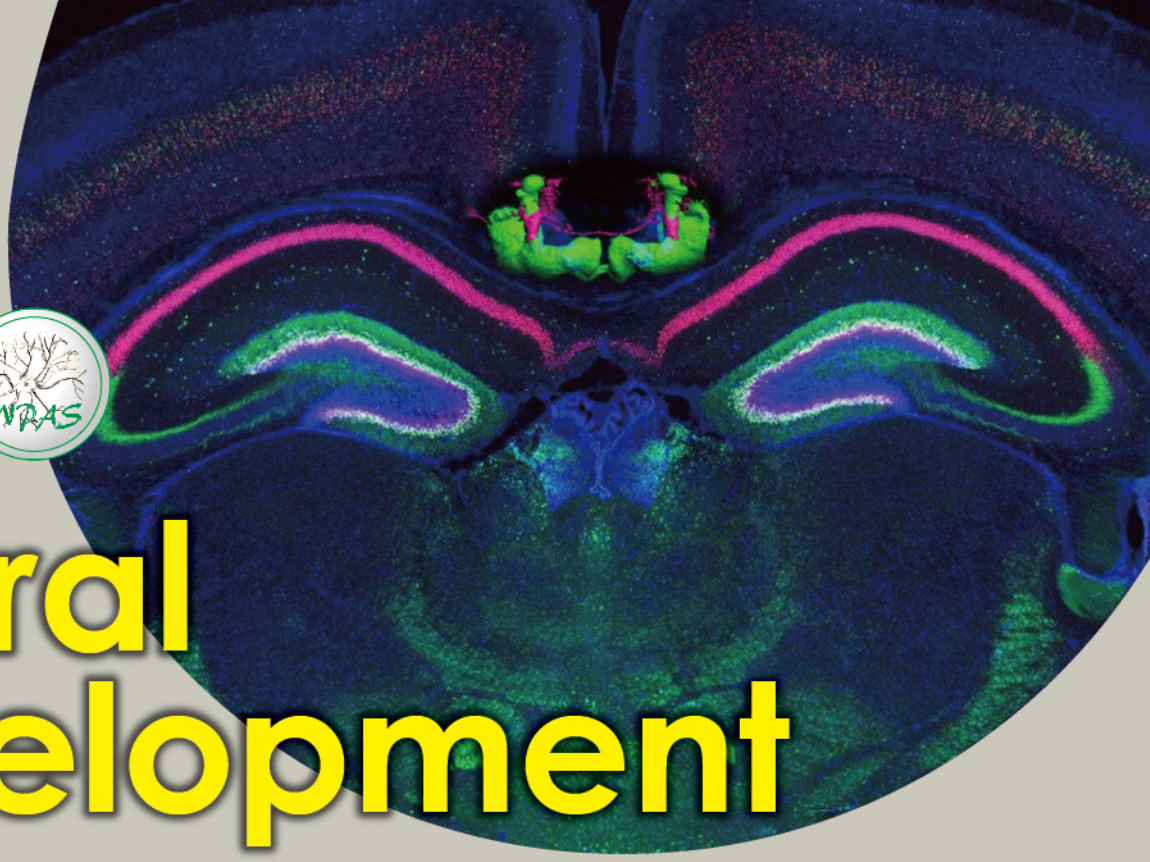

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科技部

Oral Presentation

Neural Plasticity from Synapse to Cognition

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The cognitive functions of the brain such as learning and memory depend on the ability of neural circuits to change their properties of signal processing in response to prior use. Many of these use-dependent changes ("plasticity") occur at synapses. Depending on the pattern of neuronal activities, repetitive synaptic transmission could cause long-term potentiation (LTP) or long-term depression (LTD) of the synapse in its efficacy for future transmission. I will summarize our studies on how the timing of neuronal activities (spikes) in the pre- and post-synaptic neurons determines whether a synapse undergoes LTP or LTD, a phenomenon known as "Spike Timing-Dependent Plasticity" (STDP), and how STDP may provide the mechanism for coding and storing the information on the temporal sequence and interval of sensory signals, two key elements of episodic memories. I will also discuss in general the idea that neural plasticity is the main factor that shapes the development of neural circuits, and that neural plasticity offers the potential for functional recovery from injuries and diseases of the adult brain. Finally, to argue that higher cognitive functions in humans such self-awareness may originate from experience-dependent neural plasticity, I will present our recent findings showing that mirror self-recognition, a cognitive function known to be limited only to humans and great apes, could be acquired by rhesus monkeys following training of visual-somatosensory or visual-proprioceptive association.

Novel mechanisms of neurogenesis and neural repair

Magdalena Götz

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We study the mechanisms of neurogenesis in order to implement them for neuronal repair. I will present unpublished work about the role of a novel centrosomal protein which is crucial for formation of the subventricular zone and retention of cells therein, a key event in cerebral cortex evolution. I will further present new data on the molecular function of Trnp1, a novel nuclear protein, with key roles in cerebral cortex folding and seeding of the basal radial glial cells into the SVZ in ferret (Stahl et al., 2013; Martinez-Martinez et al., 2016). Both these proteins highlight that previously considered general cell biological functions are rather cell type specific and their modulation even in subtypes of neural stem cells exerts key roles during neurogenesis and expansion of the cerebral cortex. I will then show that Trnp1 is also critical for direct neuronal reprogramming (Masserdotti et al., 2015) and up-date on the recent breakthrough in direct glia-to-neuron conversion after brain injury (Gascon et al., 2016; unpublished data). Taken together, our knowledge about basic mechanisms of neurogenesis allowed making great strides towards neuronal repair.

Stem cell heterogeneity in the adult brain

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Stem cells in the adult hippocampus produce neurones that have important functions in memory and mood control. Most adult hippocampal stem cells (AHSCs) are quiescent while a small fraction proliferate in response to various physiological stimuli or to injury. How AHSCs compute the diverse stimuli and downstream niche signals they receive to produce appropriate numbers of neurones is unknown.

We found that the transcription factor Ascl1 is essential for activation of AHSCs. Moreover Ascl1 expression is controlled by different post-translational mechanisms at different stages in the AHSC lineage. Ascl1 is transcribed in quiescent AHSCs but Ascl1 protein accumulation is suppressed by the repressor Id4, via sequestration of Ascl1 dimerisation partner and degradation of monomeric Ascl1. Ascl1 protein is also actively degraded in proliferating AHSCs, by a different mechanisms involving the E3 ubiquitin ligase Huwe1. We are characterising the niche signals that controlling Ascl1 protein levels via regulation of Id4 and Huwe1.

Active elimination of the pro-activation factor Ascl1 is essential for a fraction of proliferating AHSCs to return to quiescence. Interestingly, AHSCs that have previously proliferated and have returned to quiescence (which we call 'resting stem cells') have a unique role in maintaining homeostatic hippocampal neurogenesis. In contrast, stem cells that have not previously proliferated ('dormant stem cells') have a limited role in homeostatic neurogenesis, suggesting they may serve as a reserve stem cell population.

We are currently investigating whether resting and dormant stem cell populations are differentially activated by niche signals and by physiological and injury stimuli.

Mechanisms underlying the anterior expansion of the central nervous system

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Linkoping University, Sweden

The central nervous system (CNS) is a defining feature of bilaterally symmetric animals (bilateria), and can be grossly sub-divided into the brain and the nerve cord. A striking feature of the CNS is the anterior expansion of the brain relative to the nerve cord. This feature is evolutionarily conserved; evident in annelids, early arthropods and chordates, and becoming increasingly pronounced in vertebrates to reach its zenith in mammals, with the dramatic expansion of the telencephalon. However, the driving forces underlying this size-difference are not well understood. We are addressing this expansion in both Drosophila and mouse. We find that the brain, in both Drosophila and mouse, displays several distinguishing features that contribute to anterior CNS expansion. These include extended progenitor proliferation, more elaborate daughter cell proliferation and more rapid cell cycles. With regards to the genetic control of these features, enhanced brain proliferation is severely reduced by ectopic Hox gene expression, by either Hox misexpression or by loss of Polycomb Group (PcG) function. Interestingly, in PcG mutants, early CNS proliferation appears unaffected, whereas subsequently, brain proliferation is severely reduced. Hence, a conserved PcG-Hox program promotes the anterior expansion of the CNS. In addition, the profound differences in proliferation and in the underlying genetic mechanisms between brain and nerve cord lend support to the emerging concept of separate evolutionary origins of these two CNS regions.

Neural Development of the Cortico-Basal Ganglia Circuits

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The cortico-basal ganglia circuits are emerging as important neural circuits for speech and language as evidenced by human FOXP2 mutation studies. The circuits are also implicated in ASD pathophysioloy in which language development and function are affected. Many individuals with autism spectrum disorder (ASD) have devastating problems with spoken language. The pathological mechanisms underlying these speech and language deficits in ASD are yet unknown. The cortico-basal ganglia circuits may be a convergent site where language genes interacts with ASD-risk genes. Here, we identified ASD-risk gene Mef2c as a direct target gene suppressed by Foxp2 in the striatum. With mouse models, we show that Mef2c suppresses corticostriatal synaptogenesis and striatal dendritic spine formation. Mef2c can itself be repressed by Foxp2 through direct DNA binding. Deletion of Foxp2 de-represses Mef2c, which causes reductions in corticostriatal synpatogenesis and ultrasonic vocalization behaviors in neonatal mice. Genetic decrease of Mef2c rescues synaptogenesis and vocal communication of Foxp2-deletion mutant mice. Our study provides insights into the mechanism underlying synaptic wiring of the neural circuits related to speech and language that may be previously underestimated in the pathophysiology of ASD.

Transient moving organizers: life and death in cortical development, evolution and pathology

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The neocortex represents the brain structure that has been subjected to a major expansion in its relative size during the course of mammalian evolution. An exquisite coordination of appropriate growth and spatial patterning is required for regionalization of the cortical primordium and the formation of functional areas.

During development, progenitors expressing the Dbx1 homeodomain transcription factor are positioned at patterning centers. In mice we have shown that at the earliest stages of corticogenesis Dbx1+ progenitors give rise to subsequent waves of glutamatergic neurons which have the unique characteristics to migrate tangentially at long distance and to be transiently present during development. Cortical patterning and the fine tuning of neuronal numbers leading to the formation of functional areas depends on the migration of Dbx1-derived transient neurons. By signaling to cortical progenitors in the mitotic compartment these neurons serve as organizers during development, therefore acting as “mobile signaling units”. Our work points towards a novel general strategy for long-range patterning in large structures whereby morphogens at signaling centers induce the generation of migrating cells which by producing themselves morphogens deliver them at distant locations.

We will discuss how life and death of transient migrating neurons influence cortical function and dysfunction, in particular the molecular mechanisms controlling their generation and death in normal development and pathological conditions. Furthermore, our results indicate that the acquisition of Dbx1 expression at patterning centers and of migrating transient signaling neurons in primates might represent one of the evolutionary steps leading to increase vertebrate brain size and complexity.

Epigenetic mechanism balances human neural stem cell self-renewal and differentiation

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During neural development, neural stem cell self-renewal, specification, and differentiation require precise gene regulation. Developmental gene regulation is mediated by the combined actions of transcriptional networks, signal transduction pathways, and epigenetic modifiers. The epigenetic modifier UTX facilitates the transition from suppressed to active gene expression by removing the suppressive histone H3 lysine 27 (H3K27) methylation and promoting active chromatin structure. Dysfunction of UTX in humans and mice cause severe neurodevelopmental defects, suggesting a crucial role of UTX in neural development. However, relatively little is known about how UTX mediates chromatin structural changes and regulates gene expression during neural development.

Using immunoprecipitation-mass spectrometry, biochemical pull downs, and chromatin immunoprecipitation-deep sequencing, we discovered 53BP1 as a UTX-binding protein in human embryonic stem cells (hESCs) and neural stem cells (hNSCs). 53BP1 was initially discovered as a protein that binds the p53 tumor suppressor but now is best known for regulating DNA damage response. More than 80% of the binding sites of UTX and 53BP1 overlap genome-wide in hESCs and peak at or near transcription start sites. Neural lineage differentiation assay found that, in comparison to control hNSCs, 53BP1-knockout hNSCs and UTX-knockout hNSCs increased in proliferation and failed in neuronal differentiation. Gene expression profiling found UTX and 53BP1 co-promote genes involved in neural development. Further, 53BP1 affects H3K27 acetylation significantly more than H3K27 methylation, suggesting UTX and 53BP1 partially overlap in promoting open chromatin structure and active gene expression of neurodevelopmental genes. Our findings support a key epigenetic synergism promoting human neural development.

Modeling neural development and diseases using human iPSCs

Guo-li Ming
University of Pennsylvania

Developmental neurological disorders, such as psychiatric disorders and congenital brain disorders, are chronic and generally disabling brain disorders with a prominent genetic basis. For example, a number of susceptibility genetic loci have been identified for schizophrenia, including rare but high penetrant single gene mutations, copy number variations and polygenetic load of SNPs. How dysfunction of these genetic mutations leads to aberrant neural development and contribute to the pathophysiology of the disorder is largely unknown. Rare mutations in the DISC1 gene has been shown to confer a high risk for schizophrenia and other major mental disorders, yet its function in human neural development is largely unknown. To understand how mutation of DISC1 gene in patients impacts the development of human neurons, we generated iPSC lines from multiple patients from one family with a rare DISC1 mutation and differentiated these iPSCs into forebrain neurons in high efficiency. We have identified critical roles of DISC1 in morphological developmental and synaptic development of human neurons derived from patient specific iPSCs. Recently, we have also generated 3D organoid culture system from iPSCs and I will talk about our recent progress in using this system to model human neural development and developmental diseases.

Homeoprotein signaling from early development to adulthood

Alain Prochiantz

College de France, Center for Interdisciplinary Research in Biology

Signaling is generally understood as resulting from the interaction of membrane receptors with secreted agonists, or antagonists, followed by changes in intracellular transduction cascades and, very often, modifications taking place at the nuclear level. However, exceptions exist as some molecules, generally lipophilic, can cross the plasma membrane and bind cytosolic receptors, often taken to the nucleus to regulate transcription. In this presentation, recent findings demonstrating that homeoprotein transcription factors can slide between cells and exert direct non-cell autonomous activities will described. The focus will be on neuroepithelium aeralization, cell and axon migration, and cerebral cortex physiological homeostasis. If time permits, the consequences for a better understanding of some neurological and psychiatric diseases and the development of innovative therapeutic strategies will be discussed.

Transcription factors in cortical patterning: The role of orphan nuclear receptor NR2F1 in cortical regional patterning

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The mammalian cerebral cortex is a remarkably complex organ responsible for the perception of sensory stimuli, the execution of motor actions, learning, and consciousness. Patterning of the cerebral cortex into distinct regions is a critical developmental process for the function of the cerebral cortex. From medial to lateral, the cerebral cortex consists of three major regions: archicortex, neocortex, and piriform cortex. Each of these cortical regions has unique anatomical properties, connectivity, and specific functions. For example, the archicortex encodes spatial information, the neocortex receives and processes modality-specific sensory inputs, and the paleocortex processes olfactory inputs. So far, the molecular mechanisms for cortical regional patterning are largely unknown. The nuclear receptor NR2F1 (COUP-TF1) is expressed in a graded fashion in the cortical progenitors located in the ventricular zone, as well as in the postmitotic cortical neurons during development. We previously demonstrated that the expression level of NR2F1 determines the size and location of primary sensory areas. Here, we used Emx1-Cre to cross with NR2F1 floxed and conditional transgenic mice, and we demonstrated that the changes in the NR2F1 expression level in the developing cortices lead to changes in the position of the caudal border of the neocortex. Using additional Cre and inducible Cre lines to delete NR2F1 in the cortex at different developmental stages, we further defined the timing for NR2F1 to function in regional patterning. Our findings suggest that similar to their functions in area patterning, transcription factors expressed in graded fashions are likely to regulate cortical regional patterning.

How do glia sculpt synaptic connectivity?

Cagla Eroglu

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Our brains host billions of neurons that establish trillions of synaptic connections with each other. This complex synaptic web is organized into the neural circuits that direct our motor, sensory and cognitive functions. What are the cellular and molecular interactions that control how this complex synaptic network is weaved during development and remodeled during learning and disease? This is the main question that drives research in my laboratory. Our perspective includes glial cells, such as astrocytes, as active participants in the development, remodeling and function of synaptic circuits.

Studies in the last fifteen years have uncovered that astrocytes are powerful controllers of synapse formation, function, plasticity and elimination, both in health and disease. Research from our laboratory revealed a number of important molecular and cellular mechanisms that mediate astrocyte-neuron signaling, which control synapse formation and maturation. Currently, we are continuing to understand the function of astrocyte-neuron communication in the normal mammalian brain. Moreover, we are investigating how problems in astrocyte-neuron communication significantly contribute to the pathophysiology of neurodevelopmental disorders and neurodegeneration. Here, I will share some recent findings from my laboratory.

