Neurons, synapses & circuits | from function to disease

AUGUST 16–18, 2018

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Neurons, synapses & circuits | from function to disease

16–18 August 2018, Q Station, Sydney

DAY 1: THURSDAY AUGUST 16

6:00pm–7:00pm Session 1: Plenary speaker — Chair: David Bredt

6:00pm Richard Huganir, JOHNS HOPKINS UNIVERSITY
Regulation of AMPA receptors and synaptic plasticity in cognitive disorders

7:00pm Welcome reception and drinks

DAY 2: FRIDAY AUGUST 17

8:30am–10:30am Session 2: Disorders of the nervous system — Chair: Peter Silburn

8:30am David Bredt, JOHNSON AND JOHNSON
Getting a handle on neuropharmacology by targeting receptor-associated proteins

9:00am Susannah Tye, THE UNIVERSITY OF QUEENSLAND
Impaired metabolic capacity and cellular resilience in antidepressant resistance

9:30am Shengtao Hou, SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY
Profiling phytohormones in stroke brain—challenges and opportunities

10:00am Robert Malenka, STANFORD UNIVERSITY
Neural mechanisms of social reward

10:30am–11:00am Morning Tea

11:00am–12:30pm Session 3: Neural networks and systems — Chair: Bernardo Sabatini

11:00am Liping Wang, SHENZHEN INSTITUTES OF ADVANCED TECHNOLOGY
Optogenetic dissection of neural circuits underlying processing of innate fear

11:30am Anatol Kreitzer, UCSF
Striatal circuit dysfunction underlies motor deficits in a model of human dyskinesia

12:00pm Ehsan Arabzadeh, THE AUSTRALIAN NATIONAL UNIVERSITY
Information processing in the rodent sensory cortex: population dynamics across behavioural states

12:30pm–1:30pm Lunch

1:30pm–3:30pm Session 4: Inhibitory systems — Chair: Greg Stuart

1:30pm Julie Kauer, BROWN UNIVERSITY
Inhibitory synapses and plasticity in the ventral tegmental area

2:00pm Chris McBain, NATIONAL INSTITUTES OF HEALTH
Neuronal pentraxins control glutamate receptor driven development of hippocampal inhibitory circuits

2:30pm Pankaj Sah, THE UNIVERSITY OF QUEENSLAND
The amygdala, prefrontal cortex and hippocampal circuit in fear learning

3:00pm Bernardo Sabatini, HARVARD UNIVERSITY

3:30pm–6:00pm Afternoon free time

6:00pm–7:30pm Poster session

7:30pm–9:30pm Conference Dinner

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DAY 3: SATURDAY AUGUST 18

8:30am–10:30am Session 5: Synaptic function and plasticity — Chair: Rob Malenka

8:30am Morgan Sheng, GENENTECH
Molecular and cellular mechanisms of synapses loss in Alzheimer's disease and tauopathy

9:00am Katherine Roche, NIH
NMDA receptor regulation: clues from rare variants implicated in disease

9:30am Andres Villu Maricq, UNIVERSITY OF UTAH
A novel auxiliary protein that regulates the function of NMDA receptors

10:00am Frederic Meunier, THE UNIVERSITY OF QUEENSLAND
Neurotransmitter release machinery in a nanoscale Brownian world

10:30am–11:00am Morning Tea

11:00am–12:00pm Session 6: Plenary speaker — Chair: Pankaj Sah

11:00am Diane Lipscombe, BROWN UNIVERSITY
Cell-specific splicing of neuronal calcium channels: mechanism, function and disease

12:00pm–1:00pm Session 7: Short talks (selected from posters)

Brian Billups, AUSTRALIAN NATIONAL UNIVERSITY
Bryony Winters, UNIVERSITY OF SYDNEY
Jianyuan Sun, CHINESE ACADEMY OF SCIENCES
Tristan Wallis, UNIVERSITY OF QUEENSLAND

1:00pm–2:00pm Lunch

2:00pm–3:30pm Session 8: Therapeutics and diagnostics — Chair: Susannah Tye

2:00pm Peter Silburn, THE UNIVERSITY OF QUEENSLAND

2:30pm Elizabeth Coulson, THE UNIVERSITY OF QUEENSLAND
Cholinergic dysfunction in Alzheimer's disease

3:00pm Janet Keast, UNIVERSITY OF MELBOURNE
Mapping the visceral connectome for bioelectronic medicine

3:30pm–4:00pm Afternoon tea

4:00pm–6:00pm Session 9: Neural networks and systems (part 2) — Chair: Shengtao Hou

4:00pm Greg Stuart, THE AUSTRALIAN NATIONAL UNIVERSITY
Cellular and circuit mechanisms underlying processing of binocular visual information

4:30pm Marta Garrido, THE UNIVERSITY OF QUEENSLAND
An afferent subcortical white matter pathway to the amygdala facilitates fear recognition

5:00pm Bernard Balleine, THE UNIVERSITY OF NSW
The thalamostriatal network mediates flexible encoding for goal-directed action

5:30pm Geoffrey Goodhill, THE UNIVERSITY OF QUEENSLAND
The development of neural coding in the zebrafish brain

Closing remarks: Katherine Roche

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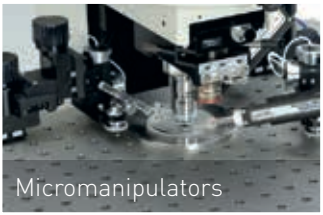

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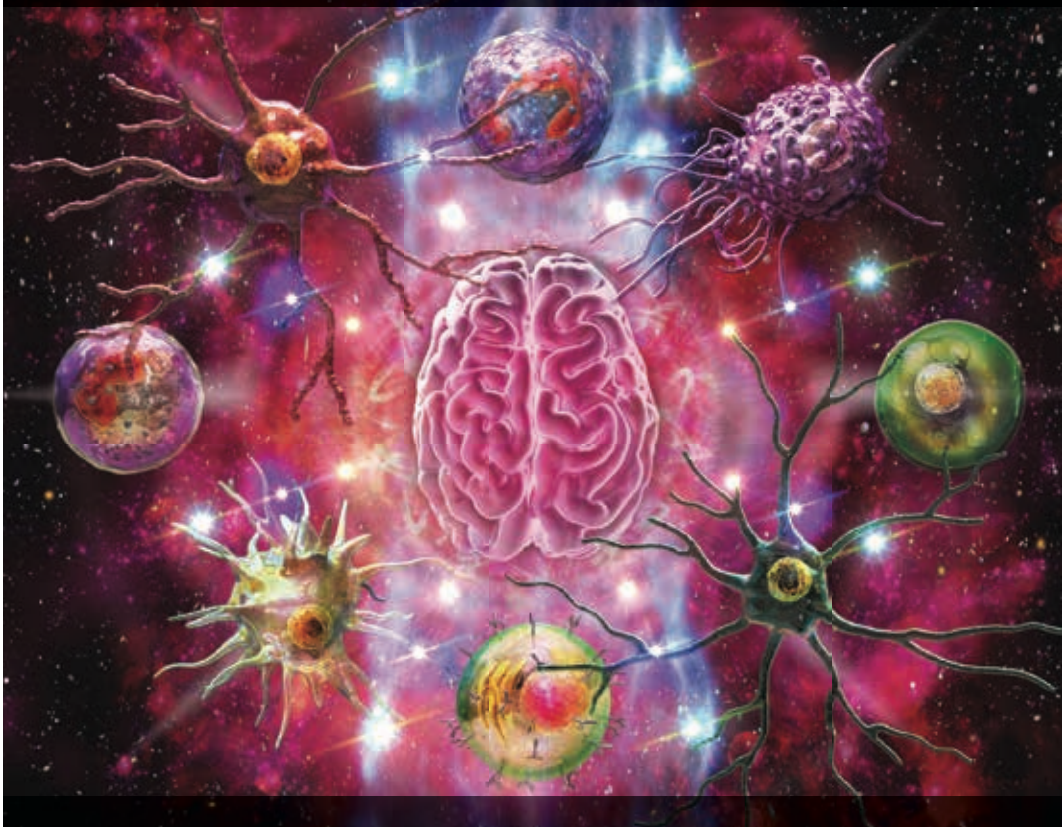

