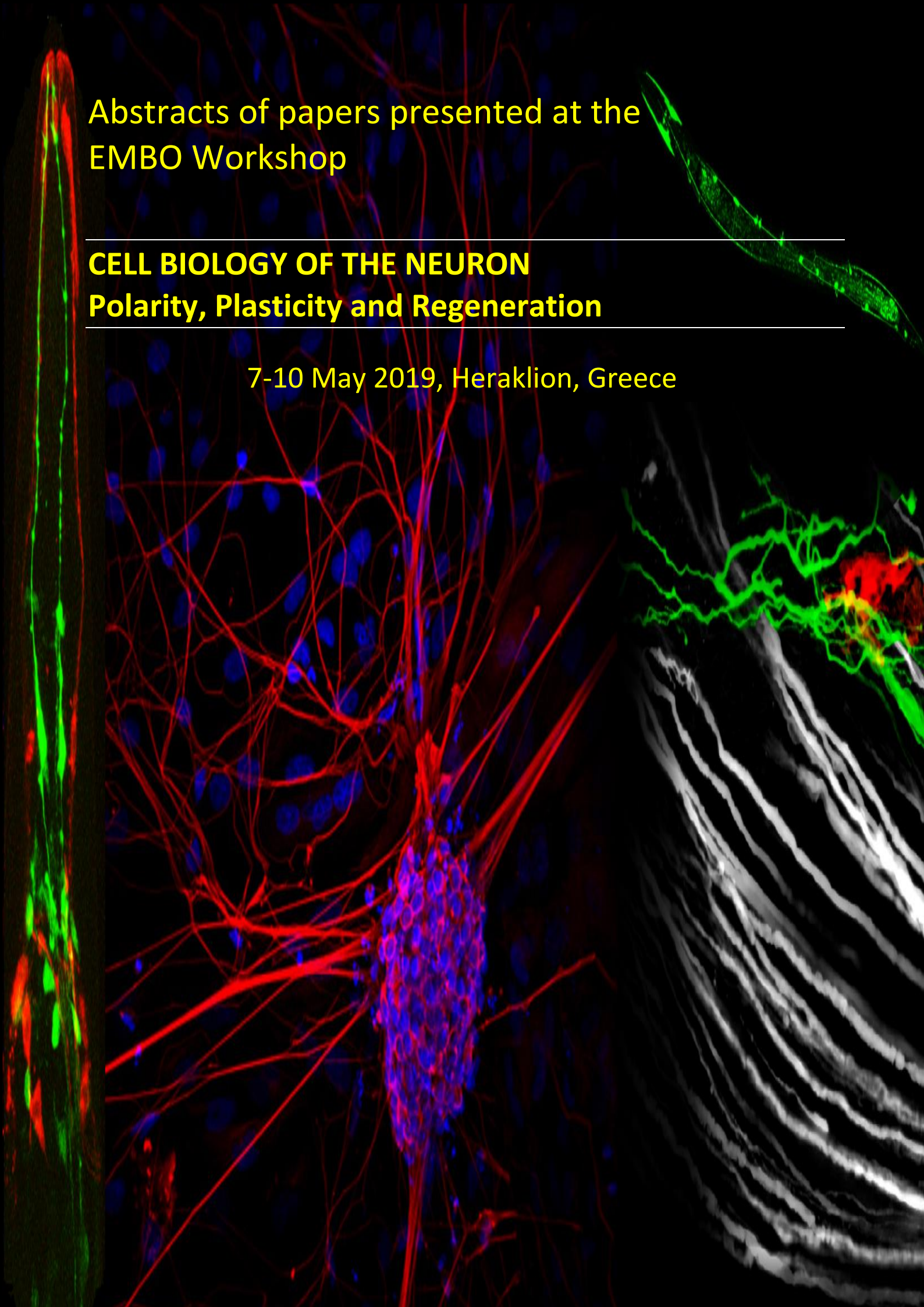
Abstracts of papers presented at the
EMBO Workshop

CELL BIOLOGY OF THE NEURON
Polarity, Plasticity and Regeneration

7-10 May 2019, Heraklion, Greece



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EMBO Workshop **Cell biology of the neuron: Polarity, plasticity and regeneration**
7–10 May 2019 | Heraklion, Greece

Programme

Tuesday, 7 May 2019	
11:00	**Registration**
13:20-13:30	Opening Remarks
Session 1: Neuronal Polarity ***Chair: Francesca BARTOLINI*** *[Columbia University, USA]*	
13:30-13.55	**Invited Talk 1: Kang SHEN** [Stanford University, USA] *Growing Tip-localized Microtubule Organizing Center Determines Microtubule Orientation in Dendrites*
13:55-14:10	Short Talk 1: **Froylan CALDERON DE ANDA** [University Medical Center Hamburg-Eppendorf, ZMNH, Germany] *Radial F-actin Organization During Early Neuronal Development*
14:10-14:25	Short Talk 2: **Ines HAHN** [University of Manchester, UK] *Polymerisation of Microtubules is Functionally Linked to their Bundled Arrangements in Axons*
14:25-14.50	**Invited Talk 2: Linda VAN AELST** [Cold Spring Harbor Laboratory, USA] *Axo-axonic Innervation of Neocortical Pyramidal Neurons by GABAergic Chandelier Cells Requires AnkyrinG-associated L1CAM*
14:50-15:30	☕ *Coffee break*
Session 2: Axon Growth & Guidance ***Chair: Nuria DOMINGUEZ ITURZA*** *[University of Lausanne, Switzerland]*	
15:30-15:55	**Invited Talk 3: Yimin ZOU** [University of California, San Diego, USA] *Wnt/planar Cell Polarity Signaling in Growth Cone Guidance*
15:55-16:10	Short Talk 3: **Aurnab GHOSE** [Indian Institute of Science, Education and Research, India] *Actin-Microtubule Coordination in the Neuronal Growth Cone: a Novel Role for Formin 2*
16:10-16:25	Short Talk 4: **Telma SANTOS** [German Center for Neurodegenerative Diseases – DZNE, Germany] *Axon Growth of CNS Neurons in three Dimensions is Amoeboid-like and Independent of Adhesions*
16:25-16:50	**Invited Talk 4: Guillermina LÓPEZ-BENDITO** [Instituto de Neurosciencias CSIC & Univ. Miguel Hernández, Spain] *Thalamic Calcium Waves Regulate the Development and Plasticity of Sensory Cortical Maps*
17:00-19:30	**Poster Session I – Red Session**
19:30-21:00	🍽 *Dinner*
21:00-23:00	*Drinks – including speed dating (poster presenters and other participants interact with main speakers*

Wednesday, 8 May 2019	
Session 3: Dendrite ***Chair: Azadeh IZADIFAR*** *[VIB-KU Leuven, Belgium]*	
09:00-09:25	**Invited Talk 5: Oren SCHULDINER** [Weizmann Institute of Science, Israel] *Adhesion Mediated Neuron-Neuron Communication Instructs Neuronal Circuit Remodeling*
09:25-09:40	Short Talk 5: **Tomke STÜRNER** [German Center for Neurodegenerative Diseases (DZNE), Germany] *In Vivo Actin Dynamics in Dendrites: A Computational Approach*
09:40-09:55	Short Talk 6: **Sebastian RUMPF** [University of Muenster, Germany] *Tissue-Derived Mechanical Force During Dendrite Pruning in the Drosophila PNS*
09:55-10:20	**Invited Talk 6: Lukas KAPITEIN** [Utrecht University, Netherlands] *Sorting Out Polarized Transport in Neurons*
10:20-10:50	☕ *Coffee break*
Session 4: Synapse ***Chair: Bharti NAWALPURI*** *[Institute for Stem Cell Science and Regenerative Medicine, India]*	
10:50-11:15	**Invited Talk 7: Peter SCHEIFFELE** [University of Basel, Switzerland] *Cell Biological Mechanisms of Synapse and Circuit Dysfunction in Rodent Models of Autism-spectrum Disorders*
11:15-11:30	Short Talk 7: **Francesca BARTOLINI** [Columbia University, USA] *Presynaptic Boutons are Hotspots for Activity-dependent Microtubule Nucleation*
11:30-11:45	Short Talk 8: **Nuria DOMINGUEZ ITURZA** [University of Lausanne, Switzerland] *The Autism and Schizophrenia-associated Protein CYFIP1 Regulates Bilateral Brain Connectivity*
11:45-12:10	**Invited Talk 8: Monica SOUSA** [University of Porto, Portugal] *Regulation of Axon Diameter by the Membrane Periodic Skeleton*
12:10-13:30	🍽 *Lunch*
Session 5: Transport ***Chair: Ines HAHN*** *[University of Manchester, UK]*	
13:30-13:55	**Invited Talk 9: Erika HOLZBAUR** [Perelman School of Medicine, University of Pennsylvania, USA] *Trafficking in Neurons: Express Shipping and Local Delivery*
13:55-14:10	Short Talk 9: **Richard EVA** [University of Cambridge, UK] *Axonal Signaling, Trafficking and Transport Mechanisms Regulating Axon Regeneration in the Adult CNS*
14:10-14:25	Short Talk 10: **Frédéric SAUDOU** [Grenoble Institut Neurosciences, France] *Vesicular Treadmilling in Axons Defines how Synapses Adapt to High Neuronal Demand*
14:25-14:50	**Invited Talk 10: Antonella RICCIO** [MRC LMCB, London, UK] *The Secret Life of 3'UTRs in Developing Neurons*
14:50-15:25	☕ *Coffee break*

Session 6: Regeneration ***Chair: Elisa SAVINO*** *[Vita-Salute San Raffaele University, Italy]*	
15:25-15:50	**Invited Talk 11: Simone DI GIOVANNI** [Imperial College London, UK] *Age-dependent Immune Control of Axonal Regeneration*
15:50-16:05	Short Talk 11: **Indrek KOPPEL** [Weizmann Institute of Science, Israel] *Subcellular Localization of the RNA-binding Protein Nucleolin for Axon Growth Regulation*
16:05-16:20	Short Talk 12: **Azadeh IZADIFAR** [VIB-KU Leuven, Belgium] *Conserved Functions of Wnk Kinases in Axon Branch Patterning and Maintenance*
16:20-16.45	**Invited Talk 12: Mark TUSZYNSKI** [UC San Diego School of Medicine, USA] *Neural Relay Formation by Neural Stem Cells after Spinal Cord Injury*
Session 7: Degeneration ***Chair: Maria SCHÖRNIG*** *[Max Planck Institute for Evolutionary Anthropology, Germany]*	
16:45-17:10	**Invited Talk 13: Marc FREEMAN** [Oregon Health & Science University, Portland, USA] *Neuron-glia Network Signaling after Nerve Injury*
17:10-17:25	Short Talk 13: **Maya MAOR NOF** [Stanford University, USA] *Uncovering Epigenetic Changes in Response to TDP-43 and C9ORF72 Dipeptide-repeat-protein Toxicity*
17:25-17:40	Short Talk 14: **Ulrich HENGST** [Columbia University, USA] *Promotion of Axon Growth and Fasciculation Through an Axonally Derived Protein*
17:40-18:05	**Invited Talk 14: Rosalind SEGAL** [Harvard Medical School, Cambridge, USA] *Transport and Translation of mRNAs that Promote Axon Survival*
18:05-20:00	**Meet the Editor/s**
20:00-21:30	🍽 *Dinner*
21:30-23:00	*Wine and Beer at Poster Room*
Thursday, 9 May 2019	
Session 8: Neuronal Plasticity ***Chair: Tomke STÜRNER*** *[German Center for Neurodegenerative Diseases (DZNE), Germany]*	
09:25-09:40	Short Talk 15: **Amrita MUKHERJEE** [University of Cambridge, UK] *Role and Regulation of Gamma Tubulin Ring Complexes (γ-TuRCs) in Dendritic Arbor Development*
09:40-09:55	Short Talk 16: **Peter SOBA**[University Medical Center Hamburg/Eppendorf (UKE), Germany] *Maintenance of Cell Type-specific Connectivity and Circuit Function Requires Tao Kinase*
09:55-10:20	**Invited Talk 15: Christina ALBERINI** [New York University, NY, USA] *A Critical Role for Autophagy in Long-term Memory Formation*
10:20-10:50	☕ *Coffee break*
Session 9: Glia ***Chair: Amrita MUKHERJEE*** *[University of Cambridge, UK]*	
10:50-11:15	**Invited Talk 16: David ATTWELL** [University College London, UK] *The Role of Capillary Pericytes in Alzheimer's Disease*
11:15-11:30	Short Talk 17: **Bharti NAWALPURI** [Institute for Stem Cell Science and Regenerative Medicine, India] *Role of GW182 during Neuronal Development*
11:30-11:45	Short Talk 18: **Martin BALASTIK** [Institute of Physiology, CAS, Czech Republic] *CRMP2 Mediates Sema3F-dependent Axon and Dendritic Spine Prunin*
11:45-12:10	**Invited Talk 17: Magdalena GÖTZ** [Ludwig Maximilians Universität München, Germany] *Novel Mechanisms of Neurogenesis and Repair*

12:10-14:00	🍽 *Lunch*
14:00-14:25	**Invited Talk 18: Nathalie ROUACH** [Collège de France, CIRB, Paris, France] *Exploring Astroglial Glutamine Transfer with Novel Dye: Where, When, How and What for?*
14:25-17:00	**Poster Session II – Blue Session**
19:00	🍽 *Dinner*

Friday, 10 May 2017

Session 10: Stem Cells

Chair: Maya MAOR NOF *[Stanford University, USA]*

09:00-09:25	**Invited Talk 19: Gaia NOVARINO** [Institute for Science and Technology, Klostemeuburg, Austria] *Epigenetic Regulators in Autism Spectrum Disorders*
09:25-09:40	Short Talk 19: **Alexandre BAFFET** [Institut Curie, France] *Acentrosomal Microtubule Organizing Centers in Mouse and Human Neural Stem Cells*
09:40-09:55	Short Talk 20: **Maria SCHÖRNIG** [Max Planck Institute for Evolutionary Anthropology, Germany] *Morphological and Functional Comparisons between Human and Chimpanzee Induced Neuron*
09:55-10:20	**Invited Talk 20: Pierre VANDERHAEGHEN** [University of Brussels, Belgium] *Deciphering Human-specific Mechanisms of Cortical Neuron Development*
10:20-10:50	☕ *Coffee break*

Session 11: Novel Methods

Chair: Telma SANTOS *[German Center for Neurodegenerative Diseases – DZNE, Germany]*

10:50-11:15	**Invited Talk 21: Valentin NÄGERL** [Université de Bordeaux / CNRS UMR 5297, France] *Super-Resolution Imaging of the Structure and Function of Tripartite Synapses*
11:15-11:30	Short Talk 21: **Fengwei YU** [Temasek Life Sciences Laboratory (TLL), Singapore] *A Microtubule Polymerase Complex is Essential for Dendritic Microtubule Polarity and Dendrite Pruning in Drosophila*
11:30-11:45	Short Talk 22: **Elisa SAVINO** [Vita-Salute San Raffaele University, Italy] *PRRT2 Function in the Regulation of Neuronal Actin Cytoskeleton*
11:45-12:10	**Invited Talk 22: Ryohei YASUDA** [Max Planck Florida Institute for Neuroscience, Jupite, USA] *Biochemical Computation in Single Dendritic Spines*
12:10-14:00	*Closing Remarks – End of Workshop* - 🍽 *Lunch*

ORAL PRESENTATIONS

Session 1: Neuronal Polarity

Invited Lectures

Growing Tip-localized Microtubule Organizing Center Determines Microtubule Orientation in Dendrites
Shen K

Axo-axonic Innervation of Neocortical Pyramidal Neurons by GABAergic Chandelier Cells Requires AnkyrinG-associated L1CAM
Gallo N, Tai Y, Wang M, Yu J-R, **Van Aelst L**

Short Talk

Radial F-actin Organization during Early Neuronal Development
Meka DP, Scharrenberg P, Zhao B, König T, Schaefer I, Schwanke B, Kobler O, Klykov S, Richter M, Eggert D, Windhorst S, Dotti CG, Kreutz MR, Mikhaylova M, **Calderon de Anda F**

Polymerisation of Microtubules is Functionally Linked to their Bundled Arrangements in Axons
Voelzmann A, Yue Q, Parkin J, Slater P, Lowery LA, Okenve-Ramos P, Sánchez-Soriano N, Prokop A, **Hahn I**

Session 2: Axon Growth & Guidance

Invited Lectures

Wnt/planar Cell Polarity Signaling in Growth Cone Guidance
Zou Y

Thalamic calcium waves regulate the development and plasticity of sensory cortical maps
López-Bendito G

Short Talk

Actin-Microtubule Coordination in the Neuronal Growth Cone: a Novel Role for Formin 2
Ghose A

Axon Growth of CNS Neurons in three Dimensions is Amoeboid-like and Independent of Adhesions
Santos TE, Broguière N, Meyn L, Zenobi-Wong M, Bradke F

Session 3: Dendrite

Invited Lectures

Adhesion Mediated Neuron-Neuron Communication Instructs Neuronal Circuit Remodeling
Bornstein B, **Schuldiner O**

Sorting Out Polarized Transport in Neurons
Kapitein L

Short Talks

***In Vivo* Actin Dynamics in Dendrites: A Computational Approach**
Stürner T, Castro A, Cuntz H, Tavosanis G

Tissue-Derived Mechanical Force During Dendrite Pruning in the Drosophila PNS
Krämer R, **Rumpf S**

Session 4: Synapse

Invited Lectures

Cell Biological Mechanisms of Synapse and Circuit Dysfunction in Rodent Models of Autism-spectrum Disorders
Scheiffele P, Hörnberg H

Regulation of Axon Diameter by the Membrane Periodic Skeleton
Sousa M

Short Talks

Presynaptic Boutons are Hotspots for Activity-dependent Microtubule Nucleation
Qu X, Kumar A, **Bartolini F**

The Autism and Schizophrenia-associated Protein CYFIP1 Regulates Bilateral Brain Connectivity
Domínguez-Iturza N, Shah D, Vannelli A, Lo AC, Armendáriz M, Li KW, Mercaldo V, Trusel M, Gastaldo D, Mameli M, Van der Linden A, Smit AB, Achsel T, Bagni C

Session 5: Transport

Invited Lectures

Dynamics of Autophagy and Mitophagy in Neurons
Holzbaur ELF

The Secret Life of 3'UTRs in Developing Neurons
Riccio A

Short Talks

Axonal Signaling, Trafficking and Transport Mechanisms Regulating Axon Regeneration in the Adult CNS
Nieuwenhuis B, Petrova V, Pearson C, Evans R, Barber A, Smith P, Reid E, Martin K, **Eva R,** Fawcett J

Vesicular Treadmilling in Axons Defines how Synapses Adapt to High Neuronal Demand
Cazorla M, Moutaux E, Aspert T, **Saudou F**

Session 6: Regeneration

Short Talks

Age-dependent Immune Control of Axonal Regeneration
Di Giovanni S

Neural Relay Formation by Neural Stem Cells After Spinal Cord Injury
Tuszynski MH, Lu P, Rosenzweig E, Brock J, Vanniekerk E, Poplawski G, Koffler J, Kimamaru H, Adler A, Dulin J, Kadoya K

Short Talks

Subcellular Localization of the RNA-binding Protein Nucleolin for Axon Growth Regulation
Koppel I, Doron-Mandel E, Oses-Prieto JA, Kawaguchi R, Abraham O, Rishal I, Alber S, Okladnikov N, Kadlec J, Jungwirth P, Coppola G, Burlingame AL, Fainzilber M

Conserved Functions of Wnk Kinases in Axon Branch Patterning and Maintenance
Izadifar A, Courchet J, Sachse S, Verreet T, Misbaer A, Yan J, Vandenbogaerde S, Ayaz D, Petrovic M, Yan B, Erfurth M-L, Dascenco D, Lewis T, Polleux F, Schmucker D

Session 7: Degeneration

Invited Lectures

Neuron-glia Network Signaling after Nerve Injury
Freeman M

Transport and Translation of mRNAs that Promote Axon Survival
Segal R

Short Talks

Uncovering Epigenetic Changes in Response to TDP-43 and C9ORF72 Dipeptide-repeat-protein Toxicity
Maor-Nof M, Shipony Z, Nakayama L, Couthouis J, Greenleaf WJ, Gitler AD

Promotion of Axon Growth and Fasciculation through an Axonally Derived Protein
McCurdy EP, **Hengst U**

Session 8: Neuronal Plasticity

Invited Lectures

A Critical Role for Autophagy in Long-term Memory Formation
Pandey K, Yu X-W, Steinmetz A, **Alberini CM**

Short Talk

Role and Regulation of Gamma Tubulin Ring Complexes (γ-TuRCs) in Dendritic Arbor Development
Mukherjee A, Brooks P, Jeske YA, Conduit PT

Maintenance of Cell Type-specific Connectivity and Circuit Function Requires Tao Kinase
Tenedini FM, Saéz Gonzáles M, Hu C, Pedersen L, Matamala Petruzzi M, Wang D, Richter M, Petersen M, Spotowicz E, Schweizer M, Sigrist S, Calderon de Anda F, **Soba P**

Session 9: Glia

Invited Lectures

The Role of Capillary Pericytes in Alzheimer's Disease
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Novel Mechanisms of Neurogenesis – from Centrosomes to Nucleoli
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Exploring Astroglial Glutamine Transfer with Novel Dye: Where, When, How and What for?
Rouach N

Short Talk

Role of GW182 during Neuronal Development
Nawalpuri B, Raman S, Muddashetty R

CRMP2 Mediates Sema3F-dependent Axon and Dendritic Spine Pruning
Ziak J, Weissova R, Jerabkova K, Janikova M, Maimon R, Petrasek T, Pukajova B, Wang M, Brill MS, Misgeld T, Stuchlik A, Perlson E, **Balastik M**

Session 10: Stem Cells

Invited Lectures

Epigenetic Regulators in Autism Spectrum Disorders
Novarino G

Deciphering Human-specific Mechanisms of Cortical Neuron Development
Vanderhaeghen P

Short Talk

Acentrosomal Microtubule Organizing Centers in Mouse and Human Neural Stem Cells
Victoria GS, Coquand LJ, Brault J-B, Martin M, Fraisier V, **Baffet A**

Morphological and Functional Comparisons between Human and Chimpanzee Induced Neurons
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Session 11: Novel Methods

Invited Lectures

Super-Resolution Imaging of the Structure and Function of Tripartite Synapses
Arizono M, **Nägerl UV**

Biochemical computation in single dendritic spines
Yasuda R

Short Talk

A Microtubule Polymerase Complex is Essential for Dendritic Microtubule Polarity and Dendrite Pruning in *Drosophila*
Tang Q, Wang Y, Rui M, **Yu F**

PRRT2 Function in the Regulation of Neuronal Actin Cytoskeleton
Savino E, Cervigni RI, Guarnieri FC, Corradi A, Benfenati F, Valtorta F

POSTER PRESENTATIONS

1. **Tropomyosin Tpm3.1 is required to maintain the Structure and Function of the Axon Initial Segment**
   Abouelezz A, Stefen H, Hardeman EC, Gunning PW, Hoogenraad CC, Fath T, Hotulainen P

2. **Region-dependent Diversity of Post-synaptic Densities in the Mouse Brain**
   Gastaldo D, Mercaldo V, Fernández E, Cencelli G, Grant SN, Bagni C, **Achsel T**

3. **Astrocyte Glutamate Transporter GLT-1 as a Possible Therapeutic Target in Rett Syndrome**
   Albizzati E, Taiarol L, Frasca A, Landsberger N

4. **Modelling Citrate Transporter Deficiency in the Human Nervous System**
   Allison K, Ísaksson HJ, Franzdóttir SR

5. **Structural Basis of Astrocytic Ca^{2+} Signals at Tripartite Synapses**
   Arizono M, Krishna Inavalli VVG, Panatier A, Pfeiffer T, Angibaud J, Stobart J, Bellocchio L, Marsicano G, Mikoshiba K, Oliet SHR, Weber B, Nägerl UV

6. **CALM Dictates GluA1-containing AMPA Receptor Endocytosis**
   Azarnia Tehran D, Kochlamazashvili G, Haucke V, Maritzen T

7. **AP-2 Prevents Amyloidogenesis via Regulation of BACE1 Trafficking in Neurons**
   Bera S, Calleja Barca E, Negrete-Hurtado A, Racho J, De Bruyckere E, Wittich C, Ellrich N, Martins S, Adjaye J, Kononenko NL