Glia-derived micro-RNA in synapse and trachea development and hypoxia response behavior

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The developmental processes of the vascular and the nervous systems are highly coordinated, and physiological functions of both systems are also interconnected through glia. To study developmental regulation of both systems, we characterized the neuromuscular junction at muscles 6 and 7 of Drosophila larvae for the interaction with tracheal branches. To identify factors that co-regulate the developmental processes between both vascular and nervous systems, we screened a collection of miRNA mutants, as miRNAs being known to be secreted for function in long distance. One mutant that deletes mir-274 shows reduced numbers in both tracheal branches and synaptic boutons. We showed that mir-274 is required in glia for these developmental processes. Also, miR-274 was detected in exosomes of the circulating hemolymph. In addition to the developmental defects, the hypoxia-induced exploratory behavior is sensitized in the mutant, and the normal behavior requires glial miR-274. We will discuss the separate targets in development and behavior. Thus, glia-derived microRNA serves as the liaison coordinating tracheal and synaptic growth, and further modulate behavior output during hypoxia.

Activity-dependent propagation of oxidative stress promotes motor neuron hyperexcitation and alters neuromuscular junction architecture upon depletion of Drosophila Eaat1

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Oxidative stress is thought as the promising risk causing amyotrophic lateral sclerosis (ALS), a devastating motor neuron degenerative disease. However, it remains unclear how oxidative stress is boosted in disease motor neurons. In ALS, perturbation in glutamate clearance via astrocyte EAATs would result in hyperexcitated motor neurons, elevating oxidative stress. Here we utilized the Drosophila larval motor system to investigate the interplay between hyperexcitation and oxidative stress. During larval locomotion, the motor neurons are activated by cholinergic interneurons but suppressed by gabaergic and glutamatergic interneurons. When Drosophila eaat1 (deaat1) is removed, excessive perisynaptic glutamate seems to desensitize glutamate-gated chloride channel expressed on the motor neurons, thereby prolonging excitation. Such hyperexcitation can elicit excessive intracellular Ca2+ elevation to generate the production of reactive oxygen species in mitochondria and cytosols in the motor neurons, similar to the ALS motor neurons. Intriguingly, we found that either genetic or pharmacological removal of oxidative stress normalizes hyperexcitation, as well as associated larval locomotion deficit. Furthermore, exposure of semi-intact larvae to H2O2 acutely triggers hyperexcitation in the motor neurons. Hence, oxidative stress indeed mediates and triggers hyperexcitation, which promotes self-propagation in an activity-dependent manner. Finally, defective function and structure of the neuromuscular junction are often associated with ALS. Consistently, we noted that increased oxidative stress can also influence normal architecture of the fly neuromuscular junction through activation of JNK signal. Together, our results shed light on the mechanism for boosting oxidative stress in the motor neuron, which may underlie the pathogenesis of ALS.

Transcriptional and epigenetic regulation of glial differentiation in Drosophila

Angela Giangrande
IGBMC, France

Multipotent stem cells produce differentiated cells through the implementation of specific transcriptional programs and the transition from multi to monopotency is associated with epigenetic regulation. Less well understood is whether cell-specific epigenetic features characterize differentiated cell populations. This has become an emerging field of investigation due to the long-term impact of epigenetic mechanisms.

Interestingly, glia and neurons of the Drosophila embryos are characterized by different levels of acetylation of the lysine 9 on the histone 3 (H3K9ac), which are low in glia, high in neurons.

The absolute amounts of this mark are strictly associated with the different cell fates in the nervous system, because the expression of the only fly gliogenic transcription factor is sufficient to induce glial differentiation and low level of H3K9ac when expressed at ectopic positions.

Because the levels of H3K9ac do not seem connected to the overall transcriptional activity of the different cell populations, we are now exploring the possibility that different epigenetic mark are associated with specific biological/molecular processes. We will present the ChIP seq and transcriptomic data obtained on sorted neurons and glia of the Drosophila embryo.

Dissecting the cell-specific regulation and role of epigenetic marks will provide a novel tool to understand neural development and physiology.

Molecular regulation of oligodendrocyte differentiation and its application on myelination

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Oligodendrocytes (OLGs) are generated from OLG precursor cells (OPCs) in the central nervous system (CNS), and produce myelin sheaths to wrap the CNS axons for the propagation of action potentials. Thus, understanding molecules that regulate OLG differentiation and maturation can bring an impact on developing effective strategy for demyelinating disorders. We previously characterized several genes enriched in tumorigenic glioma cells. B-cell leukemia/lymphoma-11b (Bcl11b) and BRCA1-BRCA2-containing complex subunit 3 (Brcc3) were found to positively control glioma tumorigenicity through either the inhibition of cell cycle arrest regulators or the upregulation of DNA repair. Recently, we showed that Bcl11b acts as a transcriptional repressor of p21 and Olig1 in OPCs. Bcl11b downregulation can promote OLG differentiation along with an increase in the expression of PLP and MOG. The progenitor cells either with Bcl11b-KD implanted into a lysolecithin-induced demyelinating animal model can efficiently differentiate into OLGs at the lesioned site of the white matter. Thus, our findings provide insight into the functional roles of Bcl11b in the negative regulation of OLG differentiation through the repression of OLG differentiation-associated genes. In contrast, Brcc3 gene knockdown (KD) in OPCs can cause the downregulation of the expression of genes (e.g. Olig1, Sox10, MBP, and Plp1) that are responsible for OLG maturation. Furthermore, we found that the Lys63-ubiquitination was increased in OLGs derived from OPCs with Brcc3-KD. While the mechanism underlying Brcc3 regulation of OPC differentiation is not fully resolved, our results demonstrate that Brcc3 is required for OLG maturation possibly through dynamic regulation of ubiquitination and deubiquitination.

Abnormal control of GABAergic signaling by astrocytes in Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by the CAG expansion in the exon 1 of huntingtin gene. The resultant mutant Huntingtin protein (mHTT) is expressed in many types of brain cells (including neurons, astrocytes, and microglia). Given that drugs targeting GABAA receptors (GABAARs) may ease a number of notorious HD symptoms (such as insomnia and anxiety), we had evaluated the function of GABA pharmacological agents and found that patients and mice with HD have an inferior function of GABAARs. Moreover, the expression of Na+-K+-2Cl− cotransporter isoform 1 (NKCC1), a key player in GABAergic signaling, was abnormally enhanced in the striatum of HD mice (R6/2 and Hdh150Q/7Q) and the caudate nucleus of HD patients. The reversal potential for GABAAR-mediated Cl− currents was also moved toward more positive potentials in striatal neurons of R6/2 mice. Importantly, astrocytic expression of mHTT was required to cause the up-regulation of neuronal NKCC1 in HD mice (R6/2). The astrocyte-conditioned medium collected from primary astrocytes stimulated with inflammatory cytokines enhanced the expression of NKCC1 in a striatal progenitor cell line. Administration with a dominant-negative inhibitor (Xpro1959) of soluble TNF-α normalized the up-regulation of NKCC1, suggesting the involvement of inflammation, and the impaired motor coordination. Taken together, these findings suggest that NKCC1 is an important therapeutic target for the treatment of motor dysfunction in HD. The dysregulated GABAergic response and signaling in HD mice appear to be authentic and may contribute to the symptoms of HD patients.

Active zone scaffold proteins tune functional diversity across brain synapses

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Recently, high throughput electron microscopy has started to reveal complete wiring diagrams of single circuits and whole brain regions, for example in the Drosophila olfactory sensory and learning center. However, efficacy, timing, and frequency tuning of synaptic vesicle release are highly diversified across the development of brain circuitry. Systematic knowledge regarding the functional features of synapse types will be required for a satisfactory understanding and functional modeling of neural circuits. Using light superresolution microscopy, we provide evidence that presynaptic active zone scaffold protein diversity controls functional diversity across Drosophila brain synapses: distinct patterns of scaffold complexes differentially recruit specific Unc13 isoforms to steer transmission dynamics in a neuron-specific manner by conferring diverse nanometer-precise positioning of vesicle release sites to Ca2+ channels. In this manner, a compositional code of such stereotypic release modules diversifies synapse response properties. Our analysis provides ‘nanoscopic molecular fingerprints’ of synapse types which helps in understanding specific synaptic features in circuit modeling.

Control of vesicle release position near receptors in single synapses

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Plasticity of synaptic function presumably arises from a combination of altering the protein content and molecular organization of individual contact sites. Recent work from many types of glutamatergic synapses has made clear that both presynaptic and postsynaptic function arise from precise organization of key molecular species within subsynaptic ensembles tens of nanometers in diameter. In the postsynaptic density, clustered scaffolding proteins create subdomains enriched in AMPA and NMDA receptors. We find that RIM and other key proteins that regulate vesicle fusion are mutually co-enriched within subregions of the AZ. By mapping sites of single-vesicle fusion events within individual AZs, we revealed that evoked fusion occurs in a confined subregion of the AZ where RIM density is highest. Consistent with this, we found using expansion microscopy that voltage-gated Ca channels are highly enriched near RIM. Remarkably, dSTORM showed that in hippocampal neurons in culture or in brain slices, the distributions of RIM and receptors are highly co-aligned across the synaptic cleft. Numerical modeling indicates that this simple organizational principle provides a means by which CNS synapses can maintain and modulate synaptic efficiency. Because numerous forms of synaptic plasticity are executed by the dense and dynamic spine actin cytoskeleton, we explored whether manipulation of actin filaments alters trans-synaptic alignment. Treatment of neuron cultures with latrunculin indeed strongly altered measures of nanoscale alignment, notably decreasing the concentration of presynaptic RIM in close registration with nanoclusters of postsynaptic PSD-95. This suggests that activity-dependent regulation of cytoskeleton may dynamically tune synaptic function via controlled transsynaptic alignment.

From god's kitchen: How the pre synapse is made

Volker Haucke[1, 2, 3], Anela Vukja[1], Ulises Rey[2, 4], Astrid Petzoldt[2], Dennis Vollweiter[1], Christoph Ott[1], Christine Quentin[2], Dmytro Puchkov[1], Eric Reynolds[2], Martin Lehmann[1], Stephan Sigrist[2], et al.

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Nervous system function relies on the polarized architecture of neurons, established by directional transport of pre- and postsynaptic cargoes. While delivery of postsynaptic components depends on the secretory pathway, the identity of the membrane compartment that supplies presynaptic active zone (AZ) and synaptic vesicle (SV) proteins is unknown. In my talk I will present our recent studies on presynaptic biogenesis. Using combined live imaging in Drosophila larvae and mouse hippocampal neurons we found that presynaptic biogenesis is mediated by axonal co-transport of AZ and SV proteins in presynaptic lysosome-related vesicles (PLVs). Loss of the lysosomal kinesin adaptor Arl8 results in the accumulation of AZ and SV protein-containing vesicles in neuronal cell bodies and a corresponding depletion of AZ and SV components from presynaptic sites leading to impaired neurotransmission. Conversely, axonal transport of AZ proteins and presynaptic function are facilitated by genetic upregulation of PLV transport. Our data reveal an unexpected function for a lysosome-related organelle as the basic building block for presynaptic biogenesis.

Dendrite Pruning of Nociceptive Sensory Neurons in Drosophila

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Selective removal of unnecessary or exuberant neuronal processes without loss of parent neurons, referred to as neurite pruning, is a crucial step of neurite remodeling during animal development. Drosophila nociceptive sensory neurons, ddaCs, selectively prune their larval dendrites in response to a late-larval pulse of the molting steroid hormone ecdysone. We have conducted two large-scale genetic screens and screened 15,000 RNAi lines. We identified a genetic pathway composed of the transcription factor Sox14 and the important cytoskeletal regulator Mical that act downstream of the ecdysone signaling to regulate neurite pruning (Kirilly D., et al., Nat Neurosci. 2009). We also identified two epigenetic factor, namely a Brahma (Brm)-containing chromatin remodeler, a histone acetyltransferase CREB-binding protein (CBP) that binds to the sox14 locus in an ecdysone-dependent manner to induce Sox14 expression (Kirilly D. et al., Neuron 2011). This RNAi screen also revealed a conserved E3 ligase that inactivates the insulin signaling pathway to regulate dendrite pruning (Wong JJL. et al., PLoS Biol. 2013). Moreover, we identified Rab5/ESCRT-dependent endocytic pathways which play crucial roles in dendrite pruning of ddaC neurons. Disruption of Rab5 or ESCRT function causes formation of enlarged endosomes and aberrant ubiquitinated protein deposits in mutant ddaC neurons. We identified a highly conserved L1-type cell adhesion molecule (CAM) Neuroglian (Nrg), which is degraded by the endolysosomal pathway prior to dendrite pruning (Zhang H., et al., Dev Cell 2014; Wang Y., et al., Development 2017). More recently, we have conducted a large-scale clonal screen and isolates new mutants with severe pruning defects.

An intimate liaison: fasciculation with axon shapes dendritic development

Chun-Hao Chen, Chun-Liang Pan

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Selective fasciculation of axons or dendrites is a pivotal mechanism that shapes neuronal trajectory and circuit connectivity. In Caenorhabitis elegans, primary dendrites of the multidendritic nociceptor PVD neuron extend horizontally in the lateral nerve cord and fasciculate with axons of the ALA interneuron. This dendrite-axon fasciculation offers an excellent opportunity to investigate signaling mechanisms that support such adhesive dendrite guidance. We will present experimental evidence suggesting the following: (1) the ALA axon functions as a pioneering guidepost for the highly stereotyped outgrowth and projection of the PVD primary dendrites, (2) adhesive signaling composed of an unusual membrane ligand (L1CAM) for a conserved guidance receptor (Robo) instructs such fasciculated dendrite growth, (3) fasciculation with the ALA axon aligns actin dynamics in the PVD growth cone for directed dendrite extension, and (4) disruption of ALA-PVD fasciculation may impair proprioception. These observations suggest that axon-dendrite fasciculation via adhesive signaling plays important roles in the wiring and proper functioning of the neural circuitry.

Haploinsufficiency of the intellectual disability-gene SETD5 disturbs developmental gene expression and cognition

Gaia Novarino, et al.
IST AUSTRIA

SETD5 gene mutations have been identified as a frequent cause of idiopathic intellectual disability. Here we elucidate the pathogenic mechanism of Setd5 haploinsuffisciency. Setd5 mutant mice show developmental defects such as abnormal brain to body size ratio and neural crest defect-associated phenotypes. Furthermore, Setd5 mutant mice show impairments in cognitive tasks and behavioral inflexibility. Learning defects in mutant animals are accompanied by abnormal histone acetylation and regulation of gene expression. Our results emphasize the decisive role of Setd5 and the Hdac3 complex in a biological pathway found to be disrupted in intellectual disability and autism spectrum disorder patients.

Using Mouse Models to Assess Developmental Deficits in Neurological Disorder

Yi-Ping Hsueh
Academia SInica

My team has been aiming to elucidate how neurons differentiate their crucial subcellular structures, including synapses, dendrites and axons, to achieve the function of receiving and delivering signals among neurons. Because appropriate neurodevelopment is essential for neural function, it is not surprising that the genes regulating neural development are associated with neurological diseases, particularly neurodevelopmental disorders. To further elucidate the molecular etiology of neurodevelopmental disorders, in addition to neuronal morphology, we have also extended our study to mouse behavior analyses, particularly those related to autism-like behaviors, and circuit characterization using tracing approaches and others. Our studies have revealed the detailed molecular regulation of neural development, as well as the molecular etiology of several neurological disorders. Our most recent research studies about neuronal signaling and autism spectrum disorders will be reported in the meeting.

Key words: Autism spectrum disorders; Neural development.

Constructing excitatory synapses at the nanoscale

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The spatio-temporal organization of neurotransmitter receptors in the postsynaptic membrane is a fundamental determinant of synaptic transmission and thus information processing by the brain. Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule superresolution imaging techniques and video-microscopy, we have established that AMPARs are not stable in the synapse as thought initially, but undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion and that AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. Recently, it has been established that these AMPAR nanodomains could be selectively localized in front of glutamate release sites.

These results open the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. Using new methods to exogenously control AMPAR surface diffusion, we have been able to demonstrate that AMPAR surface diffusion directly controls the establishment of long term synaptic plasticity. Altogether, these data suggest that regulating AMPAR surface trafficking represents a new pathway to regulate synaptic transmission and its plasticity.

We will discuss recent results on how the construction of glutamatergic synapses could depend on the nanoscale organization of neurotransmitter receptors relative to pre-synaptic release sites, and how this could be a fundamental determinant of both the amplitude and reliability of synaptic transmission.

Super-resolution imaging of brain extracellular space

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The extracellular space (ECS) of the brain provides the physical stage and signaling platform where neuronal and glial players perform in concert. While the ECS takes up a fifth of brain volume, its topology is incredibly complex and miniaturized, defying traditional investigative approaches. Consequently, despite a marked interest in the physiological roles of brain ECS, its structure and dynamics remain largely inaccessible for experimenters. We combined 3D-STED microscopy and fluorescent labeling of the extracellular fluid to develop super-resolution shadow imaging (SUSHI) of brain ECS in living organotypic brain slices. SUSHI enables quantitative analysis of ECS structure and reveals dynamics on multiple scales in response to a variety of physiological stimuli. Because SUSHI produces sharp negative images of all cellular structures, it enables unbiased imaging of unlabeled brain cells with respect to their anatomical context. Moreover, the extracellular labeling strategy greatly alleviates problems of photobleaching and phototoxicity associated with traditional imaging approaches. As a straightforward variant of STED microscopy, SUSHI provides unprecedented access to the structure and dynamics of live brain ECS and neuropil.

