

CAJAL Advanced Neuroscience Training Course on
Advanced Techniques for Synapse Biology

Bordeaux, October 13- 1 November, 2019

Projects

Project 1:

When: Block 1

Who: 2 students/block

Instructor: Camin Dean

Abstract:

I will offer expertise in imaging synapse function and protein dynamics in neuronal cultures. Several methods are available to visualize synapse activity and strength in culture. FM dye loading and unloading of synaptic vesicles (SVs) in response to activity gives a readout of endocytosis (extent of dye uptake), and exocytosis (rate and extent of fluorescence loss in response to stimulation). pHluorins (pH sensitive GFPs) attached to the luminal domain of integral vesicle proteins also give a readout of exo and endocytosis. Intracellular vesicles are normally acidified, in which the pHluorin is dark. When vesicles fuse with the plasma membrane, the pHluorin is exposed to the more basic extracellular solution and becomes bright, indicating sites of exocytosis. Synaptotagmin-4 (Syt4) is an integral dense core vesicle (DCV) protein that can be tagged with pHluorin to visualize DCV fusion. Two-color pHluorin imaging is also possible using pH sensitive GFP and mOrange2, to examine relative frequency and site of fusion of SVs versus DCVs, for example. DCVs are highly mobile in neurons. Fluorescently-tagged Syt4, or dense core vesicle cargoes, like BDNF, Chromogranin or NPY, can be used to visualize the long-range trafficking dynamics of dense core vesicles in neurons, in response to changes in neuronal activity or pharmacological perturbations. Addition of dye-conjugated antibodies against these proteins, allows visualization of vesicles that have fused (and therefore taken up antibody-conjugated dyes) and continue to traffic. In addition, we have used mRFP-tagged BDNF to visualize the transfer of BDNF from expressing neurons to neighboring neurons and astrocytes. On the post-synaptic side of the membrane, the recycling of AMPA receptors in response to neuronal activity can also be visualized using pHluorins. High frequency LTP-like stimulation causes AMPA receptors to be inserted into the post-synaptic membrane to strengthen synapses, while low frequency LTD-like stimuli causes endocytosis of receptors to decrease synaptic strength. These changes can be visualized by increases or decreases in the fluorescence of pHluorins attached to the extracellular domain of AMPA receptor subunits. In addition, expression of GCaMPs - genetically encoded fluorescent calcium indicators - give a readout of the activity of neurons (or astrocytes). Because GCaMPs allow detection of the activity of single dendritic spines they can be used to determine the spatial dynamics of activated spines in a neuron or network, for example. Individualized projects, using specific fluorescent markers or fluorescently-tagged proteins of interest, alone or in combination, are possible.

Project 2:

When: Block 1

Who: 2 students/block.

Instructor: Joris De Wit

Abstract:

Cell surface proteins are key players in establishing neuronal cell identity and specific patterns of connectivity. Synaptic adhesion molecules play important roles in linking pre- and postsynaptic neurons, mediating cell-cell recognition, and organizing the pre- and postsynaptic machinery.

In this project, we will investigate the role of synaptic cell surface interactions in synapse development in cultured hippocampal neurons. We will analyze induction of pre- and postsynaptic differentiation by synaptic adhesion molecules in neuron-HEK293T cell cocultures. We will test synaptic protein-protein interactions in direct binding assays as well as on the neuronal cell surface using proximity ligation assays. We will further study the effect of manipulating protein-protein interactions on the localization and mobility of synaptic adhesion molecules and glutamate receptors. To this end, we will use cell-surface crosslinking approaches and live-cell imaging of epitope-tagged adhesion molecules and AMPA receptor subunits, under basal conditions and during chemical induction of LTP in cultured neurons.

Project 3: Role of axonal actin structures in the transport of presynaptic components

When: Block 1

Who: 2 students

Instructor: Christophe Leterrier

Abstract:

The intricate morphology and molecular identity of axons is maintained for decades, but also continuously adapts to changes in the environment and activity of neurons. Axons fulfill these paradoxical demands thanks to a unique cytoskeletal organization that ensures the coordinated transport, anchoring and mobility of axonal components. While axonal microtubules are readily seen by electron microscopy, a number of axonal actin structures have been recently discovered, thanks to the development of optical super-resolution microscopy techniques.