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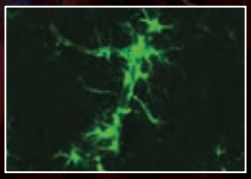

FFPE mouse brain tissue stained with astrocyte marker anti-GFAP antibody (clone SMI 25).

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Speaker abstracts

Regulation of AMPA receptors and synaptic plasticity in cognitive disorders

Richard L Huganir[1]

[1] Department of Neuroscience, Kavli Neuroscience Discovery Institute, Johns Hopkins University School of Medicine

Neurotransmitter receptors mediate signal transduction at synaptic connections between neurons in the brain and the regulation of receptor function is critical for synaptic plasticity. My laboratory has been elucidating the molecular mechanisms underlying the regulation of AMPA receptors, the major excitatory neurotransmitters receptors in the central nervous system. We have found that AMPA receptors are extensively posttranslationally modified by phosphorylation, palmitoylation and ubiquination. Protein phosphorylation is a major form of AMPA receptor regulation and the receptors are phosphorylated on serine, threonine and tyrosine residues by many different protein kinases. We have shown that phosphorylation of the receptor regulates its ion channel properties and membrane trafficking and that receptor phosphorylation is critical for the expression of several forms of synaptic plasticity and for learning and memory. We have also identified a variety of AMPA receptor interacting proteins, including GRIP1/2, PICK1, GRASP1, SNX27, KIBRA, and SynGAP that interact with AMPA receptors and are necessary for their proper subcellular trafficking. This AMPA receptor complex is important for several forms of synaptic plasticity and learning and memory. These studies indicate that the modulation of receptor function is a major mechanism for the regulation of synaptic transmission and is a critical determinant of animal behavior. Recent evidence has indicated that AMPA receptor function may be disrupted in several neurological and psychiatric disorders. Specifically, mutations in SynGAP, GRIP1 and GRASP1 have been found to be associated with cognitive disorders including intellectual disability, autism, and schizophrenia. Recently we have been characterizing some of these disease-associated mutations to examine their effect on SynGAP protein function, AMPA receptor trafficking, synaptic plasticity and behavior. These studies may help develop novel therapeutics for these devastating disorders.

Getting a handle on neuropharmacology by targeting receptor-associated proteins

David S Bredt[1]

[1] Johnson and Johnson

Targeted therapy for neuropsychiatric disorders requires selective modulation of dysfunctional neuronal pathways. Receptors relevant to CNS disorders typically have associated proteins discretely expressed in specific neuronal pathways; these accessory proteins provide a new dimension for drug discovery. Recent studies show that targeting a TARP auxiliary subunit of AMPA receptors selectively modulates neuronal excitability in specific forebrain pathways relevant to epilepsy. Other medicinally important ion channels, gated by glutamate, γ-aminobutyric acid (GABA), and acetylcholine, also have associated proteins, which may be druggable. This emerging pharmacology of receptor-associated proteins provides a new approach for improving drug efficacy while mitigating side effects.

Impaired metabolic capacity and cellular resilience in antidepressant resistance: implications and opportunities for treatment refractory psychiatric disorders

Susannah J Tye[1]

[1] The University of Queensland, Queensland Brain Institute

Deficits in synaptic plasticity contribute to treatment resistance in psychiatric illnesses, including mood, anxiety and stress disorders. Our work aims to determine the neurobiological mechanisms that functionally limit therapeutic neuroadaptations to first line antidepressant treatments in preclinical (rodent) models of stress pathophysiology. Using a genome-wide transcriptomics approach we identified molecular pathways contributing to antidepressant resistance in rats. Antidepressant-resistance was induced via chronic adrenocorticotropic hormone (ACTH; 100µg; i.p.; 14 days) treatment. Animals were allocated to forced swim test (FST) or stress-naïve conditions. The infralimbic cortex, implicated in regulation of FST responses, was dissected and global gene expression profiles obtained (Agilent). Gene set enrichment analysis was performed (DAVID) following Bonferroni correction and KEGG pathways identified (Fisher exact score $p<0.05$). Pivotal genes were validated in independent groups by RT-PCR and/or immunoblotting. The effects of deep brain stimulation, ketamine (10mg/kg), and lithium (100 mg/kg) on these behavioural and molecular responses were determined. Significant alterations were observed in key sensors of energy demand, cell division/growth, apoptosis, protein synthesis, and glucose/glycogen regulation following stress. Significantly less genes were differentially expressed following stress for ACTH pretreated animals relative to saline controls; suggestive of reduced capacity to respond under supplemental stress. Markers of oxidative stress, hypoxia, endoplasmic reticulum stress, pro-inflammatory cytokines, nutrient deprivation and DNA damage were, however, increased in the ACTH group. Mitochondria deficiency was confirmed in separate experiments and modulation of mTOR signalling was demonstrated to be associated with treatment response to deep brain stimulation, ketamine, and lithium. These data suggest that deficits in metabolic capacity and cellular resilience contribute to antidepressant resistance. Moreover, these deficits can be functionally overcome with deep brain stimulation, ketamine or lithium. Such actions may be critical for initiating longer-term neural adaptations to enable recovery from psychiatric disorders resistant to first line antidepressant treatments.