Paxillin-associated Endocytic Activity Enables Timely Neurite Initiation on a Soft Substrate Environment

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Newborn neurons develop long protrusions spontaneously on a soft tissue environment, a behavior distinct from most adherent cells and essential for establishing axonal projections and dendritic territory; however, the mechanism underlying such ability has remained unclear. Here, we report that neurons can decode mechanical properties and translate into neurite initiation on different spatial-temporal scales by a paxillin-associated molecular switch and corresponding genetic responses. We cultured hippocampal neurons on a soft substrate whose elasticity resembled that of brain tissue and discovered that neurons undergo biphasic activity as neurites emerge. The dominance of either state, which was apparent as rapidly as 5 hours after cell plating, was determined by environmental mechanical properties and cellular endocytic expression and activity. Furthermore, we found that clathrin-mediated vesicle invagination is regulated by paxillin, via direct interactions with the membrane curvature-sensing factor CIP4, a process favored on a soft substrate and required for timely neurite initiation both in culture and in vivo in the developing cortex. Interestingly, in cell culture these activities were compromised when cells were grown on stiff substrates, which favor adhesion and contraction, and resulted in unsynchronized initiation of segmented lamellipodia, which serve as neurite precursors. Such a coordinated genetic and environment response explains how neurites and, perhaps, aggressive tumors, grow preferentially on or toward soft substrates, even though those surfaces generate minimal traction forces.

GRASP1 Regulates Synaptic Plasticity and Learning through Endosomal Recycling of AMPA Receptors

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Learning depends on experience-dependent modification of synaptic efficacy and neuronal connectivity in the brain. We provide direct evidence for physiological roles of the recycling endosome protein GRASP1 in glutamatergic synapse function and animal behavior. Mice lacking GRASP1 showed abnormal excitatory synapse number, synaptic plasticity, and hippocampal-dependent learning and memory due to a failure in learning-induced synaptic AMPAR incorporation. We identified two GRASP1 point mutations from intellectual disability (ID) patients that showed convergent disruptive effects on AMPAR recycling and glutamate uncaging-induced structural and functional plasticity. Wild-type GRASP1, but not ID mutants, rescued spine loss in hippocampal CA1 neurons in Grasp1 knockout mice. Together, these results demonstrate a requirement for normal recycling endosome function in AMPAR-dependent synaptic function and neuronal connectivity in vivo, and suggest a potential role for GRASP1 in the pathophysiology of human cognitive disorders.

Activity-dependent remodelling of synaptic microenvironment

Dmitri Rusakov, et al.

UCL Institute of Neurology, University College London

Memory trace in the brain is believed to involve structural remodelling of synaptic connections. This is likely to engage ultrathin astroglial processes that often occur in the immediate proximity of excitatory synapses. Although astroglia have been emerging as an important regulator of synaptic circuitry, the causal relationships between activity-triggered synaptic restructuring and the changes in nearby astroglia remain poorly understood. We combined single-cell electrophysiology with two-photon excitation microscopy, photolytic uncaging, super-resolution techniques, and correlational 3D electron microscopy, to monitor fine astroglial morphology during the induction of synaptic long-term potentiation (LTP). We document NMDA receptor dependent-withdrawal of astroglial processes from the vicinity of synapses following LTP induction, both at the level of synaptic populations and at the level of individually monitored potentiated synapses. The reduction in synaptic astroglial coverage boosts the extra-synaptic escape of released glutamate thus facilitating NMDA receptor-mediated cross-talk among neighbouring synapses. The cellular mechanisms underlying astroglial restructuring involve local Ca2+ elevations but do not depend on metabotropic glutamate receptors, IP3-receptor signalling, aquaporins, or Ephrin-associated morphogenesis. They do require the ion exchanger NCCK1, thus pointing to the underlying ion and water homeostasis machinery. Experiments are under way to understand activity-dependent changes in the 3D nano-organisation of perisynaptically expressed signalling proteins using dSTORM imaging.

Modeling SHANK3-related autism in non-human primate

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Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impaired social communication and restricted and repetitive patterns of behaviors and interests. Mutations in SH3 domain and ankyrin repeat containing protein 3 (SHANK3) are one of the best characterized and replicated genetic defects in ASD patients. Genetically modified Shank3 mutant mice have served as valuable tools to dissect the pathophysiology of SHANK3-related ASD. However, there are significant evolutional differences between mouse and human brain and behaviors. These differences pose many challenges to assess the translational value of rodent models. Using the CRISPR/Cas9 genome editing method, we successfully introduced deleterious mutations in three monkey offspring, two aborted embryos and one live birth. SHANK3M1 showed complete deficiency of SHANK3 in the prefrontal cortex (PFC) and consequently a reduction of select postsynaptic proteins of GluN2B, PSD95, mGluR5 but normal levels of GluN1, GluA2 and Homer, in conjunction of a decrease in dendritic spine density. The number of NeuN-positive mature neurons was markedly reduced but the number of GFAP-positive astrocytes was increased in the PFC of SHANK3M1 brain, indicating a previously unknown and crucial role for SHANK3 in neurogenesis. The surviving SHANK3M3 mutant exhibited apparent behavioral and brain anatomy defects recapitulating that of ASD patients. Our findings from SHANK3 mutant monkeys are distinct from that of multiple independent lines of Shank3 deficiency mice and support the necessity and value of non-human primate model for ASD in future studies.

Defining and diversifying dendrite arbor topology

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Dendrite arbor topology determines the number, distribution and integration of neuron inputs, and neuron firing properties. Mature dendrite arbor pattern is the compound outcome of a series of branching events; with specific branches created early in dendrite outgrowth required to delineate the arbor into distinct main subtrees for arbor compartmentalization. We do not to understand the logic and molecular programs through which these fundamental topological patterning features of dendrites are defined. This is because in order to reveal this requires methods to simplifying the complexity of the differentiation process into discrete event units, and to identify which units generating specific features—an integrated approach spanning different spatiotemporal levels. Here, we reveal a specialized program underlying major branch formation show driven by a transient local upregulation of anterograde-directed microtubule nucleation at the dendrite tip. This process is separable from a continuous background of retrograde directed dendrite microtubule polymerization. By a genetic screen utilizing in vivo imaging coupled with automated dendrite feature detection and quantification, we identify the atypical myosin, Myo6, as a principal player in this process. Myo6 drives localized stabilization of single F-actin filaments at the tip, which in turn captures and target anterograde-directed microtubule polymerization events into discrete filopodia, driving tip-partition for major branch creation. Moreover we show differential use this process acts to control neuron type diversification. Overall, we reveal a tunable program that defines and diversifies critical major dendritic topological branch features that underlie circuit wiring and computation.

Cooperation among prefrontal-amygdala circuits to control fear behavior

Cyril Herry
INSERM, France

When facing danger, mammals display a broad range of fear behavior ranging from active (avoidance) to passive (freezing) fear responses. The canonical model of fear circuits posits that the basolateral amygdala directly controls fear responses through projections to the brainstem. Using state of the art behavioral, electrophysiological and optogenetic manipulations we provide evidence challenging this view. Our results indicate that in parallel to the amygdala (i) specific cell populations within the medial prefrontal cortex support different coding strategy for fear behavior and (ii) that specific manipulation of prefrontal neurons projecting to the brainstem directly regulate conditioned fear responses.

Neural dynamics of a memory trace in the hippocampal circuit

David Dupret
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Memory is critical for effective behaviour, enabling individuals to draw from past experience how to best tailor current and future actions. Principal cells of the dorsal hippocampus have been suggested to contribute to such memory-guided behaviour by providing a representation of experienced places. In my talk, I will support this view by showing that editing a hippocampal representation allows re-balancing a drug-place memory. I will further discuss how neuronal communication between the hippocampus and the nucleus accumbens underpins the behavioural manifestation of such an internal representation of space at the service of appropriate contextual behaviour.

Neuronal circuitry of retinal input to the central clock suprachiasmatic nucleus

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The suprachiasmatic nucleus (SCN) located at the hypothalamus is the master clock that controls the circadian rhythm for mammals. The SCN contains at least three different types of neurons including arginine vasopressin (AVP) expressing, vasoactive intestinal peptide (VIP) expressing, and gastrin releasing peptide(GRP) cells. Previous studies suggested that photic inputs from the retina are sent to VIP and GRP cells, which synchronized the whole population of SCN neurons for photoentrainment. The AVP cells are primary output neurons. However, anterograde labeling of intrinsically photosensitive retinal ganglion cells (ipRGC) axon showed that the whole SCN are covered. To determine the connection between the retina and the SCN, we labeled single ipRGC and reconstruct their axonal architecture in the SCN. We found that ipRGCs from different parts of the retina innervate different region of the SCN including the ventral, middle and dorsal regions, which are enriched with VIP, GRP, and AVP neuron respectively. Using ex vivo calcium imaging, we also found that AVP neuron can be directly activated by glutamate, which is mainly released by ipRGC in the SCN. Therefore, our data suggest that the retinal inputs are sent to different SCN neurons to form a multi-level of network connection instead of a linear feed-forward circuit. Thus, light may influence our clock and the clock-related physiological functions in a complex circuitry.

Water reward memory in Drosophila

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The intake of water is important for the survival of all animals and drinking water can be used as a reward in thirsty animals. Thirsty Drosophila melanogaster can associate drinking water with an odor to form a water-reward memory. Here, we found that thirsty flies form a protein-synthesis-dependent water-reward long-term memory (LTM) that requires the crammer, tequila, and radish genes. Furthermore, we found that the reinforcement of LTM requires water-responsive dopaminergic neurons projecting to the restricted region of mushroom body (MB) β′ lobe, which are different from the neurons required for the reinforcement of learning and short-term memory (STM). In addition, LTM required normal expression of the D1-like dopamine DopR1 receptors in α′β′ neurons. Synaptic output from α′β′ neurons is required for consolidation, whereas the output from γ and αβ neurons is required for the retrieval of LTM. Finally, two types of MB efferent neurons, MB-M6 (MBON-γ5β′2a) and MB-V3 (MBON-α3), retrieve LTM from γ and αβ neurons by releasing glutamate and acetylcholine, respectively. Our results therefore cast light on the cellular and molecular mechanisms responsible for processing water-reward LTM in Drosophila.

Neural Mechanics of a Hunger Circuit in Drosophila

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The fruit fly can evaluate its energy state and decide whether to pursue food-related cues. Here, we reveal that the mushroom body (MB) integrates hunger and satiety signals to control food-seeking behavior. We have discovered five pathways in the MB essential for hungry flies to locate and approach food. Blocking the MB-intrinsic Kenyon cells (KCs) and the MB output neurons (MBONs) in these pathways impairs food-seeking behavior. Starvation bi-directionally modulates MBON responses to a food odor, suggesting that hunger and satiety controls occur at the KC-to-MBON synapses. These controls are mediated by six types of dopaminergic neurons (DANs). By manipulating these DANs, we could inhibit food-seeking behavior in hungry flies or promote food seeking in fed flies. Finally, we show that the DANs receive surprisingly rich inputs of hunger and satiety signals. This work demonstrates an information-rich central circuit in the fly brain that controls hunger-driven food-seeking behavior.

The amygdala circuits in the regulation of divergent behaviors

Bo Li

Cold Spring Harbor Laboratory

The amygdala is essential for learning and expression of behavioral responses driven by either reward or aversive stimuli. How exactly distinct amygdala circuits contribute to the generation of such divergent behavioral responses remains unclear. Our recent studies in mice indicate that aversive stimulus-driven learning and stress induces distinct plastic changes in distributed amygdala circuits, including those in the extended amygdala. These changes may underlie specific learning processes or behavioral responses. Here I will report our recent findings regarding the cellular and circuit mechanisms underlying some of the behavioral roles of the amygdala.

Memory allocations in the Drosophila brain

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Creating long-term memory (LTM) requires allocation of learned information through new protein synthesis to specific neurons and synapses in a neurocircuit. To study circuit mechanisms regulating memory allocation in the whole brain, we need tools for deep-tissue imaging of learning-induced changes in synaptic proteins within a single neuron at single-molecule resolution. Recent advances in optical super-resolution microscopy allow 3D visualization of single protein molecules, but only within a limited depth because of light scattering and aberration within tissues. Here, we report a deep-tissue super-resolution (DSR) microscopy, achieved single-molecule localizations in the whole Drosophila brain. Applying an improved Bessel beam plane illumination to the optically-cleared brain, the DSR allows whole-brain localization of spontaneous blinking fluorophores tagged to the structures of interest. Successful mapping individual proteins in the whole brain showed that LTM requires allocation of newly synthesized vesicular monoamine transporter proteins to specific axonal sectors within a single DPM neuron innervating nearly the whole mushroom body, the memory center in the Drosophila brain. Together, our results reveal an intricate circuit mechanism involving axonal allocation of memory proteins to modulate neuronal activities within circuit motifs innervating specific mushroom body sectors for LTM formation.

Deciphering species-specific properties of human corticogenesis.

Pierre Vanderhaeghen

VIB/KULeuven and Université Libre de Bruxelles ULB, Belgium

The human cerebral cortex has undergone rapid expansion and increased complexity during recent hominid evolution. One striking feature of human corticogenesis is that it is highly protracted in time, from early steps of expansion of progenitor pools and neurogenesis, to later stages of neuronal maturation and wiring. This prolonged timing is thought to contribute in an important fashion to several key features of the human brain, such as cortical size and complexity. In vitro pluripotent stem cell-based models and in vivo mouse - human chimeric brain experiments indicate that the species-specific timing of key steps of corticogenesis is largely intrinsic to cortical progenitors and neurons. The underlying molecular mechanisms start to be uncovered, and include human-specific duplicated genes that can act at several steps to control species-specific features of human corticogenesis.

A Shh/Gli-driven three-node timer motif regulates temporal identity and fate of neural stem cells

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Neural stem cells often produce distinct neural progenies in a specific temporal sequence and over defined timeframes, but the key question of how time is measured in temporal patterning processes in the CNS remain unresolved. By examining a temporal lineage in the ventral brainstem, we have defined a Shh/Gli-driven three-node timer motif determining the time-point when neural stem cells terminate production of early-born motor neurons (MNs) and initiate generation of late-born serotonergic neurons (5HTNs). I will present data showing that network interactions conform an incoherent feedforward loop with a proportioner node, whereby Gli proteins promote expression of Phox2b and thereby MN-fate, but also account for a proportional feedforward-activation of a suppressive Tgfβ-node, which triggers the MN-to-5HTN fate switch by repressing Phox2b. The network architecture actively counterbalance fluctuations of Shh/Gli-input and provides remarkable robustness regarding temporal output.

Transcriptional Regulation of Neurogenesis

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Brain function depends on the orderly production of the correct numbers and subtypes of neurons during development. This in turn requires tight regulation of neural progenitor behavior to achieve an appropriate balance between proliferation and differentiation. However, the mechanisms regulating this behavior are unclear. Neurogenesis is initiated by the transient expression of the highly-conserved transcription factors known as proneural proteins. I will discuss our findings on how the transcriptional and post-transcriptional control of these factors and their activity fine tunes the behavior of neural progenitors and the orderly production and differentiation of neurons.

LncRNA Meg3 Choreographs the Epigenetic Landscape of Postmitotic Motor Neuron Cell Fate and Subtype Identity

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Long-noncoding RNAs (lncRNAs) are numerous and one of their major functions is to regulate transcription via epigenetic regulation. Epigenetic modifications have been shown to have pivotal roles in regulating motor neuron (MN) subtype specification. However, the interplay between long-noncoding RNAs (lncRNAs) and the epigenetic landscape during MN diversification remains unexplored. We systematically identified MN-enriched lncRNAs from embryonic stem cell (ESC)-derived MNs (ESC~MNs) and reveal that lncRNA Meg3 is highly expressed in MNs. Loss of Meg3 leads to ectopic expression of neural progenitor genes and caudal Hox genes. Mechanistically, Meg3 facilitates PRC2/Jarid2 interaction and targets them to non-neuronal genes to maintain postmitotic MN fate, while sculpting MN subtype identity by targeting caudal Hox genes. Absence of lncRNAs (including Meg3) from the Dlk1-Dio3 imprinted locus in embryos leads to ectopic Hoxc8 expression in the Hoxa5 domain and eroded axonal arborization in the proximal nerves of brachial spinal segments. We suggest that Meg3 is a critical lncRNA for choreographing MN cell fate and subtype identity.

An intrinsic mechanism controls reactivation of neural stem cells by spindle matrix proteins

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The switch between quiescence and proliferation is central for neurogenesis and its alteration is linked to neurodevelopmental disorders such as microcephaly. However, intrinsic mechanisms that reactivate Drosophila larval neural stem cells (NSCs) to exit from quiescence are not well established. Here we show that the spindle matrix complex containing Chromator (Chro) functions as a key intrinsic regulator of NSC reactivation downstream of extrinsic insulin/insulin-like growth factor signalling. Chro also prevents NSCs from re-entering quiescence at later stages. NSC-specific in vivo profiling has identified many downstream targets of Chro, including a temporal transcription factor Grainy head (Grh) and a neural stem cell quiescence-inducing factor Prospero (Pros). We show that spindle matrix proteins promote the expression of Grh and repress that of Pros in NSCs to govern their reactivation. Our data demonstrate that nuclear Chro critically regulates gene expression in NSCs at the transition from quiescence to proliferation.