These includes the submembrane periodic actin rings connected by spectrins, and also intra-axonal clusters called hotspots from which dynamic actin filaments rapidly grow and collapse. We want to understand the function of these new structures. For this project, we will focus on the possible role of actin structures such as hotspots in the regulation of axonal transport toward and in-between presynapses. Indeed, hotspots and trails have been suggested to be important for actin enrichment at presynapses, and the exchange of presynaptic components between presynaptic boutons depends on actin-based processes.

We will use hippocampal neurons in culture to explore these questions. We will monitor the trafficking of presynaptic components together with the dynamic of axonal actin using live-cell imaging of fluorescent reporters. We will also study the nanoscale relationship between presynaptic cargoes and actin structures using Single Molecule Localization Microscopy (SMLM), and potentially link the transports events and nanoscale architecture by correlating both approaches.

Project 4:

When: Block 1

Who: 2 students

Instructor: Ruud Toonen

Abstract:

Ruud Toonen will instruct vesicle trafficking and fusion studies in cultured mouse neurons using isolated single neurons (autapses) and mass cultured neurons (see <https://fga.cncr.nl> for more information) based on the work in progress in his team.

He aims to understand the gene networks that orchestrate secretion of diverse chemical signals such as classical neurotransmitters from synaptic vesicles and neuropeptides from large dense core vesicles (LDCVs). In this project we will investigate different aspects of secretory vesicle maturation, transport and fusion with live cell imaging experiments using fluorescent vesicle markers. Multi-color imaging will be used to identify release sites and vesicle dynamics prior to release using multiple stimulation paradigms. Data will be analysed using ImageJ and custom algorithms in Matlab. Students are invited to indicate if they would like to study the behavior of their protein of interest in this paradigm.

Project 5: Investigating the impact of kynurenic acid on the surface dynamics and synaptic stabilization of NMDAR

When: Block 1

Who: 2 students

Instructor: Julien Dupuis

Abstract:

Schizophrenia is a severe mental disorder that causes abnormal thinking (i.e. delusion) and perceptions (e.g. hallucinations). Because first-generation neuroleptics were found to act as dopamine receptor antagonists, schizophrenia was long thought to result from impairments in dopaminergic pathways. However, discovery of the psychotomimetic action of antagonists of glutamate NMDA receptors (NMDAR) such as ketamine and phencyclidine, and case studies reporting altered expression and trafficking of NMDAR in schizophrenic patients now point towards abnormal glutamatergic signaling as an additional plausible cause for the disease. Recently, this glutamatergic hypothesis gained further support from the clinical observation that abnormally elevated levels of kynurenic acid - a tryptophan metabolite synthesized and released by astrocytes that acts as a competitive antagonist of NMDAR - in the brain of patients could contribute to the symptoms schizophrenia. Nevertheless, the molecular cascades through which kynurenic acid overproduction leads to the dysfunctions of NMDAR observed in schizophrenia remain elusive.

Interestingly, several reports recently described impairments in NMDAR surface dynamics in experimental models of neuropsychiatric diseases, suggesting that they could represent an important feature of psychotic disorders. To explore this hypothesis, we will use single particle tracking methods to investigate the impact of kynurenic acid on the lateral mobility, distribution and synaptic retention of receptors at the neuronal surface.

Project 6:

When: Block 1

Who: 2 students

Instructor: Alexandre Favereaux

Abstract:

Chronic pathological pain is a major burden for most societies, with high human, social and economic cost. The development of chronic pain is largely thought to result from a malfunction the spinal neuron network. This network first process noxious stimuli originating from the periphery and then projection neurons transmit these noxious information to the brain where the pain sensation gets elaborated. Although several attempts have been made to investigate the diversity of these neurons using immunocytochemical, morphological or electrophysiological techniques, the identity of the different cellular subtypes that make-up projection neurons as well as their role in pain transmission remains poorly understood.

The recent development of patch seq techniques which enables harvesting single neuron RNA after electrophysiological and morphological characterization has recently opened the door to a multifactorial characterization of these cells based on their electrophysiological, transcriptomic and morphological properties.

Using animals whose projection neurons will be labeled through Dil or AAV injection in the parabrachial nucleus, we will investigate the transcriptomic profile of these neurons and investigate its relationship with the electrophysiological (intrinsic and synaptic) properties of these neurons. In addition to mRNA profile characterization, we will investigate if specific non-coding RNA profiles can be identified for each type of projection neurons. The techniques developed in this project may be of broad interest for the characterization of neuronal populations within the central nervous system, both in physiologic and pathologic conditions.

