1. Yossi Buskila – LBA 01

Alterations in astrocytic K+ clearance during neurodegeneration

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Neurodegeneration is a slow, progressive, and irreversible loss of neuronal cells in specific regions of the brain, leading to reduced motor and cognitive abilities. Aging and neuroinflammation are major risk factors in neurodegenerative diseases. Traditionally, most research about neurodegenerative diseases has taken a neuro-centric approach, focusing onneuronal dysfunction. However, the mammalian brain contains two major cell populations;neurons and glia, and recent genetic and transcriptomic findings strongly suggest that glia are the first cells which change with aging. This implies that glial cells play a key role in the mechanisms which dispose the aged brain to neurodegeneration, especially as ion homeostasis is a function that is primarily carried out by glial cells.

Astrocytes are type of glia that constitute the single largest population of cells in the brain, and recent advances have demonstrated that low expression of K+ channels in astrocytes is associated with age-dependent neurodegenerative diseases including Alzheimer's disease (AD) and Amyotrophic lateral sclerosis (ALS). In this study, we have investigated the ability of astrocytes to maintain K+ homeostasis in the brain via direct measurement of the astrocytic K+ clearance rate in the motor and somatosensory cortices of an ALS mouse model (SOD1G93A) and AD mouse model (5xFAD). Using electrophysiological recordings from acute brain slices, we show region-specific alterations in the K+ clearance rate, which was significantly reduced in the primary motor cortex of SOD1 mouse and hippocampus of 5xFAD mouse, but not the somatosensory cortex. These changes were accompanied by morphological alterations and were partly due to impaired conductivity via Kir4.1 channels as well as a low coupling ratio in astrocytic networks. These findings indicate that the supportive function astrocytes normally provide to neurons is diminished during disease progression and provides a potential explanation for the increased vulnerability of neurons during neurodegeneration.

2. Arpad Dobolyi – LBA 02

Molecular and network level control of social behaviour by the medial prefrontal cortex

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Social isolation induces negative behavioral changes in both humans and rodents; however, the underlying genomic and network-level mechanisms in the brain remain unclear. This study aimed to analyze and correlate behavior, gene expression levels, and molecular functions in socially isolated and pair-housed rats, focusing on the medial prefrontal cortex (mPFC). The sociability of isolated animals decreased, yet they spent more time with conspecifics in the social preference and social interaction tests compared to the social group. Elevated plus maze and open field tests revealed heightened anxiety-like behavior in isolated animals, while depression-like behavior assessed through the forced-swim test remained unchanged.

To investigate the mPFC projections involved in these behavioral changes, the study examined the role of different projection neurons in social behavior. Viral gene transfer was used to express designer receptors in neurons projecting to the medial preoptic area (MPOA) and in calcium/calmodulin-dependent protein kinase II (CaMKII)-containing neurons of the mPFC, selectively projecting to the thalamus. Prefrontal neurons projecting to the preoptic region also projected to other subcortical areas. Stimulation of these neurons resulted in decreased sociability in the three-chamber test, but direct interaction between the animals remained unaffected. CaMKII neurons projected strongly to the paratenial, mediodorsal, and submedius thalamic nuclei but not to the hypothalamus. Stimulation of CaMKII-containing cells in the mPFC reduced time spent with conspecifics in the three-chamber test and the duration of various social interaction elements. These findings indicate the role of these projection neurons in the regulation of social behaviors.

To uncover the molecular basis of mPFC alterations induced by social isolation, RNA sequencing was performed. Over 30 genes displayed differential expression between the groups, meeting the criteria of log2FC > ±1 and a correlated p-value < 0.05. KEGG pathway analysis revealed the involvement of these genes in addiction development and the regulation of behavioral and learning mechanisms. Several genes were validated through RT-qPCR, and one of them, 5-Hydroxytryptamine Receptor 2C (Htr2c), known to be expressed by mPFC projection neurons, underwent further functional examination. Rats treated with an Htr2c antagonist while housed in pairs exhibited similar behavioral changes to those observed in isolated rats. This suggests a crucial role of Htr2c in the heightened demand for conspecific interaction and increased activity resulting from social isolation.

3. Alessandro Prinetti – LBA 03

The multifaceted roles of sphingolipids in orchestrating myelin repair

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Recombinant human IgM22 (rHIgM22) effectively promotes remyelination in mouse models of multiple sclerosis. However, the target antigen and the signaling mechanisms through which rHIgM22 exerts its function are still unclear.

