in *Drosophila* Model of Parkinson's Disease

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Parkinson's disease is a common neurodegenerative disorder and the proper mechanism behind that is still not well known. Toxin induces Parkinsonism in different vertebrates as well as invertebrates like *Drosophila*. Studies revealed that different inflammatory molecules exhibit pivotal role during progression of neurodegeneration in vertebrates but, the phenomenon is still not clear in invertebrates.

Histological analysis was performed to detect neuronal lesions. Immunofluorescence (IF) study performed against Tyrosine hydroxylase (TH) to prove dopaminergic neurodegeneration. The inflammatory pathway markers were examined by western blot and qPCR analysis.

Survivability and locomotion ability were significantly decreased in treated males than females. Elevated expression of proinflamatory TNF-α found in treated males than female flies both at protein and mRNA level. Treated flies exhibited increased ROS generation as compare with control. Altered expression of TGF-β pathway found in wild type and mutant flies. Mortality and mobility assay was significantly affected in mutant than wild type fly. Altered expression level of dopaminergic neuron and glia marker was observed and expression of innate immune genes changed in both wild type and mutant.

The conserved inflammatory events in terms of expression of TNF-α have been observed more in males than females and the alteration also found in TGF-β pathway during neurodegeneration. Moreover, prominent changes have been observed in pro- and anti- inflammatory expression, neuronal and glial expression status and immune response in both wild type and mutant flies.

BACK

# Nanoscale Alignment of the Periodic Subcortical Cytoskeleton of Axon and Glia at Nodes of Ranvier

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Virtually every neuron is characterized by the presence of a subcortical periodic lattice consisting of actin, spectrin, ankyrin and other proteins along the neurites. The spacing of the lattice is ~190 nm, and hence tighter than the diffraction limit. By exploiting Stimulated Emission Depletion (STED) nanoscopy, a technique that allows imaging with sub-diffraction resolution, we show that this lattice persists in sciatic nerve fibers, underneath the myelin coat, therefore indicating that myelination does not alter its structure.

Recently, we demonstrated that oligodendrocyte precursors, which are the cells responsible for myelination in the central nervous system (CNS), also exhibit an actin/spectrin pattern similar to that of neurons. We therefore speculated whether the periodic cytoskeleton plays a role in the assembly of nodes of Ranvier, where a tight connection between the myelinating glial cell and the axon is present, and the myelin coat is interrupted to allow the saltatory propagation of the action potential.

To answer this question, we focused our attention on the organization of nodes of Ranvier in the peripheral nervous system. We found that proteins enriched at the nodal gaps, including sodium and potassium channels, show a periodic organization. However, axonal and glial nodal adhesion molecules (neurofascin-186, NrCAM) and glial actin can arrange in a more complex, 2D hexagonal-like lattice but still feature a ~190-nm periodicity. At paranodes, cytoskeletal and adhesion molecules of both the axon (betaII spectrin, Caspr) and the glia (ankyrin B, neurofascin-155) form periodic quasi–one-dimensional arrangements, with a high degree of interdependence between the position of the proteins of the different cells. Hence, these results indicate the presence of mechanisms that finely align the cytoskeleton of the axon with the one of the glial cells. Taken together, our observations reveal the importance of the lateral organization of proteins at the nodes of Ranvier and suggest a role for the periodic subcortical cytoskeleton in the organization of these complex structures.

BACK

**Poster - 024**

# Differential Requirement of Sad Kinases for Cortical and Hippocampal Development in Mouse Brain

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Neurons are highly polarized cells with a single axon and multiple dendrites. Intrinsic and extrinsic factors activate different signaling cascades to direct neuronal polarization. The AMPK-related kinases SadA and SadB play an important role in brain development *in vivo*. It has been previously reported that the loss of both Sad kinases leads to a decrease in the size of the cortex, disrupted cortical lamination and a loss of axons. The defect in axon formation has been attributed to a function of Sad kinases in the regulation of microtubule binding proteins downstream of Lkb1. The causes for the disorganization of the cortex in the *Sada*;*Sadb* double mutant have not been analyzed so far. Here we show that the decreased size of the cortex is linked to a decrease in progenitor proliferation and an increase in the apoptosis of neurons in the cortical plate but not progenitors in the ventricular zone. By contrast the hippocampus remains unaffected and no changes in progenitor proliferation or apoptosis were observed in this region. Our results also showed the importance of Sad kinases for the normal organization of the axonal cytoskeleton in the establishment of neuronal polarity. Corresponding to the defects in cultured neurons, the analysis of sections from the brain of *Sada*;*Sadb* double knockout mice revealed a loss of axon-specific neurofilament medium chain in the cortex but not the hippocampus. Taken together our results show a differential requirement of Sad kinases for the development of hippocampus and cortex.

BACK

**Poster - 025**

# The AP-2 Complex Has A Specialized Clathrin-Independent Role In Polarity Maintenance In Fungi

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Defects in microtubule-based transport are implicated in many neuropathologies. The filamentous fungus *Aspergillus nidulans* is an emerging model for studying transport owing to its unique genetic and cell biology tractability and metazoan-like dependence on microtubule-based transport for cellular trafficking, especially to polar cells like mammalian neurons. In this work we use *A. nidulans* to investigate the role of the adaptor complex AP-2 in polar growth of fungal hyphae. We show that AP-2 has a clathrin-independent essential role in polarity maintenance and growth, which is in line with a sequence analysis showing that the AP-2 β subunit (β2) of all higher fungi lacks a clathrin-binding domain, and with experiments showing that AP-2 does not co-localize with clathrin. We provide genetic and cellular evidence that AP-2 interacts with endocytic markers $SlaB^{End4}$ and $SagA^{End3}$ and the lipid flippases DnfA and DnfB in the sub-apical collar region of hyphae. The role of AP-2 in the maintenance of proper apical membrane lipid and cell wall composition is further supported by its functional interaction with BasA (sphingolipid biosynthesis) and StoA (apical sterol-rich membrane domains), and its essentiality in polar deposition of chitin. Our findings support that the AP-2 complex of dikarya has acquired, in the course of evolution, a specialized clathrin-independent function necessary for fungal polar growth. This work might also provide valuable hints on the mechanism of polarity maintenance in neurons.

**BACK**

# Optogenetic Activation of Mechanical Forces to Control Neuronal Polarisation

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During development, neurons initially form several short dynamic processes (‘neurites’) that undergo phases of growth and shrinkage. Suddenly, one of these neurites starts growing continuously and eventually becomes an axon, while the remaining neurites turn into short and highly branched dendrites. The resulting neuronal asymmetry (or ‘polarity’) is the basis for directed information flow in the nervous system: dendrites receive signals and axons transmit them. Neuronal polarisation is thus fundamental for the functioning of the nervous system, as it is the basis of perception, movement, and behaviour. However, despite the importance of this process, the mechanisms driving axon formation have not been discovered yet.

While currently no molecules are known that initiate neuronal polarisation, axon formation can be induced experimentally by applying tensile (‘pulling’) force to a neurite. Moreover, growth cones, which are specialised structures at the leading tips of neurites, constantly pull on their neurite. Based on these ‘classical’ experiments, my hypothesis is that intrinsic cellular forces determine the fate of a neurite and drive neuronal polarisation.

To test this idea, I use traction force microscopy to quantify forces in developing neurons. By correlating tensile forces, growth velocities, and maturation of neurites, I test whether growth cone-mediated forces predict which neurite will become an axon. Furthermore, I perturb local forces to control which neurites turn into axons. In order to do so, I exploit pharmacological approaches and established an optogenetic setup, which enables the local and specific recruitment of proteins thought to be crucially involved in controlling cellular force generation using fluorescent light. Finally, I study the link between the growth cone geometry and force generation, by exploiting micropatterning techniques, which allow for precise control of the shape of growth cones. This highly interdisciplinary approach will bridge the gap between biophysical and biochemical studies of neuronal polarisation, and shed new light on axon formation. It might also provide new insights into axonal regeneration, which fails in adult mammals.

**BACK**

**Poster - 027**

# Neuroepithelial Organization and Polarity Require TorsinA Regulation of the Nuclear Envelope Localized LINC Complex

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TOR1A/ torsinA mutation causes the semi-penetrant neurodevelopmental disease of DYT1 dystonia. We now examine the role of this gene using *Tor1a* null mice. This identifies semi-penetrant neural tube defects accompanied by a disorganized neuroepithelium, polarity defects and hyperproliferation of neuroepithelial cells. TorsinA is an ATPase that localizes on the inner nuclear membrane domain of the endoplasmic reticulum. This is also the site where LINC complex proteins organize the cytoskeleton and contribute to tissue architecture. We show that SUN2 is the LINC component expressed at the time when torsinA loss affects neurodevelopment. Furthermore, torsinA removes SUN2 from the nuclear envelope of cultured cells, including neuronal-type cells, and SUN2 is mislocalized in the neuroepithelium of *Tor1a* null mice. We also genetically confirm the *in vivo* importance of torsinA downregulation of SUN2 by finding that a *Sun2* null allele provides dose-dependent rescue of neural tube defects, with *Sun2*-/- fully preventing this *Tor1a* phenotype. These data show that nuclear envelope proteins organize the neuroepithelium and that their disruption causes major neurodevelopmental defects. Furthermore, finding that *Sun2* is a genetic modifier of a *Tor1a* neurodevelopmental phenotype implicates this gene and its pleiotropic functions in DYT1 dystonia pathogenesis.

**BACK**

**Poster - 028**

# The Disease Associated Protein CYFIP1 Orchestrates Axonal Outgrowth and Brain Wiring

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Autism (ASD) and schizophrenia (SCZ) are neurodevelopmental disorders characterized by defects in brain connectivity. Copy-number variants of the *CYFIP1* gene in humans have been linked to neurodevelopmental disorders, such as ASD and SCZ. CYFIP1 has a dual role, regulating actin polymerization and protein synthesis. Here, we show that *Cyfip1* heterozygous mice have decreased functional brain connectivity and exhibit a slow-growth phenotype of the callosal axons during development. While the axons eventually reach their correct target areas, defects in the callosal structure persist into adulthood. The axonal proteome revealed an imbalanced production of proteins involved in actin cytoskeleton remodeling and axonal transport that is likely causative of the delayed axonal growth. The observed delay in axonal positioning and the decreased brain connectivity caused by the *Cyfip1* haploinsufficiency might explain its genetic association to psychiatric disorders.

BACK

# Actin Cytoskeleton Disruption as a Novel Player in the Pathogenesis of Familial Amyloid Polyneuropathy

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Familial Amyloid Polyneuropathy (FAP) is a neurodegenerative disease characterized by deposition of amyloid fibrils of mutated transthyretin (TTR) in the peripheral nervous system, leading to a dying-back axon degeneration. Abnormalities in cytoskeletal organization are a common feature of many neurodegenerative disorders. In this work we investigated the hypothesis that cytoskeleton damage occurs downstream of TTR deposition. In primary cultures of mouse dorsal root ganglia (DRG) neurons, a relevant cell type for FAP studies as mutant TTR accumulates close to the DRG, axons treated with TTR oligomers presented a marked reduction of the growth cone area, with disruption of the typical morphology of the growth cone which lacked the lamellipodial actin structures. Additionally, using a FAP *Drosophila* model in which the amyloidogenic mutant TTR Val30Met is expressed in the photoreceptor cells resulting in roughening of the eye, we observed decreased axonal projection of photoreceptor neurons, that presented more compact growth cones lacking the spread distribution of filopodia and lamellipodia actin structures. A genetic screen was subsequently performed by crossing the TTR Val30Met flies with readily available fly lines for the knockdown or overexpression of candidate genes whose function is associated with cytoskeleton dynamics. In this screen we determined that the Rho GTPase family-the major regulator of actin dynamics modulates TTR-induced rough eye phenotype. Using cell based assays of DRG neurons treated with TTR oligomers, we are currently validating the results obtained in the *Drosophila* genetic screen by comparing the activity of key Rho GTPases and performing a cytoskeleton phospho-antibody array which includes 141 specific phosphorylation antibodies mainly involved in actin signaling pathways. With this work we will dissect the cascade of events that underlie alterations in axonal cytoskeleton dynamics induced by TTR.

**BACK**

# Deletion of VTI1a and VTI1b Inhibits Synaptic- and Dense-core Vesicle Fusion due to Missorting of Proteins Required for Regulated Secretion

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Regulated secretion requires sorting of signaling molecules and components of the secretion machinery into synaptic (SVs) and dense-core vesicles (DCVs) and delivery to release sites. However, these sorting steps are still largely unclear. Here, we studied the role of Vti1a and Vti1b, two ubiquitous endolysosomal SNAREs, in sorting in regulated secretory pathways. Vti1a / Vti1b double-knockout (DKO) mouse hippocampal neurons had shorter neurites, with 40% lower SV and DCV numbers. In addition, SV and DCV fusion probability upon action potential trains was 50 and 70% lower than controls, respectively, without alterations in fusion kinetics, endocytosis or calcium transients. These defects were rescued by re-expression of Vti1a or Vti1b. Synaptic levels of secretory proteins, but not cytoskeletal proteins, were also decreased in DKO neurons, with more than 50% reduction in SNAP-25 and Munc13-1 levels. In contrast, somatic levels of these proteins were unaltered and neither proteasome nor lysosome inhibition rescued the reduced synaptic levels of SNAP-25 and Munc13-1. Hence, synaptic degradation of secretory proteins is not affected in DKO neurons. We propose that Vti1a and Vti1b are involved in a general sorting step for secretory proteins and regulated organelles, whose dysregulation results in incorrect delivery to presynaptic terminals and consequent loss of secretion capacity.

BACK

**Poster - 031**

# Microtubule Guidance in Axon Outgrowth

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During axon outgrowth, dynamic microtubules (MTs) at the leading edge are indispensable for axon guidance. Even subtle pharmacological perturbations of this so-called pioneer MTs lead to loss of directed neurite outgrowth. We would like to understand how MTs themselves are regulated, and how their dynamics influence axon extension. As MT have a high turnover constantly switching from growth to catastrophe, the understanding of their regulation escapes simple models.

We investigate MT dynamics by dynamic imaging of EB3-EGFP and other cytoskeletal markers in Xenopus growth cones, where we can track individual MTs and their dynamic distribution in the periphery. We have measured how MTs follow retrograde flow by speckle microscopy and can confirm opposing forces of MT polymerization and rearward movement.

In a top-down approach, we have studied the role of CLASP/orbit, an evolutionary conserved plus-end binding protein, which is involved in response to the repellent SLIT [1]. In Xenopus embryos, CLASP is highly expressed during axon outgrowth. Using live imaging of EB3-EGFP in Xenopus growth cones, we measured CLASP's effect on MT dynamics during axon extension. In growth cones with reduced CLASP levels, MTs reached the periphery at lower speeds, similar to growth cones treated with very low doses of taxol (5nM). In both cases axons grew slower and showed defects in keeping direction [2].

To integrate data from different experiments, we use the software Cytosim [3] to model individual dynamic MTs and MT-actin interactions in realistic growth cone morphologies. The model uses parameters extracted from imaging experiments, such as growth/shrinkage rates and MT dynamicity (catastrophe, rescue). We have established a steady-state model of MT dynamics and observed good accordance with distribution of MTs in imaging data. In the model, actin bundles are necessary to allow MT ends to explore the entire growth cone periphery. As expected, the length distribution is very sensitive to parameters of dynamic instability, but also interaction with actin bundles play a decisive role: Obstacles result in a slow down of MT polymerization and therefore more frequent catastrophe [4]. This change in catastrophe contributes to localization of plus ends in the periphery, which in turn might be important for delivery of signal molecules. Our studies demonstrate an additional layer of direct mechanical regulation independent from localized binding of regulatory proteins to the MTs.

[1] H. Lee et al, Neuron 42,913 (2004)
[2] A. Marx et al., MBC 24, 195 (2013)
[3] F. Nedelec and D. Foethke N J Phys 9, 1367 (2007)
[4] M.E. Janson et al., JCB 6, 1029 (2003)

BACK

**Poster - 032**

# *FMR1* Introns Encode MicroRNA's that Provide a Window to Primate Evolution

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Three highly conserved clusters of microRNAs (miRNA's) were found in the introns of human *FMR1*, and corresponding introns in the *FMR1* genes of other primates. These clusters are ~385 nucleotides in length and encode at least 3 distinct miRNA's, including *hsa-miR-619* and *hsa-miR-5096*. Each of these miRNA's have theoretical mRNA targets involved in neurodevelopment and synaptogenesis, including *DOUBLECORTIN*, *CAMK1D*, *NEUROFASCIN*, *GRIK2*, *ATAXIN3*, *ATAXIATELANGIECTASIA*, *ETF1*, *IDE*, *CTNND1*, *TRKB*, and *RICTOR*. Stem cell derived human neurons treated with *miR-5096*-antisense showed higher mRNA levels for most of the targets listed here. Notably, these miRNA clusters were not found in *FMR1* introns of mouse, zebrafish, drosophila, or any other non-simian, and hence are called simian intron regulatory elements (SIRE). Interestingly, SIREs were identified in *FMR1* introns from all new world and old world monkeys examined, but earlier prosimians such as lemurs, galagos, or tarsiers. This temporal distribution suggests that SIRE sequences were introduced to FMR1 during the Eocene epoch, ~45-55 MYA, at the beginning of a primate radiation that saw expansion of the neocortex. Analysis of RNA folding patterns in different simians showed evolutionary progression of pre-miRNA stem loop formation that was independent of changes in protein coding sequences of *FMR1*, suggesting that SIREs may allow for allelic variations that do not affect FMR protein function. Lastly, SIREs were also found in the introns of 10 other genes linked to neurodevelopmental disorders, including *TSC1/2* (Tuberous sclerosis), *MECP2* (Rett syndrome), *LIMK1* (Williams-Beuren syndrome), *KIRREL3* & *ARHGAP32* (Jacobsen syndrome), *SHANK3* (Phelan-McDermid syndrome), *UBE3A* (Angelman and Prader-Willi syndromes), *RERE* and *NEUREXIN1-3*. The sudden appearance of SIREs in the Eocene epoch and their conservation in the introns of neurodevelopmental genes suggest important roles in human evolution and intelligence.

**BACK**

**Poster - 033**

# Excessive Synaptogenesis in Adult Hippocampus of Astrocyte-specific Ephrin-B1 Knockout Mice Impacts Synaptic Functions and Mouse Behaviors

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Impaired interactions between astrocytes and neuronal synapses are implicated in synapse pathologies associated with neurodevelopmental disorders and neurodegenerative diseases. Astrocytes are well known to control synapse formation and functions through secreted and contact-mediated factors, but the identity of astrocyte-derived factors regulating synapse pruning are still under investigation. Here we propose a new role for astrocytic ephrin-B1 as a negative regulator of synaptogenesis in the adult hippocampus, where the interactions between astrocyte-specific ephrin-B1 and neuronal EphB receptors are most likely responsible for synapse pruning by astrocytes. We found that astrocytes expressing ephrin-B1, but not its functional mutants, engulf synaptosomes containing EphB receptors. To further investigate the necessity of astrocytic ephrin-B1 in maintaining neuronal synapses, we used ERT2-CreGFAP ephrin-B1flox mice to knock down ephrin-B1 specifically in adult astrocytes. In adult hippocampus, this astrocyte-specific ablation of ephrin-B1 leads to an increase in the density of dendritic spines and vGlut1-positive pre-synaptic input on CA1 hippocampal neurons. Despite these increases in both pre- and post-synaptic excitatory inputs, CA1 pyramidal neurons show reduced postsynaptic responses accompanied by a reduction in post-synaptic AMPA receptors. This altered synaptic structure and function coincide with enhanced contextual fear memory, suggesting that the observed synaptic phenotypes may be due to the abundance of potentially silent immature spines, which are then recruited during learning. Our findings demonstrate that ablation of astrocytic ephrin-B1 in the adult hippocampus triggered an excessive formation of immature spines with overall decreased functional responses, but these potentially silent synapses may provide a substrate for enhanced long-term contextual memory. These studies suggest a new role for astrocytic ephrin-B1 in regulating learning and memory in adult mice potentially via elimination of immature synapses in the hippocampus by trans-endocytosis.

BACK

**Poster - 034**

# Synaptic Localization of β-dystroglycan Mediates Homeostatic Plasticity

**Figiel I.**, Krzystyniak A., Bijata M., Wlodarczyk J.

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Normal levels of brain activity result from a fine balance of excitation and inhibition, and disruption of this balance may cause many neurological disorders. A number of studies have demonstrated that regulated proteolysis of synaptically expressed cell adhesion molecules plays a fundamental role in the morphological reorganization of synapses underlying homeostatic plasticity. One of the major modulators of these processes is matrix metalloproteinase-9 (MMP-9), a protease known to be released by excitatory synapses. On the other hand, its main substrate, β-dystroglycan (β-DG) was shown to be present at a subset of inhibitory synapses. To explain these discrepancies in location of both proteins, we utilize various approaches allowing to investigate the subcellular localization of β-DG and its involvement in structural plasticity.

To study whether proteolytic cleavage of β-DG influences the dendritic spine shape and motility we performed life imaging of MMP-9-treated primary hippocampal cultures, previously infected with lentiviral vector (LV) coding shRNA, specifically silencing DG or LV carrying GFP. The length-to-width parameter was used to evaluate the spine shape. We observed significant increase in the spine length/width ratio in LV-SH infected neurons compared with control cultures (LV-GFP). Moreover, knockdown of DG prevented MMP-9-dependent effect on spine morphology.

