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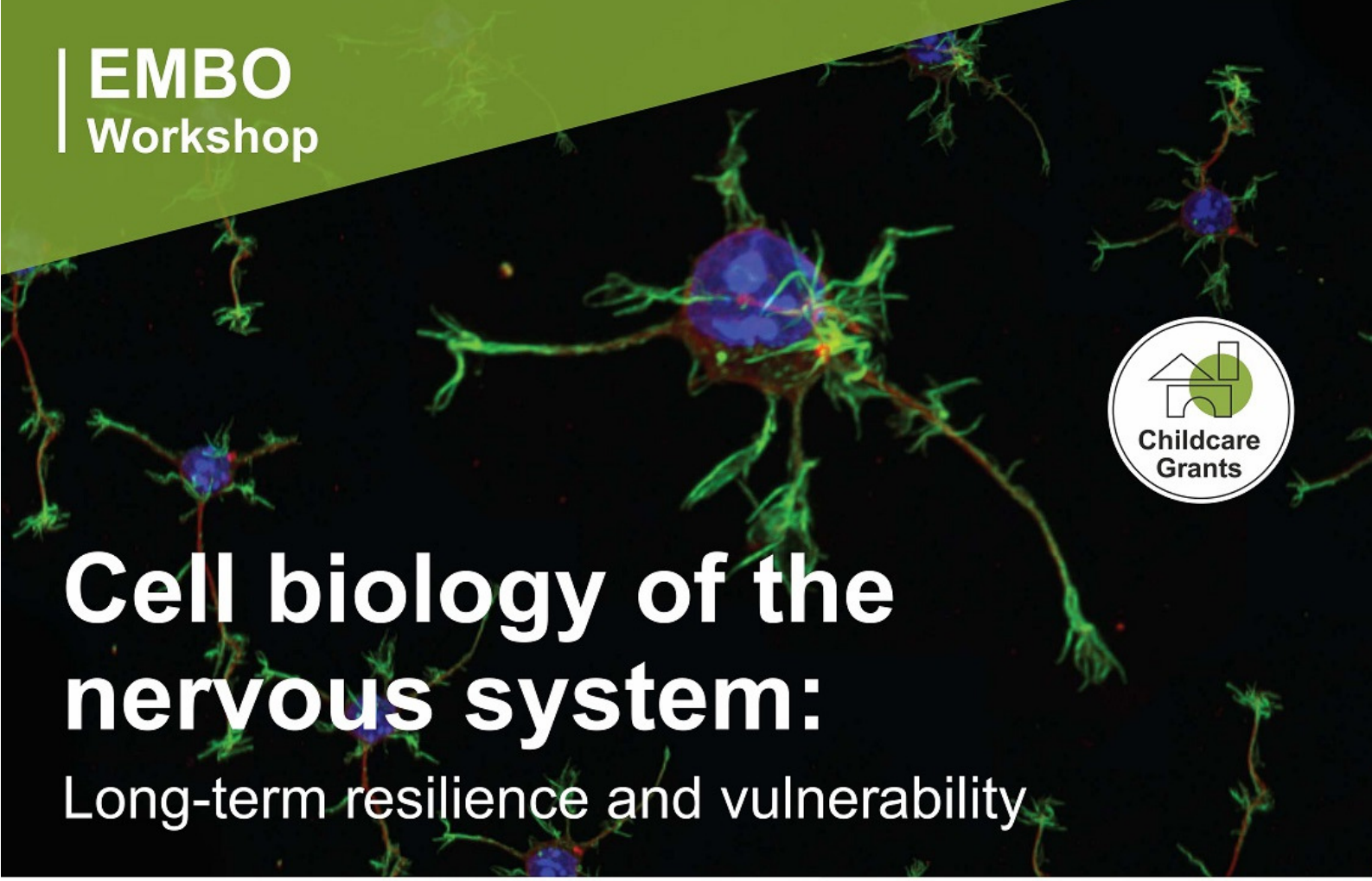

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Overexpressing elongation factor 1 alpha proteins to promote CST repair

001

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Although protein synthesis is hypothesized to have a pivotal role in axonal repair after central nervous system (CNS) injury, the role of core components of the protein synthesis machinery has not been examined. Notably, some elongation factors possess non-canonical functions that may further impact axonal repair. Here, we examined whether overexpressing eukaryotic elongation factor 1 alpha (eEF1A) proteins enhances the collateral sprouting of corticospinal tract (CST) neurons after unilateral pyramidotomy, along with the underlying molecular mechanisms. We found that overexpressing eEF1A proteins in CST neurons increased the levels of pS6, an indicator for mTOR activity, but not pSTAT3 and pAKT levels, in neuronal somas. Strikingly, overexpressing eEF1A2 alone, but neither eEF1A1 alone nor both factors simultaneously, increased protein synthesis and actin rearrangement in CST neurons. While eEF1A1 overexpression only slightly enhanced CST sprouting after pyramidotomy, eEF1A2 overexpression substantially enhanced this sprouting. Surprisingly, co-overexpression of both eEF1A1 and eEF1A2 led to a sprouting phenotype similar to wild-type controls, suggesting an antagonistic effect of overexpressing both proteins. These data provide the first evidence that overexpressing a core component of the translation machinery, eEF1A2, enhances CST sprouting, likely by a combination of increased protein synthesis, mTOR signaling and actin cytoskeleton rearrangement.

Autolysosomal Exocytosis of Lipids Protect Neurons from Ferroptosis

005

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During oxidative stress neurons release lipids that are internalized by glia and stored in lipid droplets. This process is essential to maintain the health and function of the nervous system. Defects in this coordinated process play an important role in several neurodegenerative diseases. Yet, the mechanisms of lipid release and its consequences on neuronal health are unclear. Here, we demonstrate that lipid-protein particle release by autolysosome exocytosis protects neurons from ferroptosis, a biochemically distinct form of cell death driven by lipid peroxidation. During oxidative stress in primary cell culture or fly retina, neuronal lipid release depends on the exocytic machinery; VAMP7 and syntaxin 4. We show that peroxidated lipids and iron are released through exocytosis of autolysosomes. We observe membrane-bound lipid-protein particles by transmission electron microscopy and demonstrate these particles are released from neurons using cryo-electron microscopy. Failure to release these lipid-protein particles causes lipid hydroperoxide and iron accumulation and sensitizes neurons to ferroptosis. Our results reveal how neurons use autolysosomal exocytosis to rid themselves of peroxidated lipids generated during oxidative stress. Given the number of brain pathologies that involve ferroptosis, defects in this pathway likely play a key role in the pathophysiology of neurodegenerative disease.

Functional characterization of autism and epilepsy associated EEF1A2 mutations

007

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Center for Neural Science, New York University

Protein synthesis is a fundamental process in neurons that is essential for synaptic plasticity, learning and memory. Here, we describe a neuron and muscle specific translation factor, eukaryotic elongation factor 1a2 (EEF1A2), that is mutated in patients with autism, epilepsy, and intellectual disability. We characterize three of the most common patient mutations G70S, E122K, and D252H and demonstrate that these mutations decrease de novo protein synthesis and elongation rate in HEK293 cells. In mouse cortical neurons, these mutations not only decrease de novo protein synthesis, but also alter neuronal morphology, regardless of endogenous levels of EEF1A2. This suggests that these mutations act via a toxic gain of function. We show that EEF1A2 mutant proteins display increased tRNA binding and decreased actin bundling activity, indicating that these mutations disrupt neuronal function by decreasing tRNA availability and altering the actin cytoskeleton. More broadly, these findings posit EEF1A2 as a bridge between translation and the actin skeleton, a link essential for proper neuron development and function.

Golgi-dependent Reactivation and Regeneration of Quiescent Neural Stem Cells

010

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The ability of stem cells to switch between quiescent and proliferative states is crucial for maintaining tissue homeostasis and regeneration. In Drosophila, quiescent neural stem cells (qNSCs) extend a primary protrusion, which is a hallmark of qNSCs. Here, we have unravelled that qNSC protrusions can be regenerated upon injury. This regeneration relies on the Golgi apparatus which acts as the major acentrosomal microtubule-organizing centre in qNSCs. A Golgi-resident GTPase Arf1 and its guanine-nucleotide exchange factor Sec71 promote NSC reactivation and regeneration via the regulation of microtubule growth. Arf1 physically associates with its new effector Mini Spindles (Msps)/XMAP215, a microtubule polymerase. Finally, Arf1 functions upstream of Msps to target the cell-adhesion molecule E-cadherin to NSC-neuropil contact sites during NSC reactivation. Our findings have established Drosophila qNSCs as a new regeneration model and identified a novel Arf1/Sec71-Msps pathway in the regulation of microtubule growth and NSC reactivation.

