

POSTERS ABSTRACTS

Agra de Almeida Quadros, AR

Fbxo41 Promotes Disassembly of Neuronal Primary Cilia.

Neuronal primary cilia are signaling organelles with crucial roles in brain development and disease. Cilia structure is decisive for their signaling capacities but the mechanisms regulating neuronal cilia structure are poorly understood. We identify the previously uncharacterized protein Fbxo41 as a novel Skp1/Cullin1/F-box (SCF) E3-ligase complex subunit that targets to neuronal centrioles and promotes disassembly of primary cilia. Fbxo41 targeting to centrioles requires its Coiled-coil and F-box domains and is inhibited by its C-terminal domain. In addition, Fbxo41 targeting is regulated by phosphorylation and phosphatase inhibition results in its displacement from centrioles. Levels of Fbxo41 at the centrioles inversely correlate with neuronal cilia length, and mutations that disrupt Fbxo41 targeting or assembly into SCF-complexes also disturb its function in cilia disassembly. Fbxo41 promotes cilia disassembly through Aurora A kinase activation and requires rearrangements of the actin-cytoskeleton. We propose that neurons can regulate the structure of their cilia through phosphorylation-dependent control of Fbxo41 levels at the centrioles.

Amici, M, Pope, RJP, Collingridge, GL

Novel insights into the role of PI4KII α in synaptic signalling in the hippocampus.

Synaptic plasticity events in the hippocampus are regarded as the molecular mechanisms underlying learning and memory: long term depression and potentiation are widely studied in this brain region with an ever growing number of studies unveiling the roles of many cellular components in synaptic plasticity and neuronal pathologies. Here we present preliminary unpublished data focusing on the role of a lipid kinase, phosphatidylinositol 4 kinase type II α (PI4KII α) in synaptic transmission and plasticity. PI4KII α is involved in the production of phosphatidylinositol 4-phosphate (PI4P) and in Golgi-endosomal trafficking, it localises to the trans Golgi network, to membranes of the endosomal system as well as synaptic vesicles. Non catalytic functions of this PI4 kinase have recently emerged, Robinson et al (PI4KII α phosphorylation by GSK3 directs vesicular trafficking to lysosomes. Biochemical Journal 464:145-156, 2014) demonstrated that PI4KII α can be phosphorylated by Glycogen Synthase Kinase 3 (GSK3) and that a reduction in PI4KII α expression in primary hippocampal neurons can alter the surface expression of AMPA receptors. Since GSK3 has an important role as a regulator of synaptic plasticity (Peineau et al., LTP inhibits LTD in the hippocampus via regulation of GSK3 β).

Neuron 53:703-717, 2007), we wanted to investigate the role of PI4KII α in CA1 hippocampal neurons; in order to do this we used biolistic transfection in organotypic slices to manipulate the expression level of PI4KII α . We performed patch clamp electrophysiological experiments in CA1 pyramidal cells and found evidence for a role of PI4KII α in synaptic transmission mediated by NMDA receptors.

Ashby, MC, Jackson, J, Johnson, J, Witton, J, Ahmed, Z, Hutton, ML, Isaac, JT, O'Neill, MJ
Alterations in synaptic dynamics during early tauopathy.

The pathological accumulation of tau is associated with a number of diseases including Alzheimer's Disease (AD). A severe loss of synapses occurs at the early clinical stages and has been correlated with cognitive deficits in AD patients; however the time course of this synapse loss and how the turnover of synapses is affected are relatively unknown. Here we use in vivo two-photon microscopy to assess the temporal dynamics of axonal boutons and dendritic spines in a transgenic mouse model of human tauopathy, rTg4510, which expresses the P301L tau mutation downstream of a tetracycline-operon-responsive element. Adeno-associated virus expressing GFP was injected into the layer 2/3 of the cerebral cortex to enable the visualisation of neurons and a cranial window was implanted for long-term imaging of the somatosensory cortex. GFP-labelled neurons were imaged weekly during the time period spans the onset through to severe cortical neurodegeneration in this model. The gross morphology of axons and dendrites and the dynamics of these synaptic structures were assessed in wild-type and transgenic mice of different ages as pathology develops. Gross morphological changes such as the presence of dystrophic neurites were visible as the pathology progressed. Alongside this, synapse loss and changes in the stability of synapses were also present. These synaptic and morphological changes suggest that alterations in synaptic stability characterise the earliest phases of disease, preceding neurodegeneration. Such synaptic abnormalities would likely drive altered neuronal network function.

Balik, A

F Structural properties of M3-S2 linkers and their action during glutamate receptor channel opening.

Fast synaptic transmission in CNS is mediated by ionotropic glutamate receptors (iGluRs). iGluRs are pharmacologically divided to the three major groups: AMPA, kainate and NMDA (GluN) receptors. All subunits possess a significant homology in amino acid sequence and are related in structure. Each receptor is tetramer with a conserved domain organization. The NMDAR contains two obligatory glycine-binding (GluN1) and two glutamate or glycine-binding (GluN2/3) subunits. Extracellularly the most distal part to the

membrane is the amino-terminal domain (ATD) connected to the ligand-binding domain (LBD), which is linked by three linkers to the transmembrane domain (TMD). The linker connecting third helix of the TMD (M3) and bottom domain of the LBD (S2) is central to the ion channel opening. In contrast to AMPARs the individual NMDAR subunits have linkers with distinct amino acid sequence possibly indicating their role for specific interaction between LBD and TMD. Moreover, our structure model of channel opening suggested the different contribution of GluN1 and GluN2B subunits in the course of opening. Therefore we investigated the structural properties of M3-S2 linkers of both NMDA receptor subunits for the ion channel opening. Using whole cell recording we found that (i) small shortening the length of the linker (one amino acid) have minor effect on receptor function and on affinity of both agonists (ii) however, larger shortening of the GluN1 linker near to ion channel vestibule impacted more radically on the receptor function than analogous alternations of the GluN2B linker. In parallel, we also employed homology modelling and short MD simulation to independently assess a significance of individual amino acids of M3-S2 the linkers for ion channel opening. In summary, our data suggests that the specific length of GluN1 M3-S2 linker is essential to control ion channel opening while analogous alternations of GluN2B linker have not introduced the capacity to initiate GluN1 independent ion channel opening.

Calahorro, F, Ferreiro, T, Dillon, J, O'Connor, V, Holden-Dye, L
A microfluidic platform for screening synaptic defect in Autism Spectrum Disorders, using *C. elegans* as a model.

Mutations in a specific neuronal gene, neuroligin, are associated with Autism Spectrum Disorders (ASD); understanding how neuroligin modulates neural function provides a new route to druggable targets for pharmacotherapy of ASD. We use the simple brain of the invertebrate *Caenorhabditis elegans* to express human neuroligin so that we can investigate autistic-like dysfunction and optimise a new screening platform for target and drug discovery for ASD.

We are interested in animal behavioural plasticity and this led to a focus on neuroligin and neurexin, two synaptic proteins that appear essential to integrate sensory input to produce appropriate behavioural output and which are disrupted in autism spectrum disorders (ASD). We have shown an evolutionary conservation of the neuroligin---neurexin axis from the nematode *Caenorhabditis elegans* through to human. This may extend to functional conservation of a core synaptic code that is implemented to organise animal behaviour. As the *C. elegans* nervous system is built on the same principles as the human it provides a genetically and behaviourally tractable model to study neurexin/neuroligin with potential relevance to ASD. Taking advantage of this, we have refined a microfluidic device, 'Neurochip', to use in translational neuroscience research. This device permits recording of a worm 'EFG' providing a readout of the impact of genetic

mutations or drugs on neural network activity. We have resolved electrophysiological phenotypes in *C. elegans* neuroligin mutants, which provide a discrete characterisation of the role of neuroligin in circuit function. Moreover, we have found that drugs that are used in the treatment of ASD ameliorate *C. elegans* neuroligin deficits. Here we show a highly innovative experimental platform for ASD drug discovery.

Calahorro, F, Keefe, F, Holden-Dye, L, O'Connor, V

Modelling the synaptic code for autism spectrum disorders: neuroligin/neurexin axis

Neuroligins are cell adhesion proteins that interact with neurexins at the synapse. This interaction may contribute to differentiation, plasticity and specificity of synapses. In humans, mutations in neurexin and neuroligin-encoding genes lead to neurodevelopmental disorders including autism and/or schizophrenia. For these reasons applying efforts to understand the key molecular components of the neuroligin/neurexin axis has the potential to identify drug targets for these currently intractable conditions. *Caenorhabditis elegans* is an excellent model system to address the molecular substrates of the functionality of an “autistic synapse”. Its nervous system consists of a defined set of 302 mapped neurons and their patterns of synaptic connectivity are well characterized. Its genetic tractability permits an investigation of the impact of discrete mutations on synapse formation and function to understand the synaptopathies underpinning autism; a platform to identify signalling components that emanate from the neurexin/neuroligins axis using established approaches that allow investigations across the gene to behaviour domains. We use *C. elegans* as a model to define molecular determinants of neuroligin and neurexin in synapse organisation and the consequence for the generation of aberrant behaviour. In particular we have identified behavioural deficits in *C. elegans* neuroligin mutants. We have established that mutations implicated as determinants of autistic traits in humans generate a disruption in integrative sensory behaviour. Furthermore, we have been able to re-introduce the human homologues of this gene to rescue against these genetic perturbations. Finally, through transcript analysis we highlight nlg-1 splice variants that change the intracellular domain of the gene product NLG-1. Interestingly, exon 14 encodes a cassette with two potential functional domains. One is a polyproline SH3 binding domain and the other has homology to a region encoding the binding site for the scaffolding protein gephyrin in mammalian neuroligins. This evidences a putative conservation in the intracellular scaffolding recruitment between *C. elegans* and humans.

Caldeira, GL, Louros, SR, Peça, J, Pato, CN, Chen, C, Carvalho, AL

Stargazin, a new candidate for schizophrenia - identification of new variants and targeted therapies.

Schizophrenia is a devastating disorder that affects about 1% of the worldwide population. This multifactorial disease, with a strong genetic component, is characterized by delusions, hallucinations and confusion thoughts. Furthermore schizophrenia onset and stress are inextricably related. It is well accepted that schizophrenia patients are vulnerable to changes in the environment and their ability to adapt to those changes is disrupted. Recently, synaptic networks components have been strongly implicated in this disorder as several de novo mutations have been found in these patients.

Stargazin is an auxiliary subunit for AMPAR and it is required for AMPA receptor (AMPA) trafficking to the cell surface and to synapses. Our lab recently described an important role for stargazin in experience-dependent plasticity and in scaling up of AMPAR upon blockade of activity. Interestingly, SNPs that confer susceptibility to schizophrenia were found in the CACNG2 gene, which encodes for stargazin and is located in a chromosome region strongly implicated in the disorder, 22q. Using wholegenome sequence analysis, we identified a new CACNG2 variant (Stg^{SCZ}). Stg^{SCZ}, along with Stg^{ID}, a variant previously identified in intellectual disability, failed to deliver AMPAR to synapses. Furthermore, Stg^{ID} was not able to mediate homeostatic plasticity. On the other hand, Stg^{SCZ} increased the number and decreased the length of primary dendrites, and affected the number of inhibitory synapses.

Tianeptine is a commercial memory enhancer and powerful antidepressant, recently described to increase AMPAR trapping at synapses, by increasing stargazin phosphorylation. Tianeptine treatment rescued AMPAR levels in neurons expressing STG^{SCZ}. In contrast, STG^{ID} was unable to respond to tianeptine. We are currently generating stargazin knock-in mice for these mutations and we aim to explore if tianeptine can rescue circuit dysfunction caused by STG^{SCZ} and STG^{ID}.

Carbone, A, Plested, AJR

Superactivation of AMPA receptors by auxiliary proteins.

AMPA receptors (AMPA) mediate most of the fast excitatory synaptic transmission in the brain. At synapses, AMPARs form macromolecular complexes that include auxiliary subunits, such as Transmembrane AMPA receptor Regulatory Proteins (TARPs). TARPs control the activity of AMPARs during fast synaptic transmission through a seemingly bewildering array of effects. The functional properties of AMPARs expressed at synapses shape synaptic currents and contribute to the short-term plasticity of the synaptic response. Determining the factors that control the gating of AMPARs is therefore crucial to understanding how neurons process and encode information.

Several models have been proposed to explain the mechanism of action of TARPs on AMPARs. However, none of these proposed mechanisms was able to fully describe the behaviour of these complexes.

Using a panel of GluA2 mutants, which spend very different fractions of time in the active vs. desensitised state, we developed a new simple model for GluA2-TARP complexes. Kinetic analysis showed that TARPs had a limited effect on mutants with a long-lived desensitised state. In contrast, mutants with an unstable desensitised state showed profound modulation by TARPs. These results suggest that TARPs exert their effects principally on the open state of the receptor. Our model predicts all known effects of TARPs on AMPA receptor function and also unexpected phenomena that we were then able to confirm experimentally.

A thermodynamic argument suggests that because TARPs promote channel opening, receptor activation promotes AMPAR-TARP complexes into a superactive state with high open probability. Experimental data showed that prolonged or repetitive stimulation of isolated AMPAR-TARP complexes can indeed drive the receptors into a high activity state, thus potentiating the current.

The transient positive feedback mechanism that we demonstrate has implications for information processing in the brain, because it would allow activity-dependent facilitation of excitatory synaptic transmission through a purely postsynaptic mechanism.

Carmichael, R, Wilkinson, KA, Ashby, MC, and Henley, JM

The role of MEF2A in AMPA receptor trafficking.

The myocyte enhancer factor 2 (MEF2) family of transcription factors activate numerous transcriptional programmes to regulate both universal and neuronal-specific functions, including those involved in memory formation. MEF2 activity is well established to regulate structural plasticity, and has also recently been shown to modulate synaptic plasticity via AMPA receptor (AMPA) endocytosis, though the molecular mechanisms behind this are unclear. Here, we show MEF2A is required to maintain surface AMPAR expression in cortical neuronal culture. Knock down of MEF2A results in a significant decrease in the proportion of GluA2 at the plasma membrane of cortical neurons. Additionally, MEF2A knock down prior to dihydroxyphenylglycine (DHPG) application prevents the Group I metabotropic glutamate receptor (mGluR)-dependent endocytosis of GluA2-containing AMPARs, possibly via occlusion. We are currently investigating the molecular mechanisms and transcriptional targets underlying the role of MEF2A in basal and activity-dependent AMPAR trafficking and investigating possible activity-dependent signalling that may regulate MEF2A to control surface AMPAR expression. Additionally, Small Ubiquitin-like Modifier 1 (SUMO1) modification has been reported to repress

MEF2A-dependent transcription, so we are investigating possible roles of MEF2A SUMOylation in AMPAR trafficking, via knock down/rescue experiments with MEF2A mutants exhibiting altered SUMOylation.

Carta, M, Bettadapura, S, Gorlewicz, A, Labrousse, V, Rebola, N, Mulle, C
NMDA receptors in CA3 pyramidal cells: subunit composition and plasticity.

NMDARs play a key role in the regulation of neuronal communication, synaptic function and plasticity. A single neuron can express different GluN1 isoforms and GluN2 subunits that can participate in the formation of NMDARs with various stoichiometry. In a given neuron, synaptic NMDAR subtypes may be segregated in an input-specific manner. Subunit composition is critical in defining the properties and signaling of NMDARs. In the current work we have investigated the subunit composition of NMDARs and their relevance for synaptic plasticity at two distinct excitatory inputs to hippocampal CA3 pyramidal cells (CA3-PCs), the associational commissural (A/C) and mossy fiber synapses. We have performed patch clamp experiments in acute hippocampal slices, using pharmacological tools together with KO mouse lines for different GluN2 subunits. We have found that both A/C and Mf-CA3 synapses express tri-heteromeric NMDARs composed of GluN1/GluN2A/GluN2B. However, in control condition, the proportion of GluN2B containing receptors is significantly higher at A/C inputs compared to Mf-CA3 synapses. GluN2B, but not GluN2A or GluN2D, plays a critical role in the induction and/or expression of NMDAR-LTP at Mf-CA3 synapses. Finally, we have discovered that NMDARs are enriched in GluN2B after NMDAR-LTP, rendering Mf-CA3 synapses competent for conventional AMPAR-LTP. Altogether our current results define the precise subunit composition of NMDARs in CA3-PCs and shed light on consequences for synaptic plasticity.

Chamberland, S
Distinct roles for P/Q- and N-type voltage-gated calcium channels in synchronous glutamate release.

In presynaptic terminals, calcium elevations are shaped by several key parameters, including the properties, density, combination and the spatial location of VGCCs. These features allow presynaptic terminals to translate complex firing frequencies to postsynaptic signals by regulating the amount of neurotransmitter released. For example, the number of vesicles fusing to the membrane can be amplified through mechanisms such as synchronization of multivesicular release or recruitment of additional release sites. While synchronous release relies on both P/Q- and N-type VGCCs at hippocampal

MF-CA3 synapses, the contribution of individual types of VGCCs to the mechanisms controlling neurotransmitter release remains unknown.

To dissect the roles of P/Q and N-type VGCCs, we used random-access two-photon calcium imaging and electrophysiology in combination with electron microscopy.

Our results show that calcium influx through P/Q- and N-type VGCCs differently influence glutamate release through specialized calcium dynamics. First, two-photon calcium imaging in giant mossy fiber terminals revealed that P/Q-type VGCCs mediated a larger fraction of calcium elevations than N-type VGCCs for a single action potential. Consistent with calcium imaging data, P/Q-type VGCCs showed a larger contribution to glutamate release than N-type VGCCs. However, this difference was dependent on the external calcium concentration, as decreasing the aCSF calcium concentration to 1.2 mM revealed a similar effect for both toxins. To investigate how calcium entry through N-type VGCCs can mediate a larger fraction of EPSCs in conditions of low release probability without changes in presynaptic calcium entry, we used a coefficient of variation (CV) analysis of single and train of stimuli. While blocking N-type VGCCs decreased the quantal size of EPSCs, blocking P/Q-type VGCCs reduced EPSC amplitude by reducing the number of active release sites. Furthermore, CV analysis revealed that application of ω -Agatoxin IVA or EGTA-AM had similar effects on short-term facilitation by eliminating the recruitment of additional release sites.

Altogether, our results demonstrate the highly specialized roles of P/Q- and N-type VGCCs in neurotransmitter release. While N-type VGCCs are tightly coupled to calcium sensors and provide local calcium elevations, P/Q-type VGCCs are strategically involved to support global calcium elevations and recruit additional release sites during trains of activity.

Correa, S, DaSilva, LL, Wall, MJ, Wauters, SC, de Almeida, LP, Yunan C Januário, YC, Müller, J

Arc directly interacts with clathrin-adaptor proteins to facilitate AMPAR endocytosis.