Next, using immunofluorescence staining with pre-synaptic marker antibodies (v-GAT and v-GLUT) we confirmed β-DG localization at both inhibitory and excitatory synapses. We also found significant changes in the number of β-DG-containing synapses in response to chemically induced LTP (cLTP).

To further explain association between β-DG and synapses we analyzed isolated mouse synaptosomes from P2 fraction with flow cytometry after staining them with antibodies against several synaptosomal markers including gephyrin, psd-95 and SNAP-25. We found out that β-DG is present on small subset of synaptosomes (5-10%) that exhibit expression of both post-synaptic markers (psd-95 and gephyrin). These results indicate that β-DG is associated with specific population of spines, i.e. double synapse spines, which have two different inputs – one excitatory and the other inhibitory.

Based on our findings we hypothesize that MMP-9 function in governing synaptic plasticity may be strongly associated with its effect on double synapses.

BACK

## Poster - 035

# Principles of Brain Protein Turnover

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The processes that govern protein production and degradation in the brain are particularly relevant, but poorly understood. In this complex organ, several cells including post-mitotic neurons must coordinate the turnover of thousands of proteins simultaneously. The precise regulation of these processes prevents the accumulation of potentially toxic cellular components, and protects from the appearance of pathologies.
We studied here the turnover of proteins in the mouse cortex and cerebellum. We pulsed mice with isotopically labeled lysines, and analyzed their incorporation by mass spectrometry, enabling us to determine the average lifetimes of ~2500 proteins in brain homogenates, isolated synapses, and purified synaptic vesicles. We also analyzed if modification of the brain activity through environment enrichment affects protein turnover.
We identified five basic principles of brain protein turnover. First, proteins functioning together have similar lifetimes. Second, protein abundance and lifetime are correlated, presumably to minimize the metabolic costs of turning over abundant proteins. Third, large proteins and proteins with a relatively larger surface area tend to live relatively shorter, most likely because they accumulate damage faster. Fourth, the sub-cellular environment influences turnover. For example, in neurons the presynaptic (axonal) compartment prolongs the lifetimes of the proteins. Fifth, the workload of the proteins also plays a role, since proteins are exchanged faster when they are more frequently used.
Altogether, this study establishes a general framework for the coordination of protein turnover in the brain, and offers unforeseen perspectives for the understanding of brain physiology.

BACK

# Cooperative Interactions between 480 kDa Ankyrin-G and EB Proteins Assemble the Axon Initial Segment

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The axon initial segment (AIS) is required for generating action potentials and maintaining axonal identity. The scaffolding protein 480-kDa ankyrin-G (480AnkG) is the master-organizer of the AIS but the molecular mechanisms of 480AnkG-mediated AIS formation remain unknown. Microtubules and their interactors, particularly end-binding proteins (EBs), have emerged as potential key players in AIS formation.

Here, we show that 480AnkG selectively associates with EBs and that this interaction is crucial for AIS formation and neuronal polarity in cultured rodent hippocampal neurons. EBs are essential for 480AnkG localization and stabilization at the AIS, whereas 480AnkG is required for the specific accumulation of EBs in the proximal axon.

This work thus provides insight into the functional mechanisms used by 480AnkG to drive AIS formation and thereby to establish neuronal polarity.

**BACK**

**Poster - 037**

# The Mitotic Kinase NEK7 Controls Dendritic Morphology in Neurons through Regulation of the Kinesin Eg5

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In neurons the microtubule cytoskeleton is essential for cell morphogenesis and function, for extension and compartmentalization of axons and dendrites, and for long-distance transport of cargo. Several mitotic microtubule regulators are also expressed in neurons, playing either similar or alternative roles.

Here we describe a new function for the mitotic kinase NEK7 during hippocampal neuron differentiation. We found that NEK7 is upregulated during this process and that neurons depleted of NEK7, or neurons from *Nek7* knock out mice have noticeably shorter and less branched dendrites, with fewer and thinner dendritic spines. Interestingly, these phenotypes are independent of NEK6 and NEK9, two kinases that form a signaling module with NEK7 in mitotic cells.

We identified the kinesin KIF11/Eg5 as a substrate of NEK7 in neurons, as its inhibition phenocopies NEK7 depletion/absence, and co-expression of a phospho-mimetic S1033D Eg5 mutant rescues the effects of NEK7 depletion. Phosphorylation of this residue by NEK7 is necessary for accumulation of Eg5 in dendrites, which in its turn is crucial for the stability and polarity of the dendritic microtubule array - NEK7 depletion or Eg5 inhibition decrease the levels of microtubule acetylation, and increase the percentage of minus end-distal microtubules, especially in the distal dendrite regions.

Thus, by controlling microtubule polarity and stability, the so-called mitotic kinase NEK7 regulates proper dendrite and spine morphogenesis in neurons.

Currently we are analyzing the consequences of NEK7 deficiency in vivo using *Nek7* knock out mice.

BACK

**Poster - 038**

# Differential Distribution and Function of the +TIP Proteins EB1 and EB3 in Cortical Neurons Undergoing Neuritogenesis

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To differentiate into the elaborate, polarised morphology of adult neurons, new-born neurons must first break their spherical symmetry and grow axons and dendrites. This process, referred to as neuritogenesis, begins with the emergence of a growth cone at the cell surface, which subsequently grows away from the cell body leaving behind a neurite. The dynamic interaction of both the actin and microtubule cytoskeletons is crucial for neuritogenesis. The drebrin/EB3/Cdk5 pathway is a candidate pathway for co-ordinating the dynamic behaviour of actin filaments (F-actin) and microtubules during neuritogenesis (Geraldo *et al*., 2008). Drebrin is able to directly bind F-actin and the +TIP microtubule-binding protein EB3 when bound to microtubule plus-ends. We mapped the binding site of EB3 on drebrin and used this information to derive dominant negative constructs of drebrin similar to the dominant negative constructs we have already developed for EB3 (Geraldo et al., 2008). Drebrin does not bind to EB1. In cultured embryonic cortical neurons, dynamic microtubules frequently enter the lamellipodium and occasionally run alongside F-actin bundles in filopodia during neuritogenesis. We determined the distribution of EB1 and EB3 in embryonic cortical neurons in culture by confocal fluorescence microscopy. Unexpectedly, we found that EB1 is more distally located at the plus-ends of dynamic microtubules than EB3 and occupies a shorter distance along the microtubule. Furthermore, we found evidence of a competition between EB1 and EB3 for microtubule territory as revealed by over-expression and knockdown experiments. EB1 recognises GTP-bound tubulin in the GTP-cap region at the plus-end of microtubules. The more proximal binding of EB3 is consistent with EB3 recognising GDP-bound tubulin. The differential localisation of these two +TIP proteins on the same microtubule has implications for the consequences of drebrin binding to EB3. One consequence of EB1 being more distally located on the microtubule lattice than EB3 is that microtubule growth would probably not be impeded when cross-linked through the drebrin/EB3 pathway to actin filaments at the base of a filopodium. As the microtubule extends into the filopodium, iterative proximo-distal addition of drebrin/EB3-mediated cross-links would produce a zippering-up effect of the microtubule to F-actin in the filopodium. In confirmation of a zippering-up effect we have observed a dynamic, proximo-distal extension of drebrin into filopodia.

Geraldo, S., Khanzada, U. K., Parsons, M., Chilton, J. K. & Gordon-Weeks, P. R. (2008). Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. *Nat. Cell Biol.*, **10**, 1181-1189.

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**BACK**

**Poster - 039**

# Long-distance Protein Transport Couples Extrasynaptic NMDAR-activity to Transcriptional Inactivation of CREB in Alzheimer's Disease

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Jacob is a synapto-nuclear protein messenger that encodes and transduces the synaptic and extrasynaptic origin of N-methyl-D-aspartate-receptors (NMDARs) signals to the nucleus. Nuclear import of Jacob following activation of extrasynaptic NMDARs leads to long-lasting transcriptional inactivation of CREB (CREB shut-off), a stripping of synaptic contacts, loss of dendritic arborization and finally cell death. In the present study we show that Jacob can directly bind to the bZIP domain of CREB and that it will either dock the MAP-kinase ERK or the CREB phosphatase protein phosphatase-1 (PP1) to the CREB complex. Synaptic NMDAR stimulation results in activation and binding of ERK to Jacob, phosphorylation of a serine at position 180 which increases binding to the neurofilament α-internexin that then serves as a molecular adaptor for a stable, phosphatase protected trimeric transport complex. In the nucleus this signalosome-like complex enhances plasticity related and CREB dependent gene expression. Following extrasynaptic NMDAR activation non-phosphorylated Jacob binds preferentially to the nuclear adaptor protein LIM only 4 (LMO4), a transcriptional co-activator of CREB that hinders dephosphorylation of serine 133. Jacob displaces LMO4 from the CREB complex and renders thereby CREB susceptible to dephosphorylation. Analogous to the ERK/α-internexin complex the association with LMO4 strengthens subsequent binding of Jacob to PP1 and this molecular switch in binding to an adaptor determines sustained transcriptional activation by ERK or inactivation of CREB by PP1. Thus, binding of an adaptor protein determines whether a kinase or phosphatase is bound to Jacob and which signalosome Jacob will eventually dock to CREB following long-distance trafficking.

Jacob null mutant mice show accordingly no CREB shut-off after extrasynaptic NMDAR stimulation. Jacob gene deletion in principal neurons of the forebrain, however, had no major neuroprotective effect following acute NMDA-induced excitotoxicity like for instance in brain ischemia. However, the extrasynaptic Jacob pathway kicks in when neurodegeneration is slow like in AD where Amyloid-β drives the protein into the nucleus by activation of extrasynaptic GluN2B containing NMDAR. Thus, we propose a novel molecular mechanism for the nuclear segregation of long-distance NMDAR signalling that is involved in early synaptic dysfunction and, later on, cell loss in AD.

BACK

# Aging Diminishes the Regeneration-promoting Effect of Targeting Pten in the Injured CNS

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How aging impacts axon regeneration after central nervous system (CNS) injury is not known, hampered by the very limited regenerative ability of CNS neurons. We asked whether genetic phosphatase and tensin homolog (Pten) deletion, known to promote CNS regeneration in young adults, would remain effective in older animals. We found that in both rubrospinal and corticospinal neurons, Pten deletion in older mice is effective in preventing axotomy-induced decline in neuron-intrinsic growth state, as assessed by mTOR activity, neuronal soma size and axonal growth proximal to a spinal cord injury. However, axonal regeneration distal to injury is greatly diminished, accompanied by increased expression of astroglial and inflammatory markers at the injury site. These data demonstrate that aging impairs CNS axon regeneration and have wide implications in developing strategies to counter this age-dependent decline in axonal repair after CNS injury or disease.

BACK

# Probing Physiological Relevance of Regenerating Axons after Spinal Cord Injury

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Central nervous system axons are typically unable to regenerate following injury. Several strategies have been developed to promote axon regeneration, however, little is known about the potential of these axons to form new synapses and the identity of their potential post-synaptic partners. To address this issue we are pursuing two avenues of investigation: i) Interaction of injured dorsal column (DC) axons with non-neuronal NG2+ cells, ii) Development of an anterograde trans-synaptic tracing strategy to assist in the identification of post-synaptic partner cells.

NG2+ cells proliferate after SCI and are abundant in the injury site. NG2 expressing oligodendrocyte precursor cells (OPCs) in uninjured tissue are known to receive synaptic input from neurons, thus raising the possibility that some NG2+ cells within the scar site may also synaptically interact with injured axons. Furthermore, previous studies suggest that injured axons form synaptic-like contacts with non-neuronal cells and this is speculated to play a role in preventing axon regeneration. In this study, the NG2-dsRed mouse line was used to identify NG2+ cells and AAV-GFP was used to identify DC axons. Following DC lesion, axons entering the injury site are tightly associated with NG2+ cells with axonal trajectories intertwined alongside NG2+ cell processes. Pre-synaptic proteins were present at these contact locations in some but not all of these axons. Staining with the OPC marker Olig2 revealed that the majority of these cells are not OPCs. To better understand the nature of these heterogeneous NG2 cells present specifically after injury, we performed patch clamp electrophysiological recordings in ex vivo spinal cord slices. The intrinsic electrophysiological profiles were heterogeneous with some NG2 cells displaying more complex electrophysiological properties. We have started to further elucidate potential synaptic interaction between injured DC axons and these potential postsynaptic cells.

In a parallel line of investigation we aim to identify synaptic interaction with all potential post-synaptic partners, not just those expressing NG2. The anterograde trans-synaptic tracer Wheat Germ Agglutinin was fused with Cre and expressed in a floxed Td-Tomato reporter mouse line. To increase tracing efficiency, we created an AAV expressing solely WGA-Cre and injected it directly into the dorsal root ganglion. After 4 weeks a sufficient level of trans-synaptic labeling was observed in most mice. Grey matter neurons known to receive innervation from DC axons were labeled in the uninjured spine. After injury, tracing revealed cells with heterogeneous and unique morphologies within the injury site. We are now further elucidating the identity and electrophysiological properties of these cells.

BACK

**Poster - 042**

# Using Stem Cells to Understand the Underlying Mechanisms of Polygenic Risk of Severe Mental Disorders

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The Bipolar Disorder (BD) and Schizophrenia (SCZ) are the main mental disorders that today are among the leading global causes of disability. We apply the recently developed stem cell technology (i.e. inducible pluripotent stem cells; iPSCs), allowing investigation of neuronal cells from participants. We have established an excellent infrastructure for somatic cell collection. 28 samples of more than 60 skin biopsies have been reprogrammed into hiPSCs, and differentiated into NSCs and derived cortical neurons.

Once reprogramming stage ended, several clones for each hiPSCs line were selected. The maintenance of pluripotency were checked analyzing the expression of specific pluripotent markers by qPCR and Immunocytochemistry. The hiPSCs were so ready to start the differentiation firstly in Neuroephitelial Stem Cells (or Neural Precursor Cells) and then in derived Cortical Neurons. The differentiation procedure was a two-step protocol based on a Dual-Smad inhibition. The effects of the differentiation to obtained NSCs (Neural Stem Cells) and derived cortical neurons was checked analyzing the expression of specific neural and cortical markers by qPCR and Immunocytochemistry.

We believe that the differences in timing and morphology during differentiation procedure shown in SCZ and BD cell lines compared with healthy controls can be explained analyzing the synaptic activity or plasticity. We use our large, existing in-house data of well characterized patients to identify clinical profiles associated with the polygenic risk of the new susceptibility genes. According to the polygenic profile we will select patients with high and low genetic risk, and we will focus on Ca2+ signaling pathway, other relevant cellular pathways and cell migration.

Reference:
1) Modeling psychiatric disorders: from genomic findings to cellular phenotypes. A. Falk et al., Molecular Psychiatry (2016), 1–13.
Rapid monolayer neural induction of induced pluripotent stem cells yields stably proliferating neural stem cells. K. Gunther et al., J Stem Cell Res Ther 2016, 6:6

BACK

**Poster - 043**

# In Search for Optimal Carbon Nanomaterials Coatings for Efficient Neuronal Regeneration

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Carbon nanomaterials like carbon nanotubes (CNT), carbon nanofibers or graphene for few years are considering in the area of biomaterials applications including tissue regeneration field. Not only structural and microstructural differences in particular types of carbon nanomaterials could influence on their regenerative potency but also chemical or biological functionalization and combination of various forms of carbon nanomaterials (hybrids) have also a significant impact on biological response. The aim of this study was investigation of biocompatibility and regenerative potency of some types of carbon nanomaterials deposited on titanium surfaces which have been optimized previously in respect to their physicochemical properties to obtain durable and homogenous coatings. Neuronally differentiated human neuroblastoma SH-SY5Y cells were used for this purpose. Biocompatibility was evaluated by biochemical cell viability/toxicity assays (MTT reduction and LHD release tests, respectively) and regenerative potential was estimated by analysis of morphology of CalceinAM/Hoechst3334 stained cells. As controls we used cells growing on plastic, glass and titanium surfaces. In general, the tested various types of nanomaterials: carbon nanotubes without functionalization (MWCNT) and with functionalization (MWCNT-F; MWCNT-OH) and graphene oxide (GO) in general did not evoke any toxic effects in SH-SY5Y cells (LDH release assay) after 48 or 72 h of culturing but they decreased to various extent the cell viability (MTT reduction assay), the latter probably connected with inhibition of cell proliferation and/or induction of apoptotic cell death. Further analysis of cell morphology pointed to GO as be the best coatings for neuronal regeneration. In next steps we modified GO properties by modifications with conductive polymer, polyaniline (PANI) (at ratio 1:1; 4:1 and 16:1). However, at least by using SH-SY5Y cells as a model of neuronal cells, we did not notice any significant improvement in regenerative potential of these type of modifications when compared to the efficacy of unmodified GO coating. Altogether, our study showed that neuronally differentiated human SH-SY5Y cells could be useful as a screening platform to study biocompatibility and nerve cell regenerative potential of various types of carbon nanomaterials coatings.

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BACK

# The Role of Ndr1/2 in the Regulation of Neuronal Polarity

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The kinases Ndr1 and 2 are a subgroup of the AGC group of protein kinases that is highly conserved from yeast to vertebrates and controls many important cellular processes, such as morphological changes, mitotic exit, cell proliferation and apoptosis. We have previously shown that Ndr1 and 2 play important roles in the neuronal polarity. While knockdown Ndr1 or Ndr2 alone does not result in notable defects in neuronal polarity, the simultaneous knockdown both of Ndr1 and Ndr2 leads to the formation of multiple axons. We identified Par3 is a target of Ndr kinases that is phosphorylated at Ser383. Par3 phosphorylation regulates its retrograde transport by dynein but does not affect its anterograde transport by kinesin. Ndr1 and 2 act downstream of the tumor suppressors Rassf1 and Rassf5, respectively. Rassf1 and 5 are required during the polarization of hippocampal neurons to prevent the formation of supernumerary axons. The knockdown of either Rassf1 or Rassf5, like the knockdown of Ndr1/2, leads to the formation of multiple axons. Interestingly the loss of Rassf5 also reduces the expression level of Rassf1. Our results identify a novel Rassf5/Ndr/Par3 signaling cascade that regulates the transport of Par3 during the establishment of neuronal polarity.

**BACK**

**Poster - 045**

# Coupling of Exo- and Endocytosis Mediated by Synaptic Vesicle Proteins and Lipids

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Neurotransmission involves the calcium-regulated exocytic fusion of synaptic vesicles (SVs) and the subsequent retrieval of SV membranes followed by reformation of properly sized and shaped SVs. The mechanisms by which SV membranes are endocytosed and SVs are reformed remain debated in spite of decades of research.
After fusion SV proteins stranded at the neuronal surface have to be recycled locally and sorted precisely to generate functional SV that can replenish the SV pool. Dedicated endocytic sorting adaptors have been shown to play a crucial role in regulating the correct protein composition of SVs. Especially SV proteins that are crucial for SV exocytosis like the R-SNARE synaptobrevin 2 and the calcium sensor synaptotagmin1 are recognized and sorted specifically by AP180 and stonin2, respectively. The manipulation of these selective adapters affects the recycling of synaptobrevin2 and synaptotagmin1, respectively, leading to their partial redistribution to the presynaptic cell surface. In addition, the association of SV proteins with each other ensures correct sorting into SV. Complex formation of synaptophysin with synaptobrevin 2 as well as of SV2A with synaptotagmin1 enables precise SV protein recycling. Interestingly, the deletion of adaptors only mildly affects endocytic retrieval kinetics and even accelerates the retrieval of synaptotagmin 1- pHluorin in the absence of stonin2. Based on these observations SV proteins could serve as coupling factors for SV fusion and endocytosis by regulating membrane lipids. Here we present our preliminary data regarding a putative lipid-based mechanism triggered by SV proteins on the axonal surface to initiate endocytic retrieval.

BACK

**Poster - 046**

# The Role of Pax6 in Cell Cycle Regulation

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Brain development is a very complex process, regulated by a limited number of signalling inputs and molecular pathways. Neurons proliferate, differentiate and migrate at different rates to give rise to the distinct regions of the brain. The intracellular mechanisms which are responsible for the regulation of these processes are not fully understood.

One of the main regulators involved in brain development is the transcription factor Pax6 which controls cell proliferation, migration and differentiation of neurons. Previous studies in the developing cortex have shown that one of the ways that Pax6 is able to regulate the rates of proliferation and differentiation is through the control of the cell cycle length. In particular it was shown that Pax6 inhibits the expression of CDK6, possibly lengthening the duration of G1 phase and halting cell cycle progression.

The role of Pax6 during development has been studied in the cortex, where its deletion causes shorter cycles. Very little is known about other regions of the brain, such as the diencephalon, where this gene is also highly expressed.

RNA sequence data from our group suggested that in the developing diencephalon, Pax6 might be affecting cell proliferation in an opposite way to that observed in the cortex. In this work we aim to explore whether this holds true by analysing cell cycle length changes (by incorporating fluorescent labelled Uridine analogs) after acute Pax6 deletion in three different tissues (cortex, thalamus and pre-thalamus) and across three different developmental ages (embryonic days 11.5, 12.5, 13.5). In addition, we studied the expression patterns of Cyclin D1 and D2 as CDK6 substrates and promising regulators of differentiation and proliferation rates.

