EMBO Conference on
Neural Development-
Function and Dysregulation

Date  December 04-08, 2015
Location Academia Sinica, Taipei, Taiwan

Program and Poster
# Scientific Program

**Seminar:** B1 Auditorium, Institute of Molecular Biology  
**Poster & Reception:** 1F Lobby, Interdisciplinary Research for Sciences and Technology  

## DAY 1 (Friday, Dec 4th)

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<th>Time</th>
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<tr>
<td>12:00-14:00</td>
<td>Registration</td>
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<tr>
<td>14:00-14:15</td>
<td>Welcome Remarks</td>
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<tr>
<td><strong>Session I: Synapse Formation and Plasticity</strong></td>
<td>Chair: Sue Lin-Chao, Chan-Yen Ou</td>
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</tbody>
</table>
| 14:15-14:40      | Antione Triller **INSERM, France**  
Synaptic stability and plasticity: a molecular movie |
| 14:45-15:10      | Yi-Ping Hsueh **Academia Sinica, Taiwan**  
Neuronal morphogenesis and neurodevelopmental disorders |
| 15:15-15:30      | Yi-Shuiam Huang **Academia Sinica, Taiwan**  
CPEB3-controlled translation in synaptic plasticity, memory and beyond |
| 15:35-15:50      | Pritha Majumder **Academia Sinica, Taiwan**  
TDP-43 : A regulator of Rac1 mRNA translation and hippocampal spinogenesis |
| 15:55-16:30      | **Coffee Break**                                                                     |
| 16:30-16:55      | Michela Matteoli **CNR Institute of Neuroscience and Humanitas Research Hospital, Italy**  
Immune proteins in brain development and synaptic plasticity |
| 17:00-17:15      | Jinhyun Kim **Korea Institute of Science and Technology, Korea**  
mGRASP for mapping mammalian synaptic circuit at multiple scales |
| 17:20-17:35      | Jan Pielage **FMI, Switzerland**  
Molecular mechanisms controlling synapse formation and stability |
| **Key Note I**   | Chair: Lung-Sen Kao                                                                  |
| 17:40-18:35      | Tullio Pozzan **University of Padua, Institute of Neuroscience, CNR, Italy**  
Presenilin 2 modulates ER-mitochondria coupling by tuning the inhibitory effect of mitofusin 2 on organelle tethering |
| 18:45-21:00      | **Opening Reception**                                                                |
### DAY 2 (Saturday, Dec. 5th)

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<tr>
<td><strong>Session II: Axon and Dendrite Formation</strong></td>
<td><strong>Chair: Ing Ming Chiu, Pei-Lin Cheng</strong></td>
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<tr>
<td>09:00-09:25</td>
<td><strong>Zhen-Ge Luo</strong> Chinese Academy of Sciences, China</td>
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<tr>
<td></td>
<td>Membrane trafficking mechanisms underlying axon development</td>
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<tr>
<td>09:30-09:45</td>
<td><strong>Chun-Liang Pan</strong> National Taiwan University, Taiwan</td>
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<td>Spatial control of neurite branching by Wnt-Frizzled/PCP and endosomal signaling</td>
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<td>09:50-10:05</td>
<td><strong>Yu-Chih Lin</strong> Hussman Institute for Autism, USA</td>
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<td>Cytoskeletal machinery controls actin dynamics in maintaining neuronal stability</td>
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<td><strong>10:10-10:40 Coffee Break</strong></td>
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<tr>
<td>10:40-11:05</td>
<td><strong>Kozo Kaibuchi</strong> Nogoya University Graduate School of Medicine, Japan</td>
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<tr>
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<td>Extracellular and intracellular signaling for neuronal polarity</td>
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<tr>
<td>11:10-11:35</td>
<td><strong>Cheng-Ting Chien</strong> Academia Sinica, Taiwan</td>
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<tr>
<td></td>
<td>Dendrite development and degeneration</td>
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<tr>
<td>11:40-11:55</td>
<td><strong>Pei-Lin Cheng</strong> Academia Sinica, Taiwan</td>
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<tr>
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<td>Controlled proteasome distribution in axon development</td>
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<td><strong>12:00-13:00 Lunch</strong></td>
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<tr>
<td>13:00-14:30</td>
<td><strong>Poster Session I (Odd Number)</strong></td>
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<tr>
<td><strong>Session III: Neural-Glial Interaction</strong></td>
<td><strong>Chair: Yi-Hsuan Lee, Jin-Wu Tsai</strong></td>
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<tr>
<td>14:30-14:55</td>
<td><strong>Angela Giangrande</strong> IGBMC, France</td>
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<tr>
<td></td>
<td>Of glia and blood</td>
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<tr>
<td>15:00-15:25</td>
<td><strong>Henry Sun</strong> Academia Sinica, Taiwan</td>
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<tr>
<td></td>
<td>Glia-neuron interactions in the fly visual system</td>
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<tr>
<td>15:30-15:45</td>
<td><strong>Neta Marmor-Kollet</strong> Weizmann Institute of Science, Israel</td>
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<tr>
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<td>Glial derived TGF-β instructs midline stopping via heterotypic axon-axon interactions</td>
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<td><strong>15:50-16:20 Coffee Break</strong></td>
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<tr>
<td>16:20-16:45</td>
<td><strong>Shumin Duan</strong> Zhejiang University School of Medicine, China</td>
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<td>Purine-mediated signaling in glial cells</td>
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<tr>
<td>16:50-17:15</td>
<td><strong>Yijuang Chern</strong> Academia Sinica, Taiwan</td>
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<td>Aberrant astrocytes mediate abnormal vascular reactivity in Huntington’s disease</td>
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<td>Time</td>
<td>Speaker/Title/Activity</td>
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</tbody>
</table>
| **09:00-09:25** | **Alain Prochiantz**  
College de France, France  
Assembling and regulating visual networks through homeoprotein signalling |
| **09:30-09:45** | **Shen-Ju Chou**  
Academia Sinica, Taiwan  
A novel genetic pathway provides layer 4 neurons competency to form cortical barrel |
| **09:50-10:05** | **Hung-Chih Kuo**  
Academia Sinica, Taiwan  
Programing and reprograming neural fate |
| **10:10-10:30** | **Coffee Break** |
| **10:30-10:55** | **Andrea Brand**  
Gurdon Institute, University of Cambridge, UK  
Nutritional control of neural stem cells |
| **11:00-11:25** | **Fu-Chin Liu**  
National Yang Ming University, Taiwan  
Neural development of the basal ganglia in mouse forebrain |
| **11:30-11:55** | **Lawrence W. Stanton**  
 Genome Institute of Singapore, Singapore  
In vitro modeling the life and death of human neurons |
**DAY 4 (Monday, Dec. 7th)**

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<thead>
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<th>Speaker/Title/Activity</th>
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<tr>
<td><strong>Key Note II</strong></td>
<td>Chair: Bon-chu Chung</td>
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<tr>
<td>09:00-09:50</td>
<td>Li-Huei Tsai</td>
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<td>Activity-induced DNA breaks in neuronal physiology and disease</td>
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<tr>
<td><strong>Session IV-2: Fate Specification and Stem Cells</strong></td>
<td><strong>Chair: Tang K. Tang、Shen-Ju Chou</strong></td>
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<tr>
<td>10:00-10:15</td>
<td>Jun-An Chen</td>
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<td>Investigating the functional role of non coding RNA during neurogenesis</td>
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<tr>
<td>10:20-10:50</td>
<td>Coffee Break</td>
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<tr>
<td>10:50-11:15</td>
<td>Toru Takumi</td>
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<tr>
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<td>Modeling neurodevelopmental disorders</td>
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<tr>
<td>11:20-11:35</td>
<td>Haiwei Pi</td>
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<tr>
<td></td>
<td>Actin and Actin-related protein in neurogenesis of Drosophila external sensory organ</td>
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<tr>
<td>11:40-11:55</td>
<td>Thomas Di Meglio</td>
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<td>Secreted Frizzled Related Proteins-mediated regulation of choroid plexus morphogenesis</td>
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<tr>
<td>12:00-13:00</td>
<td>Lunch</td>
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<tr>
<td><strong>13:00-14:30</strong></td>
<td><strong>Poster Session II (Even Number)</strong></td>
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<tr>
<td><strong>Session V: Circuit Formation and Function</strong></td>
<td><strong>Chair: Chi-Kuang Yao, Tzu-Yang Lin</strong></td>
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<tr>
<td>14:30-14:55</td>
<td>Daisuke Yamamoto</td>
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<tr>
<td></td>
<td>Neural basis for socially induced behavioral changes in Drosophila</td>
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<tr>
<td>15:00-15:15</td>
<td>Cheng-Chang Lien</td>
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<tr>
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<td>Inhibitory control of memory circuits</td>
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<tr>
<td>15:20-15:35</td>
<td>Thomas Hummel</td>
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<tr>
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<td>Sensory map formation in the Drosophila brain</td>
</tr>
<tr>
<td>15:40-16:05</td>
<td>Ann-Shyn Chiang</td>
</tr>
<tr>
<td></td>
<td>One-time experience is prevented from formation of long-term memory by learning-induced new proteins</td>
</tr>
<tr>
<td>16:10-16:20</td>
<td>Closing Remark</td>
</tr>
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</table>
P1  Mosaic manipulation of the cerebellum reveals novel linkage between Atoh1 and primary cilium in cerebellar development and medulloblastoma formation
Chia-Hsiang Chang1, Chih-Hsin Feng1, Alexander J. Lu1, Jin-Wu Tsai1, 2
1 Institute of Brain Science, National Yang-Ming University
2 Brain Research Center, National Yang-Ming University, Taipei, Taiwan

P2  Type VI adenyllyl cyclase negatively regulates hippocampal synaptic plasticity
Ching-Pang Chang1, 2, 4, Cheng-Ta Lee5, Wan-Hsien Hou5, Meng-Syuan Lin1, 2, 4, Hsing-Lin Lai2, Chen-Li Chien2, Chen Chang2, Pei-Lin Cheng3, Cheng-Chang Lien1, 5, Yijuang Chern1, 2, 5
1 Taiwan International Graduate Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan
2 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
3 Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan
4 Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan
5 Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan

P3  Multilineage differentiation of cortical neural precursor cell induced by electric-field
Hui-Fang Chang1, Tang K Tang2, Ji-Yen Cheng1, 3, 4, 5, 6
1 Research Center for Applied Sciences, Academia Sinica, Taipei 11529, Taiwan
2 Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan
3 Institute of Biophotonics, National Yang-Ming University, Taipei 11221, Taiwan
4 Biophotonics and Molecular Imaging Research Center (BMIRC), National Yang-Ming University, Taipei 11221, Taiwan
5 Department of Mechanical and Mechantronic Engineering, National Taiwan Ocean University, Keelung 202, Taiwan
6 Ph.D. Program in Microbial Genomics, National Chung Hsing University, Taichung 402, Taiwan

P4  Finger like structure mediated glia-axonal contact in early Drosophila visual system
Yen-Ching Chang1, 2, Y. Henry Sun1, 2
1 Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China
2 Department of Life sciences and Institute of Genome sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China

P5  CPEB3 deficiency elevates TRPV1 RNA translation in dorsal root ganglia neurons to potentiate thermosensation and inflammation-induced thermal hyperalgesia
Yu Wei Chang, et al.
Institute of Biomedical Sciences, Academia Sinica
Glia-derived ubiquitin E3 ligase dSmurf is required in controlling Drosophila locomotive behavior

Changyan Chen, Margaret S. Ho, et al.

Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China

Laboratory of Arrhythmias of the Ministry of Education of China, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China

Department of Anatomy and Neurobiology, Tongji University School of Medicine, Shanghai, China

Department of Biomedical Sciences, College of Medicine, Chang Gung University, Tao-Yuan, Taiwan

Institute of Intervention Vessel, Shanghai 10th People’s Hospital, Shanghai Key Laboratory of Signaling and Diseases Research, School of Life Science and Technology, Tongji University, 1239 Siping Road, Shanghai, 200092, China

The role of innate immunity in neuronal morphogenesis

Chiuong-Ya Chen, Hsin-Yu Liu, Yi-Ping Hsueh

Institute of Molecular Biology, Academia Sinica

NPAS proteins Trachealess and Dysfusion in synaptogenesis at Drosophila neuromuscular junctions

Pei-Yi Chen, Cheng-Ting Chien

Institute of Molecular Biology, Academia Sinica

Institute of Neuroscience, National Yang-Ming University

Ran-dependent TPX2 activation in distal neurites promotes neurite elongation and branching

Wen-Shin Chen, Yi-Ju Chen, Yung-An Huang, Eric Hwang

Department of Biological Science and Technology, NCTU, Hsinchu, Taiwan

Institute of Bioinformatics and Systems Biology, NCTU, Hsinchu, Taiwan

Center for Bioinformatics Research, NCTU, Hsinchu, Taiwan

Institute of Molecular Medicine and Bioengineering, NCTU, Hsinchu, Taiwan

The development of distinct local interneurons in Drosophila olfactory circuit

Yuh-Tarng Chen, Shih-Han Lin, Ying-Jun Chen, Ting-Han Wu, Hsin-Ju Lin, Chi-Jen Yang, Nan-Fu Liu, Tzi-Yang Lin, Ya-Hui Chou

Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 115, Taiwan

Studying the role of Huntingtin interacting protein Amphiphysin in the mechanisms of polyQ pathogenesis

Wei-Kuang Fang, Y. Henry Sun

Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan, Republic of China

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China
P12  Direct Differentiation of Human Embryonic Stem Cell to Dopaminergic Progenitors: Enrichment and Transplantation to the Hemi-Parkinson Rats
Ali Fathi1,4, Banafsheh Dolatyar2,4, Mehdi Sharifitabar1, Mehdi Mirzaei3, Mohammad Javan2,5, Ghasem Hosseini Salekdeh1,6
1 Department of Molecular Systems Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2 Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran
3 Australian School of Advanced Medicine, Macquarie University, Sydney, Australia
4 Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5 Department of Physiology, Faculty of Medical Sciences, Tarbiat Modarres University, Tehran, Iran.
6 Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran, Karaj, Iran

P13  The DCC/Frazzled chemoattractant receptor triggering collective glia migration is tightly regulated by the Gcm fate determinant
Tripti Gupta1, Arun Kumar2, Angela Giangrande1
1 Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR 7104 –Inserm U 964- Illkirch Graffenstaden, France
2 Department of Entomology, University of California, Riverside California 92521, USA

P14  LIMK2-1, a primate-specific isoform of LIMK2 associated with intellectual disability: molecular and functional characterization
BENEDETTI H1, CUBEROS H1,2, VALLEE B1, TASTET J1,2, DOUDEAU M1, GODIN F1, VOURC’H P2,3, ANDRES CR2,3
1 CNRS UPR4301, CBM, Orléans, France
2 UMR INSERM U930, Université François Rabelais, Tours, France
3 CHRU de Tours, Service de Biochimie et de Biologie Moléculaire, Tours, France

P15  Analysis of Arp6 in Drosophila sensory organ development
Yun-Ling Hsiao1,2,3, Cheng-Ting Chien3, Haiwei Pi1,2
1 Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University
2 Department of Biomedical Sciences, College of Medicine, Chang Gung University
3 Institute of Molecular Biology, Academia Sinica

P16  Dysregulations of GABAergic neurotransmission in a mouse model of Huntington’s disease.
Yi-ting Hsi1,3, Ya-Gin Chang2,3, Hui-Mei Chen3, Yijuang Chern3
1 Department of neurology, China Medical University Hospital, Taichung, Taiwan
2 Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan
3 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

P17  Postsynaptic syndecan-2 induces transsynaptic signaling via fibroblast growth factor-22 for bidirectional synaptic differentiation
Hsiao-Tang Hu1, Hisashi Umemori2, Yi-Ping Hsueh1
1 Institute of Molecular Biology, Academia sinica
2 Department of Neurology, F.M. Kirby Neurobiology Center, Boston Children’s Hospital, Harvard Medical School, Boston, USA.
**P18** Voltage-gated K+ channel Kv3.4 controls axon growth by regulating calcium activity at the growth cone  
Chia-Yi Huang, Chau-Fu Cheng, Cheng-Chang Lien, Ting-Yun Yen, Chieh-Ju Chen, Meei-Ling Tsaur  
*Institute of Neuroscience, National Yang-Ming University, Taipei, Taiwan*

**P19** Activation of AGES-RAGE axis-induced mitochondrial dysfunction: role of cyclin-dependent kinase 5 and glycogen synthase kinase 3beta.  
Hsiao-Fei Huang1, Ching-Yu Weng2,3, Lan-Ya Kang4, Nai-Kuei Huang5,6,7, Ying-Chen Yang2,8, Chuen-Lin Huang3,4  
1 Nutrition Counseling Group, An Kang District, Cardinal-Tien Hospital.  
2 EMA Program in College of Bioresources, National I-Lan University.  
3 Medical Research Center, Department of Education and Research, Cardinal-Tien Hospital.  
4 Department of Physiology and Biophysics, Graduate Institute of Physiology, National Defense Medical Center.  
5 National Research Institute of Chinese Medicine, Ministry of Health and Welfare.  
6 Institute of Biophotonics, National Yang-Ming University.  
7 Ph.D. Program for Neural Regenerative Medicine, College of Medical Science and Technology, Taipei Medical University.  
8 Department of Biotechnology and Animal Science, National I-Lan University.

**P20** Role of RBFOX3/NeuN in cognitive impairment and epilepsy  
Hsien-Sung Huang, De-Fong Huang, et al.  
*Graduate Institute of Brain and Mind Sciences, College of Medicine, National Taiwan University, Taipei, 10051, Taiwan*

**P21** Using mouse model to study the etiology of autism spectrum disorder  
Tzyy-Nan Huang, Hsiu-Chun Chuang, Yi-Ping Hsueh  
*Institute of Molecular Biology, Academia Sinica*

**P22** Astrocytic GAP43 functions to attenuate neuroinflammation and promote neuronal survival and plasticity  
Chia-Chi Hung1, Chun-Hua Lin4, Hsuan Chang2, Chen-Yu Wang2,3, Shang-Hsuan Lin2, Pei-Chien Hsu2,3, Teng-Nan Lin5, Feng-Shiun Shie7, Chih-Ming Chou1,6, Yi-Hsuan Lee2,3  
1 Graduate Institute of Medical Sciences and Department of Physiology, College of Medicine, Taipei Medical University, Taipei, Taiwan  
2 Department and Institute of Physiology, College of Medicine, National Yang-Ming University, Taipei, Taiwan  
3 Brain Research Center, National Yang-Ming University, Taipei, Taiwan  
4 Department of Nursing, Kang-Ning Junior College of Medical Care and Management, Taipei, Taiwan  
5 Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan  
6 Department of Biochemistry, College of Medicine, Taipei Medical University, Taipei, Taiwan.  
7 Center for Neuropsychiatric Research, National Health Research Institutes, Miaoli County, Taiwan.