Golgi mediated microtubule nucleation drives peripheral neuron regeneration

011

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Axon re-extension following injury of peripheral neurons is characterised by large-scale remodelling of the microtubule network. However, the mechanisms directing de-novo microtubule nucleation following peripheral nerve injury (PNI) remain unknown. Evidence from embryonic drosophila supports a role for the Golgi apparatus (GA) as a microtubule organisingcentre (MTOC) in neurons and given that mature neurons lack a centrosome, we hypothesised that the GA functions as the primary MTOC during peripheral neuron regeneration.
Using a combination of high-resolution live imaging and super-resolution fixed imaging techniques in in-vitro adult rat and human models of PNI, we have observed that striking changes in GA conformation are accompanied by the emergence of ordered microtubule architecture in regenerating peripheral neurons. This is accompanied by dynamic recruitment of the essential microtubule nucleating proteins gamma-tubulin and AKAP9 and emergence of de-novo microtubules from the GA. Furthermore, disruption of GA organisation and gamma-tubulin function results in cessation of GA-mediated microtubule polymerisation and prevents axon regeneration. These findings considerably enhance our understanding of the cellular mechanisms directing re-establishment of neuronal architecture following injury and identify the GA as a target for therapeutic strategies aimed at improving PNI regeneration.

EndoA couples activity-dependent Ca2+ influx to autophagy induction at synapses

012

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Neuronal activity causes use-dependent decline in protein function. However, it is unclear how this is coupled to local quality control mechanisms. We show in Drosophila that the endocytic protein Endophilin-A connects activity-induced calcium-influx to synaptic autophagy and neuronal survival in a Parkinson's disease-relevant fashion. Mutations in the disordered loop, including a Parkinson's disease risk mutation, render EndoA insensitive to neuronal stimulation and affect protein-dynamics: when EndoA is more flexible, its mobility in membrane-nanodomains increases, making it available for autophagosome formation. Conversely, when EndoA is more rigid, its mobility reduces, blocking stimulation-induced autophagy. Balanced stimulation-induced autophagy is required for dopaminergic neuron survival, and a variant in the human ENDOA1 disordered loop conferring risk to Parkinson's disease also blocks nanodomain protein-mobility and autophagy, both in vivo and in human-induced dopaminergic neurons. Thus, we reveal a mechanism that neurons use to connect neuronal activity to local autophagy, and that is critical for neuronal survival.

Creatine kinase B provides the starter energy for endosomal motility.

013

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Endosomes are specific organelles required for the maintenance and signaling of neuronal circuits. Their trafficking within axons is ensured by ATPase molecular motors and depends on glycolytic machinery (Zala, 2013, Hinckelmann, 2016). In addition to carry on-board their own fueling system, endosomes contain their own navigational system composed of Calcineurin and Huntingtin (HTT) that leads to their fast axonal retrograde transport to the nucleus (Scaramuzzino, Cuoc et al, 2022).

However, it is unknown how endosomes initiate their movement at the distal axon. We analyzed the proteome of isolated motile vesicles and identified the brain specific isoform of the creatine kinase (CKB). We confirmed by biochemical, high-resolution and proximity ligation assay approaches that CKB localizes on endosomes with HTT and TrkB. We used microfluidic devices (Lenoir et al 2021) and reconstituted in vitro a mature cortico-striatal circuit. We found that down-regulation of CKB inhibits the onset of endosomal motility at the synapse. We are now investigating the mechanism of CKB activation and whether such activation could be impaired in Huntington disease when HTT protein is mutated. Together, these findings reveal a new mechanism linking energy metabolism to intracellular trafficking and may reveal new therapeutic strategies for Huntington disease.

Therapeutic blocking of TDP-43 toxicity in ALS models

015

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Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal disorder characterized by the selective degeneration of motor neurons. The aggregation of the RNA-binding protein (RBP) TDP-43 is a critical pathological marker of ALS and other TDP-proteinopathies, and its dysregulation is causative of motor neuron vulnerability and death. In this study, we show that a fragment of the ALS-related RBP RGNEF is able to suppress or ameliorate the toxic phenotype induced by TDP-43 in animal models of ALS. We show that the in vivo co-expression of an amino-terminal fragment of RGNEF (NF242) with TDP-43 in fruit flies suppressed its deleterious phenotype increasing lifespan, abolishing motor defects, and preventing neurodegeneration. Using molecular docking prediction, and in vitro and live cells experimental approaches, we observed that NF242 and TDP-43 interact directly, with the RRMs domains of TDP-43 being essential for this interaction. Experiments using a virus expressing NF242 in a severe TDP-43 murine model of ALS showed improvement in the motor phenotype and a decrease in neuroinflammation markers. Here, we present a promising therapeutic strategy for TDP-43 proteinopathies such as ALS and frontotemporal dementia (FTD).

A quantitative model of sporadic axonal degeneration in Drosophila visual system

016

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In human neurodegenerative diseases, neurons undergo axonal degeneration before they die. Therefore, for potential intervention and to better understand early phases of neurodegeneration, defining the initiation of axon damage is of great importance. Invertebrate models, have significantly contributed to our understanding of neurodegenerative disorders. However, these models mainly rely on manipulation of genes identified in familial cases of neurodegenerative diseases. Nonetheless, the vast majority of cases of neurodegenerative diseases are sporadic. We developed a system modelling early degenerative events in Drosophila adult photoreceptor cells, in which mild constant light stimulation for several days overcame the intrinsic resilience of R7 photoreceptors and led to progressive axonal degeneration in the absence of cell death. Aged flies displayed an accelerated and increased vulnerability in this system and loss of synaptic integrity between R7 and its postsynaptic partner preceded axonal degeneration, thus recapitulating important features of human neurodegenerative diseases. Furthermore, we defined precisely the time window in which the axonal damage becomes irreversible. I will present our ongoing work towards a dissection of the molecular and cellular circuit mechanisms involved in the early events of axonal degeneration, allowing for a better understanding of how neurons cope with stress and lose their resilience capacities.

Heat Stress Induced Translation Reprogramming in the Mammalian CNS

017

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Neurons in the mammalian central nervous system adapt to changes in temperature through molecular mechanisms that are poorly understood. Here we show that in response to elevated temperature, neurons downregulate global polysome-dependent translation. Our studies show there is a finite window during which neurons adapt to heat stress by reprogramming translation, but beyond a set point, fail to recover translation and ultimately die. During heat stress, transcriptional activation upregulates key heat shock protein transcripts, such as Hspa1a. To our surprise, despite transcriptional activation of Hspa1a, neurons do not produce Hsp70 protein during heat stress. The lack of Hsp70 protein expression is likely caused by the preoccupation of Hspa1a transcripts with monosomes compared to polysomes during heat stress. Upon return to ambient temperature, neurons rapidly reactivate polysome-dependent translation and eventually express Hsp70 protein within two hours. With the use of Hsp70 knockout mice or pharmacological inhibitor, suppression of Hsp70 during neuronal recovery leads to caspase-dependent neuronal death. Based on these and other original findings, our central hypothesis is that neurons endure heat stress in part through translational reprogramming to prepare the system for survival in the event that recovery is an option.

Identifying genetic modifiers of prion toxicity via synthetic lethality screen

018

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Protein aggregates are thought to be toxic and major drivers of the devastating neurodegenerative diseases including Alzheimer's, Parkinson's, Hungtinton's, amyotrophic lateral sclerosis, and prion diseases. However, the underlying mechanism for the toxicity of these protein depositions remains largely unknown. Prion diseases can be faithfully modeled in vivo (mouse), ex vivo (cultured organotypic cerebellum slice), and in vitro (e.g., GT1-7 mouse hypothalamic GnRH neuronal cell line), proving us an ideal system to address the question. In this study, using cell death as a screening phenotype, we performed an unbiased genome-wide synthetic lethality screen in prion-infected GT1-7 cells via the pooled CRISPR activation sgRNA library. Enriched and drop-out sgRNA hits were identified in prion-infected GT1-7 cells compared to control cells. Furthermore, a considerable number of top hit genes were faithfully verified in multiple validation assays in vitro, including both intersected genes related with neurodegenerative diseases (e.g., Pycard, Htr6) and potentially quite novel genes (e.g., Spaca4, Slc3a1). Pharmacological inhibition of HTR6 (serotonin receptor 6) and SLC3A1 (cysteine up-take transporter) significantly rescued neurodegeneration caused by prion infection ex vivo. Collectively, our study provides a reliable screening strategy and great potential for a landscape understanding on prion toxicity.