Preliminary results are giving us insights on whether Pax6 is regulating the cell cycle in a tissue-specific manner or if the expression patterns observed are due to temporal differences during development.

We are generating models that take into account the direct regulation of CDK6 from Pax6 and the levels of cyclin Ds, in order to predict the biochemical pathways under which Pax6 acts to regulate the cell cycle. Future work, will include further elucidation of the effect of Pax6 in the cell cycle and how this affects other intracellular elements.

BACK

**Poster - 047**

# Small Molecule Stabilization of 14-3-3 Protein-protein Interactions Stimulates Axon Regeneration

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Damaged central nervous system (CNS) neurons have a poor ability to spontaneously regenerate, causing persistent functional deficits after injury. Therapies that stimulate axon growth are needed to repair CNS damage. 14-3-3 adaptors are hub proteins that are attractive targets to manipulate cell signaling. We identify a positive role for 14-3-3s in axon growth and uncover a developmental regulation of the phosphorylation and function of 14-3-3s. We show that fusicoccin-A (FC-A), a small molecule stabilizer of 14-3-3 protein-protein interactions, stimulates axon growth in vitro and regeneration in vivo. We show that FC-A stabilizes a complex between 14-3-3 and the stress response regulator GCN1, inducing GCN1 turnover and neurite outgrowth. These findings show that 14-3-3 adaptor protein complexes are druggable targets and identify a new class of small molecules that may be further optimized for the repair of CNS damage.

BACK

**Poster - 048**

# Phosphoproteomics Reveals that Phosphorylation of GAP-43 by JNK Regulates Axonal Growth

**Asami Kawasaki**[1,4], Masayasu Okada[1,4,5], Atsushi Tamada[1,4], Daiki Kobayashi[1], Shujiro Okuda[3], Motohiro Nozumi[1,4], Yutaka Yoshida[2], Kenji Sakimura[6], Hiroshi Nishina[7], Kosei Takeuchi[1,4,8], and Michihiro Igarashi[1,4]

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The molecular mechanisms of mammalian axon growth/guidance for the accurate synaptogenesis are believed to be performed through the complex signaling pathways, most of which are not understood so far. To solve these problems, since phosphorylation is the most important modification for these signaling, we performed unbiased phosphoproteomics, a comprehensive method quantitatively determining the phosphorylation sites in a given system. As results, phosphoproteomics of the axonal growth cone and identified 6,000 phosphorylation sites on 1,900 proteins. Among them, the most frequently phosphorylated site was Ser96 of GAP-43, which is a marker protein of axon growth. The phospho-specific antibody specific against this site specifically recognized the growing/regenerating axons. Genetic inactivation of Ser96 (Ser to Ala) caused abnormal axonal growth accompanying ectopic branches, and increase in excess filopodia formation. This phosphorylation was dramatically suppressed in JNK1 and MKK7 knockout mice. These results suggest that the MKK7/JNK1/GAP-43 (Ser96) pathway is a physiologically important signaling for neural circuit formation.

BACK

# Zebrafish as a Model of TSC Disease Exhibits Autistic-like Behaviour

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disorder, caused by mutation inactivating genes for proteins hamartin (*TSC1*) and tuberin (*TSC2*), which form an upstream inhibitor complex of mTORC1. As a consequence TSC also leads to overactivation of the mTORC1. TSC manifests itself by presence of benign tumors (hamartomas) in different organs (ex. brain, retina, heart, skin) although the neurological symptoms are the most influential in mortality and morbidity of this disease. In many cases, clinical manifestations of TSC include epilepsy, cognitive deficits and autism spectrum disorders (ASD). Zebrafish is convenient genetic and molecular model for vertebrate development due to its small size, rapid life cycle and embryonic transparency. Nowadays its application in neurological research is becoming more important in scientific community.
We use $TSC2^{vu242}$ Zebrafish mutant line [1] in which truncating mutation in *TSC2* gene leads to lack of TSC2 protein, that results in highly elevated mTORC1 activity as seen by phosphorylation levels of S6 protein in $TSC2^{-/-}$. $TSC2^{-/-}$ embryos in comparison with wild-type control, do not exhibit distinctive phenotype, nevertheless they die by 10 dpf (day post fertilization). Also, $TSC2^{-/-}$ have decreased motor function, which can be reverted by rapamycin (inhibitor of mTORC1) treatment which suggest that this is caused by overactivation of mTORC1 and is, in fact, mutation-dependent.
Preliminary results based on behavioral tests towards anxiety indicate increased level of anxiety in $TSC2^{-/-}$ which imply that they may exhibit autistic traits. Our research aim is to investigate social behavior, brain morphology and development in our model of TSC disease including neuronal connections and migration, searching for characteristic marks of autism disorder.

[1] Kim, Seok-Hyung, et al. "Zebrafish model of tuberous sclerosis complex reveals cell-autonomous and non-cell-autonomous functions of mutant tuberin." *Disease Models and Mechanisms* 4.2 (2011): 255-267.

BACK

# Synaptic Plasticity through Activation of AMPA-receptor Subunit GluA3

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AMPA receptors are responsible for fast excitatory synaptic transmission in the brain. In CA1 pyramidal neurons of the hippocampus two types of AMPA-receptors predominate: those that contain subunits GluA1 and GluA2, and those that contain GluA2 and GluA3. GluA1-containing AMPA-receptors have been extensively studied and are known to play a key role in several forms of synaptic plasticity and memory formation. In contrast, the contribution of GluA3 to synapse physiology has remained elusive. Here we show that GluA3-containing AMPA-receptors are inactive and contribute little to synaptic currents under basal conditions. GluA3 channel function is restored when intracellular cyclic AMP levels rise, leading to synaptic potentiation in a Ras-dependent manner. During fear, the activation of beta-adrenergic receptors triggers a massive and transient synaptic potentiation of GluA3-mediated currents. Our results indicate that the beta-adrenergic activation of GluA3-containing AMPA-receptors promotes the retrieval of fear memories.

BACK

**Poster - 051**

# Investigating the Role of the Nucleoporin Nup358 at the Axon Initial Segment of Cultured Cortical Neurons

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As highly polarized cells, neurons have a common morphology of a somatodendritic compartment and an axonal domain. The establishment of polarity occurs early during neuronal development, when one neurite differentiates to form the axon and the rest of the neurites form tapering dendrites. The outgrowth of the axon is exhibited by the formation of the axon initial segment (AIS), which is the site of input integration and action potential generation. Although the structure and protein composition of the AIS is well characterized, yet recent studies have proposed the recruitment of new proteins to this region, of which role is still under investigation. In the present study, we have addressed the clustering of the nucleoporin Nup358 to the AIS during the development of cultured cortical neurons, as it has been suggested that Nup358 might be involved in regulating neuronal polarity.

Nup358/RanBP2 is a high molecular weight protein, around 358kDa, that resides at the cytoplasmic side of the nuclear pore complex. It is involved in several cellular processes, such as the assembly of the nuclear envelop, nucleocytoplasmic transport, regulating interphase microtubules, and specifically in neurons, Nup358 was found to be essential for maintaining axon specification. In this study, we have investigated the changes in Nup358 expression profile and localization in developing mouse cortical neurons. Besides, we examine the role Nup358 has in neuronal function by stimulating or inhibiting neuronal firing with corresponding treatments.

Nup358 protein expression was analyzed in mouse cortical neurons cultured for different days in vitro. Western blotting analysis have identified an increase in the protein level relatively during neuron development. This increase in protein expression was accompanied by changes in Nup358 profile in neurons using immunofluorescence technique. At a later stage of development, neurons show a characteristic profile for Nup358: one fraction of the total pool is localized at the nuclear rim, another fraction is dispersed in the cytoplasm in the form of granules and a third fraction is extensively present in one of the processes originating from each neuron. This region is later identified to be the AIS, as cortical neurons show a co-localization of Nup358 and Ankyrin-G, an AIS marker. This localization of Nu358 at the AIS is further confirmed by detergent extraction experiment that show Nup358 resistance to extraction, a characteristic of several AIS proteins.

The distinct profile of Nup358 suggests a neuron-specific role as astrocytes show low expression level of Nup358, at both the protein and RNA level, in comparison to neurons. As for Nup358 role in neuronal activity, our results conclude that inhibiting neuronal firing with TTX does not affect Nup358 profile, whereas, the depolarization of neurons significantly suppresses Nup358 expression. While the mechanism of recruitment of Nup358 at the AIS is still unknown, the current study suggests the involvement of a nucleoporin in neuronal function.

BACK

# Ataxia Telangiectasia Mutated (ATM) Kinase Phosphorylates the Actin Binding Protein Drebrin in Response to Cellular Stress

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Human aging as well as age-related pathologies such as Alzheimer's disease are characterized by loss of synaptic connectivity. Synaptic plasticity is accompanied by changes in dendritic spine morphology which is highly regulated by actin dynamics. Several regulators of actin cytoskeleton at the synapse have been identified of which Drebrin (DBN). Progressive loss of DBN in the brain is linked to Mild Cognitive Impairment and major loss is characteristic of patients with Alzheimer disease and Down's syndrome. Given that DBN is thought to play a key role in setting the degree to which CNS synapses respond to neural activity, decreases in DBN abundance may underlie synaptic dysfunction associated ageing and/or degenerative conditions.

We recently identified DBN as a PTEN interacting protein. Our work demonstrated that the PTEN:DBN interaction is of direct nature and that it regulates a previously uncharacterized DBN phosphorylation event in the DBN c-terminus (Serine 647). Using a phosphorylation-site-specific antibody, we demonstrated that neuronal activity increases phosphorylation of DBN at S647 in hippocampal neurons in vitro and in ex vivo hippocampus slices exhibiting seizure activity. However, an assessment of the functional implication of pS647-DBN is currently missing.

We demonstrate here that phosphorylation on S647 decreases DBN protein turnover. Using AHA-labeling, we demonstrate that a S647→A mutant showed decreased protein turnover compared to the S647→D mutant and the wild type protein. Our results reveal a mechanism by which enhanced phosphorylation prolongs DBN half live. We further identify ataxia telangiectasia mutated kinase (ATM) as a kinase which can phosphorylate DBN at S647. Interestingly ATM kinase which is well known to respond to DNA damage also acts in the cytoplasm and is activated upon oxidative stress. Using *in vitro* kinase assays we show that ATM can specifically and directly phosphorylate S647-DBN. Furthermore upon oxidative stress and elevated neuronal activity ATM can increase pS647-DBN.

Altogether we propose that the phosphorylation of DBN at S647 may locally increase the stability of DBN and in turn protect the neurons from the effects of age related cellular stress.

**BACK**

**Poster - 053**

# Homeostatic Structural Plasticity Regulation by Reactive Oxygen Species

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Neurons are inherently plastic. In response to activity changes neurons adjust both their electrical properties and the size and structure of their synaptic arbors. Such adjustments are usually homeostatic promoting network stability and physiologically appropriate function. Homeostatic changes to electrical and transmitter release properties have been studied extensively. Much less is known about the mechanisms regulating structural adjustments.

Working with identified motorneurons in the *Drosophila* larva we study structural adjustments in response to elevated activity across the entire cell, both its postsynaptic dendritic arbors in the CNS and presynaptic neuromuscular junctions (NMJs). We discovered that motorneurons use Reactive Oxygen Species (ROS), a constitutive by-product of mitochondrial ATP synthesis, as a readout for neuronal activity. We find that hydrogen peroxide ($H_2O_2$) in particular is necessary and sufficient for activity-dependent regulation of synaptic terminal growth. Activity-induced structural changes to postsynaptic and presynaptic terminal arbors are adaptive and co-ordinated.

We next identified a putative redox sensor, the Parkinson's disease-linked protein DJ-1b. Redox sensitive DJ-1b, in response to elevated $H_2O_2$, inhibits the lipid phosphatase PTEN and thus increases PI3K signalling. PTEN and PI3K have been extensively linked with neuronal growth and energy metabolism and are therefore perfectly placed to expedite homeostatic growth in response to elevated neuronal activity and ROS.

Until recently, ROS were primarily considered to be a tolerated burden, rapidly removed by a range of cellular ROS-buffering and scavenger systems. Our work suggests a role for ROS during normal development and function, acting as important second messengers that regulate homeostatic synaptic terminal growth.

BACK

**Poster - 054**

# Afferent-derived Insulin and Activin Antagonistically Regulate the Dendritic Field Sizes of Amacrine Neurons in *Drosophila*


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During development, CNS neurons elaborate dendritic arbors in stereotypic patterns to synapse with appropriate partners. Little is known about how extracellular cues couple with intracellular signaling to orchestrate dendritic development and how dendritic patterning defects disrupt neural circuit assembly. We use the first-order interneuron Dm8 in the *Drosophila* visual system as a model to tackle these questions. Each Dm8 neuron elaborates a large dendritic tree in the medulla layer 6 and receives synaptic inputs from ~14 R7 photoreceptors to mediate ultraviolet light preference behavior. In a forward genetic screen, we identified that insulin/Tor (Target of Rapamycin) signaling pathway positively regulates dendritic field of Dm8. Single Dm8 neurons devoid of insulin receptor, its substrate Chico or the downstream signaling components Rheb and Tor, developed a smaller dendritic tree than wild-type did, while mutants lacking the negative regulators, Pten or Tsc1, had larger dendritic trees. Layer-specific dendritic targeting and cell fate of mutant Dm8 remained unaffected, suggesting a specific role of insulin/Tor signaling in regulating dendrite field sizes. Genetic manipulation of insulin/TOR signaling in another medulla neuron, Tm20, did not alter its dendrite morphology or size, suggesting type-specificity. Furthermore, the insulin-like peptide 2 (Dilp2) derived from lamina neurons (LNs), is delivered to the axonal terminals during development and is required for Dm8's normal dendritic development. RNAi-mediated knockdown of Dilp2 in LNs, but not in photoreceptors, reduced the dendritic field size of Dm8 with disrupted morphology. We have previously shown that R7-derived Activin negatively regulates the size of Dm8 dendritic field (Ting et al., 2014). However, mutant Dm8 devoid of both Activin and Tor signaling exhibited additive dendritic phenotype, suggesting that these two signaling pathways operate in parallel. Finally, using a novel activity-dependent receptor-based GRASP (GFP reconstitution across synaptic partners) method to probe active synaptic connections, we found that the enlarged dendritic field of *Pten* mutant Dm8 leads to aberrant connections with neighboring R7s while *Tor* mutant Dm8 receives few if any active synaptic input from R7s. In summary, the size of Dm8's dendritic field is controlled by two parallel signaling pathways operating in opposite directions: Dilp2 derived from non-synaptic partner lamina neurons positively regulates Dm8's dendritic field sizes, while Activin from Dm8's synaptic partner R7s negatively regulates Dm8.



**BACK**

# Ecm29/Proteasome Modulates Axon Initial Segment Plasticity during Early Neuronal Development

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After the axon has been determined, the site of action potential trigger zone—axon initial segment (AIS) emerges in the proximal region of axon. It is known that altering the strength of synaptic input modulates the AIS localization, which in turn changes neuronal excitability. However, the regulatory mechanisms underlying such structure-activity relationship in the developing neurons remain unclear. Here we report that the AIS position is dynamically regulated in the transition of the excitatory-to-inhibition GABA switch via a Ecm29/proteasome dependent mechanism. By using co-immunoprecipitation assays and an AIS-specific fluorescence resonance energy transfer (FRET) assay, we found that the C-terminal domain of the AIS scaffold protein ankyrin-G (AnkG) is required for its association with proteasome adaptor Ecm29 and proteasome complexes. Prior to day-7 in vitro (DIV7), Ecm29 knock-out (KO) neurons exhibited a proximal shift of AIS with a significant reduction in the distance between soma and AIS start position. In addition, DIV5-7 Ecm29 KO neurons generate significant higher GABA-evoked calcium influx than wild-type neurons, implying an excessive activity of excitatory/depolarising GABA response. Consistently, immunofluorescence staining and western blotting showed significantly higher levels of sodium potassium chloride cotransporter (NKCC1), but comparable KCC2, in Ecm29 KO neurons than wild-type neurons. Furthermore, symptoms such as hyperlocomotion and anti-anxiolytic-like behaviors were observed in Ecm29 KO mice. Together, these findings suggest a developmental regulation on AIS maturation mediated by the activity of Ecm29/proteasome, wherein may fine-tune the excitability of developing presynaptic neurons.

**BACK**

# A PTEN-associated Membrane Protein Scaffold Controls Membrane Phosphoinositides and Axon Morphogenesis

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The PTEN phosphatase represents a key node in promoting growth dependent signaling pathways. By hydrolyzing phosphatidylinositol 3,4,5-trisphosphate ($PIP_3$) to phosphatidylinositol 4,5-bisphosphate ($PIP_2$), PTEN directly antagonizes the activity of phosphoinositide 3-kinase (PI3K) and downstream Akt/mTORC1 signaling. Recently, PTEN has been highlighted as a powerful master regulator of neuronal cell growth. Specifically, inhibition of PTEN results in robust overgrowth phenotypes combining increased cell soma size, altered spine morphology, increased spine density and longer and more branched axons with more elaborate arborization patterns. These phenotypes are associated with migration deficits and ectopic axonal projections in vivo and are highly dependent upon the developmental frame of PTEN inhibition. The latter likely suggests that PTEN is recruited at distinct developmental stages, presumably by different intrinsic/extrinsic cues, to regulate localized growth.

We have identified and characterized a novel PTEN-associated membrane protein scaffold which serves specifically in regulating PTEN activity along the neuronal axon during development. We demonstrate that PRG2, a transmembrane protein belonging to the lipid phosphate phosphatase / phospho-transferase (LPP) family, inhibits PTEN activity and spatially controls $PIP_3$-mediated cellular responses in cells. In vitro, PRG2 directly inhibited PTEN activity and antagonized PTEN-induced changes in membrane $PIP_3$ levels and $PIP_3$-dependent formation of F-actin-rich protrusions. In embryonic stem cell-derived neurons, overexpression of PRG2 increased the formation of axonal branches and filopodia in a PI3K signaling-dependent manner. In vivo, PRG2 is expressed in the developing forebrain during branch formation in different cortical axon projections. Silencing of PRG2 in the progenitors of callosally projecting neurons in upper layers 2/3, greatly inhibited axon collateral formation in layer V, suggesting that PRG2 is essential for proper axonal branching, in a cell autonomous manner in vivo. This result was verified in cultured hippocampal neurons where downregulation of PRG2 resulted in a robust axonal branching defect. Our combined data suggest that association of PTEN with PRG2 along axons organizes spatially and temporally PI3K-dependent growth during neuronal development.

BACK

**Poster - 057**

# Understanding the Roles of Kinesin-1 during Axon Degeneration

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Axons are the longest cellular processes in animals which often have to be maintained for an organism's lifetime, despite the fact that their delicate structure renders axons vulnerable to degeneration. Mutations of the MT-based motor protein kinesin-1 have prominent links to axon degeneration, but the underlying pathomechanisms remain unclear. Normally, axons contain parallel bundles of microtubules (MTs) which form their structural backbones and tracks for long distance transport, whereas kinesin-1 mutant axons show swellings with disorganised MT bundles. We propose that these disorganised MTs are not a consequence but a potential cause of axon degeneration. We test this hypothesis in *Drosophila* primary neurons which we have developed into a powerful model for studying axonal cytoskeletal machinery (*Prokop et al., 2013, J. Cell Sci. 126, 2331ff.*).

In fly axons, kinesin-1 depletion leads to reduced axon growth and areas of disorganised MTs, which we propose as a potential model for axon swellings. Using point mutations in kinesin-1 or genetic depletion of specific interaction partners, we assessed the contribution of three sub-functions of kinesin-1 towards MT regulation: (1) MT sliding, (2) cargo transport or (3) mitochondrial dynamics. We found that (2) and (3), but not (1), cause MT disorganisation. Furthermore, we found that kinesin-3 contributes to those two functions, and that it strongly interacts with kinesin-1 during MT disorganisation, suggesting that the observed phenomenon might link to a wider pool of axonal transport motors.

We will discuss our newest investigation results into the underlying pathomechanisms, focussing on mitochondrial ROS, loss of OxPhos- or glycolysis-mediated ATP production and/or functional inhibition of other classes of MT regulators, such as MT stabilisers or severers. We believe that an understanding of the roles of motor proteins in MT regulation will provide fundamental new understanding of axon biology, and that *Drosophila* is the most useful tool to achieve this goal.

BACK

# Tie2-receptor is expressed in Purkinje Neurons and Contributes to the Development of its Dendritic Arbor

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The cerebellum is not only composed of different neural cell types but also contains a functional vascular network, which develops in parallel to the formation and wiring of the cerebellar neural network. Angiopoietins and their receptors are well known for their role in building and maintaining a functional vascular system, however, whether they can have a direct effect on neurons remains largely unexplored. We provide evidence that the Angiopoietin-receptor Tie2, apart of being expressed in cerebellar endothelial cells, is also expressed at low levels in Purkinje neurons. In addition, our results show that during cerebellar development the ligand Angipoietin-1 is expressed in glia cells whereas Angiopoietin-2 localizes in endothelial cells. Using *ex vivo* organotypic cerebellar slice cultures we show that stimulation with Angiopoietin-2 changes the dendritic arborization of Purkinje neurons. To verify if this effect on the dendritic morphology is due to the activation of Tie2-receptor in Purkinje cells, we generated a Purkinje cell specific Tie2 knockout mouse line (Tie2floxed x Pcp2Cre). Purkinje neurons of this line were *in vivo* labeled through viral transfection at P7 using $AAV_8$-GFP and dendritic morphology was analyzed at P21. Indeed, *in vivo* the Purkinje cells of this line show an aberrant dendritic patterning, with high number of overlapping dendritic branches. This morphological phenotype is accompanied by changes in the properties of glutamatergic synapses. Taken together, we provide evidence that the Angiopoietin-receptor Tie2 is not just expressed by endothelial cells, but also at low levels in neurons. We show that Tie2-receptor expression in Purkinje neurons concomitant with Angiopoietin-signaling during the early postnatal period, contributes to the proper dendritic development of these neurons. These results indicate that Angiopoietin-Tie signaling represents a direct mechanism of neuro-vascular communication, which need to be further investigated in the future.