Project 7: *Imaging pathological proteins in synapses in neurodegenerative disease*

When: Block 1

Who: 2 students

Instructor: Tara Spines-Jones

Abstract:

Synapse loss is a common feature of all neurodegenerative diseases, and synaptic pathology is thought to contribute to disease pathogenesis and the spread of pathological proteins through the brain. Studies of synaptic changes in neurodegeneration have been hampered by the inability to accurately study colocalization of multiple proteins at synapses in the brain. Immuno-electron microscopy can be used post-mortem to localize one or two proteins at synapses but this technique is prohibitively difficult for most groups and is very low throughput allowing only dozens to hundreds of synapses to be imaged in a given experiment. For colocalization of several markers, a typical approach is immunofluorescence labeling, but this is not appropriate for synapse studies because the axial (z-direction) resolution of light microscopes is much larger than the size of an individual synapse, preventing accurate quantification. Array tomography is a technique developed by Smith and Micheva which overcomes this axial resolution problem by physically sectioning tissue into ribbons of 70 nanometer serial sections which can be stained with multiple antibodies to determine

the proteins present at each synapse. Array tomography has resolution in the z-direction equivalent to electron microscopy and allows high throughput imaging of thousands of synapses with many more markers than was possible previously. The Spires-Jones group was the first to apply array tomography to AD mouse model tissue, finding for the first time that Ab and tau are present at synapses in the brain. Further, we extended this technique to human brain tissue and incorporated it into brain banking both at Harvard Medical School and more recently at the University of Edinburgh. With this technique, we confirmed in human Alzheimer's disease brain that Ab is present at a subset of shrunken synapses and that apolipoprotein E4 at synapses is associated with higher levels of synaptic Ab. We have also observed pathological tau in synapses in Alzheimer's, phospho-TDP43 in amyotrophic lateral sclerosis, and alpha-synuclein in dementia with Lewy Bodies. The ability to stain human brain postmortem for accurate localization of proteins at pre and post synapses is a distinct advantage over biochemical approaches to studying synaptic proteins. Homogenization of the brain allows the notoriously sticky pathological proteins like Ab to bind multiple partners that are not likely a physiological partner, a problem highlighted by the multitude of synaptic receptors that have been identified for Ab with a poor record of confirmatory studies.

In this project, students will learn the array tomography technique including tissue fixation and embedding, sectioning with an ultramicrotome, immunostaining, imaging, and image analysis. We will use a combination of array tomography and super resolution imaging to examine localization and potential interactions of proteins in synapses. Students are invited to design their own questions and either bring tissue embedded for the technique (from any species) or test their hypotheses on tissue from our brain bank of human tissue embedded for the technique. This will require some advanced planning to get ethical approvals for the study before the course. Alternatively, students can learn the technique and help with some of our ongoing questions about which synaptic receptors bind Ab in human Alzheimer's disease brain.

Project 8: Mitochondria at the synapse

When: Block 1

Who: 2 students

Instructor: Vanessa Morais

Abstract:

Mitochondria are known as the “power-house of the cell”, however in the last decades it has become evident that these organelles regulate many other crucial functions besides the production of ATP.

They are also involved in β -oxidation of fatty acids, Ca^{2+} homeostasis and apoptosis. Also, mitochondria exhibit highly dynamic properties such as fission, fusion and transport. While fusion is essential to rescue defective mitochondria through cross-exchange of proteins, lipids and mitochondrial DNA; fission is required for the distribution of mitochondria within the cell. However, when damage is irreversible, mitochondria undergo a unique and selective clearance pathway, known as mitophagy. Although these processes are of extreme importance, very little is known about mitochondrial dynamics and turnover in neurons, and particularly in the synapse. Neurons are a special case as they represent high energy demanding cells whose energy requirements rely mostly on mitochondrial oxidative phosphorylation. These cells are highly morphologically polarized and, as such, are divided into four primary compartments: the dendrites, the soma, the axons and the synapses. ATP has a limited diffusion capacity in the intracellular environment and particularly within long neuronal process. Thus stationary and intraneuronal compartment-specific mitochondria are needed to provide local sources of ATP. Actually, a non-

uniform distribution of mitochondria throughout the neuron is evident and it has been reported that synaptic are different from non-synaptic mitochondria in size and in lipid:protein contents. However, whether these mitochondria have different functions depending on their location is still elusive. Interestingly, most neurodegenerative diseases start with synaptic dysfunctions, prior to neuronal loss, and it has been widely speculated that mitochondria are at the base of most of these pathologies.