Activity-regulated cytoskeleton (Arc) is a neuron-specific immediate early gene required for learning and memory. As such, Arc protein expression is critical for several forms of synaptic plasticity by facilitating endocytosis of AMPA receptors (AMPA). To map the steps linking Arc expression to endocytosis of AMPAR, we immunoprecipitated endogenous Arc from C57BL/6 mouse hippocampal lysates and identified unknown clathrin-adaptor proteins (CAP) as Arc-binding partners. To characterize the Arc/CAP interaction we used several strategies: a) Arc co-IPs with components of the CAP in hippocampal lysates from adult C57BL/6 mice, b) recombinant Arc directly binds to the GST-containing the CAP. To determine the Arc sequence that mediates the Arc-CAP interaction, we generated Arc mutants with successive deletions from either the Nt or Ct

and performed GST pull-down assays c) to determine the subcellular location of the Arc/CAP interaction we used bimolecular fluorescent complementation assays combined with confocal microscopy.

To test whether Arc regulates AMPAR endocytosis via the interaction with newly identified CAP, we recorded AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) from primary hippocampal cultures (PHCs) at 15-18 days in vitro. PHCs co-expressed microRNAs-eGFP-tagged to knockdown the CAP with either Arc-wild-type (Arc-WT) or Arc-mutants, which do not interact with the endocytic machinery. miRNA sequences predicted not to target any known vertebrate gene were used as negative controls. The decrease in AMPAR-mediated mEPSC amplitude observed in PHC expressing Arc-WT is reduced in cells expressing Arc-mutants, suggesting that the Arc-dependent endocytosis of AMPAR requires interaction with the CAP. As expected, the increase in AMPAR-endocytosis promoted by Arc was reduced in cells where the expression of the CAP was depleted. Furthermore, disruption of the Arc/CAP interaction, by depleting the expression of CAP, dampens the Arc-mediated reduction in synaptic strength observed in homeostatic synaptic downscaling.

Our discovery that Arc directly binds to clathrin-adaptor proteins may provide the mechanism by which activity-dependent expression of Arc decisively controls the fate of AMPAR at the synapse and regulates the strength of synaptic transmission

Crisp, SJ, Vincent, A, Kullmann, DM

An Electrophysiological Study of Pathological Mechanisms of Glycine Receptor Antibodies.

Antibodies against glycine receptors (GlyR-Abs) have been found in patients with acquired neurological syndromes, which include oculomotor and autonomic disturbance, rigidity and other evidence of disturbance of spinal inhibitory circuits. However, the relationship between the identified antibody and neurological disease is based largely on circumstantial evidence.

Using whole-cell patch-clamp we have recorded spontaneous miniature inhibitory postsynaptic currents (mIPSCs) from motoneurons in rat dissociated spinal cord cultures. GABA and glycine are co-released at interneuron-motoneuron synapses, both contributing to mIPSCs. Pharmacologically isolated glycinergic currents have a shorter decay time than GABAergic currents. We use this difference in time course to separate the two components, in the absence of pharmacological blockade, to quantify the contribution of glycinergic neurotransmission to mIPSCs. We compare the contribution of glycinergic neurotransmission to mIPSCs recorded from neurons incubated in patient IgG or control IgG for up to 24h prior to recording. Our preliminary results indicate a significant

decrease in the glycinergic contribution to mIPSCs for motoneurons incubated in patient compared with control IgG from 41% to 12% ($p < 0.005$).

These results show that not only do the GlyRAbs detected in these patients bind to glycine receptors in cell based assays, and to motoneurons in slices of rodent brain tissue, but they also result in a reduction in glycinergic neurotransmission onto motoneurons. A reduction in glycinergic neurotransmission would be consistent with many of the clinical phenotypes seen in patients with GlyR-Abs. The electrophysiological findings presented here, therefore, provide strong evidence that the antibodies in these cases are pathogenic.

We plan to explore whether this functional assay can also be used to detect antibodies in sera from patients suspected to have antibody-mediated deficiency in glycinergic neurotransmission, without an identified antigenic target.

Diaz, E, Kaur, I, Chenux, G, Matt, L, Hill, TC, Yarov-Yarovoy, V, Liu, XB, Kirk, LM, Zito, K, Hell, J

Activity-dependent palmitoylation underlies SynDIG1 regulation of excitatory synapse development.

Activity-dependent remodeling of synapses is a key feature in the development of precise neural circuits that ultimately give rise to higher order cognitive skills. One mechanism underlying synapse remodeling involves variation in AMPA receptor (AMPA) content defined as 'slots' established by association with the scaffold PSD-95 in the postsynaptic density (PSD). Previously, we identified a novel AMPAR-associated transmembrane protein (SynDIG1: SD1) that regulates synaptic AMPAR and PSD-95 content in dissociated rat hippocampal neurons (Kalashnikova et al., *Neuron*, 2010, 65: 80-93), suggesting that SD1 might serve as a slot mechanism to regulate synapse remodeling.

Palmitoylation regulates localization and function of many synaptic proteins including AMPARs and PSD95. Using the acyl-biotin exchange assay, we demonstrate that SD1 is palmitoylated in both heterologous cells and in mouse hippocampus. Structural modeling of SD1 sequence suggests that the membrane associated region forms a three helical bundle with two cysteine residues located at positions 191 and 192 in the juxta-transmembrane region exposed to the cytoplasm as potential sites of palmitoylation. Site directed mutagenesis reveal that C191 and C192 in the predicted juxta-transmembrane region are palmitoylated in heterologous cells and positively regulates dendritic targeting in neurons. Finally, like PSD-95, synaptic activity blockade in rat hippocampal slice culture increases SD1 palmitoylation, consistent with our prior demonstration that SD1 localization at synapses increases upon activity blockade.

To understand the role of SD1 in vivo, we generated mice with a disruption of the SD1 gene. Ultrastructural analysis of the hippocampal CA1 region shows a decrease in the average PSD length of synapses and a decrease in the number of synapses with a mature phenotype. Intriguingly, total synapse number appears to be increased in SD1 mutant mice, possibly indicating a compensatory response or a failure to eliminate SD1 deficient immature synapses. Electrophysiological analyses show a decrease in AMPA and NMDA receptor function in SD1 deficient hippocampal neurons. Glutamate stimulation of individual dendritic spines in hippocampal slices from SD1 deficient mice reveals increased short-term structural plasticity. Notably, the overall levels of PSD-95 or glutamate receptors enriched in postsynaptic biochemical fractions remain unaltered; however, activity-dependent synapse development is abolished upon loss of SD1, supporting its importance for excitatory synapse maturation.

Altogether, these results support a model in which SD1 acts as a critical organizer of the PSD and AMPAR content during activity-dependent synapse maturation. Intriguingly, alterations in the human SD1 gene have been associated with schizophrenia, suggesting that the defects observed upon loss of SD1 might underlie aspects of synaptic remodeling deficits in psychiatric disease.

Dobson, KL, Bellamy, TC

Independence of plasticity mechanisms at synaptic and ectopic release sites in the cerebellum.

In the cerebellar molecular layer parallel fibre terminals release glutamate from both the active zone and from extrasynaptic “ectopic” sites. Ectopic release mediates transmission to the Bergmann glia that ensheath the synapse, activating Ca²⁺-permeable AMPA receptors and glutamate transporters. Parallel fibre terminals exhibit several forms of presynaptic plasticity, including cAMP-dependent long-term potentiation and endocannabinoid-dependent long-term depression, but it is not known whether these presynaptic forms of long-term plasticity also influence ectopic transmission to Bergmann glia

Whole cell patch clamp recordings from Purkinje neurons and Bergmann glia were carried out in transverse cerebellar slices from juvenile (P16-20) Wistar rats.

Stimulation of parallel fibre inputs at 16 Hz evoked LTP of synaptic transmission, but LTD of ectopic transmission. Pharmacological activation of adenylyl cyclase by forskolin caused LTP at Purkinje neurons, but only transient potentiation at Bergmann glia, reinforcing the concept that ectopic sites lack the capacity to express sustained cAMP-dependent potentiation. Activation of mGluR1 caused depression of synaptic transmission via retrograde endocannabinoid signalling but had no significant effect at ectopic sites. In

contrast, activation of NMDA receptors suppressed both synaptic and ectopic transmission.

The results suggest that the signalling mechanisms for presynaptic LTP and retrograde depression by endocannabinoids are restricted to the active zone at parallel fibre synapses, allowing independent modulation of synaptic transmission to Purkinje neurons and ectopic transmission to Bergmann glia

Domanski, APE, Isaac, JTR, Kind, PC

Bottom-Up Modeling of Network Disruption by Synaptic Defects in the Fmr1-KO Mouse.

Mechanistically dissecting circuit defects in neurodevelopmental disorders is challenging since seemingly isolated changes (e.g. intrinsic or synaptic effects) interact with each other and with developmental/homeostatic plasticity processes, manifesting both as local circuit and network-level signatures. One such example is Autism, where cellular and synaptic changes during early life critical periods are thought to perturb developmental plasticity mechanisms leaving lasting sensory and cognitive deficits. Currently we lack an understanding of how low-level circuit defects interact in the Autistic brain to affect network function – a significant impediment to the rational design of treatments and one demanding a multi-scale approach to studying cortical circuit development.

Here, we study disrupted critical period somatosensory cortex circuitry in the Fmr1-KO mouse model of Fragile X Syndrome (FXS), a prominent monogenic form of Autism accounting for ~5% of cases. Using patch-clamp and multi-electrode array slice electrophysiology we found manifold changes in the thalamorecipient circuitry of cortical Layer 4 (L4), including exaggerated neuronal excitability and short-term synaptic plasticity, reduced connectivity to the local network and slower synaptic kinetics. Moreover, imbalanced inhibitory (GABAergic) and excitatory (glutamatergic) synaptic drive at feed-forward thalamocortical synapses suggests responses to ascending sensory input by cortical neurons are disrupted in Fmr1-KOs. To investigate the effect of these compound defects on sensory processing, we developed a conductance-based thalamocortical simulation tuned by measured parameters and studied the thalamocortical integration responses of individual neurons to simulated sensory input. This “computational psychiatry” approach led to the discovery of a novel cortical circuit phenotype verified by current-clamp recordings: distorted frequency filtering of thalamic input, which likely underlies FXS patients’ tactile hypersensitivity. Furthermore we exhaustively simulated the disease model under different “parameter rescue” conditions (combinations of synaptic and cell-intrinsic treatments), showing interdependent changes suggestive of secondary, compensative effects and a highly dynamic parametric landscape. Finally, we simulate the recurrent L4 cortical circuit and reproduce features of the Fmr1-KO the thalamocortical network response, including elevated LFP gamma power

oscillations and imprecise spike timing. Overall, our bottom-up simulations of Fmr1-KO circuitry provides powerful insight into how low-level changes coalesce into network-level pathophysiology.

Dzyubenko, E, Andreas, F

A new efficient automatic method for synapse quantification in cultured neuronal networks.

Formation, maturation and modification of synapses is one of the key events during the establishment of neuronal networks. Thus, efficient and reliable estimation of synapse formation or loss in a particular in vitro model is of a clear interest for both fundamental and applied neurobiological studies. A sufficient number of synapse quantification methods have been proposed hitherto. In many cases, these methods are very labour intensive and technically demanding. Alternatively, they can be not sensitive enough to reveal subtle synaptogenesis modifications. In this work, we propose a new efficient fully automated method for synapse quantification in cultured neuronal networks. Our method is based on colocalization of presynaptic and postsynaptic markers, visualized by single-plane confocal microscopy. Thus, structurally accomplished synapses can be quantified. Previously, a similar approach was realized in the well-known Bary Wark's ImageJ plugin. Our protocol combines improved fixation and staining procedure with a new image processing algorithm. As a proof-of-concept study, we reveal the differences in GABA- and Glutamatergic synapse formation between two subpopulations of neurons in our culture – inhibitory interneurons, enwrapped with perineuronal nets (PNNs), and principal excitatory neurons.

Eckel, R, Walker, MC, Kittler, JT

Increase in Diffusion Dynamics of Synaptic GABAARs is Reversed by Inhibition of Calcineurin during Status Epilepticus.

Gamma-aminobutyric acid type A receptors (GABAARs) are the most abundant inhibitory neurotransmitter receptors in the mammalian brain. They are assembled from various subunits forming heteropentamers that contain a binding pocket for benzodiazepines between α and γ subunits. Benzodiazepines are a major target for halting prolonged seizures (status epilepticus, SE) and so preventing potentially devastating consequences including neurological and cognitive deficits. However, during SE, pharmacoresistance to benzodiazepines progressively develops. This can be explained by the observation that GABAARs undergo subunit-specific down-modulation in models of SE; here we have further elucidated the mechanisms underlying this modulation.

To determine changes in surface stability of GABAARs in an in vitro model of SE, we performed live-cell imaging using single particle tracking of Quantum-dot (QD) labelled GABAARs. This was combined with live-cell imaging of intracellular Ca²⁺ dynamics with genetically encoded calcium indicators. Using this approach, we find that under basal conditions, QD-labelled endogenous α 2-subunit containing GABAARs show specific surface dynamics, depending on whether GABAARs are inside or outside inhibitory synapses labelled with a GFP tagged intrabody to gephyrin (Gephyrin FingR). Interestingly, after induction of epileptiform activity using Mg²⁺-lacking aCSF (low Mg²⁺) we observe a time-dependent increase in the lateral mobility of synaptic GABAARs. Using the Calcineurin inhibitor FK506, we can inhibit this increase in diffusion dynamics of synaptic GABAARs. This work provides new insights into our understanding of altered GABAergic inhibition during SE and could provide the foundation for combination therapy against SE.

Fernandes, D, Ribeiro, L, Santos, S, Carvalho, AL

Homeostatic regulation of synaptic AMPA receptors by Caspr1 and the RNA-binding protein ZBP1.

Phenomena such as learning and memory rely on changes in synaptic function through several forms of synaptic plasticity including Hebbian and homeostatic plasticity. In turn, these plastic events are highly dependent on the regulation of synaptic AMPA receptors (AMPAARs), through mechanisms still poorly understood. Despite a growing list of candidates, there is still an intricate puzzle of undiscovered proteins that may contribute to the regulation of AMPA receptors upon activity. In this context, our lab has recently shown that the cell-adhesion molecule Contactin-associated protein 1 (Caspr1) is a novel AMPAR interactor, able to regulate the basal trafficking of the GluA1 subunit into synapses.

We now found evidence for an additional role for Caspr1 in posttranscriptionally regulating GluA1 mRNA and the phosphorylation of the RNA-binding protein Zipcodebinding protein 1 (ZBP1). Our results indicate that ZBP1 binds to GluA1 mRNA, an interaction that significantly decreases upon chronic changes in neuronal activity, suggesting an activity-dependent release of GluA1 transcripts to undergo translation. Indeed, we show that chronic blockade of activity significantly upregulates not only total mRNA levels and surface synaptic puncta of GluA1, but also phosphorylation levels of ZBP1. Additionally, we demonstrate that Caspr1 expression is regulated by neuronal activity, since its total protein and mRNA levels increase upon homeostatic stimuli.

This set of results suggests a requirement for Caspr1 in the regulation of homeostatic plasticity. Indeed, when we knock-down the endogenous expression of Caspr1 in hippocampal neurons, the basal synaptic content of GluA1-containing AMPARs decreases. Most important, loss of Caspr1 fully blocks the scaling up of synaptic GluA1 induced by

chronic blockade of neuronal activity. Furthermore, knock-down of the endogenous expression of the RNA-binding protein ZBP1 leads to similar effects. Loss of ZBP1 causes a significant decrease in the basal superficial expression of synaptic GluA1 and completely blocks GluA1 scaling up upon prolonged blockade of activity.

Altogether, our results identify Caspr1 and the RNA-binding protein ZBP1 as major regulators of synaptic AMPA receptors. Moreover, we start to uncover a novel activitydependent posttranscriptional mechanism that may be crucial to regulate synaptic availability of GluA1 transcripts and contribute to the expression of homeostatic plasticity phenomena.

Fivaz, M, Garcia-Alvarez G, Lu Bo, Yap, KA, Shetty, MS, Wong LC, Thevathasan JV, Lim L, Ji F, Tan KW, Mancuso JJ, Tang W, Poon SY, Augustine GJ, Sajikumar S, Bichler Z
STIM2 regulates PKA-dependent phosphorylation and trafficking of AMPA receptors.

Recent findings point to a central role of the endoplasmic reticulum (ER)-resident STIM proteins in shaping the structure and function of excitatory synapses in the brain. Here, we show that STIM2 is essential for PKA phosphorylation of the AMPA receptor (AMPA) subunit GluA1 in excitatory neurons. cAMP triggers rapid migration of STIM2 to contact sites between the ER and the dendritic plasma membrane (PM), enhances recruitment of GluA1 to these ER-PM junctions and promotes localization of STIM2 in dendritic spines. Both biochemical and imaging data suggest that STIM2 regulates GluA1 phosphorylation by coupling the AMPAR to PKA. Further, STIM2 is required for PKA-dependent surface delivery of GluA1 and regulates both long-term potentiation (LTP) and depression (LTD) in the hippocampus, two forms of synaptic plasticity that are thought to underlie learning and memory. Further, conditional deletion of both Stim genes in the forebrain results in severe deficits in spatial memory. Collectively, our findings point to a unique mechanism of synaptic plasticity driven by ER-to-synapse signaling in neural circuits encoding spatial memory.

Fouillet, A, Bigoli, M, Virdee, J, Findlay, J, Broad, L, Ursu, D
Optimisation of neuronal cultures derived from human iPSC cells as disease models for neurological disorders.

Induced pluripotent stem cells (iPSCs) are a type of stem cells that can be generated from somatic cells by retroviral expression of various reprogramming factors. By using a combination of growth factors and culture conditions iPSC cells can be further differentiated into a large variety of cellular types, including CNS-like neurons and glial cells. Although this new technology shows great promise for both academic research and drug development in the pharmaceutical industry, further work is required to improve the

maturation of these neurons. In this study, we aimed at evaluating different neuronal maturation protocols while optimizing new methods for assessing the formation of electrically active neuronal networks and the expression of synaptic proteins in the iPSC derived neurons. The initial effort focused on optimisation of immunocytochemistry (ICC) assays which allowed selection of the best antibodies against pre- and post-synaptic markers for both excitatory and inhibitory synapses. Since astrocytes are known to positively modulate neuronal development, we developed a mixed culture assay between iPSC derived neurons and rat primary astrocytes. We observed that the astrocytes co-culture markedly increased the neurite outgrowth (β 3tubulin and MAP2) in two different iPSC derived neuronal lines. We further demonstrated that various synaptic markers (VGLUT1, synaptophysin, PSD95 and gephyrin) were also increased in the presence of astrocytes. The ICC data were in agreement with our functional readouts where astrocytes co-culture strongly increased the electrically evoked calcium responses of the iPSC derived neurons. Voltage-gated Na⁺- and K⁺-currents and spontaneous synaptic activity were also evaluated by using the patch-clamp technique and demonstrated presence of a mature neuronal phenotype. Alternative protocols (3D matrix culture, custom culture media) were investigated in this study and found to further affect the maturation of cultured neurons. Altogether this study represents the ground work required for generating mature IPS derived neurons, otherwise an essential step in our endeavour to use these cellular systems as models of neurological disorders.