Profiling phytohormones in stroke brain – challenges and opportunities

Sheng-Tao Hou[1]

[1] Southern University of Science and Technology, Brain Research Centre

Stroke is a leading cause of death and disability in the world. However, protecting stroke-induced brain injury still represents one of the largest unmet medical needs. Despite of decades of efforts trying to find ways to protect neurons against ischemic insult, no clinical effective drugs are available. It is known that brain can indeed launch an internal protective response against ischemic insult, however, the exact endogenous protective mechanisms remain not well understood. Abscisic acid and phaseic acid (PA), are phytohormones regulating important physiological functions in higher plants. Here, we profiled the presence of plant stress hormones in ischemic brains and show the presence of naturally occurring (-)-PA in mouse and rat brains. (-)-PA is exclusively present in the choroid plexus and the cerebral vascular endothelial cells. Purified (-)-PA has no toxicity and protects cultured cortical neurons against glutamate toxicity through reversible inhibition of glutamate receptors. Focal occlusion of the middle cerebral artery (MCAO) elicited a significant induction in (-)-PA expression in the CSF, but not in the peripheral blood. Importantly, (-)-PA induction only occurred in the penumbra area, indicting a protective role of PA in the brain. Indeed, elevating (-)-PA level in the brain reduced ischemic brain injury, while reducing (-)-PA level using a monoclonal antibody against (-)-PA increased ischemic injury. Collectively, these studies showed for the first time that (-)-PA is an endogenous neuroprotective molecule capable of reversible inhibiting glutamate receptors during ischemic brain injury. Further understating of the internal defense system would be extremely beneficial to develop appropriate drugs against stroke in humans.

Neural mechanisms of social reward

Robert C Malenka[1]

[1] Stanford University School of Medicine, Department of Psychiatry and Behavioral Sciences

Positive prosocial interactions contribute to the development and maintenance of a range of adaptive, cooperative behaviors. Conversely, inability to participate in normal social interactions is a debilitating symptom of several prominent neuropsychiatric disorders. Although the role of neuromodulators in social behaviors, in particular oxytocin, is an active area of investigation, relatively little is known about the detailed neural mechanisms that influence sociability. We have pursued the hypothesis that the release of serotonin (5-HT) from dorsal raphe (DR) neurons in the mouse nucleus accumbens (NAc), a key node of classic reward circuitry, is critical for promoting non-aggressive prosocial interactions. We find that bidirectional modulation of 5-HT release in the NAc robustly modifies sociability in opposing directions, while having minimal effects on control behaviors. We test the importance of this mechanism in a mouse model of a relatively common genetic cause of autism spectrum disorders, a copy number variation on human chromosome 16p11.2. Genetic deletion of chromosome 7F3, which is syntenic to human 16p11.2, specifically from 5-HT neurons induces deficits in social behavior and decreases DR 5-HT neuron excitability. The decrease in sociability in 16p11.2 deletion mice can be rescued by optogenetic activation of DR 5-HT neurons, an effect requiring activation of 5-HT1b receptors in the NAc. Consistent with these results, the drug MDMA (3,4-methylenedioxy-methamphetamine), well known for its effects on promoting positive social interactions in humans, promotes sociability in mice via a mechanism that requires targeting the serotonin transporter in the NAc. These results demonstrate a surprisingly robust role for 5-HT release in the NAc in social behaviors and suggest that targeting this mechanism may prove therapeutically beneficial.

Optogenetic dissection of neural circuits underlying processing of innate fear

Liping Wang[1]

[1] Shenzhen Institutes of Advanced Technology, Brain Cognition and Brain Disease Institute (BCBDI)

The ability of animals to detect and generate emotional responses to natural threats is highly innate and conserved cross-species. Appreciated adaptive behavioral responses to different environmental cues are crucial for animal survival. Recent studies have shown that an overhead looming stimulation to mimic an approaching danger can trigger mouse flight behavior and escape to their nest (Yilmaz *et al.*, 2013) or unlearned freezing in the open-field where is "no place to hide" (Wei *et al.*, 2015). Our previous work proved the superior colliculus (SC) is the crucial brain structure for rapid responding to this looming threat signal and initialing the visually guided innate defensive responses. By using optogenetic, electrophysiology recording in freeing moving animal, we also identified a subcortical pathway from the glutamatergic projecting neurons in the medial region of the intermediate layers of the SC (ILSCm) to the lateral posterior nucleus of the thalamus (LP) mediating the innate freezing behavior. We further identified a cell specific pathway triggered by visual inputs, promoting appropriate defensive response to overhead visual threats. While expression of these responses is considered to be instinctive, their magnitude may be affected by environmental cues. However, the neural circuits underlying this modulation are still largely unknown. In current study, we found that repeated stress evoked an anxiety-like state in mice and accelerated defensive responses to looming. Stress also induced c-fos activation in locus coeruleus (LC) TH+ neurons and modified adrenergic receptor expression in SC, suggesting a possible Th::LC-SC projection that may be involved in the accelerated defensive responses. Indeed, both anterograde and retrograde neural tracing confirmed the anatomical Th::LC-SC projection and that the SC-projecting TH+ neurons in LC were activated by repeated stress. Optogenetic stimulation of either LC TH+ neurons or the Th::LC-SC fibers also caused anxiety-like behaviors and accelerated defensive responses to looming. Meanwhile, chemogenetic inhibition of LC TH+ neurons and the infusion of an adrenergic receptor antagonist in SC abolished the enhanced looming defensive responses after repeated stress, confirming the necessity of this pathway. These findings suggest that the Th::LC-SC pathway plays a key role in the sophisticated adjustments of defensive behaviors induced by changes in physiological states.

Striatal circuit dysfunction underlies motor deficits in a model of human dyskinesia

Anatol C Kreitzer[1]

[1] Gladstone Institutes, University of California, San Francisco

Abnormal involuntary movements, or dyskinesias, are seen in many neurological diseases, including disorders where the brain appears grossly normal, suggesting circuit dysfunction may be a root cause. Using unsupervised gene coexpression analysis, we identify a dyskinesia/dystonia gene module in striatum that is enriched with markers of indirect-pathway striatal projection neurons. To understand how indirect pathway neurons might contribute to dyskinesia symptoms, we used electrophysiological, optogenetic, and chemogenetic techniques in awake behaving animals to examine basal ganglia circuit function in a transgenic mouse model of human dyskinesia based on a gene within this module, paroxysmal nonkinesigenic dyskinesia (PNKD). We show that dyskinesia bouts in PNKD mice are caused by a transient loss of indirect pathway activity, which appears to be driven by alterations in excitatory synaptic input. These data provide both genetic and functional evidence for dysfunction of striatal indirect pathway neurons in the etiology of dyskinesia, and may guide development of new treatments for dyskinesias based on selective modulation of basal ganglia circuitry.

Information processing in the rodent sensory cortex: population dynamics across behavioural states

Ehsan Arabzadeh[1]

[1] Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra, Australia

Animals live in a complex and changing environment with various degrees of behavioural demands. In rodents, behavioural state can change from sleep and quiet wakefulness to active exploration of the environment, which is often manifested by whisking and locomotion. Efficient information processing is important in the active states such as during episodes of sensory decision-making. Here, I present electrophysiological and two-photon calcium imaging (GCamp6f) data that we have recorded from layer 2 and 3 of mouse primary vibrissal somatosensory cortex during a whisker vibration detection task. We characterise dynamics of population activity during correct and incorrect detections, and under different behavioural states, from quiet wakefulness, to episodes of high sensory performance. Using Information theoretic methods, we quantify how behavioural state affects the correlations among cortical neurons and the amount of information they carry about the sensory input.