8. **Signaling Between Serotonin Receptors and the Extracellular Matrix as a Key to Understanding Pathogenesis of Stress Related Disorders**
   Bijata M, Krzystyniak A, Baczynska E, Masternak J, Antoniuk S, Filipkowski R, Ponimaskin E, Wlodarczyk J

9. **Mechanisms of Microtubule Nucleation in the *Drosophila* Nervous System**
   Brooks P

10. **Ataxin 2 CAG Expansions Trigger TDP-43 Relocalization in Neurons and Alter the Phagocytic Role of Microglia in the Spinal Cord**
    Canet-Pons J, Sen NE, Döring C, Reichlmeir M, Gispert S, Auburger G

11. **MADD-4/Ce-Punctin Processing Defines Synaptic Identity at the NMJ**
    Cizeron M, Romatif O, Bessereau J-L

12. **The Microtubule Minus-end Regulator Patronin Restricts Dendritic Branching in Simple Drosophila Dendritic Arborisation Neurons**
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13. **The AMPK-related Kinase NUAK1 Controls Axon Branching through a Local Remodelling of Metabolic Pathways during Development**
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14. **Motor Neurons Harbouring the DHMN1 1.35 MB Complex Insertion: A Useful Paradigm for Understanding Genomic Organisation and Gene Regulation in Neurodegenerative Disease**
    Cutrupi AN, Perez-Siles G, Brewer MH, de Moraes Maciel R, Ly C, Nicholson GA, Saporta MA, Kennerson ML

15. **Organellar Proteomics Reveals the Mechanisms that Underlie the Neuronal Pathology of AP-4 Deficiency**
Davies AK, Itzhak DN, Edgar JR, O'Neill AC, Schessner J, Götz M, Robinson MS, Borner GHH

16. **Knockdown of HCN Channels in Mouse Hippocampal Neurons by Virus Delivered Gene-interfering Tools**
Deutsch M, Günther A, Baumann A

17. **An Axon-specific Hippo-like Pathway Regulates Axon Branching**
Edwards-Faret G, Izadifar A, Schmucker D

18. **Growth Cone Molecular Machinery in Development *vs.* Regeneration: Subcellular Transcriptome Mapping in Corticospinal Neurons**
Engmann AK, Hatch J, Kim J, Winter C, Macklis JD

19. **Anterograde Trans-synaptic Jeb-Alk Signaling is a Negative Feedback Mechanism to Stabilize Circuit Growth**
Gärtig P-A, Ostrovsky A, Manhart L, Kovacevic T, Lustig H, Chwalla B, Cachero S, Landgraf M, **Evers JF**

20. **Nrg1 Intracellular Signaling is Neuroprotective upon Stroke**
Navarro-González C, Huerga-Gómez A, **Fazzari P**

21. **Dystroglycan Affects Morphology and Function of Hippocampal Neurons**
Figiel I, Bijata M, Magnowska M, Roszkowska M, Wojtowicz T, Wlodarczyk J

22. **New Hypotheses of NeuroLSD1 Modulation: from lncRNA to Cryptic Exon Inclusion**
Forastieri C, Longaretti A, Toffolo E, Rusconi F, Battaglioli E

23. **Pontin and Reptin at the Neuro-muscular Junction**
Allison KE, Björgvinsson AÞ, Guðmundsson H, Jónsson ZO, **Franzdóttir SR**

24. ***MECP2* Mutations Affect Ciliogenesis: a Novel Perspective for Rett Syndrome and Related Disorders**
Frasca A, Spiombi E, Palmieri M, Valente M, Bergo A, Leva B, Kilstrup-Nielsen C, Bianchi F, Di Cunto F, Landsberger N

25. **The Role of RNA Binding Proteins in Amyotrophic Lateral Sclerosis: How do they contribute to Motor Neuron Degeneration?**
Garone MG

26. **The Coordination between Kinesin-3 and Dynamic Presynaptic Microtubules Specifies High Precision Delivery of Synaptic Vesicles to the Presynapse**
Guedes-Dias P, Nirschl JJ, Abreu N, Tokito MK, Janke C, Magiera MM, Holzbaur ELF

27. **Super-resolution Iimaging of Brain Tissue during Chemical Fixation**
Idziak A, Arizono M, Nägerl UV

28. **Dual Activity of RIN1 Regulates Synaptic Plasticity in Dendritic Spines**
Ignácz A, Szíber Z, Nagy-Herczeg D, Tárnok K, Hausser A, Schlett K

29. **Uniform Axonal Microtubule Organisation is established by Dynactin-mediated Microtubule Stabilisation**
Jakobs M, Franze K

30. **Deficits of Brain Protein Homeostasis Affect Social Behaviours in Flies**
Kanellopoulos AK, Mariano V, Achsel T, Bagni C

31. **Synthetic Microneurotrophin BNN27 Improves Working Memory, Ameliorates Aβ Pathology and Promotes Adult Neurogenesis in the 5xFAD Mouse Model of Alzheimer's Disease**
Karali K, Kokkali M, Efstathopoulos P, Gravanis A, Charalampopoulos I

32. **Cytoskeletal Dynamics in Mice Missing the Majority of Cortical Interneurons as a Result of Rac1 and Rac3 Deficiencies**
Kounoupa Z, Tivodar S, Theodorakis K, Karagogeos D

33. **A Super-resolution Platform for Correlative Single Molecule Imaging and STED Microscopy**
Krishna Inavalli VVG, Lenz MO, Butler C, Angibaud J, Compans B, Levet F, Tønnesen J, Rossier O, Giannone G, Thoumine O, Hosy E, Choquet D, Sibarita J-B, Nägerl UV

34. **Homeostatic Systems Control Neuropeptide and DCV Biogenesis**
Laurent P, Ch'ng QL, Jospin M, Chen C, Lorenzo JR, de Bono M

35. **Role of the Epigenetic Enzyme LSD1 in Controlling Neuronal Plasticity-related Transcriptional Programs**
Longaretti A, Forastieri C, Gerosa L, Toffolo E, Tonini R, Francolini M, Passafaro M, Rusconi F, Battaglioli E

36. **Revealing NMDA Receptor Hidden Conformational Open States that Block Electric Current under Agonist Activation in Living Cells by a Novel Single-Molecule Patch-Clamp FRET Super-Resolution Microscopy**
Sasmal D, Yadav R, **Lu HP**

37. **Neuronal TORC1 Coordinates Mood and Cognition via Serotonin Receptors**
Metaxakis T, Pavlidis M, Tavernarakis N

38. **Characterization of the Skt Gene in the Synapse: Behavioural Studies and Analysis of Synaptic Complexes**
Morellato A, Alfieri A, Angelini C, Cravero T, Pavinato L, Torelli F, D'attanasio G, Mele P, Gavello D, El-Assawi N, Mauro A, Carbone E, Eva C, Turco E, Defilippi P

39. **Identification of an axon-resident pool of vesicles regulated by neuronal activity**
Moutaux E, Benoit C, Blot B, Cazorla M, Saudou F

40. **Autophagy Lipidation Machinery Regulates Axonal Microtubule Dynamics but is Dispensable for Survival of Mammalian Neurons**
Negrete Hurtado A, Overhoff M, Bera S, De Bruyckere E, Schätzmüller K, Kye MJ, Qin C, Lammers M, Kondylis V, Kononenko NL

41. **Sensory Processing within the *C. elegans* Electrical Connectome**
Onizuka M, Laurent P

42. **PKD Controls Endocytic AMPAR Trafficking in the Synaptic Membrane**
Oueslati Morales CO, Ignácz A, Szíber Z, Gamoa A, Schlett K, Hausser A

43. **Deciphering the Mechanisms Underlying the Myoclonic Seizures Caused by the Loss of Autophagy in the Brain**
Overhoff M, De Bruyckere E, Negrete A, Bera S, Kononenko NL

44. **Extremely Low Frequency Electromagnetic Fields Enhance Neural Differentiation of Stem Cells through NMDA Receptor Activation**
Özgün A, Marote A, Salgado A, Garipcan B

45. **Neuronal Mitophagy in *C. elegans* Models of Alzheimer's Disease**
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Growing Tip-localized Microtubule Organizing Center Determines Microtubule Orientation in Dendrites

Kang Shen

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A polarized arrangement of microtubule arrays in axons and dendrites is the foundation of membrane trafficking and neuronal compartmentalization. Conserved among invertebrates and vertebrates, axons contain exclusively “plus-end-out” microtubules while dendrites contain a high percentage of “minus-end-out” microtubules. Here we show that the establishment and maintenance of polarized dendritic microtubule arrays depend on two mechanisms. First, a microtubule organizing center (MTOC) continuously resides within the tip of extending dendrites during outgrowth. This MTOC gives rise to numerous minus-end-out microtubules that populate outgrowing dendrites. Mislocalization of this MTOC to the cell body in kinesin-1 mutants causes reversed microtubule polarity in dendrites. Second, the microtubule plus-end tracking protein CLIP-1 is critical for the maintenance of dendrite polarity. CLIP-1 prevents the invasion of plus-end-out microtubules from the soma into the dendrite by binding to microtubule plus-ends and promoting their transition from polymerization to catastrophe.

BACK
PROGRAMME

Axo-axonic Innervation of Neocortical Pyramidal Neurons by GABAergic Chandelier Cells Requires AnkyrinG-associated L1CAM

Nick Gallo, Yilin Tai, Minghui Wang, Jia-Ray Yu, and **Linda Van Aelst**

Cold Spring Harbor Laboratory, Cold Spring Harbor, USA

Proper cortical network development and function is reliant on the generation, maturation, and activity of numerous cell types in addition to their complex cell-cell interactions. Essential to this process is the output of glutamatergic pyramidal neurons (PyNs), which is highly modulated by inhibitory GABAergic interneurons. One subset of interneurons that exerts powerful control over PyN spiking is the chandelier cell (ChC), which forms connections specifically at the site of action potential initiation in PyNs, referred to as the axon initial segment (AIS). Due to the unique connections formed between the terminals of ChC axonal arbors and the AISs of large populations of spiking PyNs, ChCs are physiologically poised to regulate the output of excitatory cortical networks. As a result, it is not surprising that disruptions in ChC biology have been linked to autism spectrum disorder (ASD) and schizophrenia, debilitating mental health disorders resulting from aberrant neuronal network activity. Despite the importance of ChCs, virtually nothing is known about the molecular factors governing their selective innervation at the AIS of neocortical PyNs. By performing a novel, *in vivo* RNA interference screen against PyN AIS-specific and -enriched adhesion molecules, we intriguingly revealed an essential role for the axonal cell adhesion molecule L1CAM in ChC/PyN AIS innervation. Specifically, L1CAM knockdown in neocortical PyNs was found to significantly reduce PyN AIS innervation by ChCs, thus identifying L1CAM as the only known molecule to date to regulate this process. To further elucidate how L1CAM governs selective ChC/PyN AIS innervation, we used molecular tools to perturb L1CAM/AIS cytoskeleton interactions and to manipulate PyN L1CAM levels at different developmental time points. Our results indicate that the AIS cytoskeletal proteins ankyrin-G- and βIV-spectrin are essential for proper ChC/PyN AIS innervation and demonstrate a dual requirement for L1CAM during both the establishment and maintenance of neocortical ChC/PyN AIS innervation. Together, our findings provide novel/first insight into the mechanisms governing PyN AIS subcellular innervation by ChCs.

BACK
PROGRAMME

Wnt/planar Cell Polarity Signaling in Growth Cone Guidance

Yimin Zou

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Although the major molecular guidance cues and their receptors and signaling pathways for axon wiring have been identified, the signaling and cell biological mechanisms that control the directionality of growth cone navigation are still incompletely understood. We found that the Wnt family proteins provide directional instructions via a non-canonical Wnt signaling pathway, called planar cell polarity (PCP) pathway. We also found that Wnt/PCP signaling pathway is reactivated in injured axons after spinal cord injury and regulates the sprouting of axons in adulthood. Our previous work showed that PCP proteins are asymmetrically localized in the growth cones and the Wnt receptor, Frizzled, undergo dynamic endo- and exocytosis to polarize the growth cone for turning. We now observed evidence that the PCP components also mediate growth-cone-growth-cone interactions that may coordinate the turning of groups of growth cones in brain wiring.

BACK
PROGRAMME

Thalamic calcium waves regulate the development and plasticity of sensory cortical maps

Guillermina López-Bendito

Institute of Neuroscience, San Juan de Alicante, Alicante

Our research team runs several related projects studying the cellular and molecular mechanisms involved in the development of axonal connections in the brain. In particular, our aim is to uncover the principles underlying thalamocortical axonal wiring, maintenance and ultimately the rewiring of connections, through an integrated and innovative experimental programme. The development of the thalamocortical wiring requires a precise topographical sorting of its connections. Each thalamic nucleus receives specific sensory information from the environment and projects topographically to its corresponding cortical. A second level of organization is achieved within each area, where thalamocortical connections display an intra-areal topographical organization, allowing the generation of accurate spatial representations within each cortical area. Therefore, the level of organization and specificity of the thalamocortical projections is much more complex than other projection systems in the CNS. The central hypothesis of our laboratory is that thalamocortical wiring influences and maintains the functional architecture of the brain. We also believe that rewiring and plasticity events can be triggered by activity-dependent mechanisms in the thalamus. Three major questions are been focused in the laboratory: i) the transcriptional control of thalamocortical guidance and topography; ii) the activity-dependent mechanisms involved in thalamocortical guidance and wiring and iii) the role of the thalamus and its connectivity in the neuroplastic cortical changes following sensory deprivation. Within these projects we are using several experimental programmes, these include: optical imaging, manipulation of gene expression in vivo, cell and molecular biology, biochemistry, cell culture, sensory deprivation paradigms and electrophysiology. The results derived from our investigations will contribute to our understating of how reprogramming of cortical wiring takes place following brain damage and how cortical structure is maintained.

BACK
PROGRAMME

Adhesion Mediated Neuron-Neuron Communication Instructs Neuronal Circuit Remodeling

Bavat Bornstein, **Oren Schuldiner**

Dept. of Molecular Cell Biology, Weizmann Institute of Science, Israel

In our laboratory, we study the molecular and cellular mechanisms underlying developmental neuronal remodeling. Remodeling is an essential and conserved mechanism to refine neural circuits by axon and synapse elimination, often followed by regrowth to form adult specific connections. Despite its fundamental role in neurodevelopment and proposed contribution to various neuropsychiatric disorders, the mechanisms instructing remodeling are only partially known.

The stereotypical remodeling of the *Drosophila* mushroom body (MB) during metamorphosis provides a unique system to genetically dissect distinct steps in neuronal remodeling. During the regrowth phase, MB neurons form stereotypic connections that form functional compartments that are important for appetitive and aversive memory formation. In my talk, I will focus on unpublished work in which we found how neuron-neuron communication mediated by adhesion molecules coordinates circuit reassembly and the formation of discrete axonal compartments, defined by specific neuronal connections and which are important for the function of the circuit.

BACK
PROGRAMME

Sorting Out Polarized Transport in Neurons

Lukas Kapitein

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

Neurons are highly polarized cells that depend on proper intracellular transport to deliver cargoes to either the axon or the dendrites. Proper positioning of organelles by cytoskeleton-based motor proteins underlies cellular events such as signaling, polarization, and growth. To explore how different motor proteins contribute to neuronal transport and to study the site-specific roles of different organelles, we have established optical control of intracellular transport by using light-sensitive heterodimerization to recruit specific cytoskeletal motor proteins (kinesin, dynein or myosin) to selected cargoes. In addition, to unravel how the specialized organization of the neuronal cytoskeleton guides different motor proteins to either axons or dendrites, we have developed novel approaches for optical nanoscopy. One of these, called motor-PAINT, uses nanometric tracking of motor proteins to super-resolve cytoskeletal fibers and determine their polarity. This has revealed a key architectural principle of the neuronal microtubule cytoskeleton that explains how different motor proteins can selectively transport cargoes to either axons or dendrites.

BACK
PROGRAMME

Cell Biological Mechanisms of Synapse and Circuit Dysfunction in Rodent Models of Autism-spectrum Disorders

Peter Scheiffele, Hanna Hörnberg

Biozentrum of the University of Basel, Switzerland

The assembly of functional neuronal networks during development relies on an intricate interplay of molecular programs that specify neuronal and synaptic properties. The goal of our work is to understand how autism-associated mutations modify developmental programs for synapse specification and how such synaptic alterations might impact circuit function and behavior. One focus of our studies has been on the dopaminergic neurons in the ventral tegmental area of mice as these cells control social recognition and social reward. We demonstrate that an autism-associated mutation introduced into dopaminergic cells of the ventral tegmental area results in a highly selective deficit in social recognition. This deficit correlates with alterations in neuronal plasticity and cell signalling. Pharmacological restoration of cell signaling is sufficient to recover synaptic plasticity and social recognition as assessed by behavioral assays. This work uncovers cell biological mechanisms of neuronal plasticity and circuit function that underlie the regulation of social interactions in rodents.

BACK
PROGRAMME

Regulation of Axon Diameter by the Membrane Periodic Skeleton

Monica Sousa

Instituto de Investigacao e Inovaca em Saude, Universidade do Porto, Porto, Portugal

After contacting their targets, axons increase their diameter to enable fast conduction of action potentials. In the adult, axonal caliber varies by almost 100-fold in different tracts. When considering a given axon, its diameter can further oscillate depending on organelle transport, neuronal activity, or deformations imposed by movement or degeneration. However, the fine-tune mechanisms controlling diameter throughout the lifetime of an axon, remain largely elusive. Here, we will present our most recent findings on the mechanisms regulating of axon diameter, with a specific focus on the role played by the actin-spectrin based membrane periodic skeleton, and their implications in neuronal biology.

BACK
PROGRAMME

Dynamics of Autophagy and Mitophagy in Neurons

Erika L. F. Holzbaur

University of Pennsylvania Perelman School of Medicine

Neurons are highly polarized, post-mitotic cells that must survive for decades in humans. Neurons rely on autophagy, a critical homeostatic mechanism, to maintain cellular health. Deficits in autophagic flux lead to the accumulation of protein aggregates and dysfunctional mitochondria, and are characteristic of neurodegenerative diseases such as Parkinson's, Huntington's, and ALS. Live cell imaging in neurons has revealed a dynamic pathway for axonal autophagy. This pathway functions constitutively to maintain axonal health, but is significantly impaired during aging. A second pathway that is both molecularly and spatially distinct regulates the selective removal of dysfunctional mitochondria via mitophagy. We hypothesize that both of these mechanisms are required to maintain neuronal homeostasis, and become impaired during neurodegeneration.

BACK
PROGRAMME

The Secret Life of 3'UTRs in Developing Neurons

Antonella Riccio

MRC Laboratory for Molecular Cell Biology-University College London London-UK

Understanding how cells translate extracellular cues into specific patterns of gene expression is one of the major goals of modern neurobiology. Neurons are cells with a complex morphology, which maintain their cellular structure through the compartmentalized expression of proteins essential for growth and plasticity. Asymmetric localization of RNA is an evolutionarily conserved mechanism that allows spatial restriction of protein synthesis to specific cellular compartments. Incorrect processing and delivery of mRNA causes developmental defects and severe human neurological disorders. In neurons, mRNA transcripts are transported to both dendrites and axons where they are rapidly translated in response to stimuli.

This talk will explore how transcripts localized in sympathetic neuron axons are transported, processed and translated in response to neurotrophins. Special emphasis will be given to the nature of the 3'UTRs of targeted axons and to the presence of unique elements that may determine their fate. I will also discuss our important findings indicating that the 3'UTR of localized transcripts undergo axonal cleavage and remodelling, thereby generating mRNA isoforms expressing a shorter 3'UTR, which are rapidly translated, and **a**xonally **c**leaved RNA fragments (acRNA) with yet unknown function.

BACK
PROGRAMME

Age-dependent Immune Control of Axonal Regeneration

Simone Di Giovanni

Imperial College London, Department of Medicine, London, UK

Axonal regeneration is limited or absent following injury in the peripheral or central nervous system (PNS or CNS) respectively, undermining functional recovery. The axonal regenerative ability and recovery potential further decline with ageing, which is an increasing risk factor for axonal injuries and disability. The knowledge of the cellular and molecular substrates responsible for this decline is very sparse. Previous studies showed that an age-related impairment in de-differentiation and activation of Schwann cells (SCs) limits axonal regrowth in the injured PNS. Following spinal cord injury, deletion of PTEN can partially limit the age-dependent regenerative decay in the CNS. Here, by RNAseq experiments in dorsal root ganglia (DRG), we found that 18-24 month old mice display age-dependent stark alterations in immune and cell-cell communication signaling pathways in comparison with 2 months old young mice both preceding and following a sciatic nerve injury. The main age associated molecular signature was represented by an increase in T cell activation and signaling, which was reduced by exposing mice to environmental enrichment (EE) before an injury. EE reversed the regenerative decline in aged mice back to the level of the young and offered an opportunity to identify molecular signaling that reverses age-dependent regenerative failure. Mechanistically, we found that an age-dependent increase in inflammatory cytokines activates NFkB in DRG neurons, which act as MHCI antigen presenting cells (APC) and express NFkB-dependent CXCL13 that recruits CXCR5+ T cells in proximity of neurons. Activated T cells in turn repress axonal regeneration of sensory DRG neurons by inhibiting regenerative signals. Remarkably, *in vivo* antibody-mediated specific T cell depletion or CXCL13 neutralization restore axonal regeneration of sensory neurons to the level of the young and beyond, following sciatic nerve and spinal cord injury alike. These data propose a novel mechanism restricting the axonal regenerative ability in the old. They also suggest that antibody-mediated manipulation of neuron-immune cell communication might be a clinically promising avenue to counteract regenerative decline.

BACK
PROGRAMME

Neural Relay Formation by Neural Stem Cells After Spinal Cord Injury

Mark H. Tuszynski[1,2], Paul Lu[1,2], Ephron Rosenzweig[1], John Brock[1,2], Erna Vanniekerk[1], Gunnar Poplawski[1], Jacob Koffler[1], Hiromi Kimamaru[1], Andrew Adler[1], Jennifer Dulin[1], Ken Kadoya[1]

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The injured adult central nervous system exhibits little capacity to rspontaneous egenerate after spinal cord injury. In contrast, grafts of neural stem cells to sites of spinal cord injury extend extremely large numbers of axons over very long distances. While regeneration of injured adult axons is severely restricted in spinal cord white matter, myelin *stimulates* the growth of axons emerging from neural stem cell grafts, a finding that is related to the expression of specific cell surface antigens on myelin that interact with stem cell-derived axons.

Implants of neural stem cells also enable the regeneration of injured host axons into the spinal cord lesion site (filled with a neural stem cell graft). The most important axonal system for the control of voluntary movement in humans is the corticospinal projection: when neural stem cells are driven to spinal cord fate and implanted into sites of spinal cord injury, corticospinal axons regenerate in large numbers into the lesion site. Mouse, rat and non-human primate corticospinal axons all exhibit similar responses to implants of caudalized neural stem cell grafts. Within the lesion, regenerating corticospinal axons form synaptic connections with grafted neural stem cells; these grafts in turn extend axons caudal to the injury site and form synapses with the host. In this manner, a synaptic relay is formed across the injury site that in some models supports improvement in functional outcomes.

We are currently exploring cellular and molecular mechanisms underlying this extraordinary degree of axonal growth, while attempting to develop practical methods for possible application to human clinical trials.

Supported by the Veterans Administration, NIH, Craig H. Neilsen Foundation, Wings for Life, and the Adelson Biomedical Research Foundation.

BACK
PROGRAMME

Neuron-glia Network Signaling after Nerve Injury

Marc Freeman

Oregon Health & Science University, Vollum Institute, Portland, USA

Nervous system injury has broad effects on nervous system function, but how injury signals spread through neural circuits to modify neurophysiology remains unclear. Axotomy activates dSarm/Sarm1, which promotes axon death through Axundead. MAP kinase signaling has also been implicated in axon death, but its role remains controversial. Using a simple *in vivo* preparation, we simultaneously examined the responses of severed axons and adjacent intact sensory neurons to nerve injury. Within hours after axotomy of a subset of neurons, we observed suppression of axon transport in all axons, and mechano- and chemosensory signal transduction was decreased in intact neurons. Surprisingly, dSarm was required cell-autonomously even in uninjured neurons to block axon transport, and sensory signal transduction in intact neurons, and in this context signaled with the voltage-gated calcium channel Cacophony (Cac) and MAPK, but not Axed. Intact neurons recovered within 12 hours after injury and survived, while severed axons executed axon death through dSarm/Axed signaling, independent of MAPK. Our work defines a new, early role for dSarm in promoting rapid injury-induced changes in axon physiology, shows that dSarm functions even in intact neurons after injury, and identifies a new role for Cac/dSarm/MAPK signaling *in vivo* in the spreading of injury signals that modify neural circuit function.