Concerning mitochondrial biogenesis and transport, it is thought that new mitochondria are generated in the cell body and then travel to the periphery, whereas dysfunctional mitochondria in the synapse also return to the cell body for degradation within the lysosome. However, it has been shown that biogenesis of mitochondria can occur locally in the axon. Therefore, whether quality control processes, such as mitophagy, occur at the synapse is still somewhat controversial. Moreover, it has been shown that biogenesis of mitochondria can also occur locally in the axon.

Therefore, understanding the mitochondrial transport pattern and defining the quality control pathways that are at place in the synapse will unravel new insights into mitochondrial dynamics and turnover at synapse.

In this research project, we will analyse mitochondrial movement from cell body to synapse, as well as mitochondria turnover rates in the presence of specific insults.

Project 9: Using *Drosophila* as a model to study intracellular trafficking pathways at the synapse

When: Block 1

Who: 2 students

Instructor: Sandra Soukup

Abstract:

Synapses are the communication units of the neuron and they can be located very far away from the cell body. Axonal transport of proteins is not fast enough to support the synapse especially during intense neuronal communication. Therefore, synapses have developed local mechanisms that enables them to function partially independently. Synaptic vesicle recycling is an efficient mechanism that enables the synapse to reuse proteins and lipids multiple times to maintain neurotransmitter release without immediate supply from the soma. However, after multiple rounds of recycling, dysfunctional proteins start to accumulate at the synapse. Fortunately, synapses possess various “detox processes” to disassemble and reuse dysfunctional components. Autophagy is one of these processes and like synaptic vesicle endocytosis, autophagy at the synapse is regulated by synapse specific adaptations. Importantly imbalance of autophagy at the synapse disrupts synaptic homeostasis and leads to synaptic dysfunction.

In this mini-project we will explore methods to study synaptic vesicle endocytosis, as well as autophagy and related processes. For this purpose, we will take advantage of the neuromuscular junction (NMJ) of the *Drosophila* larvae. Synapses at the NMJ are relatively big and allow live imaging of autophagosomes and endosomes that have been genetically tagged with fluorescent proteins. To do this we will learn the basics of *Drosophila* housing, genetics and neurobiology. We will then use this knowledge to express markers to visualize endosomes and autophagosomes at the synapse. We will analyse how these structures are formed in vivo using confocal live imaging. We will also explore methods to analyse synaptic vesicle recycling and methods to identify synaptic vesicle associated proteins by fluorescent dye uptake and using temperature sensitive mutants.

Project 10:

When: Block 1

Who: 2 students

Instructor: Marion Silies

Abstract:

We are interested to understand how neural circuits perform specific computations. To get a comprehensive understanding of neural computations, we aim to address this question at many different levels, linking molecular processes to circuit function and ultimately, to animal behavior. To do so, we often start from the behavioral side: We first map the circuits that are relevant for specific behaviors, we then test how individual synaptic connections contribute to functional circuit properties, and we work towards identifying the molecular mechanisms that shape the physiological properties of identified neurons or cell types.

This is only possible in an organism that allows access to all of these levels of hierarchy. In the fruit fly *Drosophila*, one can describe behavioral responses, perform physiological measurements in intact neural circuits, and combine these two with genetic manipulations of synaptic or molecular function. In the CAJAL course “Advanced Techniques for Synapse Biology” we will therefore ask the question: How are synaptic processes linked to circuit function and behavior?

Project 11:

When: Block 2

Who: 2 students

Instructor: Shigeki Watanabe

Abstract:

For neuronal communication, neurotransmitter is released from presynaptic terminals through fusion of synaptic vesicles and received by receptors in postsynaptic terminals. Efficacy of neurotransmission and connectivity of neural circuits are modulated depending on the rate and amount of release and subsequent postsynaptic activity. Despite this functional association and physical apposition, studies on presynaptic and postsynaptic architectures have not been explored until recently. Where are the fusion sites located relative to the receptors on the postsynaptic membrane? How are the fusion sites and receptors organized transsynaptically? How is the transsynaptic organization modulated with synaptic activity? How is synaptic stability maintained by transsynaptic organization? To address these questions with spatial and temporal resolutions relevant to synaptic functions, we developed several approaches in electron microscopy to track both proteins and membranes with millisecond temporal resolutions. In the course, we will use these cutting-edge electron microscopy approaches to address cellular and molecular basis of synaptic transmission and plasticity.