Frere, S

Hippocampal amyloid beta signaling and alterations of the synaptic properties in the dentate gyrus.

Accumulation of amyloid- β peptides (A β), the proteolytic products of the amyloid precursor protein (APP), induce a variety of synaptic dysfunctions ranging from network hyperactivity to synaptic depression that are postulated to cause cognitive decline in Alzheimer's disease (AD). We aim to understand the relation between peculiar synaptic dysfunctions and local A β signaling imbalance. The variable effects observed at different hippocampal synapses is not yet explained but might arise from different dynamics of APP processing and regulations of A β levels or by a cell-specific effects of A β depending on the expression of molecular determinant(s) downstream to APP processing and A β release. Understanding the relationship between the local signaling of A β and the property alterations at specific hippocampal synapses will allow us to propose a developmental model of the network dyshomeostasis resulting from AD-related insults.

Similarly to previously published, we observed the absence of expression of APP at the granule cells of the dentate gyrus (DG) in contrast to the hilar interneurons of the DG and the pyramidal cells of the CA3 area, which are highly immune-reactive for APP. How does this differential expression of the precursor and receptor for A β (Fogel, Frere et al., Cell

Report, 2014) control the effects induced by A β level changes? Surprisingly, we observed that A β level elevations at the mossy fibers decrease the release probability and increase short-term facilitation while no significant changes were observed at the inhibitory synapses of the granular cells and the at the synapses of the recurrent pathways in the CA3 area.

To similar A β challenges, different effects were measured at two excitatory and one inhibitory synapse of the DG-CA3 network. In future experiments, we will decipher what the mechanisms of the synapse-specific effects of A β are at those synapses. Exploring the complex mechanisms by which APP/A β modulate synaptic function will be critical for the understanding of the distinctive susceptibility of different cortical networks to A β injury and of the transition to AD-related pathology.

Gazit, N, Shapira, I, Slomowitz, E, Vertkin, I, Sheiba, M, Yael Mor, Y, Slutsky, I
IGF-1 receptor activity differentially regulates spontaneous and evoked synaptic vesicle release

The insulin-like growth factor-1 receptor (IGF-1R) signaling is a key regulator of lifespan, growth and development. While reduced IGF-1R signaling delays aging and Alzheimer's disease (AD) progression, whether and how it regulates information processing remains unknown. Here, we show that spontaneous, Ca²⁺-dependent local IGF-1 release activates near-synaptic IGF-1Rs, regulating basal synaptic vesicle release in excitatory hippocampal neurons. Acute IGF-1R blockade or transient knockdown suppresses spike-evoked synaptic transmission and presynaptic Ca²⁺ flux, while promoting spontaneous transmission and resting Ca²⁺. The observed changes in Ca²⁺ flux induced by IGF1R, involved alterations in Calcium balance between the mitochondria and the cytoplasm, thus, disruption of mitochondrial function mimicked the effect of IGF1R inhibition and abolished further effect by IGF1R. Suitable with the reduction in evoked release probability, diminished IGF-1R signaling boosts short-term synaptic facilitation. Finally, increased IGF-1R-mediated presynaptic modulation contributes to augmented excitatory synaptic transmission and reduced synaptic facilitation in AD mouse model.

These findings suggest that basally active IGF-1Rs regulate information processing in the hippocampus by maintaining evoked-to-spontaneous transmission ratio and ongoing spiking rate, while constraining high-pass filtering of synaptic transmission. Our data suggest a key role for mitochondrial Ca²⁺ in this process. Excessive IGF-1R-mediated signaling may contribute to AD-related hippocampal hyperactivity.

Glebov, O
Molecular clustering at the active zone controls presynaptic function

The presynaptic active zone (AZ) controls synaptic function in neurons through coordinated recruitment of voltage-gated calcium channels (VGCCs) and synaptic vesicles (SV) via multiple protein-protein interactions. AZ scaffold forms a dense environment, raising the possibility that the molecular clustering/crowding aspects of AZ organization may regulate synaptic function, but the link between nanoscale presynaptic organization and function has not been established. Here, we use stochastic optical reconstruction microscopy (STORM), ratiometric imaging and optogenetic stimulation to show that neuronal activity transsynaptically controls nanoscale clustering of the AZ scaffolding through activity-dependent presynaptic actin dynamics. In turn, AZ clustering negatively regulates presynaptic recruitment of VGCCs and SV cycling, and acute induction of AZ clustering through actin polymerization attenuates presynaptic VGCC recruitment. Dual-colour spectral-unmixing STORM demonstrates segregated distribution of the AZ scaffolding and VGCC domains within the AZ. Our study reveals a conceptually novel transsynaptic mechanism linking neuronal activity, synaptic structure and function on a nanoscale, whereby local structural plasticity of the AZ cytoskeleton and scaffold controls synaptic function through spatial restriction of presynaptic machinery recruitment. On a broader scale, our results provide the first evidence for crowding of endogenous macromolecules regulating a biological function.

Hardingham, NR, Greenhill, SD, Seaton, G, De Hann, AM, Fox, K

Acute and chronic effects of transient mutant DISC1 on layer II/III mouse barrel cortex.

DISC1 has been implicated in schizophrenia and has an important role in brain development. We have used a transgenic mouse that expresses a fragment of the DISC1 c-terminal (DISC1-cc) and acts as a dominant negative when activated for 24-48 hours by a single injection of tamoxifen. Transient activation of the mutant protein at P7 results in schizotypic behaviour in the mouse (Li et al, 2007) and a loss of experience dependent plasticity in layer 2/3 of adult barrel cortex, while activation of DISC1-cc at P28 has no effect on experience dependent plasticity.

We compared dendritic development in WT and DISC1 neurons and showed that dendritic structure in WTs was largely mature by P11. We found that dendritic growth was attenuated in DISC1s both at P11 and P14 (both $p < 0.05$) but had recovered by P21. Retarded development was observed both in basal and apical dendrites. Additionally, we measured spine density on characterised dendrites from recorded neurons to look at both acute and chronic effects of DISC1-cc. We found that at P8 there was a significant reduction in spine density on third order dendrites in DISC1 mice, and at P28 and P50 significant reductions in spine density in DISC1 mice on both second and third order dendrites. In DISC1-cc mice, we found reduced numbers of mushroom spines and increased numbers of thin spines on 2nd and 3rd order dendrites when compared to wild types (all $p < 0.05$),

We measured short term plasticity of layer 4 inputs to layer 2/3 neurons and in WTs found a similar developmental profile to the dendritic growth, while in DISC1-cc mice development of short term plasticity was retarded.

We measured levels of LTP and LTD in adult mice (P50-P70) given transient expression of DISC1-cc and in wild type littermates. We found that LTP was entirely occluded in the mutant DISC1s (comparison with WT, $p < 0.05$) and that LTD was also reduced in mutant DISC1s. We found there to be a significant difference between NMDA/AMPA ratios of WTs and DISC1s at P28 and P50 (both $p < 0.05$) but not at P14. Furthermore, NMDA currents from DISC1-cc animals were of enhanced sensitivity to ifenprodil, indicative of a more immature, NR2B mediated component when compared to wild types. In addition, 50% of EPSCs from mature DISC1 neurons had failure rates higher at -70mV than at +40mV, indicative of silent synapses. We conclude that transient expression of DISC1-cc retards development of synapses during early postnatal development that creates long lasting deficits in plasticity in adulthood.

Helm, M

The molecular anatomy of dendritic spines.

How memory is stored within the brain is still enigmatic. One hypothesis is that synaptic strength is modulated by changing the protein composition in the postsynaptic dendritic spines. Although the functions of many of these proteins have been described in detail in the synapse, an overall understanding on how information is stored is still missing. This is largely due to the fact that the molecular anatomy of the dendritic spine, meaning the nanoscale localization and the quantity of the different proteins, is unknown. Here, we use a combination of super-resolution imaging, mass spectrometry and electron microscopy to describe the nanoscale localization of postsynaptic proteins and their abundance. We investigate ~150 proteins in this way, ranging from neurotransmitter receptor organization, scaffolding proteins, ion channels and kinases. We found that proteins involved in the same physiological processes are correlating very closely, for example CaMKII and calmodulin. In total the combined techniques will enable us to create a realistic 3D model of the dendritic spine, which we can use to simulate physiological processes like ion fluxes in this complex compartment.

Hussien, M, Carvalho, AL, Peça, J

Role of GPRASP2 in mGluR-dependent signaling, regulation of neuronal morphology and spine maturation.

Autism and autism spectrum disorders (ASDs) are neurodevelopmental disorders diagnosed based on a triad of criteria: deficits in communication, impaired social

interactions, and repetitive or restricted interests and behaviours. ASDs pose an immense burden to society and are currently thought to afflict 1 out of each 68 children. Recent genetic and genomic studies have identified a large number of candidate genes for ASDs, many encoding synaptic proteins, indicating synaptic dysfunction may play a critical role in ASDs. Disease susceptibility proteins, such as those in the Neurexin/Neuroigin/PSD-95/SAPAP/SHANK macromolecular complex, converge on ionotropic and metabotropic glutamate signaling. Dysfunction in these genes has been shown to disrupt neuronal morphology, dendritic complexity and synaptic communication. Presently, several lines of evidence suggest that metabotropic glutamate receptors (mGluRs) play an important role in ASD pathophysiology. Nevertheless, research work centering on the proteins that directly regulate the trafficking and surface availability of mGluRs has not been widely explored.

The G Protein-Coupled Receptor Associated Sorting Protein (GPRASP) family regulates the trafficking of diverse classes of G-protein coupled receptors and is involved in endocytic sorting towards lysosomal degradation. GPRASPs have been shown to strongly interact with the c-terminal tails of mGluR1, mGluR5, M1 muscarinic receptor and oxytocin receptor. Interestingly, from this family of proteins, GPRASP2, located in the q22.1 band of the X chromosome and has recently been suggested as a susceptibility gene for autism. Here we describe our progress in characterizing GPRASP2, its location in subcellular compartments, expression in different brain regions and across development. We find that GPRASP2 is enriched in glutamatergic synapses where it strongly colocalizes with PSD-95, VGLUT1 and also with mGluR1/5 receptors. Testing the functional consequences of changing levels of GPRASP2 expression, our data support a role for this protein in the regulation of neuron complexity, spine density and spine maturation. We are also investigating the crosstalk between mGluR-GPRASP2 signaling and using mouse molecular genetics to understand the circuit specific deficiencies arising from mutations in this gene.

Jouhanneau, JS, Ferrarese, L, Kremkow, J, Dornn, A, Poulet, JFA

In vivo monosynaptic excitatory transmission between layer 2/3 pyramidal neurons during active cortical states.

Local, excitatory synaptic connections between glutamatergic pyramidal neurons underlie neocortical sensory processing and cognition, yet there is very limited data available on identified monosynaptic cortico-cortical connections in vivo. In particular, cortical neurons in anaesthetized animals constantly oscillate between quiescent hyperpolarized states (downstate) and active depolarized states (upstate). We know that sensory processing and perception are affected by cortical state but not how they alter synaptic integration. Here we used two-photon microscopy to make visually targeted recordings from 2-4 neighbouring (<100um) pyramidal neurons in the somatosensory cortex of the

urethane anaesthetised juvenile (P18-29) mouse. During downstates we report a sparsely connected (probability 6.5%), structured network of excitatory pyramidal neurons with an overrepresentation of bidirectional connections. Unitary excitatory postsynaptic potentials (uEPSPs) had low failure rates and were typically small in amplitude (<0.5 mV) with high trial-to-trial variability, however we observed a minority of larger amplitude (>1 mV), reliable connections. In contrast to in vitro studies, we recorded no overall paired pulse depression of uEPSP amplitude. Despite an increase in membrane potential during upstates, we did not see a change in peak amplitude. To investigate the postsynaptic properties of integration we used dendritic optogenetic stimulation to mimic synaptic inputs during down and upstates. Optogenetic responses in upstates also did not show an overall change in peak amplitude but instead a normalization of the downstates amplitude. Ongoing analysis investigates synaptic integration in active cortical states.

Kaempfer, N, Kochlamazashvili, G, Puchkov, D, Maritzen, T, Bajjalieh, SM, Natalia L. Kononenko, NL, Haucke, V
Overlapping functions of stonin 2 and SV2 in sorting of the calcium sensor synaptotagmin 1 to synaptic vesicles.

Neurotransmission involves the calcium-regulated exocytic fusion of synaptic vesicles (SVs) and the subsequent retrieval of SV membranes followed by reformation of properly sized and shaped SVs. An unresolved question is whether each SV protein is sorted by its own dedicated adaptor or whether sorting is facilitated by association between different SV proteins. We demonstrate that endocytic sorting of the calcium sensor synaptotagmin 1 (Syt1) is mediated by the overlapping activities of the Syt1-associated SV glycoprotein SV2A/B and the endocytic Syt1-adaptor stonin 2 (Stn2). Deletion or knockdown of either SV2A/B or Stn2 results in partial Syt1 loss and missorting of Syt1 to the neuronal surface, whereas deletion of both SV2A/B and Stn2 dramatically exacerbates this phenotype. Selective missorting and degradation of Syt1 in the absence of SV2A/B and Stn2 impairs the efficacy of neurotransmission at hippocampal synapses. These results indicate that endocytic sorting of Syt1 to SVs is mediated by the overlapping activities of SV2A/B and Stn2, and favor a model according to which SV protein sorting is guarded by both cargo-specific mechanisms as well as association between SV proteins.

Kalinowska, M
Dynamic remodelling of dendritic spines by group I metabotropic glutamate receptors requires actinin-4

Dendritic spines are actin-rich protrusions and sites of excitatory synaptic contacts. Spines are highly dynamic, undergoing changes in morphology and number during neuronal

development and activity-dependent synaptic plasticity. Glutamate stimulation regulates dendritic spine morphology and dynamics. Group I metabotropic glutamate receptors (mGluRs) are G protein-coupled receptors that mediate slow excitatory neurotransmission. mGluR stimulation induces elongation of dendritic protrusions and spines and we have recently shown that it can also drive protrusion turnover. At mature synapses formed on mushroom-type spines co-activation of mGluRs with NMDA receptors is required for spine shrinkage and synaptic weakening. Although it has been shown that intracellular Ca²⁺ mobilization is necessary for mGluR-mediated effects on dendritic spines, the specific molecular mechanisms by which these receptors govern structural plasticity remain unknown. We identified actinin-4, a Ca²⁺ sensitive actin-binding protein, as a new mGluR1/5 interacting partner that participates in mGluR-dependent spine remodeling. We found that actinin-4 downregulation profoundly reduced spine dynamics even though spine formation and AMPAR-mediated basal synaptic transmission remained intact. Interestingly, actinin-4 downregulation impaired spine elongation and turnover stimulated by mGluRs in the absence of perturbations in receptor surface expression or signaling capacity. Actinin-4 function in mediating spine morphogenesis was underscored by gain-of-function phenotypes in neurons where actinin-4 overexpression induced spine head enlargement. This effect was dependent on the carboxyl terminus of actinin-4 that binds to CaMKII, an interaction we showed to be regulated by mGluR activation. Strikingly, expression of an actinin-4 mutant lacking carboxyl-terminal domain induced an overabundance of immature protrusions and reduced the frequency of AMPAR mEPSCs, suggesting delayed synaptic maturation. Our findings identify actinin-4 as a critical cell-autonomous effector of dendritic protrusion dynamics that is necessary for mGluR-dependent spine remodeling and synapse maturation.

Kessels, H, Renner, MC, Albers, EHH, Gutierrez-Castellanos, N, Tessa R. Lodder, TRR, Reinders, NR, De Zeeuw, CI

Fear Elicits Synaptic Potentiation through Activation of AMPA-Receptor Subunit GluA3.

AMPA-receptors are responsible for fast excitatory synaptic transmission in the brain. In CA1 pyramidal neurons of the hippocampus two types of AMPA-receptors exist: those that contain subunit GluA1 and those that contain GluA3. GluA1-containing AMPA-receptors have been extensively studied and were shown to play a key role in several forms of synaptic plasticity and memory formation. In contrast, the contribution of GluA3 to synapse physiology and memory formation has remained elusive. Here we show that GluA3-containing AMPA-receptors are inactive and contribute little to synaptic currents under basal conditions. During fear a rise in intracellular cyclic AMP driven by norepinephrine release restores GluA3 channel and receptor function, leading to a massive and transient synaptic potentiation. When mice are in a state in which GluA3-

containing AMPA-receptors are activated, the ability to encode contextual fear memories is attenuated (or the freezing response is suppressed?). We propose that the activation of GluA3-plasticity during an anxious moment prevents memories from becoming too strong.

Koch, N, Koch, D, Sabanov, V, Krüger, S, Ahmed, T, Montag, D, Kessels, MM, Balschun, D, Qualmann, B

LTD and LTP expression, GluR1 surface organisation and internalisation requires Syndapin I.

Learning and memory formation, development and fine-tuning of neural circuitry are based on long-term changes in synaptic transmission known as synaptic plasticity. Alterations in surface-localised AMPA receptor number are prerequisite for plastic changes of synaptic transmission. Postsynaptic characterisation of Syndapin I KO mice revealed blocked LTP without spatial learning defects. However, we observed a striking anxiety-like phenotype reminiscent of GluR1 KO mice without changes in overall GluR1 level. Extensive defects in clustering and spatial organisation of surface GluR1 that profoundly affected AMPAR gating properties and led to reduced basal synaptic transmission and clearly demonstrated GluR1 malfunction. Analyses of activity-dependent plasticity processes unveiled prominent defects in GluR1 internalisation. As a consequence we observed impairments in both NMDA receptor-dependent and mGluR-dependent LTD upon Syndapin I KO. Further mechanistic investigations leading to impaired synaptic plasticity upon Syndapin I KO demonstrated a reduced NMDA receptor and mGluR signaling acting upstream of GluR1, and an increased p38 activity acting downstream of GluR1 internalisation.

Our observations introduce Syndapin I KO as a model system to analyse GluR1 functionality without changing overall GluR1 level and propose a feedforward/feedback loop generated by glutamatergic receptors, which signal upstream of GluR1 internalisation, and by p38-mediated signaling downstream of GluR1 internalisation that induces, maintains and may thus balance long-term changes in synaptic signal reception and processing.

Koerber, C, Harrach, H, Kuner, T

Extracellular matrix ensures temporally precise high frequency synaptic transmission at the calyx of held.