**P23** Characterization of mitochondrial dysfunction in drug-induced peripheral neuropathy  
Kui-Ming Hung1, Pei-Chun Chen4, Marcus J. Calkins2  
1 Department of Physiology  
2 Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan
P24  TLR8 uses cytokine-independent pathway to regulate dendrite arborization in mature neurons
Yun-Fen Hung1,2, Hsin-Yu Liu2, Chiao-Ming Huang2, Chiung-Ya Chen2, Yi-Ping Hsueh2
1 Institute of Molecular biology, Academia Sinica, Taiwan
2 Department of Life Sciences and Institute of Genome Sciences, School of Life Science, National Yang-Ming University, Taipei, Taiwan

P25  Functional analysis of CD147 in neuronal differentiation of human pluripotent stem cells
Akarachai Kongwimarn, Patompon Wongtrakoongate
Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

P26  Foxp2 regulates dendritic spine formation and synaptogenesis in medium-sized spiny neurons of the mouse striatum during development
Hsiao-Ying Kuo1, Yi-Chuan Chen1, Ulrich Bornschein2, Shih-Yun Chen1, Kuan-Ming Lu1, Hao-Yu Yang1, Gui-May Chen1, Wolfgang Enard4, Wulf Hevers2, Svante Pääbo2, Fu-Chin Liu1, et al.
1 Institute of Neuroscience, National Yang-Ming University, Taipei 11221, Taiwan
2 Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig 04103, Germany
3 Anthropology and Human Genetics, Department Biology II, Ludwig-Maximilians University, Munich 82152, Germany
4 Department of Neurology, National Hospital Organization, Tottori Medical Center, Tottori 689-0203, Japan
5 McGovern Institute for Brain Research and Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

P27  Deficiency of CPEB2-Confined ChAT Expression in the Dorsal Motor Nucleus of Vagus Causes Hyperactivated Parasympathetic Signaling-Associated Bronchoconstriction
Yen-Ting Lai, Yi-Shuian Huang
Institute of Biomedical Sciences, Academia Sinica

P28  Role for Cell cycle-related kinase in ciliogenesis and Hedgehog signaling during neural tube development
Hanky Lee1, Jonathan T. Eggenschwiler2, Hyuk Wan Ko1
1 College of Pharmacy, Dongguk University-Seoul, Goyang, 410-820, Korea
2 Department of Genetics, University of Georgia, Athens, GA 30606

P29  Characterization of Vilse/Arhgap39 in neural differentiation
Jin-Yu Lee, Mau-Sun Chang
Institute of Biochemical Sciences, National Taiwan University

P30  Muscleblind-like proteins are splicing factors essential for brain development
Kuang-Yung Lee1,2, Maurice Swanson1
1 Department of Molecular Genetics and Microbiology, Center for NeuroGenetics and the Genetics Institute, University of Florida, College of Medicine, Gainesville, Florida, USA
2 Department of Neurology, Chang Gung Memorial Hospital, Keelung, Taiwan
P31 MicroRNA Filters Hox Temporal Transcription Noise to Confer the Robustness of Boundary Formation in the Spinal Cord
Chung-Jung Li¹, 2, 5, Tian Hong³, 5, Ya-Ping Yen¹, 4, Ya-Lin Lu¹, Mien Chang¹, Qing Nie³, 6, Jun-An Chen¹, 6
¹ Academia Sinica
² Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan.
³ Center for Complex Biological Systems, University of California, 92697-2280, Department of Mathematics, University of California, 92697-3875, Irvine, California, USA.
⁴ Institute of Biotechnology, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, 106, Taiwan.
⁵ Equal contributions
⁶ Correspondence

P32 Sex Differential Effects of Neonatal Pretreatments of Fluoroacetate or MSO to Disrupt Glutamate-Glutamine Cycle on Adult Reproductive Behaviors in Rats
Chih-Yu Yeh¹, Shu-Ling Liang¹, 2
¹ Graduate Institute of Biomedical Sciences, Chang Gung University, Tao-Yuan, 33302, Taiwan
² Department of Physiology and Pharmacology, College of Medicine, Chang Gung University, Tao-Yuan, 33302, Taiwan

P33 Cdk1 and Cdk2 phosphorylate Sox2 to control neural stem cell fate
Shuhui Lim, et al.
¹ Institute of Molecular and Cell Biology (IMCB), A*STAR (Agency for Science, Technology and Research), 61 Biopolis Drive, Proteos #3-09, Singapore 138673, Singapore
Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

P34 Analysis of the molecular pathogenesis of SCA17 using transgenic mice
Chia Wei Lin, et al.
Department of Life Science, National Taiwan Normal University

P35 Reconstruction of spinal cord following transplantation of fetal spinal cord tissue in mid-cervical spinal injured rats
Chia-Ching Lin, Sih-Rong Lai, Yu-Han Shao, Kun-Ze Lee
Department of Biological Sciences, National Sun Yat-sen University

P36 Wiring diversity of bilateral local interneurons in Drosophila olfactory system
Hsin-Ju Lin, Shih-Han Lin, Kuo-Ting Tsai, Ya-Hui Chou*
Institute of Cellular and Organismic Biology, Academia Sinica, Taipei 115, Taiwan

P37 Rgs2 regulates neural crest formation and lineage determination via Wnt signaling
Sheng-Jia Lin, Huang-Yu Shih, Yi-Chuan Cheng
Department of Neurosurgery, Chang Gung Memorial Hospital at Linkou Medical Center, Taoyuan, Taiwan
P38  Deltex1 is inhibited by the Notch–Hairy/E(Spl) signaling pathway and induces neuronal and glial differentiation
Sheng-Jia Lin¹, Yun-Jin Jiang², Yi-Chuan Cheng¹
¹ Department of Neurosurgery, Chang Gung Memorial Hospital at Linkou Medical Center, Taoyuan, Taiwan
² Division of Molecular and Genomic Medicine, National Health Research Institutes, Miaoli County, Taiwan

P39  Lipophagy protects neurons from degeneration caused by neuronal accumulation of dihydroceramide
Chung-Chih Liu¹, Wei-Hung Jung¹, Yu-Chin Chang¹, Ching-Hua Kuo², Han-Chen Ho³, Chih-Chiang Chan¹
¹ Graduate Institute of Physiology, College of Medicine, National Taiwan University, Taipei, Taiwan.
² School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.
³ Department of Anatomy, School of Medicine, Tzu-Chi University, Hualien, Taiwan.

P40  Toll-like receptor7 negatively regulates dendrite outgrowth
Hsin-Yu Lü¹, Yun-Fen Hong², Chiang-Ya Chen¹, Tzuy-Nan Huang¹, Yi-Ping Hsueh¹
¹ Institute of Molecular Biology, Academia Sinica, Taipei 115, Taiwan, Republic of China.
² Faculty of Life Sciences, Institute of Genome Sciences, National Yang-Ming University, Taipei 112, Taiwan, Republic of China.

P41  CPEB2 regulates hippocampus-related long-term synaptic plasticity and memory
Wen-Hsin Lu¹, 2, 3, Yi-Shuian Huang¹, 2, 3
¹ Taiwan International Graduate Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan
² Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan
³ Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan

P42  Peripheral Nerve Implants Enriched with Developmental Signaling Cues for Peripheral Nervous Tissue Engineering
Katarzyna Nawrotek¹, Michał Tylman¹, Karolina Rudnicka², et al.
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Jhan-Jie Peng1,3, Shu-Hui Lin1, Tzu-Li Yen1, Yu-Chiu an Chang1, Yu-Tzu Liu1, Chi-Kuang Yao1,2,3  
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Hung-Yu Shih, Yi-Chuan Cheng  
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The role of Cortactin binding protein 2 in neuronal morphogenesis
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ER morphology regulation in dendritic spinogenesis and memory
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Functional characterization of steroid receptor RNA activator in human pluripotent stem cells
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Retinal Basal Glia Development in Drosophila Eye Disc
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CPEB4 regulates olfactory experience-dependent granule cell survival in the early postnatal olfactory bulbs
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P63 A long noncoding RNA cluster demarcates Hox boundary during motor neuron development
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P64  **Overconnectivity in stem cell-derived neurons of an autistic child.**
Kirill Zaslavsky¹,², Wenbo Zhang¹, Eric Deneault¹, Melody Zhao¹,², Peter Joel Ross¹, Asli Dedeagac¹, Alina Piekna¹, Peter Pascen¹, Stephen Scherer¹,², Michael Salter¹,³, James Ellis¹,²
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P65  **Spreading Depolarization Promotes Astrocytic Calcium Oscillations and Enhances Gliotransmission to Hippocampal Neurons**
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Abstracts
Key Note
Presenilin 2 modulates ER-mitochondria coupling by tuning the inhibitory effect of mitofusin 2 on organelle tethering

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Communication between organelles appears to play key roles in cell biology. In particular, physical and functional coupling of the endoplasmic reticulum (ER) and mitochondria is crucial for regulating various pathophysiological features. Here we discuss the role of two proteins, mitofusin 2 (Mfn2) and presenilin 2 (PS2), that have been shown to affect ER mitochondria interactions and the transfer of Ca2+ between the two organelles. As to Mfn2, we demonstrate that this protein plays an inhibitory role, unlike previously suggested, in the interaction between the two organelles and its down regulation causes an increase in the Ca2+ transfer between ER and mitochondria. As to PS2, whose mutations are responsible for some forms of familial Alzheimer’s disease (FAD), we show that it promotes ER-mitochondria coupling only in the presence of mitofusin 2 (Mfn2). The two proteins interact in vitro and in living cells while their homologues Mfn1 and PS1 are dispensable for this interplay. We also show that PS2 with FAD-linked mutations are more effective than the wild-type form in modulating ER-mitochondria tethering because they are more efficient at binding to Mfn2 in mitochondrial-associated membranes. We propose a revised model for ER-mitochondria interaction to account for these findings and discuss possible implications for FAD pathogenesis.
Activity-induced DNA breaks in neuronal physiology and disease

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Neurons are endowed with the remarkable ability to sense and process changes in an organism’s environment. Experiences that drive neuronal activity and lead to the adaptive behaviors require the initiation of new gene transcription programs. Yet the molecular events that govern neuronal activity-dependent gene transcription are still poorly understood. Here, I describe our discovery of an unusual mechanism of transcriptional regulation in which activity-dependent stimulation of neurons triggers the formation of DNA double strand breaks (DSBs) in the promoters of a subset of activity-regulated genes, including \textit{Fos}, \textit{Npas4}, and \textit{Egr1}. Activity-induced DSB formation is mediated by the topoisomerase, Topo IIβ, and Topo IIβ-mediated DSBs facilitate the rapid induction of specific activity-regulated genes by resolving topological barriers that suppress their expression in the absence of neuronal activity. Our observations elaborate how topological dynamics of the neuronal genome exerts a regulatory effect on crucial neuronal functions, including synaptic plasticity and learning behaviors. On the other hand, changes in the ability to either form or repair activity-induced DSBs could dysregulate activity-dependent pathways, diminish cognitive performance, and contribute to the development of neurological diseases, and I discuss these scenarios.
Oral Presentation
Synaptic stability and plasticity: a molecular movie

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The efficiency and accuracy of neurotransmission strongly depends on two apparently antagonist properties of synaptic membrane: the stability of its organization and its ability to adapt to plasticity events. In addition, the structural stability of synapses has to be reconciled with the notion that cell membranes are fluid. Membrane molecules are compelled to move within the membrane surface due to thermal Brownian agitation, which favors the homogeneous distribution of the molecules. As a result, neurons spend energy to stop or reduce these movements, and maintain molecules in certain locations via mechanisms that decrease this fluidity. We investigate the regulation of synaptic receptors dynamics by the different (structural and functional) elements that make the synapse. We have approached these conceptual paradoxes by developing new technological and analytical tools that allow the monitoring of the behavior of synaptic components at the molecular level and change of the scale of analysis. We demonstrated rapid exchanges between synaptic and extra-synaptic receptors and we showed that transient stabilization of receptors at synapses occurs by interaction with partners, such as scaffold proteins. Novel super-resolution imaging methods (PALM, STORM) gave us a precise insight on the organization of these postsynaptic structures. Thus combination of single particle tracking and super-resolution methods, open access to molecular counting and energy involved in receptor-scaffold interactions as well as on and off rate of molecular interactions. Thus beyond super-resolution methods is chemistry “in cellulo” accounting for the regulation of receptor number and consecutively that of synaptic strength. Ultimately, the dynamic regulations of receptor-scaffold and scaffold–scaffold interactions appear as a central tenet for the maintenance and plasticity-related changes of receptor numbers at synapses. These processes are likely to be deregulated in pathological situations such as in neurodegenerative diseases.
Neuronal morphogenesis and neurodevelopmental disorders

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Our research interests are to explore the molecular mechanisms of neural development. Defects in neural development alter brain circuit formations and may result in lethality, mental retardation or psychiatric disorders, including autism spectrum disorders. Thus, to investigate the physiological relevance, we also examine the potential implications of our findings in neurodevelopmental disorders. We have been studying the functions of several disease causative genes, including Cask, Tbr1, Nf1, Vcp and Ctnnb2 in neuronal morphogenesis. In this report, the newest molecular regulations of these genes in neurodevelopment and the potential etiology of related diseases will be presented and discussed.
CPEB3-controlled translation in synaptic plasticity, memory and beyond

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Maintenance of long-term plasticity requires activity-dependent synthesis of plasticity-related proteins (PRPs) to sustain morphological and functional changes of synapses that are crucial for establishing and consolidating long-term memory. Cytoplasmic polyadenylation element-binding protein (CPEB)3 is an sequence-specific RNA-binding protein that regulates translation to confine the strength of glutamatergic synapses in neurons. CPEB3 knockout (KO) mice show abnormal hippocampus-related plasticity and spatial memory that is partly caused by elevated translation of NMDA receptor subunits and PSD95. To further identify other aberrant behaviors caused by the loss of CPEB3, we analyzed fear memory in the KO animals. We found that CPEB3 deficiency imbalances NMDAR-activated CaMKIIa signaling, which consequently fails to depress synaptic strength under certain stimulatory conditions and accounts for post-traumatic stress disorder (PTSD)-like fear responses in the KO mice. Thus, CPEB3-controlled translation is critical to fine-tune plasticity underlying multiple forms of memories.
TDP-43: A Regulator of Rac1 mRNA Translation and Hippocampal Spinogenesis

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Spinogenesis of the dendrites is an integral part of the neural plasticity program of the mammalian brain. Appropriate regulation and fine tuning of this process is essential for the normal functions of the neurons. Our previous study has shown that TDP-43, a nucleic acid-binding protein implicated in multi-cellular functions and in the pathogenesis of a range of neurodegenerative diseases, is an upstream regulator of spinogenesis. Specifically TDP-43 modulate new protrusion formation and spine maturation in primary hippocampal cell. Further investigation has revealed that TDP-43 inhibit Rac1 translation and thus regulate pull of activated Rac1 in dendrites that inhibit GTP-Rac1-AMPAR pathway and formation of new active synapses. RNA-IP and RNA pull-down assays indicate that TDP-43 recruits Rac1 mRNA to a translation inhibitory complex and also regulates Rac1 mRNA transport to dendrites and local translation. TDP-43 could regulate neuronal activation through its role as a translational regulator of multiple mRNAs in the neurons.
Immune proteins in brain development and synaptic plasticity

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CNR Institute of Neuroscience and Humanitas Research Hospital Rozzano

Since the neurodevelopmental hypothesis of schizophrenia, the pathophysiological origins of many neuropsychiatric diseases are increasingly recognized to associate with environmental influences. In the last years evidence accumulated indicating that inflammation, influencing the physiology and pathology in the immature and mature brain, can modify the risk and/or severity of a variety of brain diseases. Inflammation may resolve without any harmful effects on the brain, even contributing to reparative processes, or can be shifted to a chronic state, thus contributing to injury, enhancing CNS vulnerability and/or adversely affecting brain development. Although a number of molecules involved in inflammation have been found to regulate specific neuronal processes, the possibility that inflammatory cascades, either alone or in combination with a susceptible genetic background, may impact synapse formation and plasticity, thus leading to a disease condition, has not been addressed in a systematic way. The talk will report a series of evidence indicating that immune challenges, prenatally or postnatally delivered, impact synaptic protein networks, thus resulting in neuronal modifications typical of psychiatric diseases. Results from these studies will allow the identification of new targets suitable for innovative therapeutic intervention.
mGRASP for mapping mammalian synaptic circuit at multiple scales

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Mapping mammalian synaptic connectivity has long been an important goal of neuroscientists since it is considered crucial for explaining human perception and behavior. Our new genetically controlled method to resolve synapses at the level of LM, termed mammalian GFP reconstitution across synaptic partners (mGRASP), is synapse-specific labeling with two complementary GFP components. mGRASP is based on two non-fluorescent split-GFP fragments (called spGFP1-10 and spGFP11) tethered to synaptic membranes in each of two neuronal populations. When two neurons, each expressing one of the fragments, are tightly opposed across a synaptic cleft, fluorescent GFP is reconstituted. mGRASP can relatively quickly reveal the precise locations and numbers of synapses along postsynaptic dendrites, sites responsible for determining many important characteristics of signal processing. Thus, mGRASP technology is suitable for mapping large-scale connectivity patterns at multiple scales: micro-scale for synapse-by-synapse or neuron-by-neuron analysis; and meso-scale for revealing local circuits. We performed a comprehensive fine-scale circuit mapping of hippocampal regions using the mGRASP. This mapping revealed spatially non-uniform and clustered synaptic connectivity patterns. Furthermore, synaptic clustering was enhanced between groups of neurons that shared a similar developmental/migration time window, suggesting a mechanism for establishing the spatial structure of synaptic connectivity. Such connectivity patterns are thought to effectively engage active dendritic processing and storage mechanisms, thereby potentially enhancing neuronal feature selectivity. Based on these prime connectivity characteristics, our study recently focuses on understanding synaptic connectivity profiles associated with neurological disorders using mGRASP.
Molecular mechanisms controlling synapse formation and stability

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Neuronal circuits are formed through synaptic connections between defined populations of neurons. The regulated assembly and disassembly of synaptic connections ensures precise connectivity during development and during plasticity of the mature circuit. In contrast, the inappropriate loss of synaptic connections leads to a disruption of neuronal circuits and to progressive neurodegenerative disorders. Therefore, identification of the molecular and cellular mechanisms controlling synaptic formation and stability is essential for our understanding of neuronal circuit function and plasticity in development and disease.