Contactin-2 in cortical interneuron maturation and myelination

021

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Interneurons are recently spotlighted due to their myelination pattern. Indeed, the detailed study of interneuron myelination revealed differences in composition, distribution along the axon and, as a consequence, in function. Contactin-2 is an adhesion molecule of the immunoglobulin superfamily with a dynamic spatial and temporal expression, implicated in axonal guidance and migration during development. Postnatally, it organizes myelin around the nodes of Ranvier, specifically in the juxtaparanodes of the axons of hippocampal interneurons belonging to distinct subpopulations. Importantly, apart from excitatory myelinated axons it is expressed in mature oligodendrocytes and interneurons. Its function has been implicated in disorders such as multiple sclerosis and Alzheimer's disease.
Our preliminary data with Contactin-2 deficient mice point to a reduction in the frequency of action potentials in Somatostatin(Sst)+ interneurons. We have previously shown that a significant population of Sst+ interneurons in the hippocampus are Contactin-2+. To further elucidate the role of Contactin-2 in this interneuron subpopulation, we selectively ablate Contactin-2 from Sst+-interneurons the Cre-loxP system. This genetic tool, generated in our team, is anticipated to provide us with data regarding the function and morphology of myelin in this important interneuron subpopulation with the use in vivo and ex vivo approaches.

A forward genetic screen for the modifiers of synaptic autophagy

022

Ayse Kilic

KU Leuven/VIB/Center for brain and disease

Our lab has found a new synaptic autophagy pathway under the control of LRRK2, EndoA, and Synaptojanin1. EndoA and Synj1 are unique to the synapse, and LRRK2 and Synj1 are mutated in Parkinson's Disease, while EndoA is associated with a Parkinson's risk locus. This suggests that synaptic autophagy is a process that is important in the development of this neurodegenerative disease. One of the most ambitious genetic screens in the lab was conducted in Drosophila and >25 modifier loci suppressing the neurodegeneration induced by EndoA mutations were found. Many of these suppressors map to the same pathway of neuronal connectivity. This suggests that autophagy, through neuronal network effects, causes neuronal demise. This collection of new targets puts this research in pole position to understand how synaptic autophagy elicits neuronal death. Since we aim to find common modifiers of synaptic autophagy, other PD-related genes were screened for autophagy defects. Until now, we have found one modifier, a spectraplakin protein that rescues induced autophagy caused by EndoA mutant and one of the PD genes: Rab39. This research will reveal the connections between autophagy at the synapse and neuronal demise.

A mechanism for stretch-induced growth in sensory neurons

023

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After initial elongating growth and connection to target cells, maturing neurons grow by interstitial expansion as the organism grows. The mechanisms regulating this stretch-induced growth phase are still largely unknown. We previously proposed an intrinsic length-sensing mechanism for regulation of elongating neuronal growth, mediated by dynein and axonal importin β1 (Perry et al. 2016; Rishal et al. 2012). Here we tested the possibility that stretch-induced neuronal growth might be regulated through the same mechanism. We established stretch conditions that accelerate growth and enhance protein synthesis in wild-type adult sensory neurons in culture. Striking, neurons from both dynein and importin β1 mutant mice have significantly attenuated responses to stretch. These findings suggest that stretch-induced growth acceleration might be mediated in part by dynein and importin β1 dependent axonal length-sensing.

Neural Precursor Cells as a potential therapeutic approach for Rett Syndrome.

024

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Rett syndrome (RTT) is a neurodevelopmental disorder, mostly caused by MECP2 mutations, representing the leading cause of severe intellectual disability in females. Unfortunately, no cure is available.
Considering the effectiveness of NPCs as treatment for other neurological diseases, we assessed their therapeutic potential to treat RTT. Our research demonstrated their efficacy in vitro and in vivo. Indeed, through a transwell-based co-culture system, we observed that NPCs secrete benefic factors that promote morphological and synaptic rescues in Mecp2 null neurons. In vivo, we demonstrated that NPC transplantation induce in RTT mice a significant amelioration of typical cognitive and motor defects, together with an increased lifespan.
Obtained results highlighted that NPC-mediated beneficial effects arise through paracrine mechanism; by sensing the pathological environment, NPCs secrete factors that promote immunomodulation, neuroprotection and brain plasticity. Bulk RNA-sequencing was used in vitro and in vivo to identify which positive molecular mechanisms are set in motion. One candidate molecule has been already identified and its efficacy validated, while more studies are ongoing to reveal other pathways modulated in KO neurons by NPCs.
Data will be presented to illustrate the value of this cellular approach to treat RTT and/or to identify new defective pathways with therapeutic value.

Pharmacological modulation of neuronal activity to treat Rett syndrome

025

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Rett syndrome (RTT) is a neurodevelopmental disorder, representing the most common genetic cause of severe intellectual disability in females. RTT is caused by mutations in the X-linked MECP2 gene. Given its role as master regulator of gene expression, transcriptional maturation is affected in null neurons, as well as the ability to respond to external stimuli. Neuronal activity plays a key role during brain development, thus we tested the possible causative link between immaturity and reduced activity by pharmacologically stimulating in vitro and in vivo Mecp2 null neurons within different time windows of differentiation.
To enhance activity, we used a positive AMPA receptor modulator, Ampakine CX546. By treating neurons we ameliorated their transcription and activity, highlighting the contribution of defective mechanisms of development to RTT phenotypes. In vivo the efficacy was tested by evaluating the well-being of mice and by performing motor and cognitive behavioral tests. Although the early time window suggested a prolonged benefic effect on knock-out mice, it was devoid of translational value. Therefore, to validate the possibility that ampakine might represent a safe and efficient approach for the treatment of RTT, we tested different time windows with different ampakines. Obtainedresultswillbepresented and futureperspectivediscussed.

Investigating the role of secretory autophagy in shaping the synaptic surfaceome

026

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Autophagy is renowned for its role in degradation, whereby double-membraned autophagosomes (AVs) engulf superfluous cellular components for delivery to the lysosome. However, recent findings from mammalian cell lines revealed non-canonical, yet evolutionarily conserved roles for the autophagic machinery, including a form of unconventional protein secretion (UPS) coined “secretory autophagy”. In this process, a subpopulation of AVs carrying the v-SNARE SEC22b (sAVs) traffic protein cargoes to the plasma membrane under conditions of cell stress. However, whether sAVs also exist under baseline conditions remains unknown. Given that neurons rely heavily on UPS, this raised a question of whether secretory autophagy could help shape the synaptic surface. Here, we show for the first time that secretory autophagy occurs within brain. Using confocal imaging, we confirm the existence of sAVs within neurons, where LC3 and Sec22b colocalise within dendrites. Using biochemical approaches combined with electron microscopy, we further demonstrate that SEC22b is found on the outer membrane of sAVs, facilitating their immunopurification. Proteomic profiling of these vesicles reveals the presence of several integral membrane proteins, including receptor subunits critical for synaptic function. Taken together, our results indicate that secretory autophagy exists in brain neurons as a key component of UPS, shaping the synaptic surfaceome.

Role of developmental regulators of axonal local translation in adult axons

027

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Adult axon regeneration displays numerous similarities with axon growth during development, including elongation, guidance and synaptogenesis. A key mechanism underlying all those processes, is that of local translation (LT), which provides the axons with the ability to modulate their proteome in situ and independently of the soma. Indeed, LT is one of the first processes activated after injury, and high capacity of axons for LT has been positively correlated with their intrinsic ability for regeneration. However, our understanding of the regulation of LT in adult axons is still limited. We have previously identified a ribonucleoprotein complex (Mena-RNP) that regulates axonal LT of specific mRNAs in the developing brain. Here, we investigate the conservation of the Mena-RNP in the adult nervous system (NS), to elucidate the potential role of Mena in adult axon LT and regeneration. We find that Mena-RNP components differ between the developing and adult NS and in CNS vs PNS axons. Moreover, the initial, LT-dependent axon response to injury is significantly affected by the absence of Mena in an ex vivo injury model. Using puromycin incorporation we are currently investigating the exact contribution of Mena to axonal LT, while also examining its role in axon regeneration in vivo.