In the mammalian brain, a small fraction of the neurons is surrounded by a special form of extracellular matrix, the so called perineuronal nets (PNNs). PNNs are a complex meshwork mainly composed of hyaluronan as a basic element, tenascin-R and chondroitin

sulfate proteoglycans (CSPGs) that bind to hyaluronan. CSPGs consist of a core protein to which glycosaminoglycan side chains (GAGs) are bound. These GAGs are heavily but specifically sulfated resulting in a strongly negatively charged environment. Most of the neurons described so far that bear PNNs were also found to express parvalbumin and the Kv3.1 potassium channel subunit, suggesting they are fast-spiking interneurons. Functionally, PNNs have mostly been associated with structural plasticity e.g. spine enlargement during LTP and the critical period of the visual system. However, the physiological function of PNNs in synaptic transmission under normal neuronal activity patterns remains elusive.

Contrary to the majority of brain areas, where neurons carrying PNNs are sparsely distributed, in the medial nucleus of the trapezoid body (MNTB) of the auditory brainstem, all principal neurons are surrounded by PNNs, making it an ideal system to study PNN function in synaptic transmission. MNTB principal neurons receive their main excitatory input from a single calyx of Held synapse originating from the globular bushy cells in the contralateral anterior ventral cochlear nucleus. The calyx of Held is a giant axo-somatic synapse that comprises 300-700 individual active zones and has evolved as a model system for synaptic transmission in recent years. Here, we disrupted PNNs in the MNTB by treating brainstem slices with chondroitinase (ChABC), an enzyme that specifically digests the GAGs of CSPGs leaving the protein cores intact, and examined the effects of PNN-removal on synaptic transmission at the calyx of Held-MNTB principal cell synapse. ChABC treatment led to faster synaptic short-term depression (STD) at high frequencies. However, this effect was prevented by addition of cyclothiazide and kynurenic acid to the extracellular solution. These results are suggestive of a role for PNNs in the effective clearing of glutamate from the synaptic cleft in order to prevent postsynaptic glutamate receptors from desensitization and/or saturation. We thus propose that PNNs are necessary for ensuring fast glutamate clearance during high frequency firing thereby maintaining high fidelity signal transmission.

Koopmans, F, Van Nierop P, Osumi-Sutherland D., Mi H, Lovering R C, Foulger R, Coba M P, Hussain N K, Thomas P D , Feng G, Smit A B, Verhage M
Synapse Gene Ontology and Annotation Project.

Synapses are the fundamental processing elements that form the basis for the unsurpassed computational power of our brain. For many of the major brain disorders, both neuropsychiatric and neurodegenerative, pathogenesis can be traced back to synaptic dysfunction. Synapses are dynamic structures that consist of around two thousand different types of proteins. The function of synaptic proteins in synapse function can be studied in highly standardized, reduced preparations, which allows multi-level quantitative systems analysis and full integration of genomics, proteomics and genomics can lead to an understanding of pathophysiology.

Despite the central role of synaptic genes in many brain disorders, the annotation of synaptic genes and their systematic classification is not optimal. Gene annotations in the Gene Ontology (GO) database — supported by the formal computational structure of the ontology — are widely used in the life science community and have served as the major foundation for analysis and biological interpretation of large-scale “omics” datasets, including genome-wide association studies. However, GO annotations in the area of synapse biology are incomplete, use evidence from non-synaptic studies to infer synaptic function and lack synapse-specific terms. Together, these limitations hamper efforts in computational modeling, understanding of protein network structure and pathways in health and disease, the collection of the pre- and postsynaptic gene products needs functional annotation and description in terms of logical ontologies.

We present the Synapse Gene Ontology Annotation Project (SynGO project) that aims to establish a GO-compatible annotation of pre- and postsynaptic genes. A synaptic gene annotation framework has been developed within three EU-funded FP6/7 integrated projects (EU-synapse, EUROSPIN and SynSys). The SynGO project builds on these efforts and is a collaboration between the Broad Institute, the Center for Neurogenomics and Cognitive Research (CNCR, VU University Amsterdam), and the Gene Ontology Consortium. The SynGO project is improving GO annotations so that these accurately describe cellular components, biochemical processes, and molecular functions important for synapse biology. In addition, the collection of genes that function at either the pre- or postsynaptic compartment is being defined (synaptic parts list). This parts list will initially be collected based on published research. At later stages we will integrate novel proteomics datasets and functional analyses. Pre- and postsynaptic gene products from the parts list will be annotated against the improved GO ontology. To ensure broad scientific applicability and consensus, the proposed annotations will be subjected to critical evaluation and adjustment upon consultation of the synapse research community.

Koutsikou, S, Merrison-Hort, R, Borisyuk, R, Roberts, A, Soffe, SR

Sensory pathway neurons extend synaptic excitation and slow the initiation of tadpole swimming.

Reaction times for decisions to initiate coordinated motor actions (such as eye movements or postural adjustments) in response to a stimulus can be surprisingly long (~100 ms) and the underlying processes are not well understood. In hatchling *Xenopus* tadpoles, swimming following trunk skin stimulation starts after a delay of 20---100ms. Much of the underlying neuronal circuitry has been defined: sensory Rohon---Beard (RB) neurones fire once to trunk skin touch, as do the dorsolateral sensory pathway neurones (dlis) which amplify and distribute excitation to both sides of the CNS. Reticulospinal descending neurones (dINs) with pacemaker properties excite other neurones to drive swimming.

Whole-cell recordings from dINs following skin stimulation show a build-up of EPSPs that rises slowly, delaying firing and the start of swimming. This slow build-up cannot be explained by the single firing and short synaptic delays of the known neurones in this sensory pathway. There is also no evidence of direct connections from sensory pathway neurones to dINs. We therefore propose an additional group of neurones (xINs) interposed in this pathway. Whole-cell recordings from candidate xINs, in some cases paired with dINs, showed caudal hindbrain neurones with i) short latency EPSPs and spiking to trunk skin stimulation at and just below the threshold level for swimming, ii) spiking prior to dIN firing and swimming and iii) variable firing during swimming. These xINs could produce the slow-rising build-up of postsynaptic excitation observed in dINs, which brings them to firing threshold and initiates swimming.

We test the proposal that a small, coupled, population of xINs could produce the slow build-up of excitation in dINs, using a computational model of 30 xINs with random mutual excitation. Some xINs received a single EPSP from sensory pathway dIIs; all xINs excited a single dummy dIN to monitor their summed output. The model included short-term depression at xIN-xIN synapses as a mechanism to terminate xIN activity after a delay. We explored parameters that led to the recruitment of xINs and long-lasting xIN firing which could produce the observed slow build-up of EPSPs in the dIN.

Our results suggest that xINs in this sensory pathway fire multiply following a brief stimulus and produce a slow build-up of excitation in reticulospinal neurons; these initiate swimming when their firing threshold is reached and their whole population is recruited.

Krieger, J

Computational analysis of glutamate receptor N-terminal domain function

Ionotropic glutamate receptors (iGluRs) are tetrameric ligand-gated cation channels that mediate the majority of excitatory synaptic transmission and plasticity. They have a shared domain architecture with two extracellular domains above the pore-forming transmembrane domain (TMD). The ligand-binding domain (LBD) binds agonists such as glutamate to gate the channel. Above it sits an N-terminal domain (NTD) whose function is less clear, especially in iGluRs of the AMPA and kainate subtypes. The NTD of NMDA iGluRs binds ligands such as zinc to allosterically modulate channel gating but the mechanism is unclear. We have used computational methods to analyse iGluR NTD sequence, structure and dynamics. Sequence co-evolution analyses and all-atom molecular dynamics simulations suggest a common signalling pathway through all iGluR NTDs as well as related domains such as those from metabotropic glutamate receptors. Together with coarse-grained simulations using elastic network models, these results

provide insights into NTD-mediated allostery and interdomain communication in these complex signalling machines.

Lee, Yeseul, Bortolloto, ZA, Bong-Kiun Kaang, B-K, Collingridge GL

A GSK-3 inhibitor blocks the induction of LTD in the hippocampus in vivo and enhances the accuracy of spatial memory.

Dysregulation of glycogen synthase kinase-3 (GSK-3) is implicated in various psychiatric and neurodegenerative disorders. However its physiological roles in the CNS are poorly understood. Previously we reported that GSK-3 activity is required for long-term depression (LTD) induced by the synaptic activation of N-methyl-D-aspartate receptors (NMDARs) in the CA1 region of rat hippocampal slices. Here, using in vivo recordings we show that a potent GSK-3 inhibitor, CT99021, reversibly blocks the induction of NMDA receptor-dependent form of LTD at CA1 synapses of adult mice. In addition, using behavioral tests, we also found that CT99021 facilitates learning and memory in the Morris water maze. However, hippocampus dependent fear memory and behavioral flexibility remained unaffected. These data suggest that a GSK3 dependent process, potentially NMDAR-LTD, may act as an impediment for spatial learning and memory accuracy.

Lipstein, N, Pieńkowska, K, Calloway, N, Michelassi, FE, Ryan, TA, Dittman, J, Taschenberger, H, Rhee, JS, Jans, J Brose, N

Impaired Munc13-dependent regulation of calcium channel function in a patient with neurological dysfunction.

Munc13 proteins participate in synaptic vesicle (SV) priming, and are essential for the completion of the SV cycle in the presynaptic active zone. Neurons deficient of all Munc13s show no spontaneous or evoked synaptic transmission, due to a complete loss of the readily-releasable SV pool. Munc13s are regulated directly and indirectly by Ca²⁺, and elevation of the presynaptic Ca²⁺ concentration during neuronal activity leads to an increase in the SV priming rate, and consequently to dynamic changes in the efficacy of neurotransmission.

Despite, or perhaps because of the essential role of Munc13s in neurotransmission, no human disease involving the neuronal Munc13s has yet been described. Here we describe the first Munc13-1 synaptopathy, found in a 5-year old boy suffering from multiple neurological deficiencies and autism. We characterize the single, gain-of-function point mutation found in the patient by electrophysiological recordings in autaptic hippocampal and striatal neurons. Neurons expressing the mutated protein show a marked increase in the vesicular release probability, and consequently altered evoked and spontaneous

release properties, as well as altered short-term synaptic plasticity characteristics. The elevated release probability likely results from an increase of ~60% in the action-potential evoked Ca²⁺ influx into presynaptic terminals. We conclude that an interplay exists between Munc13s and voltage-gated Ca²⁺ channel activity, that has a drastic effect on the neurological function of the human central nervous system. Our study adds Munc13-1 to the growing list of synaptic proteins involved in synaptopathies, that are emerging in recent years as the underlying cause of multiple cognitive, psychiatric and neurodegenerative diseases.

Loss, O

An investigation into the contribution of TRAK kinesin adaptors to axonal and dendritic mitochondria

Neuronal function requires regulated anterograde and retrograde trafficking of mitochondria along microtubules using the molecular motors, kinesin and dynein, to ensure supplies of energy in the form of ATP for synaptic transmission. Previous work has established that the kinesin adaptor proteins, TRAK1 and TRAK2, play an important role in mitochondrial transport in neurons. They link mitochondria to kinesin motor proteins via a TRAK acceptor protein in the mitochondrial outer membrane, the Rho GTPase, Miro. In addition, both TRAKs associate with the post-translational modification enzyme, O-linked N-acetylglucosamine transferase (OGT). Thus, TRAK1 and TRAK2 form quaternary, mitochondrial trafficking complexes composed of TRAK1 or TRAK2, kinesin heavy chain, OGT and Miro. Down-regulation of TRAK1 or TRAK2 expression as well as dissociation of the quaternary complex by a TRAK dominant negative construct both impaired mitochondrial transport in axons of hippocampal neurons in culture¹.

Recent studies found that TRAK1 preferentially controls mitochondrial transport in axons of hippocampal neurons by virtue of its binding to both kinesin and dynein motor proteins whereas TRAK2 controls mitochondrial transport in dendrites due to its binding to dynein². However, it is not clear whether the function of any of these proteins is exclusive to axons or dendrites and if their mechanisms of action are conserved between different neuronal populations and at different stages of differentiation. In this study, we have determined the distribution of TRAK1 and TRAK2 and in conjunction, the effect of TRAK1 and TRAK2 gene knock-down in axons and dendrites of both primary hippocampal and cortical neurons at different stages of differentiation, to evaluate their respective contribution to axonal and dendritic mitochondrial trafficking. These studies will help to elucidate the mechanisms regulating the molecular interplay between mitochondrial transport and energy supply in cultured neurons and may advance our understanding of neurodegenerative disorders in which defects in mitochondrial transport are implicated.

Lundgren, J, Ahmed, S, Schedin-Weiss, S, Gouras, GK, Winblad, B, Tjernberg, LO, Frykman, S

ADAM10 and BACE1 are localized to synaptic vesicles.

Background: Synaptic degeneration and accumulation of the neurotoxic amyloid β -peptide ($A\beta$) in the brain are hallmarks of Alzheimer disease. $A\beta$ is produced by sequential cleavage of its precursor protein, APP, by the β -secretase BACE1 and γ -secretase. However, $A\beta$ generation is precluded if APP is cleaved by the α -secretase ADAM10 instead of BACE1. We have previously shown that $A\beta$ can be produced locally at the synapse. However, the exact subcellular production site and the release mechanism of $A\beta$ from neurons remain elusive.

Methods: Western blot was used to analyze the levels of the APP processing enzymes and their cleavage products in highly pure synaptic vesicles isolated from rat brain by controlled-pore glass chromatography. ADAM10 and BACE1 activity was measured using commercial kits. In addition, proximity ligation assay (PLA) was used to in situ visualize the potential presence of the secretases in synaptic vesicles in intact mouse primary hippocampal neurons.

Results: The levels of ADAM10 and BACE1 as well as their cleavage products APP C-terminal fractions (APP-CTFs) were greatly enriched in synaptic vesicles compared to total brain homogenate whereas Presenilin1 was the only enriched component of the γ -secretase complex. This was further confirmed by PLA which demonstrated close proximity of ADAM10 and BACE1 with the synaptic vesicle marker synaptophysin but only sparse co-localization of active γ -secretase and synaptophysin. Moreover, ADAM10 activity was also detected in synaptic vesicles.

Conclusion: We conclude that the APP processing enzymes are present at distinct synaptic sites. The results indicate that the first step of APP processing occurs in synaptic vesicles whereas the final step is more likely to take place elsewhere.

Lyons, D, Mensch S, Baraban M, Almeida R, Czopka T, Ausborn J, El Manira, A
Synaptic vesicle release regulates myelin sheath number of individual oligodendrocytes in vivo.

The myelination of axons by oligodendrocytes markedly affects CNS function, and new evidence implies dynamic regulation of myelination in learning and memory. Indeed, many decades of work implied that myelination can be regulated by neuronal activity, but how this occurred in vivo was not known. Using zebrafish as a model, we recently found that blocking synaptic vesicle release impaired CNS myelination by reducing the number of myelin sheaths made by individual oligodendrocytes, and that stimulating neuronal activity increased myelin sheath formation by individual oligodendrocytes (Mensch et al.,

Nature Neuroscience 2015). Although these data indicate that synaptic vesicle release regulates the myelinating capacity of single oligodendrocytes, whether this is mediated by bona fide synapses between axons and mature oligodendrocytes is not known. Here I will discuss our ongoing work using in vivo Calcium imaging to characterise the functional activity of oligodendrocytes during myelination. I will also present new data that different neuronal subtypes have different extents of activity regulated myelination and discuss how this might be explained by diversity in axon---oligodendrocyte synapse formation along distinct axons. This work has implications for the concept that adaptive myelination is an important form of nervous system plasticity.

Marie, H, Pousinha, PA, Raymond, EF, Mouska, X, Willem, M
APP intracellular domain (AICD) modulates synaptic signal integration.

Amyloid precursor protein (APP) is present at excitatory synapses throughout the brain, but its function remains to be fully elucidated. APP processing is a tightly regulated process, which gives rise to the formation of soluble fragments, such amyloid-- β ($A\beta$), and the intracellular C-terminal domain called AICD. This C-terminal domain is the most conserved region of APP and related proteins (APLP1 and APLP2). As $A\beta$, AICD levels increase in brains of Alzheimer's disease patients, suggesting that this peptide could participate in hippocampus-dependent alterations in this disease. Though there is evidence that AICD harbors transcriptional activity, its contribution to neuronal function in health and disease conditions is still an open question. We addressed this issue by using in vivo virus-mediated expression of AICD in rat hippocampal pyramidal CA1 neurons. We report that enhanced in vivo AICD production increases synaptic GluN2B-mediated NMDA receptor currents. This alters synaptic NMDA receptor-SK channel negative feedback loop, correlating with frequency- dependent disruption of synaptic signal integration. While long-term depression is spared, this disturbance leads to impaired long-term potentiation, a phenotype rescued by partial antagonism of GluN2B currents. We thus identify a novel synaptic mechanism whereby AICD contributes to synaptic remodeling in the hippocampus.

Martin, C, Conforti, L, Georgiou, M, Dajas-Bailador, F
Investigating the role of miRNAs in axonal development and neuron connectivity.

Precise extension of axonal projections and formation of correct synaptic contacts during development is crucial to the function of the nervous system. The process of axon target finding is led by the tip of the extending axon (growth cone), which independently integrates cues from the local environment. In this context, local protein synthesis has

been established as a functional mechanism that permits the fast integration of specific cues needed for axon development.

As regulators of local translation, miRNAs have an important role in axon growth and synaptic development (Dajas-Bailador et al. 2012). These short, non-coding RNAs can locally repress translation or trigger degradation of target mRNAs. Although the importance of miRNAs in axon extension, pathfinding and synaptic development is now well accepted, more studies are needed in order to identify all relevant miRNAs and their respective functional mechanisms.

In our lab, we are using two experimental approaches for the screening and functional identification of miRNAs involved in neuron connectivity processes, such as axon growth, branching and synapse formation:

a) *Drosophila* as an in vivo model. Early studies have demonstrated how miRNA has a role in the development of neurons in the *drosophila* brain, in particular within the olfactory system (Berdnik et al. 2008). We are currently utilising the well-established GH146 Gal4 driver combined with recently developed UAS-miRNA lines to allow the simultaneous visualization and overexpression of selected miRNAs within the projection neurons of the olfactory system in a wild type background.

b) Compartmentalised microfluidic chambers. These devices allow the culture of neurons in conditions in which their cell body, axon and axon terminal can exist in fluidically isolated environment (Taylor et al. 2005). In this way we can provide a more accurate reflection of the in vivo environment, and generate an experimental model to separately observe and manipulate the cellular components of the neuron. We are using this approach in to manipulate the expression of specific miRNAs and predicted miRNA targets within the axon.

These models will provide a complementary and multidisciplinary approach for the identification and study of conserved miRNAs with a role in axon and synapse development.