We use the Drosophila neuromuscular junction (NMJ) as a model system to identify the molecular pathways controlling synapse formation and stability at the resolution of individual synapses. We are particularly interested in the regulatory mechanisms that link synaptic cell adhesion molecules to the presynaptic actin and microtubule cytoskeleton. Here, we will present new mechanistic insights into the processes controlling synapse formation and maintenance.
Membrane trafficking mechanisms underlying axon development

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In eukaryotic cells, the Rab family members of small GTPases is involved in various membrane trafficking events, including formation of transport vesicles, trafficking along cytoskeletal tracks, and docking and fusion with the acceptor membrane. Axon development requires membrane addition from the intracellular supply, which has been considered to be mediated by plasmalemmal precursor vesicles (PPVs). Recently, we have shown that Rab10 is associated with PPVs and Myosin V controls biogenesis of post-Golgi Rab10 carriers during axon development. Furthermore, C-Jun-amino-terminal kinase-interacting protein 1 (JIP-1) mediates the association of Rab10 with the kinesin light chain (KLC) and the Rab10/JIP-1/KLC complex mediates the anterograde transport of PPV during axonal growth. Finally, we have identified myristoylated alanine-rich C-kinase substrate (MARCKS) as a component of docking and fusion machinery for Rab10-positive PPVs and found that the MARCKS-Rab10 system is critical for membrane insertion of axonal surface proteins and axonal elongation. Thus, Rab10 and its effectors play important roles in consecutive steps during PPV-mediated polarized membrane insertion essential for axon development and elongation.
Spatial control of neurite branching by Wnt-Frizzled/PCP and endosomal signaling

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Spatial control of neurite branching is crucial for the establishment of precise connectivity of the nervous system. However, how extracellular signals specify axon branching sites remains incompletely understood. Here we examine the role of secreted Wnt glycoproteins in specifying neurite branching pattern in the bilaterally symmetric PLM touch neurons in C. elegans. The PLM has an anterior neurite that generates a single synaptic branch at a fairly invariable location. The outgrowth of the PLM branch was preceded by the emergence of a highly localized F-actin patch. In mutants that lost activity for cwn-1/Wnt, egl-20/Wnt, mig-1/Frizzled and vang-1/Strabismus/Vangl2, PLM branches developed at more proximal locations. In these mutants, we observed stronger and more dispersed F-actin signals before the onset of branch outgrowth, suggesting that Wnt-Frizzled/PCP (planar cell polarity) signaling restricted F-actin activity to the distal neurite. We propose a model in which endosomal signaling of activated Frizzleds, facilitated by VANG-1, patterns F-actin distribution in the PLM neurite and instructs PLM branching sites.
Cytoskeletal machinery controls actin dynamics in maintaining neuronal stability

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The human brain is composed of billions of neurons that have characteristic shapes, with elaborate axons and dendrites. Dendritic spines are actin-rich protrusions that extend from dendrites and serve as sites of synaptic contacts with other neurons. Alteration of dendritic spine density and shape are hallmarks of most neurological disorders. These structural defects in neurons result in cognitive and behavioral deficits that are devastating to affected individuals and their families. Several mechanisms including trans-synaptic adhesion receptor coupling, synaptic activity, and trophic signals from the presynaptic compartment are shown to play important roles in regulating the formation and the stability of dendritic spines. Although their mechanisms of action are diverse, these signaling pathways often converge within the dendritic spine on cytoskeletal regulators. Chromosomal microdeletions encompassing p190RhoGAP or its upstream regulator, the Abl2/Arg tyrosine kinase, have been observed in cases of mental retardation associated with developmental defects. Using genetic manipulation and pharmacological intervention, we show that Abl2/Arg selectively blocks Rho signaling to stabilize dendrites, and fine-tunes dendritic spine stability by regulating activity-dependent dynamics of the actin polymerization and stability regulator, cortactin. Furthermore, the kinase activity of Abl2/Arg and cortactin phosphorylation regulates the stability of dendritic spines. The interaction between Abl2/Arg and cortactin also determines the subcellular localization of these two proteins and actin dynamics. These data suggest that Abl2/Arg acts on distinct cytoskeleton regulatory pathways to synergistically control the structural stability of a neuron. Perturbation of these mechanisms may underlie the pathology of some neurological disorders, such as mental retardation.
Extracellular and intracellular signaling for neuronal polarity

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In the developing neocortex, neocortical pyramidal neurons, which are primarily generated by radial glial cells in the ventricular zone, migrate through the subventricular zone and enter the intermediate zone with multipolar morphology. These immature neurons subsequently transform from a multipolar morphology to a bipolar morphology, with a thick leading process and a thin trailing process, and migrate along radial glial fibers to the developing cortical plate. The leading process becomes the dendrite, and the trailing process becomes the axon. Our recent study revealed that a transient axonal glycoprotein-1 (TAG-1)-dependent interaction between multipolar cells and preexisting axons enables these multipolar cells to specify the preexisting axon-contacting neurite as the axon and to develop into bipolar cell (Namba et al., 2014). These findings provide molecular and cellular mechanisms for the neuronal polarization of approximately 60% of multipolar cells that extend trailing processes (nascent axons) prior to leading processes during their multipolar- to-bipolar transition. However, the remaining 40% of multipolar cells somehow specify their axon-dendrite polarity as well, likely through a complementary mechanism. Here, we report that the N-cadherin-mediated radial glia-neuron interaction determines the contacting neurite as the leading process for radial glia-guided neuronal migration and directs axon formation to the opposite side acting through the Rho family GTPases.
**Dendrite development and degeneration**

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Dendrite arborization patterns are the major feature of neurons that are distinctive to each other, and lay the groundwork for neuronal connection and circuit function. We used Drosophila dendrite arborization (da) neurons to study developmental regulation of dendrites. The da neurons that display distinct dendrite arborization patterns are classified into four classes (I-IV) according to their complexity. The da dendrites have become a model system to study development as well as degeneration.

Previously we had studied the human Parkinson’s disease gene LRRK2 in da neurons and suggested the underlying mechanism for LRRK2 mutants in causing dendrite degeneration. To follow up, we study the Drosophila homolog of Lrrk in da dendrite development. We have generated Lrrk mutants and observed dendrite arborization defects. Using confocal microscopy in dendrite live-imaging, we have found enhanced Golgi outpost movements in Lrrk mutants, which is caused by a regulation in dynein-dependent transport. We will report the cellular effect induced by LRRK2 Parkinson's mutants in da dendrites.
Controlled proteasome distribution in axon development

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The mega-size, poly-subunits 26S proteasome controls the majority of cellular protein turnover, thereby its activity and abundance are required in many aspects of neuronal development including axon/dendrite polarization and synaptic plasticity. Recently, we found a stage-dependent axonal transport of proteasome that contributes to axon development. Whether and how proteasomes transverse across the diffusion barriers at axon initial segment (AIS) remains unknown. Here we report that proteasome transport across the AIS is attributed to an activity-dependent association between proteasome adaptor Ecm29 and AIS scaffold protein Ankyrin G (AnkG). We found that the majority (~80%) of anterogradely moving proteasomes were constrained in front of or within AIS region of hippocampal neurons. This proteasome retention in AIS was released by specific siRNA-mediated down regulation of AnkG expression as well as under a hypoxic condition when AIS structure was disrupted. In contrast, no effect was observed in neither actin filament nor microtubule disruption. Co-immunoprecipitation assays and the following interaction domain mapping showed that C-terminal domain of AnkG was required for the association with Ecm29 and proteasome complexes. These findings suggest that AIS acts as a regulatory dock for axonal proteasome transport.
Of glia and blood

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Multipotent precursors are plastic cells that generate different, stable fates at the correct number, place and time, to allow tissue and organ formation. The molecular pathways driving the progression from multipotent precursors toward stable and specific identities remain poorly understood. We have found that the Drosophila glial fate determinant acts as a ‘time-bomb’ by specifically inducing its own extinction once the correct transcriptional program has been stably activated.

Our ability to respond and adapt to the internal and external environments relies on our nervous and immune systems, through the presence of glia and blood cells. In vertebrates, these two cell types have evolved from different layers and require different molecular cascades. In the simple Drosophila model, however, the same transcription factor controls the two cell populations, opening novel perspectives to understand the bases of nervous system and immune system evolution.
Glia-neuron interactions in the fly visual system

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The nervous system is composed of neuron and glia. Neurons transmit information through electrical and chemical signals. Glia plays supportive but essential roles for neural functions. We use the Drosophila visual system to study the interplay between glia and neuron in the development and maintenance of the nervous system.

In larval eye disc, a group of glia (called retinal basal glia, RBG) migrate from brain through optic stalk into the eye disc. The RBGs can be classified, based on molecular and morphological criteria, into three major subtypes: surface glia, wrapping glia and carpet glia. We established a method for long term culture and live imaging of imaginal disc. We used live imaging and clonal analysis to follow the behavior of the RBGs. Our results provided new findings on the migration, division, lineage relationship and differentiation of the RBGs.

In the adult visual system, we asked whether the survival of cells are actively maintained. We found that the adult photoreceptors send an EGFR ligand Spitz to the lamina glia to maintain their survival. In the absence of this survival signal, lamina glia have autophagosome-to-lysosome fusion defect, resulting in glia degeneration. Our finding provides the first clear in vivo evidence for the role of adult neurons in maintaining glia survival. Our finding is also the first to link EGFR signaling to the trafficking from late endosome and autophagosome to lysosome. This is a novel role of EGFR signaling in a late step in the endosome-lysosomal pathway, and also suggests a negative feedback mechanism to attenuate signaling.
Glial derived TGF-β instructs midline stopping via heterotypic axon-axon interactions

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A fundamental question that underlies the proper wiring and function of the nervous system is how axons stop growing during development. However, our mechanistic understanding of axon stopping is currently poor. The stereotypic development of the Drosophila mushroom body (MB) provides a unique system in which three types of anatomically distinct neurons (γ, α'/β', and α/β) develop and interact to form a complex neuronal structure. All three neuronal types innervate the ipsi-lateral side and do not cross the midline. Here we find that Plum, an immunoglobulin (Ig) superfamily protein that we have previously shown to function as a TGFβ accessory receptor, is required within MB neurons for the midline stopping of α/β neurons. Overexpression of Plum within MB neurons is sufficient to induce retraction of α/β axons. To our surprise, rescue experiments revealed, however, that Plum does not likely function in α/β neurons themselves; Using neuronal type specific Gal4 drivers, we found that Plum is required in α'/β' neurons to induce the subsequent stopping of α/β axons. Finally, we have identified glial derived Myoglianin (Myo) as the major TGFβ ligand that instructs midline stopping of MB neurons. We identified the Transient Interhemispheric Fibrous Ring (TIFR) as the source of Myo and therefore as a midline glia structure providing TGFβ patterning signals. Taken together, our study strongly suggest that TGFβ signals originating from the midline bind to Plum on α'/β' neurons that subsequently instruct the stopping of α/β neurons by axon-axon interactions.
Purine-mediated signaling in glial cells

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Glial cells can release signal molecules that mediate intercellular communication. In particular, ATP release from astrocytes is required for Ca2+ wave propagation among astrocytes and for feedback modulation of synaptic functions. We found that lysosomes in astrocytes contain abundant ATP and their partial exocytosis resulted in a low-level ATP release, whereas full exocytosis led to the release of a larger amount of ATP, together with lysosomal enzymes. Repetitive neuronal activity induces release of ATP/adenosine from astrocytes, which in turn causes both short-term and long-term heterosynaptic plasticity. Microglia, resident macrophages in the central nervous system, are responsible for the maintenance of brain homeostasis. Nucleotides have been also found to induce microglial chemotaxis and ingestions, leading to their scavenging of the abnormal materials. We found that nucleotides trigger microglial macropinocytosis by activation of P2Y receptors. Further evidence indicates that the purine-mediated microglial macropinocytosis plays an important role in the antigen processing and soluble beta amyloid engulfment.
Aberrant astrocytes mediate abnormal vascular reactivity in Huntington’s disease

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Huntington’s disease (HD) is an inherited neurodegenerative disease. The causative mutation is a CAG trinucleotide expansion in exon 1 of the Huntingtin (HTT) gene, which leads to mutant HTT (mHTT) protein accumulation, mHTT inclusion formation, and overall neuronal atrophy. We and several other laboratories reported earlier that brain vessel density in mice (R6/2, YAC128) and patients with HD is higher than in the controls. In the present study, we demonstrate that vascular reactivity (VR) to carbogen and carbogen-evoked cerebral blood flow were lower in HD mice than in WT mice. Immunofluorescence revealed that astrocytes of mice and patients with HD and human HD iPSC-derived astrocytes all contained more VEGF-A, which triggers endothelial cell proliferation. The inflammation-prone astrocytes in HD brains appear to mediate the low brain vessel pericyte coverage via an IKK-dependent pathway. Consistent with this hypothesis, inhibition of IκB kinase (IKK) normalized the enhanced vessel density and inferior pericyte coverage in brains from R6/2 mice. Collectively, these findings suggest that impaired cerebral autoregulation and vasodilatory capacity in the brains from mice and humans with HD may contribute to the HD pathogenesis.
Assembling and regulating visual networks through homeoprotein signalling

Alain Prochiantz

College de France

Most homeoprotein transcription factors contain two conserved regions allowing intercellular transfer, leading to the hypothesis that homeoproteins are not only transcription factors but also signalling proteins active during development and throughout adulthood.

In the visual system, this non-cell autonomous activity has been demonstrated for homeoproteins Pax6, Engrailed and Otx2 at three different developmental times, namely, and respectively, the formation of the eye anlagen, the building of the retino-tectal map and the opening - and closure - of a critical period of plasticity in the binocular visual cortex.

Beyond development, homeoprotein transfer has important adult physiological functions. Still concentrating on the visual pathways, we shall illustrate how decreasing the levels of non-cell autonomous Otx2 in the visual cortex allows one to transiently reopen plasticity and to restore normal binocular vision in adult amblyopic mice.

The more general possibility to use homeoproteins as therapeutic tools will be discussed.
A novel genetic pathway provides layer 4 neurons competency to form cortical barrel

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Mice use their whiskers to obtain spatial information and discriminate object shape and texture. This tactile sensory pathway is topographically organized from the whiskers, through the brain stem, the dorsal thalamus, then to the primary somatosensory cortex (S1). In the S1, upon receiving the thalamocortical axonal (TCA) input from the dorsal thalamus, the layer 4 (L4) neurons align to enlose the TCA input and form a ring structure, called the cortical barrel. The formation of this barrel structure was shown to require the neuronal activity from the periphery during the first postnatal week. However, not much is known about how L4 neurons perceive the TCA input to form the barrel. Here we demonstrated that Lhx2, a LIM homeodomain transcription factor, is required for these L4 neurons to form cortical barrels. The deletion of Lhx2 leads to aberrant dendritic arborization of L4 neurons and defects in barrel formation. We found Lhx2 is required for the expression of Btbd3, a BTB/POZ domain containing protein required for the L4 neuron dendritic development. We further confirmed that Lhx2 directly regulates Btbd3 promoter activity and the forced expression of Btbd3 could rescue the dendritic morphology in Lhx2 mutant cells. In conclusion, we have identified a novel function of Lhx2 in regulating barrel formation by inducing Btbd3 expression and providing L4 neurons competency to respond to TCA inputs to form cortical barrels.
Programming and reprogramming neural fates

Hung-Chih Kuo

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ES cells are clearly extremely important as they provide not only opportunity to understand the mechanisms underlying cellular differentiation during early development but also hope for the treatment of a wide range of human conditions that can be attributed to the loss or malfunction of specific cell types. The recent derivation of induced pluripotent stem cell (iPSCs) generation has offered a mean to derive patient- or disease-specific iPSCs without embryo disruption. While the potential application of iPSC is clearly recognized, the challenges in generating defined populations of specific somatic cell type from hESCs/hiPSCs has significantly compromised their applications. In my talk, I will outline our system in cell purification and present some data giving insights on how these systems can help to identify new factors important for early neural lineage specification in human. In addition to iPSCs, the recent demonstration of directly conversion of somatic cells into specific cell types by defined transcription factors, provide another source of neuronal cells for in vitro disease modeling and drug testing. I will also discuss our efforts in scratching novel combinations of transcription factors for directly convert human fibroblasts into neural progenitors (iNP).
Nutritional control of neural stem cells
Andrea H. Brand

The systemic regulation of stem cells ensures they meet the needs of the organism during growth and in response to injury. A key control point is the decision between quiescence and proliferation. Drosophila neural stem cells (neuroblasts or NSCs) enter quiescence in late embryogenesis and are reactivated post-embryonically in response to a nutrition-dependent signal from the fat body. The fat body performs many of the storage and endocrine functions of the vertebrate liver and adipose tissue and acts as a sensor, coupling nutritional state to organismal growth. We showed that the nutritional stimulus transduced by the fat body induces the expression of insulin/IGF-like peptides (dILPs) in the blood brain barrier glia, which overlie the quiescent stem cells. We found that insulin signalling is essential for NSCs to exit quiescence. Insulin signalling can also promote proliferation in vertebrate neural stem cells, suggesting that the mechanisms controlling stem cell reactivation may be conserved. We are investigating the systemic and local signals that regulate neural stem cell quiescence and reactivation.
Neural development of the basal ganglia in mouse forebrain

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The basal ganglia of the ventral forebrain is important for motor control, learning, reward, motivation and cognition. The striatum is the major input structure of the basal ganglia, as it receives extensive afferent inputs from the cerebral cortex. Despite the well documented neurological functions of the cortico-basal ganglia circuits, how the corticostriatal connectivity is established during development remains elusive. Recent studies have suggested that the language-associated gene Foxp2 is involved in neurobiological functions of the cortico-basal ganglia circuits. I will go over our studies of the biological function of Foxp2 in regulation of synaptogenesis of the corticostriatal circuits in the brains of genetically modified mice during development.
In vitro modeling the life and death of human neurons

Lawrence Stanton

Genome Institute of Singapore

Stem cells, whether derived from embryonic or adult tissues, have the defining capacities that they give rise to differentiated progeny. It is this unique ability of stem cells that garners great enthusiasm in regenerative medicine as they can be selectively driven to produce human cells and tissues that are suitable for the study and treatment of diseases. Understanding how stem cells control their cell fate decisions is critical in our quest to generate therapeutically useful cell types. My lab is primarily focused on generating human neural cell types. We have established robust methods that permit the efficient production of neural progenitors, glial cells, and neurons from human embryonic stem cells. Using genomics tools and technologies we have been elucidating the regulatory pathways that control differentiation of these neural lineages. This work has revealed several important new transcription factors, unique partnerships of known transcription factors, and non-coding RNAs that are required for neural development. Recently, the lab has been generating induced pluripotent stem cells (iPSC) from patients with severe neurological disorders. These patient-specific iPSC are then converted into various neural cell types, which provides us the opportunity to study the molecular basis of neurodevelopmental and neurodegenerative diseases.
Investigating the functional role of non coding RNA during neurogenesis

Jun-An Chen
Academia Sinica

Regulatory non coding RNAs, including microRNAs (miRNAs) and long non coding RNAs (lncRNAs), have shown to be essential for animal development and viability, yet dissecting the relevance of individual miRNA or lncRNA has been challenging for a given cell context. In addition, the role of ncRNAs for neurodegeneration is still obscure. To identify ncRNAs participated during motor neuron development and degeneration, we used mouse embryonic stem cell as a paradigm and robustly harnessed it into different motor neuron subtypes to perform strand specific RNA-seq and small RNA-seq simultaneously. We identified several novel MN signature miRNAs and lncRNAs, and systematically analyzed their functions by generating knockout mice. I will present the current progress of the characterization of these MN-ncRNAs by gain-of-function and loss-of-function studies. Collectively, these results will provide critical information for ncRNAs function and will fill in the information on how ncRNAs mediate MN development in vivo.
Modeling Neurodevelopmental Disorders

Toru Takumi

RIKEN Brain Science Institute

Autism is a complex psychiatric illness that has received considerable attention as a developmental brain disorder. Substantial evidence suggests that chromosomal abnormalities including copy number variations contribute to autism risk. The duplication of human chromosome 15q11-q13 is known to be the most frequent cytogenetic abnormality in autism. We have modeled this genetic change in mice using chromosome engineering to generate a 6.3-Mb duplication of the conserved linkage group on mouse chromosome 7. Mice with a paternal duplication display autistic-like behavioral features such as poor social interaction and stereotypical behavior, and exhibit abnormal ultrasonic vocalizations. This chromosome-engineered mouse model for autism seems to replicate various aspects of human autistic phenotypes and validates the relevance of the human chromosome abnormality. This model is a founder mouse for forward genetics of a developmental brain disorder and an invaluable tool for its therapeutic development. I will show our analyses on these mice towards understanding the molecular pathophysiology of autism spectrum disorder.
Actin and Actin-related protein in neurogenesis of Drosophila external sensory organ

Haiwei Pi

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Actin is the major cytoskeleton protein in all eukaryotic cells. Interestingly, actin and several actin-related proteins (ARPs) are also found in nucleus and function as key components in transcription and chromatin remodeling. However, less is known about the interaction between nuclear actin (and ARPs) and the tissue-specific transcriptional factors. The bHLH protein Achaetae (Ac) and Scute (Sc) are specifically expressed in the proneural clusters and are required for determination of sensory organ precursor (SOPs). We found that Ac and Sc physically associate with nuclear actin and actin-related protein 6 (ARP6). Loss-of-function studies showed that nuclear actin is required for proneural protein-dependent expression of the neural precursor genes as well as formation of SOPs. Arp6 is required for timely SOP specification. Since Arp6 is involved in H2A variant exchange in yeast and Arabidopsis, we are currently investigating the role of H2Av-dependent chromatin remodeling in neurogenesis of external sensory organs.
Secreted Frizzled Related Proteins-mediated regulation of choroid plexus morphogenesis

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The choroid plexus (CP) is a convoluted secretory organ present in each ventricle of the brain, that produces the cerebrospinal fluid (CSF) and, as such, is critical for vertebrate brain homeostasis and function. Although previous studies have demonstrated that the formation of the CP is under the influence of BMP signaling and the activity of transcription factors such as Otx2, the mechanisms underlying its morphogenesis are still poorly understood. The development of the fourth ventricle CP (IVCP) starts around E9 with the formation of the pseudostratified epithelium of the roof plate, that subsequently evolves in a folded monolayered epithelium. Here, we show that differentiation of IVCP epithelial progenitors requires a finely regulated transient activation of Wnt signaling. Indeed, artificially sustained up-regulation of Wnt signaling in epithelial progenitors prevents their differentiation and maintains their proliferative state, leading to a hyperplasic IVCP. The window of Wnt signaling activation in CP progenitors is defined by the expression of members of the secreted Wnt inhibitors known as Secreted Frizzled Related Proteins (Sfrps). Sfrp1 is expressed in the developing and differentiated IVCP cells and, together with Sfrp2 and Sfrp5, is also found in the adjacent neuroectoderm. Sfrp1-/-;Sfrp2-/- compound but not single mutant embryos exhibit a much reduced and abnormally folded IVCP, mostly composed of undifferentiated and Wnt signaling responding cells. These defects are accentuated in Sfrp1-/-;Sfrp2-/-;Sfrp5+/- embryos, indicating functional redundancy of the three proteins. Finally, Wnt/SFRPs signaling might be also controlled by the transcription factor Zic2, the absence of which impairs IVCP specification.