Inhibitory synapses and plasticity in the ventral tegmental area

Julie A Kauer[1]

[1] Brown University, Departments of Pharmacology, Physiology and Biotechnology and Department of Neuroscience

Drug-induced persistent changes in the reward pathway, including the ventral tegmental area (VTA), may precede the transition to addiction. Drugs of abuse share the common mechanism of increasing dopamine release from VTA dopamine neurons, and drug exposure fundamentally alters synaptic transmission in the VTA by enhancing excitatory and reducing inhibitory drive. For example, drug exposure induces long-term potentiation (LTP) at excitatory synapses on VTA dopamine cells, and our understanding of this mechanism has prompted possible intervention strategies for treatment of addiction. However, a second important component of dopamine cell firing rate is GABAergic inhibition, a strong brake on these spontaneously firing neurons. Our lab has been exploring how drugs of abuse and stress interact with synaptic plasticity at GABAergic VTA synapses (LTP_{GABA}); several distinct drugs of abuse block LTP_{GABA}. There are multiple long-range GABAergic projections to the VTA, and using optogenetics we have begun to unravel their control of the local VTA circuit and modification by drugs and acute stress.

Neuronal pentraxins control glutamate receptor driven development of hippocampal inhibitory circuits in health and disease

Chris J McBain[1]

[1] National Institute of Child Health and Development

Circuit computation requires precision in the timing, extent, and synchrony of principal cell firing that is largely enforced by parvalbumin-expressing, fast-spiking interneurons (PVFSIs). To reliably coordinate network activity, PVFSIs exhibit specialized synaptic and membrane properties that promote efficient afferent recruitment such as expression of high-conductance, rapidly gating, GluA4-containing AMPA receptors. We found that PVFSIs upregulate GluA4 during the second postnatal week coincident with increases in the AMPAR clustering proteins neuronal pentraxins, NPTX2 and NPTXR. Moreover, GluA4 is dramatically reduced in NPTX2(-/-)/NPTXR(-/-) mice with consequent reductions in PVFSI AMPAR function. Early postnatal NPTX2(-/-)/NPTXR(-/-) mice exhibit delayed circuit maturation with a prolonged critical period permissive for giant depolarizing potentials. Juvenile NPTX2(-/-)/NPTXR(-/-) mice display reduced feedforward inhibition yielding a circuit deficient in rhythmogenesis and prone to epileptiform discharges. Memory loss in Alzheimer's disease (AD) is attributed to pervasive weakening and loss of synapses. In a mouse model of AD amyloidosis, *Nptx2*$^{-/-}$ results in reduced GluA4 expression, disrupted rhythmicity, and increased pyramidal neuron excitability. Postmortem human AD cortex shows profound reductions of NPTX2 and coordinate reductions of GluA4. NPTX2 in human CSF is reduced in subjects with AD and shows robust correlations with cognitive performance and hippocampal volume. These findings implicate failure of adaptive control of pyramidal neuron-PV circuits as a pathophysiological mechanism contributing to cognitive failure in AD. Our findings demonstrate an essential role for NPTXs in controlling network dynamics highlighting potential therapeutic targets for disorders with inhibition/excitation imbalances such as schizophrenia and (AD).

The amygdala, prefrontal cortex and hippocampal circuit in fear learning

Pankaj Sah[1]

[1] The University of Queensland, Queensland Brain Institute

Fear conditioning is a Pavlovian Learning paradigm in which in which a neutral stimulus, the conditioned stimulus (CS), such as a light or tone, is temporally paired with an aversive stimulus, the unconditioned stimulus (US), typically a footshock. Following a small number of pairings, subjects form an association between the CS and US, and learn to respond to the CS with an avoidance response, the conditioned response (CR), which is rapidly acquired and long lasting. Subsequent presentations of the CS that are not paired with the US break this association, and lead to a gradual reduction of the CR through a process known as extinction. Studies have clearly established that the three key structures, the amygdala, medial prefrontal cortex (mPFC) and the hippocampus play a central role in fear learning and extinction. However, the neural connections between these that mediate this learning are just beginning to be elucidated. In this talk, using acute slice recordings, and behavioural analysis coupled with optogenetic stimulation, I will describe the cell types, and neural connections within and between the amygdala, mPFC, ventral hippocampus and discuss the functional roles of these circuits in fear learning and extinction.

Molecular and cellular mechanisms of synapses loss in Alzheimer's disease and tauopathy

Morgan Sheng[1]

[1] Genentech, Department of Neuroscience

Synaptic dysfunction and synapse loss are hallmarks of Alzheimer's disease (AD) and other tauopathies, yet the underlying molecular pathomechanism remains largely undefined. Here, we used unbiased proteomic analysis of postsynaptic density (PSD) proteins from wild-type versus Tau-P301S transgenic mice before the onset of overt neurodegeneration to identify early tau-dependent changes in the synapse. We identified that C1q, initiator of the classical complement cascade, is highly increased in PSDs purified from Tau-P301S hippocampus, and that C1q is localized at synapses. Tau-P301S brains showed increased engulfment of synaptic material by microglia. Moreover, C1q-neutralizing antibodies suppressed microglial synapse clearance in neuron-microglia co-cultures and *in vivo* in Tau-P301S mice. These findings suggest that tau pathology induces tagging of synapses by C1q, leading to removal of synapses by microglia, and raise the possibility that C1q-neutralizing antibodies might be a potential approach to mitigate synapse loss in AD.

NMDA receptor regulation: clues from rare variants implicated in disease

Katherine W Roche[1]

[1] National Institute of Neurological Disorders and Stroke

NMDA receptors are tetramers composed of GluN1 and GluN2 subunits. These receptors are critical for neuronal development and synaptic plasticity. GluN2A and GluN2B are highly expressed in hippocampus and cortex and are intolerant to genetic variation in the human population. In recent years, rare variants identified in GluN2 subunits have been identified in patients with a variety of neurological disorders, including autism spectrum disorders (ASDs), schizophrenia (SCZ) and epilepsy. Specifically, a large number of rare variants of GluN2B have been identified in ASD probands, whereas GluN2A variants are more commonly identified in patients with epilepsy. We have been studying variants identified within the C-terminal domain of GluN2A and GluN2B to better understand receptor structure/function in this domain and also characterize effects on binding proteins, phosphorylation and synaptic expression of receptors. Using this approach, we hope to reveal insights into the pathophysiology of neurological disorders.