BACK
PROGRAMME

Transport and Translation of mRNAs that Promote Axon Survival

Rosalind Segal

Harvard Medical School, Dana-Farber Cancer Institute, Cambridge, USA

Neuronal circuitry relies on long distance connections that are established by extensive axonal projections. Therefore, stable neuronal circuitry throughout the lifespan depends on prolonged axonal survival. Axons that do not survive undergo a complex degeneration process can be initiated by diverse types of injury, including physical trauma and chemical injuries. Here we investigate the mechanisms of axon survival and degeneration in the context of Chemotherapy induced peripheral neuropathy (CIPN), a prevalent and devastating side effect of cancer treatments. The pathological hallmark of CIPN is sensory nerve axon degeneration that occurs in a “dying back manner” starting from the distal most axonal endings. Our previous studies demonstrated that the Bcl2 family member, Bclw, promotes axonal survival, and that the chemotherapeutic agent, paclitaxel, reduces axonal Bclw levels, causes IP3R1 dependent calcium dysregulation, and results in axon degeneration. To investigate the interactions between Bclw, IP3R1, intracellular Ca++ flux and SARM1 and NMNAT2, two components of NAD metabolic pathways that have been shown to be critical in trauma-induced, Wallerian degeneration, we use compartmentalized primary DRG culture treated with paclitaxel. We find that SARM1 functions downstream of Bclw but upstream of IP3R1 in axonal degeneration pathways. Moreover, treatment with nicotinamide riboside, a NAD precursor, prevents axon degeneration caused by paclitaxel and that caused by deficiency of Bclw. Together our data suggest that there is partial convergence between the molecular mechanisms underlying Wallerian degeneration and paclitaxel induced axon degeneration, thus identifying novel therapeutic approaches for these disorders of impaired axon survival.

BACK
PROGRAMME

A Critical Role for Autophagy in Long-term Memory Formation

Kiran Pandey, Xiao-Wen Yu, Adam Steinmetz, and **Cristina M. Alberini**

Center for Neural Science, New York University, New York, USA

The formation and storage of long-term memories require waive/s of *de novo* protein synthesis, which has been observed and extensively investigated in many species and types of memories. The learning-induced *de novo* synthesized proteins cannot continuously accumulate over time and numerous learning events without altering protein homeostasis (proteostasis) and producing cellular dysfunctions. Notably, very little is known about learning-induced regulations of protein degradation. We will discuss unpublished data providing evidence that episodic learning in rats rapidly and significantly upregulates the levels of proteins involved in autophagy and lysosomal degradation as well as autophagic flux in the dorsal hippocampus, a brain region critical for episodic learning. Furthermore, pharmacological or molecular blockade of the learning-induced autophagy or lysosomal degradation processes in the hippocampus disrupts long-term memory while leaving short-term memory intact. We will discuss the conclusion that, like *de novo* translation, learning-induced autophagy and lysosomal degradation are necessary for the formation of long-term memory.

BACK
PROGRAMME

The Role of Capillary Pericytes in Alzheimer's Disease

Ross Nortley, Nils Korte, Pablo Izquierdo, Chanawee Hirunpattarasilp, Anusha Mishra, Zane Jaunmuktane, Vasiliki Kyrargyri, Christian Madry, Hui Gong, Angela Richard-Loendt, Takashi Saito, Takaomi C. Saido, Sebastian Brandner, Huma Sethi and **David Attwell**

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Brain blood flow is regulated to ensure adequate power for neuronal computation. Blood flow is increased to areas where neurons are active, and this increase underlies non-invasive brain imaging using BOLD fMRI. I will demonstrate that neuronal activity mainly increases cerebral blood flow by dilating capillaries via pericytes, that this involves signalling via astrocytes, and that dilation of capillaries and dilation of arterioles are mediated by different messengers.

Ischaemia leads to pericytes constricting and dying, thus producing a long-lasting decrease of blood flow, making pericytes a therapeutic target in stroke. I will show that a similar pericyte constriction contributes to Alzheimer's Disease: both in humans with dementia depositing amyloid β (Aβ) and in mice in a knock-in model of Alzheimer's disease, capillaries are constricted preferentially at pericyte locations. This constriction is sufficient to approximately halve blood flow and in humans increases with the severity of the Aβ deposition. Application of exogenous Aβ constricts pericytes, via a mechanism depending on reactive oxygen species and endothelin-1 release. Pericyte constriction is a therapeutic target in Alzheimer's disease.

BACK
PROGRAMME

Novel Mechanisms of Neurogenesis – from Centrosomes to Nucleoli

Magdalena Götz

Institute for Stem Cell Research, Helmholtz Center Munich and Biomedical Center, University of Munich, Germany

In order to identify crucial and novel regulators of neurogenesis, we compare genome-wide expression of astrocytes in the adult brain to neural stem cells and neuroblasts from the adult SEZ and the developing telencephalon. This resulted in the identification of novel regulators of neurogenesis. I will focus especially on a novel centrosome protein and its crucial role in neural stem cell delamination and lineage progression. As a second key player, we identified a novel nuclear protein Trnp1 that influences brain folding (Stahl et al., Cell 2013). I will present unpublished data on the molecular function of this protein as a master regulator of nuclear compartmentalization. Trnp1 influences the size and function of nucleoli thereby highlighting a novel role of this compartment in regulating neural stem cell maintenance or differentiation. These factors together with our previous work on Uhrf1 (Ramesh et al., Genes Dev. 2016) specifically repressing subsets of endogenous retroviral elements highlight the importance of sub-type specific regulators of processes previously thought to be rather general in developmental and adult neurogenesis.

BACK
PROGRAMME

Exploring Astroglial Glutamine Transfer with Novel Dye: Where, When, How and What for?

Nathalie Rouach

Collège de France, CIRB, Paris, France

The replenishment of presynaptic glutamate is fundamental to brain functions. While the neuroglial glutamate-glutamine cycle has been proposed as a local recycling mechanism particularly relevant in pathological conditions, the requirement of astroglial glutamine supply for physiological activity is still under debate. I will present data addressing the physiological conditions required for this supply to occur, its spatio-temporal features, and its involvement in physiological synaptic transmission and memory by combining design of novel fluorescent molecules for direct tracking of glutamine in live cells from brain tissues, imaging, electrophysiology and behavioral analysis.

BACK
PROGRAMME

Epigenetic Regulators in Autism Spectrum Disorders

Gaia Novarino

Institute for Science and Technology, Klostemeuburg, Austria

Neurodevelopmental disorders such as autism, intellectual disability and epilepsy affect millions of people, and are often refractory to treatments. Not infrequently autism spectrum disorder phenotypes, intellectual disability and epilepsy are coexisting, suggesting the existence of common molecular mechanisms underlying these syndromes. The causes of epilepsy and autism remain unknown for the majority of cases. Of these, a significant number have a genetic basis and many causative genes remain to be identified. With DNA sequencing being more accessible, the genomes of many patients can be analyzed and more disease-causing genes will be recognized. Even though we predict that each identified gene may represent only a tiny fraction of the total genes involved in these disorders, studying the mechanisms underlying rare inherited forms of neurodevelopmental disorders can be extremely helpful. In my talk I will describe our recent work on histone and chromatin modifier genes associated with autism and intellectual disability.

BACK
PROGRAMME

Deciphering Human-specific Mechanisms of Cortical Neuron Development

Pierre Vanderhaeghen

VIB-KU Leuven Center for Brain & Disease Research

The human cerebral cortex has undergone rapid expansion and increased complexity during recent human lineage evolution.

One striking feature of human corticogenesis is that it is highly protracted in time, from prenatal steps of neurogenesis to postnatal stages of neuronal wiring.

This prolonged development is thought to contribute in an important fashion to increased cortical size but also enhanced circuit complexity and plasticity.

Here we describe how in vitro and in vivo mouse - human chimeric brain experiments indicate that the species-specific temporal patterning of corticogenesis is surprisingly intrinsic to cortical progenitors and neurons. The underlying molecular mechanisms start to be uncovered, and include human-specific duplicated genes.

BACK
PROGRAMME

Super-Resolution Imaging of the Structure and Function of Tripartite Synapses

Misa Arizono and **U. Valentin Nägerl**

Interdisciplinary Institute for Neuroscience, University of Bordeaux / CNRS

According to the concept of the 'tripartite synapse', synaptic communication is a result of dynamic signaling between pre- and postsynaptic structures as well as perisynaptic astrocytic processes. Recent studies have shown that astrocytic calcium signals can be fast and local, supporting the possibility that astrocytes are involved in actively regulating neural circuits at the level of single synapses.

However, the anatomical basis of such specific signaling remains unclear, owing to technical difficulties in resolving the spongiform domain of astrocytes where most tripartite synapses are located.

Using 3D-STED microscopy in living organotypic brain slices, we could resolve the spongiform domain and reveal new aspects of its morphological architecture. We observed a reticular meshwork of nodes and shafts that featured rings of re-connecting astrocytic processes. The majority of dendritic spines were in contact with nodes, correlating in size with them. By FRAP experiments and calcium imaging, we showed that individual nodes were biochemically compartmentalized and hosted highly localized spontaneous calcium transients. Mapping these calcium signals onto STED images of nodes and spines confirmed that they were associated with individual synapses. We also observed these morphological structures in more intact brain tissue preparations and different brain areas (acute hippocampal brain slices, somatosensory cortex in vivo).

Our study reveals the nanoscale anatomical organization of astrocytes in live brain tissue, identifying nodes as the functional astrocytic component of tripartite synapses, which may provide the anatomical basis for synapse-specific communication between neurons and astrocytes.

BACK
PROGRAMME

Biochemical computation in single dendritic spines

Ryohei Yasuda

Max Planck Florida Insitute for Neuroscince, Jupiter, USA

Activity-dependent changes in synaptic strength and structure are believed to be cellular basis of learning and memory. A cascade of biochemical reaction in dendritic spines, tiny postsynaptic compartments emanating from dendritic surface, underlies diverse forms of synaptic plasticity. The reaction in dendritic spines is mediated via signaling networks consist of hundreds of species of proteins. Aiming to elucidate the operation principles of such signaling networks, we have developed several new techniques to measure the key properties of the signaling components. First, based on 2-photon fluorescence lifetime imaging and highly sensitive biosensors, we have developed techniques to image signaling activity in single dendritic spines. We have succeeded in monitoring activity of several key signaling proteins in single spines undergoing structural and functional plasticity. This provided new insights into how the spatiotemporal dynamics of signaling are organized during synaptic plasticity. We have developed sensitive and specific sensors for CaMKI, CaMKII, Rho GTPase proteins, Rab GTPase proteins, protein kinase C isozymes (α, β, γ etc) and the BDNF receptor TrkB. Second, based on CRISPR/Cas9-mediated gene-editing, we have developed a technique to fuse fluorescent tags to endogenous proteins in single cells *in vivo*. This technique, termed SLENDR, allows us to measure the precise localization and dynamics of any proteins. Third, we have established a molecular tool to manipulate protein activity with light. Using this technique, we measured the temporal window of CaMKII activity required for synaptic plasticity and animal's learning by inhibiting the kinase at different timing. New results obtained by these techniques provided new insights into the mechanisms underlying the spatiotemporal regulation of signaling dynamics underlying synaptic plasticity and learning and memory.

BACK
PROGRAMME

Radial F-actin Organization during Early Neuronal Development

Durga Praveen Meka[1§], Robin Scharrenberg[1§], Bing Zhao[1], Theresa König[1], Irina Schaefer[1], Birgit Schwanke[1], Oliver Kobler[2], Sergei Klykov[3], Melanie Richter[1], Dennis Eggert[4], Sabine Windhorst[5], Carlos G. Dotti[6], Michael R. Kreutz[7,8], Marina Mikhaylova[3], **Froylan Calderon de Anda**[1]

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The centrosome is thought to be the major neuronal microtubule-organizing center (MTOC) in early neuronal development, producing microtubules with a radial organization. In addition, albeit in vitro, recent work showed that isolated centrosomes could serve as an actin-organizing center , raising the possibility that neuronal development may, in addition, require a centrosome-based actin radial organization. Here we report, using super-resolution microscopy and live-cell imaging, F-actin organization around the centrosome with dynamic F-actin aster-like structures with F-actin fibers extending and retracting actively. Photoconversion/photoactivation experiments and molecular manipulations of F-actin stability reveal a robust flux of somatic F-actin towards the cell periphery. Finally, we show that somatic F-actin intermingles with centrosomal PCM-1 satellites. Knockdown of PCM-1 and disruption of centrosomal activity not only affect F-actin dynamics near the centrosome but also in distal growth cones. Collectively the data show a radial F-actin organization during early neuronal development, which might be a cellular mechanism for providing peripheral regions with a fast and continuous source of actin polymers; hence sustaining initial neuronal development.

BACK
PROGRAMME

Polymerisation of Microtubules is Functionally Linked to their Bundled Arrangements in Axons

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In ageing and certain neurodegenerative diseases, axons form pathological swellings where the prominent microtubule (MT) bundles disintegrate into disorganised arrangements of criss-crossed curling MTs. We use *Drosophila* neurons as a powerful genetic and experimental model to study the underlying mechanisms. From studies of >50 actin- and MT-binding proteins, we deduced the "local axon homeostasis" hypothesis claiming that MTs in the force-enriched axonal environment are prone to curl up, whereas MT regulators "tame" them into bundles (Voelzmann et al., 2016, BrainResBulletin 126, 226ff).

In this context, we have studied the role of MT polymerisation and uncovered two novel mechanisms. (1) Polymerisation enhances the risk of MTs going "off track". The cortical collapse factor Efa6 provides a quality control mechanism by eliminating "off track" MTs that have escaped MT bundles and polymerise towards the axonal surface; through this action, Efa6 prevents MT disorganisation and negatively regulates axon growth and branching in culture and in vivo (Qu* Hahn*, 2018, bioRXiv, 10.1101/385658). (2) Eb1 is required for spectraplakin-mediated guidance of polymerising MTs into parallel bundles (Voelzmann et al., 2017, *Sem Cell Dev Biol* **69,** 40ff). We now found that Eb1 co-operates with XMAP215 and Tau during polymerisation. Loss of either of them negatively affects axon growth and Eb1 comets (size, numbers) and, consequently, causes MT disorganisation. Regarding the mechanisms underlying co-operation, we found that Eb1 and XMAP215 are co-dependent to stay on at polymerising MT plus ends. In contrast, Tau maintains comets by preventing Eb1 from being sequestered to MT shafts. Using *Xenopus* neurons as parallel system, we find that the Eb1-Tau-XMAP215 co-operation and their links to MT bundle organisation are conserved, thus demonstrating that understanding gained in *Drosophila* neurons has promising translational potential. This will also be of interest with respect to signaling pathways and their connection to MT regulation in axon pathology, which will be briefly discussed.

Supported by BBSRC, The Leverhulme Trust and NIH.

BACK
PROGRAMME

Actin-Microtubule Coordination in the Neuronal Growth Cone: a Novel Role for Formin 2

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Formin 2 (Fmn2) is implicated in cognitive impairment and Alzheimer's disease-related pathophysiology. We have previously demonstrated the role of Fmn2 axon guidance *in vivo*. Fmn2 depletion results in slowly translocating growth cones with deficits in chemotactic responses. At the molecular level, two activities of Fmn2 subserve these functions. First, Fmn2 modulates interactions with the extracellular matrix by regulating the stability of growth cone attachments and the development of traction stresses. Fmn2 is not only capable of bundling F-actin but also contributes to the coupling of F-actin to adhesion complexes at growth cone point contacts. Regulation of the molecular clutch by Fmn2 contributes to both growth cone motility and stabilization of filopodial protrusions. Interestingly, we also find that Fmn2 can physically crosslink invading microtubules to F-actin bundles in filopodia and influence microtubule stability and capture. The latter activity appears to be critical for accurate chemotaxis by growth cones. Cell biological and *in vitro* reconstitution studies collectively suggest that Fmn2 is a novel F-actin – microtubule crosslinker with critical regulatory functions in the neuronal growth cone.

Coordination of actin and microtubule dynamics is critical to multiple neuronal processes, including circuit development, arborisation and structural plasticity of dendritic spines. This study identifies and mechanistically dissects the role of Fmn2 in mediating inter-polymer crosstalk and highlights the centrality of such co-regulatory processes in neuronal remodelling.

BACK
PROGRAMME

Axon Growth of CNS Neurons in three Dimensions is Amoeboid-like and Independent of Adhesions

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Neurons of the central nervous system (CNS) extend axons during development. It is thought that the growth cone drives axon growth by pulling along the substrate while tugging the preceding axon through actin-myosin mediated contraction. Nevertheless, most studies involving growth cone advancement are conducted in two dimensions on a stiff substrate. Here, we demonstrate that neurons in three dimensions grow more physiologically and with distinct growth cone architecture from to the conventional textbook structure. Moreover, during the axon elongation of rodent CNS neurons in three dimensions, the axon growth cone does not show pulling on the extracellular matrix and grows independently of adhesions. Unlike axon growth in two dimension conventional cultures, actin-myosin contractility does not restrain microtubules from invading the growth cone edge. Thus, axons grow using amoeboid-like movement similar to the migration modes of crawling cells to enable efficient translocation in soft environments, such as the developing CNS.

BACK
PROGRAMME

In Vivo Actin Dynamics in Dendrites: A Computational Approach

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The correct morphology of dendrites is essential for the function of the nervous system. The underlying cytoskeleton defines the shape and dynamics of dendritic branches under the control of complex protein networks. The aim of this study is to elucidate how regulators of the actin cytoskeleton define the diverse characteristic shapes and dynamics of dendritic arbors.

The dynamics of the highly actin enriched terminal branchlets of class III dendritic arborisation (c3da) neurons are essential for the neuron's function but how these dynamics are generated remains largely unknown. To elucidate the mechanisms of actin organization in branchlet dynamics, we analysed six different actin cytoskeletal proteins in *Drosophila* larva dendritic arborisation (da) neurons. A thorough literature search provided us with four actin regulators that we suspected played an important role in the dynamics of these terminal branches. We focused on the actin nucleators Arp2/3, the bundling factor Singed/Fascin, the capping protein Ena/VASP and the depolymerisation and severing factor Twinstar/Cofilin. Additionally, we performed a targeted screen for other actin regulators and found two new actin nucleators, Spire and Capu/Formin2, with exclusive functions in c3da neurons. By performing *in vivo* time-lapse recordings and developing quantification methods in the "Trees Toolbox" (http://www.treestoolbox.org/) in Matlab we found that all these actin regulators play a specific role, in part exclusively, in the formation of the terminal branchlets of c3da neurons. Mutants of the different actin regulators all show reduced number of terminal branchlets, however, their defective dendritic arbors clearly differ from each other. We thus characterised the phenotypes further with the aim of correlating the differences in dendritic structure to the function of the different proteins.

Each mutant phenotype could be quantitatively resolved on the basis of 45 morphological parameters that we defined in the Treestoolbox. Singed, for example, promotes the stabilization of tight uniparallel actin bundles and, correspondingly, we have previously shown that *singed*^{3} mutant c3da terminal branchlets display increased tortuosity. With time-lapse analysis we attempted to elucidate the etiology of the distinct dendritic phenotypes. We found that terminal branches of *singed*^{3} mutant c3da neurons have an overall increased dynamics, suggesting that bundling of actin filaments stabilizes branches by restricting their dynamics.

Moreover, the approach helps us uncover unexpected functions. While both *spire*$^{1/2F}$ or *capu*$^{1/EE}$ mutant larvae show reduced branchlet dynamics, they also have a significant defect in forming new branches, which goes in hand with their expected function in actin nucleation. However, *spire*$^{1/2F}$ mutants additionally show a reduced retraction and disappearance of branches suggesting an unforeseen role in the disassembly of actin filaments.

Taken together, we bring together elaborate morphological and molecular data in a specific type of dendritic branchlet to understand how actin regulation affects different aspects of branch dynamics. A computational approach to *in vivo* mutant analysis can reveal new mechanistic aspects of actin regulation during dendrite branching and describe the interplay between actin nucleation, bundling, severing and elongation, thereby delineating specific functions.

BACK
PROGRAMME

Tissue-Derived Mechanical Force During Dendrite Pruning in the Drosophila PNS

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Neurite pruning, the developmentally regulated degeneration of neurites without the death of the parent neuron, is an important mechanism during neuronal circuit formation, but the cell biological mechanisms underlying this process are still incompletely understood. The peripheral sensory c4da neurons of Drosophila larvae specifically prune their long and branched dendrites at the onset of metamorphosis through a mechanism involving local degeneration and dendrite severing in predetermined positions close to the soma. Dendrite pruning is induced cell autonomously by a hormone-induced transcriptional cascade. We have previously shown that local microtubule disassembly in dendrites is required for pruning, and that the uniform „plus end-in“ orientation of dendritic microtubules is required for this process, likely acting as a spatial cue that confines severing sites to proximal dendrites.

How dendrites are eventually severed from the soma has remained unclear. Here, we addressed this question using a live imaging approach. The soma of c4da neurons as well as their proximal dendrites are covered by glial processes, while their distal dendrites are in close contact with epidermal cells. We found that dendrite detachment correlates with periods of enhanced morphogenetic movements in the epidermis. Our quantitative analyses suggest that these movements can exert mechanical stress such as shear on pruning dendrites. Indeed, suppression of morphogenetic movements using genetic approaches causes partial defects in dendrite pruning. Previous evidence showed a correlation between the endpoints of glial wrapping and severing sites. Together with our evidence, these data suggest that these sites could act as rigid edges on which dendrites break. Taken together, our data suggest tissue-derived mechanical forces as a factor contributing to dendrite pruning.

BACK
PROGRAMME

Presynaptic Boutons are Hotspots for Activity-dependent Microtubule Nucleation

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Growing evidence indicates that fine control of microtubule (MT) dynamics at synapses is critical for neuronal viability and normal synaptic function. Despite the significance of these observations, virtually nothing is known about whether MT nucleation and dynamics are regulated at presynaptic contacts of pyramidal neurons. To test it, we have measured contacts of dynamic MTs with single excitatory presynaptic boutons in cultured hippocampal neurons, and found that dynamic MT plus ends preferentially grow from presynaptic boutons as a result of gamma-tubulin and augmin-dependent MT nucleation. Strikingly, presynaptic MT nucleation is promoted by neuronal activity, functionally coupled with bidirectional synaptic vesicles (SV) transport, and necessary to maintain local total SV pool, underscoring a previously uncharacterized critical role of presynaptic boutons as hotspots for activity-dependent dynamic microtubule nucleation to promote interbouton synaptic vesicle motility.

BACK
PROGRAMME

The Autism and Schizophrenia-associated Protein CYFIP1 Regulates Bilateral Brain Connectivity

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Copy-number variants of the CYFIP1 gene in humans have been linked to Autism and Schizophrenia, two neuropsychiatric disorders characterized by defects in brain connectivity. CYFIP1 regulates molecular events underlying post-synaptic functions. Here, we show that CYFIP1 plays an important role in brain functional connectivity and callosal functions. In particular, we find that *Cyfip1* heterozygous mice have reduced brain functional connectivity and defects in white matter architecture, typically relating to phenotypes found in patients with Autism, Schizophrenia and other neuropsychiatric disorders. In addition, *Cyfip1* deficient mice present deficits in the callosal axons, namely reduced myelination, altered pre-synaptic function, and impaired bilateral-connectivity related behavior. Altogether, our results show that *Cyfip1* haploinsufficiency compromises brain connectivity and function, which might explain its genetic association to neuropsychiatric disorders.