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Neural basis for socially induced behavioral changes in Drosophila

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Animals modify behavioral responses for survival and reproductive success. We attempt to disclose the link among gene mutations, neural network changes and modified behavior in Drosophila melanogaster by comprehensive neurogenetic approaches to courtship behavior.

We focus on the fruitless (fru) and doublesex (dsx) genes for their strong impacts on sex-specific traits of neural circuitries and behaviors (for review see Yamamoto and Koganezawa, 2013). Fru and Dsx proteins are two major sex-determinants that direct sex-specific development of single neurons with expression of either or both of these proteins. In an attempt to elucidate how these sexually distinct circuitries operate to generate sex-specific behaviors, we established a virtual reality paradigm (Kohatsu and Yamamoto, 2015). In this paradigm, a wild-type male fly tethered on the treadmill is stimulated with moving light spots displayed on a computer screen, while his brain neurons are stimulated via Channelrhodopsin. Wild-type males exhibited courtship pursuits toward the moving light spots only when a specific dsx-expressing neural cluster was simultaneously activated. In contrast to wild-type males, fru mutant males under similar conditions engaged in courtship pursuits without neural stimulation, provided that they were kept in a group to socialize, as a result of hypersensitive visual responsiveness to the dummy in the dsx-neurons. Thus experience modulated excitability of defined neurons to modify innate courtship behavior.

It remains to be explored how social experience induces the observed functional changes of dsx-expressing neurons and why it affects differently the wild-type and fru mutant neurons.
Inhibitory control of memory circuits

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The hippocampus plays a key role in learning and memory. The dentate gyrus (DG) serves as a gateway to the hippocampus, filtering and processing incoming afferent information from the cortex and passing output to other hippocampal areas. The DG comprises a heterogeneous population of granule cells (GCs). Among them, mature GCs, the largest neuronal population of the DG, are under tight inhibitory control by various types of GABAergic interneurons (INs) and thus display a high activation threshold. However, the causal link between identified GABAergic INs and mature GC activation in response to afferent activity from entorhinal inputs remains unknown. Here we show that pharmacological GABAA receptor blockade not only greatly enhances the sensitivity of mature GCs to afferent inputs, but also recruits a subset of non-spiking mature GCs. Analysis of input threshold and spike timing of various types of GABAergic INs suggests that feedforward inhibition originating from somatic INs and molecular layer INs limits the dynamic range of input processing. Using cell type-specific optogenetic silencing, we found that parvalbumin-expressing (PV+) INs primarily suppress the population response of mature GCs to single-shock stimulation of cortical input. By contrast, PV+ and somatostatin-expressing (SST+) INs differentially regulate mature GC dynamics in response to θ and γ frequency inputs. Notably, PV+ INs control the onset of the spike series, whereas SST+ INs regulate the late spikes in the series. Together, these results demonstrate that specific types of GABAergic INs differentially regulate mature GC input transformations in response to different cortical input patterns.
Sensory Map Formation in the Drosophila Brain

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Sensory maps in the Drosophila visual and olfactory system exhibit a high degree of synaptic specificity, in which photoreceptors and olfactory neurons segregate into distinct layers and glomeruli, respectively. Our studies are aiming at the cellular and molecular mechanisms underlying the specific recognition between synaptic partner neurons during brain circuit assembly.

In the visual system we could show that a sequence of growth cone segregation between co-projecting neurons independent of cell type recognition determines synaptic layer identity of photoreceptors. This local axon segregation is regulated by relative levels of the transcription factor Sequoia and is consolidated by position-dependent axon-target interactions via cell-type specific or general cell-surface molecules. By following ectopic R cell axons through development we show that the initial axon segregation determines final synaptic layer identity independent of cell type specific recognition molecules. Based on these data we are proposing a novel mechanistic model, in which the initial self-patterning of afferent axons, subsequently stabilized by cell adhesion molecules, determines synaptic identity in the Drosophila visual system.

In the Drosophila olfactory system, each of the 50 different functional classes of olfactory receptor neurons segregate into a distinct synaptic glomerulus. We found that the Drosophila Apaf-1-related killer (dark), an activator of the caspase dronc plays a novel non-apoptotic role in wiring specificity of ORN axons. Mutations in dark/dronc cause a specific change in the axonal projections leading to mis-targeting into ectopic glomerular structures. Intriguingly the number of neurons does not change in dark mutants and the blockade of apoptosis does not phenocopy the axonal defect proving that the connectivity phenotype is independent of apoptosis. We propose that the transition of axon pathfinding to target recognition is modulated by ORN class specific diverse caspase activity via the differential expression of cell surface molecules.
One-time experience is prevented from formation of long-term memory by learning-induced new proteins

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Long-term memory (LTM) formation requires time because information gradually accumulates across widely spaced learning episodes to a threshold that induces protein synthesis and synaptic changes. However, the neural mechanisms that determine this threshold remain obscure, particularly at the level of circuits. Here, we report that LTM formation from a one-time experience is prevented by induction of new proteins in a specific subset of neurons forming the mushroom body (MB), the learning and memory center in Drosophila. Blocking protein synthesis in early α/β MB neurons induced formation of LTM even after a single training session. Learning was accompanied by activation of molecules in early α/β MB neurons and transcription of the serotonin receptor 5-HT1A, which increased the inhibitory constraints on the storage of LTM in the downstream circuits. We propose that learning induces sequential synthesis of new proteins at three distinct locations in the brain to inhibit, enhance, and consolidate LTM.
Poster Presentation
Mosaic manipulation of the cerebellum reveals novel linkage between Atoh1 and primary cilium in cerebellar development and medulloblastoma formation

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Atoh1 is overexpressed (OX) in the Sonic Hedgehog (Shh)-type medulloblastoma (MB), the most common malignant pediatric cancer of the central nervous system. In vivo studies have shown that Atoh1 is required for cerebellar development and MB formation; however, its mechanistic contribution to these processes is still unclear. Shh signaling maintains granule neuron progenitors (GNPs) in the proliferative state; thus we hypothesized that their primary cilia, which mediate Shh signaling, are responsible for GNP differentiation delay. We developed an in vivo cerebellar electroporation technique to introduce genes of interest to GNPs. With immunostaining, we visualized the primary cilia and differentiation state of the GNPs in cerebellar slices and the distribution of small ciliary signaling molecules in response to Shh signaling. In vivo results show that Atoh1-OX is directly related to the number of proliferative cells as well as the percentage of ciliated cells. Most intriguingly, after we eliminated the primary cilium in Atoh1-OX GNPs, a majority of GNPs differentiated normally again. In addition, we found that Atoh1 altered the small-molecule distribution within primary cilia, and increased their sensitivity to Shh signaling. Preliminary follow up studies in MB model cell line confirm our in vivo findings. These data suggest a novel linkage between primary cilia and developmental regulation of the cerebellum and when deregulated, MB formation. Atoh1 may act as an oncogene in the formation of Shh-type MB, making it a promising potential therapeutic target for novel, improved treatments for Shh-type MB.
Type VI adenylyl cyclase negatively regulates hippocampal synaptic plasticity

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The type VI adenylyl cyclase (AC6) is a membrane-bound adenylyl cyclase (AC) that converts ATP to cAMP under stimulation and is negatively regulated by multiple signals (such as Gia, Ca2+, PKA, PKC, and NO). In the brain, AC6 primarily exists in neurons in many brain regions, modulates neurite outgrowth by interacting with snapin via the N-terminus of AC6, and controls basal sympathetic nerve discharge in the brainstem. Nonetheless, the physiological function of AC6 in the central nervous system remains largely unknown. In the present study, we demonstrated that AC6 negatively regulates synaptic plasticity via NMDAR-mediated signaling. Genetic ablation of AC6 leads to the elevation of dendritic spine density, alteration of neuronal excitability, enhancement of glutamate-induced cellular activities, and changes of NMDAR/AMPAR ratio in the CA1 pyramidal neurons of hippocampus. Furthermore, AC6 KO mice show enhancement of synaptic plasticity and spatial memory. Together, our study provides a novel role of AC6 to modulate the neuronal plasticity in the hippocampus.
Multilineage differentiation of cortical neural precursor cell induced by electric-field

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Neural stem/progenitor cell (NPC) in the adult mammalian brain is one of the promising candidates for developing therapeutic strategies of neuroregeneration. The differentiation of neural stem/progenitor cells (NPCs) depends on various in vivo environmental factors, such as nerve growth factor (EGF) and endogenous electrical field (EF). In this study, we demonstrated that the morphologic and phenotypic changes of mouse neural stem and progenitor cell (mNPC) could be induced simply by exposing to square-wave DC EF pulses (DC pulses, magnitude 300 mV/mm at frequency of 100 Hz). The DC pulse stimulation was lasted for 48 hours and the morphology changes of mNPCs were monitored continuously. The length of primary processes and the amount of branching significantly increased during 24 hours to 48 hours of the DC pulse stimulation. Surprisingly, after the treatment of DC pulses, the mNPC differentiate into neurons, astrocytes and oligodendrocytes simultaneously in complete medium containing epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Our results suggest that simple DC pulse treatment could control the fate of NPCs. With further studies, DC pulses may be applied to manipulate NPC differentiation and used for the development of therapeutic strategies that employs NPCs to treat nervous system disorders.
Finger like structure mediated glia-axonal contact in early Drosophila visual system

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Neuron-glia interaction has been broadly investigated in embryonic midline and longitudinal tract in Drosophila. However, little is known about the neuron-glia interaction in early development of Drosophila visual system. Retinal basal glial cells (RBG) are a group of glial cells actively involved in the very beginning developmental process of Drosophila visual system and play interactive roles such as correctly guiding axons of photoreceptor cells into the optic stalk. In our studies and studies from other group both found out lack of wrapping glia leads to the fused axon bundle. One interesting question we are asking is any role of glial cells in securing eight axons as an axon fascicle rather than a fused bundle. Therefore, we try to describe the process of how RBG temporally and spatially interact with axons. Furthermore, we find an interesting structure of cell membrane, called finger that extends toward photoreceptor cell bodies. The growth of the fingers are very dynamic. The purpose and the molecular mechanisms are not known now. When disrupting finger structure by expressing dominant negative filopodia-conserved genes, the axon fascicles are disorganized and loss the tilted arrangement in a very short amount of time which imply that functions of finger might contribute to the axonal phenotype.
CPEB3 deficiency elevates TRPV1 RNA translation in dorsal root ganglia neurons to potentiate thermosensation and inflammation-induced thermal hyperalgesia

Yu Wei Chang, et al.
Institute of Biomedical Sciences, Academia Sinica

Cytoplasmic polyadenylation element binding protein 3 (CPEB3) is a sequence-specific RNA-binding protein that downregulates translation of multiple plasticity-related proteins (PRPs) at the glutamatergic synapses. Activity-induced synthesis of PRPs maintains long-lasting synaptic changes that are critical for memory consolidation and chronic pain manifestation. CPEB3-knockout (KO) mice show aberrant hippocampus-related plasticity and memory, so we investigated whether CPEB3 might have a role in nociception-associated plasticity. CPEB3 is widely expressed in the brain and peripheral afferent sensory neurons. CPEB3-KO mice with normal mechanosensation showed hypersensitivity to noxious heat. In the complete Freund's adjuvant (CFA)-induced inflammatory pain model, CPEB3-KO animals showed moderately enhanced thermal and mechanical hyperalgesia. Translation of transient receptor potential vanilloid 1 (TRPV1) RNA was suppressed by CPEB3 in dorsal root ganglia (DRG), whereas CFA-induced inflammation reversed this inhibition. Moreover, CPEB3/TRPV1 double-KO mice behaved like TRPV1-KO mice, with severely impaired thermosensation and thermal hyperalgesia. An enhanced thermal response was recapitulated in non-inflamed but not inflamed conditional-KO mice, with cpeb3 gene ablated mostly but not completely, in small-diameter nociceptive DRG neurons. CPEB3-regulated translation of TRPV1 RNA may play a role in fine-tuning thermal sensitivity of nociceptors
Glia-derived ubiquitin E3 ligase dSmurf is required in controlling Drosophila locomotive behavior

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Higher order behavioral tasks such as motor control are mediated by complex neural circuits composed of two major cell types in the nervous system: neurons and glia. Whereas neurons have been considered to be the leading players in transducing inputs, glia, as passive as once thought, are now active in virtually all aspects of nervous system function. Despite the fact that increasing evidence has suggested the importance of glia in controlling behavior, the detailed molecular mechanism and the involved players remain elusive. The invertebrate Drosophila melanogaster is well known for its sophisticated genetic tools and diverse behavioral features, making it an excellent animal model to access glial contribution to behavioral control. Here we describe the ubiquitin E3 ligase dSmurf functions in glia to regulate Drosophila adult locomotive behavior. Whereas dSmurf was previously implicated in regulating the maintenance of germline stem cells (GSCs) via BMP signaling pathway, its functions in the nervous system remain unclear. Our results indicate that downregulation of dSmurf expression using RNAi in glia causes a decrease in adult climbing activity, and this decrease is partially rescued by the co-expression of a dSmurf transgene. Glial-specific overexpression of dSmurf results in a dominant-negative effect that also leads to a decrease in adult climbing activity. Additionally, morphology of the mushroom body (MB) is strikingly disrupted when dSmurf is overexpressed in glia. Taken together, our results indicate that dSmurf plays pivotal roles in glia-mediated MB formation and locomotive activity, reinforcing the importance of glia in neuronal function and circuit-controlled behavior.
The role of innate immunity in neuronal morphogenesis

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Viral infection during fetal or neonatal life increases the risk of neurodevelopmental disorders. Children with autism have a higher frequency of inflammation and immune dysregulation, suggesting that the immune system involves in the regulation of brain function. Innate immune system is the first line defense upon microbial attack. In the past decades, several innate immunity factors were found expressed in neuron, and neurons can act as immune cells to produce cytokines in response to pathogen infection. Activation of innate immunity Toll-like receptor 3 (TLR3) during embryonic and early postnatal stage has been setup as an animal model to investigate autism and schizophrenia. However, the role of innate immune responses in regulation of neuronal development and function needs to be further investigated. Our previous studies showed that activation of TLR7 inhibits axon and dendrite outgrowth via Myd88/c-Fos/IL-6 pathway. Some microRNAs may act on TLR7 to control neuronal development. Here, we reported that, like TLR7, TLR3 activation also negatively regulates neuronal morphogenesis. The downstream signaling of TLR3 activation in neuron is also investigated. Our study reveals the underlying mechanism of TLR3 activation caused brain dysfunction and provides an explanation of how environmental factors may influence mental health.
NPAS proteins Trachealess and Dysfusion in synaptogenesis at Drosophila neuromuscular junctions

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Neuronal PAS proteins, NPAS1-4, function as transcription factors in development and physiology of both nervous and tracheal systems in mammals. The Drosophila NPAS1/3 homolog Trachealess (Trh) and the NPAS4 homolog Dysfusion (Dys), however, are reported to have roles limited in the tracheal system. We took advantage of the tripartite synapse that intersects with trachea and glia at Drosophila neuromuscular junctions (NMJs) to elucidate the role of NPAS proteins in the nervous system. In our study, we found that partial loss-of-function mutations in trh caused abnormal bouton phenotypes with excess boutons that are bunched. As a compensatory effect, excess tracheal branches were detected in trh mutants. The phenotypes of excess tracheal branches and bunched boutons were recapitulated by rearing larvae in hypoxia condition. Also, the bunched bouton phenotype could be rescued by rearing trh mutants in hyperoxia condition. To further investigate how oxygen supply induces abnormal synaptogenesis, we study the role of Dys in glial cells that interact with NMJ boutons. Genetically, mutations in dys suppressed bunched boutons phenotype in trh mutants. Overexpression of dys in glia also caused bunched bouton phenotypes. Taken together, we propose that the hypoxia situation such as in trh mutant affects proper synaptogenesis at Drosophila NMJs through elevation of the Dys level in glia. In the future, we will investigate involvement of the hypoxia pathway and the signal transmitted between glia and NMJs.
Ran-dependent TPX2 activation in distal neurites promotes neurite elongation and branching

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A functional nervous system depends on the intricate connections between neurons, the morphogenetic process of the neuron must be carefully regulated. It has been shown that the microtubule cytoskeleton and microtubule-associated proteins are indispensable for this process. The microtubule-associated protein TPX2 plays a critical role during mitosis to generate a stable bipolar spindle. We previously identified TPX2 as one of the most abundant proteins on neuronal microtubules, suggesting that TPX2 may play a role during neuronal morphogenesis. First, we observed a decrease in neurite length and branching number in TPX2-depleted hippocampal neurons, suggesting TPX2 is involved in the neurite elongation and branching process. Second, we examined the localization of TPX2 in neurons and found that TPX2 localized primarily to a single punctum within the soma and partially colocalized with the gamma-tubulin. Additionally, TPX2 also distributed in the neurites and bound to the microtubule cytoskeleton. Next, we analyzed the dynamics of microtubules in TPX2-depleted neurons with microtubule plus-end binding protein EB3 and observed a decrease in EB3 emanating frequency specifically at the tip and the base of the neurite, suggesting that TPX2 might only be activated at these locations. Interestingly, we observed the TPX2-activating protein Ran was specifically enriched at the tip and the base of the neurite. Furthermore, the EB3 dynamics in an importin-β-Ran inhibitor (importazole) treated neurons phenocopied that in TPX2-depleted neurons. Our data suggest a model in which Ran-dependent TPX2 activation enhances microtubule nucleation and promotes neurite elongation and branching.
The development of distinct local interneurons in Drosophila olfactory circuit

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We previously found Drosophila olfactory local interneurons (LNs) are highly diverse in their innervation patterns. How do LNs establish diverse morphologies? We screened 1058 GAL4 lines to search for GAL4 specifically expressed in distinct subsets of LNs. After two rounds of screen, we narrowed down to 23 GAL4 lines for further developmental study of LNs. Through extensive investigating the development of different subpopulations of LNs by those GAL4 lines, we found larval LNs undergo distinct development processes during early pupal stages. During 0 hr after puparium formation (APF) to 36 hr APF, neurites from different subsets of LNs undergo pruning and re-extend to different regions of the AL. As expected Ecdysone signal is involved in this pruning process. Of interest, a subset of larval LNs undergoes degeneration. To our surprise, these degenerating larval LNs also express Ecdysone receptor (EcR) and ecdysone signal is required for the degeneration. Is there any biological significance of such distinct fate decisions taken by different LNs? We first found PNs and distinct LN subsets dynamically interact with each other at 24 and 36 hr APF. Disrupting protein secretion of PNs causes LN mistargeting. In addition, blocking LN pruning likely affects the targeting of PN dendrites. Our results suggest LN neurite re-extension may be partly guided by cellular cues derived from PNs. Through the mechanisms of pruning, neurite re-extension and degeneration, such differentially subregional interaction of LNs may be involved in the late stage of PN dendrite targeting.
Studying the role of Huntingtin interacting protein Amphiphysin in the mechanisms of polyQ pathogenesis