A novel auxiliary protein that regulates the function of NMDA receptors

Andres Villu Maricq[1]

[1] University of Utah, Department of Biology and Center for Cell and Genome Science

NMDA receptors (NMDARs) are a subtype of postsynaptic ionotropic glutamate receptors that have critical roles in models of learning, and are associated with a variety of neurological and psychiatric disorders, including schizophrenia, depression and Alzheimer's disease. We recently identified the first auxiliary protein (NRAP-1) for NMDARs. NRAP-1 is a presynaptic secreted protein that binds to postsynaptic NMDARs and modifies receptor gating. Our studies have revealed a novel mechanism for the regulation of neurotransmission and synaptic plasticity.

Neurotransmitter release machinery in a nanoscale Brownian world

Frédéric A Meunier[1]

[1] Clem Jones Centre for Ageing Dementia Research, Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia.

Communication between neurons relies on a process known as neuroexocytosis during which synaptic vesicles containing neurotransmitters dock, are primed and then fuse with the presynaptic plasma membrane, thereby releasing their content post-synaptically. The proteins involved in this mechanism are constantly subjected to Brownian motion and hence must be organised in functional nanoclusters to optimise the speed of the fusion process. How the inherent mobility of these proteins on the presynaptic membrane is compatible with docking, priming and ultimately fusion, is still subject to numerous investigations. We uncovered key changes in the nanoscale organization of the priming protein Munc18-1 and the SNARE protein syntaxin1A, that are critical for neuroexocytosis. Human disease mutation in Munc18-1 causing early infantile epileptic encephalopathy leads to a dramatic change in the nanocluster organisation of Munc18-1 with appearance of aggregative nanocluster on the plasma membrane. Our results suggest that perturbating the molecular network of proteins involved in neuroexocytosis initiates aggregative diffusional behaviour.

Cell-specific splicing of neuronal calcium channels: mechanism, function and disease

Diane Lipscombe[1]

[1] Brown University, Brown Institute for Brain Science

Voltage-gated calcium ion (Ca_V) channels are critical for numerous neuronal functions including triggering neurotransmitter release, rebound bursting, pacemaking, and excitation-dependent gene transcription. Ca_V channel activity is tightly controlled – from gene expression to membrane trafficking – and by the action of several signaling molecules via G protein coupled receptor activation. Mammalian Ca_V channel encoding genes are complex, containing 50+ exons and each having the capacity to generate hundreds of unique splice isoforms. Transcriptome analyses of functionally unique subsets of neurons have shown that each express a distinct pattern of ion channel splice isoforms that contribute to cell-phenotype and that change depending on cell state. A family of RNA binding proteins, including Rbfox and Nova, orchestrate cell-specific splicing across a number of genes including Ca_V channels. We have shown that cell-specific exon selection during alternative pre-mRNA splicing regulates ion channel biophysics, G protein inhibition, and drug sensitivity. Of particular interest, Trpv1-lineage nociceptors express a $Ca_V2.2$ mRNA that contains an exon that is found rarely in other neurons. This splicing event in Trpv1-lineage neurons, enhances the sensitivity of Ca_V channels to mu-opioid receptor inhibition, is disrupted following peripheral nerve injury, and when disrupted is associated with reduced effectiveness of morphine as an analgesic. In this case, the unique $Ca_V2.2$ mRNA exon composition in nociceptors is regulated by methylation of gDNA. Thus, cell-specific composition of Ca_V channel mRNAs across the nervous system is controlled by cell-specific epigenetic modification of gDNA and by a family of RNA binding proteins that collectively determine Ca_V channel function, subcellular distribution, and sensitivity to G protein modulation. This critical RNA processing step, between gene expression and mRNA export, expands the proteome to generate a deep, rich array of calcium-dependent cell functions.

Supported by NIH grant NS055251.

Mapping the visceral connectome for bioelectronic medicine

Janet R Keast[1]

[1] Department of Anatomy and Neuroscience, University of Melbourne, Australia

The resurgence of bioelectronic medicine is rapidly expanding into the visceral nervous system to develop neuromodulation approaches for treating organ dysregulation and visceral pain. This major new target requires detailed knowledge of nerve-organ relationships to design organ- and condition-selective neuromodulation devices and to repurpose existing devices by applying new protocols or accessing new surgical sites. There is a rich body of anatomy and physiology knowledge on the visceral nervous system, but many gaps still remain. In particular, many of the imaging technologies and informatics analyses that are transforming neuroanatomy and providing a foundation for the multinational brain projects have not yet been applied extensively to the peripheral nervous system or spinal cord. A major point of focus in this field to date has been the therapeutic modulation of the vagus, a mixed sensorimotor nerve that supplies the thoracic and abdominal organs. Ongoing vagal neuromodulation studies target diverse conditions, including gut inflammation, migraine, obesity, epilepsy and depression. There has been much less investigation of neuromodulation approaches for the sacral component of the visceral nervous system, which is essential for bladder and bowel voiding, and sexual function. These autonomic reflexes are unusually complex as their functions are completed only by collaboration with somatic pathways to coordinate urethral and anal sphincters, and pelvic floor muscles. In this presentation, new studies on neural circuitry regulating lower urinary tract function and pelvic pain will illustrate progress in our studies supported by the NIH Common Fund initiative, the SPARC (Stimulating Peripheral Activity to Relieve Conditions) research program. This will include summarising advances in quantitative functional mapping of this area of the nervous system, and illustrate technical approaches and challenges unique to achieving our goal of building a "lower urinary tract connectome".

An afferent subcortical white matter pathway to the amygdala facilitates fear recognition

Marta Garrido[1]

[1] The University of Queensland, Queensland Brain Institute

Our ability to rapidly detect threats is thought to be subserved by a subcortical pathway that quickly conveys visual information to the amygdala. This neural shortcut has been demonstrated in animals but has rarely been shown in the human brain. Importantly, it remains unclear whether such a pathway might influence neural activity and behaviour. We conducted a multimodal neuroimaging study of 622 participants from the Human Connectome Project. We applied probabilistic tractography to diffusion-weighted images, reconstructing a subcortical pathway to the amygdala from the superior colliculus via the pulvinar. We then computationally modelled the flow of neural activity, using functional magnetic resonance imaging, during a face-viewing task and found strong evidence for a functionally-afferent subcortical pathway. Critically, individuals with greater fibre density in this pathway also had stronger dynamic coupling and enhanced fearful face recognition. Our findings provide converging evidence for the recruitment of an afferent subcortical route to the amygdala in the human brain that facilitates fear recognition.