Project 12: Long-term imaging of active zone dynamics from single neurons in live adult fruit flies

When: Block 2

Who: 2 students

Instructor: Sha Liu

Abstract:

Although early research focused on post-synaptic mechanisms in synaptic plasticity, it is now clear that modification of synaptic strength occurs at either side of the synapses. However, as most studies focused on the post-synaptic dynamics, most methods for examining the synapses in live animals only monitor the spins and the pre-synaptic site of the synapses are largely ignored. In this short project, we try to examine the pre-synaptic changes following the behaviorally induced synaptic plasticity in live adult fruit flies.

At the pre-synaptic site, active zones play important roles in neurotransmitter release and their structural dynamics reflecting the pre-synaptic plasticity. To monitor the active zone structure changes in the brain of a living animal, we need 1) genetically labeling the active zones from single pre-synaptic neurons; 2) long-term imaging these labeled active zones in vivo. By combining MARCM and STaR strategy in *Drosophila*, we can genetically label the active zones in a dedicated neural circuit at the single neuron level. The students and the instructor will discuss and choose the target neural circuit in the fly to monitor its pre-synaptic plasticity in the project.

On the other hand, the preparation for repeated imaging the neuronal structure in the adult fly brain was developed very recently and has not been widely used in the fly neurobiology community. In this project, the students will practice this preparation and implement it on the two-photon microscope. We aim to observe the active zone dynamics from single neurons across two or three days, during which the plastic changes of the neurons will be induced by behavior manipulation.

Project 13: Multi-level experimental paradigms for the study of synaptic activity modulation by pharmacological compounds and microglial activation

When: Block 2

Who: 2 students

Instructor: Xavi Altafaj

Abstract:

Synaptic plasticity is a fundamental process allowing neuronal adaptation to intrinsic developmental programmes as well as to environmental inputs. The integration of these factors result in a plethora of molecular changes that remodelate synaptic shape and synaptic transmission efficacy. Within the excitatory glutamatergic synapse, the substrate of the main excitatory transmission, ionotropic glutamate receptors play a crucial role. Concomitantly, glutamatergic neurotransmission is regulated both by presynaptic inputs, postsynaptic signaling cascades and the homeostatic contribution of glial cells.

Along this project, we propose to profile synaptic activity-triggered subsynaptic changes occurring in the postsynaptic density and the transduction processes in the postsynaptic neuron, both using *in vitro* and *in vivo* models. Using pharmacological and biological compounds, we will manipulate regulatory pathways (post-translational mechanisms, mainly (de)phosphorylation events) involved in

primary tuning of ionotropic glutamate receptors (iGluRs) and the resulting changes in iGluR-dependent signaling pathways.

Project 14:

When: Block 2

Who: 2 students

Instructor: Keimpe Weirda

Abstract:

The CA3 region of the hippocampal plays an important role in the encoding of memory. The CA3 pyramidal neuron apical dendrites in stratum lucidum (SL) receive a very distinct form input originating from dentate gyrus (DG) granule cell (GC) axons, called the mossy fibers (MFs).

The MF axons form distinct large presynaptic specializations onto CA3 pyramidal neurons, called mossy fiber boutons (MFBs). These MFBs form complex synaptic connections with the proximal portion of the CA3 pyramidal dendrites, called thorny excrescences. In addition, filopodial extensions emerging from the MFBs exclusively contact dendritic domains of different GABAergic interneurons in the CA3. The MFBs show distinct synaptic plasticity and are shown to play a key role in processing, storage and recall of spatial information within the hippocampal network.

In addition to the MF input, CA3 also receive direct input from the entorhinal cortex (EC) via the perforant pathway (PP). Predominantly axons from layers II of the EC project to the granule cells of the dentate gyrus (DG) and pyramidal cells of the CA3 region.