**BACK**

**Poster - 059**

# Inhibition of Activity Dependent Proteolysis Controls Synaptic Plasticity

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Proteolysis is mandatory for a variety of cellular and physiological phenomena, whereas, excessive proteolytic activity is detrimental to the cells and tissues. Thus, tight control of this enzymatic activity, including function of specific endogenous inhibitors, plays fundamental biological role in preventing cell and tissue damage. Activity-dependent proteolysis at a synapse has been recognized as a pivotal factor in controlling dynamic morphological alterations in dendritic spines, bearing excitatory synapses. Here, we reveal that dendritic spine maturation is strictly controlled by the proteolytic activity at synapse, and its inhibition by Tissue inhibitor of matrix metalloproteinases-1 (TIMP-1). We show that dendritic spine maturation involves an intermediate formation of elongated spines and is concluded by the formation of mushroom-shaped spines with increased level of AMPA/NMDA ratio of glutamate receptors. We also show that, excessive proteolytic activity impairs long-term potentiation of the synaptic efficacy (LTP), and this impairment could be rescued by inhibition of protease activity. Moreover LTP is altered persistently when the ability of TIMP-1 to inhibit protease activity is abrogated, further demonstrating the role of such inhibition in the promotion of synaptic plasticity under well-defined conditions Altogether, our results identify inhibition of protease activity as a critical regulatory mechanism for dendritic spines maturation.

BACK

**Poster - 060**

# Steroid Hormone Ecdysone Signalling is Necessary for Mushroom Body Neuron Sequential Specification Mediated by Chinmo

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The functional variety in neuronal composition of an adult brain is established during development. Recent studies proposed that interactions between genetic intrinsic programs and external cues are necessary to generate proper neural diversity. However, the molecular mechanisms underlying this developmental process are still poorly understood. The three main subtypes of *Drosophila* mushroom body (MB) neurons are sequentially generated during development and represent a good example of developmental neural plasticity. Our present data propose that the environmentally controlled steroid hormone ecdysone functions as positive regulator of early-born MB neuron fate during larval-pupal transition. We found that the transcription factor Chinmo acts upstream of ecdysone signalling to promote a neuronal fate switch. Indeed, Chinmo regulates the ecdysone receptor B1 isoform expression to mediate the production of γ and
α'β' MB neurons. Finally, we provide genetic evidence for a regulatory negative feedback loop driving α'β' to αβ MB neuron transition in which the microRNA *let-7* modulates the Chinmo expression in an ecdysone signalling-dependent manner. Thus, our results uncover a novel interaction in the MB neural specification pathway for temporal control of neuronal identity by interplay between a hormonal signal and an intrinsic transcription factor cascade.

BACK

**Poster - 061**

# Molecular Mechanisms behind the Forces Driving Axon Growth

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Growth cones (GC) are motile devices at the tips of developing axons that integrate guidance cues and translate them into directional movements towards their synaptic targets. It is well accepted that GC advance requires signalling, cytoskeletal dynamics, adhesions, and the forces that this machinery generates. However, the fundamental question of whether axon growth is implemented through the pulling of GCs on axon shafts or whether axon shafts generate pushing forces that are controlled and guided by GCs, remains unresolved. To answer this question, we study GC adhesions as important hubs for signalling and force generation, and their close links to cytoskeletal dynamics. I make use of *Drosophila* primary neurons as evolutionarily well conserved models for axon growth. They provide efficient genetics for dissecting underlying mechanisms, as has been successfully demonstrated for studies of the roles and regulation of actin and microtubules (Prokop *et al.*, 2013, *J. Cell Sci.* 126, 2331ff.; Voelzmann *et al.*, 2016, *Brain Res Bulletin* 126, 226ff.). To introduce adhesions into this model, mammalian extra-cellular matrix (ECM) is not effective, but ECM harvested from *Drosophila* Kc cell lines causes a strong increase in axon growth and GC morphology, especially if further enriched for laminin. These changes are absolutely dependent on the αPS1/βPS integrin laminin receptor, but not dystroglycan or syndecan. These prominent readouts are ideal for studying the roles of integrin adhesions at GCs with access to readily available genetic tools for their downstream machinery (including talin, vinculin, paxilin, Src, FAK, RhoA, myosin II). My results so far suggest that GCs do not pull on axons to achieve extra growth, but that integrins are more likely to trigger talin-dependent signalling events which regulate microtubules behaviours, partly directly and partly through regulating actin dynamics in GCs.

Supported by the BBSRC

BACK

**Poster - 062**

# Ras and Rab Interactor 1 Controls Neuronal Plasticity by Coordinating Dendritic Filopodial Motility and AMPA Receptor Turnover

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Ras and Rab interactor 1 (RIN1) is predominantly expressed in the nervous system. RIN1 knockout animals have deficits in latent inhibition and fear extinction in the amygdala, suggesting a critical role for RIN1 in preventing the persistence of unpleasant memories. At the molecular level, RIN1 signals through Rab5 GTPases that control endocytosis of cell-surface receptors and Abl non-receptor tyrosine kinases that participate in actin cytoskeleton remodelling. Here, we report that RIN1 controls the plasticity of cultured mouse hippocampal neurons. Our results show that RIN1 affects the morphology of dendritic protrusions and accelerates dendritic filopodial motility through an Abl kinase dependent pathway. Lack of RIN1 results in enhanced mEPSC amplitudes indicating an increase in surface AMPA receptor levels compared to wild type neurons. We further provide evidence that the Rab5 GEF activity of RIN1 regulates surface GluA1 subunit endocytosis. Consequently, loss of RIN1 blocks surface AMPA receptor downregulation evoked by chemically induced long-term depression. Our findings indicate that RIN1 destabilizes synaptic connections and is a key player in postsynaptic AMPA receptor endocytosis, providing multiple ways of negatively regulating memory stabilisation during neuronal plasticity.

**BACK**

**Poster - 063**

# Dynamin Inhibitors Impair Dense-core Vesicle but not Synaptic Vesicle Exocytosis

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Dense-core vesicles (DCVs) release neuropeptides and other bioactive proteins by regulated ($Ca^{2+}$-dependent) exocytosis. While synaptic vesicles (SVs) recycle locally at the synapse, DCVs are considered not to recycle. Therefore, we hypothesized that DCV exocytosis is not blocked by inhibition of endocytosis.

In this study we used live cell imaging in hippocampal neurons expressing the DCV exocytosis marker NPY-pHluorin or the SV exocytosis marker Synaptophysin-pHluorin and two dynamin inhibitors, dynasore and dyngo-4a, to test this hypothesis. These compounds inhibit dynamin 1 and 3, essential proteins in SV endocytosis. We show that, as expected, both drugs do not affect SV exocytosis and strongly reduce SV endocytosis.

In contrast to the lack of effect on SV exocytosis, both drugs strongly reduced DCV exocytosis (dynasore > 80% reduction, dyngo-4a > 50% reduction). In addition, dynasore and dyngo-4a increased the probability of long-lasting fusion events but did not affect the location of the remaining fusion events.

Dynamin inhibition using small chemical inhibitors showed a strong impairment of dense-core vesicle exocytosis and a decreased rate of endocytosis, while leaving SV exocytosis unaffected. Hence, in addition to its function in vesicle endocytosis and control of fusion pore expansion our data suggest a role of dynamin 1 and 3 in controlling neuronal dense-core vesicle fusion.

BACK

**Poster - 064**

# CXCL12α/SDF-1 from Perisynaptic Schwann Cells Promotes Regeneration after Motor Axon Terminal Injury

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The neuromuscular junction (NMJ) is a specialized tripartite synapse composed of the motor axon terminal, covered by perisynaptic Schwann cells (PSCs), and the muscle fibre, separated by a basal lamina. It is exposed to different kind of injures such as mechanical traumas, pathogens including neurotoxins, and neuromuscular diseases such as amyotrophic lateral sclerosis and immune-mediated disorders, and has retained throughout vertebrate evolution an intrinsic ability for repair and regeneration, at variance from central synapses.

Following peripheral nerve injury, an intense but poorly defined crosstalk takes place at the NMJ among its components, functional to nerve terminal regeneration.

We have recently established a model to study NMJ degeneration and regeneration in mice based on the specific action of α-latrotoxin, a presynaptic neurotoxin isolated from the venom of the black widow spider, which targets specifically the presynaptic terminal causing its complete degeneration[3]. This toxin is a simple and controlled method to induce an acute, localized and reversible nerve terminal degeneration not blurred by inflammation, and can help to identify molecules involved in the intra- and inter-cellular signaling governing NMJ regeneration. To identify crucial factors released by PSCs and the muscle to induce nerve regrowth, we performed a transcriptome analysis of the NMJ at different time points after injection of the toxin. We report here that the mRNA encoding for the alpha isoform of the chemokine CXCL12 is increased in PSCs early after damage. Recombinant CXCL12α strongly promotes the axon growth of spinal cord motor neurons in culture dishes and in microfluidic devices. CXCL12α acts the CXCR4 receptor, which localizes at the tip of the growing axon. Either the intraperitoneal injection of a CXCL12α neutralizing antibody or of a specific CXCR4 inhibitor strongly delay recovery from the muscle paralysis induced by the neurotoxin.

These findings have important implications in the effort to promote recovery of function after different forms of motor axon terminal damage.

BACK

**Poster - 065**

# Restoring Integrin Transport in the Axon of Corticospinal Tract Neurons by Demolition of the Axon Initial Segment

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Integrins are cell surface receptors and promote axonal regeneration after injury of the nervous system[1]. It has been shown that activated integrins promote long-distance regeneration of sensory axons into the spinal cord[2]. My study aims to use the same approach of overexpression of integrin and its activator kindlin in the corticospinal tract (CST). Upper motor neurons are more difficult to regenerate however, they are key to restore function after spinal cord injury. One major difference between dorsal root ganglion and cortical neurons is that cortical neurons have an axon initial segment (AIS) that excludes growth-associated molecules from their axon, amongst others integrins[3,4]. The cytoskeletal scaffold protein ankyrin G (AnkG) is crucial to sustain the AIS[5,6]. **The objective of this study is to improve regeneration of CST axons after injury by a temporal destruction of the AIS via AnkG knock out allowing integrin transport in their axon.** We have two tools available to knock-out AnkG: (1) a clustered, regular interspaced, short palindromic repeat (CRISP)-associated endonuclease 9 from *Staphylococcus aures* (saCas9) in an single adeno-associated viral (AAV) vector that also drives the expression of the guide RNA that targets AnkG and; (2) conditional AnkG knock-out mice. We showed that the axon initial segment is demolished upon AnkG knock out by using SaCas9-AnkG *in vitro* by immunofluorescence staining against AnkG and Neurofascin (an AIS marker). Next, we will knock out AnkG *in vivo* by AAV1 mediated delivery of SaCas9-AnkG in rats. Currently, we are knocking out AnkG in conditional AnkG knockout mice by AAV1 mediated Cre-recombinase delivery into the sensorimotor cortex. If one or both *in vivo* strategies are successful we will combine this with AAV1 mediated overexpression of alpha9 integrin and kindlin to examine whether this will result in increased regeneration of the CST.

**BACK**

1. Nieuwenhuis, B. *et al.* Integrins promote axonal regeneration after injury of the nervous system. *Front. Mol. Neurosci.* (**in prep**).
2. Cheah, M. et al. Expression of an Activated Integrin Promotes Long-Distance Sensory Axon Regeneration in the Spinal Cord. J. Neurosci. 36, 7283–7297 (2016).
3. Franssen, E. H. P. et al. Exclusion of Integrins from CNS Axons Is Regulated by Arf6 Activation and the AIS. J. Neurosci. 35, 8359–8375 (2015).
4. Andrews, M. R. et al. Axonal localization of integrins in the CNS is neuronal type and age dependent. eneuro ENEURO.0029–16.2016 (2016). doi:10.1523/ENEURO.0029-16.2016
5. Hedstrom, K. L., Ogawa, Y. & Rasband, M. N. AnkyrinG is required for maintenance of the axon initial segment and neuronal polarity. The Journal of Cell Biology 183, 635–640 (2008).
6. Sobotzik, J.-M. et al. AnkyrinG is required to maintain axo-dendritic polarity in vivo. Proc. Natl. Acad. Sci. U.S.A. 106, 17564–17569 (2009).

**Poster - 066**

# The Endocytosis Coordinated with Filopodial Formation in the Growth Cone, revealed by Superresolution Microscopy

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During axon growth, the continuous reorganization of F-actin and membrane trafficking in the growth cone should be performed in its leading edge. These two events are thought to be essential to the precise navigation of an axon, however, the relationships between them are not clearly understood. To observe their coordinatory movements, we analyzed the dynamics of two endocytic components; 1) clathrin, 2) a BAR domain protein endophilin A3 (Endo3); and 3) F-actin in the growth cone of NG108-15 cells, using a superresolution microscopy 3D-SIM, and TIRFM. Whereas clathrin mainly accumulated in the basal membrane at the central domain, Endo3 appeared in the dorsal surface at the leading edge. The accumulation of Endo3 coincided with the F-actin bundling. When actin polymerization was inhibited by cytochalasin B or CK-666, Endo3 markedly reduced. RNAi against fascin, a cross-linker protein of F-actin, also reduced the numbers of Endo3 localized at the leading edge. GFP-synaptophysin (Syp) arose near the root portions of filopodia, and most of them were retrogradely moving along the actin bundles. The retrogradely moving Syp puncta were colocalized with Endo3 and dynamin 1 (Dnm1), at the leading edge, but not with clathrin. RNAi against Endo3 reduced Syp puncta from the leading edge, although Pitstop2, a clathrin inhibitor, did not affect the Syp distribution. The dominant-negative Dnm1 mutant also affected the lifetime of Syp. Interestingly, in the primary cultured neurons, Endo3 knockdown inhibited axonal growth, and reduced the growth cone size. These results suggest that there is a novel mechanism of membrane retrieval in the leading edge of a growth cone, namely, the Syp-positive vesicle production by Endo3-, Dnm1-dependent and clathrin-independent endocytosis occurred at the apical membrane there, coinciding with filopodial formation, and that these vesicles are moving along the actin retrograde flow.

**BACK**

**Poster - 067**

# Microtubule Function of Tau and Shot/spectraplakin is Essential in Synapse Formation and Maintenance during Ageing

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Parallel bundles of microtubules form the structural backbones of neurons, also crucial for axonal growth and live sustaining transport. Various factors involved in microtubule bundle maintenance (e.g. MAP1B, Tau, Kinesins, Spectraplakins, Stathmin, Spastin) are linked to neurodegenerative diseases, suggesting that aberrations of microtubule bundles are a likely cause for neurodegeneration. We use highly efficient *Drosophila* genetics to decipher their roles and regulatory networks to better understand axon biology and potential causes for neurodegeneration.

Here we report joined roles of *Drosophila* Tau (*D*Tau) and the Spectraplakin Shot in maintaining microtubule bundles with important implications also for synapse formation and maintenance: their combined loss causes radical aberrations of microtubule stability, bundling and acetylation which, in turn, causes a dramatic shift of activated JNK from synaptic axonal locations to neuronal cell bodies. This ectopic JNK activation causes a somatic roadblock for Kinesin 3-mediated axonal transport of synaptic proteins, thus starving synapses of their building blocks and inhibiting their formation during development and maintenance in ageing.

We propose JNK as a general regulator of synapse localisation, formation and maintenance, and test potential mechanisms underlying this function. To this end, we have developed an axonal model in the fly brain displaying hallmarks of ageing in unprecedented clarity. These include gradual loss of synaptic JNK, aberrations of microtubule bundles and *D*Tau localization, and the formation of *D*Tau tangle-like structures, all strongly enhanced by the expression of disease related variants of human tau and oxidative stress.

BACK

**Poster - 068**

# DNA Damage-induced Necrotic Neurodegeneration during Ageing

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Accumulation of DNA damage is a key determinant of ageing and has been implicated in neurodegeneration. Although it is well known that ultraviolet (UV) radiation induces apoptosis, the contribution of necrotic cell death to DNA damage-related pathology remains elusive. To address this question, we developed a nematode model for DNA damage-induced neurodegeneration by using UV-C irradiation to trigger DNA damage in *C. elegans* neurons. Initial observations using this model show a marked increase of cytoplasmic calcium concentration upon UV irradiation. To examine whether this acute cytoplasmic calcium elevation triggers necrosis in neurons, we exposed DNA repair-defective mutants to UV light. These mutant animals are hypersensitive to UV irradiation and exhibit widespread necrotic cell death in somatic tissues upon exposure, while neurons are particularly affected. Runaway autophagy has previously been implicated in necrotic neurodegeneration. In this context, we investigated the contribution of autophagy in DNA damage-induced cellular pathology and nuclear dynamics. Notably, we found that DNA damage induces autophagic flux and alters nuclear dynamics both in nematodes and mouse cells. We are currently dissecting the interplay between DNA damage-induced autophagy, nuclear membrane alterations and necrotic cell death, aiming to identify evolutionarily conserved molecular mechanisms interfacing these processes.

**BACK**

**Poster - 069**

# Microfluidic Platform to Investigate Long-distance BDNF Signalling in Neurodegeneration

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Neuronal processes extend over large distances forming highly organised and connected networks. To ensure the survival, growth and maintenance of these neuronal connections, growth factors are secreted, internalised and signal in an autocrine and paracrine manner. Brain-derived neurotrophic factor (BDNF) is a growth factor in the mammalian brain that can promote neuronal growth, strengthen synaptic connections and drives structural changes by binding to tropomyosin-related tyrosine kinase B (TrkB) receptor. The loss of BDNF trophic support has been implicated in many neurodegenerative diseases and as a result, upregulating BDNF has been considered a possible therapeutic approach. However, it is yet to be determined to what degree long-distance BDNF signalling can still be processed by a degenerating cell and stimulate growth-promoting effects.

Once BDNF binds to TrkB receptor, the ligand-receptor complex can signal both locally and over long distances. For example, if BDNF binds to TrkB at the distal end of an axon, the signal can undergo retrograde transport from the nerve terminal to the soma to elicit the growth-promoting effects. This type of signalling is referred to as "long-distance signalling". To investigate this in neuronal mass culture poses multiple challenges. Thus, an experimental system that fluidically isolates individual components of a cell is required. Microfluidic devices enable such fluidic isolation of the axon from the somatodendritic compartment. Therefore, changes in gene activation and signalling can be probed at the soma upon BDNF application at axonal ends in degenerating cells, allowing investigation of long-distance retrograde signalling.

To mimic degeneration in culture, we are using an EGFP-TauP301L construct that results in tau aggregation along the axon, disrupting cargo transport. This is a mutation identified in Frontotemporal-lobe dementia (FTLD). We conducted live-cell imaging of BDNF-RFP axonal transport in primary neurons to monitor axonal integrity in EGFP-TauP301L-expressing cells. We have monitored changes in TrkB receptor surface expression in TauP301L-expressing cells and will go on to analyse the possible changes in long-distance signalling using the microfluidic platform. This will allow us to understand how degenerating cells process BDNF signals and whether the signals can propagate over long distances in the brain. This will provide broader understanding of the signalling mechanisms failing in neurodegenerative diseases and provide insight into how neurotrophic factors can be used as a potential therapeutic strategy.

BACK

**Poster - 070**

# In-depth Study of the Molecular Events underlying mGluR-LTD by Combining PulsedSILAC/AHA Labeling and Phosphoproteomics

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At neuronal synapses, activation of metabotropic glutamate receptors (mGluR1/5) triggers a form of long-term depression (mGluR-LTD) that relies on new protein synthesis and the internalization of AMPA-type glutamate receptors (AMPARs). Dysregulation of these processes has been implicated in the development of mental disorders such as autism spectrum disorders (ASD), and therefore require a better understanding. mGluR-LTD has so far mostly been studied by fluorescence imaging and Western-blot approaches. It would be valuable to establish a complete overview of molecular events involved in mGluR-LTD. Here, we applied a proteomics approach to study mGluR-LTD in a detailed and comprehensive manner, using high-resolution LC-MS/MS in combination with a pulsed SILAC and AHA labeling strategy, in both wildtype and Shank knockdown hippocampal neurons. In this strategy, methionine is depleted from the medium and replaced by an analogue azidohomoalanine (AHA), which contains an azido moiety that can be used to enrich newly synthesized proteins (that incorporate AHA) and distinguish these from pre-existing ones.

Rat hippocampal neurons were stimulated with the mGluR1/5 agonist DHPG and harvested at different time points. A highly sensitive phosphoproteomics workflow with Fe(III) IMAC was applied to study the phosphoproteome during mGluR-LTD. Newly synthesized proteins upon stimulation were enriched and analyzed by applying a pulsed SILAC and AHA-LC-MS strategy.