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Aberrant expanded polyglutamine (polyQ) in specific proteins cause several neurodegenerative diseases. However, most studies have focused on neurons and less was on glia. Accumulating reports show the importance of glia in the degeneration of nervous system. Huntington’s disease is the most common polyQ disease. Drosophila has a huntingtin (htt) gene. Previously, we observed several phenotypes, such as reversed ERG, laminar degeneration and reduce locomotor ability in the flies expressing the human HTT with expanded polyQ (HTT-128Q) in glia. The reversed ERG is due to leakage in blood brain barrier (BBB). The molecular mechanisms of polyQ-induced glial dysfunctions are unknown. Yeast two hybrid data (BioGRID 3.2) identified Amphiphysin (AMPH) as a HTT-interacting protein in Drosophila. AMPH has Bin–Amphiphysin–Rvs (BAR) domain, which can regulate membrane reorganization. It is localized to actin-rich membrane domains in cells, amph mutant fly has defect in locomotor ability (Zelhof et al., 2001, Development 128, 5005-5015). We found that loss-of-function mutation of amph rescues the reversed ERG caused by expressing HTT-128Q in glia. To further investigate the mechanism of AMPH in this model, we will test whether amph mutation also rescues the other phenotype caused by glial expression of HTT-128Q, and whether other AMPH interacting protein is involved in the polyQ pathogenesis.
Direct Differentiation of Human Embryonic Stem Cell to Dopaminergic Progenitors: Enrichment and Transplantation to the Hemi-Parkinson Rats

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Self-renewal and direct differentiation characteristic points enabling hPSCs application as an excellent source for cell replacement strategies. Differentiation of hESC to dopaminergic neurons hopefully provided a new source for cell therapy in neurodegenerative diseases like Parkinson disease, progressive supranuclear palsy and dementia with Lewy bodies. To enable derivation of enriched dopaminergic population the GFP reporter gene introduced to coding sequence of LMX1A locus and progenitors were purified by sorting the positive cells. Proteomics study of dopaminergic progenitors and mature neurons carried out by shotgun approach without labeling and protein profile of purified cells compared to the control hESC cells. Novel membrane proteins were identified for DA progenitor’s purification, then enriched progenitors were characterized for expression of PITX3, FOXA2 and differentiated to functional mature neurons were expressed TH and GRIK2 and transplanted in the hemi-Parkinson rat model. DA progenitor cells on day 12 of differentiation sorted based on the CNTN2 expression and transplanted to the striatum of the rats. The behavioral results of rotation and cylinder tests showed that CNTN2+ progenitors can significantly improve motor behavior 12 weeks after transplantation compared to unsorted human DA progenitor cells. Histological experiments of the brain specimens from transplanted animals revealed that these type of the DA progenitors integrated in the host brain tissue and express DAT and TH proteins. Comparative proteome analysis of hPSCs derived midbrain dopaminergic neurons resulted followed by enrichment of DA progenitors by specific membrane proteins could promise for the development of cell-based therapies in Parkinson’s disease.
The DCC/Frazzled chemoattractant receptor triggering collective glia migration is tightly regulated by the Gcm fate determinant

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Collective cell migration is a key process during development as it allows the organization of complex tissues such as the nervous system, which comprises of a vast and complex cellular network that is essential for its proper functioning. To maintain the circuitry, neurons and glia migrate collectively from their birthplace to a distant site. Defects in this process lead to severe pathologies including mental retardation. While the role of molecules controlling cell interactions has been extensively investigated, the signaling cascades that trigger chemotropism are not understood. I have analyzed the impact of an early transcription factor and a chemoattractant in the process. The glial chain in a developing Drosophila wing provides an excellent tool to study the molecular pathway underlying collective migration.

I asked whether the transiently expressed Gcm transcription factor, which triggers the fate choice between glia and neurons, also controls collective migration. I demonstrate that Gcm directly affects migration in a dosage dependent manner by inducing the expression of Frazzled, a chemoattractant membrane Netrin receptor.

Interestingly, I found that Frazzled strongly lowers migration efficiency when downregulated in glia and enhances it when overexpressed. In contrast, Unc5 a repulsive Netrin receptor slows down migration when overexpressed. I also validated that Frazzled is a direct Gcm target.

My data demonstrate for the first time the direct role of a fate determinant on a late and collective behavior. Hence, the integration of autonomous (Gcm) and regulatory (Netrin) pathways ensures that glial migration occurs in a timely and efficient manner.
LIMK2-1, a primate-specific isoform of LIMK2 associated with intellectual disability: molecular and functional characterization

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LIMK1 and LIMK2 are Ser/Thr kinases that play key roles in actin dynamics. They act through phosphorylation and inactivation of coflin, an actin depolymerizing factor. Many studies have shown a role of LIM kinases in neurodevelopment, synaptic plasticity and neuronal death. Recently, a new primate specific isoform of LIMK2, LIMK2-1, has been identified. LIMK2-1 possesses a supplementary phosphatase 1 inhibitory domain (PP1i) at its C-terminal extremity, compared to isoforms LIMK2a and LIMK2b previously described. We showed that LIMK2-1 is expressed as a transcript in human fetal and adult brain and as a protein in human cells.

We overexpressed LIMK2-1 in a mouse motor neuron cell line (NSC-34) and observed a decrease in neurite outgrowth. In patients with intellectual disability, we identified a rare missense variation that leads to the substitution of serine 668 into proline (S668P) in the PP1i domain. Neurite outgrowth decrease is abolished when LIMK2-1S668P is transfected in NSC-34 cells, suggesting an impact of this mutation on LIMK2-1 function.

In order to get insights into the functions of LIMK2-1, we studied different truncated versions of the isoform on: (i) actin polymerization, (ii) kinase activity and (iii) interaction with PP1. Our results show that the PP1i domain of LIMK2-1 is required to promote actin polymerization and interaction with PP1. Surprisingly, LIMK2-1 appeared devoid of kinase activity on coflin.

Studies are in progress in order to know if LIMK2-1 inhibits PP1 via its PP1i domain and if this mode of action contributes to LIMK2-1 implication into cognitive functions.
Analysis of Arp6 in Drosophila sensory organ development

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A key point during Drosophila external sensory (ES) organ development is the commitment of sensory organ precursors (SOPs) from proneural clusters (PNCs) to specify neural fate. Proneural proteins achaete (ac) and scute (sc), which encode the basic helix-loop-helix (bHLH) transcriptional factors, are master controllers to this process. It is important to understand the molecular mechanisms by which cofactors interact with and modify the functions and properties of proneural proteins Ac and Sc, initiating and executing ES organ formation. Our previous report demonstrates that nuclear actin interacts with and acts cooperatively with Ac and Sc to mediate the transactivation of downstream genes to promote ES organ formation. Today, we also found that an evolutionarily conserved nuclear actin-related protein 6 (Arp6) specifically associates with proneural proteins, and is required for timely selection and patterning of neural precursors. Also, Arp6 is involved in Notch signaling pathway. In addition, a lot of studies in yeast, vertebrates and plants demonstrate that Arp6 is a component of SWR1/SCRAP/SWR1-like chromatin-remodeling complex, required for H2A variant H2A.Z deposition in the nucleosomes. In the future, we will focus on the Arp6 functions in mediating proneural protein activity during the SOP specification.
Dysregulations of GABAergic neurotransmission in a mouse model of Huntington’s disease.

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Altered synaptic balance of glutamatergic and GABAergic neurotransmission in the cortical-striatal circuit has been proposed as a potential pathophysiological process in Huntington’s disease (HD). Although the cortico-striatal excitotoxicity are well documented in HD, the pathological regulation of inhibitory neurotransmission is less known. Here, we report changes in the gene expression of GABAA receptor α1, α3 and δ subunits in the cortex and striatum of a transgenic mouse model of HD (R6/2). The alteration of GABAAR subunits became more obvious when the symptoms fully developed. The polarity of GABAAR signaling depends on the precise regulation of K+-Cl – cotransporter isoform 2 (KCC2) and Na+-K+-Cl – cotransporter isoform 1 (NKCC1). Remarkably, the expression ratio of KCC2/NKCC1 decreased in R6/2 mice, which may alter the nature of GABAAR signaling. Besides, KCC2 activity was showed to be activated by its interacting protein-brain-type creatine kinase (CKB), an enzyme in the production of ATP, which is decreased in HD mouse model and patients. Our finding suggested that decreased physical coupling between CKB and KCC2 might occur in R6/2 mice. Moreover, compared with WT mice, R6/2 mice exhibited a lower diazepam- induced motor impairment and a higher susceptibility to the neuroactive steroids-evoked sleep response. Taken together, our findings demonstrated an impairment of GABAergic signaling, which might contribute to the imbalance of excitatory and inhibitory neurotransmission in HD brains.
Postsynaptic syndecan-2 induces transsynaptic signaling via fibroblast growth factor-22 for bidirectional synaptic differentiation

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Functional synapse formation requires tight coordination of pre- and post-synaptic termini. Using heparan sulfate proteoglycan syndecan-2 (SDC2)-triggered synaptogenesis as a model, we here explore whether and how transsynaptic signaling coordinates pre- and post-synaptic formation. Our previous studies showed that SDC2 is highly concentrated in dendritic spines in mature neurons. SDC2 overexpression in cultured neurons at 2 days in vitro (DIV) promotes dendritic filopodia formation at 5 DIV, followed by dendritic spine formation at 9 DIV. It is around 9 days earlier than intrinsic process. Interestingly, postsynaptically expression of SDC2 also accelerated maturation of corresponding presynaptic termini, suggesting a transsynaptic signal promoted by postsynaptic SDC2. FGF22, a heparan sulfate binding factor, had been shown to function as presynaptic organiser of excitatory synapses. Combining biochemistry and cell biology studies, we found that SDC2 binds FGF22 and facilitates FGF22 distribution to the filopodial tips and that knockdown of FGF22 impairs the SDC2-induced presynaptic maturation. In addition to presynaptic maturation, our results also showed that FGF22 knockdown disrupts filopodia-spines (F-S) transition but not filopodial formation. Taken with the previous data that calcium influx triggered by presynaptic neurotransmitter is required for F-S transition, our studies support that postsynaptic FGF22 promotes presynaptic maturation and then feed back to promote postsynaptic maturation. In conclusion, this study provides the evidence that postsynaptic FGF22 and presynaptic neurotransmission establish a transsynaptic positive feedback to coordinate bidirectional synapse maturation.
Voltage-gated K+ channel Kv3.4 controls axon growth by regulating calcium activity at the growth cone

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Electrical activity of embryonic neurons influences axon growth and pathfinding during early development. Voltage-gated K+ channel Kv3.4 is expressed in axon tracts of the embryonic brain, but its function in the developing axons remains unclear. In this study, we detected Kv3.4 and Kv3.4-mediated currents at the growth cone of spinal commissural axons of chick embryos. Down-regulating Kv3.4 by specific shRNA impeded axon initiation and elongation in cultured dorsal spinal neurons as well as the growth of spinal commissural axons in the chick embryo and callosal axons in the rat embryo. This action of Kv3.4 was mediated by reducing Ca2+ influx due to spontaneous neuronal activity. During in vivo development, the expression of Kv3.4 in spinal commissural axons depended on the dorsal morphogen Wnt3a, which can promote axon growth. Thus, Kv3.4 channel expression is required for embryonic axon growth.
Activation of AGEs-RAGE axis-induced mitochondrial dysfunction: role of cyclin-dependent kinase 5 and glycogen synthase kinase 3beta.

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It has been considered that activation of AGEs-RAGE axis signaling results in neurodegeneration in central nervous system in diabetes. Cyclin-dependent kinase 5 (Cdk5) is involved neuron-specific function including synaptic plasticity, axonal outgrowth and neurotransmitter release and uptake. Beta-amyloid induces Cdk5 activators p35 to p25 by calpain-mediated cleavage and to cause neuronal cell death. Evidences have indicated that Cdk5 acts an upstream regulator for mitochondria fission and activator for glycogen synthase kinase 3 (GSK3) which results in hyper-phosphorylation of Tau during neuronal apoptosis. However, whether Cdk 5 is mediated in downstream of AGEs-RAGE axis signaling is still unclear. The present study demonstrate that both AGEs-BSA and methylglyoxal (MGO), a precursor product for AGEs, can cause cell death in a dose-dependent manner in human neuroblastoma SH-SY5Y cells. AGEs-BSA significantly induces increase expression of RAGE, Nox4 and phospho-Cdk5, in the other hand, it also causes the expression of p35 and p25 increase implied that AGEs-BSA indeed activate Cdk5. The similar results also occurred in MGO treatment. Both AGEs-BSA and MGO alter the mitochondrial morphology and integrity in a dose dependent-manner by using MitoTracker CMX Ros staining. Importantly, AGEs-BSA markedly decreases mitochondrial intensity and induces neurite atrophy in dopaminergic neurons. In addition, MGO treatment significantly decreased GAP43 expression suggested that MGO might suppress the neurite outgrowth and synapse formation. It will be necessary to evaluate if knockdown of RAGE or Cdk5 or GSK3beta by siRNA transfection or pharmacological inhibition of Cdk5 can attenuate the cytotoxicity of AGEs on mitochondrial dysfunction in our future study.
Role of RBFOX3/NeuN in cognitive impairment and epilepsy

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Cognitive impairment is a mental disability characterized by poor global intellectual performance and its prevalence rate varies from 3% to 29% due to differences in diagnostic criteria and sample characteristics. Epilepsy is a neurological disorder characterized by epileptic seizures and its prevalence rate is 0.7%. It is still unclear about what the exact causal mechanisms are for cognitive impairment and epilepsy. RBFOX3 (NeuN) is a neuron-specific alternative splicing regulator and its disruption has been identified in patients with cognitive impairment and epilepsy. RBFOX3 promotes neuronal differentiation through alternative splicing of Numb pre-mRNA during brain development. Despite the importance of RBFOX3 in brain development and its relevance in human brain diseases, its biological role in brain remains unclear. To address this critical question, we decided to further explore RBFOX3 in the hippocampus of mice. Our studies may provide potential molecular and cellular mechanisms underlying RBFOX3-related human brain diseases such as cognitive impairment and epilepsy.
Using mouse model to study the etiology of autism spectrum disorder

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T-Brain-1 (TBR1), a causative gene in autism spectrum disorders (ASDs), encodes a brain-specific T-box transcription factor. It is therefore possible that TBR1 controls the expression of other autism risk factors. The downstream genes of TBR1 have been identified using microarray and promoter analyses. In this study, we annotated individual genes downstream of TBR1 and investigated any associations with ASDs through extensive literature searches. Of 124 TBR1 target genes, 23 were reported to be associated with ASDs. In addition, one gene, Kiaa0319, is a known causative gene for dyslexia, a disorder frequently associated with autism. A change in expression level in 10 of these 24 genes has been previously confirmed. We further validated the alteration of RNA expression levels of Kiaa0319, Baiap2, and Gad1 in Tbr1 deficient mice. Among these 24 genes, four transcription factors Auts2, Nfia, Nr4a2, and Sox5 were found, suggesting that TBR1 controls a transcriptional cascade relevant to autism pathogenesis. A further five of the 24 genes (Cd44, Cdh8, Ctnn6, Gpc6, and Ntng1) encode membrane proteins that regulate cell adhesion and axonal outgrowth. These genes likely contribute to the role of TBR1 in regulation of neuronal migration and axonal extension. Besides, decreases in Grin2b expression and increases in Gad1 expression imply that neuronal activity may be aberrant in Tbr1 deficient mice. These analyses provide direction for future experiments to reveal the pathogenic mechanism of autism.
Astrocytic GAP43 functions to attenuate neuroinflammation and promote neuronal survival and plasticity

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Growth-associated protein 43 (GAP43) is a PKC-dependent phosphoprotein most often used to indicate activity-dependent axonal plasticity and regeneration. In this study, we found that GAP43 expression can be induced in proinflammatory lipopolysaccharide (LPS)-stimulated rat astrocytes both in vivo and in vitro, and this induction is important for the astrocyte-mediated immune regulation and synaptic plasticity. The LPS-induced astrocytic GAP43 expression is toll-like receptor 4-dependent, and the mechanisms involved are the STAT3- and NFkB-mediated transcriptional activation on the Gap43 gene P2 promoter. Reduction of GAP43 by RNA knockdown and overexpression of a S41A GAP43 mutant that mimic its PKC-dephosphorylated form both attenuated LPS-induced astrocyte hypertrophy with increased process arborization and elongation, whereas opposite effect was observed when overexpressing GAP43 or its S41D mutant mimicking PKC-phosphorylated form. Knockdown of astrocytic GAP43 was found to aggravate LPS-induced proinflammatory cytokine release and subsequently enhanced microglial activation. Furthermore, astrocytic GAP43 reduction also worsened the neurotoxicity and impaired neuronal GAP43 and postsynaptic PSD95 expression in cortical neurons treated with LPS-conditioned astrocyte medium. Notably, LPS-induced glutamate uptake mediated by the glutamate transporter EAAT2 was diminished when GAP43 was reduced in astrocytes, and the mechanism involved an actin polymerization-dependent activation of myocardin-related transcription factor 2a (MRTF2a) that targets the serum response elements-containing Eaat2 gene promoter. Together, these results suggest that astrocytic GAP43 not only mediates glial plasticity but also plays important roles in maintaining synaptic plasticity, external glutamate homeostasis, and immune regulation during neuroinflammation. Astrocytic GAP43 may serve as an indicator for beneficial astrogliosis.
Characterization of mitochondrial dysfunction in drug-induced peripheral neuropathy

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Peripheral neuropathy is a common side effect of chemotherapy and AIDS therapy. This disorder is caused by damage to nerves that transmit sensations, and it is often associated with defects related to mitochondrial DNA (mtDNA). Both polymerase gamma inhibition and mtDNA damage have been implicated in peripheral neuron cell death, but the detailed mechanism is still unknown. In our study, we characterized mitochondrial dysfunction in central and peripheral neurons treated with known neurotoxicants. We applied multiple toxicants and measured mitochondrial homeostasis in embryonic cortical neurons or adult dorsal root ganglion neurons. In order to fully investigate mitochondrial regulation during toxicity, we established a panel of endpoints including those related to biogenesis, transport, oxidative phosphorylation, recycling (dynamics) and autophagy. First, we used ethidium bromide (EtBr), which has been shown to inhibit mitochondrial polymerase gamma as a model compound. Our results showed that EtBr impaired mitochondrial biogenesis, transport and oxidative phosphorylation, but increased mitochondrial fission. Currently, we are investigating the effects of other clinically relevant compounds including NRTIs, which also inhibit polymerase gamma and chemotherapeutics, which induce mtDNA damage. Understanding the precise role of mitochondrial dysfunction in peripheral neuron toxicity may help in the development of neuroprotective strategies that can combat the development of drug-induced peripheral neuropathies.
TLR8 uses cytokine-independent pathway to regulate dendrite arborization in mature neurons