Altered cerebellum mechanics in Ataxia-Telangiectasia neurodegeneration

028

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Ataxia-Telangiectasia (A-T) is a genetic disorder characterized by progressive cerebellar neurodegeneration. A-T is caused by germline mutations that inactivate the ATM kinase, a key player in the DNA damage response. The cerebellar ataxia in A-T is due to progressive loss of Purkinje neurons (PCs); it is currently unclear why specifically these neurons are hypersensitive to ATM defects. We uncovered a novel function of ATM in protecting cells undergoing mechanical stress. ATM is activated by cell stretching and mediates recovery from mechanical stress by promoting chromatin and cytoskeleton remodelling. ATM deficient cells display altered mechanical properties, including hyper-stiffness and nuclear flattening. We investigated the mechanical properties of PCs in control and A-T patients using in vitro human iPSC-derived PCs as well as patient-derived cerebellar biopsies. We combined immunofluorescence analysis with electron microscopy and Brillouin microscopy (a type of optical elastography that can probe the viscoelastic properties of biological samples). We found that PCs nuclei are giant and euchromatic and exhibit unique mechanical features compared to other neurons. We also found that PCs nuclei in A-T patients exhibit altered nuclear morphology, increased nuclear envelope invaginations and aberrant epigenetic signatures. Altogether our data suggest that Purkinje cell mechanics is implicated in A-T neurodegeneration.

Synaptic vesicles are axonal antioxidant patrols deficient in Huntington disease

029

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Huntington's disease (HD) is an incurable and inherited neurodegenerative disease caused by a dominant mutation in huntingtin protein (mHTT). Oxidative stress is a potential key pathogenic factor in HD. Oxidative stress is an imbalance between oxidants-antioxidants, creating reactive oxygen species (ROS) that cause cell death. How mHTT induces ROS generation and neuronal death? We demonstrated that wild-type HTT protein is crucial for the transport of synaptic vesicles in neurons. I found, by proteomic analysis of motile vesicles, the presence of enzymes of the pentose phosphate pathway (PPP), a major metabolic pathway activated in response to oxidative stress. Hypothesis: Synaptic vesicles have on-board the PPP enzymes necessary to detoxify locally the ROS produced in axons. Results: Using approaches like super-resolution imaging, biochemistry and microfluidic Brain-on-a-Chip technology, I found that under stress, vesicles recruit PPP on their membranes and detoxify the axons. In HD, vesicles cannot recruit PPP, inducing ROS and neuronal death. My research demonstrates a novel and crucial role of the PPP on dynamic synaptic vesicles: vesicles carry the antioxidant machinery and act at distance to detoxify neurons during a stressful situation. G6PD activation on vesicles could lead to new therapeutic strategies in HD.

Asymmetric microtubule nucleation from Golgi within fly neurons

030

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Microtubules are polarised polymers nucleated by multi-protein γ-TuRCs. In axons, microtubules have their plus ends pointing away from the soma (plus-end-out); in dendrites, many microtubules have their plus ends pointing towards the soma. This is critical for cargo distribution and for axon and dendrite identity. Previously, we showed that γ-tubulin is localised asymmetrically to the cis face of the Golgi stacks spread throughout the soma of Drosophila sensory neurons. Microtubules nucleated from the Golgi grow with an asymmetric preference towards the axon and away from dendrites, possibly helping to regulate overall microtubule polarity (Mukherjee et al., 2020, eLife). We have now identified the Golgin proteins responsible for recruiting γ-TuRCs to the cis-Golgi and show that depleting γ-TuRCsrandomises the orientation of microtubule nucleation. The TOG domain protein CLASP localises to the trans-Golgi and its depletion also randomises the orientation of microtubule nucleation. We reason that, under normal conditions, the plus-ends of microtubules nucleated by γ-TuRCs at the cis-Golgi are stabilised by CLASP at the trans-Golgi to create the asymmetry. Consistent with this, we find that Golgi stacks are oriented cis-to-trans towards the axon. We propose that asymmetric nucleation from Golgi helps establish overall microtubule polarity in these neurons.

Elucidating the regenerative capacity of axons through a comparative study

032

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An increasing number of studies suggest that axonal mRNA translation is a crucial mechanism for neuronal survival, highly related to the intrinsic regenerative capacity of axons. Developing central nervous system (CNS) axons are characterized by high levels of protein synthesis associated to their enhanced growth capacity, but this ability is lost while transitioning from development to maturity. On the other hand, mature peripheral nervous system (PNS) axons tend to maintain high levels of local mRNA translation and are able to grow over long distances after injury, achieving fully functional regeneration in contrast to CNS neurons that can only reach a limited level of regeneration and, thus, are unable to regain full functionality. Our project's main aim is to shed light on the potential differences and similarities of the axonal mRNAs and proteins of developing and adult CNS and PNS neurons by a series of high-throughput assays and bioinformatic analyses which will allow the elaborate comparison of the aforementioned molecular repertoires. This comparison could be the key in providing an explanation regarding the low intrinsic ability of adult CNS axons to sufficiently synthesize proteins and regenerate, as well as in identifying specific molecules related to the regenerative capacity of axons.

Dendritic bubbles as Class I-specific MTOCs in Drosophila da neurons

033

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Microtubule nucleation is necessary for cells to form an organised cytoskeleton required for cell development. Microtubule nucleation is templated and catalysed by the multi-protein γ-Tubulin Rinc Complexes (γ-TuRCs), which are recruited by tethering proteins to specific microtubule organisingcentres (MTOCs). In neurons, microtubules promote neurite growth, stability and branching, as well as providing tracks for intracellular transport between the soma and the neurite terminals. In axons, most microtubules are plus-end-out while in dendrites there is a mixed polarity. Understanding how minus-end-out microtubules are generated in dendrites is a long-standing question in the field.

The primary objective of my PhD thesis is to understand the role and regulation of γ-TuRCs within Drosophila melanogaster Class I da neurons in relation to specific structures (dendritic bubbles) that may represent neuronal MTOCs. I have particularly been focussed on the role of γ-TuRCs, which localise predominantly in more distal bubbles. My results have led me to question whether γ-TuRCs are in fact required to anchor microtubules rather than nucleate them, and therefore to question which other proteins may nucleate microtubules in the absence of γ-TuRCs. Overall, I want to understand how the microtubule cytoskeleton is regulated within these dendrites to allow proper neuronal function.

BORC regulates UNC-104 & Dynein-dependent intermediate compartment distribution

034

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The precursors of synaptic vesicles (pre-SVs) are formed from post-Golgi intermediate compartments containing endolysosomal and SV proteins. We have previously identified genes, LRRK2/lrk-1 and AP-3/apb-3 that act on SV-lysosomal intermediates to mediate their separation as well as transport/localization further into the neuronal process. These intermediate compartments are present in the cell body, and only in the first 40um of the proximal neuronal process. We show that the restriction of these intermediate compartments to the proximal axon occurs via the action of both UNC-104/KIF1A and Dynein, where UNC-104 aids in the exit and Dynein aids in the restriction of these compartments. We further show that the BORC complex plays a key role in the exit of these compartments via UNC-104 leading to the accumulation of UNC-104 in the cell body. The BORC complex genetically interacts with lrk-1 and apb-3 by reducing the increased SV-lysosomal exit seen in lrk-1 and apb-3. Together our data suggest that BORC through UNC-104 may act on intermediate compartments to aid in their exit while Dynein might aid in error correction to prevent these compartments from traveling to the synapse.

snRNA-seq reveals vulnerability of cortical cell types in Huntington's disease

035

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A common feature of neurodegenerative disorders is selective vulnerability, where certain neurons succumb to disease early, while others remain spared. The molecular underpinnings of these differences are not well understood. Here, we have performed transcriptomic profiling of the motor cortex from the R6/2 mouse model of Huntington's disease (HD), an incurable hereditary movement disorder caused by a CAG repeat expansion in the Huntingtin gene, which causes degeneration of the striatum and neocortex. In the cortex, glutamatergic neurons are highly susceptible to HD, while GABAergic neurons are more resistant. Strikingly, single-nucleus RNA-sequencing revealed a clear transcriptomic separation of HD and control samples within the glutamatergic, but not GABAergic or non-neuronal cell clusters. Tissue sampling at different time points allowed us to delineate a two-stage disease trajectory with distinct changes at early and late stages. Analysis of differentially expressed genes and pathways demonstrated progressive dysregulation of neuronal cell-type identity in the HD cortex. Among the top dysregulated gene categories, we found genes related to proteostasis, ER stress and synaptic signaling. Taken together, these findings advance our understanding of neuronal vulnerability to degeneration, and lay the foundation for developing targeted therapeutic strategies against HD.