BACK
PROGRAMME

Axonal Signaling, Trafficking and Transport Mechanisms Regulating Axon Regeneration in the Adult CNS

Bart Nieuwenhuis[(1)], Veselina Petrova[(1)], Craig Pearson[(1)], Rachel Evans[(1)], Amanda Barber[(1)], Patrice Smith[(2)], Evan Reid[(3)], Keith Martin, **Richard Eva**[(1)] & James Fawcett[(1)]

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Adult CNS neurons have a weak intrinsic capacity for regeneration after injury, but the underlying cellular mechanisms preventing regeneration are not completely understood. Increasing intrinsic growth ability is a critical part of enabling brain, spinal cord and optic nerve axons to regenerate.

The work presented here is part of a large body of research into understanding the role of membrane protein trafficking and transport in the control of regenerative ability. We have found that as CNS neurons mature, there is a developmental decline in the axonal transport of integrins (cell surface adhesion and guidance molecules) and their endosomal transporters, Rab11 positive recycling endosomes. This is controlled the small GTPase ARF6 and its activator, EFA6. Switching off ARF6 in mature CNS axons leads to increases in anterograde axonal transport and regenerative ability.

By focusing on ARF6 and Rab11 we have identified two new approaches for stimulating transport and regeneration, which we have investigated in vitro using laser axotomy of mature cortical neurons, and in vivo using the optic nerve crush model of CNS axonal injury. Firstly, by investigating signaling upstream of ARF6, we have identified an isoform of PI3 Kinase which functions in a hyperactive fashion to elevate PIP3 in the distal axon and found that this enables robust regeneration in the optic nerve. This highlights the need for pro-regenerative signals within the axon as well as the cell body.

Secondly, we present exciting data from studies into a trafficking molecule which resides in the endoplasmic reticulum (ER) and demonstrate that this can be targeted to enable robust optic nerve regeneration. This molecule functions as a scaffold to stimulate regeneration through numerous mechanisms, including the mobilization of integrins and Rab11. These findings confirm integrins and Rab11 as critical facilitators of axon regeneration whilst establishing an important new role for the axonal ER in this process.

BACK
PROGRAMME

Vesicular Treadmilling in Axons Defines how Synapses Adapt to High Neuronal Demand

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Neurotransmission requires efficient axonal transport to deliver secretory cargoes and synaptic components in response to neuronal activity. In the standard model, synaptic materials are transported by vesicles that travel anterogradely from soma to synapses. However, a model with a continuous flow of anterograde vesicles fails to explain how synapses can efficiently and rapidly respond to variable phases of neuronal activity. This model would result in alternated phases of traffic jam and depletion, thus impairing the efficient and rapid delivery of materials to active synapses.

Here, using microfluidic devices and high-resolution videomicroscopy of photoconvertible cargoes, we identified a dynamic pool of axonal vesicles that circle back and forth along the axon shaft with surprisingly high regularity and homogeneity. These vesicles show extreme processivity (*i.e.* they do not randomly switch directions during transport) but specifically reverse when they reach axon extremities: anterograde-to-retrograde switch at the distal tip; retrograde-to-anterograde switch at the pre-axonal entry zone. This directional switch is actin-dependent since the vast majority of reversals take place in actin-rich domains of axon extremities and is abolished when actin dynamics is inhibited. Moreover, using microfluidic devices connected to microelectrodes, we further found that vesicular treadmilling is regulated by neuronal activity by progressively capturing vesicles at active synapses during high demand.

Based on these findings, we propose a new model for axonal transport that we dubbed vesicular treadmilling, where secretory vesicles constantly circulate back and forth along axons until their selective capture at active synapses. This dynamic, bidirectional mode of transport determines the release capacity of synapses in response to neuronal activity and defines how synapses adapt to high neuronal demand.

BACK
PROGRAMME

Subcellular Localization of the RNA-binding Protein Nucleolin for Axon Growth Regulation

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Nucleolin is a multifunctional RNA-binding protein (RBP) found in the nucleus, cytoplasm and plasma membrane of the cell. Previously we have shown that nucleolin localizes to axons through interaction with the anterograde microtubule associated motor kinesin-1 (Kif5) and that the complex transports a number of key growth-regulating mRNAs including importin β1 and mTOR. Perturbation of nucleolin-kinesin interactions leads to reduced levels of axonal nucleolin and its associated transcripts and enhances neuronal growth. Here we identify the kinesin-binding domain (KBD) in nucleolin, and show that the same domain mediates nucleolin localization to the cell cortex and plasma membrane. Heterozygous KBD-deletion mice reveal reduced axonal localization of nucleolin in dorsal root ganglion (DRG) neurons and enhanced axonal outgrowth. Homologous domains may exert similar functions in other RNA-binding proteins. The current study provides new mechanistic insights on subcellular localization of RBPs, and how changes in subcellular RBP localization regulate axon growth.

BACK
PROGRAMME

Conserved Functions of Wnk Kinases in Axon Branch Patterning and Maintenance

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In a reverse genetic screen, we discovered that loss of *Drosophila* Wnk kinase function results in developmental axon growth and branching defects of adult sensory neurons. This neurodevelopmental function of Wnk appears to be conserved in mammals. *In vivo* knockdown of Wnk1 as well as Wnk2 in mouse cortical neurons severely affects axon extension and branching. Wnk is an essential kinase, found to be misregulated in different diseases such as hypertension, cancers and a rare type of neuropathy. Yet a neurodevelopmental role of mammalian Wnk has not been described. We further discovered that a lack of Wnk function also leads to early onset axon degeneration in mature *Drosophila* sensory neurons as well as mouse cortical neurons. Moreover, we found that loss of Nmnat, a key regulator of axon survival, as well as overexpression of Axundead (Axed), an effector in axon degeneration, have axon branching defects that are remarkably similar to Wnk mutants. Both, axon branching defects and neurodegeneration (of Wnk mutant neurons) can be suppressed by gain of Nmnat and loss of Axed function. Conversely, overexpression of Wnk can suppress loss of Nmnat and gain of Axed. This epistasis analysis suggests an intriguing interrelation between Wnk, Nmnat and Axed, where Wnk enhances Nmnat function but also represses Axed activity. It further suggests an intriguing functional overlap between some developmental regulators of axon branching and factors involved in axon maintenance or degeneration. We will discuss this novel hypothesis and conserved roles of Wnk kinases in axon branch patterning as well as protection against degeneration.

BACK
PROGRAMME

Uncovering Epigenetic Changes in Response to TDP-43 and C9ORF72 Dipeptide-repeat-protein Toxicity

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease in which loss of motor neurons in the spinal cord and motor cortex leads to progressive paralysis and death. Numerous genes are linked to ALS, either genetically or pathologically, including: Tar DNA binding protein-43 (TDP-43) and Chromosome 9 open-reading frame 72 (C9ORF72). The proteins encoded by the TDP-43 gene or the dipeptiderepeat (DPR) produced aberrantly from the hexanucleotide-repeat expansion in the C9ORF72 gene share a common theme in that all are prone to aggregation and inclusion formation in distinct forms of ALS. Mutations in these two genes have been found to cause most of the common forms of familial ALS yet the majority of ALS patients suffer from a sporadic form of the disease that lacks a clear genetic component (though genetics likely underlie sporadic cases as well). We hypothesize that there is a regulatory network controlling chromatin accessibility that forms the basis of this complex multifactorial disease. We identified here the epigenomic global profile of neurons responding to cytosolic and nuclear protein aggregates, using an Assay of Transposase Accessible chromatin (ATACseq), a powerful new approach to globally profile epigenetic changes. We have found a genome-wide profile of transcription factor recognition motifs that are specifically altered by the DPR and TDP-43 toxic proteins. These transcription factors binding events orchestrate gene expression programs, leading to axonal degeneration and neuronal cell death.

BACK
PROGRAMME

Promotion of Axon Growth and Fasciculation through an Axonally Derived Protein

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Most developing axons grow along so-called pioneer axons as nerve bundles or fascicles. This process simplifies axon pathfinding for the majority of axons and is critically important for the establishment of functional circuitry. In investigating the molecular determinants and pathways controlling fasciculation, greatest emphasis has been given to adhesion molecules on the surface of axons. Additional mechanisms including the existence of axon-derived growth and fasciculation promoting factors have be suggested but are currently only weakly experimentally supported.

We found that developing axons secret a factor that supports axonal growth and fasciculation in an auto- or paracrine manner. We identified this secreted protein as the carboxy-terminal part of CREB3L2/BBF2H7. CREB3L2 belongs to the OASIS family of ER stress transducers. Its amino-terminal domain is a bZIP transcription factor that gets liberated from the ER membrane upon cleavage by the intramembrane site-2 protease (S2P). S2P is highly localized to developing axons and knockdown or inhibition of S2P resulted in reduced axon growth and fasciculation in DRG neurons. Contrary to expectation, this effect was not caused by disrupted transcription but rather by a local signaling pathway: application of the conditioned medium of control axons to S2P-deficient axons fully rescued their growth and fasciculation defects. We found that the ER luminal domain of CREB3L2 that gets generated by S2P cleavage is secreted by the axons in a Golgi-independent manner. Supplementing the secreted, luminal domain of CREB3L2 to S2P-deficient axons was sufficient to rescue the axonal growth defects. The secreted luminal domain of CREB3L2 enhances axonal Hedgehog signaling by forming a complex with Shh and its receptor Patched 1, and inhibition of Hedgehog signaling in axons mimicked the effects of S2P or CREB3L2 deficiency. Based on our results we propose that physiological ER stress triggers CREB3L2 cleavage by S2P, leading to the generation of a cytoplasmic transcription factor and a secreted luminal domain. The luminal domain activates Hh signaling leading to enhanced axon growth and fasciculation.

Together, our results reveal a novel auto-/paracrine mechanism for axon fasciculation and uncover a beneficial role for ER stress in axon development.

BACK
PROGRAMME

Role and Regulation of Gamma Tubulin Ring Complexes (γ-TuRCs) in Dendritic Arbor Development

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A fundamental question in neuronal cell biology is how are microtubules generated and how is this regulated? The major microtubule organising centre (MTOC) in mitotic animal cells, the centrosome, is thought to be dispensable and is seemingly insufficient to account for the complexity of dendritic arborisation. Microtubules are typically nucleated by multi-protein γ-tubulin ring complexes (γ-TuRCs) that are recruited and activated at MTOCs, and recently Golgi outposts located in dendrites have been suggested to recruit γ-TuRCs and regulate microtubule nucleation and polarity. The overall importance of γ-TuRCs in neurons, however, remains unclear, especially as microtubules can also be generated by microtubule severing. I have developed assays to examine the role and regulation of γ-TuRCs in *Drosophila* pupal sensory neurons. This system has a major advantage over the more popularly used larval sensory neurons, as our data from immunofluorescence experiments, western blots and from imaging endogenously tagged proteins demonstrates that the larvae carry maternally contributed proteins that negate the effect of RNAi, potentially explaining the weak phenotypes we and others have observed. Strikingly, in contrast to the effects in larval neurons, I have now found that knockdown of γ-TuRCs causes strong dendrite branching defects in pupal sensory neurons. Moreover, using the more effective pupal system, I show that knockdown of Augmin, a multi-protein complex that recruits γ-TuRCs to the sides of pre-existing microtubules rather than to MTOCs, also generates major branching defects, strongly suggesting that γ-TuRCs are regulated by Augmin in these neurons. I propose that microtubule generation in developing neurons with complex dendritic branching patterns requires Augmin-mediated microtubule nucleation and conclude that the pupal system allows a more robust analysis of the role and regulation of microtubules in *Drosophila* sensory neurons.

BACK
PROGRAMME

Maintenance of Cell Type-specific Connectivity and Circuit Function Requires Tao Kinase

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The function of a neuronal circuit is determined by its connections and synaptic strength, which allows information to flow in a specific manner to elicit behavior. Many circuits are formed during early development and undergo plastic changes including pruning and activity-dependent refinement to establish and adjust functional connectivity. While the mechanisms of circuit establishment and refinement have been extensively studied in many systems, a less well understood process is how circuits maintain functionality while the animal and nervous system are still growing. This process requires scaling growth, adjustment of synaptic strength or both to sustain functional output despite changes in input resistance due to larger dendritic trees. However, the molecular mechanisms of how neuronal networks scale proportionally during animal growth and maintain their specificity and behavioral output are not well understood.

Drosophila larvae are an excellent system to study growth-related adjustments of circuit anatomy and function: the animals dramatically increase in size and enlarge their body surface 100-fold while maintaining structural and functional connectivity of their approx. 10.000 neurons. To gain insight into the mechanisms underlying proportional circuit growth, we quantitatively investigated synaptic growth and connectivity in the Drosophila nociceptive network during larval development. We show that connectivity between primary nociceptors and their downstream neurons scales with animal size throughout larval development. We further identified the conserved Ste-20-like kinase Tao as a negative regulator of dendritic and synaptic growth, which is required for maintenance of circuit specificity and connectivity. Loss of Tao kinase resulted in exuberant addition of postsynaptic specializations and ectopic connectivity. Using functional imaging and behavioral analysis we show that loss of Tao-induced ectopic synapses are functional and alter action selection and resulting behavioral sequences in a connection-specific manner. Overall, our data show that fine-tuning of neuronal and synaptic growth by Tao kinase during animal growth is required for maintaining specificity of the neuronal network and its behavioral output.

BACK
PROGRAMME

Role of GW182 during Neuronal Development

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2) SASTRA University

Activity driven protein synthesis is an important feature of both neurodevelopment and synaptic plasticity. MicroRNA induced silencing complex (miRISC) plays an integral role in activity mediated protein synthesis by providing specific, rapid and reversible mode of regulation. miRISC composition plays crucial role in determining its reversibility and its ability to respond to external cues. This concept is particularly relevant in neurons where activity dependent changes in RISC composition direct local protein synthesis driven synaptic plasticity. Surprisingly, the importance of miRISC has not been studied in the context of neuronal development.

We investigated the temporal and spatial expression of miRISC components AGO2, GW182 and FMRP in rat hippocampus and cerebellum using imaging and biochemical techniques. Among them, GW182 revealed a distinct spatiotemporal expression pattern during neuronal development. Our results indicate that GW182 expression peaks early during post-natal neurodevelopment followed by a significant reduction in adult hippocampal and cerebellar tissues. Immunocytochemistry results show a significant reduction in dendritic localization of GW182 in adult hippocampal and cerebellar sections. This spatio-temporal pattern of GW182 expression was also recapitulated in cultured hippocampal neurons. Interestingly, as opposed to GW182, we found increased cytosolic-dendritic localization of FMRP in mature hippocampal neurons. We also found mutual exclusivity between FMRP and GW182 with respect to interaction with miRISC protein AGO2. We hypothesize that such developmental changes in miRISC composition determines the reversibility and function of miRISC and plays important role in cue driven neurodevelopment. To further delineate the function of GW182 in neuronal development, we perturbed the levels of functional GW182 in cultured neurons by overexpressing a dominant negative form of GW182 or siRNA induced knockdown of GW182. Expression of dominant negative GW182 in young hippocampal neurons (DIV3-7), results in significant reduction of neuronal growth and arborisation. Additionally, the role of GW182 in activity driven dendritic growth was studied under BDNF stimulation paradigm. Alteration of GW182 showed distinct effects on intrinsic versus cue (BDNF) driven dendritic growth. Beyond this, we are also investigating the role of GW182 in synaptic signaling. In summary, our study provides novel insights into the function of GW182 in neuronal development and function.

BACK
PROGRAMME

CRMP2 Mediates Sema3F-dependent Axon and Dendritic Spine Pruning

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The pattern of axonal connections is established in development by tightly controlled processes of axon guidance and early postnatal elimination (pruning) of inaccurate connections. Deregulation of these processes has been linked to several neurodevelopmental disorders including autism spectrum disorder (ASD) and schizophrenia. Class 3 semaphorins are key regulators of both axon guidance and pruning, but the molecular cascades that mediate their specific signals are not entirely understood. Collapsin response mediator protein 2 (CRMP2) has been shown to control axon guidance by mediating Semaphorin 3A (Sema3A) signaling and its dysfunction has been linked mainly to schizophrenia. The role of CRMP2 in synapse refinement has so far remained elusive.

Here, using newly generated *crmp2*$^{-/-}$ mice, we demonstrate that CRMP2 mediates not only Sema3A-dependent axon guidance, but also Sema3F-dependent axon- and dendritic spine- pruning. We first demonstrate that CRMP2 deficiency interferes with Sema3A signaling using compartmentalized neuron cultures and leads to defective axon guidance in corpus callosum and peripheral nerves. Furthermore, we show that *crmp2*$^{-/-}$ mice display defects in dendritic spine pruning and stereotyped axon pruning in hippocampus and visual cortex, which is consistent with impaired Sema3F rather than Sema3A signaling, and with autism spectrum disorder (ASD) rather than schizophrenia-like phenotype. Using hippocampal neuron cultures we demonstrate that CRMP2 indeed mediates Sema3F-induced axon retraction. Finally, we show that *crmp2*$^{-/-}$ mice display ASD-like early postnatal changes of ultrasonic vocalization and adult cognitive dysfunction. In conclusion, we demonstrate that CRMP2 is essential regulator of class 3 semaphorin-dependent synapse formation and refinement and its dysfunction shares histological and behavioral features of ASD.

BACK
PROGRAMME

Acentrosomal Microtubule Organizing Centers in Mouse and Human Neural Stem Cells

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The development of the mammalian neocortex relies on a tightly regulated program of proliferation and differentiation of neuroepithelial stem cells. While significant differences exist between the mouse and human progenitor pools, both the major progenitor types in mice (apical radial glia, aRGs) and in humans (basal radial glia, bRGs) contain a long basal process that extends toward the pia and terminates in a basal endfoot. This structure provides support for migrating newly-born neurons and may mediate signaling. It is also linked to cell fate decisions: the inheritance of the basal process at mitosis is believed to confer progenitor fate to the recipient daughter. This is proposed to be due to the specific asymmetric segregation of progenitor determinants, such as CyclinD2 mRNA, to the basal endfoot. However, how determinants are asymmetrically transported to the basal process is unknown. To address this question, we first characterized the organization of the microtubule (MT) cytoskeleton, which mediates long-range transport inside the cell. We have developed an approach for high spatio-temporal resolution live imaging within thick embryonic brain slices, allowing us to image and track growing microtubule plus ends. Surprisingly, although microtubules in the apical process of aRG cells are uniformly polarized, microtubules in the basal process display a mixed polarity, resembling the dendritic microtubule organization. These microtubules largely emanate from “swellings” or varicosities of the basal fiber, which appear to act as acentrosomal MT organizing centers (MTOCs). We showed that these swellings contain Golgi satellites that we hypothesize serve as MT nucleating centers. Using live imaging of human fetal brain slices, we demonstrated that this process is conserved in human bRG cells. We are currently performing functional experiments to confirm the role of these Golgi satellites, focusing on a variety of components involved in Golgi-dependent MT nucleation in other systems. Together, this work defines for the first time the intracellular organization of the MT cytoskeleton in embryonic neural stem cells in situ.

BACK
PROGRAMME

Morphological and Functional Comparisons between Human and Chimpanzee Induced Neurons

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To gain insights into similarities and differences between human and ape neuronal functions, we use human, chimpanzee and bonobo induced pluripotent stem cells to generate mature induced excitatory neurons under forced expression of the transcription factor neurogenin-2 (Zhang et al., 2013). We focus on neurite outgrowth, electrophysiological properties at single cell and network levels and single cell RNAsequencing. Our results show that over-expression of neurogenin-2 in induced pluripotent stem cells leads to generation of similar neuronal cell types in human and ape cultures as judged by single cell RNAsequencing. However, over three weeks of differentiation, chimpanzee and bonobo iNeurons generate more dendrites, longer axons and greater total neurite length than human iNeurons. Similarly, ape iNeurons show more spontaneous excitatory activity than human iNeurons. This is compatible with that human iNeurons differentiate slower as has been shown for neuronal progenitor differentiation *in vitro* and *in vivo* (Otani et al., 2016; Marchetto et al., 2019). In contrast, preliminary results suggest that the timing of the establishment of neuronal polarity does not differ between the species. We are currently using the single cell RNAsequencing data to gain further insight into the rate and mode of iNeuron differentiation.

Zhang et al. (2013): Rapid single-step induction of functional neurons from human pluripotent stem cells. Neuron 78 (5), 785-98

Otani et al. (2016): 2D and 3D Stem Cell Models of Primate Cortical Development Identify Species-Specific Differences in Progenitor Behavior Contributing to Brain Size. Cell Stem Cell 18 (4), 467-90

Marchetto et al. (2019): Species-specific maturation profiles of human, chimpanzee and bonobo neural cells. eLife; 8: e37527, 1-23

BACK
PROGRAMME

A Microtubule Polymerase Complex is Essential for Dendritic Microtubule Polarity and Dendrite Pruning in *Drosophila*

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Pruning that selectively eliminates unnecessary or exuberant neuronal processes without causing death of the parental neurons is a crucial step for sculpting the mature nervous system. In *Drosophila*, class IV dendritic arborization neurons, C4da or ddaC neurons, selectively prune their larval dendrites but maintain their intact axons, a process known as dendrite-specific pruning. Developmental dendrite pruning shares a number of features with pathological neurite degeneration occurring in adult-onset neurological disorders and therefore also serves as an excellent model for understanding molecular and cellular mechanisms of neurodegeneration. Our lab has identified several key regulators of dendrite pruning (Kirilly et al., *Nature Neurosci.* 2009; Kirilly et al., *Neuron* 2011; Wong et al., *PLOS Biology* 2013; Zhang et al., *Developmental Cell* 2014; Wang et al., *Development* 2017; Zong et al., *PLOS Biology* 2018).

Microtubule (MT) disassembly is a key step that precedes membrane fission during pruning (Watts et al., *Neuron* 2003; Williams et al., *Development* 2005). However, to date, all MT depolymerization/severing factors that have been tested are dispensable for dendrite pruning in ddaC neurons (Stone et al., *Cell Reports* 2014; Lee, et al., *PNAS*, 2009), with the exception of Kat-60L1. Here, we show that Kat-60L1 unlikely functions as a MT-severing factor. In the current study, we demonstrate that instead of MT depolymerization/severing factors, MT polymerization factors play key and unexpected roles in dendrite pruning. We show that Mini spindles, a Msps/XMAP215/ch-TOG MT polymerase family protein (Brouhard et al., *Cell* 2008), regulates neuronal remodeling. Further, we have identified TACC as a Msps-interacting protein that is also required for dendrite pruning in ddaC neurons. Msps and TACC stabilize each other in ddaC neurons and adult neurons. Mechanistically, Msps-TACC MT polymerase complex plays a novel role in orienting minus-end-out MT arrays in dendrites of ddaC sensory neurons. Anterograde EB1-GFP comets that mark the MT plus ends are significantly increased in the dendrites of *msps* and *tacc* mutant neurons, indicating impaired MT polarity in these mutant dendrites. Furthermore, we demonstrate that excessive MT depolymerization is attributed to both dendritic MT polarity and pruning defects in *msps*-depleted ddaC neurons. Finally, MT depolymerization, induced by using MT destabilizing drugs or katanin overexpression, perturbs dendritic MT polarity and dendrite pruning, resembling those *msps* or *tacc* mutant phenotypes. Thus, our unpublished study provide genetic, cell biological and pharmacological data demonstrating that Msps-dependent MT polymerization is essential to control minus-end-out MT orientation in the dendrites and thereby facilitate dendrite pruning in ddaC neurons.

BACK
PROGRAMME

PRRT2 Function in the Regulation of Neuronal Actin Cytoskeleton

Elisa Savino[1], Romina Inès Cervigni[1], Fabrizia Claudia Guarnieri[1], Anna Corradi[2], Fabio Benfenati[2,3] and Flavia Valtorta[1]

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Introduction: Mutations in the PRoline-Rich Transmembrane protein 2 (PRRT2) gene have been reported in several paroxysmal neurological disorders, including paroxysmal kinesigenic dyskinesia, hemiplegic migraine and benign infantile epilepsy. PRRT2 is expressed in the synapses of the neurons where it participates in the regulation of neurotransmitter release. We are currently deciphering novel functions of PRRT2 in the regulation of actin cytoskeleton remodelling both during development and in mature neurons.

Methods: We used primary hippocampal neurons in culture transduced with lentiviral vectors for silencing or overexpressing PRRT2, as well as hippocampal neurons derived from a PRRT2 knock-out (KO) mouse model. We applied biochemical and imaging approaches to characterize cellular phenotypes and intracellular pathways induced by alteration of PRRT2 expression levels.