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Toll-like receptors (TLRs), the most well-known pattern recognition receptors, are critical for triggering innate immune response. In addition to defending foreign pathogens, recent studies showed that TLRs sense endogenous ligands to regulate neuronal morphogenesis and neurogenesis. Among various TLRs, both TLR7 and TLR8 recognize single-stranded RNA (ssRNA). Our study has demonstrated that TLR7 regulates axon and dendrite outgrowth through the Myd88-c-Fos-IL-6 pathway. In TLR7\textsuperscript{-/-} neurons, TLR8 is noticeably upregulated, suggesting a compensatory effect of TLR8 on TLR7 deficiency. In this report, we aim to investigate whether TLR8 is also involved in regulation of neuronal morphology. We first found that the TLR8 expression levels were gradually increased during neuronal maturation, indicating a role of TLR8 in mature neurons. Using TLR7\textsuperscript{-/-} neurons, we identified TLR8 specific agonist. Combining the specific agonist and knockdown and overexpression of TLR8, we found that TLR8 activation inhibited dendrite growth, but not axon extension, in both young TLR7\textsuperscript{-/-} and mature wild-type cortical neurons. It suggests a role of TLR8 in dendritic arborization. Similar to TLR7, MYD88 was required for TLR8 to control dendritic growth. In contrast, cytokines were not involved in the TLR8 pathway to restrict dendritic growth. Our data suggest that although TLR8 and TLR7 share the similar ligand specificity and both use MYD88 as a key signalling adaptor, these two molecules use distinct signal pathways and mechanisms to control neuronal morphology.
Functional analysis of CD147 in neuronal differentiation of human pluripotent stem cells

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Neurogenesis is a process by which neurons are generated from their stem cells and progenitors. This process is regulated via many signaling pathways. However, cell signaling and molecular mechanisms that regulate this process have been elusive. The highly abundant, single-transmembrane protein CD147 has been shown to play a versatile role in many cellular behaviors, both in normal and disease states. It has been revealed to regulate signaling pathways such as MEK/ERK, p38 and Wnt signaling, and to control differentiation of lymphocytes and trophoblasts. We are interested in the role of CD147 in neuronal differentiation of human pluripotent stem cells. The human pluripotent embryonal carcinoma (EC) stem cell line NTERA2 has served as an in vitro model to study early human embryonic development. Moreover, it has also been widely used to investigate neuronal differentiation. Immunofluorescence staining using two different monoclonal antibodies revealed localization of CD147 on the plasma membrane as well as in the cytoplasm of NTERA2. Reverse transcription quantitative PCR showed that CD147 transcript is not differentially expressed during retinoic acid-induced differentiation of NTERA2. In contrast, CD147 is differentially expressed at protein level as determined by western blot. Moreover, we also observed differential levels of glycosylated forms of CD147 during neural differentiation of NTERA2. Our ongoing work aims at depletion of CD147 to ascertain its role in neuronal differentiation of the human pluripotent stem cells.
Foxp2 regulates dendritic spine formation and synaptogenesis in medium-sized spiny neurons of the mouse striatum during development

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FOXP2 is the first gene identified to be involved in speech and language. Previous studies have suggested that FOXP2 functions through the cortico-basal ganglia circuits to regulate production of spoken language. In the present study, we studied the neurobiological function of the mouse homologue Foxp2 which differs three amino acids from human FOXP2. We focused on the spine formation and synaptogenesis of medium-sized spiny neurons (MSN). The Golgi’s staining showed that the dendritic spine density in MSNs was significantly reduced in Foxp2 knockout (KO) striatum at postnatal day (P) 12. In parallel to the decreased dendritic spine density, the postsynaptic markers of PSD95 and PSD95 and GluR1, and the pre-synaptic marker of VGlut1, was also reduced in Foxp2 KO striatum. As humanized version of Foxp2H/H protein, which carries human-specific two amino acids substitutions in mouse Foxp2 gene, has been suggested to functions more efficiently than mouse Foxp2 protein in regulating neuronal morphology and function of MSNs (Enard et al., 2009), we also assayed the spine formation and synaptogenesis of MSNs in humanized Foxp2H/H mice at P14. In contrast to Foxp2 KO mice, dendritic spines and synaptic markers of PSD95, GluR1 and VGlut1 were increased in MSNs of Foxp2H/H striatum. In summary, our study suggests that Foxp2 plays an important role in regulation of dendritic spine and synapse formation of MSNs in the mouse striatum during development. This work was supported by NSC99-2321-B-010-002, NSC102-2911-I-010-506, MOST103-2321-B-010-009.
Deficiency of CPEB2-Confined ChAT Expression in the Dorsal Motor Nucleus of Vagus Causes Hyperactivated Parasympathetic Signaling-Associated Bronchoconstriction

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Cytoplasmic polyadenylation element binding protein 2 (CPEB2) is an RNA-binding protein and translational regulator. To understand the physiological function of CPEB2, we generated CPEB2 knockout (KO) mice and found that most died within 3 days after birth. CPEB2 is highly expressed in the brainstem, which controls vital life functions such as breathing and heartbeat. Echocardiography and whole-body plethymography revealed that KO neonates had normal heart rate but aberrant respiration with frequent apnea. Nevertheless, the morphology and function of respiratory rhythm-generating center and diaphragm neuromuscular junctions appeared normal. In vivo respiratory pattern analyses suggested that KO mice had increased airway resistance under both normoxic and hypercapnic conditions. Upregulated translation of choline acetyltransferase (ChAT) RNA in the CPEB2 KO dorsal motor nucleus of vagus (DMNV) resulted in hyperactivation of parasympathetic signaling on bronchi, as evidenced by increased pulmonary content of acetylcholine. Consequently, the elevated airway constriction induced by cholinergic transmission in KO neonates may account for the respiratory defect and mortality.
Role for Cell cycle-related kinase in ciliogenesis and Hedgehog signaling during neural tube development

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Most of mammalian cells, including neuronal cells, have primary cilia which are key regulator of embryogenesis and adult tissue homeostasis. Physiological importance of primary cilia in mammals remains enigmatic for a past century and recent advance in cilia biology research enlightened the crucial roles of primary cilia in animal development and disease. Previously our lab identified the novel Hedgehog (Hh) signaling pathway component, Bromi, and loss of its expression in vivo causes dorsalized neural tube and diminished Hh signaling activity in mouse neural tube development. To better understand the function of BROMI in neural tube development, we identified Bromi interacting protein, Cell cycle-related kinase (CCRK), which is a homologue of long flagellar mutant 2 (LF2) in Chlamydomonas. We generated CCRK knock out mice and analyzed the developmental defects. We observed that downregulation of Hh signaling and abnormal ciliogenesis caused dorsalized neural tube formation in CCRK mutant embryos. Abnormal ciliogenesis in CCRK mutant shows disrupted GLI3 processing indicating that total GLI transcription factor activity was compromised during neural tube patterning. Moreover, multiple developmental organogenesis defects were manifested in mutant mice embryos with misregulation of Hh signaling. These results support the idea that primary cilia play a central role in mammalian specific Hh signaling and neurodevelopment.
Characterization of Vilse/Arhgap39 in neural differentiation

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Rho GTPases are key regulators of cell dynamics, intracellular membrane trafficking, neuronal migration and polarity. They are regulated by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs) to cycle between an active GTP-bound and an inactive GDP-bound state. The balance among GTPases as well as GAPs and GEFs is critical for a switch from cellular proliferation toward neuronal differentiation and neurite outgrowth.

Vilse/CrGAP/Arhgap39 is a newly identified GAP that contains a conserved RhoGAP domain accompanied with two protein-protein interaction domain, namely WW domain and MyTH4 domain. Recent studies have demonstrated that Vilse interacts with Robo receptor through WW domain and acts downstream of Robo receptor to regulate Rac/cdc42-dependent cytoskeletal changes, thereby mediating the midline repulsion in Drosophila. Additionally, Vilse associates with connector enhancer of KSR-2 (CNK2) through WW domain to modulate Rac cycling during spine morphogenesis in hippocampal neurons.

To investigate the role of Vilse in neurogenesis, we constructed WW, MyTH4, and RhoGAP-deleted mutants of Vilse. Introduction of these mutants to neuroblastoma N2a cells showed an unexpected results that MyTH4-deleted mutant accumulated in the cytoplasm, thereby significantly impairing the neurite outgrowth of N2a cells in response to retinoic acid (RA)-mediated differentiation. To decipher its function in vivo, homozygous ablation of Vilse mice has been generated and macroscopic examinations indicate an essential role of Vilse for embryonic survival. We used hippocampus specific knockout mouse to do Morris water maze experience, the result suggested that knockout Vilse impaired learning competency of mouse.
Muscleblind-like proteins are splicing factors essential for brain development

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Muscleblind-like (MBNL) proteins are RNA binding proteins that regulate alternative splicing during development. There are three paralogs in MBNL family with distinct spatial and temporal expressions. While Mbnl3 is mostly expressed during embryonic stage, Mbnl2 and Mbnl1 are abundantly expressed in the adult brain and critical for developmental transition from fetal to adult. Myotonic dystrophy (DM) is a multi-systemic disorder involving a variety of organs including muscle and brain. Patients develop brain symptoms/signs including hypersomnia, cognitive dysfunction, visual spatial learning deficits, tauopathy and white matter lesions. DM is also a microsatellite expansion disorder with small tandem repeats of C(C)TG in the 3’-UTR region of DMPK gene (DM type 1) and intron 1 region of CNBP gene (DM type 2), respectively. These expansions result in the expression of C(C)UGexp mutant RNAs that sequester MBNL proteins. To test the hypothesis that MBNL loss of function is the primary event in DM pathogenesis, we created Mbnl knockout (KO) mouse models. While Mbnl2 KO mice recapitulate REM sleep misregulations and memory impairment in DM, neuron-specific Mbnl1 and Mbnl2 compound KOs show severe motor deficits accompanied with complete reversal of splicing back to fetal pattern. Importantly, mis-splicing of microtubule-associated protein tau (MAPT) exon 2, 3 and 10 in DM patients were also reproduced in these models, indicating their potential in elucidating DM CNS pathogenesis. We conclude that Mbnl KO mice are reliable models to characterize the molecular events leading to DM brain symptoms and MBNL proteins are critical during brain development.
MicroRNA Filters Hox Temporal Transcription Noise to Confer the Robustness of Boundary Formation in the Spinal Cord

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The rostrocaudal (RC) patterning of the developing spinal cord is controlled by extrinsic signals that act on early neural progenitors within the nascent neural tube. The initial RC patterning leads to differential expression of Hox genes in postmitotic motor neurons (MNs) and specification of MN subtype identity and connectivity. Here we revealed that several 3’ Hox genes, and notably Hoxa5, is expressed in progenitors in a noisy manner, and Hoxa5 protein is only detectable in postmitotic MNs. We found miRNA biogenesis impairment via Dicer deletion leads to precocious and propagates the noise of Hoxa5 at protein level, resulting in a rough Hox5-Hox8 boundary in vitro and in vivo.

Using in silico simulation to explore the topology of Hox gene and miRNA network, we predicted that the loss of feed-forward Hox-miRNA loops may account for the precocious and noisy Hoxa5 expression, as well as the ill-defined boundary phenotype of Dicer mutants. Finally, we identified mir-27 as a major regulator coordinating the temporal delay and spatial collinearity for Hox protein expression via gain- and loss-of-function studies. Our results describe a novel Hox-miRNA genetic circuit that filters the noise and controls timing of protein expression to confer robust individual neuron identity.
Sex Differential Effects of Neonatal Pretreatments of Fluoroacetate or MSO to Disrupt Glutamate-Glutamine Cycle on Adult Reproductive Behaviors in Rats

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Neonatal estradiol exposure permanently determines the neonatal sex differences in astrocytic morphology and spine densities in the hypothalamus and their expressions of adult reproductive behaviors. Estradiol upregulates astrocyte glutamate-glutamine cycle (GGC) enzymes activities. We hypothesized that the GGC efficacy between astrocytes and neurons during neonatal sensitive period determines the different expressions of the adult sexual behaviors.

All pups were received two injections of either fluoroacetate or MSO to disrupt GGC on the sensitive period [Postnatal day 0 (PN0) and PN1] and non-sensitive period (PN13 and PN14). Animals were then divided into two groups: one group was sacrificed 5 days after the injections for examination the GGC enzyme proteins using western blot and the other group was raised until PN42 for sexual behavior testing.

Neonatal males showed higher spinophilin and GGC enzyme proteins, glutamine synthetase and glutaminase in the hypothalamus than the females during the sensitive but not non-sensitive period. The pretreatments significantly reduced GS protein in all the brain areas of both sex, yet only male pups showed reductions of glutaminase and spinophilin proteins in the hypothalamus during the sensitive period, in contrast, the females showed an increase or no effect on the protein expressions. The sensitive period but not the non-sensitive period pretreatments significantly reduced reproductive behaviors of the males but not the females. The pretreated animals showed no change on all the steroid receptor protein expressions.

We conclude that neonatal disruption of GGC will interrupt expressions of male but not female adult sexual behaviors.
Cdk1 and Cdk2 phosphorylate Sox2 to control neural stem cell fate

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In neurodevelopment, the switch from proliferation to differentiation of neural stem cells (NSCs) is a highly coordinated event that we have previously shown to be controlled by cyclin-dependent kinases (Cdks). However, the precise mechanism through which NSCs couple differences in Cdk activity to changes in cell fate remain elusive. Sox2 is a SRY-related HMG-box transcription factor with important functions in neuronal cells. Here, we identify a conserved Cdk phosphorylation site on Sox2 (serine 39; S39) that is specifically targeted by Cdk1 and Cdk2. In NSCs, phosphorylation of S39 enhances the ability of Sox2 to negatively regulate neuronal differentiation, while loss of phosphorylation is necessary for chromatin retention of a truncated form of Sox2 generated during neurogenesis. We further demonstrate that non-phosphorylated cleaved Sox2 specifically activates proneural genes and promotes neurogenic commitment in vivo. Our present study sheds light on how Cdks directly regulate Sox2 to tip the balance between self-renewal and differentiation in NSCs.
Analysis of the molecular pathogenesis of SCA17 using transgenic mice

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Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant neurodegenerative disease caused by the CAG/CAA expansion in the TATA binding protein (TBP) gene. Clinical symptoms of SCA17 include ataxia, spasticity, chorea and cognitive decline. The neurological feature of SCA17 is Purkinje cell loss and gliosis. However, the molecule mechanism of SCA17 is not clear. Our laboratory generated the SCA17 transgenic mice that express human TBP with 109 CAG repeats. These SCA17 mice showed ataxia and Purkinje cell loss, which mimic the patients’ phenotypes and are suitable for study of SCA17 pathomechanism to investigate the molecular mechanism of SCA17 disease. Western blot and immunofluorescent analyses were performed to identify several molecules, including calbindin, GFAP, heat shock proteins, autophagy, ERK1/2 pathway and inflammatory cytokines in mouse cerebellum at 2-24 weeks old. We also examined the downstream targets of ERK1/2 pathway, including pCREB, p90RSK, and pElk. We found a significant reduction of calbindin expression at SCA17 transgenic mice since 4 weeks old. The overexpression of GFAP and TBP aggregation were also observed since then. We also found the pERK level in SCA17 transgenic mice was higher than wild type mice, especially after G-CSF treatment. Our study suggest that G-CSF could protect SCA17 transgenic mice via activated pERK expression. Immunofluorescence staining analysis showed a significant increase of pERK expression in mouse cerebellum after 2 months old.

Further studies in the relationship between ERK pathway and Purkinje cells degeneration might provide a new insight of the pathogenesis of SCA17.
Reconstruction of spinal cord following transplantation of fetal spinal cord tissue in mid-cervical spinal injured rats

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Mid-cervical spinal cord injury results in respiratory impairment due to interruption of respiratory pathways and damage of motoneurons. Currently, there is no effective treatment to restore function following spinal injury. Accordingly, the present study investigated whether transplantation of neural progenitors derived from the fetal spinal cord (FSC) can be a promising therapeutic strategy to replace lost cells and reconstruct neural circuits. Hank’s balanced salt solution or embryonic day 14 FSC expressing green fluorescence protein were transplanted into the lesion cavity in rats with unilateral hemisection of 4th cervical spinal cord at 1 week post-injury. Cervical spinal cord was dissected and sectioned to evaluate the viability and differentiation of FSC-derived cells at 8 weeks post-transplantation. The result demonstrated that GFP-positive cells were detected in all layers of the cervical spinal cord, suggesting graft derived from FSC can survive and fill the lesion cavity. Immunofluorescence staining demonstrated that some GFP-positive cells also appeared positive for neuron-specific nuclear protein (NeuN) or glial fibrillary acidic protein (GFAP). In addition, the connectivity between the graft and phrenic nucleus was evaluated by painting a transsynaptic tracer (wheat germ agglutinin; WGA) on the diaphragm 8 weeks post-transplantation. Cervical spinal cord sections were evaluated at 3 days after tracer painting. Phrenic motoneurons were robustly labeled by WGA, and a few WGA-positive cells were detected within the graft region. These data suggest that graft derived from FSC can survive and differentiate in the injured spinal cord and may have potential to reconstruct the spinal cord circuit.
Wiring diversity of bilateral local interneurons in Drosophila olfactory system

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Local interneurons (LNs) in Drosophila olfactory system provide horizontal connections inside the antennal lobe (AL) and are essential in neural computation and information processing. Our previous work demonstrated olfactory LNs are either unilateral LNs or bilateral LNs. Those olfactory LNs are highly diverse and variable in their arborization patterns in the AL. LNs are also diverse in their neurotransmitter expressing profiles. Of interest, majority of the bilateral LNs are either GABAergic or Glutamatergic. However, compared to unilaterally innervating LNs, bilaterally innervating LNs have been far less studied. Here we focused on Drosophila bilateral LNs and tried to ask if they also possess morphological diversity and variability and if there is a correlation between their innervation patterns and neurotransmitter profiles. Genetic technique MARCM (Mosaic Analysis with a Repressible Cell Marker) was employed to characterize bilateral LNs in single cell resolution. So far, we have analyzed bilateral LNs in fourteen twelve-hour time windows covering larval and pupal development and obtained >1078 single cell clones. We found these bilateral LNs have either roughly symmetric or asymmetric innervation patterns in the ipsilateral and contralateral AL. Their innervations are also highly diverse. Currently, we are analyzing the innervation patterns of individual bilateral LNs to see if those bilateral LNs also exhibit morphological variability. After quantifying individual bilateral LNs’ innervation profiles and their neurotransmitter identities, we will be able to know whether GABAergic and glutamatergic bilateral LNs have any preference of specific innervations in the AL or not. We will focus on several types of bilateral LNs to investigate their development and potential biological functions.
Rgs2 regulates neural crest formation and lineage determination via Wnt signaling

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Neural crest cells are multipotent progenitors. They arise at the border between the neuroectoderm and epidermis and subsequently delaminate, migrate, and differentiate into a diverse array of cell types distributed throughout the vertebrate body plan. Here, we isolated a zebrafish regulator of G-protein signaling 2 (rgs2) and showed that Rgs2 deficiency induced the formation of neural crest progenitors. Disruption in the expression of Rgs2 by a morpholinos and a dominant-negative construct also induced the non-ectomesenchymal derivatives as the compensatory mechanism for ectomesenchymal derivatives, suggesting that Rgs2 regulates the fate determination of neural crest lineages. Furthermore, we found that Rgs2 depletion induced the expression of many components of the canonical Wnt signaling pathway. This study revealed Rgs2-Wnt signaling as a novel regulatory mechanism for neural crest formation and lineage determination.
Deltex1 is inhibited by the Notch–Hairy/E(Spl) signaling pathway and induces neuronal and glial differentiation