We showed that rIHgM22 binds in vitro to sulfatide, but also to phosphatidylinositol, phos-phatidylserine and phosphatidic acid. Moreover, changes in the composition of the lipid microenvironment of the target antigen were able to modulate the affinity of the antibody, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity.

In rat mixed glial cells (MGCs), rHIgM22 induced a proliferative response in all the cells in the culture, with the most significant response associated with astrocytes and oligodendrocyte precursor cells (OPCs). Treatment also induced the production and release of sphingosine 1-phosphate (S1P) by microglia, suggesting that rHIgM22 indirectly influences the proliferation of astrocytes and OPCs via microglia-released S1P. However direct effects of rHIgM22 on astrocytes and other glia cell types cannot be excluded.

We studied rHIgM22 on the glycosphingolipid metabolism of different cultured glial cells. rHIgM22 had no significant effects on the lipid patterns in pure astrocytes, while in OPCs, oligodendrocytes (OLs) and microglia we observed a significant increase in the levels of GM3 and GD3 gangliosides. In addition, we observed a significant decrease in cholesterol levels in differentiated OLs upon rHIgM22 treatment. No changes in phospholipid contents or distribution were observed in all cell types when treated with rHIgM22. We hypothesize that rHIgM22 myelin-repair activity could be at least in part mediated by alterations of lipid-dependent membrane organization in OPCs, OLs and microglia. The finding that rHIgM22 treatment affected in opposite ways two sphingolipid metabolic enzymes further supports this hypothesis. rHIgM22 reduced the activity of the acid sphingomyelinase (a key player for the detrimental effects of ceramide in MS) in differentiated OLs. On the other hand, S1P treatment in MGCs induced an increased expression of the galactocerebrosidase (known to be important to preserve the efficiency of myelin repair).

In conclusion, rHIgM22 exerts its protective effects by acting directly or indirectly on different glia populations involved in the mechanism of myelin repair, with sphingolipids being always key players.

4. Joaquín Sánchez Gómez – LBA 04

Role of NMDARs in spike timing-dependent plasticity at layer 2/3-2/3 synapses of the mouse somatosensory cortex during development.

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Spike timing-dependent plasticity (STDP) mediates synaptic changes that support circuit plasticity in sensory cortices during development. Synaptic plasticity in layer 2/3-2/3 synapses of the somatosensory cortex participates in the integration and modulation of sensory information. NMDA-type glutamate receptors (NMDARs) are necessary for the induction of STDP in diverse excitatory synapses of the central nervous system. The mechanisms of L2/3-L2/3 plasticity are not yet well-known. The objective of this work was to determine whether NMDARs are involved in t-LTD and t-LTP at L2/3-2/3 synapses at different developmental stages (P13-21, P22-25 and P26-35). For this, we performed electrophysiological recordings from layer 2/3 neurons in slices using the whole-cell configuration of the patch-clamp technique. To induce t-LTD and t-LTP, post-pre or pre-post pairing protocols were applied after a stable EPSP baseline period of 10 min. To determine the involvement of NMDARs, we applied the aforementioned pairing protocols under specific pharmacological blockade of NMDARs with DAP5 or MK801 and in the presence of antagonists of NMDARs containing specific subunits (GluN2A, GluN2B, and Glun2C/D using Zn2+, Ro256981 and PPDA, respectively) in P13-21 mice. Additionally, we applied these plasticity protocols in older mice (P21-25) to determine the developmental profile of this form of plasticity. Our results indicate that t-LTP requires NMDARs that contain GluN2A and GluN2B subunits, whereas t-LTD requires NMDARs containing GluN2B and GluN2C subunits. Moreover, while t-LTP is present until P35, t-LTD disappears at P21-25. Thus, both t-LTD and t-LTP, are present at these synapses at the postnatal age P13-21, and each is mediated by different NMDARs.