Results: In primary hippocampal neurons PRRT2 knockdown (KD) strongly affects synaptogenesis and spine formation, suggesting a possible role of PRRT2 in actin cytoskeleton dynamics within the synaptic compartment. Indeed, following manipulation of PRRT2 levels of expression, we observed alterations in the organization of the actin network, which was linked to modifications in the activity of key actin-binding proteins. Moreover, it was reported that PRRT2 downregulation by *in utero* electroporation causes a delay in neuronal precursor migration during mouse brain development (Liu et al., 2016). To verify whether PRRT2 could regulate actin remodelling also during neuronal development and migration, we assessed PRRT2 KO hippocampal neuron growth and morphology at 1-3 days *in vitro*. The involvement of PRRT2 in cytoskeleton remodelling was further supported by studies in non-neuronal cell lines, where heterologous expression of PRRT2 induces actin-related phenotypes.

Conclusions: Our results reveal that PRRT2 modulates the actin cytoskeleton. This activity may underlie the role of PRRT2 in both neuronal development and synapse formation and maintenance. These newly characterized functions could be relevant to address the role of the protein in the pathophysiology of neurological paroxysmal diseases.

BACK
PROGRAMME

Poster - 01

Tropomyosin Tpm3.1 is required to maintain the Structure and Function of the Axon Initial Segment

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The axon initial segment (AIS) is a specialized region marking the boundary between the axon and the somatodendritic domain in vertebrate neurons. The AIS plays a major role in establishing and maintaining neuronal polarity and is the site of action potential initiation. Recent work revealed the details of the structure of the AIS, which comprises periodic sub-membranous rings of actin filaments connected via spectrin tetramers. The precise function of these actin rings, however, as well as the details of their regulation and dynamics, are unclear, and the mature AIS is insensitive to the inhibition of actin polymerization.

We found that the tropomyosin isoform Tpm3.1 decorates a specific population of relatively stable actin filaments in the AIS, that are part of the AIS actin rings and actin patches in the AIS. Tpm3.1 stabilizes actin filaments by reducing the rate of depolymerization and regulating interactions with other actin-binding proteins. The pharmacologicial inhibition of Tpm3.1 in rat hippocampal neurons in culture led to the loss of AIS structure, including the loss of the clustering of sodium channels and the AIS vesicle filter. Our data suggests that the actin cytoskeleton in the AIS plays a role more complex than previously appreciated.

BACK

Poster - 02

Region-dependent Diversity of Post-synaptic Densities in the Mouse Brain

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The post-synaptic part of glutamatergic synapses consists of the receptor field and the first layer of molecules involved in signal processing, which are organized together with the receptors in a tight structure called post-synaptic density (PSD). While this structure and its key components are ubiquitously expressed across the mammalian brain, it is becoming more and more apparent that glutamatergic synapses are not uniform. To get a first grasp of their structural and functional diversity, we purified post-synaptic densities from *Dlg4*$^{TAP/TAP}$ knock-in mice that express a TAP immunoaffinity tag at the C terminus of PSD-95, a core component of the PSDs. We prepared PSDs from three different brain regions during brain development and in the context of intellectual disability, using the *Fmr1*$^{y/-}$ mouse model of the Fragile X syndrome. To our surprise, quantification of the mass spectroscopy analysis revealed that the *Fmr1* genetic background had little or no impact on PSD composition, while the expression of core PSD components and associated proteins appears to be quite different between brain regions. These results point towards a regional divergence in the functional characteristics of glutamatergic synapses.

BACK

Poster - 03

Astrocyte Glutamate Transporter GLT-1 as a Possible Therapeutic Target in Rett Syndrome

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Rett syndrome (RTT; OMIM# 312750) is a rare devastating neurodevelopmental disorder that with an incidence of ~ 1:10,000, represents the most common genetic cause of severe intellectual disability in girls. Mutations in the X-linked methyl-CpG-binding protein 2 gene (*MECP2*), encoding for a multifunctional protein mainly acting as a transcriptional regulator, have been reported in over 95% cases of classical forms of RTT. No cure is currently available and patients are treated only to ameliorate secondary phenotypes. It has, however, been established that phenotypic rescue is possible in *Mecp2*-deficient mice upon reactivation of the endogenous *Mecp2* gene, therefore raising the possibility of therapeutic intervention.

Initial studies have supported an exclusive neuronal role for MeCP2 in RTT; however, recent data suggest that astrocytes play a major role in the disease throughout a non-cell autonomous effect. In particular, it was demonstrated that wt astrocytes exert a positive effect on *Mecp2* null neurons and the exclusive re-expression of *Mecp2* in astrocytes significantly improves the phenotype and lifespan of the otherwise null mice. Although these studies prove that RTT involves impairments in both neurons and astrocytes and support the possibility of targeting astrocytes as a complementary strategy for improving the RTT condition, to date it remains obscure which key molecules in astrocytes affect neuronal structure and function and the molecular alterations that characterize RTT astrocytes.

In order to fill this gap of knowledge, we molecularly characterized *Mecp2* KO astrocytes, reporting a significant reduction of the glutamate transporter-1 (GLT-1) both at the transcriptional and translational levels. This molecular defect observed in cultured astrocytes was confirmed in brain of younger and symptomatic *Mecp2* KO mice. Considering the importance of glutamate homeostasis for proper neuronal maturation and functioning we tested the ability of selected drugs to revert '*in vitro*' and '*in vivo*' GLT-1 defective expression. Our data report the capability of these drugs to rescue GLT-1 expression, thus emerging as an interesting avenue to be tested in preclinical studies.

BACK

Poster - 04

Modelling Citrate Transporter Deficiency in the Human Nervous System

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Citrate transporter disorder (CTD) is a very rare, recently discovered genetic disorder characterized by early onset epileptic encephalopathy, impaired motor functions, developmental delay, lack of speech and tooth hypoplasia. The disease is caused by recessive mutations in the SLC13A5 gene, which encodes a sodium dependent citrate transporter. Although rare in other countries, a disease mutation has been found to be unusually common in Iceland, leading us to initiate this project.

It is unknown how the transporter dysfunction leads to the disease phenotype seen in CTD and very little information exists on the expression of the gene in the human central nervous system. Our current work is focused on analyzing where in the brain and on what cell types the SLC13A5 protein is expressed. Our aim is to provide a detailed map of expression in healthy individuals of different ages. This information will be used to better tailor an *in vitro* disease model which we are developing through neural induction of iPSCs, yielding both monolayer systems as well as organoid cultures. The model will provide a means to test hypotheses to explain the seizure activity seen in patients and the interplay between different cell types in this context.

BACK

Poster - 05

Structural Basis of Astrocytic Ca^{2+} Signals at Tripartite Synapses

Misa Arizono[1,2], V. V. G. Krishna Inavalli[1,2], Aude Panatier[1,4], Thomas Pfeiffer[1,2], Julie Angibaud[1,2], Jillian Stobart[5], Luigi Bellocchio[1,4], Giovanni Marsicano[1,4], Katsuhiko Mikoshiba[3], Stéphane H.R. Oliet[1,4], Bruno Weber[5], U. Valentin Nägerl[1,2]

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According to the concept of the 'tripartite synapse', synaptic communication is a result of dynamic signaling between pre- and postsynaptic structures as well as perisynaptic astrocytic processes. Despite the wide recognition of the concept, the fundamental aspects of tripartite synapse signaling remain unclear, owing to technical difficulties in resolving the spongiform domain of astrocytes where most tripartite synapses are located.
We overcame this problem by using 3D-STED microscopy in living brain slices, which unambiguously resolved the spongiform domain of astrocytes revealing a reticular meshwork of hyperfine processes. These processes often displayed loop-like structures and formed 'nodes' at branch points. The majority of spines formed contact with nodes and their sizes were correlated. By FRAP experiments and Ca^{2+} imaging, we showed that individual nodes were biochemically compartmentalized and hosted highly localized spontaneous Ca^{2+} transients. Finally, by mapping these Ca^{2+} signals onto STED images of nodes and spines, we demonstrated that nodes exhibit Ca^{2+} signals unique to individual synapses.
Taken together, our findings indicate that nodes are the functional astrocytic component of tripartite synapses, serving as anatomical basis for synapse-specific communication between neurons and astrocytes.

(This work is in revision at Neuron)

BACK

Poster - 06

CALM Dictates GluA1-containing AMPA Receptor Endocytosis

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Fast excitatory neurotransmission at synapses of the central nervous system is mainly mediated by AMPA receptors (AMPARs). During neuronal activity, AMPAR trafficking induces long-term changes in synaptic strength including long-term potentiation (LTP) and long-term depression (LTD). AMPARs are heterotetramers of different combinations of the subunits GluA1-4. Their specific subunit composition determines both their functional properties and trafficking. Subunit-specific protein interactors, auxiliary proteins and post-translational modifications modulate the removal of AMPARs from the post-synaptic plasma membrane during LTD, which is fundamental for higher cognitive functions such as learning and memory and affected in several neurological diseases. Although intensively investigated, the molecular mechanisms underlying LTD remain poorly understood.

Here, we report that the endocytic adaptor protein CALM localizes at endocytic zones adjacent to the post-synaptic sites where AMPAR endocytosis is thought to occur. Both in CALM-deficient hippocampal neurons and upon lentivirally-mediated CALM knockdown, the surface levels of GluA1 are increased and its endocytosis is impaired, leaving AMPAR exocytosis and lateral movement to and from post-synaptic sites intact. In line with these results, we found a significantly decreased LTD expression in our neuron-specific CALM KO mice, while basal synaptic transmission and LTP remained unaltered.

Collectively, our data suggest that CALM may function as a novel regulator to specifically control GluA1-containing AMPAR trafficking efficiency and synaptic plasticity.

BACK

Poster - 07

AP-2 Prevents Amyloidogenesis via Regulation of BACE1 Trafficking in Neurons

Sujoy Bera[1], Elena Calleja Barca[1], Albert Negrete-Hurtado[1], Julia Racho[1], Elodie De Bruyckere[1], Christoph Wittich[1], Nina Ellrich[1], Soraia Martins[2], James Adjaye[2] & Natalia L. Kononenko[1*]

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Cleavage of amyloid precursor protein (APP) by BACE-1 (β-site APP cleaving enzyme-1) is the rate-limiting step in amyloid-β (Aβ) production and a neuropathological hallmark of Alzheimer's disease (AD). Despite decades of research, cellular mechanisms of amyloidogenic APP processing remain highly controversial. Here, we show that in neurons amyloidogenesis is prevented by the endocytic adaptor protein complex-2 (AP-2), which regulates intracellular sorting, while the BACE1 endocytosis is mostly dispensable of AP-2. AP-2 is decreased in iPSCs- derived neurons from patients with late-onset AD, while conditional AP-2 knockout (KO) mice suffer from increased Aβ generation, resulting from accumulation of BACE1 within the late endosomes and autophagosomes. Deletion of BACE1 decreases amyloidogenesis and mitigates synapse loss in neurons lacking AP-2. Taken together, these data suggest a mechanism for BACE1 intracellular trafficking and degradation via an endocytosis-independent function of AP-2 and reveal a novel role of endocytic proteins in amyloidogenesis and open a new therapeutic window for the prevention of AD.

BACK

Poster - 08

Signaling Between Serotonin Receptors and the Extracellular Matrix as a Key to Understanding Pathogenesis of Stress Related Disorders

Bijata M.[1], Krzystyniak A.[1], Baczynska E.[1], Masternak J.[1], Antoniuk S.[1,2], Filipkowski R.[3], Ponimaskin E.[2], Wlodarczyk J.[1]

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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-HT_7R,) the matrix metalloproteinase-9 (MMP-9), the hyaluronan receptor CD44, and the small GTPase Cdc42. We find that 5-HT_7R 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-HT_7R-mediated activation of MMP-9, which leads to CD44 cleavage followed by Cdc42 activation. Pharmacological/genetic suppression of this pathway rescues the 5-HT_7R-induced synaptic changes and the deficit in LTP. Our results thus reveal causal interactions in a previously unknown molecular mechanism regulating neuronal plasticity.

The direct evidence for the possible behavioral role of described 5-HT_7R/MMP-9 pathway was an intriguing issue. We were wondering if 5-HT_7R/MMP-9/Cdc42 signaling pathway also exists in brain of adult animals and how does it affect animal behavior. Our results demonstrate that application of 5-HT_7R agonist prompts a significant increase of MMP-9 activity, whereas application of 5-HT_7R antagonist leads to MMP-9 decrease. Changes in MMP-9 activity reflected also behavior in animals: increased immobility time in tail suspension test (depressive behavior) after application of 5-HT_7R agonist and decreased immobility time in tail suspension test application after application of 5-HT_7R antagonist. These new data provide the first indication for the behavioral importance of 5-HT_7R/MMP-9 signaling.

BACK

Poster - 09

Mechanisms of Microtubule Nucleation in the *Drosophila* Nervous System

Paul Brooks

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Most complex animals have evolved to interact with their environment using a nervous system. These systems range in complexity from the relatively simple *C.elegans*, with only 302 neurons, to the almost unfathomable 100 billion neurons of *H.sapiens*, and while these networks differ greatly in their complexity, the fundamental makeup of a neuron remains largely similar throughout most species.
In order for the neuron to function properly, it is crucial that dendritic arbor development is accurate and well regulated. This process relies heavily on the dynamic cytoskeleton which likely provides pushing forces for the dendritic branches to grow and elaborate. In addition, neuronal microtubules also determine dendrite and axon polarity and help maintain axon and dendritic branching. While there is a plethora of data on cytoskeleton regulation in dividing cells and, to a lesser degree, the neuronal axon, little is known about how microtubules are generated or regulated within the dendritic arbor.

Using sensory neurons in *Drosophila melanogaster*, our work aims to understand microtubule regulation in the dendritic arbor, including what regulatory factors are important to maintain arbor morphology, and how microtubules dynamics are controlled within the complex dendritic tree.

I have used a targeted RNAi screen and identified that the microtubule minus-end stabilising protein Patronin (mammalian homolog CAMSAP) as essential for maintaining dendritic arbor morphology. Knockdown of Patronin in the sensory neurons effects dendrite complexity and induces a partial reversal of microtubule polarity within subsections of the dendritic arbor. Interestingly, loss of Patronin effects both dynamic and stable microtubules differently in neurons with simple (DaI) and complex (DaIV) arbors, possibly reflecting the morphological differences in complexity observed between the two arbor types. These data suggest that Patronin is more than just a simple minus-end microtubule stabilising protein and highlights its role in the development and maintenance of different types of dendritic arbor.

BACK

Poster - 10

Ataxin 2 CAG Expansions Trigger TDP-43 Relocalization in Neurons and Alter the Phagocytic Role of Microglia in the Spinal Cord

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Ataxin-2 (ATXN2) is a RNA Binding Protein highly conserved during evolution. In humans, it contains an unstable polyglutamine (polyQ) domain encoded by a CAG repeat, whose expansions beyond 32 units causes Spinocerebellar Ataxia type 2 (SCA2), while intermediate expansions are a known risk factor for Amyotrophic lateral sclerosis (ALS). In 2017 it was reported that the depletion of ATXN2 extends lifespan in a TDP-43 mouse model of ALS, revealing a RNA-mediated interaction between these two proteins (Becker *et al*. Nature).

In order to better understand the pathology caused by the expansions in the *Atxn2* gene, we generated a novel *Atxn2*-CAG100-knock-in mouse line that shows a strong phenotype starting at 6-7 months of age and recapitulates the SCA2 pathology in the cerebellum. Interestingly, we could also observe that TDP-43 coaggregates with ATXN2 in the spinal cord progressively. To gather further insight into RNA toxicity mechanisms, we performed high-density global transcriptome profiling via Clariom D Affymetrix microarray with spinal cord samples from old animals. Curiously, the highest upregulations concerned RNAs enriched in microglia, pointing to pathological activation of synaptic pruning. Upon double immunofluorescence, the presence of ATXN2 aggregates in microglia of the spinal cord was detected. This suggests that microglia are not only responding to the neuronal dysfunction, but also suffering from the toxic polyQ expansion.

Thus, this mutant is the first mouse model that shows TDP-43 relocalization without modifying its expression levels. Moreover, the mutant allows us to characterize the typical pattern of neuronal cell loss, and also to define the vulnerability of other cell types to SCA2 pathogenesis.

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Poster - 11

MADD-4/Ce-Punctin Processing Defines Synaptic Identity at the NMJ

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In *C. elegans*, body-wall muscle cells receive both excitatory (cholinergic) and inhibitory (GABAergic) inputs. MADD-4/Ce-Punctin is an extracellular matrix protein, secreted by motoneurons, which specifies the type of receptors to cluster at each type of neuromuscular junction (NMJ). Indeed, two MADD-4 isoforms are differentially expressed at NMJs: a small isoform (MADD-4S) is expressed at both types of synapses whereas a long isoform (MADD-4L) is exclusively expressed by cholinergic motoneurons. While the long isoform is necessary for the correct localization of cholinergic (ACh) receptors in front of corresponding ACh terminals, the short isoform is required for the clustering of GABA receptors at inhibitory inputs. However, this model does not account for the fact that, in the absence of the long isoform, GABA receptors remain in front of GABA terminals, even though MADD-4S is expressed at cholinergic and GABAergic NMJs.

Our recent results show that MADD-4S, but not MADD-4L, is cleaved at least into two fragments that localize differentially at NMJs. The N-terminal part of MADD-4S exclusively localizes at GABA synapses, whereas the N-terminal part of MADD-4L is restricted to ACh synapses. Moreover, the C-terminal fragment, which is common to both isoforms, is enriched at cholinergic synapses. When expressing MADD-4S tagged at both N- and C-termini either in GABA or in ACh motoneurons, N- and C-terminal fragments differentially localize as in the wild type, regardless of the type of motoneuron from which the pro-protein is secreted. This suggests that MADD-4S fragments diffuse in the extracellular matrix and are differentially trapped at specific NMJs by yet unidentified partners. We approximately mapped MADD-4S cleavage site and experiments are ongoing to identify the protease involved in MADD-4S processing and to characterize its functional significance.

BACK

Poster - 12

The Microtubule Minus-end Regulator Patronin Restricts Dendritic Branching in Simple Drosophila Dendritic Arborisation Neurons

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Microtubules are polarised polymers that are essential for neuronal growth and function. We aim to understand how microtubule formation and organisation are regulated in neurons and we use *Drosophila* dendritic arborisation (da) neurons as a powerful *in vivo* model system. Different classes of da neurons exhibit different complexities in their dendritic arbors, and this is regulated by transcription factors that drive the expression of effector genes, many of which remain unknown. We have performed a candidate screen for regulators of microtubules that are required for simple class I da neurons to restrict their dendritic arbor pattern. The major hit from this screen is a protein called Patronin, which is known to bind and stabilise the minus-end of microtubules in both flies and mammals. In human cells, Patronin homologues stabilise microtubules at the Golgi complex during interphase, and in *Drosophila* class I da neurons fragments of Golgi distributed throughout the dendrites called Golgi outposts were shown to help restrict dendritic branching by controlling the orientation of microtubule nucleation. We find, however, that *Drosophila* Patronin does not localise to Golgi in da neurons, including to the Golgi outposts. After knocking down Patronin in class I da neurons, we see a striking increase in dendritic branching and an upregulation of microtubule growth (marked by EB1-GFP), all of which is due to increased anterograde growth (away from the cell body). The microtubules in these neurons are known to be oriented minus-end out, and while EB1-GFP typically binds growing microtubule plus ends, EB1-GFP has been shown to bind growing microtubule minus ends in fly cultured cells under certain conditions. We therefore propose that in class I da neurons Patronin protects the microtubule minus ends from polymerisation to restrict dendritic branching. If proved correct, our model will not only highlight Patronin as a key effector gene that restricts dendritic branching in simple class I da neurons, it will also be the first *in vivo* example of Patronin functioning to suppress minus end growth, rather than minus end depolymerisation.

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Poster - 13

The AMPK-related Kinase NUAK1 Controls Axon Branching through a Local Remodelling of Metabolic Pathways during Development

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The growth and branching of the axon is a highly compartmentalized cellular process involved in the formation of long-range synaptic connections. The precise regulation of the cellular mechanisms underlying axonal morphogenesis is essential to the formation of functional neuronal networks whose disruption is linked to socially-devastating neurodevelopmental disorders. We previously identified a two kinases signaling pathway including the tumor-suppressor LKB1 (STK11) and the AMPK-related kinase NUAK1 (ARK5) as central regulators of terminal axon branching in mouse cortical pyramidal neurons (PNs) through its ability to control mitochondria trafficking (Courchet, Lewis et al. Cell 2013; Courchet et al., Nature Comm. 2018). These studies raised an important question: how do local mitochondrial function regulate axon branching during development? Here, we focused on the metabolic activity of mitochondria in the developing axon at distinct stages of axonal development. We observed that in cortical neurons, mitochondria are recruited after, and not during or prior to collateral branch formation. Through time-lapse imaging and Chromophore Assisted Light Inactivation (CALI) of mitochondria, we uncovered a correlation between branch stabilization and the local presence of functional mitochondria. We subsequently observed that the inactivation of NUAK1 impairs mitochondria function and ATP concentration in the axon. Through Seahorse analyses, we observed a dose-dependent reduction in mitochondrial respiration, but not glycolytic capacity, in NUAK1 deficient neurons. Finally, we developed strategies to upregulate mitochondrial metabolism and observed that metabolic rescue is sufficient to rescue axonal branching in NUAK1 deficient neurons *in vitro* and *in vivo*. Altogether, our results indicate that a local, mitochondrial-dependent remodeling of metabolic homeostasis is an important step for axon morphogenesis.

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Poster - 14

Motor Neurons Harbouring the DHMN1 1.35 MB Complex Insertion: A Useful Paradigm for Understanding Genomic Organisation and Gene Regulation in Neurodegenerative Disease

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Distal hereditary motor neuropathies (DHMN) are a group of neurodegenerative diseases with length-dependent axonal degeneration of the lower motor neurons leading to chronic disability. Our group has mapped an autosomal dominant form DHMN to chromosome 7q34-q36 (DHMN1: OMIM %182960) in a large Australian family (Family-54). - Using whole genome sequencing we recently reported a novel 1.35 Mb complex insertion within the DHMN1 locus after having excluded all coding mutations in the linkage region. We hypothesise the DHMN1 insertion is likely to cause peripheral neuropathy through gene dysregulation resulting in axonal degeneration. To address this problem, we have utilised induced pluripotent stem cell motor neurons (iPSC-MN) derived from patients (MN^{DHMN1}) and controls (MN^{CTRL}). Motor neuron identity was confirmed by staining with NF68, TUJ1 as well as pan-neuronal markers ISLET1 and HB9. Chromatin Conformation Capture and paired-end sequencing (Hi-C) of MN^{DHMN} has revealed an altered topologically associated domain (TAD) profile suggesting a disrupted gene regulatory landscape in mutant vs wild-type iPSC-MNs. RNA-seq and quantitative PCR (qPCR) has shown gene dysregulation as a result of the DHMN1 complex insertion. Preliminary assessment of high priority candidate genes (*DPP6*, *MNX1/HB9*, *ACTR3B* and *UBE3C*) has revealed that the *MNX1/HB9* is upregulated in MN^{DHMN1} when compared to MN^{CTRL}. Time-lapse imaging show that MN^{DHMN1} has aberrant and delayed axonal out-growth when compared to MN^{CTRL}. The DHMN1 structural variation is providing a useful paradigm to understand genomic organization, chromatin interactions, and gene dysregulation in the broader context of neuronal development and neurodegeneration.

BACK

Poster - 15

Organellar Proteomics Reveals the Mechanisms that Underlie the Neuronal Pathology of AP-4 Deficiency

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Adaptor protein complex 4 (AP-4) is one of five related adaptor protein complexes, which selectively incorporate transmembrane cargo into transport vesicles. In humans, AP-4 deficiency causes a severe neurological disorder, indicating an important role for AP-4 in neuronal development and homeostasis. The pathomechanisms that underlie the neuronal pathology in AP-4 deficiency are currently unknown, but as AP-4 is proposed to function in protein sorting at the trans-Golgi network (TGN), AP-4 deficiency is most likely a disease of missorting.