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Developing a functional nervous system requires a precise balance of cell proliferation, differentiation, and apoptosis to generate a sufficient population of various neural cell derivatives. Notch signaling is one of the major factors in neural development and brain tumors; however, regulators in Notch signaling have not been fully identified and examined. Deltex proteins have been discovered and shown to be noncanonical components in Notch singling. However, the function of these proteins and their role in Notch signaling remains unclear. We identified zebrafish Deltex1 (Dtx1) and demonstrated that the Notch downstream transcription factors Hairy/E(Spl) bind directly to dtx1 and inhibit its transcription. Dtx1 overexpression was necessary for promoting neuronal and glial differentiation. In addition, we reduced Dtx1 expression by using a Dtx1 deletion variant that lacked the RING domain and dtx1 morpholino knockdown approaches. These two methods produced an identical phenotype, which reduced neuronal and glial differentiation, demonstrating the essential function of the RING finger domain and confirming the knockdown specificity. Cell proliferation and apoptosis were unaffected, even after Dtx1 expression was altered. This study revealed a novel regulatory mechanism that explains how Notch signaling regulates neural differentiation without affecting proliferation and apoptosis.
Lipophagy protects neurons from degeneration caused by neuronal accumulation of dihydroceramide

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Infertile crescent (Ifc) is an evolutionary conserved dihydroceramide desaturase which plays a crucial role in de novo ceramide (Cer) synthesis by converting dihydroceramide (DHC) to Cer in Drosophila. While elevated levels of Cer have been associated with several neurodegenerative diseases, the neuronal function of its precursor DHC remains largely unknown. Here, we reveal an in vivo function of DHC in neuronal maintenance. To genetically manipulate DHC levels, we generated ifc knockout flies (ifc-KO) utilizing the CRISPR/Cas9 system. Sphingolipidomic analysis of ifc-KO indicated increasing DHC and decreasing Cer levels. Loss of ifc resulted in lethality during larval development, which could be rescued by neuron-specific expression of its human ortholog, DEGS1, demonstrating tissue specificity and evolutionary conservation. Clonal analysis of ifc-KO photoreceptors upon 3-day light stimuli resulted in the accumulation of lipophagic structure and the increase of atg8/LC3 puncta, suggesting the activation of lipophagy. Prolonged light stimuli led to activity-dependent neurodegeneration and apoptotic cell death, which were rescued by treatment with the autophagy inducer Rapamycin, suggesting that enhanced lipophagy plays a protective role in ifc-dependent neurodegeneration. Interestingly, genetically reducing DHC levels inhibited the functional degeneration of photoreceptors and rescued the larval lethality of ifc-KO, pinpointing the toxicity caused by the accumulation of DHC, but not Cer, in neuronal function and organism survival. In summary, we identified the neuronal function of Ifc in vivo, and uncovered the bioactive role of DHC in lipophagy and neuronal survival.
Toll-like receptor7 negatively regulates dendrite outgrowth

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Toll-like receptor (TLR) family has been known as a critical danger sensor in innate immune system through recognition of both pathogen- and damage-associated molecular patterns to against invading pathogens. In addition to immune cells, some of TLRs are expressed in neurons and involved in the regulation of neurogenesis, neurite growth and neurodegeneration. However, the downstream signal pathways and effectors for TLRs in neurons are still controversial. From our TLR agonists screening, we identified that TLR7 agonist impaired dendritic growth of cultured mouse cortical neurons. To investigate the function of Tlr7 in neuronal development, we measured the difference of neuronal morphology between wild-type (WT) and Tlr7 knockout (KO) neurons in primary cortical cultures, and we found that Tlr7 KO neurons have longer dendrites and primary axons. Similarly, Tlr7 knockdown neurons also showed more complex dendritic arborization and misoriented of apical dendrites in vitro and in vivo. In addition, lower exploratory activity of juvenile Tlr7 KO mice were observed in an open field, indicating that the changes of neuronal morphology also influence mouse behaviors. Furthermore, we identified the specific TLR7 endogenous ligands, let7c and miR21, as well as synthetic agonists, CL075 and Lox, in primary neuronal culture. Moreover, we found that TLR7 activation induced the expression of IL-6 and TNF-α cytokines as well as c-FOS transcription factor. Using different deficient neurons, including Myd88, Trif, IL-6, Tnf-α and IL-1R1 KO neurons, demonstrated that the MYD88 and IL-6 are essential for TLR7 signaling to restrict dendritic growth.
CPEB2 regulates hippocampus-related long-term synaptic plasticity and memory

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Activity-dependent de novo protein synthesis in either axons and/or synapto-dendritic compartments is necessary to sustain long-lasting modification of synapses and consequently support long-term memory. Cytoplasmic polyadenylation element binding protein 2 (CPEB2) is a translational regulator and widely expressed in the central nervous system, so we examine whether CPEB2-controlled translation is important for learning and memory. Because of respiratory distress-associated neonatal lethality in CPEB2 knockout (KO) mice, the conditional CPEB2 wild-type (cWT) and KO (cKO) mice of which cpeb2 gene is ablated in neurons expressing Cre recombinase under the control of CaMKII promoter, were used for behavioral and electrophysiological analyses. Although CPEB2 cKO mice performed normally in anxiety-related tests, including open field and elevated plus maze, they exhibited memory consolidation deficit in hippocampus-associated Morris water maze and contextual fear conditioning tests. Moreover, certain forms of synaptic transmission, including long-term depression (LTD) and long-term potentiation (LTP), were impaired in the Schaffer collateral-CA1 region of cKO hippocampal slices. To identify the molecular and cellular defects underlying aberrant memory performances and electrophysiological responses, we used WT and KO cortical/hippocampal neurons to examine dendritic spine morphology and miniature excitatory postsynaptic current (mEPSC). CPEB2 KO neurons exhibited elongated spines and reduced mEPSC amplitude. Using the RNA-immunoprecipitation approach, we identified a list of CPEB2-bound mRNAs. In combination with literature knowledge regarding of the abnormal responses identified from the aforementioned experiments, we are examining whether several candidate RNAs are dysregulated in the absence of CPEB2, thereby leading to memory defects in the cKO mice.
Peripheral Nerve Implants Enriched with Developmental Signaling Cues for Peripheral Nervous Tissue Engineering

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Peripheral nerve injuries are one of the most influential damages to our body. There are reported over 600,000 cases in Europe and in the United States annually. Many of such nerve injuries cause gaps between nerve stumps. Without intervention, they can lead to the formation of a stump neuroma.

The current approaches to regeneration of damaged peripheral nerves include: autografting, allografting, and, last but not least, the implantation of polymeric conduits between nerve stumps. Nerve autografting is considered as the “gold standard” technique for the repair of peripheral nerve discontinuities, but it has a number of limitations, such as the requirement for the second surgical procedure to harvest the graft tissue, the donor site morbidity, additional injuries and scarring as well as the increased recovery time. Allografts and xenografts can be an alternative to autografts, but their main drawback lies in the high possibility of an undesirable immune response. The most promising materials for peripheral nerve conduits preparation are natural biopolymers. They exhibit similar properties to the tissues they are replacing and good cell adhesion. Furthermore, they tend to accelerate regeneration processes due to specific chemical interactions within the human body, e.g. with extracellular matrix molecules.

The purpose of our study is to create a conduit with properties that will mimic the ones of the extracellular matrix of the peripheral nervous system. Our focus is put on natural polymers, especially chitosan. In addition, we use developmental signaling cues which exhibit properties beneficial in regeneration of the peripheral nervous tissue.
Derivation of GnRH neuron-like cells from hESC-derived neural crest progenitor cells

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Neural crest (NC) cells emerge at the interface between neural and non-neural ectoderm, and migrate extensively to form a variety of NC derivatives such as peripheral neurons, glia, melanocytes, endocrine cells and mesenchymal precursor cells. NC cells possess various unique properties and are capable of undergoing cell fate decisions across multiple tissues and germ layers. In zebrafish and mice, GnRH neurons are reported to arise also from NC. Here we showed that GnRH neuron-like cells could be obtained from NC cells, which are derived from human pluripotent stem cells (hPSCs). We first induced NC fate from hPSCs by our recently published protocol; these hPSC-derived NC cells expressed NC-specifier genes, including MSX2, PAX3, SLUG, TWIST1 and SOX10, and were multipotent. We next differentiated hPSC-derived NC cells toward neuronal lineage, which resulted in upregulation of a set of neuronal genes including TUJ1, MASH1 and NGN2. In addition, peripheral sensory neurons and sympathetic neurons were detected by immunocytochemistry. Importantly, GnRH neuron developmental genes, EBF2, DCC and VAX1, were increased upon neuronal induction of hPSC-derived NC cells. GnRH1 expression did not not increase significantly; however, GnRH1-immunopositive cells could be detected among TUJ1-positive neurons. In addition, the resulting GnRH1-positive cells were co-localized with NC markers, such as SOX1, p75(NGFR) and HNK1. Altogether, our data show that GnRH expressing cells can be generated from hPSC-derived NC cells. We are currently investigating the implications of these findings with Kallmann syndrome patient-derived induced pluripotent stem cells.
Screening of compounds with neurogenic induction property using human pluripotent stem cells as a platform

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Pluripotent stem cells (PSCs) are cells that possess the ability to give rise to all cell types of the three germ layers via differentiation process, and also exhibit the capacity for unlimited self-renewal in vitro. Because of their ability of differentiation into several tissue-specific cell types, human PSCs (hPSCs) offer a platform for modeling human pathology, for regenerative medicine and for drug discovery. Human pluripotent embryonal carcinoma stem cells (ECCs) are derived from teratocarcinomas, and can be used as a model system for testing drugs or toxicological substances. Recently, a growing number of bioactive compounds have been revealed as modulators of stem cell fates as well as inducers of neurogenesis. In order to identify herb-derived compounds that can induce neural differentiation of hPSCs, we have employed the pluripotent ECC line NTERA2 for their initial screening. SSEA-4, a cell surface marker of hPSCs, was utilized to determine stem cell state after the treatments. We found that Galangin, Geraniol and Piperine possess a positive effect on self-renewal of hPSCs, while the plant-derived small peptides HY10-2, HY11-1, HY11-2, HY11-3, and HY11-4 but not KL-12 possess a negative effect on self-renewal the stem cells. Ongoing work has been conducted to determine neurogenic induction property of these small peptides during differentiation of NTERA2 toward neural stem cells.
The role of AIM2 inflammasome in neuronal morphogenesis

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Recent researches suggest that a large number of immune proteins are expressed in the central nervous system (CNS) and play an important roles in brain development regulation. Previously, our lab found that TLR 7 agonist (Cl075) induce interleukin-1β (IL-1β) gene expression in neurons, but we didn’t detect IL-1β protein in cultured medium. IL-1β is defined as an alarm cytokine to mediate inflammation and need to some processes, like inflammasome to promote cleavage and secretion. Until now, the exact role of inflammasome in neurons is unclear. First, we analyzed the inflammasome-related genes by quantitative-PCR and data showed that AIM2 inflammasomes sensing double-stranded DNA (dsDNA) are the major inflammasomes in cultured neurons as well as mouse brains. Furthermore, we test to activate AIM2 inflammasome by treatment of poly dAdT, a synthetic dsDNA, and found that IL-1 secretion was an AIM2-dependent manner. If neuron pretreated with Cl075, activation of AIM2 inflammasome promote more IL-1β secretion. Next, we want to know whether activation of AIM2 inflammasome affect dendrite outgrowth. We found that AIM2 inflammasome activation inhibits dendrite outgrowth but promote axon growth. Whether AIM2 inflammasomet inhibited dendrite outgrowth through IL-1β needed be further confirmed. We also measured the different of neuronal morphology between wild-type (WT) and Aim2 knockout (KO) neurons and Golgi staining in vivo, and found that AIM2 influences neuronal development by promoting axon growth but restricting dendrite growth. According to above phenomena, AIM2 might play a regulator role in neuronal development.
Glutamate clearance by astrocyte-like glia modulates motor rhythm and ensures proper synaptic growth at the Drosophila neuromuscular junction

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Central pattern generators (CPG) establish rhythmic firing pattern in motoneurons, which is crucial for repeated, highly coordinated behaviors, such as walking, respiration, and mastication. Astrocytes emerge as the key modulator for such motor rhythm by releasing ATP and Ca2+ buffering protein. In Drosophila, coordinated larval locomotion is under tight control of CPG that resides in the ventral nerve cord. We currently investigated the role of glutamate uptake by Drosophila astrocyte-like glia (ALG) in the integrity of CPG. Excitatory amino acid transporter 1 (Eaat1) is the sole high-affinity glutamate transporter in flies and expressed in ALG of the larval central nerve system. Like its mammalian counterparts, we found that Eaat1 is enriched in ALG’s cellular processes, which closely associates with glutamatergic synapses. Our results from GCaMP6 imaging and intracellularly muscular recording further revealed that loss of eaat1 causes aberrant motoneuron firing rhythm featured with less burst frequency but prolonged burst duration. We also observed that the propagation of posterior to anterior segmental motor rhythm is severely disrupted. Physiologically, these abnormalities impaired larval locomotion. Our work therefore highlights a role of Eaat1 in motor rhythm. Intriguingly, the firing burst pattern in motoneurons varied in different eaat1 mutants. These distinct firing properties can even contribute to differential activation for ROS/JNK and BMP signaling pathways, which leads to different types of synaptic bouton outgrowth. Hence, in addition to coordinated locomotion, proper modulation of CPG by astrocyte-mediated glutamate clearance is essential for synaptogenesis of motoneurons. Furthermore, our work indicates that the type of neuronal firing pattern may confer the specificity for the activation of activity-dependent cellular pathways.
mir-17~92 functions as intrinsic vulnerability modifier of limb-innervating motor neurons in ALS

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Amyotrophic lateral sclerosis (ALS) is an age onset neurodegenerative disease, and the hallmark of ALS is the gradual loss of lower motor neurons (MNs). Importantly, motor neuron subtypes undergo different degrees of degeneration, and the earliest degenerated MNs in ALS patients are lateral motor column MNs (LMC-MNs), which innervate limb muscles. However, the underlying mechanism for this selective vulnerability of LMC-MNs is still unknown. Previously we revealed that the expression of a microRNA cluster, mir-17~92, is enriched in developing LMC-MNs, and the target deletion of mir-17~92 in motor neurons lead to the selective apoptosis of LMC-MNs in embryos. While overexpression of mir-17~92 in MNs prevents naturally occurring cell death during neural development by attenuating PTEN expression and its nuclear import.

Here, we further examined if mir-17~92 and PTEN subcellular localization affects the selective susceptibility of LMC-MN degeneration. We first profiled the expression pattern of mir-17~92 and PTEN from control and SOD1G93A mouse, as well as embryonic stem cell (ESC)-derived MNs. In both in vivo and in vitro systems, we revealed a reduction of mir17~92 expression, with a concomitant nuclear PTEN accumulation in SOD1G93A ALS MNs, compared to control WT MNs. We are testing if manipulation of mir-17~92 in SOD1G93A mice can prolonged the survival as well as ameliorate ALS pathology in vitro and in vivo.
MATERNAL IMMUNE ACTIVATION PROVOKES HIGHER SUSCEPTIBILITY TO EPILEPSY IN THE OFFSPRING

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Synapses are fundamental brain structures that mediate information transfer between neurons and control cognition, attention and learning. Recently, evidence accumulated indicating that immune deregulation or inflammation are linked to several types of psychiatric diseases (Patterson, 2009). Accordingly, perinatal infections have been associated with increased risk for neurodevelopmental disorders (Meyer et al., 2006; Harvey and Boksa, 2012), including schizophrenia (Miller et al., 2013) and depression/anxiety (Enayati et al., 2012). Interestingly, recent evidence was provided demonstrating that prolonged inflammation during pregnancy is associated with increased hippocampal excitability in the offspring in an animal model (Pineda et al., 2013). By using the Poly I:C (polyinosinic-polycytidylic acid) model of inflammation, we addressed the possibility that a single inflammatory event during pregnancy may alter neurodevelopment, leading to neurologic disorders in the offspring.

Our results demonstrate that a single injection of Poly I:C at gestation day 9 causes higher susceptibility to kainate-induced seizures in the offspring. This is associated with increased permeability of the Blood-Brain Barrier (BBB), due to alterations in tight junctions, as demonstrated by a reduction of Claudin-5 expression levels.

No changes in the expression levels of synaptic proteins was observed in the offspring of Poly I:C-treated mothers. Electrophysiological recordings of neuronal cultures obtained from mouse embryos from Poly I:C-injected mothers and relative controls showed no defects in the excitation-inhibition balance.

Our results thus open to the possibility that inflammation during early pregnancy may increase susceptibility to epilepsy by interfering with normal brain vascularization and BBB formation.
Role of Kv2.1 channel in corticostriatal circuitry during neuronal stress

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Striatal function is heavily modulated by dopamine, and it controls a variety of functions including locomotor activity, cognition, emotion, food intake, and endocrine function. Many types of neurological dysfunction have been linked to a dysregulation of dopaminergic transmission. However, the striatum does not function in isolation, being interconnected with many other brain regions. Disruption of neural networks that include the striatum has been associated with several disease states. Kv2.1, a voltage gated potassium channel, is thought to be involved in the innate protective response of neurons. Paradoxically, enhanced K+ efflux has been shown to be an essential mediator in programmed cell death. Our hypothesis is that a toxic insult may increase K+ efflux via Kv2.1 channel but lead to different outcomes in the same neural circuit. Because mitochondrial dysfunction is thought to induce hyperexcitability of neurons, we employed mitochondrial toxins and examined the neuronal coping mechanisms, focusing on Kv2.1 channels in corticostriatal circuitry. We used two mitochondrial inhibitors for this purpose, 3-nitropropionic acid (3-NP) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). We found 3-NP dose dependently increased Kv2.1 channel expression using immunoblotting and quantitative PCR in cortical neurons and SH-SY5Y cells. In vivo, we observed a change of Kv2.1 channel expression in the cortex and striatum from mice chronically injected with 3NP or MPTP. We determined whether manipulation of Kv2.1 channel expression affects neuronal survival after 3NP or MPTP insult. From these experiments we expect to gain a better understanding of Kv2.1 modulation and the functional consequences of that modulation in corticostriatal circuitry during neuronal stress. (This work is supported by MOST 1032320B006005MY2)
Role of IKca and Kv2.1 potassium channels in neuronal differentiation

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Previous work on ion channel activity during neuronal differentiation has established that K+-channel currents are highly regulated. At early stages of neuronal differentiation, action potentials have relatively long duration and the inward current is carried primary by Ca2+, but this duration becomes shorter in more differentiated neurons as the inward current is carried mainly by K+. We are interested in investigating the regulation of two distinct K+ channels during this process. The intermediate conductance calcium-activated potassium channel (IKCa) was recently found to be localized to neurons within the CNS. Recent studies have shown that IKCa regulates neuronal precursor cells (NPCs) migration to the olfactory bulb in adult rodent brains, but it becomes undetectable in these differentiated/mature neurons. The other K+ channel is Kv2.1 which is the major subtype of voltage-gated potassium channels in the cortex and hippocampus. According to previous studies, Kv2.1 channel expression increases with neuron differentiation. We observed a decrease of IKca channels but an increase of Kv2.1 channels in mRNA, protein and channel activity using quantitative PCR, immunoblotting and patch clamp recording in an differentiated motor neuron like cell line. We are currently conducting experiments to test whether IKCa and Kv2.1 channels participate in driving neuronal differentiation using primary cortical and sensory neurons as well as NPCs. Concurrently, we are investigating the signaling pathway that regulate IKCa and Kv2.1 channel expression and their effect on neurite outgrowth. We expect that identifying the role and regulatory signaling pathway of IKCa and Kv2.1 channels in neuronal differentiation could provide more understanding in neuronal development.
Bmp5 regulates neural crest cell survival and proliferation via two different signalling pathways

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The BMP signalling cascade can initiate canonical and non-canonical pathways to govern cellular responses; however, the underlying mechanisms remain unclear. Here we show that zebrafish bmp5 is expressed in neural crest progenitor cells and that it activates the Smad and Erk signalling pathways to regulate cell survival and proliferation, respectively. Loss-of-function analysis showed that Bmp5 was required for cell survival and this response is mediated by the Smad–Msxb signalling cascade. However, the Bmp5–Smad–Msxb signalling pathway had no effect on cell proliferation. In contrast, Bmp5 was sufficient to induce cell proliferation through the Mek–Erk–Id3 signalling cascade, whereas disruption of this signalling cascade had no effect on cell survival. Taken together, our results demonstrate an important regulatory mechanism for BMP-initiated signal transduction in cellular function and also reveal a critical pathway for neural crest development.
The transcription factor hairy/E(spl)-related 2 induces proliferation of neural progenitors and regulates neurogenesis and gliogenesis