The thalamostriatal network mediates flexible encoding for goal-directed action

Bernard Balleine[1]

[1] University of New South Wales, Sydney

The dorsomedial striatum (DMS) is an essential structure for encoding the specific action-outcome associations that underlie goal-directed action. These associations can fluctuate with changes in the environment and recent research suggests that cholinergic interneurons (CINs) in the DMS play a key role in minimizing interference between old and new learning, allowing goal-directed actions to change and adapt. CINs receive excitatory signals from the parafascicular thalamic nucleus (Pf) and it has been demonstrated that the Pf–DMS pathway is vital for this integration of action memories. The Pf input to pDMS declines with aging, Parkinson's disease dementia and Lewy body dementia. Using an innovative animal model, we assessed the influence of neurodegeneration and neuroinflammation in the thalamo-striatal pathway, and its consequential loss of cholinergic function, in the learning and memory processes that contribute to goal-directed action. We show that the burst firing activity of CINs is related to the integration of new and existing memories and that inflammation in the Pf interferes with thalamo-striatal pathway function, affecting DMS CIN burst firing activity and the integration of action memories. Interestingly, selegiline, a monoamine oxidase B inhibitor normally used as a treatment in combination with levodopa in the early stage of Parkinson disease, was able to recover the loss of CIN burst firing activity in the DMS and partially restore the normal integration of action memories.

The development of neural coding in the zebrafish brain

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I will discuss our recent experimental and computational analyses of how functional connectivity and spontaneous and evoked activity change during the early development of the zebrafish tectum. This includes comparing algorithms for detecting neural assemblies in calcium imaging data, and a Hebbian learning model which produces neural assemblies without afferent input.

Notes

Poster abstracts

GluA1 uiquitination mediates amyloid-β-induced loss of surface AMPA receptors

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The accumulation of soluble amyloid-beta (Aβ) peptides produces profound neuronal changes in the brain during the pathogenesis of Alzheimer's disease. Excessive levels of Aβ disrupt excitatory synaptic transmission by promoting the removal of synaptic AMPA receptors (AMPARs), dendritic spine loss and synaptic depression. Recently, activity-dependent ubiquitination of the GluA1 subunit has been shown to regulate the intracellular sorting of AMPARs toward late endosomes for degradation. However, whether this ubiquitin signalling pathway mediates Aβ-induced loss of surface AMPARs is unknown. In this study, we demonstrate that acute exposure of cultured neurons to soluble Aβ oligomers induces AMPAR ubiquitination concomitant with the removal of AMPARs from the plasma membrane. Importantly, expression of the GluA1 ubiquitin-deficient mutants inhibited the adverse effects of Aβ on the surface expression of AMPARs in neurons. Furthermore, we revealed the cross-talk between GluA1 ubiquitination and phosphorylation, in particular phosphorylation at Ser-845, which is crucial for AMPAR recycling and is known to be dephosphorylated in the presence of Aβ. Our data showed that the GluA1 ubiquitin-deficient mutant enhances GluA1 phosphorylation on Ser-845. Conversely, the GluA1 S845D phosphomimetic mutant reduced binding with Nedd4-1, and hence the ubiquitination of AMPARs. Importantly, the GluA1 S845D mutant also prevented Aβ-induced removal of surface AMPARs. Taken together, these findings provide the first demonstration of the dynamic cross-modulation of GluA1 ubiquitination and phosphorylation, a process that is perturbed by Aβ, in regulating the membrane sorting decision that ultimately determines the number of AMPARs on the cell surface.

Organization of a reverberating cell assembly in the rodent basolateral amygdala

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Synchronized activity in neurons organized as a cell assembly is hypothesized to generate oscillations for cognitive processes. However, how diverse subsets of neurons are operate during synchronized activity is unclear. We investigated microcircuits and mechanisms for generation of Sharp wave ripple oscillations (SWR), a default offline network oscillation, in the rodent basolateral amygdala (BLA), *in vitro*. In our preparations, BLA circuits spontaneously generated SWRs with characteristic time-locked compound synaptic inputs in interneurons (INs) and principal neurons (PNs). We found that a single spike in a unique subset of interneurons, Chandelier neurons (Chns), initiated SWRs as a reverberating activity through GABAergic excitation at the axon initial segment of PNs. Chn evoked reverberations showed consistent temporal and synaptic properties, a defining feature for a cell assembly like network. In the BLA, PNs made large monosynaptic glutamatergic inputs to INs. When the Chn excited PNs, strong glutamatergic connections facilitated fast recruitment of INs including other Chns. A single spike in a Chn recruited PNs and INs to provide feedback and feedforward inputs that occurred as compound synaptic inputs in SWRs. Based on these findings, we suggest Chns orchestrate a reverberating cell assembly in the BLA to generate SWRs.

Presynaptic glutamine transport regulates synaptic transmission in the brainstem and hippocampus

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To maintain synaptic transmission it is essential that glutamate released from neurons is rapidly replenished. Recycling glutamate via astrocytic uptake of released glutamate, conversion of glutamate to glutamine and subsequent return of glutamine to neurons for ongoing glutamate synthesis (the "glutamate-glutamine cycle") is commonly believed to be critical for maintaining presynaptic glutamate supply. However, the amino acid transporter that sequesters glutamine from the extracellular space into presynaptic terminals has not been identified, and its role in replenishing synaptic glutamate under physiological levels of neurotransmission has not been determined. To investigate presynaptic glutamine transport we have performed whole-cell patch-clamp recordings from presynaptic terminals in the brainstem (the calyx of Held) and in the hippocampus (mossy fibre boutons) in acutely isolated brain slices. In both preparations, we show that glutamine is sequestered into presynaptic terminals by an electrogenic neutral amino acid transporter. The role of this transporter in synaptic transmission was investigated by recording postsynaptic responses in principal cells from the medial nucleus of the trapezoid body upon stimulation of the Calyx of Held synapse, and by recording CA3 pyramidal cell responses upon mossy fibre stimulation. We find that during physiological levels of synaptic stimulation, inhibiting this presynaptic glutamine transport reduces miniature EPSC amplitude and causes a run-down of stimulated EPSCs. This indicates that the presynaptic supply of neurotransmitter is rapidly depleted when recycling via glutamine is impaired. These results are the first direct recordings of presynaptic glutamine transporter activity in individual forebrain neurons, and clearly demonstrate that they play a role in recycling glutamate at excitatory synapses. It is therefore clear that developing pharmacological inhibitors of presynaptic glutamine transport represents a novel and promising way of modulating glutamatergic synapses.

Dementia-related deficits in decision-making

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The role of the dorsal hippocampus (DH) in goal-directed action is not well defined. Neuroimaging studies in humans have linked hippocampal activation to goal-directed processes, whereas lesion studies in rodents have found that these processes to be independent of DH. Here we used variants on an outcome devaluation design to reconcile these findings. First, we gave rats a single training session in which the left and right levers uniquely earned pellet and sucrose outcomes, respectively (counterbalanced). The next day, we fed rats to satiety on one of these outcomes to reduce its value, then gave rats a choice between the levers in extinction. Saline controls showed intact devaluation; responding selectively on the lever that had previously earned the valued outcome (valued > devalued). Devaluation was impaired (valued = devalued) however, for rats that received DH inactivations via GABAA agonist muscimol infusions prior to training or test. In a second experiment, when rats received specific action-outcome contingencies over 6 days of training, performance was intact for all rats, regardless of DH inactivation. We explored the implications of these findings using a J20 transgenic mouse model of Alzheimer's disease that over-express human amyloid precursor protein (hAPP), and exhibit a number of AD-like neuropathologies in DH. We again found devaluation performance to be initially impaired relative to wildtypes, then restored by additional training. Postmortem assessment of microglia marker Iba1 in the dorsal CA1 region of hippocampus demonstrated a negative correlation for J20 mice with initial devaluation performance, suggesting that more microglia was associated with poorer performance. No such relationship was detected for wildtype mice, or for either group on the test for which goal-directed action was intact. Together, these data suggest that DH inactivation causes initial deficits in goal-directed actions that can be overcome by additional training, which could have important implications for AD patients.