We recently developed a proteomic method called 'Dynamic Organellar Maps' that provides protein subcellular localisation information at the whole proteome level. The method is highly reproducible, allowing the resulting cell maps to be used comparatively to identify proteins that change localisation in one condition relative to another. Using this approach, three ubiquitously expressed transmembrane cargo proteins, ATG9A, SERINC1 and SERINC3, were found to be mislocalised in AP-4-deficient HeLa cells. Further orthogonal proteomic approaches confirmed these findings and identified two novel AP-4 accessory proteins, RUSC1 and RUSC2. ATG9A, a core autophagy protein, accumulates at the TGN in several AP-4-deficient cell lines, including fibroblasts from AP-4-deficient patients, neuroblastoma-derived SH-SY5Y cells and human iPSC-derived neurons. In addition, RUSC2 facilitates the microtubule plus-end-directed transport of AP-4-derived, ATG9A- and SERINC-positive vesicles from the TGN to the cell periphery. Microtubules are unipolar in axons, with distally localised plus-ends, and the distal axon is an important site of autophagosome biogenesis in neurons. Thus, our data suggest that AP-4 deficiency is caused by inefficient delivery of ATG9A to the distal axon and hence by spatially dysregulated autophagy. We are now testing this model and searching for additional neuronal-specific AP-4 cargo proteins by applying our proteomic approaches to AP-4 deficient iPSC-derived neurons and in an AP-4 knockout mouse model.

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Poster - 16

Knockdown of HCN Channels in Mouse Hippocampal Neurons by Virus Delivered Gene-interfering Tools

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Pacemaker ion channels, also known as hyperpolarization- and cyclic nucleotide-gated (HCN) ion channels, are frequently expressed in various neuronal tissues. On the cellular level they contribute to the regulation of the resting membrane potential, integration of synaptic input at the dendrites, regulation of presynaptic neurotransmitter release, as well as generation of rhythmic activity. Thus HCN-channel dysfunction or altered gene expression levels are considered to be involved in several pathological conditions including epilepsy, neuropathic pain, or an age-related decline in the working memory.

To investigate consequences of HCN-channel dysfunctions in single neurons and neuronal networks, we interfered with HCN-channel expression levels. Therefore, we specifically targeted the different channel isoforms using two independent gene-interfering techniques. First, we took advantage of a cell-autonomous RNA-interference process. It mediates the breakdown of target mRNA by the application of short-hairpin RNAs. As a second approach we used an enzymatically inactive Cas9 variant. This protein binds specifically to transcriptional start regions of *hcn* genes, thereby directly interfering with the gene transcription. Both techniques were delivered to hippocampal neurons in a primary cell culture system and in an organotypic slice culture system by recombinant adeno-associated viruses (AAVs). We monitored the specificity and efficacy of *hcn* gene knockdown by immunological, and quantitative PCR assays. By electrophysiological recordings of virus transduced neurons, we validated changes of HCN-current responses and neuronal activity. The knowledge achieved by these experiments provides further insight in HCN-channel functions, in particular their contribution to the activity of neuronal networks.

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Poster - 17

An Axon-specific Hippo-like Pathway Regulates Axon Branching

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In genetic screens and loss of function analysis, we discovered that multiple core components of the Hippo pathway are essential for axon growth and branching of mechanosensory neurons in *Drosophila*. Currently we characterize the upstream signals that specifically control in postmitotic neurons this important growth regulatory pathway. Importantly, we also investigate whether the identified Hippo-like regulatory network is functioning in multiple neuron types and whether it is also utilized in vertebrates (i.e. conserved). Furthermore, in a comparative study we examine in vivo how components of a neuron-specific Hippo-like pathway control molecular mechanisms underlying axon growth and branching in *Drosophila* and *Xenopus tropicalis.*

We are testing loss-of-function and overexpression of key components of this pathway in different neuron types that exhibit complex morphologies and highly branched compartments. Specifically we are testing the Wts kinases (Lats1/2 in vertebrates) and the transcriptional effector Yki (Yap/Taz in vertebrates). Axons projections and branching are analyzed in DCN motion detection neurons as well as multiple different medulla neurons (e.g. DM 1, 6, and TM 9) of the visual system of mutant flies. Our analysis confirmed already that Yki loss-of-function in DCN neurons and DM 6 results in aberrant branching suggesting that this signaling factor have important functions in multiple postmitotic neuron types. Our next step is to perform the loss-of-function studies in a developmental single cell analysis.

We are also testing the function of Lats1/2 and Yap/Taz in vertebrate neurons by using single cell analysis in *Xenopus tropicalis*. The loss of function of Yap/Taz and Lats1/2 are being perform by single retinal ganglion cell electroporation by targeting the eye at stage 30. Axon branching in single mutant neurons is analyzed at stage 46 by confocal imaging and 3D reconstruction. We confirmed that morpholinos against Yap and Lats1 decreases the protein levels in *Xenopus*, and suggests that are required for normal retinal ganglion cells axon branching in the Tectum. In follow up studies, we use LOF analysis by generating cell specific somatic homozygous knockouts using CRISPR/Cas9 techniques already established in the lab.

Recent work in our lab has suggested the possibility that several developmental axon branching regulators are also necessary for initiating axon growth in response to injury. Given the fact that the Hippo pathway constitutes a major injury response pathway, we plan to further examine whether Lats1/2 and Yap/Taz are also important during regenerative axon growth and targeting in *Xenopus tropicalis*.

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Poster - 18

Growth Cone Molecular Machinery in Development *vs.* Regeneration: Subcellular Transcriptome Mapping in Corticospinal Neurons

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Neurons of the central nervous system (CNS) grow axons over remarkably long distances during development - generating exquisitely precise functional circuitry. The exact same neurons, however, fail to regenerate in the context of the adult, injured CNS. Deep research over decades has identified that both a growth inhibitory environment as well as failed activation of intrinsic growth machinery prevent axonal regeneration in the injured CNS. Despite this knowledge, no effective, causal treatment exists for traumatic injuries to the CNS. Because of limitations of available approaches, mechanistic studies to-date in the developing and lesioned CNS focus on molecular changes in neuronal cell bodies (somata).

Neurons, however, are unique, specialized cells with extreme polarity, spatial expanse (10^4-10^5 soma diameters), and a complex internal organization into functionally and spatially distinct subcellular compartments. In development and regeneration, growth cones (GCs) are the subcellular units effecting or limiting axonal growth, guidance, and circuit/synapse assembly. Until very recently, molecular states of developmental and regenerative GCs were not experimentally accessible. New experimental and analytic approaches developed recently in our lab now enable direct access to the RNA and protein molecular machinery of subtype-, stage-, and state-specific GCs (and their own parent somata) directly from mouse brain. For purification of subtype-specific GCs and somata, we combine specific neuronal and GC molecular labeling, biophysical GC and soma isolations, density gradient ultracentrifugation for GC enrichment, and newly developed fluorescent small particle sorting for GCs and FACS for somata. The application of this newly developed analytic, quantitative "subcellular RNA-proteome mapping" approach to GCs and somata of callosal projection neurons in the developing cortex of mouse has identified hundreds of subtype-specific, GC-enriched RNAs/proteins [Poulopoulos et al., Nature 2019].

Using a combination of subtype-specific GC purification, high-depth GC-soma molecular analyses, and quantitative "subcellular RNA mapping", we are currently investigating the molecular machinery of corticospinal GCs *in vivo* during development. Corticospinal neurons reside in layer V of cortex and send axons over remarkably long distances to the midbrain, brainstem, and spinal cord. They play a key role in several clinical conditions, including spinal cord injury. Using genetic labeling, we are currently specifically purifying corticospinal GCs (and their parent somata) at distinct developmental stages. Deep RNA sequencing enables direct comparative analysis of subcellular localization and enrichment of transcripts (and proteome in parallel) across developmental stages.

We are now expanding this work to regenerative GCs following spinal cord injury, aiming at identifying molecular similarities and differences between the local transcriptome (and proteome) of developing (growth-permissive) and regenerating (growth-abortive) corticospinal GCs.

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Poster - 19

Anterograde Trans-synaptic Jeb-Alk Signaling is a Negative Feedback Mechanism to Stabilize Circuit Growth

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The brain adapts to a changing environment or growing body size by structural growth and synaptic plasticity. To maintain neuronal circuits functional, these alterations are tightly coordinated between synaptic partner neurons. Feedback loops are widely deployed when such interacting processes need to be reciprocally tuned to establish a stable, but adaptive response to external input. Several positive feedback mechanisms have been characterized that synchronize structural expansion and synapse proliferation between partner neurons. Negative feedback mechanisms that operate to keep such reciprocal growth promotion at bay have so far remained elusive.

Here, we analyzed the interplay between neuronal growth dynamics and synaptogenesis by in vivo imaging and quantification of endogenous synaptic protein accumulation at nanometer resolution in the motor system of *Drosophila* larvae. We show that anterograde trans-synaptic Jeb-Alk signaling inhibits proliferation of post- but not presynaptic specializations, and promotes stabilization of nascent dendritic branches. Alk dependent retrograde trans-synaptic signaling represses presynaptic filopodia formation and consequently leads to a reduction in postsynaptic growth.

Our findings demonstrate that Jeb-Alk signaling is a negative feedback mechanism to implement adaptive, but stable neuronal growth and synaptogenesis in response to other growth promoting signals specifically during postembryonic circuit expansion.

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Poster - 20

Nrg1 Intracellular Signaling is Neuroprotective upon Stroke

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Nrg1 is a schizophrenia risk gene that controls the formation of excitatory and inhibitory synapses in cortical circuits. While different Nrg1 isoforms are expressed during development, adult neurons primarily express type III Nrg1 that is characterized by a highly conserved intracellular domain (Nrg1-ICD). We and others showed that the Nrg1 intracellular signaling promotes dendrite elongation and excitatory connections during neuronal development. However, the role of Nrg1 intracellular signaling in adult neurons and in pathological conditions remains largely unaddressed. Here, we investigated the role of Nrg1 intracellular signaling in neuroprotection in aging and stroke.

Our bioinformatic analysis showed that Nrg1 intracellular domain is extremely conserved during evolution and that NRG1 expression significantly decreases with age in the human frontal cortex. Hence, we first tested weather Nrg1 signaling may affect pathological hallmarks of aging such as DNA damage and cellular stress in an *in vitro* model of neuronal aging. Our data did not suggest a role for Nrg1 in this context.

Previous studies showed that the soluble EGF domain of Nrg1 could alleviate brain ischemia, a pathological process that involves the generation of free radicals, reactive oxygen species and excitotoxicity. Hence, we tested the hypothesis that Nrg1 intracellular signaling could be neuroprotective in stroke. We found that Nrg1 expression significantly increased neuronal survival upon oxygen-glucose deprivation, an established *in vitro* model for brain ischemia. Notably, the specific activation of Nrg1 intracellular signaling by expression of the Nrg1 intracellular domain was sufficient to protect neurons from ischemia. Time-lapse experiments further confirmed that Nrg1 intracellular signaling increases the survival of ischemic neurons at different time points.

Finally, we investigated the relevance of Nrg1 intracellular signaling in stroke *in vivo*. Using viral vectors, we expressed Nrg1 intracellular domain in cortical neurons that were subsequently challenged by focal hemorrhagic stroke. We found that Nrg1 intracellular signaling could improve neuronal survival in the infarcted area.

Altogether, our data showed that Nrg1 intracellular signaling is neuroprotective upon ischemic lesion both *in vitro* and *in vivo*. The neurotoxic effect of stroke is complex and involves different mechanisms such as the generation of reactive oxygen species, excitotoxicity and inflammation. Therefore, further studies will be required to determine the molecular bases of the neuroprotective effect of Nrg1 intracellular signaling. In conclusion, our work indicates that the stimulation of Nrg1 intracellular signaling may by a promising target for the treatment of cortical ischemia.

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Poster - 21

Dystroglycan Affects Morphology and Function of Hippocampal Neurons

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Many studies have shown that regulated proteolysis of synaptically expressed cell adhesion molecules plays a fundamental role in the morphological reorganization of synapses underlying homeostatic plasticity as well as learning and memory processes. In this study we focus on dystroglycan (DG), which is cleaved by matrix metalloproteinase-9 (MMP-9) in response to enhanced neuronal activity. DG is a cell adhesion receptor composed of α- and β-subunits that form a link between the extracellular matrix (ECM) and intracellular actin cytoskeleton. Although it has been demonstrated that deletion of DG in neurons impairs hippocampal long-term potentiation (LTP), detailed knowledge of DG action in neuronal cells is still lacking.

Our previous studies revealed that DG controls dendritic outgrowth and arborization of hippocampal neurons in vitro (Bijata et. al, 2015). However, its effect on synapse remodeling remains unknown. To explain the role of DG in structural plasticity of dendritic spines we use multiple approaches including modification of DG gene expression in neuronal cells using plasmid and viral vectors, immunofluorescence and biochemical techniques, and visualization of MMP-9 activity by FRET-based biosensor. The length-to-width parameter was used to evaluate the spine shape. We observed significant increase in the spine length/width ratio in hippocampal neurons infected with lentiviral vector expressing shRNA for down-regulation of DG compared with control cells. Interestingly, we noticed a similar effect in neurons overexpressed β-DG with a mutation in the MMP-9 cleavage site. These results indicate that DG exerts an influence on dendritic spine structure in MMP-9-dependent manner. Since DG has the potential to modulate neuronal plasticity, we have examined the electrophysiological properties of neurons exhibiting impaired DG expression. Our results indicate that DG is an important cell adhesion molecule which affects structural and functional plasticity of hippocampal neurons.

BACK

Poster - 22

New Hypotheses of NeuroLSD1 Modulation: from lncRNA to Cryptic Exon Inclusion

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Behavioral outcome in response to adverse contingencies can vary in an individual-specific manner accordingly to life experiences and genetic background in the frame of genotype x environment interactions (GxE). The transcriptional corepressor Lysine-Specific Demethylase 1 (LSD1), along with its neuronal specific alternative splicing variant, neuroLSD1, represent a remarkable example of environmental stimuli transducer in the mammalian brain. LSD1 is a negative modulator of stress-plasticity and memory formation, while neuroLSD1, acting as LSD1 dominant negative isoform, promotes stimuli-dependent transcription of plasticity-related genes in response to environmental stimuli serving as a drive to encode experience memory traces. Interestingly, LSD1 and neuroLSD1 relative level can be directly modified by stressful events, impacting LSD1-dependent H3K4 demethylase activity. In conclusion, tuning LSD1/neuroLSD1 relative ratio can be considered a process of transcriptional homeostasis pivotal to environmental adaptation at the behavioral level.

NeuroLSD1 isoform differs from the ubiquitously expressed LSD1 isoform only for the presence of the tetra-peptide Asp-Thr-Val-Lys, coded by the 12 nucleotides-long micro-exon E8a. The regulation of this alternative splicing event ensures fine control on neuroLSD1 expression level during all developmental stages, and is mainly exerted by two neurospecific splicing regulators, nSR100 and NOVA1. An important question is to clarify how this splicing event is modulated in the hippocampus, in response to stimuli. We will present data on the identification of a further layer of splicing regulation, involving the long non-coding RNA MALAT1 as stress-induced negative regulator of micro-exon E8a inclusion and neuroLSD1 expression.

Recently we discovered the presence of a primate specific 77-bp long cryptic exon, that we named exon E8b, whose inclusion into LSD1 mature transcript is regulated by a different neurospecific splicing factor recently identified as one of the most important genetic loci associated with neuropsychiatric disorders. The exon E8b sequence inclusion, cause Non-sense mRNA Mediated Decay (NMD). Since in the human brain exon E8b is included preferentially in exon E8a-containing transcript, NMD might be a further mechanism to specifically regulate neuroLSD1 level. This primate-restricted neuroLSD1 splicing tuner could therefore contribute to the complexity of cognitive and social abilities that distinguishes higher primates from other mammals but might also represents a vulnerability factor for stress-related neuropsychiatric disorders.

BACK

Poster - 23

Pontin and Reptin at the Neuro-muscular Junction

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The AAA+ ATPases Pontin and Reptin are highly conserved in Eukaryotes and have been associated with numerous multi-unit complexes in a multitude of cellular processes. Although their roles in each case have not been fully explained it is becoming evident that the proteins often play a chaperoning or scaffolding role in complex assembly. In multicellular animals the two proteins which generally act together sometimes have opposing effects. Both Pontin and Reptin associate with microtubules and Pontin is required for the formation of the mitotic spindle. We and others have found interactions between Pontin, Reptin, tubulins and motor protein subunits in various species, and recently it has been shown that Pontin is required for axonemal Dynein assembly. We are interested in the cytoplasmic roles of Pontin and Reptin in multicellular and developmental context, in particular in the nervous system, where mitotic roles can be separated from other functions. Both proteins are expressed in the *Drosophila melanogaster* developing and adult nervous system and pan-neuronal knock-down of either protein results in pupal lethality with few survivors, which are shaky and show rapid loss of fitness. A range of phenotypes is also observed in the larval CNS. In motor neurons, where we find that conditional knock-down of either Pontin or Reptin leads to reduced survival, motor deficits and progressive loss of fitness, suggesting neurodegenerative effects. Here we will present our findings on Pontin and Reptin at larval neuro-muscular junctions.

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Poster - 24

MECP2 Mutations Affect Ciliogenesis: a Novel Perspective for Rett Syndrome and Related Disorders

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Rett syndrome (RTT; OMIM 312750) is a progressive X linked neurodevelopmental disorder that, because of its incidence (1:10000 females), represents the most common cause of severe intellectual disability in girls worldwide. RTT is caused by mutations in methyl-CpG-binding protein 2 (MeCP2), a protein expressed mainly in the nucleus where it modulates the expression of several genes. Although mostly functioning as an epigenetic transcriptional repressor, MeCP2 is a multifunctional protein revealing several activities. We have recently demonstrated that MeCP2 associates with centrosome, therefore affecting its function. Primary cilia are "sensory antennae" that originates from centrosomes and protrude from several cells, including neurons and astrocytes. Cilia receive and integrate extracellular signals, affecting neuronal migration, dendritic arborization, neural circuits integration, learning and memory. Defective primary cilia are associated with a growing list of "ciliopathies" characterized by several symptoms in common with RTT syndrome.
Considering all above, we investigated and demonstrated that MeCP2 deficiency affects ciliogenesis in cultured cells, including Rett patients' fibroblasts, and in *Mecp2* null brains. We also demonstrated that the cilia-related Sonic Hedgehog pathway, essential for proper brain development and functioning, is altered in MeCP2 deficient cells and cerebellum. We demonstrate that microtubule instability caused by excessive activity of HDAC6 participates in the MeCp2 defective cells to the observed cilia defects. Indeed, the pharmacological inhibition of HDAC6 by tubacin, restores ciliogenesis and cilia functioning in MeCP2 deficient cells. Together, our data propose the alteration of primary cilia as a pathogenic mechanism participating to the clinical features of MECP2 related disorders.

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Poster - 25

The Role of RNA Binding Proteins in Amyotrophic Lateral Sclerosis: How do they contribute to Motor Neuron Degeneration?

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Motor neuron (MN) is any cell that carries the signal outside the central nervous system (CNS) to control essential voluntary muscle activity. Normally, this signal is transmitted from nerve cells in the brain (upper motor neurons) to nerve cells in the brainstem and spinal cord (lower motor neurons) and from them to particular muscles. MNs are the primarily affected cell type in some neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). This is an idiopathic and fatal neurodegenerative disorder of the motor system, caused by progressive degeneration of MNs in the brain and spinal cord. ALS is a complex disease, in which the contributes of environmental signals and genetic predisposition are still poorly understood. Identification of the pathogenic mechanisms responsible for motor neuron degeneration is hampered by the lack of appropriate model systems. An important help is provided by induced pluripotent stem cells (iPSCs) that are widely used to model neurodegenerative diseases in vitro, thanks to their pluripotent character. In fact, iPSCs offer the possibility of generating any type of adult cell in vitro.

My laboratory studies the causal relationship between the alteration of RNA metabolism and ALS in human motoneurons, generated by the differentiation of iPSCs carrying pathological mutations in the FUS gene. FUS is a predominantly nuclear DNA/RNA-binding protein with functional roles in transcriptional regulation, splicing, pre-microRNA processing and messenger RNA transport and stability. Autosomal mutations in its gene were identified in ALS, linking genetics and pathology with neurodegeneration. Through the transcriptome analysis, we have identified members of the ELAVL family among FUS mutant targets. These genes encode for RNA-binding proteins involved in neural development, function and survival but never associated with ALS before. Furthermore, we have observed an interaction between mutant FUS and ELAVL4 at the protein-protein level and their co- localization in cytoplasmic granules in oxidative stress conditions.

Moreover, we have conducted a proteomics analysis that has revealed differential expression of proteins involved in motor axon biology, such as axon development, growth cones, cytoskeleton parts, neuron projections. Through microfluidic devices, we have analyzed these differences at morphological levels. Preliminary data show an increase of arborization parameters about branches, junctions, triple points and quadruple points, and a decrease of maximum branch length and longest shortest path in MNs carrying FUS mutation.

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Poster - 26

The Coordination between Kinesin-3 and Dynamic Presynaptic Microtubules Specifies High Precision Delivery of Synaptic Vesicles to the Presynapse

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Neurons in the central nervous system establish thousands of *en passant* synapses along their extensive axonal arbors. Replenishment of these presynaptic sites with new synaptic vesicles in a timely and spatially-precise manner is critical for sustaining reliable neurotransmission. However, the mechanisms specifying the local delivery of synaptic vesicle precursors (SVPs) to presynaptic sites remain unclear. By employing live-cell fluorescence microscopy and single-molecule reconstitution assays, we found that delivery of SVPs to *en passant* synapses in hippocampal neurons occurs with high precision and is specified by an interplay between the kinesin-3 KIF1A motor and presynaptic microtubules. We identified presynaptic sites as hotspots of dynamic microtubules rich in GTP-tubulin, and found that KIF1A binds weakly to GTP-tubulin and competes with EB proteins for binding to the microtubule lattice. The enrichment of dynamic microtubules at the presynapse effectively defines a localized SVP unloading zone and ensures a supply rate of SVPs at the presynapse that is in tune with the estimated synaptic vesicle lifetime. Additionally, we identified a human disease-causing mutation within KIF1A motor domain that abates the differential binding of KIF1A to the GTP and GDP microtubule lattices. Expression of this mutation specifically disrupts SVP delivery to presynapses and reduces presynaptic strength in hippocampal neurons. Together, we show that microtubule dynamics and organization along the axon provide a spatial code that specifies presynaptic delivery of KIF1A-SVPs and controls presynaptic strength in hippocampal neurons.

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Poster - 27

Super-resolution Iimaging of Brain Tissue during Chemical Fixation

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Chemical fixation using paraformaldehyde (PFA) is a commonly used strategy to preserve biological samples for immunohistochemistry and electron microscopy. Even though it is widely used in biological research, it is well known that chemical fixation is prone to 'fixation artifacts', which may grossly disturb important cellular details of the tissue. In the case of brain tissue, the obvious problem is the nearly complete depletion of the extracellular space (ECS) following PFA fixation, whereas quantitative analyses based on biophysical approaches and super-resolution microscopy in live settings indicate that the ECS occupies about a quarter of the volume of the brain. With the growing interest in brain connectomics, tissue clearing and expansion microscopy, which inevitably involve tissue fixation, there is an urgent need to shine light on this issue.

To understand better the impact of chemical fixation on brain tissue, we established a protocol to image in real-time changes in the micro-anatomical organization of organotypic brain slices induced by chemical fixation. It is based on the recent SUSHI (super-resolution shadow imaging) technique, which can visualize the ECS in living brain tissue with nanoscale spatial resolution. By combining time-lapse SUSHI with positive labeling of neurons and astrocytes, our approach makes it possible to identify the cell types and compartments that swell, shrink or structurally remodel during the process of chemical fixation, accounting for the net changes in ECS.

Our study provides the first detailed characterization of the response of brain tissue to chemical fixation, providing a quantitative methodology to assess the nature and extent of tissue compromisation during chemical fixation and thus help establish and validate improved tissue fixation strategies.

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Poster - 28

Dual Activity of RIN1 Regulates Synaptic Plasticity in Dendritic Spines

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Actin cytoskeletal remodelling and the turnover of synaptic receptors in the postsynaptic membrane are controlled in an activity-dependent manner during synaptic plasticity, but it is still poorly understood how these processes are synchronised.

We recently showed that the Rab5 GEF activity of the Ras and Rab interactor 1 (RIN1) is essential for AMPA receptor endocytosis upon chemically induced long-term depression (cLTD) in hippocampal neurons. RIN1 controls actin remodelling through Abl kinase activation, but it has not yet been addressed whether RIN1-dependent cytoskeletal rearrangements also contribute to synaptic plasticity.