5. Uri Ashery – LBA 05

Dissection of the Cellular and Molecular mechanisms of cAMP--dependent synaptic plasticity in the hippocampus

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Cyclic adenosine monophosphate (cAMP) is a crucial second messenger that was shown to be involved in the process of synaptic plasticity in many brain regions including the hippocampus, cerebellum and nucleus accumbens. Despite decades of research, the mechanisms by which cAMP induces synaptic plasticity are still largely unknown. This is supported by the fact that, until recently, the only known pathway for cAMP signaling in the nervous system was via the activation of protein kinase A (PKA). However, recent studies have started providing evidence for PKAindependent cAMP-regulated molecular pathways relevant to neuronal processes. One of these non-canonical cAMP-dependent pathways, is mediated by a cAMP-dependent protein, called RapGEF2, and was shown to mediate neuritogenesis in neuroendocrine cells. Another exchange protein that is directly activated by cyclic AMP (Epac) represents a family of novel cAMP-binding effector proteins and have been shown to be involved in synaptic transmission. Nevertheless, the contribution of each one of these pathways to synaptic plasticity is not clear. We have developed a unique system that allows dissecting the contribution of cAMP either presynaptically or postsynaptically and the contribution of the different cAMP dependent targets. In addition, we show that RapGEF2 mediates cAMP dependent synaptic potentiation in the hippocampus.

6. Illana Gozes – LBA 06

Protection against tauopathy is significantly influenced by sex

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We are developing davunetide (NAP, CP201, AL-108), derived from activity-dependent neuroprotective protein (ADNP). Essential for brain development/survival, ADNP is de novo mutated in ADNP syndrome children suffering from intellectual disabilities1 and somatically mutated in Alzheimer's disease (AD) brains, driving tauopathy. The cytoplasmic localization of ADNP through 14-3-3 promotes sex-dependent neuronal morphogenesis, cortical connectivity, and calcium signaling2. ADNP shows reduced expression in female hippocampus (humans and mice), and further suppresses the expression of the major AD risk gene ApoE in female and not in male mice, presenting striking sexual dichotomy and regulating sex steroids. Davunetide, an essential part of ADNP, fortifies ADNP/Tau interactions with microtubules (MT)/autophagy/SH3/actin and WNT signaling4,5 In two original ADNP mutated/deficient mouse models, exhibiting early onset tauopathy, mimicking ADNP syndrome and AD, davunetide protected against tauopathy/cognitive/social/verbal/motor impairments in a sex-dependent manner5. Lastly, post-hoc clinical trial analysis in the tauopathy, progressive supranuclear palsy (PSP), suggested davunetide as a first-of-its-kind drug candidate addressing sex-related tauopathy6.

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Davunetide, under patent protection, is licensed for development.

7. Ildiko Pal – LBA 07

A novel positive modulator of alpha7 nAChR enhances cholinergic neurotransmission and promotes intracellular calcium responses in hippocampal interneurons

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Homomeric alpha7 nicotinic acetylcholine receptors (nAChRs) are expressed in the central nervous system in cognition-relevant areas including the prefrontal cortex and the hippocampus. Numerous studies indicate the potential of nAChR ligands to improve cognitive functions. Despite the extensive efforts in the nAChR field, effective treatments remain an unmet medical need. The observed suboptimal efficacy of various alpha7 nAChR-selective agonists and partial agonists in clinical trials may be partially attributed to the desensitization-driven nAChR loss of function. Therefore, we have developed novel alpha7 nAChR selective positive modulator compounds.

Modulators were selected by their effect on intracellular Ca2+ elevation of a selective alpha7 nAChR agonist using plate-reader based fluorometry and on the kinetics of current evoked by choline using patch clamp in recombinant cells expressing human alpha7 nAChR. Intracellular Ca2+ concentration changes were measured simultaneously by multiphoton imaging in interneurons from rat hippocampal slices.

Our compound (RGH-560) elicited a significant increase in both the potency and the efficacy of choline to evoke responses showing decelerating effect on current decay of choline induced current, up to 100 nM without agonist effect. From 1 µM it evoked an inward current. RGH-560 significantly enhanced choline-evoked intracellular Ca2+ responses in the dendrites of hippocampal interneurons. Moreover, it promoted the action potential firing of the interneurons evoked by choline. Spontaneous neuronal activity was also elevated showing network level effect of the compound. All these effects could be blocked by the alpha7 nAChR selective antagonist MLA.

The compound was shown to be effective in vivo. RGH-560 produced a significant reversal of a scopolamine-induced cognitive deficit in the mouse place recognition test at 10 mg/kg p.o. and proved to be effective in delay-induced natural forgetting (novel object recognition) test in rats from 3 mg/kg p.o.

Our in vitro data uncover unique and promising properties of this novel positive modulator of alpha7 nAChR. Furthermore, RGH-560 displays in vivo efficacy in animal models of cognition, validating the targeted molecular mechanism of action. Further development of these compounds may provide an efficient strategy for treatment of cognitive disorders.