CircRNAs in the pathogenesis of amyotrophic lateral sclerosis

037

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RNA metabolism alterations have been described in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease of motor neurons. Here, we studied circular RNAs (circRNAs) in human spinal cord in ALS and in a neuronal cell line after ferroptosis, a cell death mechanism that mediates selective death of motor neurons in ALS. We performed RNA-sequencing of spinal cord samples of ALS and control individuals and observed dysregulation in the expression of ~25% of circRNAs, most of which are up-regulated and encoded within the protein coding sequence of genes. Within the group of top altered circRNAs, we confirmed the dysregulation of five circRNAs using qPCR and compared these alterations with those observed in SH-SY5Y cells differentiated with retinoic acid after ferroptosis. Then, we used FISH/IF to study the subcellular localization of circRNAs and observed that circRNAs are distributed in granular structures in the nucleus and the cytoplasm, including neurites, but they barely colocalize with classical markers of RNA granules. RNA pull-down/MS experiments identified protein interactors for a subgroup of circRNAs suggesting key roles in neuronal function. These findings and future experiments will help to uncover novel functions of circRNAs in neuronal death and survival, and trace RNA network defects associated with ALS.

Chromatin remodeler CHD2 in a mouse model of ALS

038

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the degeneration of motor neurons. Alterations in RNA metabolism are a hallmark of the disease and the dysregulation of the protein TDP-43 the most relevant in the pathogenesis of ALS. To date, the epigenetic changes related to this phenomenon have been not explored in ALS. Here, we analyzed the expression of the chromatin remodeler CHD2 (chromodomain helicase DNA-binding protein 2) when TDP-43 was dysregulated in vivo and in vitro when cells were stressed by methylglyoxal (MG). Using an ALS mouse model generated by the inducible expression of human TDP-43-ΔNLS, we observed a decrease in the levels of CHD2 in the brain. When HEK293 cells were stressed using MG, we observed a reduction in the levels of CHD2 mRNA and protein. The level of phospho-TDP-43 and phospho-H2A.X were used as controls of the pathology. We conclude that alterations in the expression of TDP-43 are associated with a dysregulation in the expression of CHD2 in vivo and in vitro. And suggest a mechanism for epigenetic modifications of gene expression associated with the pathogenesis of ALS.

Unravelling the role of atg101 in the brain: generation of the first (hypomorph)

039

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Atg101 is a core autophagy protein expressed specifically in non-budding-yeast eukaryotes, substituting Atg29 and Atg31. It participates in the ULK1 complex by directly binding to Atg13 via HORMA domain interactions. In non-neuronal cells, it is indispensable for autophagy initiation under starvation, as it prevents the proteasomal degradation of Atg13. However, its role in neuronal physiology and function remains elusive. To delineate these functions, we have generated the first atg101 knock-in mouse, where the atg101 gene is flanked by two loxP sites and N-terminally tagged by a 3xFLAG and Avi-tag. Validation of these animals demonstrates that atg101 expression levels are negatively affected, producing a hypomorphic mouse. Homozygotes for the tag present with growth retardation and a shorter lifespan, hind limb-clasping and spontaneous seizures. Macroscopic comparison between ATF-atg101F/F and atg5F/F hypomorphic mice indicate that Atg101 has a yet unexplored, non-degradative function responsible for the severe phenotype. MassSpec comparative analysis of the hypomorphs further indicate a non-canonical role of Atg101 in the nucleus of neurons. Further EM and electrophysiology experiments will shed light on the unique functions of this core autophagy protein in brain physiology and function.

SLC13A5 in the human brain – a road map to in vitro models

040

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Citrate transporter disorder (CTD) is a severe neurological disorder caused by recessive mutations in the SLC13A5 gene. One such mutation, G219R, has been found to be almost 50x more common in the Icelandic population than the rest of the world.
The SLC13A5 gene encodes a sodium-coupled citrate transporter (NaCT), the orthologue of which can be knocked out in experimental animals without deleterious neurological effects. The neurological symptoms seen in CTD in humans include severe epilepsy, ataxia, hypotonia and lack of speech. This highlights a need for a human-based model system for CTD.

The project presented here began with a histological analysis of human brain samples from non-affected individuals. We examined several brain areas, determining the localization of the protein on a tissue scale as well as on the cellular level. This information is then used as a guide in the generation and utilization of in vitro model systems.

For the model systems I use patient-derived iPS cells and an isogenic control line to test hypotheses on cell types generated through neural induction and differentiation. We analyze differences in cell morphology, neuronal outgrowth and biochemical processes and have generated co-cultures to examine the interplay between these cells.

Characterizing activity-dependent neuroligin-3 cleavage

041

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Neuroligins (NLGNs) are a family of post-synaptic single-pass transmembrane proteins that, along with neurexins, their pre-synaptic binding partner, aid in the formation, maintenance, and function of synapses. NLGN1-3 have at least 98% homology between humans and mice, making mouse models a translatable method for investigation. NLGN3 is located on the X-chromosome and is located at both glutamatergic and GABAergic synapses. There are many well-characterized patient derived mutations from people with ASD on NLGN3, and it was recently discovered that NLGN3 is potent mitogenic factor in glioblastomas.

Recent work by our lab and others demonstrated that NLGN3 undergoes activity-dependent cleavage, resulting in an extracellular domain (ECD) product and a C-terminal fragment (CTF). The NLGN3 ECD has been investigated in the context of glioma, but the physiological role of the NLGN3 ECD and CTFs remain unknown. We have discovered differential synaptogenic effects of the NLGN1 and NLGN3 ECD when bath applied to hippocampal neurons. Additionally, we have identified multiple species of NLGN3 CTF that vary with PKC activity and protease inhibitor treatment. This work is critical to our understanding of the physiological role of NLGN3 and future work on ASD patient derived mutations could lead to possible therapeutic treatments.

Dendrimer-Mediated Delivery of Therapeutics Across the Blood-Brain Barrier

042

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Neurodegenerative diseases have emerged as a global health concern, with current therapeutics often unable to cross the blood-brain barrier (BBB) due to its role in regulating transport between blood and the central nervous system. Recent advances in nanotechnology have led to the development of dendrimers as a promising tool for the transport of biologically active compounds across the BBB.

This study aimed to investigate the genotoxic effects, internalization efficiency, and quality of cell adhesion and integrity in an in vitro model of the BBB affected by dendrimers and their complexes with siRNA targeted against genes implicated in the development of Alzheimer's disease. The genotoxic properties were assessed using the Fast Halo Assay, internalization efficiency was assessed using confocal microscopy. The quality of brain microvascular endothelial cell (HBEC-5i) adhesion and integrity in the BBB in vitro model was evaluated by real-time cellular impedance analysis.

Our findings demonstrate that the tested dendrimer has significantly genotoxic effects only in relatively high concentrations. Complexation with nucleic acid significantly mitigated the genotoxic effects of the dendrimer. It was observed that the studied dendrimer efficiently internalizes to cells and significantly induce co-internalization of siRNA. The tested nano-carriers and their siRNA complexes can effectively reduce barrier integrity.

Repurposing Drug Combinations for Glioblastoma in Zebrafish Larval Models

043

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Glioblastoma multiforme (GBM), a type of aggressive brain cancer, makes up more than 50% of all brain cancer incidents annually. Yet radiotherapeutic drugs have limited use, hence new treatment modalities are needed. Zebrafish, a widely-used vertebrate disease model, provides advantages in testing neurotoxicity and anticancer activity of drugs due to its high fecundity and genome similarity to mammals. In this project, we tested the repurposing potential of FDA approved drugs and/or their combinations in glioblastoma in zebrafish larvae; these drugs were trifluoperazine (TFP), an antipsychotic, sorafenib (SFB), a kinase inhibitor, and panobinostat, a pan-HDAC inhibitor. We found that the combination of TFP and SFB induced a drastic decrease in the viability of GBM cells. In addition, panobinostat was found to be effective even at very low concentrations, indicating the potency of these drugs for repurposing. To assess the ability of our treatments, morphological assessments and behavioral assays were performed in zebrafish larvae. Next, an orthotopic xenograft model was generated to test changes due to drug treatment in vivo with respect to tumor burden. Our results are promising for future personalized medicine applications of GBM therapy.

Investigating α-Synuclein mediated coordination of RNA metabolism

044

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Parkinson's disease (PD) is a severe neurodegenerative disorder. The disease is linked to the aggregation of a small amyloid protein, alpha-synuclein (aSyn), which is implicated in synaptic vesicle trafficking and neurotransmitter release, with an as yet undefined role. In PD, aSyn is found in brain inclusions in neurites and in Lewy bodies. A well characterized mutation of aSyn (G209A), encodes for A53TαSyn protein that exhibits faster aggregation kinetics and is directly linked to the familiar type of PD. In this study, we use a toolkit of neuronal cell line with stable expression of A53TαSyn, primary hippocampal neurons from transgenic A53T mice and patient derived hiPSC-neurons. Our transciptomics and proteomics analysis of hiPSC derived neurons revealed altered expression levels of core molecules involved in RNA metabolism linked to A53T mutation. Combining "-omics" approaches with high end microscopy and single molecule RNA FISH (smFISH), we aim in investigating how the expression of A53TαSyn affects RNA dynamics in neurons. Initial data bridge aSyn biology to RNA granule organization and imbalanced metabolism of RNA machinery triggered by the presence of A53T aSyn in cellular models of PD.