Diet-induced obesity causes 'addiction-like' synaptic plasticity in the nucleus accumbens

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Overconsumption of highly palatable 'junk' foods is a primary contributor to the development of obesity. There is increasing evidence that the pathological overeating which often underlies obesity is compulsive in nature and therefore contains elements of an addictive disorder. However, direct physiological evidence linking obesity to synaptic plasticity akin to that occurring in addiction is lacking. We have accumulated evidence using a rat model of diet-induced obesity which suggests that the propensity to diet-induced obesity is associated with addiction-like behavior and hallmark synaptic impairments in the glutamatergic input to the nucleus accumbens core (NAcore) previously observed in rodent models of drug addiction. Diet-induced obese and resistant rats were assessed for operant self-administration of palatable food pellets using fixed and progressive ratios in order to determine whether they would display features of 'addiction-like' behavior towards palatable food – heightened motivation as assessed by progressive ratio, excessive intake, and the persistence of responding during periods where pellets were unavailable. Subsequently, NAcore brain slices were prepared and we tested for changes in the ratio between AMPA and NMDA currents (AMPA/NMDA) and the ability to exhibit long-term depression (LTD). Obesity prone rats were more 'food addiction' vulnerable than their obesity resistant counterparts as displayed by i) heightened motivation ii) excessive consumption and ii) increased food-seeking as measured by lever-pressing during periods where palatable food was unavailable. Moreover, rats prone to diet-induced obesity exhibited deficits in the ability to induce LTD in the nucleus accumbens core as well as increased potentiation at these synapses as measured by the ratio of AMPA/NMDA currents. These are hallmark synaptic impairments that have been observed in the brains of animals that have self-administered drugs of abuse such as cocaine, nicotine and heroin. Our results show overlap between the propensity to obesity and synaptic changes associated with drug addiction, supporting partial coincident neurological underpinnings for obesity and drug addiction.

Transitions in choice behavior over the course of both discrimination and reversal learning

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Discrimination learning and flexible decision-making/action selection allow us to survive and thrive in dynamic and uncertain situations. Little is known about how these functions operate and integrate with each other in the complex scenarios where behaviour is normally generated and experienced. To investigate how learning and decision-making behaviour manifest in a more naturalistic setting, we have developed detailed and automated assessments of these processes that can be deployed in the home cage of group-housed mice. Groups of four adult male C57BL/6 mice were co-housed in an IntelliCage for the duration of the experiment and accessed water by engaging in an instrumental visual cue discrimination (VD) task in nightly sessions of self-initiated trials. Once an individual reached performance criterion (2 consecutive sessions of >80% correct), the learned VD rule was reversed (RR). Individual cumulative records of performance (correct or incorrect) and action selection (choosing the left or right nosepoke port) underwent a thorough change point analysis to characterise the acquisition profiles for the VD and RR tasks. Change point analyses revealed that mice exhibit distinct phases of both performance and action selection during both VD and RR tasks. Furthermore, transitions between these phases appear to be abrupt, suggesting rapid changes in behavioural control as animals learn and adapt to change. Our novel approach allows for a highly detailed examination of the evolution of choice behaviour across entire epochs of discrimination learning and adaptation to change in complex and naturalistic scenarios. The abrupt transitions in behavioural control will be further interrogated both computationally and neuronally.

Dendritic spikes along the apical obliques of layer 5 cortical pyramidal neurons

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Dendritic spikes are regenerative potentials that are able to enhance neuronal output, and may play a critical role in coincidence detection during synaptic input. In cortical pyramidal neurons, dendritic spikes have been reported to occur in basal dendrites and in the nexus of apical tuft, which is known to be the calcium-spike initiation zone, as well as in apical tuft branches. Here, we report the generation of dendritic spikes in thin oblique dendrites that can be evoked by low-frequency bursts of back-propagating action potentials (bAPs). Using *in vitro* two-photon calcium imaging in cortical L5 pyramidal neurons, we observed a step-wise increase in calcium influx into specific oblique dendrites during 30 to 50Hz bursts of bAPs. A similar increase in calcium influx was observed in the oblique dendrites of morphologically-realistic models of layer 5 pyramidal neurons during around 30 Hz of bAP train. In these models, this non-linear increase in calcium influx was specific to: (1) thin oblique dendrites (~1 micron diameter); and (2) oblique dendrites that strongly attenuates the first bAP. These results suggest that apical oblique dendrites are capable of generating local dendritic spikes during low frequency action potential bursts.

Effects of GluN2A and GluN2B epilepsy mutations on synaptic currents mediated by diheteromeric and triheteromeric NMDA receptors in artificial synapses

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Epilepsy is a spectrum of neurological disorders with many causal factors. The NMDAR is a major genetic target for some forms of heritable human epilepsies. To date, however, there is little information available on how mutations affect the function of NMDAR-mediated excitatory postsynaptic currents (EPSCs). Such information is essential for understanding epileptogenic mechanisms and designing optimal therapies for particular epilepsy genotypes. Accordingly, the aim of this study was to define the basic biophysical and pharmacological properties of EPSCs mediated by mutant NMDARs in a cortical neuron-HEK293 cell co-culture assay. Here we evaluated the effects of three missense mutations, GluN2A N615K (early-onset epileptic encephalopathy), GluN2B N615I and V618G (West syndrome), on EPSCs mediated by the diheteromeric GluN1-GluN2A and GluN1-GluN2B isoforms and the triheteromeric GluN1-GluN2A-GluN2B isoform, that are the most prevalent stoichiometries in native synapses. The three mutant diheteromeric channels produced inverse Mg^{2+} sensitivity relative to wild-type NMDARs and only the GluN2B V618G mutation eliminated memantine block of EPSCs. After confirming the expression of triheteromeric NMDARs within the synapse, we found that only GluN2B V618G-containing channels exhibited no Mg^{2+} block. In addition, all three mutant triheteromeric receptors exhibited altered EPSC properties. These results provide new clues as to how these mutations lead to different types of epilepsy.