Stimulation of mGluR1/5 triggered a long-lasting decrease in AMPAR surface expression. The applied proteomics approach allowed for the first time to study the molecular events underlying mGluR–LTD in a comprehensive matter in both normal and autism related Shank knockdown neurons. We observed a very high correlation between phosphorylation dynamics and protein synthesis upon mGluR stimulation. We identified several phosphorylation changes in pathways leading to mTORC (e.g.Tsc2 was strongly phosphorylated upon DHPG stimulation) and Erk translational regulation. Furthermore changes in the AMPA receptor endocytosis pathway in both protein synthesis and phosphorylation upon LTD were identified. We thereby identified a novel player in this pathway, which was validated to play an important role in AMPA trafficking during mGluR-LTD. Combining these results enhanced the understanding of cellular pathways leading to mGluR-LTD in general and in an Autism phenotype, this can facilitate in better understanding the molecular events involved in ASD and other intellectual disabilities.

BACK

**Poster - 071**

# The Role of Neuronal DEG/ENaC Ion Channel Family Members in Organismal Stress Responses

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The integration of sensory stimuli to appropriately modulate behavioral responses to environmental signals is critical for organismal survival. The molecular mechanisms that underlie such responses are not fully understood. Dopamine signaling is involved in several forms of behavioral plasticity. In Caenorhabtitis elegans the functionality of the dopamine and serotonin pathways can be easily assessed by monitoring specific locomotory responses to environmental food availability cues, termed basal and enhanced slowing. We found that degenerin ion channel proteins expressed in dopaminergic and other sensory neurons modulate basal and/or enhanced slowing responses. Degenerin effects are largely influenced by stress conditions, such as heat and starvation. Notably, the stress response transcription factors DAF-16/FOXO and SKN-1/Nrf couple degenerin ion channel function to environmental conditions and behavioral output.

BACK

**Poster - 072**

# Overexpression of Protrudin in Primary Cortical Neurons results in Altered Neuronal Morphology and in Improved Regeneration after Laser Axotomy

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During development, neurons are fully equipped with growth machinery to extend long axons, reach their target cells and form functional connections. Once these connections have been established, the elongation capacity of neurons declines dramatically. One reason why adult CNS axons have poor regenerative capabilities might be that a developmental change occurs where essential growth molecules such as integrins become excluded from axons. Our laboratory is particularly interested in elucidating the transport mechanisms and the machinery needed to transport integrins and other growth-associated molecules to the tip of injured axons in order to design strategies to promote regeneration. Protrudin, a newly discovered member of the ZFYVE family of zinc-binding proteins, is a peripheral membrane protein involved in neurite outgrowth and directional membrane trafficking in HeLa and PC12 cells (Shirane *et al.*, 2006). Interestingly, phospho-protrudin binds to a small GTPase, Rab11 which is involved in selective trafficking of growth-associated cargo along axons and this interaction is necessary for neurite outgrowth. Here, we hypothesise that promoting the association of protrudin with Rab11 (by creating phosphomimetic forms of protrudin) will result in an increased anterograde axonal transport of growth molecules which will potentially lead to increasing the regenerative capacity of mature cortical neurons. Firstly, the localisation of endogenous protrudin in cortical neurons was studied with maturation – as neurons mature, protrudin seems to be downregulated in axons compared to dendrites, which is a phenomenon observed with integrins and Rab11 distribution as well. Interestingly, protrudin seems to be localised to the proximal part of the axon in mature cortical neurons. Furthermore, overexpression of the phosphomimetic forms of protrudin resulted in morphological changes in dendrites by creating a complex dendritic branching in the form of "hairy" structures. Wild-type and phospho-protrudin both resulted in increased (60-70%) and faster (4-6hrs) regeneration compared to control neurons (30%, 8-10hrs) in 14-17DIV cortical neurons. The effects of phospho-protrudin overexpression on Rab11-dependant integrin transport and microtubule dynamics are currently being studied.

Shirane M., Nakayama K. (2006) Protrudin induces neurite formation by directional membrane trafficking. *Science*, **314:** 818-821.

**BACK**

**Poster - 073**

# Differential Distribution and Function of the +TIP Proteins EB1 and EB3 in Cortical Neurons Undergoing Neuritogenesis

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To differentiate into the elaborate, polarised morphology of adult neurons, new-born neurons must first break their spherical symmetry and grow axons and dendrites. This process, referred to as neuritogenesis, begins with the emergence of a growth cone at the cell surface, which subsequently grows away from the cell body leaving behind a neurite. The dynamic interaction of both the actin and microtubule cytoskeletons is crucial for neuritogenesis. The drebrin/EB3/Cdk5 pathway is a candidate pathway for co-ordinating the dynamic behaviour of actin filaments (F-actin) and microtubules during neuritogenesis (Geraldo *et al*., 2008). Drebrin is able to directly bind F-actin and the +TIP microtubule-binding protein EB3 when bound to microtubule plus-ends. We mapped the binding site of EB3 on drebrin and used this information to derive dominant negative constructs of drebrin similar to the dominant negative constructs we have already developed for EB3 (Geraldo et al., 2008). Drebrin does not bind to EB1. In cultured embryonic cortical neurons, dynamic microtubules frequently enter the lamellipodium and occasionally run alongside F-actin bundles in filopodia during neuritogenesis. We determined the distribution of EB1 and EB3 in embryonic cortical neurons in culture by confocal fluorescence microscopy. Unexpectedly, we found that EB1 is more distally located at the plus-ends of dynamic microtubules than EB3 and occupies a shorter distance along the microtubule. Furthermore, we found evidence of a competition between EB1 and EB3 for microtubule territory as revealed by over-expression and knockdown experiments. EB1 recognises GTP-bound tubulin in the GTP-cap region at the plus-end of microtubules. The more proximal binding of EB3 is consistent with EB3 recognising GDP-bound tubulin. The differential localisation of these two +TIP proteins on the same microtubule has implications for the consequences of drebrin binding to EB3. One consequence of EB1 being more distally located on the microtubule lattice than EB3 is that microtubule growth would probably not be impeded when cross-linked through the drebrin/EB3 pathway to actin filaments at the base of a filopodium. As the microtubule extends into the filopodium, iterative proximo-distal addition of drebrin/EB3-mediated cross-links would produce a zippering-up effect of the microtubule to F-actin in the filopodium. In confirmation of a zippering-up effect we have observed a dynamic, proximo-distal extension of drebrin into filopodia.

Geraldo, S., Khanzada, U. K., Parsons, M., Chilton, J. K. & Gordon-Weeks, P. R. (2008). Targeting of the F-actin-binding protein drebrin by the microtubule plus-tip protein EB3 is required for neuritogenesis. *Nat. Cell Biol*., **10**, 1181-1189.

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BACK

**Poster - 074**

# Subcellular RNA-Proteome Mapping Reveals TOP Motifs and mTOR Are Specific to Axon Growth Cones

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The formation of circuits throughout the nervous system relies heavily on molecular machinery localized in growth cones (GCs), the subcellular specializations at the tips of growing axons. Subsets of each neuron's transcriptome and proteome localize to GCs to implement extension and guidance of axon projections toward their specific targets. Direct access to these sub-transcriptomes and sub-proteomes with circuit-specificity has remained experimentally challenging. Toward these ends, we developed a new approach termed "GC sorting and RNA-Proteome mapping" to investigate the local networks of molecules in nascent projections in the brain. We achieve this by combining in utero labeling, subcellular fractionation, small particle sorting, RNA-seq, and Mass-spec to obtain ratiometric measurements of transcriptome and proteome distributions from select native projections. We applied this new approach to investigate the molecular machineries driving circuit development in long-range projections connecting the two hemispheres of the mouse cerebral cortex. We observed that native cortical GCs possess the molecular constituents for local synthesis, folding, and turnover of select protein classes. We additionally identified that mTOR, a hub molecule regulating cell growth, specifically localizes to axon GCs in developing cortical neurons, and that mRNA classes broadly distribute within these neurons based on their sensitivity to mTOR for translation. mTOR-hypersensitive transcripts, which contain TOP motifs and mostly encode ribosomal proteins, map specifically to GCs and not to cell bodies, while mTOR-resistant transcripts map inversely. Given the importance of mTOR for axon growth and regeneration, this subcellular organization may have implications for future regenerative strategies in the nervous system. Additionally, multi-color GC sorting enabled us to differentially compare local molecular networks in projections having diverging targets, an approach that can be applied to a broad range of circuits, to specific projections in distinct genetic backgrounds, or to native vs. reprogrammed neuron subtypes in order to identify circuit-specific substrates of wiring, miswiring, and rewiring.

BACK

**Poster - 075**

# Novel Concepts of Cytoskeleton Regulation during Neuronal Growth, Maintenance and Degeneration

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Axons are key lesion sites during ageing, injury, and many neurodegenerative diseases, not least due to their unique architecture which has usually to be sustained for an organism's lifetime. Unsurprisingly, we gradually lose 50% of our axons towards higher age. The structural backbones of axons are formed by parallel bundles of microtubules (MTs) which also serve as the highways for long distance transport. Loss of this bundled organisation is one likely cause for axon decay and, accordingly, axon swellings with disorganised MTs can be observed in ageing and neurodegenerative diseases. We study the mechanisms that form and maintain axonal MT bundles, focusing on different classes of MT-binding proteins, as well as cross-regulation through the actin cytoskeleton. To decipher this complex machinery we capitalise on efficient *Drosophila* genetics which has enabled us to obtain functional data for ~50 MT- and actin-binding proteins so far.

From this work, we deduced the model of local axon homeostasis. It proposes that MTs in axons are disorganised by default, and only through the role of MT regulators are they 'tamed' into bundled arrangements (Voelzmann et al., 2016, Brain Res Bulletin 126, 226ff.). For this, a key mechanism we discovered is provided by the spectraplakin Shot, a large actin-microtubule linkers which guides the extension of polymerising MTs along cortical F-actin, thus directly laying axonal MTs out into parallel bundles. Accordingly, axon swellings with disorganised MTs are a hallmark of degenerating neurons in mutant mice lacking the spectraplakin dystonin (a model for type VI HSAN; OMIM #614653). Here we propose two further mechanisms of bundle maintenance: (1) A membrane-anchored cortical collapse factor serves as a check point, eliminating "off track" MTs that have escaped the guidance mechanism. (2) MT polymerisation and turnover is required to maintain healthy axonal bundles, and we identified roles of Shot and regularly spaced cortical actin rings which both sustain MT polymerisation events through independent mechanisms.

Supported by the BBSRC

**BACK**

**Poster - 076**

# Fine Tuning Dendritic Arborization – The Role of Regulated Protein Synthesis

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Dendritic arborization of neurons is a critical stage during the development of a nervous system wherein the basic cellular computational machinery is laid out. Although initially thought as a cell autonomous process, recent studies have shown that dendritic morphology is significantly affected by external cues including trophic factors and synaptic inputs. Such morphological changes are predominantly driven by actin cytoskeletal rearrangement mediated by various Actin Binding Proteins. We found that in young cultured neurons BDNF (Brain Derived Neurotrophic Factor) induced change in cofilin activity-a key determinant of actin dynamics- is dependent on new protein synthesis. BDNF has a significant effect on the dendritic arborization which requires extensive actin modifications. Our results indicate the role of activity mediated protein synthesis fine tuning the early neuronal development through regulating actin dynamics. Interestingly, on BDNF stimulation total cofilin levels was unchanged implying of the role of its upstream regulators which are translationally regulated in this process. We studied the translational profile of several upstream actin modulators and found that LIMK1, the kinase responsible for cofilin activity is up regulated in response to BDNF, explaining the dependence of cofilin activity on new protein synthesis.

BDNF is an important trophic factor involved in neuronal survival, neurite maturation and synaptic plasticity, and is known to induce translational changes in mature dendritic spines. But, it is not understood whether immature dendrites use similar ways of regulation during its growth and branching. Our data shows that BDNF also regulates translation of actin modulators in young neurons. We also observed a significant increase in total neurite length in response to BDNF treatment, and we are currently investigating the molecular players involved in translation induced actin dynamics mediating this physiological outcome. Interestingly, even at a basal state some of the actin modulator proteins appears to be turned over rapidly, implying that not only their synthesis but also their degradation kinetics are tightly controlled- thus suggesting a temporal window of regulation. Our findings points out that dendrite patterning is a highly regulated process; and give insights for better understanding of the cellular pathology of some neurodevelopmental disorders, where there is a known defect in translational regulation.

**BACK**

**Poster - 077**

# Modeling Collective Axon Growth from *in vivo* Data reveals the Importance of Physical axon-axon Interactions

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Neurite extension is essential to establish complex neuronal circuits during brain development. In particular, neurons extend long cytoplasmic projections, the axons, in a crowded environment to reach target territories and connect to specific partners. Much work has been performed on cultured isolated neurons, focusing on the response of axon growing ends to chemical guidance cues. However, the cellular mechanisms involved in the growth of axon groups in their natural physical environment (here, the brain), are still poorly understood. Our objective is to shed light on collective axon growth and branching processes in a complex environment, with the help of mathematical modeling.

To study these mechanisms in the context of a living organism, we use *Drosophila* mushroom body gamma neurons as a paradigm. This population of neurons represents a good model for collective axon growth, as their adult axonal processes grow synchronously, in a relatively short time scale (around 15-20 hours), in a constraint environment (i.e. medial lobe of the Mushroom body inside the central brain). To feed our mathematical model we use an average pattern of medial lobe obtained from biological samples, and a database composed of confocal images of individual wild-type and mutants gamma neurons labeled with GFP.

Growth of individual axons is modeled by a Gaussian Markov chain in a 3D space, which depends on two main parameters estimated from real data: i- axon rigidity and ii- attraction to the target field. Furthermore, we hypothesize that axon tips pause when encountering a mechanical obstacle (i.e. other neurons or the lobe limits), and associate this behavior with the birth of long terminal branches. We show that the proposed mechanistic branch generation process is plausible. More importantly, our model predicts that branch formation in response to mechanical interactions enhances the probability that axons reach their final destination at the population level, and that axon density influences arborization patterns. Indeed, about 40% of simulated axons do not grow properly in the absence of branching in a wild-type environment, a result that is validated by biological data obtained with mutant neurons, in which axon growth defects are associated with defective branching.

To further validate the model hypotheses concerning branch formation, we are currently collecting and analyzing live-imaging data sets from brains with GFP-labeled growing axons.

We hope that combining cell biology, imaging and mathematical modeling will help us better understand the process of axon growth at the population level, in both normal and pathological contexts.

BACK

**Poster - 078**

# The Sorting Receptor SorCS1 Controls Axonal Targeting of Neurexin

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Precise targeting of synaptic surface receptors is required for synaptogenesis and neurotransmission. Neurexin-1 (Nrxn1) is a presynaptic cell adhesion protein that engages in trans-synaptic interactions with several postsynaptic ligands and plays a critical role in the function of synapses. We recently showed that SorCS1, a dendritic endosomal sorting receptor, binds Nrxn1 and controls its synaptic abundance. However, the mechanism by which SorCS1 regulates the trafficking and synaptic abundance of Nrxn1 is still unknown.

In cultured mouse cortical neutrons, Nrxn1 is polarised to the axonal surface, but is also present in the somatodendritic compartment, consistent with previous reports. Synchronized release experiments of Nrxn1 from the Golgi apparatus reveals that Nrxn1 is first trafficked to dendrites from the soma. Blocking endocytosis in cultured neurons increases dendritic surface expression of Nrxn1 and disrupts its axonal targeting. When we interfere with the formation of early and recycling endosomes the same phenotype is obtained, indicating that axonal targeting of Nrxn1 requires endocytosis and transport via early/recycling endosomes in dendrites. These are hallmarks of transcytosis: axonal proteins are first sorted in the trans-Golgi network to the somatodendritic domain and redirected to the axon after endocytosis. Indeed, overexpression of wildtype SorCS1, but not of an endocytosis-defective mutant, removes Nrxn1 from the dendritic surface and promotes its axonal targeting. Conversely, loss of SorCS1 increases dendritic surface levels of Nrxn1, while decreasing its levels on the axonal surface. Consistent with decreased axonal trafficking of Nrxn1, synapse formation and presynaptic function are impaired in Sorcs1 KO neurons. Overall, these data indicate that SorCS1 is required to redirect Nrxn1 to the axon from the dendrites

BACK

**Poster - 079**

# Crosstalk at the Neuromuscular Junction Driving Nerve Terminal Regeneration

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The neuromuscular junction (NMJ) is one of the few human tissues capable of complete regeneration after major damages[1]. Regeneration relies on an intense interplay among the nerve, the glia, the muscle and the basal lamina, which is currently poorly characterized in its molecular details[2].

We recently set up two reliable models of acute and reversible nerve terminal degeneration in mice based on -Latrotoxin (from the black widow spider venom) or on immune-complexes (anti-polysialoganglioside antibodies *plus* complement). Both agents induce a localized and reversible motor axon terminal degeneration, mainly due to calcium overload, and their action mimics the cascade of events that leads to nerve terminal degeneration in injured patients and in other neurodegenerative conditions[3,4]. Anti-polysialoganglioside autoimmune antibodies are found in patients affected by a group of immune-mediated peripheral neuropathies indicated as Guillain-Barré syndrome (GBS). GBS subtypes can have highly different prognosis, from spontaneous complete recovery to a poorer outcome[5]. We found that the complex between anti-polysialoganglioside antibodies and the complement system reproduces the reversible degeneration that takes place in patients affected by a number of GBS subtypes[4].

Neurons injured by -Latrotoxin or by these immune-complexes release *alarmins* (hydrogen peroxide, mitochondrial DNA, cytochrome c and ATP), which in turn activate important signaling pathways in perisynaptic Schwann cells ($Ca^{2+}$, cyclic AMP; MAP kinases, CREB), of crucial importance for regeneration to occur[3-4,6-7].

In additon, we have recently defined the transcriptome profile of murine NMJs during degeneration and regeneration induced by the spider neurotoxin, and are currently investigating a set of candidate molecules that perisynaptic Schwann cells may produce to stimulate axonal regrowth and NMJ recovery of function. These studies could have important implications in human therapy, to stimulate motor axon terminal regeneration in a number of peripheral neuropathies.

*1. Brosius Lutz A, Barres BA. Contrasting the glial response to axon injury in the central and peripheral nervous systems. Dev Cell. (2014) 28:7-17*

*2. Rigoni M, Montecucco C. Animal models for studying motor axon terminal paralysis and recovery. J Neurochem. (2017). In press.*

*3. Duregotti E, Negro S, Scorzeto M, Zornetta I, Dickinson BC, Chang CJ, Montecucco C, Rigoni M. Mitochondrial alarmins released by degenerating motor axon terminals activate perisynaptic Schwann cells. Proc Natl Acad Sci U S A. (2015) 112:497-505*

*4. Rodella U, Scorzeto M., Duregotti E, Negro S, Dickinson BC, Chang CJ, Yuki N, Rigoni M, Montecucco C. An animal model of Miller Fisher syndrome: Mitochondrial hydrogen peroxide is produced by the autoimmune attack of nerve terminals and activates Schwann cells. Neurobiol Dis. (2016) 96, 95-104*

*5. Yuki N, Hartung HP. Guillain-Barrè syndrome. New Engl J Med. 2012 366, 2294-2304*

*6. Negro S, Begamin E, Rodella U, Duregotti E, Scorzeto M, Jalink K, Montecucco C, Rigoni M. ATP released by injured neurons activates Schwann cells. Front Cell Neurosci. (2016) 10, 134*

*7. Rodella U, Negro S, Scorzeto M, Bergamin E, Jalink K, Montecucco C, Yuki N, Rigoni M. Schwann cells are activated by ATP released from neurons in an in vitro cellular model of Miller Fisher syndrome. Dis Model Mech. 2017 Jan 6. pii: dmm.027870. doi: 10.1242/dmm.027870*

**BACK**

**Poster - 080**

# Gene-specific Translation Initiation is Important for Dendrite Pruning in *Drosophila*

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Remodeling of neuronal connectivity is crucial for the establishment of the mature nervous system of both invertebrates and vertebrates. During metamorphosis, Drosophila Class IV dendritic arborization neurons (C4da), sensory neurons lying in the larval body wall, undergo selective elimination of their dendrites (Williams and Truman, 2005). This process of dendrite pruning is initiated by the steroid hormone ecdysone which induces the expression of pruning genes, among them the actin severing enzyme Mical (Kirilly, 2009). While transcriptional regulation of c4da neuron dendrite pruning is well documented, little is known about post-transcriptional regulatory mechanisms during this process.

We performed a candidate RNAi screen for RNA-binding proteins required for c4da neuron dendrite pruning. In this screen, we identified a regulator of translation initiation. Expression analyses and genetic interaction experiments reveal that this factor is required for expression of a subset of ecdysone target genes, in part via regulation at the level of 5' untranslated regions (5'UTRs). Our results suggest an additional layer of pruning regulation through translation regulation of certain ecdysone target genes.

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**Poster - 081**

# Angiomotins, a Novel Family of Proteins Involved in Neuronal Networks Organization

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Understanding of molecular processes that govern organization of the neuronal networks is in the center of attention of the neurobiological research. Here, we investigated the expression and function of Angiomotin family of proteins comprising of Amot, Amotl1 and Amotl2 in the central nervous system (CNS).