8. Ginny G. Farías – LBA 08

ER organization in Axonal Identity

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Establishment of neuronal polarity depends on local microtubule (MT) reorganization and organelle dynamics. The endoplasmic reticulum (ER) consists of cisternae and tubules and, like MTs, forms an extensive network throughout the entire cell. How the two networks interact and control neuronal development is an outstanding question. We found that the interplay between MTs and ER tubules is essential for neuronal polarity. MTs are essential for axonal ER tubule stabilization, and, reciprocally, ER tubules are required for stabilizing and organizing axonal MTs. Recruitment of ER tubules into one minor neurite initiates axon formation, whereas local ER tubule disruption prevent neuronal polarization. In addition to ER – MT interactions, the ER forms contact with other organelles. How this interaction contributes to the organization, dynamics and polarized distribution of other organellesin neurons remains unclear.

We found that local somatic ER tubules contribute to the axonal distribution of lysosomes. Somatic ER tubule disruption causes enlarged lysosomes accumulated in the soma, preventing their entrance to the axon. We identified a somatic ER tubule array, enriched at a pre-axonal region, which ensures co-stabilization of ER tubule – MT – lysosome contacts to promote kinesin-1-driven lysosome fission and their translocation into the axon.

Lastly, ER cisternae, involved in translation of transmembrane and secretion proteins are absent along the axon. Unexpectedly, we found axonal ER tubule in contact with ribosomes indeveloping neurons. This interaction is regulated by neuronal stimuli and is required for proper local axonal translation. Disruption of ER tubule – ribosome contact impairs axonal translation.

Thus, neuronal ER tubule plays an essential role in maintaining axonal identity, by organizing MTs, and regulating both axonal lysosome availability and local axonal translation

9. Ulysse Thivisol – LBA 09

Hippocampal Protein Trajectories across Mouse Adolescent Development

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Many neuropsychiatric disorders emerge during adolescence, a vulnerable developmental window for brain maturation. However, the normal trajectories of brain protein changes during adolescence are poorly understood. In this study, we aimed to map the trajectories in hippocampal protein expression between early adolescence to young adulthood in mice. The hippocampus is a region critical for cognition and is highly impacted in schizophrenia and other neuropsychiatric disorders. We chose select candidate proteins including markers for major cell types, synapses, myelin, immune response, and plasticity-related proteins, which were semi-quanti□ied by western blot analyses. Hippocampal tissue from male and female mice was analysed at postnatal ages 4.5, 6, 8, 12, and 14 weeks, n=6 per sex per timepoint. We found that most protein trajectories exhibit a signi□icant age effect (2-way ANOVA), with signi□icant differences emerging between time points (Tukey's multiple comparisons). Several proteins show signi□icant decline from 6-8 weeks, including PSD95, GFAP, MBP, C3, and IFNg, highlighting an important period of adolescent hippocampal development. In comparison, NeuN, CNPase, CREB, and mBDNF showed no signi□icant age-related changes. We also observed sex-speci□ic differences in several protein trajectories, illustrating the importance of sex in neurodevelopment. Our results provide a foundation for future work to examine how these trajectories may deviate in animal models for neuropsychiatric disorders. Moreover, they are the foundation for our current research, investigating how these protein trajectories are affected by hippocampal-dependant spatial memory training.

10. Liliana Rojas-Charry – LBA 10

Synaptic mitochondrial impairments in autism spectrum disorder

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The etiology of autism spectrum disorders (ASD) remains unknown. Although synaptic genes represent the best candidates for ASD pathogenesis, the complexity of these disorders implies that other cellular functions are compromised. A good example is the increased prevalence of mitochondrial disease in cohorts of individuals with ASD. Mitochondria participate in crucial biological processes in the synapses involving classical energy production, calcium buffering, and regulation of reactive oxygen species (ROS). Here, we hypothesized that ASD alters mitochondrial bioenergetics and the redox and metabolic state of the synapses. We assessed mitochondrial respiration with high-resolution respirometry in different brain regions of an ASD mouse model and simultaneously measured the production of ROS with amplex red. Subcellular fractionation was performed to isolate synaptic and non-synaptic mitochondria for protein expression analysis, and mitochondrial morphology was studied with electron microscopy. We found functional and morphological mitochondrial impairments, represented by reduced respiration in the striatum of ASD mice and altered shape characterized by smaller and abnormal mitochondria. Several differences in the redox state of mitochondrial and ASD-associated proteins were identified through redox proteomics analysis. Additionally, mitochondria and endoplasmic reticulum contacts were reduced. The expression of phosphorylated pyruvate dehydrogenase and the mitochondrial calcium uniporter was also affected in synaptic mitochondria obtained from the striatum of our ASD mouse model. Our results demonstrate that mitochondrial functional impairments are present in the striatal synapses of ASD mice. These could affect critical cellular processes and regulatory pathways determining synaptic activity and neuronal metabolism. The understanding of mitochondrial roles in the synapse can provide helpful knowledge in the treatment of ASD.