Reprogramming neural fates

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The development and success of pluripotency reprogramming technology have inspired and encouraged researchers to explore the possibility of directly converting somatic cells into other types of somatic cell. At present, various cell types, including neural progenitors (NPs), can be directly induced from fibroblasts. The ability to induce expandable NP populations with the potential to form multiple types of neuron is particularly important, as it would enable the generation of disease-specific neural cell types on a scalable level for disease modeling, drug discovery, and cellular therapy. We identified a panel of neural TFs (nTFs), which were highly enriched in hESC-derived NPs as compared to fibroblasts, by comparative gene expression profiling. We defined nTF combinations, whose overexpression can efficiently convert human fibroblasts into expandable multipotent induced ENPs (iNPs). Also, we demonstrated that the iENP populations acquired the common characteristics of NPs, and the ability to give rise to astrocytes, oligodendrocytes, and functional CNS and PNS neurons upon in vitro and in vivo differentiation. By employing the iNP paradigm described in this study, we successively generated neurodegenerative disease-specific iNPs, and demonstrated that the neurons differentiated from iNPs derived from Huntington's disease and Alzheimer's disease patients exhibited disease-relevant phenotypes. Collectively, our studies demonstrate a novel paradigm for direct conversion of multipotent iNPs from human somatic cells by hESC-derived NP enriched neural TFs. This system will allow rapid generation of large quantities of expandable iNP populations with desirable neural differentiation propensities, and facilitate the discovery of novel mechanisms and drugs for neurodegenerative disease treatment and regenerative medicine.

EMBO Workshop

Neural Development

Poster Presentation

The level of COUP-TF1 expression regulates cortical regional patterning

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The cerebral cortex represents the brain region that has undergone a major increase in size and complexity during the course of evolution. Patterning of the cerebral cortex into distinct regions is a critical developmental process for the function of mammalian cerebral cortex. From medial to lateral, the cerebral cortex consists of three regions: archicortex, neocortex and piriform cortex. Different regions in the cerebral cortex have unique anatomical and functional properties. The molecular mechanisms for cortical regionalization are largely unknown. COUP-TF1 (NR2F1), a nuclear receptor, is expressed in the cortex during development. COUP-TF1 was shown to regulate the size and location of primary sensory areas. Here, we studied the function of COUP-TF1 in regional patterning of the cortex, focusing on the formation of the boundary between archicortex and neocortex.

RBM4 modulates radial migration via alternative splicing of Dab1 during cortex development

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RNA-binding motif 4 (RBM4) modulates cell differentiation via its role in alternative splicing regulation. RBM4 is enriched in ventricular zone/subventricular zone at embryonic day 12.5 and becomes profuse in the cortical cortex later during brain development. Rbm4a knockout brain exhibited delayed migration of late-born neurons. Using in utero electroporation, we confirmed that knockdown of RBM4 impaired cortical neuronal migration. We searched for RBM4-associated transcripts in the mouse embryonic brain using RNA immunoprecipitation-sequencing, and identified Disabled-1 (Dab1), encoding a Reelin signaling adaptor protein, as a potential target transcript of RBM4. Rbm4a knockout embryonic brain showed altered Dab1 isoform ratios. Overexpression of RBM4 promoted the inclusion of Dab1 exons 7 and 8 (7/8), whereas its antagonist PTBP1 acted in an opposite manner. RBM4 counteracted the effect of PTBP1 on exon 7/8 selection by competing for PTBP1 binding to intron 7. Finally, we showed that the full-length Dab1, but not exon 7/8-truncated Dab1, rescued neuronal migration defects in RBM4-depleted neurons, indicating that RBM4 plays a role in neuronal migration via modulating the expression of Dab1 splice isoforms. Our findings imply that RBM4 is necessary during brain development and that its deficiency leads to developmental brain abnormality.

Localization of actin blobs prefigures dendrite branching

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The actin cytoskeleton provides structural stability and adaptability to the cell. Neuronal dendrites frequently undergo morphological changes by emanating, elongating and withdrawing branches. However, the knowledge about actin dynamics in dendrites during these processes is limited. By performing in-vivo imaging of F-actin markers, we found that F-actin was highly dynamic and heterogeneously distributed in dendritic shafts, with enrichment at terminal dendrites. A dynamic F-actin population, we named as actin blobs, propagated bi-directionally at an average velocity of 1 μm/min. Interestingly, these actin blobs stalled at sites where new dendrites would branch out in minutes. Over-stabilization of F-actin by the G15S mutant abolished actin blobs and dendrite branching. We identified the F-actin severing protein Tsr/cofilin as a regulator of dynamic actin blobs and branching activity. Hence, actin blob localization at future branching sites represents a dendrite-branching mechanism to account for highly diversified dendritic morphology.

Investigating the role of mitochondria in neuronal migration using induce pluripotent stem cells from patients suffering from Periventricular heterotopia

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Cortical malformations encompass a wide range of neurodevelopmental disorders and are major causes of intellectual disability and epilepsy. Periventricular heterotopia (PH) is a neurodevelopmental defect caused by multiple genetic mutations and is characterized by failure of neurons to migrate to the cortical plate. Data from patient-derived induced pluripotent stem cells iPSCs and mouse models have demonstrated that the cadherin receptor-ligand pair DCHS1 and FAT4 plays an important role in the correct neuronal migration and development of the cerebral cortex. Single-cell RNA-sequencing analysis from patient-derived organoids bearing mutations in DCHS1 and FAT4 suggest that these mutations alter the expression levels of mitochondrial genes and thereby may contribute to neuronal migration defects. Moreover, single-cell RNA-sequencing analysis also highlights alterations in the expression levels of genes involved in axon development and synapse formation. Here I am to test whether abnormalities in mitochondria underlie the migration defects on neurons with mutations in DCHS1 or FAT4 and to study the electrophysiological properties of these mutant neurons. To address this, I will employ neuronal and cerebral organoids derived from patient-derived iPSCs. Finally, this research line will also give more insights into the role of mitochondria in neurodevelopment and help towards a better understanding of the molecular machinery that is altered in PH.

A pronucleotide probe unravels AMPylation as hallmark of human neuronal differentiation in cerebral organoids

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Posttranslational modification (PTM) of proteins represents an important cellular mechanism for controlling diverse functions such as signalling, localization or protein-protein interactions. Numerous PTMs have been shown to play a role in brain development and neuronal function, regulating for example the dynamics and stability of the microtubule cytoskeleton that undergoes rapid changes in neuronal migration. AMPylation has been recently discovered as a prevalent PTM regulating protein activity. In human cells, the adenosine monophosphate transfer onto serine, threonin and tyrosin is catalyzed by FICD, so far the only known AMPylator. The functional elucidation of AMPylation is still in its infancy. Here, we introduce a cell-permeable probe for infiltrating cellular AMPylation pathways and report distinct modifications in intact cancer cells, NPCs, neurons, and cerebral organoids via LC-MS/MS, as well as imaging methods. A total of 165 AMP modified proteins were identified with neuronal targets being unique and the most numerous. Functionally, we found that AMPylation inhibited cathepsin activity by modification of serine residues close to its active site. Furthermore, accelerated differentiation of progenitor cells into mature neurons and corresponding increase of AMPylation was observed in cerebral organoids carrying a FICD overexpression plasmid. These results suggest that AMPylation represents a so far unknown trigger of neuronal progenitor differentiation.

Chromatin remodeling complex and Notch signalling pathway in apoptosis of Drosophila larval neural stem cells

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Most of the genes and biological pathways are conserved across all species. We choose Drosophila melanogaster as our model organism to understand how interplay between Transcription Factors (TFs), signalling pathway , epigenetic memory together with niche orchestrate the complex central nervous system (CNS) development. We have chosen the hox gene Deformed (Dfd) to understand the patterning of CNS.

We have recently shown that the combinatorial action of three transcription factors(TFs) namely Hox, Extradenticle and Grainyhead on a 717bp enhancer is required for transcriptionally activating the death genes and initiate larval neural stem cells (Nbs) apoptosis (Khandelwal et al,2017). To further understand role of niche and epigenetic regulation, we did an in-vivo RNAi screen for identifying the Nbs fate regulators. Further, we have identified signalling pathway and chromatin remodelers that regulates apoptosis and differentiation of Nbs.

The Notch pathway plays a crucial role in organism development. We have found that Notch signaling for programmed cell death of larval Nbs is independent of endocytosis mechanism for internalization of Notch intracellular domain. We find that chromatin remodeling complex members are important for apoptosis of larval neural stem cells in Drosophila. Our genetic experiments with chromatin remodeling complex and Notch confirm that they work together to controlling the apoptosis of larval neural stem cells. We further observe that chromatin remodeling complex and Notch signaling are important for maintenance of the apoptotic enhancer, which in turn is important for activating the downstream apoptotic genes. Taken together, our results indicate that fine tuning between TFs, epigenetic regulators & niche is important for programmed cell death of larval Nbs.

Control of hilar mossy cell excitability regulates emotional behaviors and pattern separation

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Hilar mossy cells (MCs), the glutamatergic neurons located in the hilus of the hippocampal dentate gyrus (DG), have been known to play an important role in network function and complex information processing such as pattern separation. However, their role in emotional behaviors remains largely unexplored, especially in active form. To address this question, we manipulated the activity of MCs in the DG using a designer receptors exclusively activated by designer drugs (DREADDs)-based chemogenetic approach. Selective expression of DREADDs was achieved by injecting a virus encoding Cre-dependent DREADDs into a mossy/CA3-Cre driver, a mouse line specifically expressing Cre recombinase in the hilar MCs and CA3 cells. We found that decreasing the activity of MCs by specifically expressing inhibitory DREADDs (i.e., hM4Di receptor) on the membrane of the MCs increased the mouse innate-anxiety level. Conversely, elevating the activity of MCs by expressing excitatory DREADDs (i.e., hM3Dq receptor) decreased their innate- and defensive-related anxiety level. In addition, we tested the effect of manipulating the MC activity during and after contextual fear conditioning. We found that decreasing the MC activity induced the fear-like behavior in the retrieval phase, although mice performed normally during learning phase. Conversely, elevating the MC activity reduced the freezing level in the retrieval phase. Notably, both decreasing and elevating the activity of MCs impaired short-term function of memory formation. In summary, using the chemogenetic approach, we demonstrated that MCs in the DG may participate in controlling the anxiety-like, fear-like behaviors and memory formation.

Neurogliovascular alterations triggered by early developmental chronic consumption of methylphenidate

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Methylphenidate (MPH) is the first-line of pharmacological treatment for attention deficit hyperactivity disorder (ADHD), the most prevalent childhood neurobehavioral disorder that often persist into adulthood. Concerns have been raised about ADHD overdiagnosed and the long-term neurological consequences of MPH treatment. Thus, the purpose of this study was to understand the chronic effect of MPH use during a critical development period.

To mimic a misuse condition, healthy rats were treated with MPH (1.5 or 5 mg/kg/day, per os) from P28-P55. Our results reveal that 5 mg/kg/day MPH increases hippocampal blood-brain barrier permeability by vesicular transport together with peripheral immune cells infiltration. In addition to endothelial alterations, MPH highly interfered with astroglial morphology, namely the length and degree of cell ramification. Interestingly, we verified that a low-dose of MPH increased astrocytic processes and promoted the expression of synaptic proteins related with neurite outgrowth, axonal sprouting and signaling pathways involved in synaptic plasticity, which culminated in the improvement of working memory. By contrast, we found that a higher dose of MPH induced atrophy of astrocytes expressing less and thinner processes and reduced gliovascular interaction. These effects were coincident with the downregulation of several synaptic proteins and impairment of AKT/CREB signaling, together with working memory deficit.

To conclude, this work clearly shows that an early chronic exposure to MPH has a dose-dependent effect being harmful at higher doses. These findings not only have implications for children misdiagnosed with ADHD but also for those who deliberately misuse MPH.

Inflammatory cytokines and serotonin were involved in depression-like behaviors of SPAK null mice

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SPAK (SPS1-related proline/alanine-rich kinase) is a master regulator to control the activity of Na+/K+/Cl-- and K+/Cl--cotransporters, to maintain the ions homeostasis and cell volume of the GABA neurons. However, the role of SPAK in the CNS is still unknown. Our previous studies have demonstrated that SPAK null mice displayed superior performance in prepulse inhibition- and novel object recognition test in isolation rearing, a schizophrenia-like animal model, suggesting SPAK might play a role in neurological disorder of CNS. SPAK null mice significantly dispalyed depression-like behaviors such as tail suspension test (TST) and force swimming test (FST) comparing with wild type mice. However, it is still unknown whether SPAK null mice-displayed depression-like behaviors are different between sex. Both male and female of SPAK null mice displayed markedly immobility time than wild type mice in TST. In addition, female SPAK null mice exhibited more significant immobility time but not male mice in FST. Deficiency of serotonin and BDNF as well as chronic inflammation in the brain have been reported to cause depression. Treatment with escitalopram, a selective serotonin reuptake inhibitor, can markedly remedy depression-like behavior of SPAK null mice implied that SPAK might play a role in serotonin neurotransmission, especially in depression. Interestingly, serum inflammatory cytokines of SPAK null mice, IL1beta and IFNgama, were obviously higher than wild type mice. Escitalopram treatment can significantly attenuate IL1beta but not IFNgama. To access these relevant issues to figure out the role of SPAK in depression will be necessary and important.

Whole-brain single-molecule imaging reveals memory allocation within a single neuron in Drosophila

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Memory formation requires allocation of learned information to specific neurons and synapses in a neurocircuit though new protein synthesis. To study mechanisms regulating memory allocation, we need tools to directly image and manipulate learning-induced synaptic proteins within a single neuron at single-molecule resolution. Recent advances in optical super-resolution microscopy allow 3D visualization of single protein molecules, but only within a limited depth because of light scattering and aberration within large-tissue volumes. Here, we report a deep-tissue super-resolution microscopy, called Clearing Light-Sheet Localization Microscopy (CLLM), achieved single-molecule localizations in the whole Drosophila brain. Applying the Bessel beam plane illumination to the optically-cleared brain, the CLLM allows deep-tissue localization of spontaneous blinking fluorophores tagged to any nanostructures of interest. Successful mapping individual proteins in the whole brain showed that memory formation requires synthesis of new vesicular monoamine transporter proteins distributed at specific axonal sectors within a single DPM neuron innervating the whole mushroom body, the memory center in the Drosophila brain. Thus, the developed deep-tissue super-resolution imaging allows quantitative localization of single molecules within a large-tissue volume and opens a new way to address how the brain translates behavioral training into synaptic allocation of learning-induced new proteins to encode memory.

Repression of Dlx1/2 Signaling by Nolz-1/Znf503 is Essential for Parcellation of the Striatal Complex into Dorsal and Ventral Striatum

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The striatal complex comprises the caudoputamen of the dorsal striatum and the nucleus accumbens and olfactory tubercle of the ventral striatum. The dorsal and ventral striatum control diverse functions of motor, reward learning, and motivation. A fundamental question is how striatal subdivisions are differentially specified during development. Here, we first performed genome-wide comparisons of gene expression patterns of dorsal and ventral parts of the LGE. We identified Nolz-1/Znf503 gene as a dorsal striatum-enriched gene. Nolz-1 null mutation not only resulted in a loss of cell type identity within the striatal complex but also resulted in an enlarged ventral striatum at the expense of the dorsal striatum. Deficits were evident in Isl1+ lineages of striatonigral neurons that exhibited decreased expression of striatonigral-enriched genes and increased expression of striatopallidal-enriched genes. Mechanistically, Nolz-1 mutation resulted in de-repression of Dlx1 and Dlx2, which promoted aberrant cell migration from the dorsal toward ventral striatum. Knocking down Dlx1/2 in Nolz-1 mutant striatum partially rescued aberrant cell migration and differentiation phenotypes. Conversely, over-expression of Dlx2 in E13.5 wildtype striatum promoted aberrant migration of striatal cells toward the ventral striatum, reminiscent of the Nolz-1 mutant phenotype. In conclusion, we have identified Nolz-1 as a novel transcriptional regulator that controls not only specification of cell type identity but also the balanced cell migration between the dorsal and ventral striatum.

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Neuroprotective effect of Embelin in primary rat hippocampal culture model of neurotoxicity

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder that has a high impact on millions of older people and their families. The exact cause of AD is still unknown. At present there is no treatment that can cure Alzheimer's disease. Streptozotocin (STZ) is an experimental tool to induce AD like condition. Key features of this model that mimic clinical AD are Aβ fragments, Aβ deposits and total tau protein. In our preliminary study, embelin is found to be a promising compound as a memory enhancer underscore the cholinergic hypothesis of Alzheimer's disease. Primary rat hippocampal neuronal cells were toxicated with STZ in the absence or presence of embelin at various concentration for 24 hours. Cells were treated with embelin 2 hours before STZ exposure. These treated cells were evaluated for neurotoxocity, cell viability and gene expression. It was found that embelin is neuroprotective against STZ induced toxicity and significantly modulate the mRNA expression of genes related to AD. These present study found that embelin is a potential candidate drug and provide therapeutic benefits in neurodegenerative disease.