Fundingsource: aSyn EPANEK-ESPA (T2EΔK-02813& MIS 5131418; to RM)

Modeling group 3 medulloblastoma with ARHGAP36/MYC induction in mice.

045

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Rho GTPase-Activating Protein 36 (ARHGAP36) suppresses PKA activity by driving protein degradation via a lysosome-mediated process and acting as a pseudosubstrate. Through PKA inhibition, ARHAP36 induces ectopic activation of SHH signaling and cell proliferation leading to abnormal development of the cerebellum in mouse embryos. Notably, despite its inductive activity on SHH pathway, ARHGAP36 expression is higher in group 3 and group 4 medulloblastoma (MB), rather than in the SHH subgroup, and high levels correlate with poor prognosis. Modeling group 3-4 MB in mouse models has proven difficult and could be achieved by overexpression of selected gene combinations in the postnatal mouse brain. We demonstrate that intracranial transfection of ARHGAP36 together with MYC in mouse pups (P0) generates MB that molecularly and phenotypically belongs to the group 3 subtype. Tumorigenesis depends on ARHGAP36-mediated PKA inhibition providing druggable targets for treatment of group 3 MB.

Mechanism of low NAD+-induced photoreceptor neuron degeneration in Drosophila

046

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Nicotinamide adenine dinucleotide (NAD+) is a vital cofactor and coenzyme. Downregulated NAD+ homeostasis is observed in many neurological pathologies, including chronic and acute degenerative conditions. Conversely, dietary supplementation or genetic manipulation resulting in bolstered NAD+ homeostasis prevents neurodegeneration and harbors beneficial outcomes. However, how a decrease in NAD+ mechanistically results in neurodegeneration remains unknown.

We present a novel Drosophila model to manipulate NAD+ homeostasis specifically in photoreceptor neurons. NAD+ levels are lowered by the expression of a constitutively active NAD+ hydrolase dSarm lacking its autoinhibitory ARM domain (dSarm∆ARM). The photoreceptor neuron-specific dSarm∆ARM expression triggers their degeneration, resulting in a small adult compound eye serving as a proxy for neurodegeneration. Our model is sensitive to detect changes induced by dosage-dependent dietarily-supplemented NAD+ precursors, compounds targeting NAD+-consuming enzymes, and autonomous genetic manipulations of NAD+ metabolism; all moderately reversed the small-eye phenotype. We present our preliminary characterization of mutants isolated in a forward genetic pilot screen fully reverting the small-eye phenotype. These candidates likely suppress neurodegeneration induced by low NAD+ levels. Impaired NAD+ flux triggers a broad range of metabolic disorders. Evolutionarily conserved genes identified in our Drosophila model might provide druggable targets to ensure robust NAD+ homeostasis in mammals.

Chaperoning proteostasis in axons

047

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A key to neuronal resilience and longevity is the maintenance of active proteostasis. The pace of production and destruction of proteins must be tightly regulated and misfolded peptides and aggregates rapidly cleared in order to prevent accumulation of transport material in neurites, especially the axon. The interaction between proteostatic processes, such as the translational machinery, the ubiquitin-proteasome system and autophagy is important to ensure rapid responses and coupling in compensatory processes under stressful conditions. Furthermore, these processes depend on the cellular transport machinery, in particular microtubule transport.

We study the roles of an ATPase complex in setting up and maintaining healthy motor neurons in Drosophila melanogaster. We have found that this complex is required for constraining synaptic bouton growth at the neuromuscular junction (NMJ) during larval development, and loss of function leads to progressive loss of motor skills, accompanied with shortened life span and the accumulation of cellular materials and polyubiquitinated proteins in axons and at the NMJ, indicating failure in proteostasis and/or transport. As the proteins also show conserved interactions with parts of the proteostatic machinery, as well as with microtubules, we are further investigating their potential role at the intersection of proteostatic processes and transport of aggregating material.

CRMP2 isoforms in microtubule dynamics, brain development and autism

048

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Deregulation of microtubule associated proteins has been associated with several neurodevelopmental disorders. We have shown that deficiency in CRMP2 results in defective axon guidance, synapse pruning and histological and behavioral changes associated with autism spectrum disorder (ASD). CRMP2 is spliced into 2 isoforms. While CRMP2B isoform has been extensively analyzed, little is known about the low-abundant CRMP2A.

Here, we show that CRMP2A binds microtubules and, compared to CRMP2B, significantly increases tubulin polymerization velocity and rescue rate. Moreover, CRMP2A preferentially forms tetramers interacting with microtubules. To assess the biological role of CRMP2A, we generate isoform-specific CRMP2A-KO mice and found that they show similar defects in axon guidance, spine pruning and ASD-linked behavior as the full CRMP2-KOs.
CRMP2 autoantibodies were detected in human maternal ASD sera. We demonstrate that maternal ASD sera show altered affinity to CRMP2 isoforms and that CRMP2 antibodies, or CRMP2, change cortical neuron migration and axon growth by activating NMDA receptors.

Our work demonstrates that the low-abundant CRMP2A is essential in development, promoting microtubule and axon growth, synapse pruning, and that its deficiency leads to ASD-related phenotype. Furthermore, we present the first evidence and mechanism how CRMP2 autoantibodies alter brain development and may contribute to ASD.

Active zone plasticity optimizes brain aging

049

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The brain as a central regulator of stress integration determines what is threatening, stores memories, and regulates physiological adaptations across the aging trajectory. While sleep homeostasis seems to be linked to brain resilience, how age-associated changes intersect to adapt brain resilience to life history remains enigmatic. We here provide evidence that a brain-wide form of presynaptic active zone plasticity(“PreScale”), characterized by increases of active zone scaffold proteins and synaptic vesicle release factors, integrates resilience by coupling sleep, longevity, and memory during early aging of Drosophila. PreScale increased over the brain until mid-age, to then decreased again, and promoted the age-typical adaption of sleep patterns as well as extended longevity, while at the same time it reduced the ability of forming new memories. Genetic induction of PreScale mimicked early agingassociated adaption of sleep patterns and the neuronal activity/excitability of sleep control neurons. Spermidine supplementation and pharmacological induction of sleep for 2 days in mid-age flies reset PreScale, restored memory formation, and rejuvenated sleep patterns. Our data suggest that early along the aging trajectory, PreScale acts as an acute, brain-wide form of presynaptic plasticity to steer trade-offs between longevity, sleep, and memory formation in a still plastic phase of early brain aging.

The role of FMRP in glioblastoma

051

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Converging evidence indicates that the Fragile X Mental Retardation Protein (FMRP) can modulate the aggressiveness of cancer. While FMRP functions have been extensively studied and partially uncovered in brain development, its involvement in the biology of brain tumors remains largely unexplored. Here we show that FMRP expression directly correlates with worse outcome in patients with glioblastoma (GBM). Furthermore, high FMRP expression levels promotes brain infiltration and tumor growth in xenografts generated with human GBM stem-like cells (GSCs). Finally, the FMRP-regulated transcriptome of human GSCs highlights the canonical Wnt/βcatenin and the non-canonical signaling pathways involved in the proliferation of this tumor. Our findings support a key role for FMRP in GBM cancer progression, acting via regulation of Wnt signaling.

Detachment of tau from microtubules drives mechanical stress as an early event o

052

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Tau is a microtubule binding protein involved in stabilising the cytoskeleton, especially in axons. One of the earliest events associated with MAPT mutations is the destabilisation of the microtubule skeleton. My working hypothesis is that cytoskeletal stress caused by tau defects leads to changes in the mechanical properties of brain cells, by affecting tissue viscoelasticity and nuclear envelope integrity, ultimately activating an innate immune response. We have developed a novel human MAPT knock-in (KI) mouse model displaying hyperphosphorylation (but no aggregation), synapse loss and cognitive deficits, which closely phenocopies the earliest events in tau pathogenesis. Interestingly, the mutant line shows tau-dependent nuclear envelope invagination, ruptures, DNA damage accumulation and cytoskeletal distress compared to the wild-type KI. We see that tau affects cytoskeletal dynamics, leading to impaired brain viscoelasticity, nuclear mechanics and ultimately inflammation. This work will help us understand how pathological tau exerts toxicity in the very earliest stage of frontotemporal dementia and other tauopathies.