11. Frederic A Meunier – LBA 11

Phospholipase A1 (DDHD2) interacts with STXBP1 to mediate long-term memory via the generation of myristic acid

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Unveiling a lipid marker of learning and memory is one of the holy grail of Neurochemistry. The phospholipid and free fatty acid (FFA) composition of neuronal membranes plays a crucial role in learning and memory, but the mechanisms through which neuronal activity affects the brain's lipid landscape remain largely unexplored. Saturated FFAs, particularly myristic acid (C14:0), strongly increase during neuronal stimulation and memory acquisition, suggesting the involvement of phospholipase A1 (PLA1) activity in synaptic plasticity. Here, we show that genetic ablation of the DDHD2 isoform of PLA1 in mice reduced memory performance in reward-based learning and spatial memory models prior to the development of neuromuscular deficits, and markedly reduced saturated FFAs across the brain. DDHD2 was shown to bind to the key synaptic protein STXBP1. Using STXBP1/2 knockout neurosecretory cells and a haploinsufficient *STXBP1*+/- mouse model of STXBP1 encephalopathy that is also associated with intellectual disability and motor dysfunction, we show that STXBP1 controls the targeting of DDHD2 to the plasma membrane and the generation of saturated FFAs in the brain. Our findings suggest key roles for DDHD2 and STXBP1 in the lipid metabolism underlying synaptic plasticity, learning and memory.

12. Shanley Sanders – LBA 12

Synapsin2a tetramerization selectively controls the presynaptic nanoscale organisation of reserve synaptic vesicles.

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Neurotransmitter release relies on the regulated fusion of synaptic vesicles (SVs) that are tightly packed within the presynapse of neurons. The mechanism by which SVs are anchored at the presynapse while preserving their ability to dynamically recycle thereby supporting neuronal communication remains unknown. Synapsin2a tetramerization was recently suggested to cluster SV in presynapses. Here, we used Dual-pulse sub-diffractional Tracking of Internalised Molecules (DsdTIM) to simultaneously track SVs from the recycling and reserve pools, in live hippocampal neurons. The reserve pool displays a lower presynaptic mobility compared to the recycling pool and exhibits a more mobile axonal pool. Synapsin1-3 triple knockout (SynTKO) selectively increased the reserve pool mobility. Re-expression of wild-type Synapsin2a, but not the tetramerization-deficient mutant K337Q, fully rescued these effects. Tracking Synapsin2aK337Q-mEos3.2 revealed altered synapsin activity-dependent presynaptic translocation and nanoclustering. Synapsin2a tetramerization therefore controls its own presynaptic nanoclustering allowing dynamic immobilisation of the reserve pool at the presynapse.

13. Maria A Garcia – LBA 13

ROLE OF HYPOTHALAMIC HYDROCABOXYLIC ACID RECEPTORS IN METABOLIC GLIA-NEURON COMMUNICATION

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Introduction: The hypothalamus is the primary local and peripheral signal integrator in the brain, and home to a diverse population of cells, including orexigenic and anorexigenic neurons and glial cells, such as astrocytes and tanycytes. The body´s energy level is mostly determined by the metabolic interaction between glia and neurons via nutrient sensing, but the underlying metabolic and molecular mechanisms have not been elucidated. Recently, we showed that activating the lactate receptor (HCAR1) by L-lactate increases the electrical activity of anorexigenic pro-opiomelanocortin (POMC) neurons. However, we discovered that HCAR1 is not expressed in POMC neurons although it is expressed in hypothalamic astrocytes. Here, we explored if HCAR1 activation in astrocytes induces intracellular calcium [(Ca2+)i] increase that allows astrocyte glutamate release.
Methods: We used in situ hybridization and immunocytochemistry for evaluated HCAR1 and HCAR2 hypothalamic localization. Tanycyte cultures transduced with adenoviral particles (Ad) were used to evaluate lactate generation in response to glycolytic activators or inhibitors. Hypothalamic astrocyte cultures were incubated with a HCAR1-specific agonist, 10mM L-lactate, and tanycyte conditioned media to evaluate [(Ca2+)i] increase and glutamate release.
Results: Using in situ hybridization and immunocytochemistry, we detected hypothalamic astrocytic HCAR1 localization and NPY- neuron HCAR2 localization. Conditioned media from Ad-shMCT1-transduced tanycytes contained less lactate concentration than that from Ad-control transduced tanycytes. In an oppositive way, -conditioned media from Ad-shGKRP-transduced tanycytes had more lactate than the control. The last one, L-lactate, and a HCAR1-agonist induced [(Ca2+)i] increase and glutamate release from astrocytes.
Conclusion: Lactate produces by tanycytes acts on its receptor localized in hypothalamic astrocytes, allowing an extracellular supply of glutamate.
Acknowledgements: FONDECYT regular 1221508.