We have discovered that all three proteins are widely expressed in CNS neurons where Amotl1 and Amotl2 localize to the synaptic compartments and Amot is distributed in axons and dendrites. Our functional experiments on cultured neurons reviled that Amot regulates dendritic tree arborization and growth. Mass spectrometry analysis of neuron-specific interactors demonstrated that Amot interplays with actin organizing proteins and components of the Hippo signaling pathway while Amotl1 and Amotl2 interact with synaptic proteins e.g. Ephrin-B3, Homer1, TANC and Magi 1-3.

To study the function of Amot in vivo we generated conditional knockout mice with neuron-specific deletion of Amot. Mutant mice had abnormal cerebellar morphology and exhibited defects in motor coordination in behavioral tests. To analyze neuronal morphology in vivo we crossed mice to Thy1-GFP transgenic line allowing for sparse labeling of cells or injected brains with low tighter GFP-expressing AAV viruses. Interestingly, deletion of Amot in neurons in vivo caused abnormalities in the development of the dendritic tree, similarly to the phenotype observed in Amot KD experiments in vitro. In contrast to Amot mutant mice, neuronal deletion of Amotl1 did not affect motor coordination, but led to abnormalities in social behavior including nest building. Collectively, our research identified a novel family of proteins that regulate neuronal organization and behavior of living animals. This research was supported by the National Science Center (NCN) grants Sonata-Bis 2012/05/E/NZ3/00487, Preludium 2015/19/N/NZ3/02346.

BACK

**Poster - 082**

# Microtubule Disassembly during Drosophila Sensory Neuron Dendrite Pruning

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The elimination of unwanted or unspecific synapses, axons, or dendrites, a process also known pruning, is an important specificity mechanism during neuronal morphogenesis. Drosophila sensory class IV dendritic arborization (c4da) neurons prune their larval dendrites at the onset of metamorphosis, while their axons stay intact. C4da neuron dendrite pruning is induced by the steroid hormone ecdysone and occurs by a mechanism involving local dendritic degeneration and dendrite severing in proximal dendrites. Early events during dendrite pruning include local loss of dendritic microtubules and destabilization of dendritic plasma membrane by increased endocytosis. Little is known about the genetic basis of these events, the epistasis between the pathways and about the spatial regulation. Here, we show that the kinase PAR-1 regulates microtubule disassembly in dendrites. We show that PAR-1 acts to inhibit the microtubule-associated protein tau in dendrites, thus leading to microtubule destabilization. Furthermore, we also found that microtubule motors of the kinesin family are required for dendrite pruning, and our data suggest that kinesins are required for dendritic microtubule removal through the regulation of dendritic microtubule orientation, thus contributing to spatial selectivity. Finally, we found that local loss of microtubules is a prerequisite for membrane remodeling through endocytosis. Our data suggest a comprehensive model where the interplay between kinase activities, microtubule-associated proteins and microtubule orientation leads to disassembly of microtubules during dendrite pruning and suggest a mechanism for the spatial coordination of these activities.

**BACK**

**Poster - 083**

# The Role of Sharpin in Formation and Maturation of Neurites

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Sharpin is a multifunctional adaptor protein which was originally identified as a **direct interactor and activator of Shank proteins in the post-synaptic density of excitatory neurons in the brain**. This interaction is thought to crosslink Shank proteins, which plays a role in enteric nervous system function. Sharpin plays a role in several signalling pathways. For example, Sharpin inhibits integrin cell adhesion receptors and is a member of the linear ubiquitination complex (LUBAC), which promotes NF-κB signalling. Not surprisingly, Sharpin null mice have a complex phenotype and suffer from chronic autoinflammation and chronic proliferative dermatitis. Furthermore, recent data from our group shows that in addition to its other functions, Sharpin promotes function of Arp2/3 complex, which is responsible for actin branching (manuscript under revision). This interaction promotes Arp2/3-dependent lamellipodia formation and cell migration.

Differentiation of neuronal cells includes neuritogenesis, during which neurites sprout to mature later into dendrites and neurites. Importantly, neurite outgrowth has a central role in neuronal migration and differentiation, and its dysregulation is implied in many neurogenerative disease and even autism and schizophrenia. In addition, formation and maturation of synapses and maintaining synaptic plasticity is pivotal, since abnormal dendritic spines and synapses associate with various neurological disorders including psychiathric and neurodevelopmental disorders.

**Importantly, several of Sharpins interactors are involved in neurological processes**. Activity of Shank family proteins is linked to formation of synapses and mutations of Shank family proteins lead to synaptic dysfunction which is typical to autism spectrum disorders. In addition, integrin activity has been shown to regulate neurite outgrowth. Furthermore, dysregulation of integrin signalling associates with progression of Alzheimer's disease. Furthermore, Arp2/3-mediated lamellipodia formation has a role in neurite outgrowth and it regulates formation of dendritic spines and synapses, dendritic maturation and stability, formation of filopodia growth cone motility and neuritogenesis.

Shank family proteins, integrins and Arp2/3 have well-defined roles in neuronal processes including neurite outgrowth and formation of neuronal protrusions. Since Sharpin regulates activity of all of them, we hypothesize **that Sharpin may have a role in neurite outgrowth**. We are currently addressing whether Sharpin truly has a role in neuritogenesis using neuroblastoma cell lines and brains of Sharpin null mice to see if they have any defects or morphological changes.

The EMBO Cell biology of the neuron: Polarity, plasticity and regeneration Conference would be an optimal place for meeting the experts on this field, to learn more useful techniques and experiments that would be relevant for us and that would support our aim to study the role of Sharpin in formation and maturation of neurites. It would also provide a great platform to meet possible future collaborators.

BACK

**Poster - 084**

# Axonal Growth in 3 Dimensions

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The physiological growth of neurons happens in 3 dimensions and yet the conventional cultures are made on a flat surface. It has been shown in other dynamic cell types that the dimensionality affects the cytoskeletal organization, molecular pathways and cell dynamics. This work's aim is to characterize a new *in* vitro method that includes the physiological dimensionality of the neuron in a controlled environment. We demonstrate here that neurons growth more in a 3D matrix compared to the conventional glass coverslips. The way they growth is also altered, where they explore the surroundings in a rapid grow-retraction manner. We observed a different architecture of the growth cone. They are smaller and spiky, consistent with its more dynamic behavior. Moreover, we show that the neuronal mode of growth don't rely on pulling forces on the matrix unlike other cell types, namely fibroblasts. With this work we demonstrate the importance of a more physiological environment for the intrinsic study of neuronal growth and its characterization.

BACK

**Poster - 085**

# The GTPase TC10 Controls Dendritic Tree and Spine Morphogenesis via the GIT/βPIX/PAK Complex

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As central to the regulation of cytoskeleton dynamics, Rho GTPases contribute to various aspects of neuronal differentiation, such as the morphogenesis of dendrites and spines. Purkinje neurons have the most spectacular dendritic tree among neurons of the central nervous system and thus represent a good model system in which to decipher the molecular signalling underlying these processes.

In order to identify novel regulators of dendritogenesis among members of the Rho GTPase family, we performed a gene expression profiling of all mammalian Rho GTPases, using real-time quantitative PCR on mRNA from FACS-purified murine Purkinje cells, at different postnatal stages. We found a strong increasing expression of TC10 (RhoQ), a GTPase of the Cdc42 subfamily, during the period corresponding to the morphogenesis of dendrites and spines. Depletion of TC10 in cerebellar organotypic cultures or in cultured hippocampal neurons led to reduced dendritic branching and absence of spines along the dendrites. This effect was rescued by reintroducing TC10 into the neurons. By mass spectrometry analysis, we further identified as downstream effectors of TC10 the complex composed of βPIX/GIT1/PAK, which is known to play key roles in the formation of dendritic spines, by activating Rac1. We show that TC10 co-immunoprecipitated with βPIX, GIT1 and PAK3 in the PSD95-positive synaptosomal fraction and co-localised with the complex in spines. By live imaging, we show that TC10 is present in motile vesicles in the dendritic shaft, consistent with its role in vesicular trafficking, and that TC10 and GIT/βPIX travel together in these vesicles along the dendritic shaft and in spines. Interestingly, depletion of TC10 resulted in the same phenotype as depletion of either βPIX, GIT1 or PAK3. Finally, the effect of TC10 overexpression on the morphology of the spines was attenuated by depletion of βPIX or GIT.
Altogether, our results show that TC10 plays an essential role in the formation of dendrites and spines. Our current working model is that TC10 is required for the targeting of the GIT/βPIX/PAK complex to the spines, leading to localised Rac1 activation to allow the correct formation of spines.

**BACK**

**Poster - 086**

# Two Differentially Regulated Synaptic AP-2/Clathrin Endocytic Pathways Contribute to Synaptic Plasticity

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AP-2 mediated endocytosis plays an important role in SV recycling. The mouse ‚knock-out' of the AP-1 σ1B adaptin revealed that also the AP-1 plays a role in SV recycling and in addition in synaptic early endosome maturation into late, multivesicular body endosomes. σ1B -/- synapses have a slower SV recycling rate and fewer SV, while early endosomes are enlarged. Despite the lower SV recycling rate and SV numbers, these synapses contain twice as many AP-2 CCV than wt synapses. We characterized those AP-2 CCV biochemically, which revealed the existence of two synaptic AP-2 CCV pools in σ1B -/- synapses. Their protein compositions differ from the respective CCV pools of wt synapses. One of the two AP-2 CCV pools in σ1B -/- synapses is stabilized, demonstrated by the increase in AP-2 phosphorylation. AP-1/σ1B-deficiency induces the upregulation of two different endocytic AP-2 CCV pathways and the stabilization of the CCV coat in only one of the pathways. This delays CCV uncoating and thus the delivery of those AP-2 cargo proteins into early endosomes. Thus the two synaptic AP-2 endocytic pathways are differentially regulated.

**BACK**

**Poster - 087**

# Meta-analysis of RNA-sequencing Data Supports Distinct Regulation of RNA Editing in Alzheimer's Disease Patients

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Alzheimer's disease (AD) is a common disorder characterized by progressive neuronal loss, memory deficit and cognitive impairment. RNA editing is a post-transcriptional modification, which introduces epigenetic DNA-RNA differences (RDDs). Deregulation of this process is associated with cancer and various neurological diseases. ADARs and APOBEC1 are the two enzyme families that mediate A-to-I and C-to-U conversion, respectively.
The purpose of our study is to establish the detailed global RNA editing profile (editome) of AD patients compared to healthy controls with the aim to elucidate the contribution of RNA editing in AD pathogenesis and disease progression. Publicly available RNA sequencing data (SRA060572) of frontal cortex tissue (healthy individuals n= 7, AD patients n=8) have been processed using an 'in-house' pipeline and REDItools software platform. Initially we defined the total number of editing positions for both ADAR and APOBEC1 (7604 and 3143 respectively). Next, we proceeded to identify unique AD-related editing sites that could potentially indicate a connection between RNA editing dysregulation and AD pathology. The newly identified RNA editing positions have been classified into 3 phenotype-dependent groups. The majority of the positions are found either uniquely in AD patients (3733), or in non-demented controls (2269). Common editing sites were identified independently of disease phenotype. Next, we performed Pearson correlation analysis of edited mRNAs with mRNA expression profiles. Genes with profoundly differential expression were subjected to pathway analysis using DAVID, leading to the identification of all dysregulated pathways potentially contributing to AD pathology.
Our results demonstrate for the first time, a twofold increase in the number of editing positions for both ADAR and APOBEC in AD. Binary phenotype comparison underlined the significantly distinct editome profiles present in AD patients. Lastly, differentially expressed mRNAs that harbor an editing event, at the 3'Untransalted region (3'UTR) or at exonic regions, and are also enriched in the implicated pathways, were selected as candidate genes for subsequent functional validation. Currently, we are analyzing RNA edited molecular targets to verify the occurrence of the editing conversion, while inter-individual variations are aborted by analyzing both DNA and RNA from the same individual. More importantly, edited transcripts are being analysed for their expression profiles in human AD frontal cortex tissue samples to determine the functional role of target genes in the progression of AD.

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BACK

**Poster - 088**

# Synaptic Scaling and GluA2 Expression in Hippocampal Neurons are regulated by an Activity Responsive microRNA

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Activity-dependent changes in synaptic strength are the cellular correlate for learning and memory. Synaptic scaling is a homeostatic mechanism through which neurons maintain their neuronal firing rate in a physiological range. Through this mechanism, neurons can perceive their overall excitability and scale glutamate receptors in synapses accordingly. The number and strength of synapses are altered in an activity-dependent manner that relies on long-term modifications in gene expression and local protein synthesis, although these mechanisms are still largely unclear. MicroRNAs are key posttranscriptional regulators that can be modulated by neuronal activity in neurons, controlling local translation and, therefore, mechanisms underlying synaptic scaling.

Here, we characterize novel activity regulated microRNAs with an impact in synaptic function in the hippocampus. We performed gene expression microarray analysis of primary cultures of rat hippocampal neurons under blockade of neuronal activity. Focusing on a limited group of altered genes with crucial roles in plasticity, we predicted putative microRNA regulators and tested their expression levels in cultures of hippocampal neurons subjected to manipulation of neuronal activity. This screening allowed us to identify several activity-regulated microRNAs. We focused our attention on a specific microRNA that exhibits a dramatic activity-dependent change in its expression levels, and whose putative targets are enriched in dendrites. We found that this microRNA targets GluA2, and demonstrated that its overexpression or downregulation bidirectionally regulate surface and synaptic expression of GluA2 and affect synaptic scaling in hippocampal neurons. These results elucidate a miRNA-mediated mechanism for activity-dependent regulation of AMPA receptor subunits and synaptic scaling.

**BACK**

**Poster - 089**

# The Switching of the PHF10/BAF45a Isoforms, the Subunits of the PBAF Chromatin Remodeling Complex, is correlated with the Neural Progenitor Differentiation

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The composition of chromatin remodeling BAF/PBAF complexes determine the patterns of gene remodeling in neural differentiation. During embryogenesis and neural development BAF/PBAF complex changes its subunit composition, and thus its properties. Expression of BAF53a, SS18 are characteristic for proliferating progenitors. After mitotic exit these subunits are exchanged for BAF53b and CREST in the BAF complexes. The alteration of complex's composition leads to change in patterns of active genes required for neuronal differentiation and specialization.

BAF45a/PHF10 is a subunit of PBAF mammalian complex and is expressed as four isoforms that are different by their N- and C-end domains. PHF10 isoform containing PHD domains (PHF10-Pl isoform) is necessary for proliferation of neural progenitors. In mice expression of PHF10-Pl is decreased after birth in every part of the brain, correlating with mitotic exit in neuronal progenitors. We demonstrated that parallel with this process these cells start to express of another isoform - PHF10-Ss (short isoform without PHD's). PHF10-Ss has a different pattern of phosphorylation than PHF10-Pl and is predominantly expressed in Purkinje cells in the brain of adult mouse.

Both PHF10-Pl and PHF10-Ss are subunits of 2MDa PBAF complexes, but complexes containing PHF10-Pl and PHF10-Ss differ in charge and additional associated partners. After multistep purification of PHF10-Pl and -Ss contained complexes and subsequent MALDI-TOFF we determined core subunits of TFIID complexes associated with PHF10-Ss BAF complex. With co-immunoprecipitation we confirmed the interaction between TFIID and PHF10-Ss containing PBAF complexes and increased levels of PBAF and TFIID complexes at promoters of Purkinje cells marker genes.

We conclude that neuronal differentiation of Purkinje cells is accompanied by exchange of PHF10-Pl isoform for PHF10-Ss isoform in the PBAF chromatin remodeling complex, leading to recruitment of TFIID complex to promotors and subsequent activation of Purkinje-specific genes.

The project was supported by President Grants for young scientists (MK-7512.2016.4).

BACK

**Poster - 090**

# Synaptic Vesicle Endocytosis Occurs on Multiple Timescales and is Mediated by Formin-dependent Actin Assembly

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Neurotransmission is based on the exocytic fusion of synaptic vesicles (SVs) followed by endocytic membrane retrieval and the reformation of SVs. Recent data suggest that at physiological temperature SVs are internalized via clathrin-independent ultrafast endocytosis (UFE) within hundreds of milliseconds, while other studies have postulated a key role for clathrin-mediated endocytosis (CME) of SV proteins on a timescale of seconds to tens of seconds. Here we demonstrate using cultured hippocampal neurons as a model that at physiological temperature SV endocytosis occurs on several timescales from less than a second to several seconds, yet, is largely independent of clathrin. Clathrin-independent endocytosis (CIE) of SV membranes is mediated by actin-nucleating formins such as mDia1, which are required for the formation of presynaptic endosome-like vacuoles from which SVs reform. Our results resolve previous discrepancies in the field and suggest that SV membranes are predominantly retrieved via CIE mediated by formin-dependent actin assembly.

BACK

**Poster - 091**

# Quantitative Map of Proteome Dynamics during Neuronal Differentiation Reveals NCAM1 as Regulator of Dendritic Morphogenesis

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Neuronal differentiation is a multistep process that shapes and re-shapes neurons by progressing through several typical stages, including axon outgrowth, dendritogenesis and synapse formation. To systematically profile proteome dynamics throughout differentiation we took cultured hippocampal neurons at different developmental stages (DIV1, DIV5, DIV14) and monitored changes in protein abundance using a combination of stable isotope labeling and high-resolution tandem mass spectrometry (LC-MS/MS).

Almost one third (1793) of all 4500 proteins quantified underwent a more than two-fold expression change during neuronal differentiation, indicating extensive remodeling of the neuron proteome. This analysis revealed six clusters of distinct expression profiles with proteins upregulated during differentiation in clusters 1, 2 and 3, and proteins downregulated present in clusters 4, 5 and 6. We mainly focused our attention on cluster 3, which contains proteins whose expression levels are highly up-regulated between DIV1 and DIV5 of neuronal development. According to gene ontology classification, most of the proteins present in this cluster are transmembrane proteins that could play a role by modulating dendritic outgrowth and branching.

To highlight the strength of our resource, we further characterized neural cell adhesion molecule 1 (NCAM1) as a novel regulator for dendritic outgrowth during neuronal development. The transmembrane isoform of NCAM1, NCAM180, is strongly upregulated during dendrite outgrowth and is highly enriched in dendritic growth cones. AP-MS experiments revealed that NCAM180 interacts with a large variety of actin binding proteins. These observations suggest that NCAM1 can act as a scaffold for multiple cytoskeleton components and that multiple actin-binding proteins can amplify the interaction between NCAM1 with the actin cytoskeleton. Interestingly, inducing actin polymerization by Jasplakinolide treatment rescues the NCAM1 knockdown phenotype. These results indicate that NCAM180 stimulates dendritic arbor development by promoting actin filament growth at the dendritic growth cone and actin stabilization is one of the driving forces that act during dendrites morphogenesis.

Based on the obtained data on NCAM1, we anticipate that our quantitative map of neuronal proteome dynamics constitutes a rich resource that may be used in the future for a better understanding of any specific molecular mechanism involved in neurodevelopment.

BACK

**Poster - 092**

# The Role of Cofilin in *Drosophila* Mushroom Body Axon Branching

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Axon branching allows individual neurons to connect to multiple synaptic targets. However, the mechanisms regulating this process are not well understood. Axonal morphogenesis of the *Drosophila* mushroom body (MB) γ neurons involves initial axon outgrowth to form larval-specific projections, which are pruned during metamorphosis. Developmental axon regrowth follows, to generate medially projecting adult γ axons that also bear several branches. The whole process can be visualised at single cell resolution, and is amenable to combinatorial genetic manipulation, making it an excellent system to identify regulators of axon branching and the molecular mechanisms they employ.

The generation of a branch from an axon shaft would involve reorganization of cytoskeletal structures at the branch point. We found that cofilin (encoded by *twinstar* known as *tsr*), a regulator of the F-actin cytoskeleton which severs microfilaments, is required for MB γ axon branching during regrowth. Although previously known to be required for γ axon growth, we find by looking at single neurons that $tsr^{-/-}$ MB γ neurons exhibit compromised branching as well as defective regrowth. To our surprise, the overexpression of *tsrS3A*, which encodes a variant of cofilin that cannot be phosphorylated by its inhibitor, LIM Kinase1, and is therefore considered to be constitutively active, restores branching in $tsr^{-/-}$ γ axons, but not their growth. We also see reduced branching in a neurite sprouting assay of dissociated $tsr^{-/-}$ pupal neurons, although the sprouting and growth of primary neurites is affected to a much lesser effect.

We are currently studying actin dynamics in WT and $tsr^{-/-}$ axons during developmental regrowth, and during neurite sprouting in culture using F-actin reporters. We also plan to monitor the effect of *tsr* loss-of-function on the microtubule (MT) network. Work in the lab has identified other actin regulators to be important for the developmental regrowth of γ axons. We plan to examine whether they play a part in axon branching as well, either on their own, or in conjunction with Tsr. Together, we hope to uncover how the axonal cytoskeleton is reorganized in order to form branches.