In this project we will examine synaptic input from the PP and/or MF pathway to the CA3 pyramidal neurons and/or GABAergic interneurons. We will use whole cell recordings in combination with optical and/or electrical stimulation of the axonal pathways. We will use Rbp4-Cre⁺ mice that express a ChR2 variant specifically in the granule cells of the dentate gyrus of the hippocampus allowing specific light-induced activation of the MF pathway, which can be combined with electrical stimulation of the perforant pathway.

Project 15: *Impact of membrane phospholipid composition on dopamine D2-receptor dependent signaling*

When: Block 2

Who: 2 students

Instructor: Pierre Trifilieff, Maria Florencia Angelo, Etienne Herzog & Véronique De Smedt-Peyrusse

Abstract:

Several psychiatric disorders such as schizophrenia, bipolar disorders or major depression have been shown to display alterations in lipid metabolism. In particular, a decrease in the phospholipid content in polyunsaturated fatty acids (PL-PUFAs) has been consistently described, but the implication of such endophenotype in the etiology of the diseases remain to be established. PL-PUFAs are main constituents of neuronal membrane bilayer and constitute roughly 30% of the brain dry weight. Their particular biophysical properties have been shown to impact membrane dynamic, vesicular trafficking, as well as the activity of transmembrane proteins. The instructors have conducted research over the last years that demonstrate that membrane PL-PUFA composition

particularly impact signaling at the dopamine D2 receptor, leading to behavioral alterations that resemble characteristic symptomatic dimensions of psychiatric disorders. These results suggest that decreased PL-PUFA biostatus could be involved in the etiology of psychiatric disorders through alterations of D2-dependent signaling.

The students will investigate how PL-PUFA manipulation impact the recruitment of signaling effectors at the D2 receptor. They will focus on arrestin-dependent signaling and receptor trafficking, in heterologous system as well as synaptosome preparations.

Techniques: Synaptosome preparation from mouse striatum, tagging and imaging of synaptic vesicles and receptors, proximity-ligation assay, in cellulo assay for arrestin recruitment at the D2 receptor, etc, etc...

Project 16: *In utero electroporation to study spine/synapse formation in the cerebral cortex and hippocampus*

When: Block 2

Who: 2 students

Instructor: Paccary Emilie

Abstract:

In utero electroporation is a rapid and powerful technique to study neuronal development in different brain areas. This approach presents several advantages over others methods to study specific steps of neuronal development in vivo, from proliferation to synaptic integration. During this practical course, students will learn how to perform in utero electroporation of cortical and/or hippocampal progenitors in mice. Analysis will be then performed in fixed sections at postnatal stages (around P21). Students will electroporate constructs that allow to visualize spines/synapses and/or to manipulate their development.

The individual steps necessary to carry out the technique will be described in details. In addition the variations that can be implemented to target different cerebral structures and study specific steps of development will be highlighted.

Project 17: *Tuning of GABAergic synaptic plasticity by Cl⁻ second messenger signaling pathway*

When: Block 2

Who: 2 students

Instructor: Sabine Levi

Abstract:

We recently demonstrated the contribution of a novel signaling pathway, the chloride (Cl⁻) sensitive WNK1 kinase and of its downstream effector the SPAK kinase, in the rapid homeostatic control of GABAergic inhibitory synapses of the hippocampus (Heubl et al., Nature Communications 2017 Nov 24;8(1):1776). In this regulation Cl⁻ acts as a second messenger to tune the activity of the main neuronal K⁺/Cl⁻ KCC2 cotransporter, and to regulate thereby neuronal chloride homeostasis.

We wish now to investigate the contribution of the WNK/SPAK signaling pathway and of the second messenger Cl⁻ in the direct regulation of GABAA receptors in the context of synaptic plasticity in primary cultures of hippocampal neurons. For this purpose, we will use a combination of

pharmacological (WNK and SPAK inhibitors) and genetic tools (shRNA against WNK and SPAK) to inactivate WNK and SPAK kinases and to test their impact on inhibitory long term potentiation (iLTP) and depression (iLTD). In particular, we will study rapid changes in GABAA receptor number at synapses using state-of-the-arts imaging techniques such as quantum-dot based single particle tracking and super-resolution PALM/STORM approaches. We will then characterize the molecular pathway leading to the activation of the kinases and linking WNK and SPAK to the regulation of receptor trafficking. In particular, we will test the possibility that chloride acts as a second messenger in the regulation of iLTP and iLTD to activate the Cl-sensitive WNK kinase. Altogether, this work may help to identify a novel signaling pathway controlling the synaptic plasticity of inhibitory GABAergic synapses.