14. Fumilayo Olopade – LBA 14

Vanadium Administration Ameliorates Cortical Structural and Functional Changes in Juvenile Hydrocephalic Mice

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Vanadium, a prevalent transition metal, is neurotoxic but has therapeutic potentials in some neurological conditions. Hydrocephalus results in neuronal degeneration, causes motor deficits and thus poses a major clinical burden. The primary treatment for hydrocephalus (shunting) has complications including infection and blockage; alternative drug-based therapies are therefore necessary. This study investigates the function and cytoarchitecture of motor and cerebellar cortices in juvenile hydrocephalic mice following treatment with varying doses of vanadium.
Fifty juvenile mice were allocated into five groups (n=10); controls, hydrocephalus-only, low (0.15mg/kg), moderate (0.3mg/kg) and high (3.0mg/kg) dose vanadium groups. Hydrocephalus was induced by intra-cisternal injection of kaolin and sodium metavanadate administered by intraperitoneal injection 72hourly for 28 days. Neurobehavioral tests: Open field, hanging wire and pole tests, were carried out to assess locomotion, muscular strength and motor coordination, respectively. The mice were euthanized and their brains processed for Cresyl violet staining and immunohistochemistry for neurons (NeuN) and astrocytes (GFAP). Data was analysed and compared using ANOVA.
Horizontal and vertical movements in open field test and latency to fall in hanging wire test were significantly reduced, while latency to turn and to descend in pole test were prolonged in hydrocephalic mice, suggesting impaired motor ability in them; this was improved in all vanadium-treated groups. Pyknotic cells, NeuN-positive cells with signs of neurodegeneration and reactive astrocytes were observed in the motor and cerebellar cortices of the hydrocephalic mice; this was mitigated in the vanadium-treated mice.
These results demonstrate the neuroprotective potential of vanadium in hydrocephalus, including, the high dose, which is usually toxic in normal animals. We however recommend the low to intermediate dose in order to avoid possible complications due to vanadium accumulation in the body.

15. Vedrana Montana – LBA 15

Vesicular glutamate release modulation by presenilin from cultured astrocytes

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Alzheimer disease, AD is a progressive neurodegenerative disease characterized by severe disruption of functional connectivity of neural networks. All cells in the brain undergo changes that affect brain functions. Astrocytes are implicated to have a central role in the cellular phase of AD. Astroglia is responsible for maintaining connectivity by releasing gliotransmitters and controlling neurotransmitter homeostasis. Thus, it is essential to study mechanisms which underlie vesicle-based signaling. Gliotransmitters are secreted by astrocytes and their release is Ca2+-dependent. Ca2+ is delivered from both intracellular and extracellular sources.

Presenilins (PS) are subunits of γ-secretase that cleaves amyloid precursor protein of amyloid plaques found in brains of AD patients. Additionally, PS function as leak Ca2+ channels while residing in the ER membrane. A number of mutations affect Ca2+ permeability of PS and result in throwing intracellular Ca2+ dynamics out of balance affecting various functions and signaling pathways. Although astrocytes express both forms of presenilin, PS1 and PS2, the role of their mutated forms on Ca2+ homeostasis, Ca2+ dynamics and signaling pathways, such as Ca2+ -dependent gliotransmitter release, has not been extensively studied.

Here, we have used primary mouse cultured astrocytes and co-expressed three different PS mutants, PS1ΔE9, PS1M146V and PS2N141I together with reporter dsRed2-C1. Using fluorescently based glutamate dehydrogenase assay, we measured glutamate release, which was significantly reduced in the presence of PS mutants. These changes were consistent with drop in bradykinin-invoked Ca2+ response suggesting that dysregulation of intracellular Ca2+ due to the presence of mutants on the ER membrane has profound effect on functional output. Furthermore, spontaneous mobility of vesicles was also reduced implicating changes in function of intracellular Ca2+ stores. We tested capacity and dynamics of the stores. While mutants presented similar dynamics, the capacity was dramatically reduced.