BACK

**Poster - 093**

# The Analgesic and Anxiolytic Effect of Souvenaid, a Novel Nutraceutical, Is Mediated by Alox15 Activity in the Prefrontal Cortex

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Pain and anxiety have a complex relationship and pain is known to share neurobiological pathways and neurotransmitters with anxiety. Top-down modulatory pathways of pain have been shown to originate from cortical and subcortical regions, including the dorsolateral prefrontal cortex. In this study, a novel docosahexaenoic acid (DHA)-containing nutraceutical, Souvenaid was administered to mice with infraorbital nerve ligation-induced neuropathic pain and behavioral responses recorded. Infraorbital nerve ligation resulted in increased face wash strokes of the face upon von Frey hair stimulation, indicating increased nociception. Part of this response involves general pain sensitization that is dependent on the CNS, since increased nociception was also found in the paws during the hot plate test. Mice receiving oral gavage of Souvenaid, a nutraceutical containing DHA, choline and other cell membrane components, showed significantly reduced pain sensitization. The mechanism of Souvenaid's activity involves supraspinal antinociception, originating in the prefrontal cortex, since inhibition of the DHA-metabolizing enzyme 15-lipoxygenase (Alox15) in the prefrontal cortex attenuated the antinociceptive effect of Souvenaid. Alox15 inhibition also modulated anxiety behavior associated with pain after infraorbital nerve ligation. The effects of Souvenaid components and Alox15 on reducing central sensitization of pain may be due to strengthening of a known supraspinal antinociceptive pathway from the prefrontal cortex to the periaqueductal gray. Together, results indicate the importance of the prefrontal cortex and DHA / Alox15 in central antinociceptive pathways and suggest that Souvenaid may be a novel therapeutic for neuropathic pain.

Shalini, S. M., Herr, D. R., & Ong, W. Y. (2016). The Analgesic and Anxiolytic Effect of Souvenaid, a Novel Nutraceutical, Is Mediated by Alox15 Activity in the Prefrontal Cortex. *Molecular Neurobiology*, 1-14.

BACK

**Poster - 094**

# The Formin DAAM Coordinates the Actin and the Microtubule Cytoskeleton during Axonal Development

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Directed axonal growth is known to be strictly dependent on proper coordination of the actin and microtubule cytoskeleton. However, despite of the relatively large number of proteins implicated in actin-MT cross-talk, the mechanisms whereby actin polymerization is coupled to MT stabilization and advancement in the peripheral growth cone remained largely unclear. The neuronal cytoskeleton is hardly accessible *in vivo*, therefore we use primary neuronal cell cultures, which offer excellent opportunities for research into cellular mechanisms. The *Drosophila* formin *DAAM* (Dishevelled Associated Activator of Morphogenesis) is an important and evolutionarily well conserved actin regulator. Previously we have shown that dDAAM plays an essential role in differentiation of the embryonic nervous system and later on in development of the adult brain. *In vitro* and *in vivo* studies revealed that dDAAM behaves as a *bona fide* formin, i.e. it nucleates actin filaments and supports their elongation by remaining processively attached to their barbed ends. Here we show that *DAAM* also plays a pivotal role in regulation of the axonal microtubule cytoskeleton. The absence of *DAAM* leads to abnormal microtubule organization. Moreover, we found that *DAAM* partially colocalizes with the microtubules in neurons, whereas *in vitro* analysis confirms that *DAAM* is able to interact both with actin and microtubules, and consistently, it has the ability to crosslink these two types of filaments. Live imaging revealed that *DAAM* regulates the dynamics of axonal microtubules. Together these findings suggest that *DAAM* is involved in the co-alignment/crosslinking of the actin and microtubule cytoskeleton, and in this way it represents a potential novel mechanism of actin-microtubule coordination in neuronal growth cones.

**BACK**

**Poster - 096**

# Ral GTPase Regulates Axonal Caliber and Structural Plasticity at the *Drosophila* Neuromuscular Junction

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Neurons are the most morphologically diverse cell type whose morphology determines many functional aspects of a neuronal network. The primary shape of a neuron is established during axon and dendrite outgrowth and synapse formation, but is subject to subsequent modifications by physiological events. In response to changes in synaptic activity, neurons can alter both pre and postsynaptic elements of the synapse. Defects in synaptic morphology and in activity-dependent plasticity are a hallmark of several neurodegenerative and cognitive disorders. Microtubules (MT) play important roles during the development of neuronal morphology, and for plasticity to occur, and a dysfunctional MT network may lead to degeneration. Here, we show that the small GTPase Ral regulates axonal caliber and mediates activity-dependent structural plasticity at the *Drosophila* neuromuscular junction (NMJ): Ral mutants have thicker axons and defective structural plasticity. Additionally, these mutants have increased levels of acetylated tubulin suggesting that MTs may be altered. Ral has been shown to interact with several different signaling pathways. We are testing whether and how Ral interacts with these pathways to regulate the number of MTs, their dynamics, and their contribution to axonal thickness and plasticity, and whether its function is required in neurons and/or glia. The efficacy of axonal transport relies on proper MT function and Ral may contribute to this important aspect of neuronal development and function. Our findings uncover new functions for Ral GTPase in neurons, implicating this GTPase in the regulation of MTs. Given the striking similarities between plasticity and cancer metastasis, it is possible that our work will shed light on the mechanisms by which Ral participates in its well-described role in cancer progression.

**BACK**

**Poster - 097**

# The Roles of Pax6 in Regulating Neuronal Morphogenesis during Embryonic Development of the Prethalamus

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During neuronal morphogenesis, newborn neurons undergo active migration to reach their final destination, acquire their specific morphology and eventually form synapses with particular partners. Understanding the mechanisms regulating neuronal morphogenesis is thus important as it underlies functional neural circuitry formation.

The transcription factor Pax6 is a pleiotropic player during neural development. In the central nervous system, Pax6 is mostly expressed by neural progenitor cells, in which its functions have been most extensively studied. However, in the anterior diencephalon, the prethalamus, Pax6 is expressed in both the neural progenitor cells and post-mitotic cells. The functions of Pax6 in these post-mitotic cells are seldom addressed.

Our RNAseq data has revealed that when Pax6 was removed from the prethalamus, genes involved in various aspects of neuronal morphogenesis, e.g., neuritogenesis, establishment of neuronal polarity, axon elongation etc., were significantly differentially expressed. This project aims to investigate how Pax6 might be regulating neuronal morphogenesis in the prethalamic post-mitotic cells, and whether such regulation is cell-autonomous or not. To this end, we performed dissociated cell cultures of prethalamus at embryonic day 13.5. Aspects of neuronal morphogenesis, such as number of neurites, length of longest neurite, length of total neurites and rate of neurite elongation, were analysed in these primary prethalamic neurons cultured for 1-6 days in vitro (DIV).

Preliminary results showed that the Pax6-null prethalamic neurons consistently displayed fewer neurites from 1-6 DIV. At earlier stages of cell culture (1-3DIV), lengths of longest neurite and total neurites were shorter in the Pax6-null prethalamic neurons. Rate of neurite elongation appeared to be slower from 1-3DIV but then overshot at later stages of cell culture (4-6DIV), during which the Pax6-null prethalamic neurons exhibited longer length of longest neurite and total neurites.

These results pointed to a cell-autonomous regulation of neuronal morphogenesis by Pax6 in the prethalamic post-mitotic cells. The next step would be to further investigate the underlying mechanism of such regulation.

BACK

**Poster - 098**

# Dynamic Palmitoylation Targets MAP6 to the Axon to Promote Microtubule Stabilization during Neuronal Polarization

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Microtubule-associated proteins (MAPs) are well known to stabilize neuronal microtubules, playing an important role in establishing axon-dendrite polarity. However, how MAPs are selectively targeted to specific neuronal compartments remains poorly understood. Here, we show specific localization of microtubule-associated protein 6 (MAP6) / stable tubule-only polypeptide (STOP) throughout neuronal maturation and its role in axonal development. In unpolarized neurons, MAP6 is present at the Golgi complex and in secretory vesicles. As neurons mature, MAP6 is translocated to the proximal axon, where it binds and stabilizes microtubules. Further, we demonstrate that dynamic palmitoylation controls shuttling of MAP6 between membranes and microtubules, and is required for MAP6 retention in axons. We propose a model in which MAP6's palmitoylation mediates microtubule stabilization, allows efficient organelle trafficking and controls axon specification *in vitro* and *in vivo*.

**BACK**

**Poster - 099**

# Metabolic Regulation of Axonal Growth during Development

**Valeria Ulisse**[1], Adi Minis[1], Einav Peri[1], Irena Gokhman[1], Vered Shacham[1], Calanit Raanan[2] and Avraham Yaron[1]

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Developing neurons use a significant amount of energy to rapidly grow axons during development. However, the mechanisms regulating the coupling between energy homeostasis and axonal growth are largely unknown. We have found that ablation of Liver Kinase B1 (LKB1), a master regulator of energy homeostasis, in sensory neurons is associated with axonal degeneration during development *in vivo* and reduced axonal growth *in vitro*. Biochemical analysis of LKB1 KO neurons revealed a metabolic aberration characterized by reduction in ATP levels and drop in the activity of the LKB1 downstream kinase AMPK. Through genomic approach, we found that ablation of LKB1 leads to strong reduction in the poorly characterized mitochondrial $Ca^{2+}$-binding protein, mitocalcin. To explore the role of mitocalcin as downstream effector of LKB1 in sensory neurons, we generated a mitocalcin KO mouse by CRISPR. Analysis of this mouse reveled reduced axonal growth *in vivo* during development. Strikingly, we detected hyper activation of LKB1 and AMPK in sensory neurons of the mitocalcin KO mouse both *in vitro* and *in vivo*. Importantly, sensory neurons from the mitocalcin KO mice showed hypersensitivity to pharmacological inhibition of AMPK *in vitro*.

Overall, our work uncovers a new metabolic pathway that is required for axonal growth during development.

**BACK**

**Poster - 100**

# ARHGAP36 Integrates Morphogen Signals to Instruct Spinal Motor Neuron Identity

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Neural progenitor cells generate a vast number of highly specialized classes of neurons in response to molecular gradients that pattern the developing nervous system. It is unclear whether these instructive signals are maintained into adulthood and to what extent they contribute to the homeostatic control of neuronal connections later in life and in response to injury.

We are studying how motor neurons of the spinal cord acquire and preserve unique molecular properties and connectivity that enable them to relay the flow of sensory inputs and motor commands to peripheral organs for the control of body movements and physiology.

Gene profiling of spinal motor neurons revealed high and restricted expression of *ARHGAP36*, a putative RhoGAP up-regulated in human medulloblastomas. Forced expression of *ARHGAP36* in spinal cord progenitors in chick and mouse models affects neural fate specification and patterning, with a prominent expansion of motor neuron domain. These effects are consistent with a GAP-independent role for ARHGAP36 in mediating crosstalk between WNT and Shh (Sonic Hedgehog), which exert opposite effects on motor neurons.

Our studies identify ARHGAP36 as a novel regulator of cell-type specific responsiveness to morphogen signals that instruct neuronal fates in the developing nervous system. Since expression of ARHGAP36 persists in adult motor neurons, it is possible that these regulatory mechanisms are needed past fetal development to ensure motor neurons maintain their connections and react effectively to damage.

**BACK**

**Poster - 101**

# Investigating the Location Dependence of mGluR5 in mGluR-LTD: Combining Protein Translation and Phosphoproteomics

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Long term depression (LTD) is a form of synaptic plasticity characterized by an activity regulated reduction in synaptic strength. Stimulation of the metabotropic glutamate receptor 5 (mGluR5) in the hippocampus induces a form of synaptic LTD that ultimately results in AMPA-type glutamate receptor internalization. This process is independent of transcription activities, but rather relies on rapid translation of local dendritic mRNAs.

mGlu5 receptors are abundantly expressed in brain areas involved in memory and learning, and have therefore extensively been studied in combination with synaptic plasticity. Classically, focus has been on the extracellularly located mGluR5, which are found on the outer edges of the post synaptic density. More recently however, numerous types of G protein coupled receptors (GPCRs) have been found to potentially play an active role in cell signaling processes when they are located inside the cell. Active mGluR5 have been located on the endoplasmic reticulum (ER) and on the nucleus, where they have proved to contribute to LTD via the activation of known G protein-dependent signaling routes, but also non-canonical, G protein-independent routes. To date however, it is not known to which extent these intracellular mGluR5 are essential for LTD induction and maintenance.

This study aims to identify and quantitate newly synthesized proteins upon mGluR-LTD induction by the mGluR group I agonist DHPG, and to elucidate the relative contribution of intracellular mGlu5 receptors in LTD induction through stimulation with the mGluR class I and AMPAR agonist quisqualic acid. To this aim, we used a combination of a pulsed stable isotope labeling of amino acids in culture (pSILAC) and a click-chemistry based enrichment of newly synthesized proteins using azido homo alanine (AHA) labeling. Moreover, we employed a highly sensitive mass spectrometry-based phosphoproteomics workflow for the analysis of phosphorylation regulated signaling dynamics in LTD. All experiments were performed using primary rat hippocampal neurons.

So far we found that the combination of pSILAC and AHA labeling allows for selective enrichment and quantification of newly synthesized proteins, even after short labeling pulses. We observed a high correlation between phosphorylation dynamics and protein synthesis upon mGluR stimulation, allowing the study of mGluR-LTD in a detailed and comprehensive manner.

BACK

**Poster - 102**

# Deciphering Proteome Dynamics during Neural Development

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The human brain is a complex system and disruptions during the development causes many neurological disorders. Studying such disorders require model systems that allow the comparison of development of healthy neurons versus neurons derived from patients. The rapid generation of functional neurons derived from induced pluripotent stem cells hold great promise to study neurogenesis.

Neural development describes the cellular and molecular mechanisms involving initial polarization, neurite outgrowth, axon formation, dendrite formation, synaptogenesis and further maturation. To date, many studies characterize the resulting neurons at the end stage but the pathways underlying the differentiation remain unclear. Next generation mass spectrometry-based proteomics is highly sensitive and enables the identification and quantification of thousands of proteins in a high throughput manner. The research presented here quantitatively monitors the proteomic landscape at 10 different time points during neurogenesis.

We identified 6215 proteins and quantified 4417 proteins from two biological replicates from which 128 proteins show more than three-fold upregulation during the differentiation. These proteins are found to play a role in nervous system development and other neuronal processes. 42 proteins are found to be downregulated during the differentiation. These proteins are involved in the germ layer formation and specification. In addition to known neuronal and iPSC proteins, we also identified new proteins associated with differentiation and loss of pluripotency. Several proteins were also identified revealing a day specific pattern with different biological functions. Furthermore, 6 clusters with distinct expression profiles could be generated revealing defined waves of the proteomic landscape.

These data can be used to study the cellular and molecular phenotypes associated with neurological disorders.

BACK

**Poster - 103**

# Neuronal Polarization is Independent of Centrosomal Microtubule Nucleation

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The centrosome is the major microtubule organizing center (MTOC) in metazoan cells. However, in many differentiated cell types including neurons, the centrosome loses its functions as a MTOC. In agreement, γ-Tubulin Ring Complexes (γ-TuRCs), which nucleate microtubules (MTs), are gradually depleted from the centrosome during neuronal maturation. Nevertheless, the role of the centrosome in young neurons, when the centrosome is still active, remains unclear.

To address the question whether the centrosomal MT nucleation is involved in the initial establishment of neuronal polarity, we used truncated mutants of centrosomal proteins Nedd1 and of the γ-TuRC activator protein Cdk5rap2 to displace γ-tubulin and Cdk5rap2, respectively, from the centrosome in cultured rat embryonic hippocampal neurons.

Analysis of MT nucleation confirmed that the activity of the neuronal centrosome decreases with time. Surprisingly, unlike in epithelial cells, displacement or overexpression of full length Cdk5rap2 did not change centrosomal MT nucleation. On the other hand, simultaneous displacement of γ-tubulin and Cdk5rap2 from the centrosome led to a decrease of both centrosomal γ-TuRCs and centrosomal MT nucleation. Neuronal polarity as assessed by morphological analysis was not different in these cells than in control.

Our data indicate that the frequency of centrosomal MT nucleation in young neurons is proportional to the amount of γ-TuRC at the centrosome and does not rely on activation by Cdk5rap2. Finally, downregulation of centrosomal MT nucleation does not affect the establishment of neuronal polarity. Thus, neuronal polarization occurs independently of centrosomal microtubule nucleation.

BACK

**Poster - 104**

# Axonal Transport and Cargo Delivery Mechanisms in Health and Neuro-Degenerative Disease

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Neurons rely on active transport to move cargo along their axons to synapses. This process, driven by molecular motor proteins, is crucial for proper synapse formation and plasticity. In Alzheimer's disease, impaired axonal transport emerges as one of the earliest pathogenic events. Yet, detailed mechanistic understanding of axonal transport and cargo delivery to the synapse in health and disease is lacking. Especially the transport of secretory vesicles, which supply neurotrophic factors and other peptides essential for proper synapse function, remains poorly understood.

In this study we aim to investigate the transport of secretory vesicles to their fusion sites, and their activity-dependent stalling upon calcium influx. We employ microfluidic chambers to look at isolated axons with known polarity, and also investigate the potential of optogenetic activation to induce local calcium elevations. Furthermore, we investigate downstream modulators of calcium influx in order to test how several calcium sensors impact axonal transport and synaptic plasticity.

We thereby aim to increase understanding of secretory vesicle transport and to identify possible new therapeutic targets for relieving axonal transport deficits in Alzheimer's disease.

BACK

**Poster - 105**

# Dynein Regulator NDEL1 Controls Polarized Cargo Transport at the Axon Initial Segment

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Neurons rely on the differential targeting of cargo to the axon or dendrites. Vesicle filtering takes place at the axon initial segment (AIS), a proximal region of the axon where somatodendritic cargo is deterred while axonal cargo proceeds into the axon. However, it remained unclear which molecular mechanisms facilitate cargo specificity at the AIS.

We found that NDEL1, a regulator of the cytoplasmic dynein molecular motor complex, localizes to the AIS via a stable interaction with the AIS-scaffolding protein Ankyrin-G. We show that NDEL1 and the NDEL1-associated dynein regulator LIS1 are required for polarized trafficking of somatodendritic cargo, and that artificial recruitment of dynein to vesicles can drive cargo transport out of the axon. We therefore present a dynein-based model for axonal exclusion of specific cargos, wherein NDEL1 is concentrated in the AIS to locally mediate LIS1-signaling and thereby boost somatodendritic cargo reversal.

BACK

**Poster - 106**

# Interplay Between S-palmitoylation and S-nitrosylation in the Chronic Stress Disorder

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Chronic stress exposure is a key environmental factor for development of neuropsychiatric disorders such as major depression and anxiety disorders. A number of studies have demonstrated that chronic stress-related emotional and cognitive impairment is associated with alternations in synaptic organization. One of the best described mechanism of synaptic proteins regulation are post-translational modifications (PTMs) that occur on a protein, after its translation is complete. Some of these modifications have been the subject of intensive research, and their biological role is extensively characterized. In contrast, understanding of other modifications such as S-palmitoylation or S-nitrosylation is still elusive. S-palmitoylation is the covalent lipid reversible modification of cysteinę side chain with 16 carbon fatty acid palmitate via thioester which regulates diverse aspects of neuronal protein trafficking and function. The reversible nature of palmitoylation allows proteins to associate with membranes, what regulate their sorting, localization and functions. Intracellular protein S-palmitoylation is controlled by a newly described family of protein S-acyl transferases and palmiotyl tioestrases responsible for protein depalmitoylation. Recent study shows alternative mechanism of S-palmitoylation regulation. Other cysteine posttranslational modification, S-nitrosylation was described as regulator of S-palmitoylation dynamic. S-nitrosylation - the covalent modification of a protein cysteine thiol by a nitric oxide (NO) group has emerged as an important mechanism by which NO acts as a signaling molecule. Accumulating evidence indicates important roles for S-palmitoylation and S-nitrosylation both in normal physiology and in a broad spectrum of human diseases by affecting a number of specific protein targets. S-nitrosylation may alter S-palmitoylation either via competition for target cysteines or by direct displacement of palmitoyl moieties on S-palmitoylated cysteines.

We propose a hypothesis that abnormal PTMs of proteins that control synaptic plasticity, resulting in destabilization and loss of synaptic connections and leads to depression.

Using mass spectrometry based (MS-based) proteomic approaches we profiled endogenous S-nitrosylation and S-palmitoylation of postsynaptic density proteins from control and mouse models of chronic stress. Our MS-based approach allows for differential analysis of S-palmitoylation and S-nitrosylation at the level of exact sites of modification. Functional enrichment analysis linked differentially S-palmitoylated and S-nitrosylated proteins to various cellular pathways, including: synaptic transmission, neurotransmitter release or protein localization.

Summarizing, our results suggest that altered mechanism of interplay between S-palmitoylation and S-nitrosylation of proteins involved in synaptic transmission, protein localization and regulation of synaptic plasticity might be one of the main events associated with chronic stress disorder, leading to destabilization in synaptic networks, confirming the previous observations.

BACK

**Poster - 107**

# Microtubules Instruct F-actin Dynamics during Neuronal Polarization

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Breaking cell symmetry during neuronal polarization is reflected by different dynamics of microtubules and filamentous actin (F-actin). Axonal microtubules are more stable than those in the remaining neurites, while dynamics of F-actin in axonal growth cones clearly exceeds those in their dendritic counterparts. However, whether a functional interplay exists between microtubules and F-actin dynamics in growing axons and whether this interplay is instrumental to break cellular symmetry is currently unknown. Here we show that microtubules instruct F-actin dynamics during neuronal polarization. Specifically, we found a negative correlation between microtubules and F-actin dynamics, where an increment on microtubules stability or number of microtubules associate with increased F-actin dynamics. Moreover, we show that drebrin E, which bind F-actin and plus ends microtubules, mediates this cross talk. Drebrin E segregates to more dynamic growth cones where F-actin is unstable and more plus end microtubules are found. Interruption of the interaction of drebrin E with microtubules decreases F-actin dynamics and arrests neuronal polarization and axon extension. Collectively the data show that microtubules instruct F-actin dynamics for initial axon extension during neuronal development.