Ceramide Synthase at the Knot of Neurodegeneration

054

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The brain is very susceptible to disturbances in lipid metabolism. In particular, impaired ceramide metabolism was associated with a number of severe neurological disorders including Progressive Myocolic Epilepsy 8 (PME8). PME8 is caused by a loss of Ceramide synthase (CerS) activity and patients suffer from epileptic seizures, mental retardation, and gait impairments. Here we have established an in vivo model for studying PME8 using Drosophila larval c4da neurons. Those show very strong dendrite growth defects and morphological degeneration when expressing the disease-causing allele. Our shot-gut lipidomic analysis shows that cerS mutant c4da neurons suffer from reduced amounts of complex membrane lipids and at the same time accumulating signaling lipids such as (dh)S1P and C16-ceramides. Using genetics we show that all the above-mentioned metabolic changes on their own already negatively affect c4da neuronal morphology. Our research led us to develop a model in which the lack of membrane lipids in cerS mutant neurons may primarily cause neurodevelopmental growth defects while accumulating signaling lipids may become toxic over time and would be the driving force for an early onset of neurodegeneration. Taken together we revealed the complex metabolic alterations in cerS deficient neurons which offers also new possibilities for treating PME8 patients.

Microtubule networks are a key lesion site in ageing neurons and a key driver of

055

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Nerve cells have an extreme morphology, demanding metabolism and must survive for our entire lifetime. Unsurprisingly, ageing neurons display gradual axonal and synaptic decay, become more vulnerable to environmental or genetic stressors and are at risk of developing pathologies reminiscent of neurodegenerative diseases. However, it is very little understood what mechanisms cause neuronal ageing.

To deal with the complexity of the cell biology of neuronal ageing, we introduced a new cellular model within the Drosophila brain. We could show that microtubule properties change during ageing driving other ageing hallmarks including appearance of axonal swellings, reduction in axonal calibre and morphological changes at synaptic terminals. Furthermore, we identified specific cytoskeleton regulators necessary for the maintenance of axons and synapses, which drive microtubule deterioration and neuronal decay during ageing. Our data demonstrates that their activity decreases with age and this is driven by oxidative stress, as typically occurring in ageing; Preventing oxidative stress or boosting these cytoskeleton regulators not only improves microtubule decay but also axonal and synaptic atrophy. Our research suggests that microtubules offer promising opportunities to improve neuronal health in advanced age.

Supportedby the WellcomeTrust and the BBSRC.

Spectraplakin Couples Microtubule Orientation to Actin During Dendritic Pruning

056

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Neurite pruning, the elimination of specific axonal or dendritic branches, is an essential mechanism to refine developing neural circuitry. Following local microtubule and actin disassembly, Drosophila sensory c4da neurons prune their dendrites during metamorphosis. We previously found that the uniform plus end-in orientation of dendritic microtubules is required for efficient pruning by enabling their coordinated disassembly. How dendritic microtubule organization is established is only incompletely understood. Here, we show that the spectraplakin short stop (Shot), an actin-microtubule crosslinker interacting with plus- and minus-end binding proteins, is required for c4da neuron dendritic pruning. We find that Shot genetically interacts with known factors governing dendritic microtubule organization, and loss of Shot itself misorients dendritic microtubules. A putative self-interaction domain of Shot as well as the actin binding ability of Shot impinges on microtubule orientation. Using structured illumination microscopy, we show that Shot forms parallel tracks along dendrites. We propose that Shot tracks act as a template to orient microtubules in dendrites, facilitating pruning.

SREBP modulates the NADP+/NADPH cycle to control night sleep in Drosophila

057

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Sleep behavior is conserved among evolution, and sleep disturbances are a frequent comorbidity of neuropsychiatric disorders(1). However, the molecular basis remains elusive(2). Using a model for neurodevelopmental disorders, the Drosophila Cytoplasmic FMR1 interacting protein haploinsufficiency(3), we identify a novel mechanism modulating sleep. We show that increased activity of the sterol regulatory element-binding protein (SREBP) in Cyfip85.1/+ flies increases the transcription of wakefulness-associated genes, such as the malic enzyme (Men), causes disturbances in the daily NADP+/NADPH oscillations reducing sleep pressure at the night-time onset. Modulation of these genes rescues the metabolites and sleep deficits, indicating that these genes act downstream Cyfip to regulate sleep. Our work identifies a novel molecular pathway worth exploring for its therapeutic potential in sleep disorders(4).

1 Missig G et al. Sleep as a translationally-relevant endpoint in studies of autism spectrum disorder. Npp, 2020.
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Autophagy limits glycolytic metabolism in cerebellar Purkinje cells

059

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The housekeeping role of autophagy in selective protein degradation is believed to be important in neurons, because these cells are not able to dilute detrimental proteins and organelles by cell division. However, recent studies show that neurons are resistant to autophagy-deficiency in-vivo, with an exception of cerebellar Purkinje cells, which are selectively vulnerable to autophagy loss compared to forebrain GABAergic neurons. The mechanisms underlying the selective vulnerability of cerebellar Purkinje cells to autophagy deficits are currently elusive. Using a combination of proteomic, metabolomic and ex-vivo imaging techniques, we find that the cerebellum of conditional ATG5 KO mice is highly glycolytic, whereas these changes are not observed in ATG5-deficient cortex. We could further identify that these alterations depend on glucose transporter 2 (GLUT2), which is degraded in Purkinje cells by autophagy. Loss of ATG5 stabilizes GLUT2 levels, a phenotype that leads to elevated levels of glycolysis-derived ATP without affecting mitochondrial respiration. Taken together, our data indicate that postmitotic neurons are able to rewrite their metabolism to adapt to autophagy-deficient conditions.

Novel role of endocytic kinase AAK1 in regulation of autophagosome trafficking

060

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Clathrin-mediated endocytosis (CME) is highly important for recycling of synaptic vesicles during neurotransmission. CME is initiated by the recruitment of the adaptor protein complex-2 (AP-2) to the plasma membrane followed by the assembly of clathrin. It is known that phosphorylation of AP-2 is critical for its membrane association by adaptor-associated kinase-1 (AAK1). Apart from its role in CME, AAK1 also regulates dendritic branching, plays a role as a positive regulator of Notch pathway and is involved in regulation of β-catenin-dependent WNT signaling pathway. In spite of this, the consequences of AAK1 loss-of-function in the brain have not been yet explored. Here, by combining mouse genetic and biochemical approaches we reveal that AAK1 is not essential for neuronal survival. AAK1 KO mice are hyperactive and reveal deficits in motor coordination. Interestingly, AAK1 KO brains are smaller and show dysregulated autophagy pathway, a phenotype which becomes more severe with age. Using a combination of proteomic and phosphoroteomic approaches we reveal novel AAK1 substrates, which are potentially important to regulate autophagosome trafficking in neurons. Taken together our data suggest that AAK1 has a novel role in regulation of neuronal autophagy and is a promising target for autophagy modulation in the brain.

Phosphorylation of NL2 by PKA regulates cell surface abundance and synapse.

063

JaturonKwanthongdee et al.

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γ-Aminobutyric acid type A receptors (GABAARs) are the principal mediators of fast synaptic inhibition and play a key role in controlling the balance between neuronal excitation and inhibition in the brain. Therefore, defects in GABAARs are implicated in inhibitory neurotransmission efficacy and neurological conditions.

The trans-synaptic adhesion molecule Neuroligin-2 (NL2) is a transmembrane protein that interacts with presynaptic neurexins, and which can, in addition recruitscaffold protein gephyrin, to stabilize GABAARs in the postsynaptic domain. However, the molecular mechanisms regulating synaptic stabilization of NL2 remain poorly understood. Here, we investigated the molecular mechanisms that control NL2 phosphorylation and its effect on NL2 dynamic behavior.

We found that phosphorylation of NL2 by cAMP-dependent protein kinase (PKA) can promote NL2 dispersal from the synapse, a reduction in NL2 surface levels and a loss of synaptic GABAARs. Thus PKA plays a key role in regulating NL2 function and GABAergic inhibition.

A proteostasis-mechanics-ECM-junctions crosstalk ensures astroglialarchitecture

065

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Circuit function relies on faithful assembly and resilient maintenance of circuit architecture. Failure to preserve circuit integrity upon environmental challenge results in neuropathology. Neurons and glia both sculpt circuits, and glial architecture defines connectivity. Yet, molecular players ensuring their in vivo long-term integrity, crosstalk with extracellular matrix (ECM) and the environment, remain understudied. C. elegans offers a powerful setting for single-cell-resolution investigation, with well-characterized anatomy, connectivity, genetic tractability and glia largely dispensable for neuronal viability. We dissect lifelong maintenance of circuit architecture, by combining advanced genetics, quantitative imaging of (sub)cellular features, ECM and biomechanics.