Taken together, our data reveal that the presence of PS mutations reduces Ca2+ capacity and dynamics in conjunction with consequent glutamate release and vesicle mobility. These effects could have profound impact in early stages and progression of AD as astroglia play an active role in synaptic strength and integration of synaptic processes by monitoring and modulating neuronal activity at tripartite synapse.

16. Carlo Sala – LBA 16

Transcriptomics and functional activity of ketamine on neurons derived from human induced pluripotent stem cells (hiPSCs).

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Patients with Major Depressive Disorder (MDD) develop treatment-resistant depression (TRD) in approximately 30% of cases. Among the various factors that make TRD challenging in both clinical and research contexts, a fundamental role is played by the inadequate understanding of MDD pathophysiology and the limitations of current pharmacological treatments. However, the field of psychiatry is facing exciting times. In combination with recent advances in genome editing techniques, human induced pluripotent stem cell (hiPSC) technology offers new and unique opportunities in both disease modeling and drug discovery. Moreover, the field is approaching the advent of (es)ketamine, a glutamate N-methyl-Daspartate (NMDA) receptor antagonist, claimed as one of the first and exemplary agents with rapid antidepressant effects (within hours), even in TRD patients. Although ketamine appears poised to transform the treatment of depression, its exact mechanisms of action are still unclear but highly demanded, as the resulting knowledge may provide a model to understand the mechanisms behind rapid-acting antidepressants, which could lead to the discovery of novel compounds for depression treatment. Therefore, to better clarify the mechanism of action of ketamine, we used hiPSC-derived neurons that have been characterized for transcriptomic and functional changes induced by acute ketamine treatment. Our results aim to better understand the activity of ketamine on human neurons in order to develop new therapeutic approaches for TRD patients.

17. Holly Melland – LBA 17

Delineation of the pathogenic mechanisms of synaptotagmin-1 variants

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Synaptotagmin-1 (SYT1) is an essential synaptic vesicle protein that acts as the major calcium sensor mediating fast, synchronous neurotransmitter release in the cerebrum. In addition, SYT1 clamps spontaneous neurotransmitter release and modulates endocytic recycling of synaptic vesicles. Heterozygous variants in SYT1 give rise to a neurodevelopmental disorder with a spectrum of severity and features including intellectual disability, motor delay and disordered movement. We have shown that SYT1 variants cause a dominant-negative impairment of action potential-evoked synaptic vesicle exocytosis, with a preliminary genotype-phenotype relationship. However, it is unknown whether disease-associated variants perturb other aspects of synaptic vesicle dynamics. We therefore aimed to determine the synaptic processes contributing to the pathogenesis of this disorder. To monitor synaptic vesicle cycling in individual boutons, we performed live cell imaging of cultured mouse hippocampal neurons transfected with SYT1 variants conjugated to pH-sensitive pHluorin reporters. In the absence of action potentials, spontaneous synaptic vesicle exocytosis was not altered by SYT1 variants. Moreover, synaptic vesicle endocytosis was also unaffected by these variants. We conclude that the auxiliary functions of SYT1 in spontaneous release and endocytosis are not impacted in a dominant-negative manner by disease-associated SYT1 variants and therefore confirm that defective evoked exocytosis is the major pathogenic mechanism of SYT1-associated neurodevelopmental disorder. This further clarifies mechanistic targets for treatment and could improve confidence in predicting and preventing off-target therapeutic effects. Furthermore, we used both patient and strategic mutations to explore the molecular mechanics of calcium-binding by SYT1 and the underpinnings of genotype-phenotype associations in this disorder.