CPEB2 Activates GRASP1 mRNA Translation and Promotes AMPA Receptor Surface Expression, Long-term Potentiation and Memory

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Activity-dependent synthesis of plasticity-related proteins is necessary to sustain long-lasting synaptic modifications and consolidate memory. Regulated mRNA translation contributes to synaptic plasticity, so we investigated the role of the translational regulator cytoplasmic polyadenylation element binding protein 2 (CPEB2) in learning and memory. Forebrain-restricted CPEB2-conditional knockout (cKO) mice exhibited impaired hippocampus-dependent memory in the contextual fear conditioning and Morris water maze tests. CPEB2-cKO hippocampi showed impaired long-term potentiation in the Schaffer collateral-CA1 pathway. Reduced surface but not total expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs) in CPEB2-KO neurons led us to identify that CPEB2 enhanced the translation of GRASP1 mRNA to facilitate recycling and maintain the surface level of AMPARs. Ectopic expression of CPEB2 or GRASP1 in CA1 areas of CPEB2-cKO mouse hippocampi rescued long-term potentiation and spatial memory in the water maze test. Thus, CPEB2-regulated GRASP1 mRNA translation is pivotal for AMPAR recycling, long-term plasticity and memory.

Knockout of infertile crescent (ifc) causes neurotoxic accumulation of dihydroceramide and facilitates neuroprotective lipophagy

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Dihydroceramide desaturases are evolutionarily conserved enzymes that convert dihydroceramide (dhCer) to ceramide (Cer). While elevated Cer levels cause neurodegenerative diseases, the neuronal activity of its direct precursor, dhCer, remains unclear. Here, we show that knockout of the fly dhCer desaturase gene, infertile crescent (ifc), results in larval lethality with increased dhCer and decreased Cer levels. Light stimulation leads to apoptotic cell death in ifc-KO photoreceptors, resulting in activity-dependent neurodegeneration. Reducing dhCer synthesis prevents photoreceptor degeneration and rescues ifc-KO lethality. These results pinpoint that dhCer accumulation is responsible for ifc-KO defects. Also, we demonstrate that dhCer accumulation increases ROS level and activates lipophagy in ifc-KO photoreceptors. Enhancing lipophagy reduces lipid droplet accumulation, decreases ROS levels, and rescues ifc-KO defects, indicating that lipophagy plays a protective role. This study demonstrates a novel requirement for dhCer desaturase in neuronal maintenance in vivo and shows that an increase in ROS induces lipophagy, and lipophagy activation can reduce the level of ROS and prevent the activity-dependent degeneration of ifc-KO photoreceptors.

Gap junctions between mushroom body neurons are involved in consolidated memory retrieval in Drosophila

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The communications between neurons are most commonly mediated by chemical synapses. However, gap junctions, the electrical synapses provide another way for neuronal communications. Gap junctions play the role for the synchronization of activity in neuronal networks but their contributions to cognition has not been well characterized. Here we show that knocking down on of the gap junction gene, innexin5 (inx5), in mushroom body (MB) disrupted olfactory aversive anesthesia-resistant memory (ARM), while leaving anesthesia-sensitive memory (ASM) and initial learning intact. Whole-mount brain immunohistochemistry indicates that INX5 proteins preferentially express in somas and the calyx regions of MBs. Adult-stage-specific silencing of inx5 in αβ neurons disrupts ARM suggesting the exclusive requirement of INX5 in αβ neurons. Moreover, the MB α-branch specific 3-hour memory trace is diminished in inx5 knockdown flies. Finally, silencing the neuronal activities in MB r memory trace is diminished in indicateRM), while halorodopsin (eNpHR), a light-gated chloride pump, also disrupts ARM. Together, our results suggest the gap junction channels composed by INX5 are essential for ARM retrieval in MB αβ neurons.

TLR3 activation alters Disc1 expression via MYD88 to control neuronal morphology

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Viral infection during fetal or neonatal stages increases the risk of developing neuropsychiatric disorders such as schizophrenia and autism spectrum disorders. Although neurons express several key regulators of innate immunity, the role of neuronal innate immunity in psychiatric disorders is still unclear. Using cultured neurons and in vivo mouse brain studies, we show here that Toll-like receptor 3 (TLR3) acts through myeloid differentiation primary response gene 88 (MYD88) to negatively control Disrupted in schizophrenia 1 (Disc1) expression, resulting in impairment of neuronal development. Cytokines are not involved in TLR3-mediated inhibition of dendrite outgrowth. Instead, TLR3 signaling suppresses expression of several psychiatric disorder-related genes, including Disc1. The impaired dendritic arborization caused by TLR3 activation is rescued by MYD88 deficiency or DISC1 overexpression. In addition, TLR3 activation at the neonatal stage increases dendritic spine density but narrows spine heads at postnatal day 21, suggesting a long-lasting effect of TLR3 activation on spinogenesis. Our study reveals a novel mechanism of TLR3 in regulation of dendritic morphology and provides an explanation for how environmental factors influence mental health.

Cap Methyltransferase 1 (CMTR1) Regulates Dendritic Development

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Eukaryotic mRNAs are capped with a 7-methylguanosine (m7G) via triphosphate bond at the 5' end of mRNAs. The cap structure has numerous roles, such as increasing stability, directing nuclear export, and enhancing cap-dependent translation of mRNAs. The cap structure in higher eukaryotic organisms is more complicated with additional modifications at the first two nucleotides. Cap methyltransferase 1 (CMTR1) catalyses 2'-O-ribose methylation of the first transcribed ribonucleotide adjacent to the m7G cap to generate cap1 (m7GpppNmN). A previous study reported that knockdown of CMTR1 in human cell lines evokes innate immune responses due to elevated cap1-deficiency mRNAs, which are recognized as non-self molecules by retinoic acid-inducible gene-I (RIG-I). Knockdown (KD) of CMTR1 in neurons caused abnormal dendritic arborization that could be rescued by ectopically expressing wild type hCMTR1 but not the mutants with the loss of catalytic activity (K239A). These results suggest that cap1 modification of mRNAs by CMTR1 in the nucleus is crucial for neuronal development. Moreover, the neuron morphology defect is not caused by innate immune response-activated type I interferon (IFN) signalling in CMTR1-KD neurons. Using RNAs isolated from control and CMTR1-KD neurons for micro-array analysis, I have identified several candidates whose expression is altered in CMTR1-KD neurons. For example, calcium/calmodulin-dependent protein kinase II alpha subunit (CaMKIIa), which plays an important role in neuronal maturation, is downregulated in CMTR1-KD neurons. Whether and how CMTR1-meditaed cap1 modification affects target-specific gene expression to promote dendritic arborization is currently under investigation.

Rab27 anchors S6K for dendritic protein synthesis in αβ posterior neurons of mushroom body to regulate lifespan extension

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The mushroom body (MB) is the regulatory center for learning, memory, and sleep in Drosophila. Whether MB regulates lifespan is unknown. MB is composed of five distinct lobes, Kenyon cells, and calyces. Here, we show that Rab27 controls lifespan within the pioneer subset of the αβ lobes (αβp) of MB. Rab27 is an evolutionarily conserved Rab GTPase mediating the exocytic machinery in neuroendocrine cells. We show that both rab27 knockout flies and αβp-specific knockdown of rab27 significantly extend lifespan. To reveal the signal transduction, reduction of rab27 leads to decreased phosphorylation of S6 (p-S6), a downstream component of target of Rapamycin (TOR) signaling. Consistently, suppression of TOR signaling by feeding Rapamycin or expressing Tsc2 does not further increase the longevity of rab27KO, indicating that Rab27 functions through TOR signaling. The molecular evidence shows that loss of rab27 leads to mislocalization of S6K within the αβ pioneer neurons. We discovered a novel node of TOR signaling in a specific brain region for lifespan extension.

C9orf72 is Essential for Neurodevelopment and Motility Mediated by Cyclin G1

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Hexanucleotide repeat expansions in the C9orf72 gene are a common genetic cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). However, the function of C9orf72 in neural development and the pathogenic mechanism underlying neurodegeneration are unknown. In this study, we disrupt C9orf72 expression in zebrafish embryos by using C9orf72 constructs that lack the complete DENN domain with reduced GTPase activity. These C9orf72-deficient zebrafish embryos exhibited impaired axonogenesis and motility defects which are identical to ALS symptoms. The C9orf72 deficiency also caused neuronal apoptosis in brain that could be rescue by Tp53 knockdown. The effect on C9orf72-deficient embryos was phenocopied by knocking down endogenous C9orf72 expression by using morpholinos. We further generated C9orf72-deficient transgenic line by transposed C9orf72 constructs into zebrafish genome. The C9orf72-deficient transgenic fish showed less social behavior in adulthood which mimicking the phenotype in FTD. Moreover, we identified Cyclin G1 as a downstream target for C9orf72, and downregulation of Cyclin G1 in C9orf72-deficient embryos rescued all the phenotypes including those with axonal, motility defects, and cell apoptosis. In summary, the results of this study reveal a novel mechanism of C9orf72 in neural development and aid in interpreting the cellular processes underlying ALS–FTD.

Etv5a regulates neural progenitors via targeting sox2 and foxm1

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The neural progenitor cells (NPCs) are self-renewal, proliferative, and multipotent cell population, which generate diverse types of neurons and glia to build the nervous system. Transcription factors have critical roles in regulating various cellular responses of NPCs, however, the transcription factors in regulating neural progenitor potency and differentiation have not been fully characterized. In the present study, we found zebrafish ets variant 5a (Etv5a, the homologue of mammalian ETV5) is expressed in d NPCs. We examined the physiological roles of Etv5a by morpholino and a truncated form to down-regulate the endogenous Etv5a function. Suppressed Etv5a function increased the proliferation of NPCs accompanying with decreased neurogenesis and gliogenesis. These phenotypes in Etv5a-depleted embryos could be rescued by co-injection of etv5a cRNA. Furthermore, Etv5a overexpression reduced sox2 expression, and we identified the putative binding sites of Etv5a in the 5' untranslated region of sox2. The directly binding of Etv5a to the regulatory elements of sox2, was affirmed by chromatin-immunoprecipitation. These data reveal the Etv5a directly suppresses the sox2 expression to reduce neural progenitor identity. In addition, the expression of foxm1, a cell cycle regulator and a putative target gene of Etv5a, was up-regulated in Etv5a-deficient embryos, and suppression of FoxM1 function by a selective inhibitor FDI-6 or dominant-negative construct restored the up-regulated proliferation of NPCs caused by Etv5 deficiency. Taken together, our result revealed that Etv5a is essential for inhibiting different transcription factors to reduce the proliferation and promote the differentiation of NPCs.

Anisotropic surface topography can preferentially guide axon outgrowth and enhance axon regeneration

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During neuron development, the axon travels across a long distance to reach the target to form a functional connection. It is known that this process is regulated by both chemical and physical cues. While the mechanism of chemical guidance of neuron is well studied, relatively few studies were aimed at studying the physical cues. To address this question, we established an anisotropic surface topography to study the contact guidance on development and the post-injury response. We have successfully generated different surface topographies on a biocompatible polymer using thermoplastic nanoimprinted lithography (T-NIL) technology. We found that neurons from both the central nervous system (cortical neurons) and peripheral nervous system (dorsal root ganglion neurons) were able to survive on the anisotropic surface topography. Moreover, we observed that the direction of axon growth strikingly followed the submicrometer-sized grooves. We also discovered that by controlling the stiffness and the width of the grooves, the surface topology could specifically guide the axons, but not the dendrites. This specific axon effect may serve as a platform for investigating axon versus dendrites contact guidance. In addition, we observed that neurons grown on the grooved surface are capable of regeneration and have a better regeneration after on-site injury. Together, our data demonstrate that the anisotropic surface topography guides axon growth and promotes axon regeneration. We will also present results on how cytoskeleton effects the axon guiding behavior.

GTP-bound Ran in the cytoplasm promotes acentrosomal microtubule nucleation in neurons

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The proper formation of microtubules (MTs) is essential for neuronal developmental processes, such as polarity, migration and differentiation. The generation of MTs in post-mitotic neurons highly depends on nucleation by acentrosomal MT-organizing centers (MTOCs). While Golgi outposts have been shown to promote acentrosomal MT formation in dendrites, the acentrosomal MTOCs in axon remain elusive. We have previously shown that TPX2 is an important component of the acentrosomal MTOC in neurons. TPX2 distributes along the neurite shaft and binds to the MTs. In addition, TPX2 depletion in neurons impairs MT formation at the tip and the base of the neurite. Because it has been shown that the small GTPase Ran regulates TPX2 activity and MT nucleation around the chromosomes during mitosis, we set out to examine whether Ran also regulates MT nucleation in neurons. Here we show that the active GTP-bound Ran concentrates in regions similar to TPX2 (i.e. at the tip of the neurite and in the soma). Additionally, acentrosomal MT formation decreases when importazole, a Ran signaling interrupting molecule, is applied. When a constitutively active Ran mutant (RanQ69L) is overexpressed in neurons, the level of RanGTP increases in the nucleus and neurite tip. Furthermore, both of the neurite length and the microtubule nucleation frequency increase. These data suggest that cytoplasmic RanGTP activates TPX2 and promotes MT nucleation in neurons. Finally, we will report an optogenetic system (RanTRAP) to reversibly control Ran activity in neurons.

Regulator of G protein signaling 2 (Rgs2) regulates neural crest development through Pparδ-Sox10 cascade

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Neural crest cells are multipotent and migratory progenitors, which differentiate into various derivatives. These characterizations of neural crest cells take them becoming an attractive model for studying various aspects of cell progression. Many ligand–receptor-activated signaling pathways and transcription factors that control neural crest development have been characterized; nevertheless, the modulators that link the ligand–receptor signaling pathways to the nucleus transcription regulation is still far from clear. In the present study, we used dominant-negative constructs and morpholino knockdown to downregulate Rgs2 function, and found that Rgs2 is a crucial factor in governing the generation of neural crest progenitors and ectomesenchymal or nonectomesenchymal derivatives. Moreover, Rgs2 deficiency increased a downstream target for Wnt signaling, Pparδ. Dominant-negative construct or Pparδ antagonist treatments rescued the neural crest defects in Rgs2-deficient embryos. Further analysis found the Pparδ upregulates the transcription of neural crest specifier, sox10, through directly binding to its promoter regions. Our study uncovered the Wnt-Rgs2-Pparδ-Sox10 signaling axis is essential for neural crest development.

Activity-induced oxidative stress propagation perturbs motor circuit rhythmicity and neuromuscular junction architecture upon depletion of Drosophila Eaat1

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Hyperexcitability is a common risk contributing to neurodegenerative diseases, particularly Amyotrophic Lateral Sclerosis (ALS). Dysregulation of glutamate transmission and intrinsic neuronal excitability can trigger hyperexcitability. Yet it remains unclear how hyperexcitability is established in the motor neuron and how it eventually causes neurodegeneration. Here we utilized the Drosophila larval motor circuit as the system to tackle these questions We found that depletion of Drosophila excitatory amino acid transporter (eatt1), encoding a high-affinity glutamate transporter which recycles presynaptic glutamate to astrocytes, results in excessive perisynaptic glutamate which seems to desensitize glutamate-gated chloride channel on the motor neuron, thereby resulting in hyperexcited motor neurons. Similar to ALS motor neurons, hyperexcitability leads to massive Ca2+ influx and generation of oxidative stress in mitochondria and cytosols in the fly motor neurons. To our surprise, removing oxidative stress by either genetic or pharmacological way significantly normalizes hyperexcitability phenotype, as well as associated larval locomotion deficit. In addition, acute treatment of hydrogen peroxide sufficiently mimics hyperexcited motor neuron phenotype associated with loss of eaat1. These findings indicate that oxidative stress mediates hyperexcitability and further its own propagation. In addition, we noted that elevated oxidative stress can also alter architecture of the neuromuscular junction, a common feature seen in most ALS patients. We are currently looking into the underlying mechanism of oxidative stress propagation. Overall, our results reveal a neuronal activity-dependent mechanism that can boost up oxidative stress in the motor neuron, which may provide new therapeutic strategies for neurodegenerative diseases.

MicroRNA Filters Hox Temporal Transcription Noise to Confer the Robustness of Boundary Formation in the Spinal Cord

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During organism developments, cell lineages are specified by several factors. In the rostrocaudal (RC) patterning of spinal cord development, extrinsic signals act on early neural progenitors within the nascent neural tube. The initial RC patterning lead to differential expression of Hox genes in postmitotic motor neurons (MNs) and specification of MN subtype identity and connectivity. Although several 3' Hox genes are expressed in progenitors in a noisy manner, these Hox proteins are not expressed in the progenitors and only become detectable in postmitotic MNs. We found microRNA biogenesis impairment leads to precocious expression and propagates the noise of Hoxa5 at the protein level, resulting in an imprecise Hoxa5-Hoxc8 boundary. Using in silico simulation, we uncovered two feed-forward HoxmiRNA loops accounting 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 boundary of Hox protein expression. Our results describe a novel trans Hox-miRNA circuit that filters transcription noise and precisely controls the timing of proteins expression to confer robust individual motor neuron identity.