McMillan, L

mAbp1 regulates WAVE1-mediated actin dynamics in dendritic spines

Mammalian Abp1 (mAbp1) is an F-actin binding, SH3 domain-containing protein, which is highly enriched in the brain. It is known that mAbp1 is involved in key neuronal developmental stages including early dendrite morphogenesis and dendritic spine formation. These processes critically rely on organization and dynamics of the actin cytoskeleton. We have shown previously that mAbp1's role in these processes involves its association with key actin nucleation machinery such as the Neural Wiskott-Aldrich

syndrome protein (N-WASP) and the actin nucleator, Cobl. Here we reveal a novel interaction of mAbp1 with WAVE1. WAVE1 is a major activator of Arp2/3-mediated dynamics at the cell cortex via its C-terminal VCA domain. WAVE1 is predominantly found in the brain and particularly in the hippocampus and cerebral cortex. The Arp2/3 activation activity of WAVE1 is highly regulated. WAVE1 is typically found in a complex with its associated regulatory proteins: Nck-Associated Protein (Nap1), Abl-interactor 1 (Abi1), the hematopoietic stem progenitor cell 300 (HSPC300) and the specific Rac1-associated protein (Sra1). Behavioural and electrophysiological studies in WAVE1 knockout mice suggest an importance of WAVE1 in hippocampal learning and memory processes and on the cellular level display a decreased dendritic spine density as well as an abnormal synaptic structure. However, the molecular details governing WAVE1's importance in dendritic spines and its role in spine dynamics are not fully understood. Our results show that mAbp1 and WAVE1 colocalize in dendritic spines in rat primary hippocampal cultures. Moreover, mAbp1 associates with WAVE1 together with its complex components in the brain. Membrane-targeting experiments suggest that mAbp1 may recruit WAVE1 to membranes via its SH3 domain. Loss-of-function and rescue experiments reveal that loss of Abp1 and WAVE1 leads to dramatic changes in dendritic spine morphology. We hypothesized that Abp1 is a regulator of WAVE1-mediated, Arp2/3-dependent actin dynamics in dendritic spines. We addressed this in loss-of-function experiments using FRAP of GFP-actin, which reveal that knockdown of Abp1, together with WAVE1 alters the turnover of F-actin in dendritic spines. This data strongly suggests that Abp1 may be a critical regulator of WAVE1-dependent control of local actin dynamics mediating proper dendritic spine formation and function.

Mercier, M, Kullmann, DM

Hebbian long-term potentiation in feed-forward hippocampal interneurons.

Long-term potentiation (LTP) of excitatory transmission in hippocampal principal cells is thought to play an important role in memory encoding. Within area CA1, LTP in pyramidal cells depends on Ca²⁺ influx through postsynaptic N-methyl-D-aspartate (NMDA) receptors, and the subsequent phosphorylation of the α isoform of Ca²⁺/calmodulin-dependent kinase type II (α CaMKII). Interestingly, a similar form of Hebbian LTP has been described in CA1 feed-forward inhibitory interneurons, located in stratum radiatum, which serves to maintain the temporal fidelity of synaptic integration in pyramidal cells (Lamsa et al., 2005). Unlike pyramidal cell LTP, however, this form of plasticity is only observed in ~50% of interneurons, and depends on a non- α isoform of CaMKII (Lamsa et al., 2007).

The perforated-patch clamp technique is necessary to observe this form of interneuron LTP, as whole-cell recordings have been found to induce rapid run-down of NMDA receptor-mediated signaling in these cells (Lamsa et al., 2005). Alternatively, feed-forward

interneuron LTP can also be detected indirectly using whole-cell recordings from pyramidal cells, where an increase in disynaptic inhibition can be seen. In order to evoke and record such disynaptic responses, acute mouse hippocampal slices were taken and whole-cell voltage clamp recordings made from CA1 pyramidal cells, located distant (>1 mm) from the stimulation site at the Schaffer collateral pathway; cells were held close to the reversal potential for glutamate receptors in order to selectively detect inhibitory postsynaptic currents (IPSCs). In line with previous work, a pairing protocol designed to induce Hebbian LTP in local stratum radiatum interneurons led to a persistent increase in disynaptic IPSC amplitude in pyramidal cells. This enhancement of inhibition was blocked by D-(-)-2-amino-5-phosphonopentanoic acid (AP5), importantly confirming its dependence on NMDA receptor signaling. Interestingly, the potentiation was only evident in mice aged P20 or older, suggesting that the expression of Hebbian LTP in feed-forward interneurons may be developmentally regulated.

Further experiments will probe the signaling pathways and mechanisms involved in this relatively unknown form of LTP, and seek to identify other feed-forward interneuron subtypes which may similarly express long-term plasticity.

Milosevic, I, Gowrisankaran, S

Rabconnectin-3 - endophilin-A interaction is important for the maturation of synaptic vesicles

Synaptic vesicles (SVs) recycling is essential to sustain high rate of neurotransmission, however, it is not well understood how and when the newly endocytosed vesicle transform into mature SV. Clathrin-mediated endocytosis is essential for internalization and recycling of proteins and membranes. RabGTPases play an important role in SV recycling and endosome trafficking. Rab3a, a member of Rab GTPase family, is a marker for mature SV and is actively cycled during SV recycling. Rabconnectin-3 (RC3), a novel 340kDa synapse-enriched protein with unknown function, was reported to interact with Rab3 GTP/GDP exchange factors (Rab3 GEF) and Rab3 GTPase activating protein (Rab3 GAP).

We have cloned human RC3 and expressed in cell lines and primary hippocampal neurons. Our live imaging studies in cells suggest a role for RC3 in clathrin-mediated endocytosis, as seen from the co-localization with clathrin and adaptor proteins. RC3 has also a role in membrane trafficking as seen from the co-localization of RC3 with early endosomal markers EEA1 and Rab5, and the late endosomal/lysosomal marker LAMP1. Further, we found that RC3 interacts directly with key endocytic proteins endophilin A1 and A3, and this interaction is mediated through the endophilin's SH3-domain. Overexpression of RC3 in primary neurons confirms its synaptic localization, and knock-down studies reveal its importance in SV recycling. We propose that RC3 is a scaffolding molecule that interacts

with key proteins of endocytic pathway and the Rab3 family members, thereby playing an important role in SV recycling and formation of new SVs. Given that mutations in RC3 are reported as a cause of polyendocrine-polyneuropathy syndrome, our results may have broader implications for diseases.

Mubarak, B, Jeans A, Emptage N

The role of Cav2.1- and 2.2-type voltage-gated calcium channels in mediating homeostatic synaptic plasticity.

Homeostatic synaptic plasticity (HSP) is a form of biological regulation aimed at constraining neuronal electrical activity within a stable physiological range critical for normal brain function. Recent developments in the field suggest that disruption in HSP could lead to a variety of neurological and neuropsychiatric diseases. HSP can involve changes in both pre- and/or postsynaptic function; however, while the latter has been extensively studied, much less is known about the presynaptic mechanisms of HSP. Available evidence indicates that the homeostatic changes in presynaptic function in response to alterations in network activity occur primarily through changes in the stimulus-linked influx of calcium which drives neurotransmitter release. However, many aspects of the process remain poorly understood and, in particular, the relative contributions of the main presynaptic voltage-gated calcium channel subtypes (Cav2.1 and Cav2.2), and how these changes influence release, is completely unknown. Here we address this utilizing a high resolution optical approach that combines genetically encoded Ca²⁺ indicator localized to presynaptic terminals (SyGCaMP5) and SypHy2x, a pH-sensitive GFP-based reporter of neurotransmitter release. These reporters allow direct measurements of Ca²⁺ influx and neurotransmitter release in response to single action potentials at single synapses in dissociated rodent hippocampal cultures subjected to chronic perturbation of neural activity. Our findings will help gain a better understanding of the processes driving HSP in health and disease and have the potential to identify novel therapeutic targets.

Nistor, P, May, PW, Caldwell, MA

Conducting Diamond Electrodes for Synaptic Activity Investigation.

The gold standard for recording the electrical component of synaptic neuronal activity is patch clamping. This decades-old technique still provides the best signal-to-noise ratio. However, the method has significant drawbacks including being very labor intensive, the fact that the number of neurons that can be studied simultaneously is very limited, and that the clamping itself damages the cells, which limits the time over which data can be collected.

In recent years many devices have been proposed with the aim of scaling up and automating the electrophysiological recording. However, most of the proposed techniques have not greatly improved the time over which recordings can be made, a fundamental drawback, especially in the study of key synaptic plasticity events like long-term potentiation and depression; the most important reason why these phenomena are still being studied almost exclusively in vivo. The main reasons for these failures were the inability to maintain the neuronal cultures for extended periods of time, coupled with electrode toxicity.

We have recently proposed a protocol for long-term culture of de novo, in vitro differentiated human neurons on a diamond substrate [1]. Diamond is chemically, biologically and immunologically inert [2-4], while new chemical vapour deposition (CVD) methods allow diamond films to be deposited on a variety of substrates. Although diamond is electrically insulating, CVD diamond films can be deposited with near-metallic conductivity by boron doping. We have shown that the presence of boron has no adverse effects on neuronal differentiation, proliferation and long term survival.

We propose here a diamond-based multi electrode array for long-term recording from human neurons.

O'Connor, V, Dalliere, N, Bhatla, N, Holden-Dye, L, Horowitz, H R , Walker, RJ
Distinct pharyngeal glutamate determinants underpinning the context dependent modulation of feeding behaviour.

Pharyngeal pumping mediates the filter feeding of *C.elegans*. In the presence of bacteria, worms maintain high pump rates (250 pumps per minute). Although the modalities that control this sensory dependent increase in pumping are unknown, removal of bacteria reduces pumping. Our investigations suggest that the reduced pumping OFF food is not simply loss of an activating ON food cue. Mutants that exhibit high pump rates (>100 pumps per minute) OFF food suggest an important transmitter-mediated reduction of pumping. *eat-4* mutants, deficient in the neurotransmitter glutamate, produce an antonymous regulation in the ON and OFF food contexts. Compared to N2, *eat-4* mutants have a reduced pumping ON food while OFF food their pumping is raised. The reduced pump ON food is consistent with the loss of glutamate release from the pharyngeal neuron M3. Screening a number of glutamate receptor mutants ON food identified that the pharyngeal muscle receptors *glc-2* and *avr-15* have a reduced pumping. In addition, the *ceh-2* mutants, disrupted in M3 motor neuron function, phenocopy *eat-4* ON food. This reinforces that M3 release of glutamate onto inhibitory glutamate-gated chloride channels that relax muscle after contraction acts as the critical determinants of pumping ON food. In contrast, these established determinants of pharyngeal glutamate function do not change the OFF food pumping. Surprisingly, a distinct glutamate gated chloride

receptor, *avr-14*, that has no effect on the pumping ON food, replicate the OFF food phenotype of *eat-4*. This receptor was not known to be expressed in the pharynx, but re-analysis indicates it is expressed in a number of pharyngeal neurons. OFF food signalling has been identified at the level of the extrapharyngeal sensory nervous system but our studies indicate that the pharyngeal neuron I2 is a dominating determinant in the OFF food glutamate dependent response. Selective expression of *eat-4* in I2 suppresses the elevated pumping of *eat-4* mutants, restoring OFF food pumping to wild-type levels. Further, selective ablation of this neuron imparts an *eat-4* like phenotype OFF food. This suggests that I2-mediated glutamate release underpins the OFF food imposition of an inhibitory tone. This conclusion is reinforced by the observation that optogenetic activation of I2 mediates a powerful reduction in pharyngeal pumping occluded in the OFF food context. Overall our data indicate that major determinants of ON and OFF food behaviour are mediated by discrete microcircuits contained within the pharyngeal circuit. Although proprioceptive feedback inhibition from mechnosensory cues in the gut explain how glutamate transmission is engaged ON food the upstream modality for the distinct OFF food response is unknown.

Orav, E, Shintyapina, A, Lauri, SE

Neto1 expression is required for tonic KAR activity in the immature hippocampus.

Kainate type glutamate receptors (KARs) are important modulators of synaptic transmission in the central nervous system. Presynaptic KARs act as autoreceptors to regulate neurotransmitter release, while postsynaptic KARs participate in modulating neuronal excitability and contribute to synaptic transmission. They are composed of 5 subunits (GluK1-5) assembled as homo- or heterotetrameres. KAR auxiliary receptors NETO1/2 are important for several aspects of KARs such as receptor trafficking, biophysical properties, and receptor affinity.

KARs are highly expressed in the developing hippocampus where they are tonically activated by ambient glutamate to inhibit synaptic transmission. To date, the role of NETO1/2 in regulation of KAR functions in neonatal brain remains unclear.

Using in situ hybridisation and qPCR we demonstrate that both NETO1 and NETO2 are expressed in the developing (P4) hippocampus. Their physiological roles in the developing circuitry were studied in vitro using electrophysiological recordings from CA1 pyramidal neurons from neonatal (P4-P6) NETO1 and NETO2 deficient mice.

We show that the frequency of AMPA-R mediated miniature EPSC in immature CA1 pyramidal neurons was significantly lower in NETO1^{-/-} but not NETO2^{-/-} mice as compared to wild-types. Application of GluK1 antagonist (200nM ACET) led to a significant increase in mEPSC frequency in WT and NETO2^{-/-} slices, due to block of tonic presynaptic

KAR activity. In contrast, ACET had no effect on mEPSC in *Neto1*^{-/-} CA1 pyramidal cells suggesting that tonic activity of GluK1 is absent in *NETO1*^{-/-} mice. Tonic inhibition by GluK1 subunit containing KARs is manifested as short-term facilitation of transmission at immature CA3 – CA1 synapses. This facilitation of transmission was not detected in *NETO1*^{-/-}, while significant facilitation was observed both in WT and *NETO2*^{-/-} slices. Finally, in contrast to WTs, evoked EPSC amplitude was not changed in *Neto1*^{-/-} mice in response to GluK1 agonist (1 μ M ATPA).

Taken together, our data suggest that *Neto1* is necessary for tonic activation of presynaptic KARs in the area CA1 of developing hippocampus. Tonic KAR activity has been suggested to be critical for maturation of the CA3-CA1 circuitry, thus the loss of this mechanism might contribute to the observed reduction in glutamatergic input to CA1 pyramidal neurons in the *NETO1*^{-/-} mice.

Padamsey, Z

Glutamate release is inhibitory and unnecessary for presynaptic LTP

Long-term potentiation (LTP) and long-term depression (LTD) of transmitter release probability (*Pr*) are thought to be triggered by the activation of glutamate receptors. Here we demonstrate that glutamate release at Schaffer-collateral synapses is in fact inhibitory and unnecessary for an increase in *Pr*. Instead, at active presynaptic terminals, postsynaptic depolarization alone can increase *Pr* by promoting the release of nitric oxide from neuronal dendrites in a manner dependent on L-type voltage-gated Ca²⁺ channels. The release of glutamate, in contrast, decreases *Pr* by activating presynaptic NMDA receptors (NMDAR). Thus, net changes in *Pr* are determined by the level of glutamate release and postsynaptic depolarization that accompany presynaptic activity, and do not directly depend on the activation of postsynaptic NMDARs. Our findings demonstrate that the processes of LTP and LTD operate jointly to tune *Pr*, and reveal a mechanism by which presynaptic terminals that release little or no glutamate can be efficiently potentiated by Hebbian activity.

Palacios-Filardo, J, Mellor, JR

Regulation of the temporoammonic pathway in the hippocampus by acetylcholine.

The release of acetylcholine in the hippocampus during awake behaviour is important for encoding memory. Within the hippocampal network, acetylcholine has diverse effects: it increases neuronal excitability, controls synaptic strength and regulates the induction of synaptic plasticity. However, these effects are not ubiquitous and instead are exhibited at individual neurons and synapses within the network with each effect mediated by specific subtypes of acetylcholine receptor. The temporoammonic (TA) pathway carries spatial

information from grid cells in entorhinal cortex layer III (EC LIII) to CA1 hippocampal place cells synapsing onto the distal dendrites. It is not currently known how acetylcholine regulates synaptic transmission in the temporoammonic pathway or which acetylcholine receptors mediate this regulation.

To determine how acetylcholine regulates the temporoammonic pathway we made whole cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from adult mice. Electrical stimulation in the Stratum Lacunosum Moleculare elicited monosynaptic excitatory and polysynaptic inhibitory synaptic responses. The acetylcholine receptor agonist carbachol (10 μ M) reduced both excitatory and inhibitory synaptic responses and increased paired-pulse ratio for excitatory responses, indicating a presynaptic locus of action. The reduction in synaptic response for excitatory and inhibitory responses was similar for both but the increase in paired pulse ratio for excitatory responses produced a facilitation of excitatory-inhibitory balance in response to repetitive stimulation. The reduction in synaptic responses caused by carbachol was blocked by atropine but not mecamylamine, indicating a role for presynaptic muscarinic receptors. However, the reduction was not replicated by a selective muscarinic M1 receptor agonist (GSK-5). Instead, the reduction in synaptic response induced by carbachol was blocked by a muscarinic M3 receptor antagonist (DAU5884 1 μ M). We conclude that acetylcholine modulates the temporoammonic pathway onto CA1 pyramidal neurons by presynaptically located M3 muscarinic receptors.

Park, P, Sanderson, T, Amici, M, Zhuo, M, Bong-Kiun Kaang, B-K, Collingridge, GL
Stimulus parameters determine the role of calcium-permeable AMPARs in the induction of LTP at hippocampal CA1 synapses.

Long-term potentiation (LTP) is considered to be the best cellular correlate of learning and memory. In the hippocampal CA1 synapses, three different forms of LTP can be readily distinguished over the first few hours following induction. These are commonly referred to as short-term potentiation (STP), early-LTP and late-phase LTP. For reasons discussed elsewhere (Park et al, 2014), we refer to these processes as LTPa, LTPb and LTPc, respectively.

In agreement with previous work (Nguyen and Woo, 2003; Kim et al., 2010) we recently reported that a compressed train of HFS resulted in LTP that was independent of both PKA and protein synthesis (i.e., LTPb) whereas a spaced train of HFS yielded an additional component that required both PKA and protein synthesis (i.e., LTPc) (Park et al, 2014). In the present study we have used theta burst stimulation (TBS) and compared a train of 25 pulses (5 shocks at 100 Hz delivered at 5 Hz) presented 3 times with an inter-train interval of either 10 s (cTBS) or 10 / 20 min (sTBS). Consistent with earlier reports we found that the cTBS generated at LTP that was independent of PKA and protein synthesis (i.e., LTPb)

whereas sTBS again elicited an additional component that required both PKA and protein synthesis (i.e., LTPc). Since calcium permeable-AMPA receptors (CP-AMPA receptors) lead to distinct signaling in synaptic plasticity (Cull-Candy et al., 2006; Wiltgen et al., 2010; Lee, 2012), any involvement of CP-AMPA receptors in the mechanism was subsequently tested. Here we found that blockers of CP-AMPA receptors (philanthotoxin 433 (5 - 10 μ M), NASPM (30 μ M) and IEM 1460 (30 μ M)) had no effect on LTPb induced by cTBS. However, they all inhibited LTPc when present during or shortly following the delivery of sTBS. Consistent with the insertion of CP-AMPA receptors we found, using simultaneously whole-cell and field potential recording, that sTBS resulted in an increase in inward rectification of pharmacologically-isolated AMPA-EPSCs.