**BACK**

**Poster - 108**

# Cell Autonomous Regulation of Neuronal Fatty Acid Synthesis is required for Dendritic Development and Maintenance

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The brain is our fattiest organ. It is believed that glial cells are in charge of lipid synthesis and neuronal fat supply. However, it is unknown to what extent neurons themselves contribute to lipid production to ensure regular development and the maintenance of their elaborate neurites. To shed light on this question we down-regulated the expression of lipid metabolism-related genes in neurons and monitored their morphological development over time. We compared two morphologically different types of neurons: small dendritic arborization Class I neurons (CI-da neurons) and large Class IV (CIV) sensory da-neurons.

Our screen data suggested that the transcription factor Sterol Regulatory Element Binding Protein (SREPB) is a critical regulator of dendritic morphology in the complex and large CIV-da neurons. SREBP promotes the expression of genes involved in *de novo* fatty acid synthesis. Hypomorph or null mutant CIV-da neurons showed severe developmental defects. Loss of SREBP function and thus fatty acid depletion led to a clear simplification of the dendritic tree while the cell body and axon seemed largely unaffected. This effect was specific to the large-scale CIV-da-neurons since morphology of the small CI-da-neurons was not altered by SREPB down-regulation. SREBP was strongly expressed only in CIV-da neurons and not in da-neurons of smaller size, supporting the specificity of the phenotype.

We next investigated the effects of SREBP overexpression in da neurons using full-length SREBP or a constitutive active form (SREBP-CA) that leads to an accumulation of free fatty acids. Overexpression of full length SREBP caused the opposite phenotype of SREBP down-regulation: CIV-da-neurons were unaffected but CI-da-neurons increased in dendritic length and branching. However, the expression of SREBP-CA led to the loss of dendrites in both classes of da-neurons, suggesting neurotoxic effects of excess fatty acids.

Taken together we show that SREBP is required for proper dendritic development in large-scale CIV-da neurons, in which it is specifically expressed. Our future work is directed towards understanding the molecular pathways linking SREBP expression to neurite growth and maintenance.

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# CONFERENCE INFO


# Survivor's Guide…

- **A small piece of advice:** *Better take your POSTER onboard rather than leave it in your luggage; the effort putting your roll under your seat or above your head is worth avoiding the risk of your presentation traveling to some exotic destination on its own…*

## Transportation from / to Heraklion Airport "N. Kazantzakis" [HER]

Transportation is not provided by the organizers.

**TAXI**

Taxi ride from Heraklion "Nikos Kazantzakis" Airport [HER] to the Hotel at Fodele costs around 45 Euros. TAXIs are outside the arrival room on the left.

There is an offer from our partner organizer CCBS-Greece. For the same price (45 € per taxi), the TAXI driver will wait for you holding a sign with your name on it. The price is fixed from Heraklion to Fodele Beach. If you wish to take advantage of the service you are kindly requested to send an e-mail directly to sales@ccbsgreece.gr, Ms Katerina Koronaiou, subject: "EMBO Conference-2017", indicating your name, your arrival flight number and your arrival date and time in Heraklion airport.

**PUBLIC TRANSPORT**

Heraklion airport is close to the city center; only 15 minutes by any city bus (blue bus), which leaves from just outside the airport. You need to buy a 1,20 € ticket (colored orange) at the bus stop kiosk; you will enter at the front door and the bus driver will tear half of your ticket apart. Ask the driver to drop you off at the "KTEL for Chania", which is the bus station for the RETHYMNO-CHANIA intercity coach (green bus). Buy a ticket to FODELE BEACH inside the station (for ~3,00 €), and tell the driver on the bus where to stop (FODELE BEACH). The short ride should take no more than 30 minutes. It's a final 300m walk to the hotel from there (public transportation plan – see page 4).

**Bus schedule:** City buses leave from the airport to the city centre every 10 minutes or so. Intercity coaches leave from the KTEL bus station near the port every hour on the half hour from 5.30 until 18.30 and after that, at 20.00 and 21.00 (city map and location of KTEL - see page 5).

Please, be careful, the public bus station across the street from airport arrivals, does not serve West Crete and Fodele.

## Arriving at the Hotel / Registration on the 7th

- Upon arrival at the hotel you must check-in your room. You don't need any voucher. Just state your name at the reception. You will get the "all inclusive" colored bracelet put on your wrist (list of "all inclusive service" attached – see page 6); also attached is a plan of the hotel (see page 7)
- Since your reservation is made through the meeting organizers, you shouldn't pay any extras at the hotel. For settling any pending accommodation extras, please contact the person from CCBS-Greece during registration and throughout the Course. Additional charges (e.g. telephone, internet access, mini bar, etc) are not included in your accommodation included in the registration fees. Please remember to take care of these directly at your hotel, upon checking out.

- Upon registration (starting at 11.00 on Sunday, 7 May) you will be given an EMBO bag containing the following:
  - Course Program
  - Posters List
  - Name badge
  - Badge strip
  - Notepad / Pen
  - Leaflets

  * The Abstract Book will be distributed electronically

**Registration will take place at the Course Secretariat, outside the Conference Room**
(No 35 at the hotel plan – see page 7)

**If you are a speaker:**

There will be a data projector connected to a PC (Windows – Microsoft Office XP) so kindly prepare your presentation file(s) accordingly. There will be assistance inside the conference room, at your disposal for any technical assistance. Please ask the secretariat for more detailed information. You should not forget to contact the assistant during the break *prior to* your presentation's session and hand over to them your presentation data (CD, memory stick, notebook /laptop). Assistants will wear yellow colored badges.

- If you are a Mac user, please don't forget to bring the cable required to connect your machine to the projector.

**If you are presenting a poster:**

**Poster Room: No 34 "Games Room" at the hotel plan (see page 7)**
Poster boards will be ready for presentation mounting. You are not allowed to use push-pins or any other mount material which could damage boards. Therefore, you should ask for proper mounting material (blue tack or scotch tape) at the desk located inside the poster room. Remember to consult the detailed poster presentation guidelines (attached, see page 8). **POSTERS SHOULD BE PORTRAIT ORIENTED**.
Posters should be up for display by Sunday, 7 May at 16:30. All posters will remain mounted for the whole duration of the meeting.

There are 2 Poster sessions: **Monday, 8 May 2017 @ 15:30 – ODD NUMBERS** and **Tuesday, 9 May 2017 @ 15:45 – EVEN NUMBERS**. There will be a poster list where you can check the poster session you present in and your poster number/board. You will also be notified for your poster session during registration.

## ❖ Frequently Asked Questions

**Will I have Internet access during the conference?**

For wireless internet access inside the conference room and the rest of the conference venue, you will need to buy an access card from the Reception of the hotel. The prices are: 1 day → 2,00 €, 3 days → 5,00 €, 7 days → 12,00 €.

Free internet, low standard bandwidth, is provided in the central square outside the conference and poster room, close to Starlight Bar (No 18 in the Hotel Plan), in the Water Park (No 38 in the Hotel Plan) and the Beach.

**What if I want to keep my room after 12 noon on checkout day?**

12.00 is the regular check-out time. If you wish to keep your room past 12.00, you should notify the reception of the hotel in advance to check for availability. In case of extended day stay, you can contact the course secretariat before the end of the course. Bear in mind that after 18.00 a full overnight stay charge is applicable.

**Do I have any options outside the conference area?**

The person in charge from CCBS-Greece, Ms. Katerina Koronaiou, will be happy to advice you on short visits or day schedules all around Crete and will be more than willing to arrange such off-conference activities for you and/or your escorts.

- Last but not least, unless you are unlucky, weather should be perfect as usual in early May; warm and sunny, even tempting for a swim. Check this link http://bit.ly/dEvFpC and bring you swimsuit!

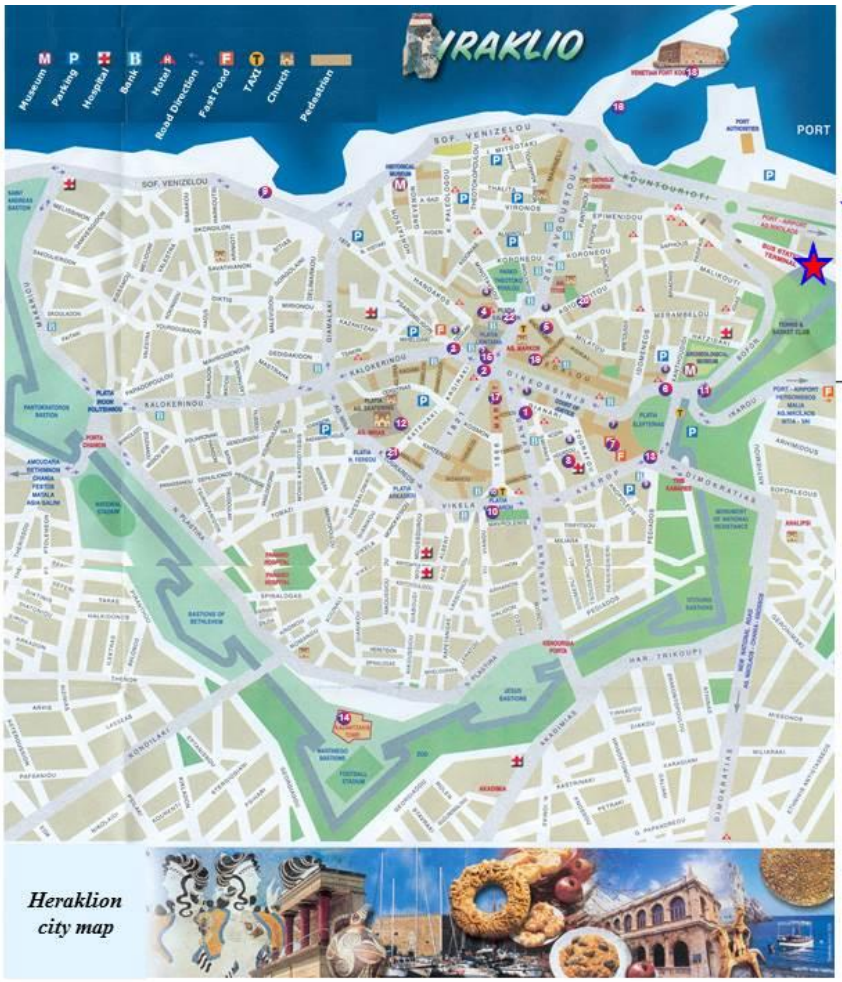
We are all looking forward for a very interesting conference!

The organizers,

Casper Hoogenraad, Frank Bradke, Nektarios Tavernarakis

# Public Transportation

# HERAKLION CITY MAP




Here is where you catch the (green) bus to Chania. You should ask the driver to stop to Fodele Beach Hotel. You will have to walk about 300 meters to reach the hotel from the bus stop.

**Price per way:** ~ 3,00 Euros.

**TAXI terminals**
Airport to the hotel: around 45 Euro.

1. Tourist Police
2. Police
3. Traffic Police
4. Telephone Office
5. Loggia - Town Hall
6. Ntl Tourist Organization
7. Daskalogianni Square
8. Post Office
9. Natural History Museum
10. Valide tzami
11. Archaelogical Museum
12. St. Ekaterini Museum
13. Eleftherias Square
14. Grave of N. Kazantzakis
15. Morozini Fountain
16. Old Venetian Harbour
17. Open Market
18. Koules Venetian Castle
19. St. Marcus Church
20. St. Titus Church
21. St. Minas Church
22. El-Greco Park

## Fodele Beach and Water Park Holiday Resort

### All Inclusive Description

#### Food & Beverage Services

**Main Restaurant**

**All meals are served in a buffet style**

- **Early Breakfast**: (05:00-07:00) Main Restaurant available upon request one day in advance at reception.
- **Continental Breakfast** (07:00-07:30) Main Restaurant "Amadeus"
- **Full Buffet Breakfast** (07:30-10:00) Main Restaurant "Amadeus"
- **Late Continental Breakfast** (10:00-11:00) at Snack Bar "Margherita"
- **Lunch Buffet + Show Cooking** (12:30-14:30) Main Restaurant "Amadeus"
- **Children Dinner** (from 01/07 until 31/08, upon arrangement with the chief animator)
- **Dinner Buffet + Show Cooking** (18:30-21:30) Main Restaurant "Amadeus"
- **Late Dinner** (21:30-23:00) Light Buffet – Snacks ,Main Restaurant "Amadeus"
- **Lunch Baskets**: available upon request at reception one day in advance until 19:00.
- **Greek Night**: Once a week in our Main Restaurant "Amadeus" (music & dance in the hotel )
- **4 theme buffets in 2 weeks stay** (Greek, Asian, Mediterranean and International , in our Main Restaurant "Amadeus"

**Drinks**, during meals in main restaurant: water, soft drinks, juices, local beer & local wine.

**A La Carte Restaurants* (19:00 - 21:30) supplement required**

1. **Cretan Restaurant *Elia*** (Cretan cuisine)
2. **International Restaurant *Ambrosia*** (International Cuisine)

**Accompanied by water, soft drinks, juices, local beer & local wine.**

**upon reservation one day in advance at the Main Restaurant.*

**Snacks Outlets**

**A. *Margarita Snack* Bar** at the main swimming pool (10:00 -18:00).
**B. *Windmill Snack* Bar*** at the Hotel's Water Park open on specific hours
**C. *After Dark* Snacks** in the Main Restaurant (21:30 -23:00).
**Accompanied by water, soft drinks, juices, local beer & local wine.**
** Open air, weather permitting*

**Bars**

1. ***Belvedere Cocktail* Bar** at the Hotel's lobby, self service. Belvedere Coctail Bar is open from17:30 -01:00.All inclusive is valid though till 23:00.
2. Offering a big variety of International and Local Alcoholic Drinks, Cocktails, Soft drinks, Juices, Aperitifs, Local Beer, House Wine and Coffee.
3. ***Starfish Beach* Bar*** at the beach, self service (10:00 -18:00). Soft Drinks, Juices, Aperitifs, Local Beer, House Wine, Filter Coffee and Light Snacks.
4. ***Starlight* Bar*** at Hotel main plaza, self service. Starlight Bar is open from 17:30-24:00. All Inclusive is valid till 23:00. Offering a big variety of International and Local Alcoholic Drinks, Soft drinks, Juices, Aperitifs, Local Beer, House Wine and Coffee.

**open air, weather permitting.*

**Sports Activities***

- **Floodlit Tennis Court** 08:00 – 20:00 (One hour a day/reservation required one day in advance / free equipment)
- **Mini Golf**: please contact our reception desk
- **Water Park**: with 7 slides and 6 Pools
- **Table Tennis**: just outside the snack bar
- **Mini Football (on the beach):** Please contact our animation team.
- **Basketball court**
- **Beach Volley**
- **Archery**
- **Boccia**

****There might be a time limit in the use of the sports facilities and equipment.***

**Animation - Entertainment**

Day & Evening Animation programme six days a week, for more details please check the information board.

Water polo, Darts, Gymnastics, Aerobics, Aqua Gym, Evening Shows, Quiz, Games , ance competitions, Bingo, Various tournaments, Mini Club 4-12 years old, Mini Disco (20:30 -21:30, 6 days a week)

**Beach Facilities (Sandy Beach) 10:00 - 18:00**

Sunbeds and umbrellas, Showers – Changing cabins – WC, Lifeguard on duty 1st June – 30th September

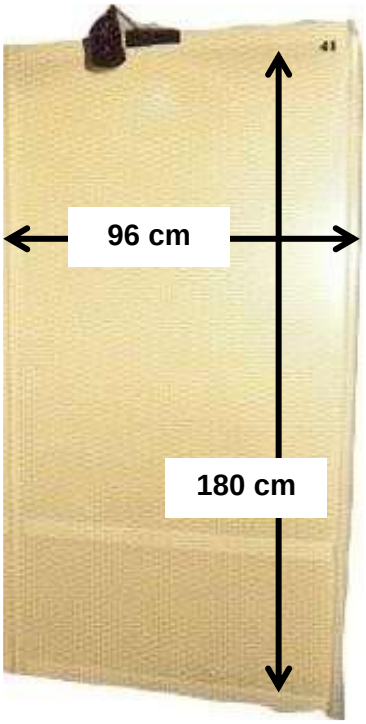
**Water Sports**

Pedaloes, Canoe, Wind surfing, Water Skiing, Scuba diving , Diving lessons, Banana

# HOTEL PLAN


1 MAIN ENTRANCE
2 MAIN BUILDING ENTRANCE
3 RECEPTION
4 MAIN BAR
5 MAIN RESTAURANT
6 INTERNET CORNER
7 A LA CARTE RESTAURANT "AMBROSIA"
8 SNACK BAR
9 BEACH BAR
10 DISCO
11 MINI CLUB
12 MEDICAL CENTER
13 FITNESS ROOM (GYM)
14 SAUNA
15. MAIN SWIMMING POOL
16 HEATED SWIMMING POOL
17 PLAZZA
18 STAR LIGHT BAR
19 SWIMMING POOL
20 MINI GOLF
21 TENNIS COURTS
22 BASKET BALL COURT
23 MINI MARKET
24 HAIRDRESSING SALON
25 RENT A CAR
26 BEACH
27 SEA SPORTS
28 AMPHITHEATRE
29 LUGGAGE ROOM
30 PARKING
31 ARCHERY
32 BEACH SOCCER
33 BEACH VOLLEY
34 GAMES ROOM
35 CONFERENCE ROOM
36 DIVING CENTER
37 ATM
38 WATERPARK
39 SNACK BAR "WINDMILL"
40 BOCCIA
41 A LA CARTE RESTAURANT
FODELE BEACH HOLIDAYS RESORT
P.O.BOX 1354
GR - 71500 HERAKLION
CRETE - GREECE
Tel . +302810521251/5
+302810521351/4
Fax. +302810521249
www.fodelebeach.gr
e-mail: fodele@fodelebeach.gr
FODELE BEACH
HOTEL PLAN

# POSTER PRESENTATION




✓ Each author will have a board space of (HxW) 180 cm x 96 cm (5ft 10in x 3ft 2in), on which to mount the poster. The poster should be designed to summarize current research in graphic forms. Presentations should be self-explanatory so that the author is free to supplement and discuss particular points. For easy identification, provide a poster heading, listing its title and author(s), identical to that on the official program.

✓ The poster board **(PORTRAIT ORIENTATION)** is double-sided with one presentation on each side. You will find your number on the poster board. The boards will be arranged in numerical order outside the conference hall.

✓ Poster materials may be mounted on thin poster paper or cardboard and attached to the poster board with the material provided by the organizers.

✓ **DO NOT USE PUSH-PINS OR GLUE** (materials for attaching illustrations will be available at the help desk inside the poster room).

✓ **DO NOT PAINT OR WRITE ON THE BOARD**

✓ Do not mount your poster on heavy or thick backing, as it may be difficult to fasten to the board. If you require assistance with mounting or removing your poster, there will be assistants inside the room.

✓ Each author is responsible for assembly and removal of his/her own presentation.

✓ Please remove your poster promptly. Materials left on the poster boards after the removal deadline will be taken down. The organizers or the hotel staff has no responsibility for materials that may be lost or damaged.

✓ The poster sessions have a designated time in which the poster presenters are requested to be available at their poster to discuss their research with the meeting attendees.

***Administration - Conference Secretary***

Mrs. Georgia Choulaki
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY
IMBB-FORTH
Tel. +30 2810 391110
gh@imbb.forth.gr
http://www.imbb.forth.gr

***Partner Organizer:***

**C**retan **C**onference & **B**usiness **S**ervices Greece
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Tel: +30 2810 331010 - Fax: +30 2810 390606
e-mail: info@ccbsgreece.gr
http://www.ccbsgreece.gr

EMBO Conference

# Cell biology of the neuron:
## Polarity, plasticity and regeneration

7 – 10 May 2017 | Heraklion, Greece

### ORGANIZERS

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German Center for Neurodegenerative Diseases (DZNE), DE

**Casper Hoogenraad**
Cell Biology, Faculty of Sciences, Utrecht University, NL

**Nektarios Tavernarakis**
Institute of Molecular Biology and Biotechnology, GR

### REGISTRATION

Application deadline
31 January 2017
Abstract submission deadline
31 January 2017
Academia (single room) .....480 EUR
Academia (shared room)....375 EUR
Industry (single room).....650 EUR
Industry (shared room) .....530 EUR

### CONTACT

INFO@EMBO-NEURO2017.GR

embo-neuro2017.gr

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KU Leuven, BE

**Hugo Bellen**
Jan and Dan Duncan Neurological Research Institute, Houston, US

**Azad Bonni**
Washington University School of Medicines, St. Louis, US

**Elena Cattaneo**
University of Milan, IT

**Mike Fainzilber**
Weizmann Institute of Science, Rehovot, IL

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**Volker Haucke**
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**Nobutaka Hirokawa**
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**Corette Wierenga**
Utrecht University, NL

**Xiaowei Zhuang**
Harvard University, Cambridge, US

EMBO excellence in life sciences
FORTH
Universiteit Utrecht
DZNE Deutsches Zentrum für Neurodegenerative Erkrankungen in der Helmholtz-Gemeinschaft