Glycosphingolipids regulates active zone assembly and synaptic bouton formation and at the Drosophila neuromuscular junction

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Glycosphingolipids are known to participate in many cellular processes. However, very little is known about their role in neurons. Here we used Drosophila larval neuromuscular junction as the system to investigate the role of glycosphingolipids in synaptic development and plasticity. We generated a Drosophila mutant collection covering major glycosylation modification pathways and conducted a forward genetic screen to identify potential genes that regulate synaptic bouton formation and active zone assembly at the Drosophila larval neuromuscular junction. In this screen, we analysed the formation and architecture of synaptic bouton, the integrity of the active zone, and the organization of postsynaptic glutamate receptor. We found that the mutations in egghead (egh), encoding beta-1,4-mannosyltransferase activity which adds mannose to glucosylceramide, the early precursor of glycosphingolipid, results in a significant reduction in the number of synaptic bouton. Moreover, our immunostaining results reveal that the intensity of Bruchpilot (Brp), a key active zone scaffold protein, is decreased. Consistently, our preliminary TEM assay shows that small and aberrant T-bar ultrastructure is associated with loss of egh. This suggests that proper glycosphingolipid biogenesis or proper level of glucosylceramide or even upstream precursors is required for these important synaptic events. We are now doing tissue-specific transgene rescue and RNAi downtown experiments to understand in which tissue egh is required. Functionally, we are using electrophysiology to test the impact of these defects on synaptic transmission. We are also looking into the mechanism underlying these phenotypes. These results will be discussed in the meeting.

Supt4h is essential for the embryonic development of mouse neurons critical for perinatal respiration

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Supt4h, a transcription elongation factor, is required for expression of genes containing repetitive nucleotide sequences and has been implicated as a potential therapeutic target against neurological disorders. In earlier studies, we found that conventional Supt4h homozygous knockout of mice results in an embryonic lethality. Here, using a line of Nestin-Cre driven Supt4h conditional knockout mice, we would like to understand the biological function of Supt4h in the subject of neural development. We found that the genetic ablation has a great impact in neonatal lethality, stemming from the inability of mice to initiate respiration. By analysis of embryos at E18.5, we found that these mice have a normal innervation and neuromuscular junction formation in diaphragm, but lack the action of phrenic bursts. The respiratory generator, parafacial respiratory group (pFRG) remains intact. However, the pre-Bötzinger complex, a cluster of interneurons essential for the generation of respiratory rhythm in mammals is absent. Our results suggest that, during the developmental stage, Supt4h is directing a subset of neural cells toward their cell fates and plays an important role in perinatal respiration and survival.

Glial response to hypoxia in trachealess mutants induces synapse remodeling

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Synaptic structure and activity are highly sensitive to environmental alteration. The modulation of synaptic morphology and function is often induced by signals from glia. However, the process that synapses via glia respond to environmental perturbation such as hypoxia remains unknown. Here, we report that in the trachealess (trh) mutant synaptic boutons become smaller and form clusters named bunch boutons at Drosophila larval neuromuscular junctions (NMJs). The trh mutant is defective in tracheal branching with reduced oxygen supply. The bunch bouton phenotype in the trh mutant was suppressed by hyperoxia, and could be phenocopied in wild-type larvae raised in hypoxia. Furthermore, reducing the activity of the hypoxia responding gene similar (sima) in glia suppress the appearance of bunch boutons in the trh mutants, while glial overexpression of sima recapitulated this phenotype. Up-regulated Wingless (Wg) and modified microtubule structures to bring on the abnormal synaptogenesis. Under the severe hypoxia condition, the quantal content of synaptic transmission failed to retain. Taken together, we propose that Wg, as a hypoxia transducing signal, induced by hypoxia signal Sima in glia modulates the microtubule structure and function of synapses.

Dual role of Atoh1 in the regulation of OFD1 stability in the context of sonic hedgehog signaling in mouse granule neuron progenitors

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Cerebellar development requires tight regulation of primary cilia, which transduce sonic hedgehog (SHH) signaling, in the granule neuron progenitors (GNPs). Centriolar satellite/basal body protein OFD1 involved in the formation and disassembly of primary cilia through cerebellar development timeline works as a negative regulator of early neuron differentiation. Our preliminary data indicated that Atoh1, a downstream bHLH transcription factor required for ciliogenesis in GNPs, maintained the primary cilia and enhanced SHH signaling in GNPs, and knockout of Gli2, a downstream molecule of SHH-Atoh1 signaling, promoted autophagy level and reduced OFD1 abundance. Previous hypothesis argued that autophagy selectively removed Ofd1 from centriolar satellites and promoted ciliogenesis. However, the mechanism of SHH-Atoh1-Gli2 signaling regulating Ofd1 stability or expression and the competitive dual functions of Atoh1 in maintaining the primary cilium and promote autophagy remains elusive.

Our result indicated that SHH stimulation slightly enhanced Ofd1 mRNA expression, while did not significantly elevate OFD1 abundance and LC3II protein level in GNPs. In NIH3T3, Atoh1 promoted LC3II abundance and reduced OFD1 protein level under starvation stress with lysosomal inhibitor chloroquine, while maintain OFD1 under serum starvation. In NIH3T3 Gli2-/-, ubiquitination degree of Ofd1 seems to be strengthened, implied an independent ubiquitin-proteasome pathway of Ofd1 degradation. These data suggest that posttranslational modification of Ofd1 may play an important role in their interaction with SHH-, Atoh1- or Gli2-induced autophagy and proteasome degradation. We expect to shed light on the molecular network of cilia proteins and SHH signaling as well as underlying functions in neural development.

Mir-17~92 Governs the Regional Vulnerability of Motor Neuron Subtypes in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, and the hallmark of ALS is the differential degree of spinal motor neuron (MN) subtype degeneration: the earliest degenerated MNs are lateral motor column MNs (LMC-MNs) innervating limb muscles. However, the underlying mechanism for this selective vulnerability of LMC-MNs is still unknown. Previously we reported that the expression of microRNA cluster, mir-17~92, is enriched in developing LMC-MNs, and the target deletion of mir-17~92 in motor neurons leads to the selective apoptosis of LMC-MNs in embryos. As overexpression of mir-17~92 in MNs prevents naturally occurring cell death during neural development by attenuating PTEN expression and its nuclear import, we therefore exploited the hypothesis that if the dysregulation of mir-17~92/nPTEN pathway attributing to the motor neuron subtype vulnerability in ALS pathology. In this study, we revealed that a significant reduction of mir17~92 expression, with a concomitant nuclear PTEN accumulation in LMC-MNs from ESC derived MNs of SOD1G93A-ALS, whereas other subtypes remain relatively unaffected. This is further evidenced by SOD1G93A mice at disease onset stage. Finally, we demonstrated that overexpression of mir-17~92 in the MNs of SOD1G93A mice can prolong the survival as well as ameliorate ALS motor deficit pathology. We are currently testing if the same pathology is recapitulated in human ALS-iPSC derived MNs and if overexpressing mir17~92 by scAAV9 or using small molecule to enhance miRNA biogenesis could be applicable for ALS treatment.

USP11 deubiquitinates and stabilizes Sox11 to promote cortical neurogenesis.

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The ubiquitin-proteasome pathway plays a key role in neural development. Although the functions of several ubiquitin ligases in this process have been characterized, little is known about the role of deubiquitinating enzymes. Here, we showed that deubiquitinating enzyme USP11 is abundantly expressed in the developing central nervous system and is enriched in the cortical plate of embryonic neocortex. Compared with wild type mice, USP11 knockout mice display a reduction in neocortex thickness by a decrease of deep layer neurons. To analyze the differentiation and migration of cortical neurons, we labeled NPC with EdU at different developmental stages and followed the fates of EdU positive cells. This analysis revealed that USP11 knockout impairs NPC differentiation into early-born neurons and delays the migration of late-born neurons. To identify USP11 substrates responsible for these effects in corticogenesis, we carried out comparative ubiquitylome and proteome analyses on NPC with and without USP11 overexpression, as USP11 expressed in NPC similarly promotes neurogenesis in vitro. Through this analysis, we identified Sox11, a transcriptional factor known to promote neuron differentiation and migration during corticogenesis, as a USP11 substrate candidate. Subsequent biochemical studies demonstrated that USP11 interacts with and deubiquitinates Sox11, thereby promoting its stabilization. Furthermore, Sox11 protein level in cortical neurons is greatly diminished by USP11 knockout or knockdown. Together, our findings identify the critical roles of USP11 in corticogenesis by promoting neuron differentiation and migration and suggest an involvement of Sox11 stabilization in these functions of USP11.

High precision and transient stimulation from advanced automated laser tracking and optogenetic manipulation system (a-ALTOMS) manipulation neuronal circuit during operant restraining memory

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In this study, we developed an advanced automated laser tracking and optogenetic manipulation system (a-ALTOMS), a system that provided a useful single platform, which enables neural manipulation simultaneously on different body part and a zoom view window of freely-moving larval and adult fly. To address precision and transient laser stimulation from a-ALTOMS manipulating, we selected 12862-Gal4 to drive both ChR2 and NpHR expression in the giant fiber neurons. These results suggest that laser irradiation effectively triggers ChR2 and NpHR and that high precision and transient manipulation giant fiber neuron within different body part during online tracking, and provides an effective method for determining the information processing from interneuron. Taking the advantage of a-ALTOMS, we created a operant restraining memory assay in unrestrained flies. Applying 1064-nm IR laser irradiation as an aversive unconditioned stimulus, a courting male could quickly learn to stay away from a freely moving virgin female. By behavioural screening, operant restraining memory requires olfactory system (or47b neurons)、dopamine neurons (MB-MV1 neurons) and pain neuronal circuits. In addition, optogenetic manipulation of neuronal activities with NpHR inhibition revealed an ensemble of brain neurons orchestrating this distance-restraining social memory. These results show that γ lobe of MB were involved in acquisition of the restraining learning and α'/β' lobe of MB were involved in retrieval of the restraining learning. Thus, a-ALTOMS offers opportunities to systematically map operanr restraining circuits that orchestrate specific Drosophila behaviors.

Inherited mutations of CTTNBP2 in autism spectrum disorders impair dendritic spine formation of neurons

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Neuronal morphogenesis is a critical step of brain development. Many neuropsychiatric disorders, such as autism spectrum disorder (ASD), are caused by defects of brain development. Cortactin binding protein 2 (CTTNBP2), a brain specific cytoskeleton regulator, controls dendritic arborization and synapse formation through the regulation of microtubule and cortactin, respectively. Using whole exon sequencing, multiple mutations of CTTNBP2 gene had been identified in ASD patients. To explore how ASD mutations disrupt the function of CTTNBP2, we here analyzed the morphological impact of those mutations using cultured hippocampal neurons. Biochemical, molecular and cell biology approaches were combined to examine the effects of these mutations. We found that different ASD mutations exhibit different biochemical properties. Moreover, different ASD mutants manifest different phenotypes at different neuronal developmental stages. These results suggest that mutations in a single ASD causative gene are possible to result in diverse neuronal deficits, echoing the various phenotypes in patients.

Perturbation of Central Amygdala Neuron Excitability Reduces Pain- and Anxiety-like Behaviors

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Chronic pain disorder is associated with anxiety- and depression-like behavior. Although the amygdala is thought as a key node of the neural circuits mediating emotions, it also serves a major receiver of purely nociceptive signals. However, the circuit mechanisms by which the amygdala contributes to the pain-related anxiety has remained unclear. Here, we investigated circuit mechanisms underlying comorbid symptoms in chronic pain mouse models, including acid-induced muscle pain and spinal nerve ligation-induced neuropathic pain. We first found that the phosphorylated ERK (pERK) level increased in the lateral subdivision of central amygdala (CeL) after chronic pain development. To address the role of the CeL in chronic pain, we attempted to manipulate CeL neurons using chemo- and opto-genetic approaches. We hypothesized that silencing of somatostatin-positive (SOM+) neurons in the CeL, which may activate CeL output neurons (i.e., SOM- neuron) and thereby suppresses the CeM projecting neurons, which reduces mechanical sensitivity and chronic pain-related behavior. In chemogenetic part, selective expression of designer receptors exclusively activated by designer drugs (DREADDs) was achieved by injecting a virus encoding Cre-dependent inhibitory DREADDs (i.e., hM4Di receptor) into a SOM-Cre driver, a mouse line specifically expressing Cre recombinase in a major population of the CeL. In optogenetic part, we expressed inhibitory halorhodopsin (eNpHR) in SOM+ cells in the CeL. Consistent with this hypothesis, we found that both chemo- and opto-genetic silencing of SOM+ neurons in the CeL reduced mechanical sensitivity and comorbid anxiety-like behavior.

Activations of endosomal TLRs control diverse transcriptomic profiles of neurons and differentially regulate neuronal morphology

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Neuroinflammation is associated with diverse neurological disorders. Like other cells, neurons express various Toll-like receptors (TLRs) to detect danger signals, and this regulates neuronal morphogenesis. Here, we show that TLR3, TLR7 and TLR8, three well-known endosomal TLRs, control different transcriptomic profiles related to innate immunity, signaling and neural development to conduct differential activity in neuronal morphogenesis. These three TLRs all require signaling adaptor MYD88, but exhibit non-identical effects on neuronal morphology. Unlike TLR3 and TLR7, TLR8 only controls dendritic pruning, and not axonal growth. Similar to TLR3, but not TLR7, TLR8 acts independently of secreted factors. RNA-seq analysis indicates differential gene expression profiles upon different TLR activation and also suggests involvement of MAPK in the TLR8 pathway. We further confirm an essential role for P38 in the dendritic pruning induced by TLR8 activation. In conclusion, neuronal TLRs use various downstream pathways to differentially control neuronal morphology, which likely contribute to developmental processes and pathological responses.

Genetic screens identified loss-of-function mutations in genes required for sensory cilium formation confer resistance to the nematicidal mushroom Pleurotus ostreatus

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The oyster mushroom Pleurotus ostreatus is a nematophagous basidiomycete which is known to produce toxin and paralyze nematodes within minutes. To understand the molecular mechanisms of the mushroom caused paralysis, we conducted forward genetic screens to isolate mutants resistant to P. ostreatus. Approximately 30 mutants were isolated after 3 rounds of screens with the coverage of ~200,000 genomes. Genetic mapping and whole-genome sequencing identified 10 mutants have independent loss-of-function alleles in osm-1, osm-6, daf- 6 and dyf-7. Complementation test with fosmid clones containing these genes rescue the phenotype. Dye filling experiments showed that all mutants isolated from the screens were unable to uptake the dye (DiI). These results demonstrated that mutants defected in the sensory cilium formation confers resistance to the mushroom, suggesting that the toxin uptake likely occurs at the sensory cilium which then caused muscle hyper contraction in the body wall muscle of C. elegans via a still unclear mechanism.

Olfactory Experience- and Developmental Stage-Dependent Control of CPEB4 Regulates c-Fos mRNA Translation for Granule Cell Survival

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Mammalian olfactory bulbs (OBs) require continuous replenishment of interneurons, mainly granule cells (GCs), to support local circuits throughout life. Two spatiotemporally distinct waves of postnatal neurogenesis contribute to expanding and maintaining the GC pool. Although neonate-born GCs show higher survival rate than adult-born GCs, the molecular mechanism underlying this survival remains unclear. Here, we identify RNA-binding protein cytoplasmic polyadenylation element-binding protein 4 (CPEB4) as a survival factor exclusively for early postnatal GCs. During the first 2 postnatal weeks, olfactory experience initiated CPEB4-activated c-Fos mRNA translation. In CPEB4-deficient OBs, c-FOS insufficiency reduced the neurotrophic signaling to impair GC survival and thus cause the OB hypoplasia. The maldevelopment led to the functional defect in odor discrimination. Both cyclic AMP responsive element binding protein (CREB)-dependent transcription and CPEB4-promoted translation support c-FOS expression early postnatally but disengage in adult OBs. Activity-related c-FOS synthesis and GC survival is thus developmentally controlled by distinct molecular mechanisms to govern OB growth.

Ubiquitination of MBNL1 is Required for its Cytoplasmic Localization and Functioning in Promoting Neurite Outgrowth

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The Muscleblind-like (MBNL) protein family plays an important role in regulating the transition between differentiation and pluripotency and in the pathogenesis of myotonic dystrophy type 1 (DM1), a CTG expansion disorder. How different MBNL isoforms contribute to the differentiation and are affected in DM has not been investigated. Here we show that the MBNL1 cytoplasmic but not nuclear isoform promotes neurite morphogenesis and reverses the morphological defects caused by expanded CUG RNA. Cytoplasmic MBNL1 is polyubiquitinated by lysine 63 (K63). Reduced cytoplasmic MBNL1 in the DM1 mouse brain is consistent with the reduced extent of K63 ubiquitination. Expanded CUG RNA induced the deubiqutination of cytoplasmic MBNL1, which resulted in nuclear translocation and morphological impairment that could be ameliorated by inhibiting K63-linked polyubiquitin chain degradation. Our results suggest that K63-linked ubiquitination of MBNL1 is required for its cytoplasmic localization and that deubiquitination of cytoplasmic MBNL1 is pathogenic in the DM1 brain.

Differential Glutamate and GABA Release onto Dentate Gyrus Cells by Supramammillary Nucleus Neurons

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The hippocampus, a key brain structure for cognitive and emotional behaviors, comprises multiple subregions. Among these subregions, the dentate gyrus (DG) is the first information processor which receives sensory inputs from the entorhinal cortex (EC) through the perforant path (PP). Cortico-hippocampal pathways are known to be crucial for memory processing and spatial navigation. However, little is known about the functional relevance of subcortical inputs to the hippocampus. The supramammillary nucleus (SuM) is a hypothalamic structure, in which a subset of neurons project substantially to the DG and CA2/CA3a areas. Despite that the SuM-DG pathway is known to regulate hippocampal theta oscillations, learning, REM sleep and explorative locomotor activities, its neurotransmitter signaling and synaptic properties remain elusive.