We focused on how RIN1 influences the motility and morphology of dendritic protrusions. Our results obtained from RIN1ko hippocampal neurons transiently expressing GFP- RIN1 constructs demonstrate that RIN1 i) increases filopodial motility through activation of Abl kinases; ii) increases the ratio of thin protrusions in expense for mushroom spines; and iii) is required for the morphological alterations accompanying cLTD. Finally, actin-FRAP experiments revealed that RIN1 enhances actin dynamics in filopodia but not in mature dendritic spines.

Taken together our results indicate that RIN1 regulates synaptic plasticity in a dual manner. Besides controlling the activity-dependent morphological transitions of dendritic protrusions, it also regulates AMPA receptor endocytosis. Both of these steps are important during LTD and might explain why RIN1 knock-out (RIN1ko) mice show reduced fear extinction.

Research was supported by the National Brain Research Programs (KTIA_NAP_13-2-2014-0018 and 2017-1.2.1-NKP-2017-00002), the VEKOP-2.3.3-15-2016-00007 grant by NRDIO, and by the German Research Foundation (DFG HA-.3557/11-2).

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Poster - 29

Uniform Axonal Microtubule Organisation is established by Dynactin-mediated Microtubule Stabilisation

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The microtubule (MT) cytoskeleton in neuronal axons is highly oriented with almost all MTs pointing with their growing end (+end) away from the cell body (+end out). Molecular motor proteins rely on this orientation to efficiently move cellular cargo to the far distal regions of the axon. Despite 30 years of research, the mechanism that establishes this unique MT configuration remains unknown. We here analysed MT growth behavior in primary *Drosophila melanogaster* neurons with a novel machine learning algorithm; the *KymoButler*. The unmatched precision of this approach led us to the discovery that +end out MTs grow for longer times than –end out MTs. Together with an analytical model of microtubule growth, this observation predicts dramatic differences in average MT length, so that –end out MTs are short and unstable while +end out MTS become very long. Additionally, we found evidence that dynactin is responsible for the difference in growth times by promoting growth at the periphery of the cell through a molecular gradient. These findings suggest a simple mechanism that organises axonal MTs. First, +end out MTs are stabilized by distally located dynactin. Subsequently, the short –end out MTs may get transported out of the axon, depolymerize, or reorient, leaving mostly +end out MTs in the axon. Our results pave the way towards a deeper understanding of how the cytoskeleton in neurons orients to support molecular transport along the axon, potentially shedding light on pathologies that are characterized by axonal transport deficiencies such as Alzheimer's disease.

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Poster - 30

Deficits of Brain Protein Homeostasis Affect Social Behaviours in Flies

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Social deficits are a hallmark of intellectual disabilities (IDs) such as autism spectrum disorder (ASD) and schizophrenia (SCZ). Those disorders are caused by defects in various shared pathways, but their interplay has not yet been elucidated. A number of mutations in genes encoding synaptic proteins are associated to IDs and appear to confer abnormal social behavior in different biological models. However the machinery/ies responsible for sociability remain unclear.

Here we identified the circuitry and molecular mechanisms underlying social interactions in Drosophila melanogaster. Proteomic profiling reveals that deficits in protein synthesis and specific metabolic pathways contribute to impaired social interaction.

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Poster - 31

Synthetic Microneurotrophin BNN27 Improves Working Memory, Ameliorates Aβ Pathology and Promotes Adult Neurogenesis in the 5xFAD Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by progressive neuronal loss and cognitive decline. The major pathological hallmark of AD is the accumulation of the β-amyloid (Aβ) peptide within the brain. There is now strong evidence that the reduction of neurotrophin Nerve Growth Factor (NGF) is also involved in cell death and reduced neurorestoration. BNN27 is a newly developed 17-spiro-steroid analog that mimics the neuroprotective effects of NGF, acting as selective activator of its receptors, TrkA and p75NTR (microneurotrophin), promoting neuronal survival. We examined the ability of BNN27 to ameliorate AD-related cognitive deficits and neuropathology, reducing Aβ toxicity as well as promoting the proliferation and/or survival of neuronal precursors in vitro and in vivo.

BNN27 pellets were sub-dermally applied to 5xFAD mice, which harbor five familial AD mutations, prior to the development of any Aβ pathology (1.5 months of age). The pellets allowed a slow release of the compound over 6 weeks. BNN27 treatment significantly improved working memory tested in the T-maze in the 5xFAD animals. Moreover, BNN27 decreased the formation of Aβ plaques and the loss of synapses within the hippocampus of the 5xFAD animals. Additionally, BNN27 effectively promoted adult hippocampal neurogenesis, significantly increasing the number of doublecortin (DCX) positive neurons, a marker of neuronal precursor cells and immature neurons, within the dentate gyrus of the hippocampus. BNN27 partially reduced the accumulation of oligomeric Aβ1-42 in the hippocampus of 5xFAD mice. It is of note that oligomeric Aβ1-42 reduces the proliferation of hippocampal neural stem cells. Furthermore, the integrity of myelin, axons and cholinergic neurons was also investigated. No significant changes of myelin and axonal integrity were observed in the hippocampus after treatment of 5xFAD mice with BNN27. However, BNN27 reduced cholinergic atrophy in basal forebrain of 5xFAD mice, significantly increasing the mean soma size of Choline Acetyltransferase (ChAT) positive neurons.

Our findings suggest that microneurotrophin BNN27 blocks amyloid deposition and promotes hippocampal neuroregeneration in the 5xFAD mice, most probably affecting the pathways downstream to NGF receptors.

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Poster - 32

Cytoskeletal Dynamics in Mice Missing the Majority of Cortical Interneurons as a Result of Rac1 and Rac3 Deficiencies

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GABAergic interneurons provide the main source of inhibition in cortical microcircuits. Impaired interneuronal function results in severe neurodevelopmental disorders such as schizophrenia, epilepsy and autism. Interneuron migration from their origin to the neocortex is a pivotal process and is determined by extracellular factors which modify their leading processes through activation of intracellular pathways. Rac-proteins are intracellular mediators of numerous developmental processes such as cytoskeleton organization, vesicle trafficking, transcription, cell cycle progression in diverse cell types. We focus in the role of the ubiquitous Rac1 and neural-specific Rac3 in interneurons derived from the medial ganglionic eminence (MGE), a population comprising the majority of cortical interneurons.

We previously revealed a cell autonomous and stage-specific requirement for Rac1 activity within proliferating interneuron precursors using Cre/loxP technology (Vidaki et al, 2012). We have also generated Rac1/Rac3 double mutant mice (Tivodar et al, 2014) and found that in the absence of both Racs, the embryonic migration of MGE-derived interneurons is impaired, resulting in an 80% loss of cortical interneurons, postnatally. Rac1/Rac3-deficient interneurons show gross cytoskeletal defects when grown *in vitro*, including nuclear shape alterations, reduction of axon length, splitting of the leading process, abnormal growth cone formation and reduction of microtubule stability. In addition their migratory behavior is severely perturbed as a number of motility parameters such as velocity, amplitude and frequency of nuclear translocation are decreased significantly in *ex vivo* time-lapse imaging assays. There is also a delay in principal neurite outgrowth. RNA seq data indicate putative downstream effectors that are under investigation. In summary, in the absence of Rac1/Rac3, cortical interneurons fail to migrate to the cortex due to defects in actin and microtubule cytoskeletal dynamics.

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Poster - 33

A Super-resolution Platform for Correlative Single Molecule Imaging and STED Microscopy

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Super-resolution microscopy offers tremendous opportunities to unravel the complex and dynamic architecture of living cells. However, current super-resolution microscopes are proficient at revealing either protein distributions or cell morphology, but not both. Here, we present a new super-resolution platform that permits correlative single molecule localization microscopy (SMLM) and STED imaging. We demonstrate that this multi-modal approach gives access to both kinds of information by visualizing on a nanometer spatial scale the positions and movements of proteins and their morphological context in live cells.

The SMLM/STED combination was constructed around a commercial inverted microscope where one port provided access for STED laser-scanning and two others for single-molecule imaging. The STED part used pulsed lasers for fluorescence excitation and stimulated emission and confocal fluorescence detection. Single-molecule imaging was implemented by full-field excitation under oblique illumination and fluorescence collection using an EMCCD camera.

We tested the performance of the microscope by imaging fluorescently labeled cell adhesion molecules in transfected mouse embryonic fibroblasts (β3-integrin and paxillin). We then visualized neuronal morphology and several prominent cytoskeletal/synaptic proteins (actin, PSD-95, GluA1 and GluA2) in rat hippocampal cell cultures. While pre-and postsynaptic structures (axonal boutons and dendritic spines) were imaged by 3D-STED of cytosolic GFP or super-resolution shadow imaging (SUSHI) using calcein as extracellular dye, the proteins were labeled with mEos and Atto647N for single-molecule imaging.

We demonstrate proof-of principle that by combining deterministic and stochastic super-resolution microscopy techniques it is possible to gain access to quantitative morphological and molecular information on a nanometer-scale in a live setting, extracting novel biological information and relationships.

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Poster - 34

Homeostatic Systems Control Neuropeptide and DCV Biogenesis

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Neuropeptides are used by a broad phylogenetic spectrum to create, maintain, or amplify neuronal network states. After neurosecretion, neuropeptides store must be reconstituted by de-novo synthesis followed by neuropeptide sorting into Dense Core Vesicles (DCVs). Therefore, the precise tuning of the neuronal network states requires a tight control over neuropeptide and DCVs production. To explore their cellular biology, we defined PQR, URX and BAG neurons as model responding to O2 or CO2 by persistent calcium entry and tonic secretion of neuropeptides. In these neuronal types, several observations suggest that homeostatic systems couple the exocytosis of DCVs with DCVs biogenesis. I will provide two examples. 1) Neurosecretion promotes the recycling of membrane proteins to new DCVs, providing a mechanism to maintain DCV production. 2) Neurosecretion is coupled to a tight transcriptional control of neuropeptides and DCV genes.

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Poster - 35

Role of the Epigenetic Enzyme LSD1 in Controlling Neuronal Plasticity-related Transcriptional Programs

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The acquisition and maintenance of the specific neuronal functions underlying learning, memory and cognition require the induction of plasticity-related transcriptional programs. The epigenetic enzyme lysine-specific demethylase-1 (LSD1) and its neurospecific splicing variant neuroLSD1 are key players in this process. Our work aims at characterizing the involvement of the transcriptional corepressor LSD1 and its dominant negative isoform neuroLSD1 in the process of activity-dependent transcription in the brain.

Remarkably, LSD1 plays a dual role in information processing, being this epigenetic enzyme instrumental to transduce environmental stimuli under the form of inherent neuronal plasticity, but also representing a target whose function is directly modulated by these same challenges. In mouse hippocampus, the ratio between the transcriptional corepressor LSD1 and its dominant negative isoform neuroLSD1 is dynamic and responds to different paradigms of neuronal activation such as psychosocial stress. In primary rat hippocampal neurons, we showed that neuroLSD1 is downregulated upon activation of the NMDA receptor and that a low dose of neuroLSD1 generates a defective activity-dependent transcription of the IEGs egr1, c-fos and npas4.

Moreover, lack of neuroLSD1 determines decreased mean dendritic spine number in the CA1 area of the hippocampus, reduced post synaptic density thickness and length and aberrant plasticity-related phenotypes such as reduced memory and low anxiety. Electrophysiological measurements highlight an impaired LTP induction in neuroLSD1 deficient mice.

These findings are relevant in the context of two well-known forms of long-lasting neuronal plasticity, LTP and LTD, both instrumental to learning and memory formation, and that entail opposite modifications of the structure of dendritic arbor as well as changes in the number and shape of dendritic spines. In this regard, while LTP correlates with an increased number of spines, LTD has been shown to induce concomitant shrinkage and retraction of dendritic spines. Thus, it seems that bidirectional changes in synaptic efficacy correlate with bidirectional structural modifications.
We suggest that the modification of the ratio between LSD1 and neuroLSD1 splicing isoforms is an epigenetic mechanism that might contribute to coupling environmental stimuli to the modification of structural plasticity of hippocampal neurons.

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Poster - 36

Revealing NMDA Receptor Hidden Conformational Open States that Block Electric Current under Agonist Activation in Living Cells by a Novel Single-Molecule Patch-Clamp FRET Super-Resolution Microscopy

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Conformational dynamics plays a critical role in the activation, deactivation, and open-close activities of ion channels in living cells. Such conformational dynamics is often inhomogeneous and extremely difficult to be directly characterized by ensemble-averaged spectroscopic imaging or only by single channel patch-clamp electric recording methods. We have developed a new and combined technical approach, single-molecule patch-clamp FRET microscopy, to probe ion channel conformational dynamics in living cell by simultaneous and correlated measurements of real-time single-molecule FRET spectroscopic imaging with single-channel electric current recording (1-4). Our approach is particularly capable of resolving ion channel conformational change rate process when the channel is at its electrically off states and before the ion channel is activated, the so-called "silent time" when the electric current signals are at zero or background. We have probed NMDA (N-Methyl-D-Aspartate) receptor ion channel in live HEK-293 cell, especially, the single ion channel open-close activity and its associated protein conformational changes simultaneously. Furthermore, we have revealed that the seemingly identical electrically off states are associated with multiple conformational states, including the desensitized states. Based on our experimental results, we have proposed a new multistate clamshell model to interpret the NMDA receptor open-close dynamics. Technically, our new method has a great potential to provide new structure-function analysis for understanding the function, activity and mechanism of glutamate receptor ion-channels.

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Poster - 37

Neuronal TORC1 Coordinates Mood and Cognition via Serotonin Receptors

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mTORC1 (mechanistic target of rapamycin complex 1) is a nutrients sensing protein complex that regulates protein synthesis and autophagy. Moreover, it has been extensively implicated in cognitive impairment and mood stabilization. However, the relevant mechanisms are not fully understood. Here, we investigated the cellular and molecular mechanisms by which mTORC1 affects behavior and cognition in two animal models: flies and zebrafish.

Combined behavioral and biochemical analysis revealed that acute rapamycin treatment, known to induce autophagy through mTORC1 inhibition, impaired memory and changed behavioral patterns in both species. Similarly, genetic mTORC1 upregulation evoked opposite effects on memory formation and behavior. We find that mTORC1 exerts its effects at the serotonergic cells of ellipsoid bodies in *Drosophila*, hence identifying ellipsoid bodies as the brain domain responsible for the coordination of behavior and cognition in flies. Specifically, acute mTORC1 inhibition was found to alter intracellular localisation of serotonin transporter via *atg1* (autophagy-related gene 1), thus leading to altered serotonergic signaling that, through specific serotonin receptors at the neighboring nmda2 receptor-expressing neurons, regulates cAMP signaling and Tyr1472 phosphorylation of NMDA2 receptors. Our preliminary results indicate that neuronal *atg1* coordinates mood and cognition through an evolutionary conserved interplay between serotoninergic and glutamatergic – specific cAMP signaling pathways.

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Poster - 38

Characterization of the Skt Gene in the Synapse: Behavioural Studies and Analysis of Synaptic Complexes

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Aims:
In this work we want to analyze the possible role of a novel protein, highly expressed in the synapse, called SKT/KIAA1217 (the human homolog of murine sickle tail, Skt, gene) through the behavioral and electrophysiological characterization of mice Skt -/-. We also studied the functional molecular interactions of SKT/KIAA1217 with the principal post-synaptic component.

Methods:
Primary hippocampal and cortical neuronal cultures. Behavioural analysis: Open Field Test, Barnes Maze test, Puzzle Box Test, Burrowing Test. Microelectrode Analysis (MEA), Electroencephalogram (EEG) analysis, co-immunoprecipitation (Co-IP) from crude synaptosomes fraction, Co-IP from HEK293T cell line transfected with tagged constructs; Immunofluorescence (IF) in primary neuron culture and IF in COS7 cell line transfected with tagged constructs.

Results:
We found that primary cultured neurons from Skt -/- showed a decreased number in total dendritic spines, with a reduction of mature, mushroom, spines and concomitant increase in immature filopodia spine structure. MEA analysis from hippocampal primary cultures showed a delay on neuronal synchronization and maturation. Moreover, Puzzle Box test and EEG revealed that mice Skt -/- present an impaired in cortical function. Through biochemical approaches, we found that SKT/KIAA1217 interacts with the PDZ domain of PSD-95 and with the Shank family member Shank3.

Conclusions:
Our data suggest a possible impairment on cognitive process and memory establishment; moreover, the interaction between SKT/KIAA1217 and two of the key structural protein in the synapse (PSD-95 and Shank3) propose us that SKT/KIAA1217 could be a new player in structural and functional organization of this compartment and that its absence could be linked to neurological alteration.

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Poster - 39

Identification of an axon-resident pool of vesicles regulated by neuronal activity

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During postnatal development, long-range axonal projections reach their target to form branches and synaptic contacts. This process highly depends on neuronal activity and requires large amounts of secretory materials. Axonal transport of secretory vesicles is responsible for addressing these cargoes to high demand sites. However, mechanisms that control the preferential targeting of axonal vesicles to active sites are unknown.

Here, we identify an axon-resident reserve pool recruited upon neuronal activity to rapidly distribute secretory materials to presynaptic sites. Using an *in vitro* system compatible with high-resolution videomicroscopy that combines microfluidic chambers and microelectrode arrays (MEA), we reconstituted a physiologically-relevant cortico-cortical network in which neuronal activity can be controlled. Using this approach, we showed that neuronal activity instantly recruits a pool of anterograde vesicles tethered along the axon shaft. We found that neuronal activity induces Calcium-Induced Calcium Release from ryanodine receptor after activation of Voltage Gated Calcium Channel in the axon, which triggers the recruitment of tethered vesicles. We are now investigating the precise molecular mechanism responsible for vesicle recruitment.

Using 2-photon live microscopy of cortical axons in acute slices, we confirmed that a pool of axon-resident static vesicles is recruited by neuronal activity *in vivo* with similar kinetics. We are now investigating the functional relevance of this axonal reserve pool to promote fast supply of synaptic materials during axon branching and synaptogenesis.

BACK

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Autophagy Lipidation Machinery Regulates Axonal Microtubule Dynamics but is Dispensable for Survival of Mammalian Neurons

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Neurons maintain axonal homeostasis via employing a unique organization of microtubule (MT) cytoskeleton, which supports axonal morphology and provides tracks for intracellular transport. Abnormal MT-based trafficking hallmarks the pathology of neurodegenerative diseases, but the exact mechanism regulating MT dynamics in the axon remains enigmatic. Here we report on a novel regulation of MT dynamics by autophagy, a pathway previously linked to lysosomal degradation. We find that AuTophaGy(ATG)-related proteins required for LC3 lipid conjugation are dispensable for survival of excitatory neurons and instead regulate MT stability via controlling the abundance of MT-binding protein CLASP2. This function of ATGs is independent of their role in protein degradation and requires the active zone protein ELKS1. Our results highlight a novel role of autophagy in neurons and suggest that pharmacological activation of autophagy may not only promote the degradation of bulk cytoplasmic material, but also impair axonal integrity via altering the MT stability.

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Sensory Processing within the *C. elegans* Electrical Connectome

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A central question of systems neurobiology is to understand how a given neuronal circuit process sensory information to coordinates behavioral responses. The fully described chemical and electrical connectome of the nematode *C. elegans* is a well-established model for that purpose. Every one of the 118 neuron classes of the *C. elegans* hermaphrodite makes electrical synapses with 1 to 30 synaptic partners. How is the information processed by such a widespread electrical connectome? To address that question, we plan to observe how neuronal activity spread in the syntaxin null-mutant *unc-64(js115)*, lacking synaptic transmission. Several hub interneurons display electrical connections with numerous partners. The well-described RMG hub-and-spoke system will be explored first. Then we plan to focus on a circuit made of three interneurons (AVK, PVP and PVT) connected by GAP-junctions made of 8 innexins. Several of these neurons have not yet been explored with modern genetic tools because of the lack of specific promoter. We first defined specific promoter for each neuron to express genetically encoded calcium sensors, tetanus toxin, channelrhodopsin or the histamine-mediated chloride channel in order to visualize neuronal activity, block neurotransmission, activate or inhibit the neurons. Finally, based on any synchronized activity we would observe in our model, we will assess the contexts where the gap-junction network might be involved. This information should allow us to better understand how GAP junctions-connected neurons contribute to the processing of informations.

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PKD Controls Endocytic AMPAR Trafficking in the Synaptic Membrane

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The protein kinase D (PKD) family of serine/threonine kinases affects dendritic development and maintenance, intracellular transport as well as the modulation of transmembrane receptors. Here, we investigated a potential role of PKD in the regulation of AMPA receptor turnover in the synaptic membrane. We used mouse embryonic hippocampal neuronal cultures and blocked PKD activity by the selective inhibitor CRT0066101. Surface expression of the AMPAR subunit GluA1 was assessed through biotinylation and Western blot analysis upon agonist-induced internalization and chemically induced LTP. Short-term inhibition of PKD increased the amount of GluA1 on the cell surface indicating that PKD is required for proper endocytic turnover of GluA1. This finding was further corroborated by antibody feeding and quantitative microscopic evaluation within the PSD of dendritic spines. To test the dynamics of PKD-mediated AMPA receptor loss from the synapses, we expressed super-ecliptic pHluorine tagged GluA1 (SEP-GluA1) in the neurons and analyzed FRAP in the dendritic spines. Our data revealed that inhibition of PKD significantly increased the recovery half time of surface GluA1. Taken together our results indicate a role for PKD in promoting activity-dependent endocytosis of AMPA receptors. Currently, we are investigating the contribution of the PKD substrate Rabaptin-5 to this process.

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Poster - 43

Deciphering the Mechanisms Underlying the Myoclonic Seizures Caused by the Loss of Autophagy in the Brain

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Macroautophagy is a highly evolutionary conserved process to remove damaged organelles and toxic protein aggregates within autophagosomes for lysosomal degradation. In neurons, the process of autophagy is particularly important because of their highly polarized structure and long live span. A key step in the initiation of autophagy is the cleavage of microtubule-associated protein 1 light chain 3 (LC3) that allows the recruitment of it to the growing autophagophore by a complex composed of AuTophaGy (ATG) proteins 5, 16 and 12 (ATG5-ATG16L1-ATG12). Accumulation of autophagosomes hallmarks the pathology of neurodegenerative disorders, while neuronal-confined knockout of several ATG- related genes causes neurodegeneration. Here we describe the occurrence of late-onset myoclonic seizures in mice lacking either ATG5 or ATG16L1 specifically in neurons. Interestingly, already a hemizygous deletion of either of two proteins was sufficient to cause the appearance of myoclonic seizures. Behavioral assessment reveals that myoclonus is startle-induced, while histological analysis highlights the loss of parvalbumin (PV)-positive ($^{+}$) interneurons, but not the principal excitatory cells in the cortex of these mice. These alterations on the network levels are accompanied by the changes in protein aggregation, marked by the levels of polyubiquitin-binding protein Sequestosome1 (SQSTM1) in two classes of autophagy-deficient neurons. We find that although PV^{+} neurons undergo neurodegeneration in autophagy deficient mice, they fail to accumulate SQSTM1-positive aggregates. This phenotype was exclusively confined to inhibitory neurons, as SQSTM1-positive inclusions were abundant in excitatory autophagy-deficient cells. Taken together, our data indicate that autophagy prevents myoclonic seizures via maintaining the survival of GABAegic neurons in a protein-aggregation independent manner. Currently, we are investigating the precise molecular mechanism by which autophagy regulates the function of interneurons and prevents epileptogenesis.

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Poster - 44

Extremely Low Frequency Electromagnetic Fields Enhance Neural Differentiation of Stem Cells through NMDA Receptor Activation

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Exposure to extremely low frequency (ELF) electromagnetic fields (EMFs) is known to enhance neural differentiation of stem cells. Although this effect promises translation into clinical applications for treatment of neural pathologies, the lack of knowledge about underlying mechanisms prevents further studies. In this study, we report that ELF EMFs enhance neural differentiation by activating N- methyl D- aspartate (NMDA) receptors.

We induced differentiation of human embryonic neural progenitor cells in vitro under ELF EMF exposure and showed that the number of cells positive for neuronal markers do not change significantly. However, western blot results show increased expression of neuronal markers under exposure. This effect is eliminated when cells are treated with memantine, an NMDA receptor blocker. This observation is attributed to activation of EGFR cascade by NMDA receptor activation through a disintegrin and metalloproteinase 10 (ADAM10) enzyme upregulation.