We study glia analogous to astrocytes in molecular content, functions, and ramified, synapse-ensheathing architecture. We isolated mutants displaying age-progressive, environment-dependent defects in glial architecture. Glial-membrane defects trigger mispositioning of neurons, axons and synapses. Thus, glia ensure maintenance of circuit architecture across spatial scales. Synapse healthspan and animal's lifespan are also affected. An underlying HSP70-chaperone malfunction causes abnormal ECM deposition, hypersensitivity to temperature and mechanical stress, and defective glial architecture following ECM- and cell-attachments. Brillioun microscopy reveals that mutants suffer decreased tissue viscosity. Importantly, modifying environment's viscosity, ECM composition, or cell junctions, safeguards glial integrity. An interplay of proteostasis, ECM and mechanics, ensures robust circuit integrity.

Molecular basis of clinical onset in familial Alzheimer's disease (FAD)

066

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KU Lueven

FAD, caused by mutations in Presenilin (PSEN1/2) and APP genes, is associated with an early age at onset (AAO) of symptoms. AAO is relatively consistent between carriers of the same mutations, but differs markedly between individuals carrying different mutations. Gaining a mechanistic understanding of why certain mutations manifest symptoms several decades earlier than others is extremely important in elucidating the foundations of pathogenesis and AAO. Pathogenic mutations affect the protease (PSEN/γ-secretase) and the substrate (APP) that generate Aβ peptides. Altered Aβ metabolism has long been associated with AD pathogenesis. However, its relationship with the AAO is still unclear. Here, we investigated this central aspect of AD pathophysiology via comprehensive analysis of 25 PSEN1 FAD-linked Aβ profiles generated in vitro by rescuing MEF deficient in PSEN1/2 with the respective gamma secretase mutants/wt. Our data demonstrate linear correlations between mutation-driven alterations in Aβ profiles and AAO. In addition, our studies show that the Aβ (37 + 38 + 40) / (42 + 43) ratio offers predictive value in the assessment of ‘unclear’ PSEN1 variants. This study thus delivers valuable assays for fundamental, clinical and genetic research as well as supports therapeutic interventions aimed at shifting Aβ profiles towards shorter Aβ peptides.

Heterogeneous SMN Protein Levels Driving Selective Motor Neuron Vulnerability

067

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Selective degeneration of specific neuronal subtypes is a hallmark of all neurodegenerative diseases, but its molecular basis remains unresolved. This knowledge gap hampers the discovery of efficacious treatments. This striking degenerative pattern also characterizes the motor neuron (MN) disease spinal muscular atrophy (SMA), a leading genetic cause of infant mortality caused by low levels of SMN, an essential homeostatic protein involved in many housekeeping processes such as mRNA splicing. Previously, we found that MNs display heterogeneous SMN levels, regardless of genetic background. By employing genome-engineered SMN:CloverhiPSCs, live longitudinal imaging, and automated single-cell quantification, we demonstrated a direct correlation than can estimate MN survival time from SMN Level. This method revealed that MNs are classifiable as, “constant” or “increasing,” depending on whether they harbor steady or rising SMN protein levels as stress increases. Furthermore, “increasing-MNs” survive longer than “constant-MNs” indicating that increasing SMN protein confers an intrinsic resistance to degeneration. These results support SMN protein heterogeneity as a driver of selective neuronal vulnerability and suggest that SMA may not be a MN disease once intrinsically low-SMN MNs are lost. In such case, current treatments would need to be reevaluated. Additionally, understanding single-cell SMN expression regulation could reveal therapeutically relevant avenues.

Mitochondria and the ER as opposing dynamics players in pruning

068

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The University of Texas at Arlington

The precise role of how subcellular structures influence cell pruning is an intriguing area of study. We discovered a novel cell elimination program, Compartmentalized Cell Elimination (CCE), in two cell types in the developing C. elegans embryo. Here, three cell domains-the soma, proximal process segment and distal process segment -are dismantled differently. CCE is highly ordered and stereotyped showing hallmarks of two types of neurite pruning, fragmentation and retraction. We present CCE as a novel in vivo setting to better understand the molecular mechanism of pruning. Our forward genetic screens illuminate how CCE is initiated and executed and implicate subcellular organelles with opposing roles. Our data suggest retrograde trafficking of mitochondria is essential for CCE and that caspase function is required to confine mitochondria in the cell body, identifying a conserved kinesin as an in vivo caspase target. We also find that the ER is dynamically localized across development and that conserved ER shaping and microtubule severing genes linked to neurodegeneration promote CCE execution by facilitating organized microtubule disassemble. Our studies suggest mitochondria and ER have opposing roles, as cell protective and cell regressive respectively, in CCE, and by extension pruning.

Peroxisomal biogenesis as a potential target for polyglutamine disorders.

070

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Polyglutamine disorders, such as Huntington's disease and certain types of Spinocerebellar Ataxia are the result of abnormal expansion of CAG glutamine codon repeats in important genes. This expansion leads to the synthesis of elongated polyglutamine (polyQ) chains in the affected protein and the formation of aggregates. In patients, this is associated with the gradual development of severe neurodegenerative phenotypes such as motor and cognitive deficits. Studies in patients and animal models have suggested that lipid and oxidative metabolism are connected to the emergence of these symptoms. They have also specifically implicated PPAR-δ and PGC-1α as important factors mediating disease pathogenesis. Taken together, these findings suggest that peroxisomes, with their prominent auxiliary role in lipid metabolism and their central role in ROS regulation could also be key players in polyQ-induced neurodegeneration. To test this hypothesis, we examined the effects of manipulating peroxisomes on the formation of polyQ aggregates in C. elegans models of the disease. We demonstrate that the inhibition of peroxisomal biogenesis during development can significantly delay the formation of large polyQ inclusions. We are currently dissecting the cellular and molecular mechanisms underlying these neuroprotective events.

Neuronal atg1 Coordinates Autophagy Induction and Physiological Adaptations

071

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The mTORC1 nutrient-sensing pathway integrates metabolic and endocrine signals into the brain to evoke physiological responses to food deprivation, such as autophagy. Nevertheless, the impact of neuronal mTORC1 activity on neuronal circuits and organismal metabolism remains obscure. Here, we show that mTORC1 inhibition acutely perturbs serotonergic neurotransmission via proteostatic alterations evoked by autophagy inducer atg1. Neuronal ATG1 alters the intracellular localization of the serotonin transporter, which increases extracellular serotonin and stimulates the 5HTR7 postsynaptic receptor. 5HTR7 enhances food-searching behaviour and ecdysone-induced catabolism in Drosophila. Along similar lines, pharmacological inhibition of mTORC1 in zebrafish also stimulates food-searching behaviour via serotonergic activity. These effects occur in parallel with neuronal autophagy induction, irrespective of autophagic activity and protein synthesis reduction. In addition, ectopic neuronal atg1 expression enhances catabolism via insulin pathway downregulation, impedes peptidergic secretion, and activates non-cell autonomous cAMP/PKA. The above exert diverse systemic effects on organismal metabolism, development, melanization, and longevity. We conclude that neuronal atg1 aligns neuronal autophagy induction with distinct physiological modulations, to orchestrate a coordinated physiological response against reduced mTORC1 activity.

Altered striatal actin dynamics drives behavioral inflexibility in FXS mice.

072

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The proteome of glutamatergic synapses is diverse across the mammalian brain and involved in neurodevelopmental disorders (NDDs). Among those is fragile X syndrome (FXS), an NDD caused by the absence of the functional RNA-binding protein FMRP. Here, we demonstrate how the brain region-specific composition of postsynaptic density (PSD) contributes to FXS. In the striatum, the FXS mouse model shows an altered association of the PSD with the actin cytoskeleton, reflecting immature dendritic spine morphology and reduced synaptic actin dynamics. Enhancing actin turnover with constitutively active RAC1 ameliorates these deficits. At the behavioral level, the FXS model displays striatal-driven inflexibility, a typical feature of FXS individuals, which is rescued by exogenous RAC1. Striatal ablation of Fmr1 is sufficient to recapitulate behavioral impairments observed in the FXS model. These results indicate that dysregulation of synaptic actin dynamics in the striatum, a region largely unexplored in FXS, contributes to the manifestation of FXS behavioral phenotypes.

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