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The study of molecular regulation in neural development provides information to understand how diverse neural cells are generated. It also helps to establish therapeutic strategies for the treatment of neural degenerative disorders and brain tumors. The Hairy/E(spl) family members are potential targets of Notch signaling, which is fundamental to neural cell maintenance, cell fate decisions, and compartment boundary formation. In this study, we isolated a zebrafish homolog of Hairy/E(spl), her2, and showed that this gene is expressed in neural progenitor cells and in the developing nervous system. The expression of her2 required Notch activation, as revealed by a Notch-defective mutant and a chemical inhibitor, N-[N-(3,5-difluorophenacyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT). The endogenous expression of Her2 was altered by both overexpression and morpholino-knockdown approaches, and the results demonstrated that Her2 was both necessary and sufficient to promote the proliferation of neural progenitors by inhibiting the transcription of the cell cycle inhibitors cdkn1a, cdkn1ba, and cdkn1bb. Her2 knockdown caused premature neuronal differentiation, which indicates that Her2 is essential for inhibiting neuronal differentiation. At a later stage of neural development, Her2 could induce glial differentiation. The overexpression of Her2 constructs lacking the bHLH or WRPW domain phenocopied the effect of the morpholino knockdown, demonstrating the essential function of these two domains and further confirming the knockdown specificity. In conclusion, our data reveal that Her2 promotes progenitor proliferation and maintains progenitor characteristics by inhibiting neuronal differentiation. Together, these two mechanisms ensure the proper development of the neural progenitor cell pool.
The role of Cortactin binding protein 2 in neuronal morphogenesis

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Dendritic spines are important subcellular structure for neuron to receive the input from presynaptic neuron. Specifically, dendritic spines are the major locations of excitatory synapses in mammalian brain. Previous studies indicated that the development defects of dendritic spines may result in various neuropsychiatric diseases. Recently, we identified a neuron-specific gene, CTTNBP2, which regulates the formation and maintenance of dendritic spines by regulate Cortactin protein mobility. Besides, CTTNBP2 controls synaptic distribution of the protein phosphatase 2A. Here, we show that in addition to associating with F-actin cytoskeletons via the interaction with Cortactin, CTTNBP2 also associated with microtubules, increased microtubule stability and consequently regulated dendritic development. We identified that the middle (Mid) region of CTTNBP2 associated with microtubules. However, the association with the Mid region is not sufficient for microtubule regulation per se. The homo-oligomerization through the N-terminal NCC region of CTTNBP2 is also necessary. In cultured hippocampal neurons, knockdown of CTTNBP2 or expression of the Mid or NCC domain alone reduced the acetylation levels of microtubules and impaired early stage dendrite outgrowth and dendritic arborization. Our study suggests that CTTNBP2 influences both the F-actin and microtubule cytoskeletons and thus performs two distinct functions in neuronal morphogenesis: dendritic spine formation and dendritic morphogenesis.
ER morphology regulation in dendritic spinogenesis and memory

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Valosin-containing protein (VCP; also known as p97), the causative gene of frontotemporal dementia (IBMPFD) and amyotrophic lateral sclerosis, encodes a hexameric AAA+ ATPase that functions as a chaperon to control diverse cellular processes, including endoplasmic reticulum (ER) and Golgi morphogenesis, ER-associated protein degradation (ERAD), the ubiquitin-proteasome system (UPS) and others. However, it is unclear which pathway downstream of VCP controls dendritic spinogenesis. Using confocal laser scanning and 3D-structured illumination microscopy (3D-SIM) to analyze cultured neurons and in utero electroporated brains, our results demonstrate that VCP, together with its cofactor P47, regulate tubular endoplasmic reticulum formation and thus control dendritic spine formation. The ER formation disturbance and the consequence of physiological imbalance may as a pathogenic hallmark of a range of neurological disorders.
Functional characterization of steroid receptor RNA activator in human pluripotent stem cells

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Human pluripotent stem cells possess an ability to give rise to essentially all cell types in the body, thus occupying pluripotent state. Because of their pluripotency, they have been employed as a model to study early embryonic development including early neurogenesis, which has been shown to be controlled by various epigenetic mechanisms. Yet, how epigenetic processes regulate neurogenesis is not well understood. We found that the long non-coding RNA steroid receptor RNA activator (SRA) associates with trithorax group (TrxG) and polycomb repressive complex 2 (PRC2). TrxG and PRC2 complexes establish bivalent modification comprising of histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 27 trimethylation (H3K27me3), respectively. Expression of SRA and its binding partner RNA helicase p68 is transiently down-regulated during early retinoic acid-induced differentiation of human pluripotent stem cells NTERA2. Similar to previous reports using other cell lines, the majority of p68 is localized within nucleus of NTERA2. Short hairpin RNA interference of SRA led to down-regulation of OCT4, while PAX6 is up-regulated. However, in contrast to PAX6, expression of other lineage-specific genes including GATA6, T and CDX2 is down-regulated. Our results therefore suggest the role of SRA as an attenuator of neural differentiation in human pluripotent stem cells.
Retinal Basal Glia Development in Drosophila Eye Disc

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The retinal basal glia (RBG) play important roles in neuronal development in the eye of Drosophila. In 2nd instar larvae, there are about 6 to 20 RBGs in the optic stalk. RBG cells migrate into the eye disc at the 3rd instar larval stage while the photoreceptor cells (R cells) begin to differentiate. Three major types of RBG, namely surface glia (SG), wrapping glia (WG) and carpet glia (CG), have been identified based on morphological and molecular criteria. Silies et al. (2007) proposed a “sequential differentiation model” that SGs migrate along the CGs and come in contact with the photoreceptor cell when they reach the anterior edge of CG, and then the migratory SGs differentiate into WGs for wrapping axon of R cell. However, we used the twin-spot mosaic analysis with repressible cell markers (twin-spot MARCM) method and showed that there were cell-lineage decisions in both WG and SG. It is inconsistent with the sequential differentiation model. We also performed live imaging of eye disc to monitor the behavior of RBGs. In previous published studies, the eye disc can be cultured only for a few hours. We have improved the method and can culture the eye disc for more than 16 hours and showed that the disc developed normally under such condition. This new method allowed us to monitor the migratory and proliferative behavior of RBGs and has provided new insights into RBG differentiation.
CPEB4 regulates olfactory experience-dependent granule cell survival in the early postnatal olfactory bulbs

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Cytoplasmic polyadenylation element binding protein 4 (CPEB4) is a sequence-specific RNA-binding protein, which promotes polyadenylation-induced translation of target mRNAs. Although CPEB4 is distributed widely in the brain, the neuronal function of CPEB4 remains to be revealed. In this study, we found that 3-month-old CPEB4 knockout (KO) mice had smaller olfactory bulbs (OBs), in which the granule cell layer was significantly reduced. CPEB4 null mice with normal olfactory sensitivity and memory displayed impaired olfactory discrimination, which may result from the reduction of granule cells. Since OBs continue to replenish with new interneurons from adult neurogenesis to maintain its size, we first examined if any defect in this process. Unexpectedly, no difference in adult neurogenesis between wild-type (WT) and KO littermates was found. Instead, CPEB4 deficiency-induced OB hypoplasia resulted from increased apoptosis in granule cells during the early (i.e. the first two weeks) postnatal OB development. Moreover, sensory deprivation by naris occlusion enhanced granule cell apoptosis and reduced OB size in WT but not CPEB4 KO mice, indicating that CPEB4 governs OB growth in an olfactory experience-dependent manner. Furthermore, using RNA immunoprecipitation coupled with microarray, several RNA candidates bound by CPEB4 were identified. Currently, we are investigating which RNA targets are indeed translationally regulated by CPEB4 to contribute to the granule cell survival during OB development.
Mir-17~92 governs motor neuron subtype survival by mediating nuclear PTEN

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Motor neurons (MNs) are unique because they project their axons outside of the CNS to innervate the peripheral muscles. Limb-innervating lateral motor column MNs (LMC-MNs) travel substantially to innervate distal limb mesenchyme. How LMC-MNs finetune the balance between survival and apoptosis while wiring the sensorimotor circuit en route remains unclear. Here, we show that the mir-17~92 cluster is enriched in embryonic stem cell (ESC)-derived LMC-MNs and that conditional mir-17~92 deletion in MNs results in the death of LMC-MNs in vitro and in vivo. mir-17~92 overexpression rescues MNs from apoptosis, which occurs spontaneously during embryonic development. PTEN is a primary target of mir-17~92 responsible for LMC MN degeneration. Additionally, mir-17~92 directly targets components of E3 ubiquitin ligases, affecting PTEN subcellular localization through monoubiquitination. This miRNA-mediated regulation modulates both target expression and target subcellular localization, providing LMC-MNs with an intricate defensive mechanism that controls their survival.
Molecular mechanisms for zinc-induced allosteric potentiation of GlyR α1 receptors

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The inhibitory glycine receptor (GlyR)-mediated fast synaptic transmission is involved in motor control and other physiological processes. Deficits in channel functions of GlyR α1 subunit are linked to human hyperekplexia. Previous studies and our recent findings have revealed that the lack of positive zinc modulation at W170 site on GlyR α1 is sufficient to affect normal synaptic transmission and leads to human hyperekplexia. However, it remains unknown how this W170 constitute a molecular pathway that mediates the micro-structural changes and transduces conformational changes from zinc binding to allosteric potentiation of GlyR. In the present study, we found that mutation of L195 adjacent to W170, reduced the sensitivity of zinc-mediated potentiation. W170C mutation abolished zinc-mediated potentiation, also dramatically reduced glycine-induced current responses of GlyR α1. Interestingly, cysteine cross-link between W170C and L195C not only restored glycine-induced currents comparable to the wild type receptor, but also partially rescued zinc-mediated potentiation of GlyR α1. Furthermore, either W170C or I210C single mutations impaired the glycine-induced current response, which was, however, totally rescued by double mutations of W170C and I210C. Unlike the Cys cross-link of W170C/L195C, the cross-link of W170C/I210C did not rescue zinc-mediated potentiation on GlyR α1. Our results indicate that W170 acts as a hub between the zinc modulation and the receptor activation. These findings also suggest that the connection between β8 and β10 strands is required for agonist-induced channel opening, whereas the connection between β8 and β9 strands is crucial for signal transduction from the allosteric modulation to the channel activation.
The mechanism of resveratrol in preventing paraquat-induced mitochondrial dysfunction on a cellular model of Parkinson’s disease

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Herbicides paraquat (PQ) is considered to be the main culprit of causing PD, it is well known that PQ-induced neuronal cell death, however, PQ how to cause the mitochondrial dysfunction is still unclear. We are trying to evaluate if resveratrol (Res) can against PQ-induced neuronal cell damage especially in mitochondrial function by using cell cultures of SH-SY5Y cells and dopaminergic neurons. The toxic effects of PQ on SH-SY5Y cells show that the inhibition of PQ is a dose dependent manner and the IC50 of PQ is about 0.15 mM. Interestingly, pretreatment with higher concentrations of Res can significantly rescue the PQ-induced inhibition implicated that the Res displays protective action on SH-SY5Y cells from PQ's toxicity. PQ can markedly induce mitochondrial morphology from smooth pattern change to circle-like by observation from mitotracker staining and mito-EGFP plasmid transfection. Importantly, pretreatment of Res can attenuate PQ-induced circle-like mitochondria, however, this phenomenon is not occurred in pretreatment with dibuacine and cyclosporin A, a mitochondria outer membrane permeabilization inhibitor and a mitochondria permeability transition pore inhibitor, respectively, both can effectively rescue PQ's toxicity. Interestingly, PQ caused total Drp1 expression increase, a promoting mitochondrial fission molecule, but this phenomenon can be reversed by Res. PQ induces cytosolic Bax and Bak increase and pretreatment with Res can reduce PQ-induced Bak increase. Finally, these results of PQ's toxicity and protective action of Res reappear in the primary culture midbrain dopamine neurons. From our results indicating that Res might offer another therapeutic strategy for preventing PQ-induced neuronal cell damage.
N-terminal Gcm Confers Specific Degradation Signals That Regulate Drosophila Gliogenesis

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A fundamental issue during nervous system development is how individual cells are formed from the undefined precursors. Differentiated neurons and glia, two major cell types mediating neuronal function, are acquired from immature precursors via a series of explicit controls exerted by transcription factors such as proteins in the family of Glial Cells Missing (Gcm). In mammals, Gcm proteins are involved in placenta and parathyroid gland development, whereas in the invertebrate organism Drosophila, Gcm proteins act as fate determinants for glial cells, regulate neural stem cell (NSC) induction and conversion, and promote glial proliferation. In particular, Gcm protein levels are carefully tuned for Drosophila gliogenesis and their stability is under precise control via the ubiquitin-proteasome system (UPS). Here we describe a versatile form of Gcm, which contains a N-terminus amino acid substitution Arginine to Leucine (R59L) associated with the mammalian disease hypoparathyroidism. Our results show that GcmR59L protein is less stable and exhibits a shorter half-life, suggesting that GcmR59L is a faster degrading form of Gcm. The instability inferred from R59L is altered in the presence of a proteasome inhibitor and due to hyperubiquitination, indicating a potential involvement of UPS. Interestingly, GcmR59L proteins exhibit an altered profile for intrinsic phosphorylation status, leading to abnormal degradation. Finally, in-vivo analysis shows that GcmR59L is less competent in inducing Drosophila gliogenesis, reinforcing the significance of protein stability in the contexts of transcription activation and disease-related mechanism.
Laminin-A Regulates Synaptic Plasticity at Drosophila Neuromuscular Junctions

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Neurons have the abilities to change their synaptic functions and structures in response to the experiences of hyper-activation or hypo-activation. This ability has been suggested as the cellular basis for learning and memory. Despite of its importance, the downstream mechanisms for synaptic activity to mediate the change remain elusive. LanA, an alpha subunit of laminin, has been shown to regulate the synaptic growth of Drosophila larval neuromuscular junctions (NMJs). Drosophila larval NMJs exhibit high degree of structural plasticity, forming new boutons in response to acute synaptic activation. We were able to assay the effect of LanA on the induction of new boutons, and showed that LanA levels are negatively correlated with the activation threshold required for new boutons induction. Furthermore, synaptic activation acutely down-regulates the level of LanA at NMJs, and this down-regulation can be blocked by treatment of inhibitor of matrix metalloproteases (MMPs), which have also been shown to suppress the induction of new boutons. Thus, I propose a model, in which activity-dependent MMP activation mediates the clearance of LanA at NMJs, thus releasing its inhibition on structural plasticity, promoting new boutons to form after hyper-activation of motor neurons.
A long noncoding RNA cluster demarcates Hox boundary during motor neuron development

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Long noncoding RNAs (lncRNAs) are an emerging mediator that regulates gene expression by controlling epigenetic regulation. It has been proposed that the collinear expression of Hox genes is mediated by H3 lysine 27 tri-methylation (H3K27me3), and the mutually exclusive expression of different set of Hox genes control motor neuron (MN) subtype identity along the rostrocaudal axis of the developing spinal cord. However, whether lncRNAs coordinate the epigenetic landscape to control MN subtypes in vivo is remains unexplored. Here, we investigated functions of MN-enriched lncRNAs during ESC derived MN differentiation by performing strand specific RNA-seq, and revealed that lncRNAs Meg3, Rian, and Migr encoded within Dlk1-Dio3 imprinted locus were highly expressed in MNs in vitro and in vivo. To screen the targets of this lncRNA cluster, we derived a maternal deletion of intergenic differentially methylated region (IG-DMRMatΔ/Pat+) ESCs manifesting abrogated expression of these lncRNAs. We profiled gene expressions by microarray and observed that compared to wild-type ESC-differentiated MNs, the expressions of posterior Hox genes (Hox7~13) were increased in mutants. In addition, we found that Hoxa5 reduction in MNs causes a concomitant Hoxc8 expansion in the brachial segment in IG-DMRMatΔ/Pat+ spinal cords. We are currently examining the molecular mechanism underlying the elevation of caudal Hox gene expression in the IG-DMRMatΔ/Pat+ embryos. Collectively, these results provide critical information for lncRNAs function and will fill in the information on lncRNA-mediated MN development.
Overconnectivity in stem cell-derived neurons of an autistic child.

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De novo deletions and nonsense mutations of SHANK2 (SH3-domain and ankyrin repeats) have been implicated in several autism spectrum disorder (ASD) cases. To determine how ASD-associated SHANK2 mutations affect human neuronal function, we have generated induced pluripotent stem cells (iPSC) from two children with ASD, one harboring a nonsense mutation and the other a deletion, as well as their parental controls. To isolate the effect of SHANK2, we have corrected the nonsense mutation in ASD iPSCs and knocked out SHANK2 in iPSCs from an unrelated neurotypic individual using CRISPR/Cas9 technology. Using directed differentiation by inhibition of BMP, Activin/Nodal, and Wnt signaling, we have generated neural precursor cells (NPCs), which we use to generate cortical neurons. To assess synaptogenesis, four week-old neurons are re-seeded on mouse astrocytes to promote synapse formation. Five weeks after re-seeding, electrophysiological and imaging approaches are used in parallel to assess synaptic connectivity and cell morphology. Both synapse number and the frequency of AMPAR miniature excitatory postsynaptic currents are increased in neurons of the autistic individual with the nonsense mutation, suggesting the cells make an overabundance of functional synaptic connections. This increase is driven primarily by increases in dendrite length and complexity, as synapse density does not change. We are currently investigating whether SHANK2 knockout and deletion cells share the same phenotype, and whether SHANK2 correction in ASD iPSCs rescues it. Using these approaches, we aim to determine how development and function are perturbed in neurons specific to children with ASD and SHANK2 mutations.
Spreading Depolarization Promotes Astrocytic Calcium Oscillations and Enhances Gliotransmission to Hippocampal Neurons

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Spreading depolarization (SD) is a pathophysiological phenomenon, which manifests as propagating waves of profound depolarization of neurons and glia in the grey matter. SD is involved in various neurological conditions, including migraine, traumatic brain injury, subarachnoid hemorrhage, and malignant stroke. Under these conditions, SD causes cell swelling, disrupted brain metabolism, and compromised neurovascular coupling, leading to SD-related secondary brain injury. SD could induce neuronal hyperexcitability and this might contribute to the symptomatology of migraine attacks. Although the propagation of SD wave is mainly mediated by neuronal signaling, astrocytes exhibit concurrent Ca\textsuperscript{2+} waves associated with the propagating SD wave. However, it remains unknown how astrocyte activities are modulated and how astrocytes contribute to modulation of neuronal excitabilities after SD. In the present study, we report that after the initial astroglial Ca\textsuperscript{2+} wave that has been reported to co-occur with SD propagation, SD also enhanced astrocyte activities by promoting a secondary period of Ca\textsuperscript{2+} oscillations. The SD-induced Ca\textsuperscript{2+} oscillations were originated from non-synaptic transmissions and were independent of activation of metabotropic glutamate or ATP receptors. The secondary Ca\textsuperscript{2+} oscillations likely resulted from enhancement of PLC- and IP3 receptor-dependent intrinsic astrocyte activities and were originated from different mechanisms as compared to the SD-accompanied initial Ca\textsuperscript{2+} waves. Furthermore, the elevated astrocytic signaling increased the number of NMDA receptor-mediated slow inward currents (SICs) in hippocampal pyramidal neurons. We conclude that SD enhances activities of the astrocyte network that further promote gliotransmissions and neuronal excitabilities.