Complete elimination of the effect by memantine treatment implicates NMDA receptors as the exclusive player. This result may be translated to in vivo and clinical applications for selective and non-invasive activation of NMDA receptors. This work was partially conducted within the framework of EMBO short-term fellowship (STF 7502).

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Poster - 45

Neuronal Mitophagy in *C. elegans* Models of Alzheimer's Disease

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Mitochondria are indispensable and highly dynamic, energy-generating organelles in all eukaryotic cells that also play essential roles in fundamental cellular processes. Neuronal cells depend, perhaps more than any other cell type, on proper mitochondrial function. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's disease. Interestingly, accumulation of damaged mitochondria has been observed in post-mortem brain of Alzheimer's disease patients. Although disease-associated tau and amyloid β are known to deregulate mitochondrial function, it remains elusive whether they also directly influence the efficiency of mitophagy. 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 their metabolic state. However, little is known about the role of mitophagy in the pathogenesis of Alzheimer's disease. To address this question, we developed an *in vivo* imaging system to monitor mitophagy in neurons. Neuronal mitophagy is impaired in *C. elegans* models of Alzheimer's disease. Urolithin A- and nicotinamide mononucleotide-induced mitophagy ameliorates several pathological features of Alzheimer's disease, including cognitive defects. Mitophagy stimulation restores memory impairment through PINK-1-, PDR-1/Parkin- or DCT-1/BNIP3-dependent pathways. Our findings suggest that impaired removal of damaged mitochondria is a pivotal event in Alzheimer's disease pathogenesis highlighting mitophagy as a potential therapeutic intervention.

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Poster - 46

Post-translational Modifications of α-tubulin in Alzheimer's Disease: Focus on Tyrosination/Detyrosination Cycle

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Alzheimer disease, as the most common neurodegenerative disease, is characterized by progressive accumulation of intracellular neurofibrillary tangles (NFTs) and extracellular senile plaques, composed of amyloid-β peptide. Neither senile plaques nor NFTs are involved in early events of Alzheimer development, when cognition impairment begins. Synaptic dysfunction could be related to changes in microtubule dynamics, known to participate in synaptic plasticity, maintaining dendritic spines and synapse morphology. Our team established causal links between microtubule dynamics and tubulin tyr/detyrosination state. Furthermore, we recently identified the carboxypeptidase complex (VASH1/SVBP and VASH2/SVBP) that removes the C-terminal tyrosine of long lived (stable) microtubules. After depolymerization, the tyrosine is re added by the ligase (TTL) to re-build dynamic tyr-microtubules. We wondered if perturbation on the tyr/detyrosination cycle of tubulin could be an early event preceding synaptic dysfunction and cognition impairments at the beginning of Alzheimer disease. We investigate the levels of TTL and modified tubulins in four regions of human brains known to be affected sequentially in Alzheimer disease at different stages of the pathology evolution. TTL level decreased is highly correlated with detyrosinated and Δ2 tubulin accumulation in all studied regions and in function of the disease progression. Behavioral tests performed on TTL +/- mice (mimicking TTL reduction as observed in Alzheimer patients) lead to cognitive impairment. Moreover, neurons from TTL +/- mice showed a decreased dendritic spine density (*in vitro* as *in vivo*). Ectopically controlled expression of TTL in TTL +/- cultured neurons protects dendritic spine loss induced by Aβ oligomers. Additionally, the suppression of each member of the carboxypeptidase complex in neurons (VASH1, VASH2 or SVBP), induce the same protective effect against Aβ toxicity.

All together our results highlight the importance of the tyrosination/detyrosination cycle of tubulin in Alzheimer disease and highlight TTL, VASH1, VASH2 and SVBP as a new potential targets for drug design.

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

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Organisms receive and process external information to adapt their behavior to an ever-changing environment. The integration of sensory stimuli to appropriately modulate behavioral responses to environmental signals, either stressful or not, 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 reward processing and in the control of motor output. 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. By implementing molecular genetic manipulation technics and behavioral assays we identified three degenerin ion channel proteins to participate in sensory integration through modulation of the dopaminergic pathway. Utilizing advanced imaging technics, we found that degenerins DEL-2, DEL-3 and DEL-4 are expressed in mechanosensory, chemosensory and motor neurons and do not adopt a synaptic localization pattern. These ion channel proteins modulate basal and/or enhanced slowing responses and respond to gustatory stimuli. They act through dopamine receptors and affect the signaling at the neuromuscular junction. 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.

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Poster - 48

Mechanical Regulation of Chemical Signalling during Axon Growth and Guidance in the Developing *Xenopus* Brain

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During nervous system development, growing neurons respond to chemical as well as mechanical signals in their environment. We found that axons of the optic tract grow along stiffness gradients in the developing *Xenopus* brain. Mechanosensitive ion channels (MSCs) are key players in transducing these mechanical cues into intracellular signals. Pharmacological blocking of MSCs and knockdown of the MSC, Piezo1, caused severe pathfinding errors in the developing *Xenopus* optic tract. In addition to directly impacting axon growth, downregulation of Piezo1 also dramatically altered the expression of semaphorin3A (Sema3A), a chemical guidance cue known to be critical in optic tract axon pathfinding. Our results thus indicate that the expression of chemical guidance cues may be modulated by tissue mechanics during development. As mechanical changes occur throughout development and during ageing and injury, this study could allow us to better understand how chemical signalling may be influenced by tissue mechanics during these processes.

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Postsynaptic DCC/UNC-40 Recruits a Scaffolding Complex for GABA Receptors in *C. elegans*

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Positioning type A GABA receptors ($GABA_A$Rs) in front of GABA release sites sets the strength of inhibitory synapses and consequently the excitability of neuronal networks. The *C. elegans* inhibitory neuromuscular junction (NMJ) provides a genetically tractable model to analyze the clustering of $GABA_A$Rs because muscle cells receive inhibitory innervation from GABAergic motor neurons. We previously identified an original synaptic organizer, Ce-Punctin/MADD-4, that governs the molecular composition of postsynaptic domains at the NMJs. A short isoform of Punctin is secreted by the inhibitory motoneurons and localizes in the extracellular matrix at NMJs. Punctin has a dual role: on one hand, it binds and clusters neuroligin (NLG-1) in the postsynaptic membrane in register with GABAergic boutons. On the other hand, it locally activates the netrin receptor UNC-40/DCC (Deleted in Colorectal Cancer), which in turn promotes the interaction of $GABA_A$Rs with neuroligin. It was recently shown that LIN-2/CASK, a membrane-associated guanylate kinase well documented for its role in presynaptic assembly, and FRM-3/FARP, a FERM domain protein, stabilize $GABA_A$Rs clusters.

Using imaging and biochemistry techniques, we show that UNC-40 directly binds and recruits FRM-3/FARP at postsynaptic sites. The FERM and FERM adjacent (FA) domains of FRM-3/FARP are necessary and sufficient for binding to UNC-40/DCC and for $GABA_A$R clustering. FRM-3/FARP, controls the postsynaptic localization of LIN-2/CASK, which, in turn, binds to the $GABA_A$R and to NLG-1/neuroligin, and stabilizes $GABA_A$R clusters at GABAergic synapses. Altogether, our work defines a novel pathway for $GABA_A$R clustering controlled by UNC-40/DCC. Since the mammalian orthologs of the *C. elegans* proteins involved in this pathway are present at postsynaptic sites in the central nervous system, this pathway might be evolutionarily conserved.

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Studying Neuron Differentiation and Regeneration in hiPSC-Derived Neurons

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Human induced pluripotent stem cells (hiPSCs) offer a very useful tool to study genes involved in differentiation and regeneration of human neurons. Although there is currently no cure for neurodegenerative diseases and stroke, regenerative therapies hold new promise for these diseases. We optimized a protocol to generate cortical neurons from human induced pluripotent stem cells. Our aim is to find regenerative therapies for ischemic cortical stroke, which is the most common type of stroke in patients.

We successfully generated neurons from hiPSCs combining two protocols published in Nature: Shi et al 2014 and Qi et al 2017. This differentiation protocol included three phases: 1) neuronal induction, 2) differentiation and 3) maturation (up to 3 weeks). We optimized the protocol for coating, seeding density, maturation media and inhibitor concentrations. We characterized the cells in different time points by staining with immature and mature neuron markers as well as cortical neuron markers. This protocol yielded very high percentage of neurons, as a vast majority of the cells were DCX+/TUJ1+ on day 32 of differentiation, and DCX+/MAP2+ on day 7 after maturation. The neurons sent long processes and formed connections with other neurons. We also identified a time range, when immature neurons did not proliferate anymore but could still be passaged and transplanted to murine brain. With this protocol, we can study genes that are suspected to have an effect on neuron differentiation and regeneration. Immature neurons can also be transplanted to murine brain after cortical stroke to investigate, whether they would form new connections with the existing neurons or facilitate recovery from stroke.

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Novel Roles of Microtubule Networks in Neuronal Ageing

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Ageing of the nervous system is accompanied by progressive axonal atrophy and synaptic decay contributing to a decrease in cognition and mobility. However, what causes axonal and synaptic atrophy remains largely unknown. We study the cell biology of neuronal ageing by focussing on the pathological mechanisms that lead to the decay of axonal microtubules (MTs) based on the rationale that (1) they form the structural backbones of axons determining and maintaining their morphology, (2) they are crucial for the transport of organelles and molecular cargoes, and (3) alterations in MT networks and their regulatory proteins have strong links to neurodegenerative diseases.

To carry out this research, we developed a quantitative cellular model based on defined axons in the adult Drosophila brain where ageing phenotypes are prominent after 4 weeks already. This model reproduces hallmarks of physiological ageing known from primates: (A) axonal atrophy including axonal swellings (often displaying MT disorganisation), decreased MT density, (B) protein aggregation, (C) synaptic alterations and (D) decreased neuronal activity. Using this model complementary to primary neuronal cultures, we investigate how typical ageing pathways affect MT regulation and, in turn, how aberrant MTs then contribute to axonal decay. Here we will present our most recent results and discuss how our findings open up new avenues to unravel mechanisms of neuronal ageing in normal and disease conditions.

Supported by the BBSRC and the Wellcome Trust

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AGRP Neurons-specific CPT1A Deletion Increases Energy Expenditure by Enhancing Brown Adipose Tissue Activity

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Food intake and whole-body energy balance are regulated by the brain through a sophisticated neuronal network located mostly in the hypothalamus. In particular, the hypothalamic arcuate nucleus (ARC) is a fundamental sensor for the hormones and nutrients that inform about the energy state of the organism. The ARC contains two populations of neurons with opposite functions: anorexigenic proopiomelanocortin (POMC)-expressing neurons and orexigenic Agouti-related protein (AgRP)-expressing neurons. Activation of AgRP neurons leads to an increase in food intake and a decrease in energy expenditure. It has been suggested that lipid metabolism in the ARC plays an important role in the central control of whole-body energy balance. Yet it is unclear whether lipid metabolism regulates the activity of AgRP neurons specifically. To answer this question, we studied mutant mice lacking carnitine palmitoyltransferase 1A (CPT1A) specifically in AgRP neurons. CPT1A regulates the rate-limiting step in the mitochondrial oxidation of fatty acids (FAs) and therefore plays a central role in the metabolism of lipids. The results of our research demonstrate that the deletion of Cpt1a in AgRP neurons: 1) reduces body weight, 2) increases energy expenditure and the activity of brown adipose tissue (BAT), 3) decreases white and brown adiposity and increases the expression of genes involved in lipolysis in BAT and 4) at central level, reduces AgRP protusions. Altogether, our results suggest that CPT1A and fatty acid oxidation in AgRP neurons impact peripheral energy balance and highlight this pathway as a possible target for therapeutic strategies to decrease body weight.

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Poster - 53

MECP2 Mutations Affect Ciliogenesis: a Novel Perspective for Rett Syndrome and Related Disorders

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Rett syndrome (RTT; OMIM 312750) is a progressive X linked neurodevelopmental disorder that, because of its incidence (1:10000 females), represents the most common cause of severe intellectual disability in girls worldwide. RTT is caused by mutations in methyl-CpG-binding protein 2 (MeCP2), a protein expressed mainly in the nucleus where it modulates the expression of several genes. Although mostly functioning as an epigenetic transcriptional repressor, MeCP2 is a multifunctional protein revealing several activities. We have recently demonstrated that MeCP2 associates with centrosome, therefore affecting its function. Primary cilia are "sensory antennae" that originates from centrosomes and protrude from several cells, including neurons and astrocytes. Cilia receive and integrate extracellular signals, affecting neuronal migration, dendritic arborization, neural circuits integration, learning and memory. Defective primary cilia are associated with a growing list of "ciliopathies" characterized by several symptoms in common with RTT syndrome.

Considering all above, we investigated and demonstrated that MeCP2 deficiency affects ciliogenesis in cultured cells, including Rett patients' fibroblasts, and in *Mecp2* null brains. We also demonstrated that the cilia-related Sonic Hedgehog pathway, essential for proper brain development and functioning, is altered in MeCP2 deficient cells and cerebellum. We demonstrate that microtubule instability caused by excessive activity of HDAC6 participates in the MeCp2 defective cells to the observed cilia defects. Indeed, the pharmacological inhibition of HDAC6 by tubacin, restores ciliogenesis and cilia functioning in MeCP2 deficient cells. Together, our data propose the alteration of primary cilia as a pathogenic mechanism participating to the clinical features of MECP2 related disorders.

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PHACTR, a New Regulator of Neuronal Function?

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The **PH**osphatase and **ACT**in **R**egulator proteins (PHACTR) control Protein Phosphatase 1 (PP1) activity and F-actin remodeling. Among the 4 members of the PHACTR family, Scapinin is highly expressed in human brain although its functions in neurons are largely unknown. F26H9.2, the unique *C. elegans* homolog for the vertebrate PHACTR family is almost exclusively expressed in the nervous system of the adult. We explored the potential roles of F26H9.2 in development and behavior. *F26H9.2(tm2453)* mutation has a weak effect on axon growth. To challenge the actin network, we used the temperature sensitive mutant for the actin gene *act-2(or621)* causing an excess in acto-myosin contractility, embryonic death and abnormal branching of neurites. These phenotypes are worsened by the *F26H9.2(tm2453)* mutation. In addition, several behavioral and pharmacological responses are weakly affected in *F26H9.2(tm2453),* suggesting that *F26H9.2* affects neuronal function.

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Poster - 55

Activity-based Checkpoints for Interneuron Maturation and Connectivity in the Olfactory System

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Olfactory circuits function at birth, yet are continuously remodeled by the integration of postnatally-born interneurons into the olfactory bulb in a manner that preserves perception throughout adult life. The mechanisms that regulate the development of interneurons and ensure circuit stability in this dynamic context remain poorly understood. Since granule cell (GC) interneurons sculpt the excitatory output of mitral and tufted (MT) neurons to the olfactory cortex, we predicted that MT neurons instruct interneuron integration in the adult brain. By blocking synaptic transmission from MT neurons using genetically-encoded tetanus toxin, we show that MT neuronal activity is critical to maintain olfactory bulb integrity and interneuron survival. Single cell tracing analyses of GCs showed these interneurons exhibit decreased dendritic branching as well as spine density, resembling immature neurons. Furthermore, inhibiting interneuron cell death uncovered a second activity-dependent checkpoint regulating GC dendrite morphology. In contrast, MT neurons and downstream cortical circuits remain stable in the face of the degenerating olfactory bulb architecture. Finally, transcriptome analyses of mature GCs and those stalled at developmental milestones identify gene regulatory networks that correlate with activity-dependent maturation and functional integration. These studies identify a circuit-specific role for non-sensory activity in regulating integration of neurons into the adult brain, as predicted by previous computational models.

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Poster - 56

Huntingtin Phosphorylation at Serine 421 regulates Axonal Transport of Synaptic Vesicle Precursors and Mouse Behavior

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Huntingtin (HTT), the protein that when mutated causes Huntington's disease, is a large protein interacting with more than 300 partners. These interactions are responsible for HTT physiological roles and are modulated by post-translational modifications, such as phosphorylation. One of HTT function is to facilitate the axonal transport of the main brain neurotrophic factor, Brain Derived Neurotrophic Factor (BDNF). HTT-mediated BDNF transport is crucial for the maintenance of corticostriatal network, altered in HD, resulting in a reduced trophic support and striatal degeneration. Interestingly, HTT phosphorylation at serine 421 (S421) promotes BDNF vesicle anterograde transport. Here we investigated whether HTT capacity to transport vesicles is restricted to BDNF vesicles.

We found that HTT also regulate the axonal transport of synaptic vesicle precursors (SVPs). Using microfluidics reconstituting corticostriatal network on-a-chip, we showed that axonal transport of SVPs depends on HTT S421 phosphorylation: while neurons carrying S421A mutation, that mimics the absence of phosphorylation, promotes retrograde transport of SVPs, S421D that mimics constitutive phosphorylation promotes their anterograde transport. We found by electron microscopy that synaptic vesicle (SVs) quantity changes according to S421 phosphorylation status. We also found by electrophysiology that S421 phosphorylation regulates synaptic transmission and facilitation in the striatum. Interestingly, SVPs contain neurotransmitters that are crucial for neuronal plasticity and memory. Interestingly, we found that procedural memory is altered in mutant S421D mice.

We are now investigating the consequences of specific modulation of axonal transport of SVPs in the corticostriatal network in vivo using viral-mediated expression or silencing of specific molecular motors and of phosphorylated HTT on this behavior.

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Cellular Extensions in Quiescent Neural Stem Cells are required for their Reactivation

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The balance between proliferation and quiescence of stem cells is crucial in maintaining tissue homeostasis. Quiescent neural stem cells (qNSCs) in *Drosophila* extend a cellular extension and retract this extension before their reactivation (exit from the quiescence). We previously showed that the cellular extensions in qNSCs are enriched for microtubules marked by □-tubulin. However, the structure and function of these cellular extensions remain unknown. Here, we demonstrate that microtubules are polarized in these protrusions of qNSCs. Microtubule plus ends are in the protrusions, while the microtubule minus ends are anchored at the opposite side of the qNSCs. We also found that a couple of microtubule-binding proteins are present in these cellular extensions. Loss of these microtubule-binding proteins resulted in the formation of shorter protrusions, leading to a delay in reactivation. Taken together, we show that microtubules are polarized in the protrusions of the qNSCs and the formation of these protrusions are important for NSC reactivation.

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CONFERENCE INFO

EMBO Workshop **Cell biology of the neuron: Polarity, plasticity and regeneration**
7–10 May 2019 | Heraklion, Greece

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]

Note: Transportation is not provided by the organizers.

TAXI

A 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, with subject: "EMBO Workshop-2019", indicating your name, your arrival flight number and your arrival date and time at 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 center 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

❖ Upon arrival at the hotel you must check-in your room. You don't need any voucher. Just give 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)

- ***Together with your room key, you will receive an internet access card for 3 days (one device)***

❖ 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 Workshop. Additional charges (e.g. telephone, extra internet access, mini bar, etc.) are not included in your accommodation or the registration fees. Please remember to take care of these directly at your hotel, upon checking out.

- Upon registration (starting at 11.00 on Tuesday, 7 May) you will be given an EMBO bag with the following:
  - Workshop Program with Poster List
  - Posting materials for poster presenters
  - Name badge
  - Badge strip
  - Notepad / Pen
  - Leaflets

  * The Abstract Book will be distributed electronically

Registration will take place at the Workshop 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) 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 (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 during registration. Remember to consult the detailed poster presentation guidelines (attached, see page 8). **POSTERS SHOULD BE PORTRAIT ORIENTED**.
Posters should be up for display by **Tuesday, 7 May at 15:30.** All posters will remain mounted for the whole duration of the meeting.

There are 2 Poster sessions: **Tuesday, 7 May 2019 @ 17:00 – RED SESSION** and **Thursday, 9 May 2019 @ 14:25 – BLUE SESSION**. 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?

Upon check-in, you will receive an internet access for 3 days.

For extra days 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 the weather and bring you swimsuit!

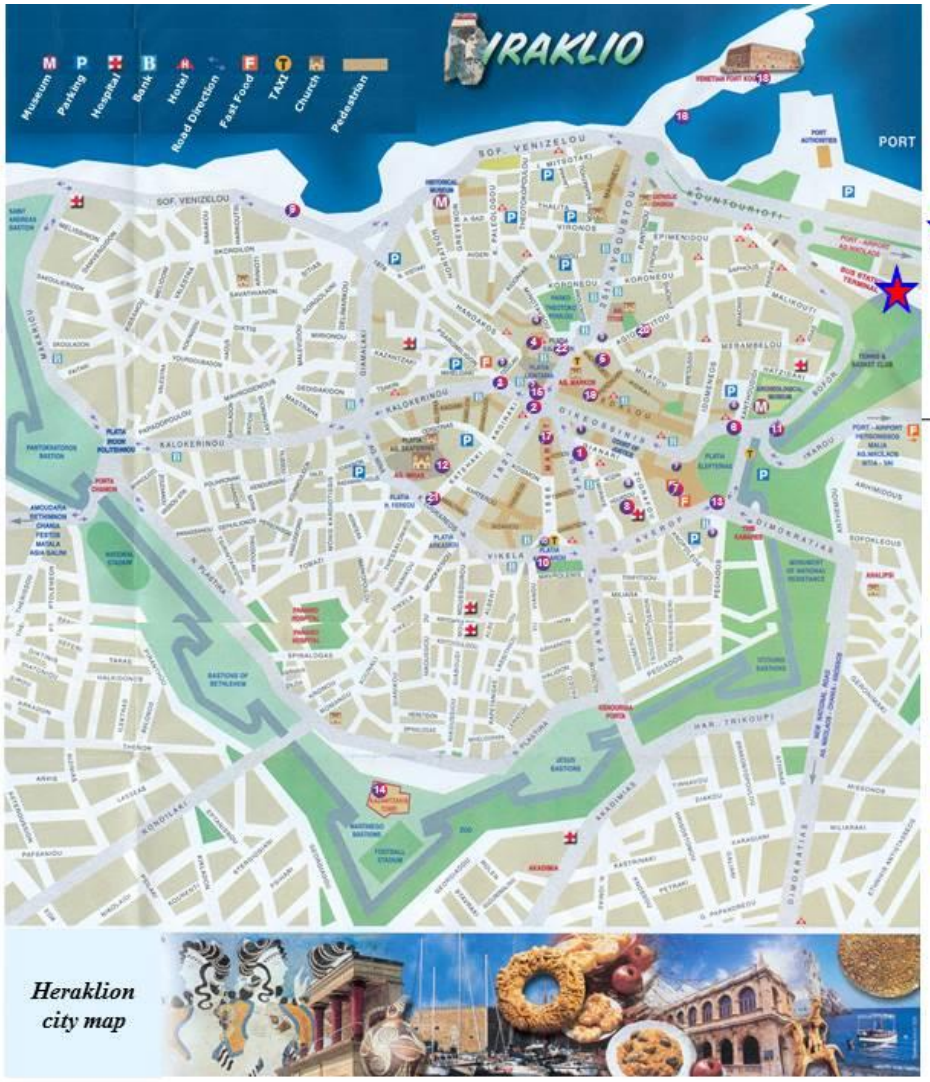
We are all looking forward to a very interesting conference!

The organizers,

Claudia Bagni, 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
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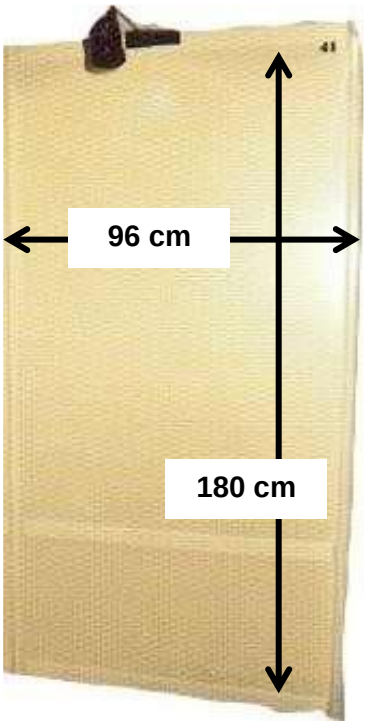
FODELE BEACH
HOTEL PLAN

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

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