By combining optogenetic tools, electrophysiological and pharmacological approaches, we found that DG-projecting SuM neurons preferentially innervate the DG granule cell layer, and form functional connections with granule cells (GCs), mossy cells (MCs) and GABAergic interneurons (INs). Optogenetic activation of channelrhodopsin2 (ChR2)-expressing SuM-DG terminals elicits monosynaptic responses of both glutamate and GABA onto single GCs and INs. Short-term plasticity of these two components are almost identical, suggesting co-release of two transmitters. Further analysis of individual connections revealed that SuM-GC and SuM-fast spiking IN synapses are dominated by GABAergic transmission whereas SuM-non-fast spiking INs synapses are largely mediated by glutamatergic transmission. Our finding suggests differential co-release of glutamate and GABA onto DG neurons by SuM neurons or differential innervation by distinct glutamatergic and GABAergic neurons within the SuM.

Elucidating the role of LHX2-DACH1-Mir-215 axis in early human cerebellar corticogenesis using human ESC-derived 2D neural culture and 3D cortical organoids

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With their ability to undergo self-renewal and pluripotent differentiation in vitro and in vivo, human pluripotent stem cells (hPSCs), provide an opportunity to study the mechanisms underlying cellular differentiation during early development. These stem cells are the only human biological sources with which to study early human development in the absence of complex ethical issues. Several laboratories have demonstrated that hPSCs have an intrinsic tendency to form three-dimensional polarized cerebral cortex tissues and cerebral organoids that resemble the embryonic cortex. The ability to grow these organoids offers an excellent opportunity to further investigate the detailed molecular mechanisms of human corticogenesis. In this study, we demonstrated that LHX2, a LIM-HD transcription factor, has a critical role in regulating forebrain neural transcription factors, such as PAX6, and a WNT signaling component, CER1, to modulate the formation of early forebrain lineages. Furthermore, our results indicate that several transcription factors, which are highly expressed in early hESC-derived neural progenitors and co-expressed with LHX2 and/or PAX6, affect early cortical gene expression. Among these transcription factors, DACH1, a nuclear factor that is expressed during mouse neural development, is involved in regulating the patterning of hESC-derived cortical structures. In this context, we propose that the LHX2-PAX6-DACH1 axis and its associated genetic factors, including non-coding RNAs are likely to be important for modulating cortical development in human. Consequently, the interplay between the genetic factors and the machinery controlling the orientation and mode of cell division may also play a role in cortical cell fate determination and cortex formation.

Investigate the role of mir-34/449 during motor neuron development

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Although the role of miRNAs during embryonic development has been intensively characterized over the past decade, their functions during early neural induction and patterning remains elusive. By taking advantage of an embryonic stem cell (ESC)-derived differentiation approach, we have systematically identified several miRNAs that are enriched at each stage of motor neuron (MN) differentiation during development. Among these miRNAs, the miR-34/449 family, which comprises six miRNA members located at three separate genomic loci, manifests dynamic expression patterns in MN progenitors and post-mitotic MNs. To remove the redundancy of this miRNA family, we applied CRISPR/Cas9 together with conventional knockout (KO) approaches to generate the triple KO models of miR-34/449 both in vitro and in vivo, and aim to investigate the functional importance of miR-34/449 during MN development, from molecular characterization, progenitor patterning, neuronal specification, to the cell survival and differentiation. Preliminarily, we observed significantly decreased number of MNs derived from the miR-34/449 KO ESCs, which was not caused by the programmed cell death. In the future, we will further uncover the underlying mechanism of the phenotypes and perform microarray analysis to identify the MN-context-dependent targets of miR-34/449.

Direct conversion of human fibroblasts into pre-oligodendrocytes by small molecules

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Oligodendrocytes are responsible for generating myelin sheath to wrap around the axons. Myelin sheath is important for insulation and increasing electrical conduction in the central nervous system. In previous studies, human oligodendrocyte lineage cells are derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). However, the differentiation protocol required more than 45 days which is labor consuming and high cost. The ESC/iPSC-derived cells may have the biosafety risks of tumor formation and virus insertion mutagenesis. For these reasons, small molecules/chemicals have been utilized to alter cell fate. Here, we demonstrated, only with 4-5 chemicals, is sufficient to convert human dermal fibroblasts into oligodendrocyte-like cells directly. It is a footprint-free method with a two-step induction protocol that only required 3 days. These induced pre-oligodendrocytes (iPOCs) have a significant morphological change in comparison to the parental fibroblasts. To further define the cell stage, we tested the molecular signature of these chemically induced cells by QRT-PCR and immunofluorescence. Our results indicate that these iPOCs express the specific marker of pre-oligodendrocyte GPR17, oligodendrocyte transcription factor Olig2, and oligodendrocyte marker O4, O1 and MBP. The conversion rate of iPOCs is over 30% on average. Moreover, we proved that the iPOCs have the myelination ability after co-culture with mouse dorsal root ganglion cells (DRGs). To prove the myelination ability in vivo, iPOCs will be further injected into the shiverer mice which is immunodeficiency (demyelination mouse model). Overall, our findings reveal a new method to generate iPOCs with high efficiency, which has major implications for broader contribution in stem cell research and regenerative medicine.

Calpain mediates Muscleblind-like 2 degradation in neurodegeneration

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The Muscleblind-like (MBNL) protein family plays an important role in regulation of developmental alternative splicing, polyadenylation transitions and in the pathogenesis of myotonic dystrophy type 1 (DM1). DM1 is the most common form of muscular dystrophy, together with multisystemic disorder. Cognitive deficits, including mental retardation, excessive daytime sleepiness, psychiatric disorders and neurodegeneration, are found in a high percentage of individuals with DM1. The genetic basis of DM1 is an expansion of CTG trinucleotide repeats in the 3' untranslated region (3'UTR) of Dystrophia Myotonica Protein Kinase (DMPK) gene. Mutant DMPK mRNA binds and sequesters MBNL proteins, resulting in their loss-of-function. Although nuclear sequestration by RNA foci is widely accepted as the causal mechanism for loss of MBNL2 function, we found reduced MBNL2 expression in association with neurodegeneration in the EpA960/CaMKII-Cre mouse brain in which expanded CUG RNA was postnatally expressed in the forebrain neurons. In an attempt to study the causal mechanism of neurodegeneration associated MBNL2 reduction, we adopted an in vitro system for studying neurodegeneration using glutamate to induce excitotoxicity in the cultured hippocampal neurons. We showed that MBNL2 protein level is reduced by NMDA signaling. Pharmacological and genetic knockdown approaches further identified that the cysteine protease calpain 2 mediates MBNL2 degradation via NMDA receptor (NMDAR) activation induced calcium influx. Furthermore, aberrant MBNL2-regulated alternative splicing events were detected in the neurons with excitotoxicity. Together, our results provide a mechanism for MBNL2 down-regulation via NMDAR-mediated calpain activation.

New role of ATM in hippocampal neurons during development

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The establishment of the correct equilibrium between inhibitory and excitatory synaptic transmission (I/E balance) is emerging as a fundamental principle underlying a variety of neuropsychiatric and neurodevelopmental disorders. Recently, we found that Ataxia Telangiectasia mutated (ATM) protein kinase plays an important role in the development of GABAergic inhibition by controlling the potassium/chloride co-transporter KCC2 expression. Taking advantage of neuronal cultures, we found in ATM heterozygous hippocampal neurons a significant I/E imbalance toward inhibition as indicated by: the higher frequency of mIPSCs, the increased number of GABAergic synapses and a more precocious development of the inhibitory system (i.e. excitatory to inhibitory GABA switch). In vivo, we found a more complex phenotype characterized by the enhancement of both excitatory and inhibitory synaptic transmission. Thus, in order to investigate these apparently conflicting results, we assessed the neuronal intrinsic excitability by current clamp recordings in hippocampal slices. Interestingly, even in presence of comparable passive properties, a reduced neuronal excitability was found as indicated by the lower action potential frequency generated in response to high-current intensity stimuli. Moreover, we dissected the glutamatergic components by electrophysiology (AMPA-NMDA and Kainate currents) and we unmasked a potentiated Kainate-receptor (KAR) mediated current in ATM heterozygous slices. Accordingly, levels of the KAR GluK2 subunit were significantly higher in heterozygous hippocampal tissues. Finally, heterozygous animals displayed an increased KA-induced toxicity upon i.p. injection. All together these results demonstrate that halved amounts of ATM, by affecting the brain inhibitory component during development, impact hippocampal functioning both in vitro and in vivo.

Autism spectrum disorder mutation in Cttnbp2 gene alters microtubule association of CTTNBP2 and neuronal morphology of hippocampal neurons

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Cortactin binding protein-2 (CTTNBP2), a high-risk autism spectrum disorder (ASD) gene, encodes a brain specific cytoskeleton associated protein. It associates with both F-actin and microtubule cytoskeletons in neurons. In mature neurons, CTTNBP2 is highly concentrated at dendritic spines, in which it modulates cortactin mobility and control formation and maintenance of dendritic spines. Before forming dendritic spines, it is present along dendrites, where it associates with microtubules and promotes microtubule bundling. Consequently, dendritic arborization is influenced by CTTNBP2. Human genetic studies had identified several ASD mutations in CTTNBP2 gene. We found that one of C-terminal mutations influenced subcellular distribution pattern of CTTNBP2. Expression of this C-terminal mutated protein regulated microtubule stability and also exhibited better ability to associate with microtubule, even this mutated residue is not located within the microtubule-association domain. In BP2-depleted primary cultured neuron, this C-terminal mutation impairs spine targeting of CTTNBP2 and reduced dendritic spine density. Our study suggests that CTTNBP2 ASD mutation impairs the activity of CTTNBP2 in association with microtubule and regulation of neuronal morphology. It is intriguing to further compare the effect of this C-terminal mutation with other ASD mutations. Our study will reveal how CTTNBP2 controls neuronal morphology and activity and the relevance with etiology of ASD.

Brain-specific actinfilin that is associated with infantile spasms modulates dendrite arborization and spine formation

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Dendritic spines, the actin-enrich protrusions emerged from dendrites, are subcellular locations of excitatory synapses in the mammalian brain. Many actin-regulating molecules modulate dendritic spine morphology and synapse plasticity in response to neuronal activity. Actinfilin (AF), an actin-binding protein, is predominantly expressed in brain. It contains N-terminal Broad-Complex, Tramtrack and Bric a brac (BTB) domain, which is involved in CUL3-dependent ubiquitination, and C-terminal kelch domain, which interacts with F-actin. Human genetic study indicated the association of AF with infantile spasms, a rare childhood epilepsies and related to mental retardation, suggesting that AF plays a role in brain development and function. Here, we used cultured hippocampal neurons to explore the role of AF in neuronal morphogenesis and synaptic plasticity. We found that AF is enriched at dendritic branching site and dendritic spines. Its expression was regulated by neuronal activity. Using miRNA knockdown approach, we found that loss of AF impairs dendritic arborization, spinogenesis and functional synapse formation. We further suggested that loss of AF decreased F-actin intensity on dendritic spine and show impairment of activity-induced spine enlargement. In addition to knockdown of AF expression, overexpression of AF fragments was also performed to disrupt the function of endogenous AF in neurons. We found that both AF-N terminal and AF-C terminal truncated mutants impaired F-actin rearrangement and synapse formation. In conclusion, our results provide the evidence that AF, a gene associating with infantile spasms, contributes to neuronal morphogenesis and synapse plasticity.

Revealing the pathological mechanism of Alzheimer's Disease (AD)-linked mutation using induced neuron from patient derived induced pluripotent stem cell (iPSC)

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Alzheimer's Disease (AD) is the common form of dementia, primarily caused by the accumulation Aβ, which is produced by sequential cleavages of amyloid precursor protein (APP). The mutations of APP can alter the Aβ production, thus cause the early onset Alzheimer's Disease (EOAD). APP D678H heterozygous point mutation (APPD678H/WT) was identified from two EOAD Taiwanese families.

We investigated the pathogenic mechanism of APP D678H mutation using patient-derived induced pluripotent stem cells (iPSC). However, the availability of appropriate control with the same genetic background has become a great concern. We thus occupied the CRISPR/Cas9 system to edit this APPD678H/WT mutation site into isogenic control (APPWT/WT) and isogenic mutation (APPD678H/D678H) in iPSCs.

Next, we differentiated those iPSCs into excitatory neurons. Several confirmation experiments showed that these induced neurons expressed neuronal marker genes, potassium and sodium channel, and capable to generate the repetitive action potential on 21st day after differentiation.

The patient derived APPD678H/WT induced neuron, isogenic controls and isogenic mutation were used to study the pathological mechanism of AD in detailed manner. We found out that Aβ42, the toxic form of Aβ, and neurofibrillary tangled (NFT) were not significantly different between APPD678H/WT group and the APPWT/WT group. Nevertheless, the treatment of oxidative stress and endoplasmic reticulum (ER) stress altered the Aβ42 and NFT level in APPD678H/WT group that is comparable to the APPD678H/D678H group. Together, our study to understand the disease mechanism using iPSCs could be one way to discover a new effective treatment for these patients.

ST8SIA3 mediates sialylation of striatal proteins and functions as a signal coordinator in the striatum

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ST8SIA3 is highly expressed in the striatum and responsible for transferring sialic acids to the N- or O-linked glycan chains of glycoproteins. To investigate the pathophysiological function of ST8SIA3, we generated a St8sia3-knockout (KO) mouse model using the CRISPR/Cas9. St8sia3-KO mice exhibited reduced performance on the rotarod test and a smaller striatum via magnetic resonance imaging. iTRAQ-based proteomic analyses identified an alteration of cell signaling proteins and glycomic analyses demonstrated St8Sia3-KO mice had fewer disialylated and trisialylated terminal glycotopes on N- and O-glycans in the striatum. To identify the protein substrates of ST8SIA3, we evaluated several striatum-enriched proteins in St8sia3-KO mice. Immunoblotting analyses suggested that the A2A adenosine receptor (A2AR), the type V adenylyl cyclase (AC5), the D2 dopamine receptor (D2R) and the D1 dopamine receptor (D1R) are substrates of ST8SIA3 because these proteins in St8sia3-KO mice traveled faster in SDS-PAGE gels and the differences were eliminated after treatment with sialidase. Consistently, locomotor activity analyses suggested that the motor responses to caffeine, an A2AR antagonist (SCH 58261), an A2AR agonist (CGS 21680), a D1R agonist (SKF 81297) and a D2R antagonist (L-741626) were altered in St8sia3-KO mice. Furthermore, the motor coordination, sialylation patterns of substrates and motor stimulant effects were rescued by treatment with adeno-associated virus (AAV)-mediated striatal expression of ST8SIA3 in St8sia3-KO mice. Collectively, ST8SIA3 appears to play a critical role in the striatum by mediating sialylation of specific striatal proteins and subsequently modulate striatal functions.

Interhemispheric connectivity controls amygdala-dependent memory and social interaction

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Impairment of interhemispheric functional connectivity, particularly between homotopic brain regions, is commonly found in various psychiatric disorders such as autism spectrum disorders and schizophrenia. However, how interhemispheric connectivity influences brain function remains elusive. Here, we show that basolateral amygdalae (BLA) in the two brain hemispheres contralaterally innervate and activate each other via the anterior commissure to regulate amygdala-dependent behaviors. Using unilateral optogenetic stimulation, electrophysiological approaches, surgical lesion of the anterior commissure and unilateral pharmacological inhibition, we demonstrate that BLA neurons directly innervate and activate projection neurons of the contralateral BLA. The BLA neurons that innervate the contralateral amygdala are widely distributed, rostrally to caudally, in BLA. Moreover, contralateral afferents and innervating targets of a particular BLA neuron tend to be different. Thus interhemispheric connections amplify neural activation in amygdalae. Both disconnection of the anterior commissure and unilateral inhibition of amygdalar neurons are sufficient to induce abnormal reciprocal social interaction and impaired amygdala-dependent memory. In conclusion, our study suggests that interhemispheric connectivity is essential for amygdalar activity and function.

Modeling amyotrophic lateral sclerosis (ALS) with iPSC reveal potential role of LncRNA NEAT1 in TDP43-associated disease pathology

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease and patients ended up with respiratory failure with no effective treatment. Cytoplasmic accumulation and aggregation of TAR DNA binding protein–43 (TDP-43) are the pathologic hallmarks of ALS. Here, we have corrected TDP-43 mutations in iPSCs derived from ALS patients with CRISPR/Cas9. To explore motor neuron (MN) specific disease mechanisms, we generated GFP-tagged HB9 reporter lines in both ALS and corrected iPSCs with validation by immunostaining of MN markers including ISL-1, HB9, SMI32 and ChAT. Recapitulation of MN death was validated by TUNEL assay. The progression of TDP-43 pathogenesis was validated by mislocalization, oligomerization and aggregation at different time points. From purified HB9+ MNs derived from ALS and corrected iPSCs, we have identified that lncRNA NEAT1 was upregulated and partially localized in ALS MNs. In our system, we have shown that cytoplasmic TDP-43 aggregation was associated with NEAT1. Knocking-down NEAT1 could not only reduce TDP-43 aggregation, but also rescue MN death. By promoting expression of endogenous NEAT1 in normal neuron progenitors, TDP43 aggregates and neuronal cell death were increased. In the cell-free system, NEAT1 directly interacted with TDP-43 and increased insolubility which leads to aggregation. Thus, we provide a new platform for ALS drug screening as well as identified potential candidates for preventing ALS disease progression. Therefore, our data suggest a toxic noncoding NEAT1 as a target for disrupting TDP-43 aggregation and provide a potential candidate for rescuing motor neuron degeneration in ALS.