In conclusion, our findings, that may reconcile previous discrepancies (Plant et al, 2006; Adesnik and Nicoll, 2007), suggest the following mechanisms for the induction of LTPb and LTPc. The first train of TBS induces LTPb, which does not require either PKA or protein synthesis and has the potential to last many hours. The first train also primes the synapse such that a second (or more) TBS delivered within a critical time window, rapidly induces a PKA and protein synthesis-dependent component of LTP (i.e., LTPc). Here we show that the induction of LTPc requires CP-AMPA receptors.

Pegasiou, C-M, Gomez-Nicola, D, Sri, S, Zolnourian, A, Ahmed, AI, Bulters, D, Perry, VH, Vargas-Caballero, M

Need and opportunity for the analysis of adult human neurons: Understanding the function and dysfunction of excitatory synapses.

Objectives: Synaptic plasticity, widely accepted as a major cellular mechanism for encoding of memories, has been extensively investigated in the rodent brain, with some of its key features, such as the requirement for NMDA-receptor dependent coincidence detection, recently being confirmed in human neurons. However, there is still a big gap in translating knowledge of disease mechanisms from rodents to humans. To address this issue, we have established a collaboration with the Wessex Neurological Centre to study human tissue resected during neurosurgery. We present our data on baseline characterisation of glutamatergic cortical synapses.

Materials and methods: We obtained patch-clamp recordings from adult cortical human neurons from tissue resected during neurosurgery. We analysed inputs to cortical pyramidal Layer II-III neurons for NMDA and AMPA currents. To determine NMDAR composition at the synapse we performed whole-cell voltage clamp prior to and following pharmacological block of GluN2B subtype NMDARs using a specific GluN2B subunit blocker, Ro-256981. We grouped the results according to different categories, including age, gender and the underlying pathology necessitating neurosurgery.

Results: We have recorded from a total of 75 neurons from 9 cases spanning 20 to 70 years of age. The average NMDA/AMPA ratio under control conditions in human neurons is highly comparable to what we have observed in mice. We are building a database with our observations and although more data are needed, we observe a trend towards a larger GluN2B-mediated NMDA component in younger patients. In addition, we observe a further trend for tissue from patients with epilepsy to show a smaller NMDA component compared to those harbouring glial tumours.

Conclusions and ongoing work: Cortical neurons from human tissue obtained from neurosurgical cases are highly amenable to physiological recording and survive for 24 hours or longer following resection, during which, functional and biochemical analysis can be undertaken. Ongoing work focuses on investigating basal levels of synaptic plasticity and the response of human tissue to acute disease manipulations, such as incubation with amyloid beta species known to cause synaptic plasticity impairment in rodents. We suggest that human brain tissue from neurosurgery cases will allow a deeper understanding of the factors that impact synaptic composition in human neurons.

**Pelucchi, S, Musardo, S, Passafaro, M, Gardoni, FE, Marcello E, Di Luca, M
CAP2, a regulator of actin filament dynamic, is a novel ADAM10 Interactor.**

Alzheimer's disease (AD) is a progressive and neurodegenerative disorder characterized by increased levels of amyloid β -peptides ($A\beta$) and their deposition as senile plaques. $A\beta$ has been shown to play a central role in AD being responsible for synaptic dysfunction and cognitive deficits. $A\beta$ derives from the Amyloid Precursor Protein (APP), which is sequentially cleaved by the protease α BACE1 and by the γ -secretase to produce $A\beta$. In the non-amyloidogenic pathway, α -secretase (ADAM10), cleaves A within the $A\beta$ domain, thus preventing $A\beta$ generation. The correct spatial localization of ADAM10 in the postsynaptic membrane is pivotal for an efficient APP α -secretase cleavage, thus the mechanisms regulating the trafficking of ADAM10 to the synapse play a key role in the modulation of its activity. The results of a yeast two-hybrid screening, carried out using ADAM10 C-terminal tail as a bait, revealed CAP2 as a new ADAM10 binding partner. CAP2 is regulator of actin filament dynamics and could be involved in the modulation of ADAM10 subcellular distribution in neurons. The main aim is to analyse the role of CAP2 in the modulation of ADAM10 localization and activity towards APP in neurons.

Here we confirmed ADAM10-CAP2 interaction by biochemical approaches and we identified the domain responsible for the association. Moreover, we defined the CAP2 sequence involved in actin binding and we analysed the effect of this interaction on ADAM10 synaptic localization.

The characterization of ADAM10-CAP2 complex could favour the development of new experimental approaches to promote ADAM10 neuronal activity thus limiting A β generation.

Penn, A, Georges, F, Carbone, AL, Espana, A, Royer, L, Breillat, C, Groc, L, Plested, A, Choquet, D

Interplay between AMPA receptor surface diffusion and desensitization regulates postsynaptic short-term plasticity at CA1 hippocampal synapses.

Occurrence of post-synaptic AMPA receptor (AMPA) desensitization during basal transmission has been dismissed when potentiators like cyclothiazide had no effect on short-term plasticity (STP). However, between release events, desensitized AMPARs may escape rapidly from the synapse by surface diffusion, therefore hiding the effects of cyclothiazide on STP. To address whether AMPARs desensitize at Schaffer collateral synapses, we measured STP after preventing AMPAR surface diffusion. Cross-linking AMPARs reduced the paired-pulse ratio (PPR) under high release probability conditions and caused a long-lasting decrease in PPR for mutations that slowed AMPAR resensitization. In contrast, cross-linking had no effect when AMPAR desensitization was prevented by mutation or with cyclothiazide. Together, these results show that at CA1 synapses, AMPARs desensitize to synaptically released glutamate and that AMPAR surface diffusion counterbalances desensitization to limit use-dependent synaptic depression. Hence, postsynaptic mechanisms have under-appreciated roles in regulating STP.

Perrins, R, Yong, L, Pickering, AE

Noradrenergic inhibitory synaptic transmission by the locus coeruleus: optogenetic characterisation.

The locus coeruleus (LC) is a dense cluster of noradrenergic neurons in the pons. These neurons release noradrenaline (NA) within the LC as part of an inhibitory auto-feedback mechanism via activation of α 2-adrenoceptors^{1, 2}. We have used optogenetic techniques to study the release of NA and subsequent activation of α 2-adrenoceptors in the LC and onto post-synaptic targets in the midbrain central grey.

All procedures conformed to the UK Animals (Scientific Procedures) Act 1986 and had local ethical approval. NAergic neurons were transduced with a canine adenoviral vector expressing Channelrhodopsin2-mCherry under the control of the PRS promoter (CAV-PRS-ChR2-mCherry). Rats (p21) had unilateral injection of CAV directly into the LC under recovery anaesthetic 1-2 weeks prior to experimentation. Whole-cell patch clamp recordings were made from neurons in acute horizontal pontine slices. Neurons were

activated by LED illumination via a fibreoptic (473nm, 0-10mW). All data mean \pm standard error.

Both inhibition of action potential discharge (in current clamp) and corresponding outward currents (in voltage clamp) were observed in LC neurons following brief, pulsed optoactivation of transduced LC neurons. The NA reuptake inhibitor reboxetine (10 μ M) reversibly enhanced these outward currents in amplitude (9.0 ± 1.2 to 14.7 ± 1.3 pA, $P < 0.001$, $n=6$) and duration (5.7 ± 0.9 to 22.4 ± 2.7 seconds, $P < 0.001$, $n=6$) while the $\alpha 2$ -receptor antagonist atipamezole (5 μ M) blocked the currents (amplitude from 12.6 ± 1.6 to 3.8 ± 0.4 pA, $P < 0.001$, $n=6$). The outward currents are therefore due to the release of NA within the LC.

The outward currents showed inward rectification at V_{hold} above -60mV and reversed at -100mV. The currents were blocked by internal Cs⁺ (86% block after 15 minutes, from 10.5 ± 2.3 to 1.7 ± 0.4 pA, $P < 0.01$, $n=4$). This suggests the NA current is due to the outward movement of K⁺ ions through an inwardly rectifying K⁺ channel, likely to be GIRK as previously shown for NA transmission within the LC3.

The voltage-gated Na⁺ channel blocker tetrodotoxin (TTX) partially blocks action potentials in LC neurons, leaving a large Ca²⁺ spike⁴. TTX (0.5 M) reduced the outward current by only 25% (from 31.3 ± 6.3 to 23.2 ± 5.1 pA, $P < 0.05$, $n=6$). This suggests Ca²⁺ spikes in LC neurons may be capable of causing NA release within the LC.

Having characterised the $\alpha 2$ -mediated autoinhibition within the LC, we then sought post-synaptic targets outside the LC. We recorded from neurons in the midbrain central grey near fluorescent LC axons. These neurons showed a clear outward current and/or a hyperpolarisation of the membrane potential during optoactivation ($n=7$). These outward currents showed similar electrophysiology and pharmacology to those observed in the LC.

These results demonstrate that optogenetic activation of the LC produces time locked, synaptic release of NA both locally within the LC and onto post-synaptic targets.

Pickford, J, Apps, R, Bashir, ZI

Muscarinic acetylcholine receptors regulate synaptic activity of neurons of the cerebellar nuclei.

It is unclear how the cerebellum carries out any particular function, even for its well-established involvement in motor control. While many studies have focussed on the properties of inputs to Purkinje cells (PCs) of the cerebellar cortex, little is known about how PCs communicate information to their target neurons in the cerebellar nuclei (CN). The CN are responsible for extra-cerebellar projections, so studying their synaptic inputs will enhance our understanding of cerebellar outputs.

In this study visualised whole-cell patch clamp recordings were performed on CN neurons in juvenile rat cerebellar slices. Electrical stimulation of the white matter surrounding the CN was used to elicit a postsynaptic response. Inhibitory GABAergic inputs from PCs to CN were examined during pharmacological blockade of excitatory transmission. Excitatory glutamatergic inputs from mossy fibres and climbing fibres were examined during pharmacological blockade of GABAergic transmission.

Bath application of the cholinergic receptor agonist carbachol (10 μ M) produced long-term depression (LTD; 29.4 \pm 11.9%) of the inhibitory postsynaptic currents evoked by electrical stimulation of PC axons. This effect was inhibited by the muscarinic receptor antagonist scopolamine (10 μ M). Carbachol also produced LTD (41.9 \pm 11.9%) of excitatory postsynaptic currents resulting from the stimulation of mossy fibre and climbing fibre axons. Similarly, this LTD was inhibited by scopolamine. Under whole-cell current clamp recording conditions spontaneously firing CN neurons underwent depolarisation of the membrane potential (6.04 \pm 1.17 mV) and on average doubled the action potential frequency in response to carbachol; both of these effects were prevented during co-application of scopolamine.

It has been shown previously in anatomical studies that cholinergic fibres project to both the cerebellar cortex and CN (Jaarsma et al. 1997). Here we provide evidence of a functional cholinergic influence on the CN which appears to be mediated by muscarinic acetylcholine receptors. Further experiments will investigate the consequence of physiological stimulation of cholinergic inputs to CN and in vivo techniques will also be used to probe behaviours potentially mediated by the cholinergic system in the cerebellum.

Privitera, L, Morè, L, Arthur, SJ, Frenguelli, BG

MSK1 is required for experience-dependent enhancement of hippocampal plasticity and cognition.

By providing additional sensory, motor, cognitive and social stimulation, an enriched environment (EE) causes profound changes in neuronal structure and function and strongly influences the connectivity and development of the brain.

The neurotrophin BDNF is one of the key factors in converting sensory experience into enduring changes at the cellular and behavioural level and exerts its influence on neuronal structure and function via the activation of a number of intracellular signaling pathways. One of these involves the nuclear enzyme mitogen- and stress-activated protein kinase 1 (MSK1), which regulates gene transcription via phosphorylation of downstream targets such as CREB and histone H3. We have previously demonstrated that mice carrying an inactivating kinase-dead (KD) knock-in point mutation of the MSK1 gene failed to show

enhancement of synaptic transmission in response to EE and showed a blunted increase in dendritic spines [1]. This suggests that MSK1 is a key regulator of experience-dependent synaptic adaptation, an action that likely revolves around its ability to directly influence transcription. Here, we explored whether EE improved hippocampus-dependent synaptic plasticity and cognition in an MSK1-dependent manner. To this end, we reared wild-type (WT) and MSK1 KD mice in standard housing (SH) or in EE from birth to 2.5-5 months of age.

MSK1 KD mice displayed smaller fEPSPs compared to WT mice but had similar presynaptic fibre volley amplitudes and paired-pulse facilitation. These parameters were not influenced by housing status. In contrast, long-term potentiation (LTP), which was no different between mutant ($126 \pm 4\%$, $n=7$) and WT ($130 \pm 4\%$, $n=8$) slices under SC, was enhanced ($p<0.008$) by EE in slices from WT mice ($153 \pm 6\%$, $n=9$), but not MSK1 KD mice ($125 \pm 4\%$, $n=8$).

Behavioural investigation of hippocampus-dependent spatial reference and working memory showed that the kinase activity of MSK1 is required for the majority of the cognitive-enhancing effects of EE: compared to wild-type mice, MSK1 KD mice showed less improvement in hippocampus-dependent spatial working and reference memory as measured by spontaneous alternation and the water maze tasks, respectively.

Taken together these data implicate MSK1 as transducer of positive environmental stimulation into long-lasting structural and functional neuronal adaptations that underpin the enhanced cognition associated with enrichment.

Qiu, J, McQueen, J, Bilican, B, Dando, O, Livesey, M, Haghi, G, Cezard, T, Burr, K, Patani, R, Rajan, R, Sheppard, O, Kind, PC, Simpson, I, Tybulewicz, VLJ, Wyllie, DJA, Gharbi, K, Fisher, E, Chandran, S, Hardingham, GE

Conservation and divergence of the activity-dependent transcriptome of mouse and human neurons.

Many neuronal processes including development, survival and plasticity, rely on activity-dependent changes to the transcriptome. Whilst much has been learnt from studies of rodent neurons, the degree of conservation between rodent and human neurons is unknown. Using RNA-seq, we compared the activity-dependent transcriptome of mouse cortical and cortical-patterned human ES cell-derived neurons in response to L-type Ca^{2+} channel activation. This revealed considerable overlap in the activity-responsiveness across species. However, a substantial number of genes were uniquely regulated in either mouse or human neurons. Species-specific gene induction was confirmed using mouse cortical neurons from Tc1 mice, which carry human chromosome-21, allowing for direct comparison of mouse and human gene expression from the same neurons. Analysis of

the promoter regions of uniquely regulated genes in both mouse and human neurons revealed differences in transcription factor binding sites that could underlie species-specific gene induction. Mutation of the differential transcription factor binding sites confirmed their role in species-specific gene induction. The level of conservation in the activity-dependent transcriptome across species suggests that rodent neurons are good model for studying activity-dependent early gene expression, however, the divergences identified in this study may impact on how these neurons functionally interpret electrical activity.

Reinders, N, Pao, Y, Renner, MC, van Huijstee, A, Malinow, R, Kessels, HW
AMPA-receptor subunit GluA3 is crucial for A β -mediated synaptic and memory deficits.

During the early stages of Alzheimer disease (AD), patients show memory impairments. The prime suspect to cause these AD-related memory deficits is a small peptidic fragment called amyloid- β (A β). Experiments with AD-mouse models show that an excess of A β in the brain is sufficient to cause memory impairment and a loss of synapses. Previous studies showed that A β -oligomers weaken synapses by removing AMPA- and NMDA-type glutamate receptors from the post-synaptic membrane. In this study we will investigate which synaptic components make a synapse susceptible to a β oligomers.

Excitatory neurons mainly express two types of AMPA-receptors: AMPA-receptors consisting of subunits GluA1 and GluA2 (GluA1/2) and those consisting of GluA2 and GluA3 (GluA2/3). To determine whether GluA1/2s or GluA2/3s are removed from synapses by A β oligomers, we transfected organotypic hippocampal slices from GluA3-deficient mice with APP-CT100, the precursor to A β . We found that these neurons were fully protected against A β -mediated synaptic depression: both AMPA-receptor and NMDA-receptor currents remained unaffected. This experiment indicates that GluA3 is required for A β -mediated synaptic depression.

To assess whether A β -driven memory deficits also depend on GluA3 expression, APP/PS1-transgenic mice were crossed into a GluA3-deficient background and tested for contextual fear memory performance. While APP/PS1 mice showed significant memory impairment at 6 months of age, memories in littermate GluA3-deficient APP/PS1 mice were intact. In contrast, when we crossed APP/PS1-transgenic mice into a GluA1-deficient background, we found that GluA1-deficient APP/PS1 mice showed more severe memory deficits compared to their APP/PS1 littermates. These experiments demonstrate that A β selectively targets synapses dependent on their AMPA-receptors subunit composition.

Rojek, K, Niewiadomski, P, Bernadzki, K, Doleżyczek, H, Rylski, M, Kaczmarek, L, Jaworski, J, Prószyński, T

Expression and function of Angiomotin family of proteins in the brain.

Proper organization of synaptic connections is important for the transmission of information in the central and peripheral nervous systems (CNS and PNS). Synaptic remodeling is a process whereby synapses are rewired to form functional neuronal networks. The molecular mechanisms underlying this process are still poorly understood. We have recently identified the scaffold protein Amotl2 as a potential regulator of neuromuscular junction (NMJ) remodeling. Interestingly, many proteins involved in NMJ remodeling are also implicated in the plasticity of synapses in the brain. Therefore, we investigated the expression and function of Amotl2 and closely related proteins Amot and Amotl1, collectively called Angiomotins, in CNS neurons. We demonstrated that all three angiomotins are widely expressed in the brain. In cultured rat hippocampal neurons and mouse brain slices Amotl2 and Amotl1 localize to the synaptic compartment, whereas Amot is distributed in neurites. Amot depletion in neurons leads to reduced dendritic tree arborization and malfunction of the axon initial segment as reflected by aberrant localization of its main components ankyrin G and neurofascin.

Thus, our experiments identify a novel group of proteins that play a critical role in the organization of neurons and may regulate synaptogenesis both in the CNS and PNS. Amot may play a role in dendrite outgrowth and is critical for the establishment of the axon initial segment, suggesting a role in the maintenance of polarity in neurons.

De La Rosa, M, Karpova, A, Kreutz, MR

Mechanisms and dynamics of long-distance protein transport of Jacob from synapse-to-nucleus.