Project 18:

When: Block 2

Who: 2 students

Instructor: Martin Muller

Abstract:

Neddylation is a posttranslational modification that controls the cell cycle and cellular proliferation by covalently conjugating Nedd8 to specific target proteins. However, its role in nonreplicating postmitotic cells and neural function has remained enigmatic. Recent evidence suggests that neddylation regulates the maturation, stability and function of dendritic spines (Vogl et al., 2015). Neddylation has not been studied in the context of presynaptic function.

The major aim of this project is to investigate the role of neddylation in controlling neurotransmitter release. Preliminary data suggest that neddylation regulates presynaptic function under baseline conditions and during presynaptic homeostatic plasticity at the larval *Drosophila* neuromuscular junction (NMJ). In this project, we will employ a combination of electrophysiology, STED microscopy and genetics at the *Drosophila* NMJ to elucidate (1) which synaptic parameters are controlled by neddylation, (2) how neddylation intersects with the molecular mechanisms underlying homeostatic plasticity, and (3) if and how neddylation affects the organization of synaptic proteins at the nanometer scale.

Project 19:

When: Block 2

Who: 2 students

Instructor: Vassiliki Nikolettou

Abstract:

Autophagy is a highly conserved process that delivers cellular constituents to the lysosome for degradation. Genetic ablation of key autophagy genes in the entire brain leads to late-onset neurodegeneration across different brain areas, indicating that autophagy is indispensable for neuronal maintenance and brain health. Moreover, impairment of autophagy in neurons also leads to synaptic and behavioural deficits. This is not surprising, given that many forms of plasticity require precise protein turnover. Indicatively, autophagy deficient dopaminergic neurons display increased

dopamine release and more rapid pre- synaptic recovery, while post-synaptically, autophagy has a critical role in the developmental pruning of dendritic spines.

However, the identity of proteins that are degraded in neurons by autophagy, remains largely elusive. A major step forward towards understanding the neuronal autophagic cargo is the ability to purify intact autophagic vesicles (AVs) from the mouse brain or from cultured neurons, which can be used for unbiased proteomic analyses. In this workshop, we will demonstrate a method for the purification of autophagic vesicles from the mouse brain.

The following experiments will be carried out:

- a) Dissection of mouse brains
- b) Homogenization and purification of AVs using 3 consecutive discontinuous gradients
- c) Biochemical characterization of the purity of the AV preparation
- d) Confirmation of AV integrity by proteinase K protection assay
- e) Biochemical separation of AV membranes and soluble content

Project 20: Kinetics of polarized protein degradation in mammalian neurons

When: Block 2

Who: 2 students

Instructor: Natalia Kononenko

Abstract:

Autophagy and lysosomal degradation regulate neuronal homeostasis by controlling the turnover of a wide variety of synaptic proteins and organelles, including synaptic vesicles itself and postsynaptic AMPA receptors. Although autophagy-dependent lysosomal degradation is well established in both axons and dendrites, one particular feature of neurons complicates the accomplishment of this type of protein turnover. Lysosomes are found mostly within the cell body and proximal dendrites, while axons are mostly devoid of functional lysosomes. This raises the question of whether the kinetics of lysosomal degradation upon autophagy induction differ in axons and dendrites. Since selective axonal degeneration is a common feature of autophagy-dysfunction associated neurodegenerative disorders, delayed kinetics of protein turnover in axons can explain why this neuronal compartment is most vulnerable to stress-induced condition i.e. autophagy induction.

To test the kinetics of protein targeting to lysosomes during autophagy induction in axons and dendrites we will employ a construct expressing a protein of interest C-terminally tagged with monomeric Keima-Red (mKeima). mKeima is a coral-derived acid-stable fluorescent protein with a bimodal excitation spectra dependent on the surrounding pH. In neutral pH, Keima has an excitation peak at 438 nm, and in the acidic compartment, its excitation peak shifts to 586 nm. Therefore, the amount of Keima delivered to the lysosome over time can be estimated by the ratio of signal strength excited at 550 nm divided by that excited at 438 nm. We will transiently express a protein of interest tagged with mKeima in cultured primary neurons and analyze its lysosomal delivery under autophagy-inducing conditions using live-cell confocal microscopy.