Zika virus infection worsen the pathological phenotypes in neural progenitors derived from neurodegenerative disease iPSC

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It is well known that Zika virus (ZIKV) infection causes an increased DNA damage in the developing neural progenitors. Also, increased DNA double-strand breaks (DSBs) have been linked to pathogenesis in neurodegenerative diseases, such as Huntington disease (HD). DNA damage in HD is caused by dysfunction of mutant huntingtin protein in DNA repair. Thus, it would be of great interest to know whether ZIKV infection can cause a greater impact on the developing HD neural progenitors. Here, our results show that ZIKV infection promotes the progression of HD-associated pathological phenotypes in the HD-iPSC-derived neural progenitors. Combined ICC and immunoblotting analysis demonstrated that HD-iPSC-derived-NPCs exhibited a higher DNA damage response, based on the expression of γH2AX in ZIKV inoculation. Next, we examined whether ZIKV can induced cell death in iPSC-derived NPCs, we found that the expression of cleaved caspase-3, an apoptosis marker, was also higher in HD-iPSC-derived NPCs than the control subjects. Our mechanismic analysis revealed that the expression level of Ku70, a main component of the NHEJ pathway that repairs DNA DSBs, was significant lower in HD-iPSC-derived NPCs than control subjects suggesting ZIKV involved in the HD-associated DNA damage pathway to compromise the survival of HD-NPC. Collectively, these finding support our hypothesis that HD-iPSC-derived NPCs were more susceptible to ZIKV infection through the defective DNA repair machinery.

Regulation of interneuron distribution by the fate of cortical projection neurons

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Mammalian cerebral cortex is comprised of distinct cortical regions, including six-layered neocortex that processes vision, audition and somatosensation, and three-layered paleocortex, including piriform cortex (PC) that processes olfaction. Neocortex (NC) and piriform cortex both arise predominantly from dorsal telencephalic (dTel) progenitors of an Emx1 lineage that express Lhx2, a LIM homeodomain transcription factor in a graded, position-dependent level. We showed previously that Lhx2 controls a binary fate decision of dTel progenitors to generate NC versus PC. When Lhx2 is deleted by Emx1-Cre in the dorsal telencephalon in the Lhx2 conditional knockout (cKO), the lateral neocortex is refated to form an ectopic piriform cortex (Chou et al., 2009). Inhibitory cortical interneurons (INs), which use γ-amino butyric acid (GABA) as their main neurotransmitter, are primarily generated in the medial ganglionic eminence (MGE) of ventral telencephalon (vTel) and enter the cortex by tangential migration. The migration of INs from the MGE to cortex is controlled by a complex combination of long-range and short-range attractant and repelling signals. The mechanisms regulating the migration of INs to their final destination are not fully understood. Here we used a panel of IN makers to investigate the distribution of INs in Lhx2 cKO cortex. We observed changes in IN distribution in the cKO cortex when compared with wild type littermates. This suggests that the fate of cortical projection neurons plays a role in regulating IN distribution.

Thirst control of water memory expression in Drosophila

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Abstract: -

Motivation is an internal drive that stimulates the desire to do a particular behavior. It also interacts with memory systems so that memory expression has been shown to be more efficient under relevant motivational states. Thirst is one of the primary motivational states, which is essential for survival and conserved in every animal towards the continuity of life. Studies have shown that thirsty flies actively seek water and can be trained to associate odors with drinking water to form water reward memory. The formation of water reward memory requires dopaminergic neurons innervating specific substructures of the mushroom body, an olfactory learning and memory center in Drosophila. Whether water memory expression is regulated by thirst, and, if so, what the underlying cellular and molecular mechanisms are, remain to be elucidated. Our results show that water reward memory in fruit flies was repressed by satiety and promoted by thirst. We also identified one potential neuropeptide responsible for thirst dependent naïve water seeking as well as thirst control of water memory expression. Blocking the expression of the potential neuropeptide receptor in dopaminergic neurons results in water memory expression defect, suggesting our neuropeptide of interest regulates water memory expression via the mushroom body circuit. However, which specific subtypes of the dopaminergic neurons receive this neuropeptide signal and how this signal modulates the mushroom body circuit in order to promote water memory expression need to be studied. Our study provides a fundamental entry point for understanding how motivational state controls and coordinates with memory systems.

Tbr1 haploinsufficiency impairs olfaction discrimination

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In previous studies, the Tbr1 heterozygous mice were exhibited defects of anterior commissure to cause loss connection of amygdala and sociality defects and seem to be a model in autism spectrum disorder. However, the function of olfaction in Tbr1 heterozygous mice was unclear until now. Olfaction not only plays an important role in social behaviors but also anterior commissure contains decussating fibers from the olfactory tracts. In this study, we recognized the olfactory function in Tbr1 heterozygous mice. First, we confirmed the olfaction discrimination between wild type and Tbr1 heterozygous mice. Tbr1 heterozygous mice were showed poor ability of olfaction discrimination. Then, we checked c-fos signals after odor stimulated in olfaction co-related brain areas. The numbers of c-fos positive cells in piriform and entorhinal cortex were significantly reduced in Tbr1 heterozygous mice. Final, the cell types in olfactory bulb were compared between wild type and Tbr1 heterozygous mice. The Tbr2 positive cells were increased but the calretinin positive were decreased in Tbr1 heterozygous mice. It could be the reasons of the olfaction discrimination defeat in Tbr1 heterozygous mice.

Neural-derived Angiopoietin-1 regulates oligodendrocyte precursor specification via a "blood vessel-crosstalk mechanism"

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Research over the past decade has revealed that the vascular and central nervous systems (CNS) influence reciprocally via an intricate crosstalk for assuring proper development. However, signals linking oligodendrogenesis with vascular crosstalk are poorly understood.

Angs are angiogenic molecules that signal via Tie2, an endothelial cell (EC)-expressed tyrosine kinase receptor, crucial for vascular maturation and homeostasis. Recent evidence, mostly in vitro, suggests that Angs influence neurogenesis, neurite organization, and outgrowth. However, most efforts for understanding Angs/Tie role have circumvented the significance, especially in vivo, that this pathway may exert in CNS development. Here, we unravel Angiopoietins (Angs)/Tie2 axis as a key neurovascular pathway for developing mouse spinal cord (SC) oligodendrocyte precursor (OLP) specification.

Neural progenitors from the motor neuron progenitor domain (pMN) give rise to OLPs from E12.5. We show that the pMN express Ang1 in a dynamic spatiotemporal manner and that Shh regulates Ang1 expression, regulation required for Shh-induced OLP specification. Tie2 is exclusively expressed by ECs.

To assess Ang1 role in OLP development, we ablated Ang1 in the CNS or in pMN. To demonstrate neural Ang1-to EC signaling, we generated Tie2 EC-ablated embryos. Our results show a consistent reduction of newly specified OLPs. Additionally, we currently study signals triggered by EC-Tie2 activation that induce OLP specification. Preliminary results suggest TGF-β as a candidate to mediate this vascular-to-pMN crosstalk for OLP differentiation.

This study does not only suggest Ang1 as an OLP-fate signal but also provides new mechanistic insights as an angiocrine factor, shaping and regulating CNS development.

Comprehensive Classification of Spinal Motor Neurons by Single Cell Transcriptomics

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Spinal motor neurons convey commands to muscle fibers to control movements. In order to precisely coordinate movements, motor neurons are topographically arranged along the rostrocaudal axis of the spinal cord. Spinal motor neurons are grouped according to their corresponding muscle targets, termed motor pools. Motor pool identity provides the positional and innervation cue which is invaluable but far from sufficient. Until now we know that a dozen of transcription factors defines motor pool identity. Therefore, a comprehensive understanding of the molecular profile of each group of motor neurons would provide further insight into how motor neuron diversity is established, and help us elucidate how each subtype carries out specific function and how they work together to coordinate movement and locomotion properly.

Here we aim to map the transcriptome of rodent embryonic spinal motor neuron in single cell resolution. We hope to identify novel motor neuron subtypes and marker genes based on differential gene expressions. We would then characterize how these novel subtypes contribute to motor function. Ultimately, we also hope to extend the impact of our study by investigating the conservation of these novel subtypes in human. Our study would help us understand MN diversity with unprecedented resolution and could also shed some light on how each group of MN are influenced and exhibited differential vulnerability in neurodegenerative disease such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS).

Postmitotic neurons determine the formation and plasticity of barrel cortex in early development

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During development, the central nervous system acquires information via intrinsic as well as extrinsic factors. These information, in a critical period, make the wiring in nerve system adaptable, resulting in developmental plasticity. Many studies have shown that experience-induced activity is involved in plasticity during development, however the intrinsic factors that regulate cortical plasticity and the mechanism are still unclear. Barrel cortex is a good model to study the formation of wiring. Here we showed that Lhx2 in postmitotic cortical neuron is critical for barrel cortex formation and regulates the expression of several activity-related genes, suggesting Lhx2 has the potential role to modulate the plasticity during neuronal circuit development. We also investigated the function of Lhx2 in barrel formation plasticity in both structural and functional aspects.

Modeling Motor Unit in Spinal Muscular Atrophy with Human iPS-derived Cells

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Spinal Muscular atrophy is characterized by the loss of spinal motor neurons and muscle weakness. It is the leading genetic cause of infant death, and SMA patients suffer to rely on mechanical ventilation due to compromised motor function. Though the causative gene SMN1 was identified, how the deficiency of SMN protein leads to the specific motor dysfunction is still unclear. This is largely attributed to the inaccessibility of human nervous tissue. To understand the mechanism underlying selective vulnerability of spinal motor neurons and to dissect individual dysfunction in motor circuit, we utilized a versatile stem cell-based system to yield human spinal motor neuron, and built a motor neuron-skeletal muscle fiber co-culture system to recapitulate a motor unit in vitro. We then aim to identify disrupted regulatory mechanisms and function with this system to provide insight into SMA, which might also apply to the pathogenesis of other genres of neurodegenerative diseases.

Modeling ALS by stem cell-derived motor neurons to investigate the role of mir-17~92 in motor neuron degeneration

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The hallmark of Amyotrophic lateral sclerosis (ALS) is gradual loss of motor neurons (MNs). Importantly, and the earliest degenerated MNs in ALS patients are lateral motor column MNs (LMC-MNs), which innervate limb muscles. We recently revealed a significant reduction of mir-17~92 expression, with a concomitant nuclear PTEN accumulation in spinal LMC-MNs before disease onset in SOD1G93A ALS mice. The importance of mir-17~92 is further supported by the evidence that overexpression of mir-17~92 in spinal MNs can prolong the life span and ameliorate symptoms of SOD1G93A ALS mice. Here, we acquired mouse ALS ESCs (SOD1G93A+/Tg), human iPSCs of fALS patients (SOD1+/L144F and SOD1+/A4V) and their isogenic controls (SOD1+/L144L and SOD1+/A4L). These lines can be successfully differentiated into MN subtypes following a developmental temporal order and can recapitulate ALS MN degeneration under stress conditions. The current system provides a useful platform to examine early change and regulatory mechanisms of mir-17~92 pathway in ALS MN contents with homogenous genetic backgrounds.

Spatial and temporal regulation of Drosophila glial miR-274 on development and behavior

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The system of glia-coupled neuronal synapses to blood vessels maintain physiological and metabolic conditions in nervous systems and is compromised in some neurological and neurodegenerative diseases. Here, we used the Drosophila larval neuromuscular junction, which exhibits glial coupling of synaptic boutons to respiratory tracheal terminals, as a basic model to study their developmental and physiological interactions. We screened the microRNA mutant collection for defective glia-synapse-trachea coupling, and identified miR-274 mutants exhibiting both reduced synaptic boutons and tracheal branches. The expression of miR-274 was required in glia to regulate growth of synaptic boutons and tracheal branches, a non-cell autonomous effect suggesting that miR-274 might be secreted. Indeed, miR-274 was packaged into the secretory exosomes through the ESCRT-dependent pathway. Secreted miR-274 targeted sprouty (sty) in trachea and synaptic bouton and affected the MAPK signaling, which is required for growth. We further found that the exploratory behavior of miR-274 mutant larvae was enhanced under the hypoxia condition. Elevation of nitric oxide synthesis (Nos) expression in glia, one of the miR-274 targets, resulted in larvae hypersensitive to reduced oxygen levels, while the Nos mutant allele suppressed miR-274 mutant larval exploratory behavior, suggesting miR-274 functions cell autonomously to regulate Nos expression in glia. The developmental and physiological roles of miR-274 could be decoupled as transient induction of miR-274 expression in the mutant larvae was sufficient to rescue their behavioral deficit without restoring the anatomical changes in synapses and trachea.

An effective screen for dendrite morphogenesis identifies cell-intrinsic mechanisms including the CCT chaperonin

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The morphology of dendrite is crucial for neuronal functions. Large-scale screens have been performed to understand mechanisms underlying dendrite development. Clonal genetic screens in Drosophila have been used to identify dendrite genes. However, it is time-consuming due to low frequency and tedious heat-shock manipulation in clone generation. Here, we conducted an improved screen that generates neuronal clones at high frequency. The collection of mapped P-element insertions that have been shown to disrupt the eye morphology was screened for impairments of dendritic morphology. 19% mutants exhibited dendritic defects, and over half of them lost more than 50% of control dendritic branches. It indicates that the screen is highly effective in finding important genes for dendrite morphogenesis. By database analyses, complementation tests, and RNAi validations, we identified 38 genes previously undescribed in Drosophila dendrite development. Intriguingly, 12 molecular and cell biological mechanisms were confirmed as key mechanisms for dendrite development, because each of them has two or more components found in the screen. We highlights several poorly understood and currently unexplored mechanisms in Drosophila dendrite, including Nogo signaling, protein phosphatase 2A complex, and protein folding machinery. Specifically, our findings indicate that the chaperonin containing TCP-1 (CCT) complex, which facilitates folding of cytoplasmic proteins, localizes within dendrites and regulates dendrite development via modulating microtubule organization. Thus, our study shows a more effective screening strategy for finding dendrite regulators in vivo and provides novel insights into the molecular basis of dendrite morphogenesis.

Ablation of the LIM only 4 gene in parvalbumin interneurons disrupts excitation/inhibition balance and leads to autism-like behaviours in mice.

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Autism spectrum disorders (ASD) include impaired social communication, repetitive stereotyped behaviours, and cognitive deficits. The cause of ASD is likely multifactorial: many genetic mutants as well as toxicants during development are thought to disrupt the excitatory and inhibitory (E/I) balance in neural circuits and contribute to the manifestation of ASD. In particular, deficits and/or alterations of parvalbumin (PV) GABAergic neuron maturation and development have been tied to ASD and therapeutic interven¬tions that target GABA inhibitory pathways in ASD are currently undergoing clinical trials. However, toxicity in young ASD subjects remains a concern. A better understanding of the mechanisms underlying ASD may reveal better and safer therapeutic targets.

ASD subjects have high prevalence of metabolic syndrome with insulin resistance and 25% have a seizure disorder. Insulin resistance is tied to hyperactivity of the tyrosine phosphatase PTP1B that extinguishes insulin signaling. The LIM only domain 4 protein (LMO4) is an endogenous inhibitor of PTP1B. We found that mice with LMO4 ablated in PV GABAergic neurons have reduced inhibitory inputs to cortical pyramidal neurons, increased susceptibility to absence seizures, and ASD-like behaviours: reduced social interaction, repetitive behaviours, and enhanced prepulse inhibition (PPI) of the acoustic startle response. Given that a PTP1B inhibitor was recently shown to alleviate insulin resistance and ASD-like behaviors in Rett syndrome mice, our studies further suggest that PTP1B might be a therapeutic target for ASD-like behaviours.

Differential Dependence of GABAergic and Glutamatergic Neurons on Glia for the Establishment of Synaptic Transmission

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In the mammalian cortex, GABAergic and glutamatergic neurons represent the 2 major neuronal classes, which establish inhibitory and excitatory synapses, respectively. These cells differ in their anatomy, physiology and developmental origin. Nevertheless, both cell types require support from glial cells, particularly astrocytes, for their growth and survival. Recent experiments indicate that glutamatergic neurons also depend on astrocytes for synapse formation. However, it was not clear if the same holds true for GABAergic neurons. To address this point, we developed highly pure GABAergic cell cultures, established through fluorescent activated cell (FAC) sorting. By studying the development of these purified cultures, we find that GABAergic neurons depend on glial secreted factors for their growth and survival, but not synapse formation. In contrast, we find that glutamatergic neurons collectively depend on glia for growth, survival and synapse formation. Our results demonstrate a fundamental difference in the way GABAergic and glutamatergic neurons depend on glia for the establishment of synaptic transmission, a finding that has important implications for our understanding of how neuronal networks develop.