In order to maintain long-lasting changes in synaptic efficacy excitatory synapses communicate to the nucleus in response to NMDAR activity that likely influence transcription via synapto-nuclear protein messengers (Jordan and Kreutz, 2009; Karpova et al., 2012; Panayotis et al., 2015). In previous work we found that GluN2B containing NMDAR activity-dependent Jacob synapse-to-nucleus redistribution after LTP inducing stimuli (Dietrich et al.2008; Behnisch et al., 2011). Jacob directly associates with the GluN2B subunit of NMDAR and activation of synaptic NMDARs unequivocally leads to activation of the MAPK ERK1/2 (Ivanov et al., 2006). Activated ERK then binds and phosphorylates a crucial serine in Jacob at the position 180. Phosphorylation of Jacob at this residue is essential for dissociation of Jacob from dendritic spine synapses. In the nucleus pS180Jacob is involved in the regulation of the plasticity related genes arc and BDNF (Karpova et al., 2013). We now addressed which signaling events downstream of the GluN2B receptor lead to Jacob phosphorylation by activated ERK1/2 and its subsequent

nuclear import. It has been shown recently that CaMKII- α is critically involved in GluN2B-dependent activation of ERK1/2 through a direct interaction with GluN2B (Gaamouch et al., 2012). Employing the CaMKII- α inhibitor KN-93 we observed reduced nuclear import of phosphorylated Jacob in response to enhanced synaptic activity in hippocampal neurons at DIV16 and DIV 23 as compared to non-treated controls indicating a role of CaMKII- α in mediating Jacob nuclear import during synaptic NMDAR activity. It has been shown that Ras-MEK-ERK pathway is linked to the GluN2A containing NMDAR via RasGRF2 (Li et al., 2006) and that RasGRF2 activation of ERK1/2 contributes to LTP maintenance only in mature animals (Li et al., 2006). Using a shRNA RasGRF2 KD approach we found no differences in panJacob/pS180Jacob nuclear IRs as well as panERK/pERK upon synaptic NMDAR stimulation compare to scrambled control in 16DIV hippocampal neurons. Interestingly, we found that RasGRF2 downstream signaling is required for pS180Jacob/pERK nuclear accumulation in more mature neurons (DIV23). Taken together, our data suggest that upon synaptic activation of NMDAR CaMKII- α mediates Jacob translocation to the nucleus in developing and mature neurons, however, Ras-GRF2 is important for Jacob nuclear accumulation only in mature neurons.

Rost, B, Schneider, F, Grauel, MK, Wozny, C, Bentz, C, Blessing, A, Rosenmund, T, Jentsch, T, Schmitz, D, Hegemann, P, Rosenmund, C
Optogenetic Control of Synaptic Vesicle Acidification.

Optogenetic applications have emerged as versatile tools to control, record and analyse cellular activity. However, the power of optogenetic actuators has not frequently been applied to address molecular processes on the subcellular level. Here, we present the development of pHoenix, an optogenetic tool that allows controlling as well as monitoring of pH in synaptic vesicles. Under physiological conditions photoactivation of pHoenix provides additional driving force for transmitter uptake. After pharmacological inactivation of endogenous proton pumps, pHoenix rapidly induces synaptic vesicle acidification followed by neurotransmitter filling and restoration of synaptic transmission. Light-controlled acidification allowed us to titrate vesicular neurotransmitter content, revealing that incompletely filled vesicles exhibit a lower release probability compared to full vesicles. These data demonstrate that synaptic transmission is assured by releasing completely filled vesicles with the highest efficiency. The targeting strategy for pHoenix can be transferred to other organelles, as demonstrated for a variant that allows for light-activated acidification of lysosomes.

Sabec, M, Warburton, CE, Bashir ZI
Nicotinic receptors exert bidirectional modulation of hippocampal-medial PFC synaptic plasticity.

Nicotinic acetylcholine receptors (nAChR) are expressed in the medial prefrontal cortex (mPFC), where they play essential roles in cognitive functions such as attention and working memory (Wallace and Bertrand, 2013). Several lines of evidence have shown that working memory processes are also dependent on functional connectivity with the hippocampus (Godsil et al., 2013). Activation of nAChR in the mPFC can have a variety of effects, for example presynaptic nAChR on glutamatergic afferents enhance spontaneous excitatory release onto layer V pyramidal neurons, whilst nicotine has also been shown to increase the threshold for intracortical synaptic plasticity induction at these same cells (Couey et al., 2007, Lambe et al., 2003). Thus, the modulation exerted by nAChR is complex and the outcome of nAChR activity may depend on the precise synapses targeted. The presented work investigates the influence of the major nicotinic receptor subtypes on synaptic transmission and plasticity specifically at the hippocampal-mPFC pathway.

Nicotinic modulation of hippocampal-mPFC transmission was investigated through in vitro electrophysiology. Responses evoked through selective stimulation of hippocampal afferents were measured in whole cell recordings taken in layer V pyramidal neurons from the prelimbic region of rat mPFC slices (Parent et al., 2010). Synaptic plasticity of the pathway was investigated using a protocol of paired pre- and postsynaptic activity during bath application of selective agonists for homomeric $\alpha 7$ and heteromeric $\alpha 4\beta 2$ nAChR subtypes.

Short-term potentiation produced by pre-post pairing in control conditions was converted to a stable long-term potentiation of transmission by selective activation of $\alpha 7$ nAChR. In contrast, $\alpha 4\beta 2$ nAChR activity converted the short-term potentiation into long-term depression of transmission. These data indicate that nicotinic receptors can selectively gate the induction of LTP and LTD at the hippocampal-mPFC pathway, with the polarity of plasticity evoked dependent on the specific nAChR subtype activated.

Sanderson, T, Kim, SJ, Collingridge, GL

DHPG-induced long term depression is not input-specific but requires Schaffer collateral stimulation.

The activity dependent strengthening or weakening of synapses is thought to underlie the encoding of memories. Here we have studied a long lasting reduction in synaptic strength known as long term depression (LTD) in area CA1 of the hippocampus that is induced by the group I metabotropic glutamate receptor agonist dihydroxyphenylglycine (DHPG-LTD). Most forms of synaptic plasticity are input specific, meaning that the synaptic strength only changes at synapses that directly experience the conditioning stimuli. In DHPG-LTD the input specificity is difficult to determine since bath application of the agonist will result in similar exposure of all synapses to the drug. Also whether stimulation

of the Schaffer collateral is required for DHPG-LTD is controversial, as conflicting reports have been reported in the literature. To determine whether electrical stimulation is required for DHPG-LTD and whether this results in input specific LTD we performed two pathway recordings in organotypic hippocampal slices and found that the magnitude of DHPG-LTD was $59 \pm 9 \%$ and $62 \pm 9 \%$ in the two pathways, respectively. If both pathways were paused during DHPG application however LTD was not induced (responses were $103 \pm 20 \%$ and $96 \pm 15 \%$), indicating a requirement for stimulation. However, if one pathway was paused during DHPG application, but the other stimulated at twice the original frequency (to maintain the original number of stimulations) both pathways underwent LTD although with a lower overall magnitude (responses were $73 \pm 11 \%$ and $77 \pm 10 \%$, respectively). Thus in organotypic hippocampal slices DHPG-LTD requires Schaffer collateral stimulation, but the resulting LTD is not input specific. In this preparation DHPG-LTD is dependent on mGluR1 but not mGluR5, as relative to interleaved control LTD that reduced responses to $51 \pm 6 \%$, it was blocked by the mGluR1 antagonist LY367385 (responses were $87 \pm 9 \%$ 30 min following DHPG washout) but not the mGluR5 antagonist MPEP ($45 \pm 8 \%$). DHPG-LTD was also not accompanied by a change in paired pulse facilitation ($99 \pm 11 \%$, 30 mins following DHPG washout) indicating it occurs without a significant change in probability of glutamate release. These results indicate that DHPG-LTD is dependent on electrical stimulation and may result in a cell wide reduction in synaptic transmission, most likely due to post synaptic signaling and expression mechanisms.

Schmidt, J, Ferreira, JS, Carvalho, AL

Role of ARHGAP8, a novel Rho GAP, in regulating excitatory synapses

Normal brain function is strongly dependent on the correct development and assembly of the neuronal cytoskeleton. Failure to achieve the correct neuronal layout or to form the right interconnections between nerve cells underlies disorders involving cognitive deficits and mental disability. During development and other processes that include changes in cell architecture, as seen for example in plasticity, neurons have to undergo in some cases extensive reorganization of their actin scaffolding. Synaptic plasticity is associated with mechanisms such as the dynamic changes in spine size and number and the ability of excitatory glutamate-activated synapses to alter their strength in response to changes in activity patterns. Members of the Rho small GTPase subfamily have been shown to orchestrate many cellular processes involving intraneuronal actin dynamics. Essentially working like binary switches, they cycle between inactive GDP-bound states and active GTP-bound states, to which they are driven by GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), respectively. A variety of these regulators have by now been shown to play critical roles within neurons. We identified ARHGAP8 as a novel Rho GAP which is localized to spines in a GluN2B-NMDA receptor dependent

manner. Using quantitative mass spectrometry, we have found ARHGAP8 to be absent from postsynaptic densities (PSDs) of mouse neurons that lack the developmentally regulated NMDA-type glutamate receptor subunit GluN2B, while it is identified in PSDs isolated from wild-type neurons. We are currently characterizing its function within neurons and our data from biochemical and immunocytochemical experiments suggest a brainwide expression pattern and a partial localization to active synapses. Using a fluorescence resonance energy transfer (FRET) assay we are investigating the Rho GAP activity of ARHGAP8 in spines. Furthermore, ARHGAP8 overexpression in rat neuronal cultures affects spine maturation. Overall our data suggest that ARHGAP is a novel regulator of excitatory synapses.

Smejkalova, T

Selective inhibition of tonically over synaptically activated NMDA receptors by pregnane derivatives

Phasic activation of NMDA receptors by synaptically released glutamate plays a key role in synaptic plasticity and can be neuroprotective, but excessive tonic NMDAR activation by elevated extracellular glutamate mediates excitotoxicity. Therefore, there is much interest in pharmacological agents capable of selectively blocking tonically activated NMDARs, while leaving phasically/synaptically activated NMDARs intact. Theoretical considerations predict that, although NMDA inhibitors with use-dependent onset and offset of inhibition (“trapping” blockers), exemplified by memantine, would show some preference for tonically vs. phasically activated NMDARs, the strongest selectivity would be achieved by inhibitors with use-dependent onset but use-independent offset of inhibition (“foot-in-the-door” block). We have previously shown that an endogenous neurosteroid 20-oxo-5 β -pregnan-3 α -yl sulfate (pregnanolone sulfate; PA-S) is an NMDAR inhibitor with a “foot-in-the-door” mechanism of block. Therefore, we tested PA-S, as well as a series of novel synthetic PA-S analogues, for their selectivity for inhibiting tonically over phasically/synaptically activated NMDARs.

First, we used heterologously expressed GluN1/GluN2B receptors activated by fast agonist application and examined the peak current (phasic receptor activation) and the steady-state current (tonic activation) both in the absence and in the continuous presence of the steroid. We found that PA-S was ~2-fold more potent at inhibiting the steady-state current vs. the peak. Remarkably, certain synthetic PA-S analogues showed even stronger selectivity for tonically over phasically activated NMDARs, with 5 β -pregnan-20-on 3 α -yl-2'-hemipimelate (PA-hPim) exhibiting over 10-fold preference for inhibiting the steady-state vs. the peak current.

We confirmed that this selectivity holds for native NMDARs in autaptic hippocampal cultures activated either synaptically or tonically by long agonist applications. PA-S was

~3-fold more potent at inhibiting tonically activated NMDARs than the NMDAR EPSC. This difference was even more pronounced for PA-hPim, which showed no inhibition of NMDAR EPSC at a concentration that inhibits the tonic current by over 70%. These results provide a unique opportunity for the development of new therapeutic neurosteroid-based ligands to treat diseases associated with the dysfunction of the glutamate system.

Smith, K, Jones, KA, Kopeikina, KJ, Burette, AC, Copits, BA, Hanley, JG, Swanson, GT, Richard J. Weinberg, RJ, Penzes, P

A role for autism-associated cadherin-10 at excitatory and inhibitory synapses.

Synaptic adhesion is a key mechanism in synapse formation, stability and synaptic plasticity at both excitatory and inhibitory synapses. Cadherin-associated complexes have been shown to be important in synaptogenesis and functional and structural plasticity. The first genome-wide association study for autism spectrum disorder (ASD) identified six ASD-associated single nucleotide polymorphisms, between the genes encoding cadherin-9 and cadherin-10. Cadherin-10 has strong expression in the frontal cortex, in contrast with the uniformly low expression of cadherin-9, suggesting that cadherin-10 may play an important role in frontal cortical regions, which are heavily implicated in ASDs. However, the sub-cellular localization and functions of cadherin-10 in cortical neurons are unknown.

Here we examine the localization and function of cadherin-10 in mature cortical neurons using a combination of EM, confocal and super-resolution microscopy, and electrophysiological techniques. We find that cadherin-10 is abundant at both inhibitory and excitatory synapses in cortical neurons, showing extensive association with both gephyrin and PSD-95. We utilize structured-illumination microscopy to examine the nanoscale localization of cadherin-10 at synapses with respect to gephyrin and PSD-95. We find that cadherin-10 forms nanodomains that display a similar distribution at inhibitory and excitatory synapses, forming both a centrally synaptic and perisynaptic pool. Cadherin-10 knock-down reduces dendritic spine size and glutamatergic synapse function, whilst concurrently increasing GABAA receptor clustering and synaptic strength, which is controlled by interactions with synaptic scaffolds. Therefore, cadherin-10 has functional roles at inhibitory and excitatory synapses that may be relevant for understanding the regulation of excitatory/inhibitory balance in the brain and contribute to our understanding of the pathogenesis of ASDs.

Srivastava, DK, Michael Deans, PJ, Halai, S, Raval, P, Warre-Cornish, K, Sellers, K, Cocks, G, Price, J

A novel role for the schizophrenia susceptibility protein ZNF804A at synapses in human neurons?

Variation in the gene encoding zinc finger binding protein 804A (ZNF804A) exhibits genome-wide significant association with schizophrenia and psychosis more broadly. Previous studies have suggested that ZNF804A is a regulator of gene transcription and is only present in the nucleus of neural progenitor cells (NPCs). Consistent with this, functional studies have demonstrated that manipulation of ZNF804A levels results in altered expression of multiple genes, including those involved in cell adhesion, neurite outgrowth, synapse formation and several genes associated with schizophrenia. However, a detailed examination of ZNF804A distribution in neurons have yet to be performed. Determining the exact subcellular distribution of the protein may reveal previously unknown functions.

In order to gain an insight into the potential biological functions of the ZNF804A, we have studied the subcellular localisation of the protein in neurons differentiated from a human NPC line or human induced pluripotent stem cells (iPSCs) as well as in primary rat cortical neurons. This has revealed that ZNF804A is localized along distal portions dendrites, where it colocalized with putative synaptic markers. Critically, superresolution microscopy has demonstrated that ZNF804A localizes to subsynaptic domains within dendritic spines of mature rat cortical neurons, where it co-localizes with the synaptic proteins PSD95 and GluN1, in nanodomains within the head of spines. Subsequent studies are aimed at determining the functional impact of manipulating ZNF804A expression levels on neurite outgrowth and synapse formation in human neurons.

These data reveal a novel subcellular distribution for ZNF804A within somato-dendritic compartments and at excitatory synapses in human and animal neurons. Critically, this suggests that the transcriptional regulatory actions of ZNF804A may not be the full extent of its biological function, and that this protein may also have a functional role at excitatory synapses.

Styr, B

Interplay between population firing stability, single neuron dynamics and inhibition in hippocampal networks.

Neuronal circuits' ability to maintain the delicate balance between stability and flexibility in changing environments is critical for normal neuronal functioning. However, to what extent individual neurons and neuronal populations maintain internal firing properties remains largely unknown. We show that distributions of spontaneous population firing rates and synchrony are subject to accurate homeostatic control following increase of synaptic inhibition in cultured hippocampal networks. Reduction in firing rate triggered

synaptic and intrinsic adaptive responses operating as global homeostatic mechanisms to maintain firing macro-stability, without achieving local homeostasis at the single-neuron level. Adaptive mechanisms, while stabilizing population-firing properties, reduced short-term facilitation essential for synaptic discrimination of input patterns. Thus, invariant ongoing population dynamics emerge from intrinsically unstable activity patterns of individual neurons and synapses. The observed differences in the precision of homeostatic control at different spatial scales challenge cell-autonomous theory of network homeostasis and suggest the existence of network-wide regulation rules. We further elucidate the key factors governing network-wide regulation, by targeting specific inhibitory synaptic targets and cell types and testing their role in the ability of the network to perform homeostatic adaptations, enabling us to describe the basic relationships governing the regulation of inhibitory input, population dynamics and neuronal adaptive mechanisms.

Szabadics, J, Neubrandt, M, Brunner, J

Single physiological burst-evoked amplification of unitary mossy fibre synaptic responses in CA3 feedforward inhibitory cells.

Hippocampal mossy fibres (MF), the axons of DG granule cells (GCs) provide a major synaptic input to the CA3 network. GCs are mostly quiescent and their sparse activities consist of either single action potentials or short, high-frequency bursts. Mossy fibres innervate more GABAergic cells than pyramidal cells, and their synaptic outputs show diverse postsynaptic cell type-specific short-term plasticity. We asked how single spikes and short, high-frequency burst firing affects the synaptic output of single MFs using paired recordings of anatomically identified postsynaptic CA3 neurons, and single presynaptic MFs, either direct recording from giant MF boutons or from CA3 GCs. Intriguingly, single bursts in single presynaptic MFs (15AP at 150Hz) increased the unitary amplitudes by $288 \pm 12\%$ for a relatively long period (0.8-8 sec after the burst) in a subset of postsynaptic GABAergic cells including ivy, fast-spiking basket, axo-axonic and regular-spiking basket cells. Importantly, these cell types are crucial elements of the feed-forward inhibitory circuits between the DG and CA3 networks. The single burst-induced amplification developed only after several hundreds of milliseconds, but the augmentation was not permanent as the amplitudes returned to their initial levels within 60 seconds. Shorter bursts were similarly effective ($319 \pm 34\%$ after 4-7 APs at 150Hz). In postsynaptic pyramidal cells and other GABAergic neurons the effect of the bursts only resembled classical post-tetanic-potential. Changes of the short-term dynamics of the connections onto GABAergic cells suggest the involvement of presynaptic changes following the single bursts. Furthermore, the results of the experiments where the initial release probabilities is changed by varying the extracellular calcium concentration also indicate a presynaptic mechanism. The phorbol-ester-sensitivity suggests that

maximization of the vesicle priming after the single presynaptic bursts underlies the amplification but most likely it is achieved without the direct involvement of PKC and munc13 proteins. These results highlight that single, physiologically relevant short bursts of single MFs nearly maximize the efficacy of the synaptic transmission for almost ten seconds in a specific subset of postsynaptic GABAergic cells, which provide powerful feed-forward inhibition to the CA3; thus, the feed-forward network between the DG and CA3 are efficiently rearranged after a single MF burst.

Tigaret, C, Olivo, V, Sadowski, JHLP, Ashby, MC, Mellor, JR
Distinct spine Ca²⁺ sources and mGluRs encode hippocampal Spike Timing-Dependent Plasticity.