18. Anibal Gallardo-Recio – LBA 18

Synaptic plasticity throughout development at CA3-CA2 synapses of mouse hippocampus

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Synaptic plasticity mediates processes of great importance such as memory, learning and the maturation of neuronal circuits. In the CA2 region of the hippocampus this plasticity seems to be limited compared to CA1. CA2 has an important role in the processing of information in the hippocampus. In the present work, synaptic plasticity has been studied at CA3-CA2 synapses of mice at different ages (P13-21, P35-42, and P>60). For this, two different protocols have been used: High Frequency Stimulation (HFS: 100 Hz, 1 s) and Low Frequency Stimulation (LFS: 1 Hz, 15 min). HFS protocol induced long-term potentiation (LTP). Similar to what occurs at CA3-CA1 synapses, an increase in the magnitude of the LTP induced by this protocol was observed during development. Interestingly this increase is delayed at CA3-CA2 synapses when compared to CA3-CA1. On other hand, the LFS protocol induced long-term depression (LTD) in young mice (P13-21) that depends on NMDA, but not on AMPA neither GABAA receptors. Moreover and similar to what occurs at CA3-CA1 synapses, LTD switch to LTP with maturation. Interestingly, this switch is also delayed at CA3-CA2 synapses when compared to CA3-CA1. Furthermore, the use of the mutant dnSNARE mouse indicates that astrocyte-derived gliotransmitters are not required for the switch of LTD to LTP at CA3-CA2 synapses as this switch is observed in these mice. Finally, A1 adenosine receptors seems to be necessary for LTP induced by LFS in old mice as this LTP is prevented in the presence of the A1R antagonist 8-CPT.

19. Matthew MacDonald LBA -19

Multilevel proteomics links aberrant synaptic proteostasis and kinase signaling to dendritic spine pathology in schizophrenia.

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Schizophrenia is a leading source of lifetime disability, in which impairments of cognitive and sensory processing contribute much to functional impairment. The formation and persistence of dendritic spines, the post-synaptic component of most glutamatergic synapses in the cerebral cortex, are critical to the acquisition and maintenance of cognitive and sensory information. Thus, unsurprisingly, postmortem studies find fewer cortical dendritic spines in individuals with schizophrenia. Spine formation and persistence depend on protein expression, trafficking, local translation, and posttranslational modifications. Therefore, we evaluated multidimensional proteomes in the adult human auditory cortex, specifically protein levels, synaptic localization, and protein phosphorylation, and how they relate to dendritic spine measurements of layer 3 of the auditory cortex. Comparing tissue homogenate from schizophrenia and control individuals, we find significant differences in levels and phosphorylation of certain proteins. Nonetheless, more substantive, and often distinct differences emerge for proteins localized to the synapse. These alterations implicate a broad array of pathways critical for synaptic function. For example, the markedly different phosphoproteome implicates kinome dysregulation in schizophrenia, especially CDK5. Using correlated abundance to form subnetworks of proteins, and then extracting their leading eigenvectors, identifies several discrete biological functions both altered in schizophrenia and associated with decreased dendritic spine density. Causal modeling uncovered dysregulated cytoskeletal proteostasis as a possible upstream driver of dendritic spine pathology. Together, our findings demonstrate that synaptic proteome pathology in schizophrenia extends beyond glutamate receptor signaling impairments, which is the current-day target of therapies, specifically nominating cytoskeletal proteostasis and kinase hyperactivity as potential novel therapeutic targets.

20. Jordi Duran - LBA 20

Role of glycogen in the pathophysiology of amyotrophic lateral sclerosis

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Glycogen, a branched polymer of glucose, is the only energy reservoir of the brain and it plays important roles in this organ. However, in some conditions, glycogen accumulates abnormally in the brain. The most striking example of this is Lafora disease (LD), a neurodegenerative condition characterized by the accumulation of glycogen aggregates in the nervous tissue. We previously demonstrated that the accumulation of glycogen in astrocytes underlies neuroinflammation in LD. Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease characterized by the progressive loss of motor neurons in the spinal cord. Glial cells, including astrocytes and microglia, have been shown to contribute to neurodegeneration in ALS, and metabolic dysfunction plays an important role in the progression of the disease. Importantly, glycogen accumulation has been reported in the spinal cord of human ALS patients and mouse models. Using the SOD1G93A mouse model of ALS, we show that glycogen accumulates in the spinal cord and brainstem during symptomatic and end stages of the disease and that the accumulated glycogen is associated with reactive astrocytes. To study the contribution of glycogen to ALS progression, we generated SOD1G93A mice with reduced glycogen synthesis (SOD1G93A GShet mice). SOD1G93A GShet mice had a significantly longer lifespan than SOD1G93A mice and showed lower levels of the astrocytic pro-inflammatory cytokine Cxcl10, suggesting that the accumulation of glycogen is associated with an inflammatory response. Supporting this, inducing an increase in glycogen synthesis reduced lifespan in SOD1G93A mice. Altogether, these results suggest that glycogen in reactive astrocytes contributes to neurotoxicity and disease progression in ALS.