Induction of spike timing-dependent plasticity (STDP) at mature Schaffer collateral (S/C) synapses onto CA1 hippocampal pyramidal neurons requires synaptic input time-correlated with postsynaptic spikes to generate postsynaptic NMDA receptor (NMDARs) – dependent Ca²⁺ transients (EPSCaTs) in dendritic spines. The Ca²⁺ hypothesis for synaptic plasticity proposes that the size of spine EPSCaTs determines the magnitude and direction of synaptic plasticity, and predicts that the strongest spine Ca²⁺ signals are evoked by stimuli that induce spike timing-dependent long-term potentiation (STD-LTP) as opposed to stimuli that induce long-term depression (STD-LTD). However, this prediction has not been directly tested in the mature hippocampus. We evaluated the rules for NMDAR-dependent STDP induction at S/C– CA1 synapses in acute hippocampal slices from adult rats, by pairing pre-synaptic stimuli in stratum radiatum with somatically-evoked postsynaptic spikes. Using two-photon Ca²⁺ fluorescence imaging we show that the amplitude of EPSCaTs induced by STDP stimuli does not match the observed plasticity induction rule. Recordings were performed in whole-cell current clamp, at 36°C, under GABAA receptor inhibition (50 μM picrotoxin). In contrast, induction of NMDA receptor-dependent LTP by time-correlated pre- and post-synaptic spikes requires the sequential activation of NMDARs followed by voltage-sensitive Ca²⁺ channels within dendritic spines. Furthermore LTP requires mGlu1-dependent inhibition of SK channels to promote NMDAR activation. We conclude that induction of LTP by time- correlated pre- and post-synaptic activity requires the activation of distinct sources of Ca²⁺ and the recruitment of an mGluR1-dependent inhibition of a negative feedback loop that targets the activation of NMDARs.

Truckenbrodt, S
The age of the synaptic vesicle determines its ability to release.

Old organelles can become a hazard for cellular function, due to the accumulation of damaged molecules. Mechanisms that measure organelle age, and prevent old organelles from participating in cellular reactions, are therefore necessary. The prevailing assumption is that organelles are functionally employed in cells until degradation via damage response mechanisms. We have identified an additional mechanism, which measures the usage of synaptic vesicles and functionally inactivates them long before degradation. Using cultured hippocampal neurons, we found that newly synthesized vesicles are preferentially employed in neurotransmitter release. They recycle only ~270 times, over ~24 hours. During recycling, they become contaminated with a molecule from the plasma membrane, which interferes with a vital component of the synaptic vesicle release machinery. This process renders the old vesicles less competent to release than their newly synthesised counterparts. The inactivation can be accelerated by increasing the activity of neurons, suggesting that the cell uses this mechanism to directly measure the number of times a vesicle has been used – determining its functional age rather than its chronological age. The old vesicles are eventually targeted for degradation, but their functional inactivation precedes degradation by up to several days. We conclude that the contamination is a timing mechanism needed to ensure that old vesicles are not used in neurotransmitter release. Synaptic transmission is presumably too sensitive to tolerate accumulation of damage on synaptic vesicles until they can be recognised by classical damage response mechanisms, necessitating this additional functional inactivation.

Udakis, M, Wonnacott, S, Mansvelder, H, Bailey, C

Synaptic plasticity in the medial prefrontal cortex: role of $\alpha 7$ nicotinic acetylcholine receptors.

The medial prefrontal cortex (mPFC) is a key brain region implicated in drug-related associative learning (1). $\alpha 7$ nicotinic acetylcholine receptors (nAChR) have a modulatory role in the mPFC (2) and $\alpha 7$ nAChR antagonism can inhibit reinstatement to opioid drug seeking (3). We have explored the mechanisms by which $\alpha 7$ nAChRs modulate excitation in the mPFC as a pre-requisite to investigating their influence on drug-associated learning.

We measured spontaneous EPSCs and IPSCs in brain slices from drug naïve mice, in response to $\alpha 7$ nAChR positive allosteric modulator (PNU-120596), selective agonist (PNU-282987) and antagonists (MLA). Functional somatic $\alpha 7$ nAChRs on inhibitory interneurons and presynaptic $\alpha 7$ nAChRs on glutamatergic terminals were discriminated. This enables $\alpha 7$ nAChRs to play a complex dynamic role in both excitation and inhibition in the mPFC, with endogenous tonic acetylcholine preferentially enhancing excitatory transmission via $\alpha 7$ nAChRs. Using field recordings to study stimulus-induced plasticity in mPFC slices, both inhibition and activation of $\alpha 7$ nAChRs reduced stimulus-induced LTP, whilst MLA enhanced LTD. Optogenetic strategies are currently being applied to define

the glutamatergic input pathways bearing $\alpha 7$ nAChRs. These studies will pave the way for an analysis of the roles of $\alpha 7$ nAChRs in the PFC in drug-related learning.

Vertkin, I

GABA(B) receptor deficiency causes failure of neuronal homeostasis in hippocampal networks

Stabilization of neuronal activity by homeostatic control systems is fundamental for proper functioning of neural circuits. Failure in neuronal homeostasis has been hypothesized to underlie common pathophysiological mechanisms in a variety of brain disorders. However, the key molecules regulating homeostasis in central mammalian neural circuits remain obscure. Here, we show that selective inactivation of GABAB, but not GABAA, receptors impairs firing rate homeostasis by disrupting synaptic homeostatic plasticity in hippocampal networks. Pharmacological GABAB receptor (GABABR) blockade or genetic deletion of the GB1a receptor subunit disrupts homeostatic increase in synaptic vesicle release induced by neuronal inactivity. GABABRs mediate adaptive presynaptic hyperactivity by two principle mechanisms. First, neuronal silencing promotes syntaxin-1 switch from a closed to an open conformation to accelerate SNARE complex assembly. Second, it boosts spike-evoked presynaptic calcium flux. In both cases, neuronal inactivity removes tonic block imposed by the presynaptic, GB1a-containing receptors on syntaxin-1 and calcium flux to enhance release probability. We identified the GB1a intracellular domain essential for the presynaptic homeostatic response by tuning inter-molecular interactions between the receptor, syntaxin-1 and the CaV2.2 channel. The presynaptic homeostasis was accompanied by scaling of excitatory quantal amplitude via the postsynaptic, GB1b-containing receptors. Thus, GABABRs sense chronic perturbations in GABA levels and transduce it to homeostatic changes in synaptic strength. Our results reveal a novel role for GABABR as a key regulator of population firing stability and propose that disruption of homeostatic synaptic plasticity may underlie seizure's persistence in the absence of functional GABABRs.

Wagner, M, Staras, K

Synapse-specific determinants of single-vesicle recycling kinetics in central presynaptic terminals.

Efficient stimulus-driven fusion and recycling of neurotransmitter-filled synaptic vesicles in small central synaptic terminals is critical for the maintenance of synaptic performance. In recent years considerable effort has been invested in understanding these processes, facilitated by advances in imaging techniques and development of improved fluorescent reporters of synaptic function. It is widely accepted that synapses exhibit striking

heterogeneous activity across populations but the factors that underlie this variability are poorly understood. Here we exploited high-sensitivity imaging approaches to examine whether expression of synaptic profiles was predictable at the level of individual release events and the rules that underlie this. Specifically, we imaged synaptophysin-2x-pHluorin (syHy2x)-expressing synapses in dissociated rat hippocampal neurons. We collected response profiles using minimal stimulation protocols and generated frequency distribution plots. These were highly quantized, allowing us to sub-divide profiles into quantal and multi-quantal events using simple custom-written algorithms. We found that the timecourse of individual traces was highly variable across the whole population of synapses. Nonetheless, the expression of different profiles was not random but instead determined by factors related to the identity of individual terminals. This relationship was true for single vesicle retrievals but became less robust in the case of multiple fusion events. By inducing a form of homeostatic plasticity we were able to manipulate these parameters and change vesicle kinetics in predictable ways, suggesting that they are highly linked. Our findings offer new insights into the rules that determine single vesicle retrieval events in individual synapses.

Wentzel, C, Müller, M

Homeostatic control of presynaptic protein turnover and synaptic transmission

Neurons function robustly within neural circuits throughout the lifetime of an animal. At the same time, the proteins determining neural activity are turned over on time scales of hours to weeks. It is therefore remarkable that robust neural function, and thus animal behavior, can be achieved and maintained at all. Several studies have implicated homeostatic processes in stabilizing nervous system function, but the underlying molecular signaling systems are largely unknown.

The *Drosophila* neuromuscular junction (NMJ) has emerged as a powerful model system to uncover genes that are required for homeostatic modulation of neurotransmitter release. An electrophysiology-based genetic screen has implicated the first genes in this form of synaptic plasticity. However, it is entirely unclear how the proteins encoded by the identified genes participate in homeostatic stabilization of synaptic efficacy.

Here we link presynaptic proteolysis through the ubiquitin proteasome system (UPS) to homeostatic modulation of release. We found that acute, pharmacological proteasome inhibition induces a rapid and marked increase in neurotransmitter release at the *Drosophila* NMJ on the minute time scale. Moreover, acute UPS perturbation occludes homeostatic modulation of release. Importantly, presynaptic expression of a dominant-temperature sensitive proteasome mutation (UAS-DTS-5/7) potentiates release and occludes homeostatic potentiation, while postsynaptic expression does not. Furthermore, we detect increased presynaptic ubiquitinated protein levels at

homeostatically potentiated synapses using an antibody against ubiquitinated proteins. Together, these results imply that the presynaptic UPS has the capacity of rapidly modulating presynaptic protein turnover and neurotransmitter release at the *Drosophila* NMJ, and that this process is required for homeostatic potentiation of release.

To unravel the underlying molecular mechanisms, we are currently systematically analyzing UPS-dependent regulation of neurotransmitter release in mutants that have been linked to homeostatic plasticity. Interestingly, UPS-dependent release modulation is blocked in some mutants (*dysbindin*, a gene linked to schizophrenia), while proceeding normally in others (*rim-binding protein*, *rbp*). This implies that UPS-dependent modulation of release involves specific presynaptic proteins. Moreover, the implication of a major schizophrenia susceptibility gene (*dysbindin*) in UPS-dependent regulation of release may link this form of synaptic regulation to neural pathology.

Williams, S, Pavlov, I, Williams, R S, Walker, M C

The role of AMPA receptors in the treatment of epilepsy.

Postsynaptic ionotropic receptor mediated currents are often targeted for the treatment of epilepsy. Many anti-epileptic drugs act on GABA_A receptors to potentiate inhibitory currents. Recently, AMPA receptor inhibition has been a target for antiepileptic drug therapy, and the non-competitive AMPA receptor antagonist Perampanel has recently been approved for clinical use in epilepsy. AMPA receptors play a critical role in the generation and maintenance of seizure activity.

We have also recently shown that Decanoic acid, a key component of the medium chain triglyceride ketogenic diet used in the treatment of refractory epilepsy, acts as a non-competitive AMPA receptor antagonist. Here, we explored this mechanism further, and determined whether other AMPA receptor antagonists are also effective anti-seizure agents.

We found that the decanoic acid's AMPA receptor antagonism is conserved in human neurons and is not use dependant. Unexpectedly, decanoic acid also reduces the intrinsic excitability of neurons, indicating that it may have multiple targets.

We then tested novel AMPA receptor antagonists structurally related to fatty acids in an *in vitro* model of seizure-like activity, and demonstrated that they too have potent anti-seizure effects.

These compounds are now being tested in an *in vivo* model of electrically induced status epilepticus (prolonged seizure activity) model; decanoic acid given as a bolus has minimal effects on status epilepticus, possibly because the liver extensively metabolises straight

chain fatty acids. Other compounds identified may have more favourable pharmacokinetics and so more promising in vivo therapeutic effects.

Winters, B, Gregoriou, GC, Wells, OAA, Bagley EE

Regulation of Amygdala Intercalated Cells by Endogenous Opioids: Implications for Anxiety Disorders.

The intercalated cells (ITC) of the amygdala are small, densely populated GABAergic interneurons that form a large nucleus surrounding the basolateral amygdala (BLA) and provide a significant inhibitory interface between the BLA and central amygdala (CEA). ITCs play an important role in fear memory and the modulation of ITC activity is thought to affect amygdala output and formation of anxiety-related behaviour. In particular, ablation of ITC control has been shown to impede fear extinction, a memory process that is thought to be impaired in anxiety disorders such as post-traumatic stress disorder. These cells can be identified by their high expression of both the μ -opioid receptor and enkephalin, an endogenously expressed opioid peptide. ITCs receive multiple synaptic inputs including: glutamate afferents from the BLA and medial prefrontal cortex and local GABAergic inputs within the ITC nucleus. Convergence of these synaptic inputs and intrinsic opioid signalling likely plays an important role in gating ITC activity. Indeed, opioid-dependent signalling has been implicated in the acquisition and extinction of fear memory. However, the effects of these neuromodulators on ITC-associated synaptic activity are unknown. Using whole-cell, voltage-clamp electrophysiology we recorded electrically evoked synaptic currents from ITCs in coronal slices of the amygdala. We show that exogenous application of met-enkephalin (ME) reduces the amplitude of excitatory postsynaptic currents (EPSC) at both BLA- and cortical-ITC synapses by decreasing presynaptic glutamate release. Similarly, ME reduced inhibitory postsynaptic currents (IPSC) at local ITC-ITC synapses. Further, following modest field stimulation and inhibition of enkephalin-related peptidases, we show the opioid antagonist naloxone increases EPSCs at BLA-ITC associated synapses indicating endogenous opioids are present and acting to reduce glutamate release. Together, these data indicate endogenous opioids have the capacity to be significant modulators of ITC function and may participate in regulating key circuitry underlying fear memory.

Younts, T, Klein, ME, Hannah R, Monday, HR, Jordan, BA, Castillo, PE

Presynaptic protein synthesis is required for long-term depression of inhibition in the hippocampus.

It is known that postsynaptic protein synthesis can support local and persistent changes in synaptic function during long-term potentiation (LTP) and depression (LTD). However,

a role for presynaptic protein synthesis during long-term plasticity in the mature mammalian brain has not been demonstrated. In murine hippocampus, endocannabinoid-mediated long-term depression of inhibition (iLTD) is expressed presynaptically at a subset of inhibitory interneuron terminals, and iLTD is mediated by presynaptic type-1 cannabinoid receptor (CB1R) activation. We found in primary neurons using fluorescent non-canonical amino acid tagging (FUNCAT) that CB1R activation increases protein synthesis. Using ultra long-term paired electrophysiological recordings and single-cell manipulations in an intact hippocampal slice preparation, we demonstrated that iLTD was completely abolished when cap-dependent protein synthesis was interrupted exclusively in the presynaptic neuron. Mechanistically, presynaptic CB1Rs rapidly signal via PKA and the mTOR pathway to engage the translation initiation complex eIF4F. The requirement for protein synthesis was specific to long-term plasticity because when translation was blocked basal synaptic transmission and short-term plasticity remained intact. Furthermore, iLTD was independent of somatic transcription and microtubule-based trafficking, suggesting translation occurred locally in presynaptic compartments. Consistently, CB1R activation increased translation in isolated axons. Our results show that presynaptic protein synthesis is essential for iLTD, and strongly suggest that CB1Rs rapidly signal to the cap-dependent translation machinery to persistently modify neurotransmitter release in the mammalian brain. Presynaptic translation in long-term plasticity is thus evolutionarily conserved from invertebrates to vertebrates.

Lenkey, N, Holderith, N, Kiriz, T, Nusser, Z

CCK+ perisomatic and dendritic axon terminals are morphologically and functionally different

The properties of axon terminals of basket and dendritic layer innervating (DLI) cholecystokinin-containing (CCK+) interneurons in the CA3 region of the hippocampus were compared by performing Ca²⁺ imaging experiments and electron microscopy (EM). We have found that DLI cells have ~30% larger action potential-evoked [Ca²⁺] transients in their axon terminals compared to basket cells. This difference can be the consequence of either a different calcium buffering capacity, or distinct active zone/bouton volume ratio, or different type and/or number of voltage-gated Ca²⁺ channels between the two cell types. The endogenous buffer capacity is most likely similar in basket and DLI cells, since the decay kinetics of the [Ca²⁺] transients showed no difference between the two cell types. Three- dimensional EM reconstruction of CCK+ axon terminals in strata pyramidale and radiatum showed that basket cells have on average 70% larger bouton volumes and similar active zone (AZ) areas, resulting in a 40% smaller AZ / bouton volume ratio. SDS-digested freeze- fracture replica immunolabeling for Cav2.1 and Cav2.2 Ca²⁺ channel subunits revealed the exclusive presence of the Cav2.2 subunit in both types of terminals. In addition, almost complete block of [Ca²⁺] transients was found in both cell

types following the application of the N-type Ca²⁺ channel (Cav2.2) blocker 1 μM ω-Conotoxin-GVIA.

Assuming that the Ca²⁺ channels are restricted to the AZ and have similar densities in basket and DLI cells, our results demonstrate that the difference in the bouton volume alone can account for the detected differences in the Ca²⁺ concentration.

Ribeiro, F, Catarino, T, Santos, S, Esteban, J,, Carvalho, A
Constitutive activity of the ghrelin receptor controls AMPARs synaptic insertion in the hippocampus

Ghrelin is a peptide mainly produced by the stomach and released into circulation. Ghrelin binding to its G protein-coupled receptor, GHSR-1a, promotes appetite and affects energy balance and memory retention. We have recently shown that ghrelin triggers the synaptic incorporation of AMPA receptors, and enhances long-term potentiation in the hippocampus. The ghrelin receptor shows unusually high constitutive activity in the absence of the ligand in several systems but the biological relevance of this activity or its requirement for higher brain functions is largely unknown. Herein, we explore if the constitutive, ligand-independent activity of the ghrelin receptor modulates excitatory synapse function in the hippocampus.

We found that blocking the constitutive activity of the ghrelin receptor in hippocampal neurons, using the receptor inverse agonist, decreases the synaptic content of AMPARs. Accordingly, blocking ghrelin receptor constitutive activity in hippocampal slices decreased the AMPAR/NMDAR ratio of synaptic responses, suggesting that the basal activity of the ghrelin receptor controls AMPARs-mediated synaptic transmission in the hippocampus. Furthermore, the ghrelin receptor inverse agonist blocked the activity-induced synaptic delivery of AMPAR upon chemical LTP. Ghrelin receptor knockdown in hippocampal cultures decreased the total and synaptic intensity of GluA1, suggesting that genetic deletion of ghrelin receptor produces the same phenotype as the pharmacological blockade of the constitutive activity of the receptor. Moreover, we show that treatment with the inverse agonist significantly decreases activation of CamKIV and phosphorylation of GluA1 in Serine845 in the hippocampus, suggesting that ligand-independent activity of ghrelin receptor triggers the activation of a signaling pathway through CamKIV, and affects GluA1 phosphorylation.

This study points to a previously unrecognized neuromodulatory function for the constitutive activity of the ghrelin receptor in regulating glutamatergic transmission in the hippocampus.

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