Impact of superior colliculus on cortical processing of somatosensory (whisker) input

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Superior colliculus (SC) is an evolutionary ancient midbrain structure, which is highly conserved across species. It is well established that SC receives direct input from the primary sensory cortices. What is not known is whether (and if so how) SC modulates information processing in primary sensory cortex. To address this question, we combined optogenetic activation of SC with recordings in primary vibrissal somatosensory cortex (vS1) of adult C57BL6/J mice. Adeno-associated virus containing ChR2 was injected into SC to induce ChR2 expression (n=48). Two to four weeks after injection, under urethane anesthesia, we identified whisker responsive neurons located in the intermediate layers of SC and confirmed their reliable activation with blue light. We then performed *in vivo* whole-cell and extracellular recordings from vS1 while activating SC via an optic fiber. Consistent with the idea that SC can have an impact on cortical processing, photo-activation of ChR2 in SC led to light-evoked responses in vS1 neurons. These responses were manifested in the local field potential, as well as depolarization of the membrane potential and increased spiking activity. Furthermore, photo-activation of SC led to a shift in the input-output relationship of cortical neurons, increasing action potential firing at low stimulus intensities. Given the absence of a direct anatomical projection from SC to vS1, we investigated two potential pathways involved in this functional modulation: (i) a projection from SC to facial nucleus, which is responsible for whisker movements though the facial nerve and (ii) an indirect thalamic pathway from SC to vS1 through the rostral sector of the posterior nucleus of the thalamus (Pom). Monitoring the whiskers under high-speed camera revealed whisker protractions after photo-activation of SC. These movements were abolished after cutting the facial nerve, however, this procedure had no impact on vS1 responses following photo-activation of SC. Consistent with the idea that SC modulates vS1 via Pom, extracellular recordings indicated that photo-activation of SC led to increased firing of Pom neurons. Taken together, our results suggest that SC, which plays a key role in attention, modulates sensory processing in vS1 via an indirect pathway through the thalamus.

Illuminating axon tension with a beta spectrin-based sensor in organotypic brain slices

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Ultrasonic neuromodulation is a novel technique proposed as a therapeutic treatment of neurological disorders, though the mechanism of action is unclear. There is evidence that changes in membrane stress and activation of mechanosensitive ion channels are important components of the effect of ultrasound on neurons. To further study this effect we are developing a spectrin-based tension sensor that will enable measurement of cytoskeletal strain. Recently, a FRET-based tension-sensor module (TSM) was incorporated into *C. elegans* beta-spectrin, UNC-70, showing the spectrin cytoskeleton is required for touch sensation[1]. Given the high conservation of beta-spectrin across species, we investigate whether the same sensor can be used to visualise cytoskeletal tension in mammalian neurons. Methods: The UNC70-TSM construct and associated controls were subcloned into the pcDNA3.1 vector and transfected into organotypic hippocampal slices prepared from P6 rats. Expression in neurons (MAP2), and localisation to the cytoskeleton (beta II spectrin) was determined through immunofluorescence via confocal microscopy. Results: The expression pattern of UNC70-TSM is distinct from the cytosolic TSM control, and is similar to that of beta II spectrin staining. Co-staining of UNC70-TSM transfected cells with beta-spectrin antibodies indicates colocalisation. Conclusions: The *C. elegans* beta-spectrin homolog is incorporated into the mammalian cytoskeleton. This will allow us to analyse changes in axonal tension through FRET imaging.

[1] Kreig *et al.*, 2014, doi:10.1038/ncb2915

Neurodevelopmental disorder-associated mutations in synaptotagmin-1 cause presynaptic dysfunction

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Synaptotagmin-1 (syt1) is an essential synaptic vesicle protein that acts as the Ca^{2+}-sensor for fast, synchronous neurotransmitter release. Syt1 has also been implicated in other aspects of synaptic physiology including the endocytic retrieval of synaptic vesicles. Recently, whole exome sequencing of a child with a distinct neurodevelopmental disorder revealed the first known human mutation in syt1 (I368T). We have now identified an additional 4 distinct mutations in syt1 in a total of 11 individuals exhibiting a phenotypic spectrum of symptoms including motor delay and intellectual disability. These missense mutations are all clustered within the C2B domain, a Ca^{2+}-binding region central to syt1 function in both exocytosis and endocytosis. To investigate the physiological effects of these variants, the homologous mutations were induced in pHluorin-tagged rat syt1 and expressed in cultured mouse hippocampal neurons. Syt1 variants express at approximately equal levels to endogenous wild type syt1 and correctly localise to nerve terminals at rest, with the exception of one mutant ($p<0.01$, $n=3-4$). pHluorin imaging revealed that syt1 mutants significantly slow the rate of exocytosis ($p<0.05$, $n=5-7$). Importantly, these deficits could be ameliorated by increasing $[Ca^{2+}]$; however, even at high $[Ca^{2+}]$, exocytosis proceeds slower in the presence of disease-associated syt1 variants compared to WT ($p<0.05$, $n=7-8$). Our findings reveal that a syt1-associated neurodevelopmental disorder is caused by mutations that affect the Ca^{2+}-dependent function of syt1, and further highlight the importance of syt1 functionality in human cognitive and motor development.

Influence of modulatory, long-projecting GABA neurons expressing relaxin-family peptides on synapses and circuits in health and disease?

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This conference aims to review advances towards a fundamental understanding of how neuronal activity and synaptic function produce, and are shaped by, behaviour, as better treatments for mental health and neurological disorders will rely on gaining a clear appreciation for how brain activity is coordinated at synaptic, circuit and system levels. As highlighted recently[1],this includes establishing the roles many, highly-conserved peptides play in control of complex circuits and behaviours, which means going beyond use as markers for neurons such as cortical GABA neurons[2]. Eventually, the therapeutic potential of altering the largely GPCR-mediated effects of key peptides to improve symptoms of relevant clinical disorders should be assessed. Our research has explored both these aspects by assessing the impact of relaxin-3 signalling via a type-1 GPCR, RXFP3 on cognitive/social behaviour, and identifying RXFP3-target neurons to facilitate studies of their physiological/synaptic actions[3]. Briefly, relaxin-3/GABA neurons in the hindbrain innervate the entire limbic system including hippocampus, amygdala and frontal cortex, and relaxin-3 acts via RXFP3 on GABA/somatostatin, GABA/parvalbumin and other GABA neurons in these areas. RXFP3 signalling also has a regulatory effect on magnocellular and parvocellular oxytocin (OT) and Arg-vasopressin (AVP) neurons[3]. Relaxin-3 neurons are activated by neurogenic stress and modulated by stress/arousal-related peptides, CRF, orexin and MCH[3,4]. In contrast, the 'hormone' relaxin is expressed by small populations of cortical GABA neurons and acts via the type-C, LGR-containing GPCR, RXFP1 that is topographically distributed within deep layers of cingulate, somatosensory, visual, entorhinal cortex and in claustrum and subiculum. Based on a recent transcriptome analysis of cortical GABA neurons[2], it is proposed relaxin/RXFP1 signalling is involved in modulation of arousal/sleep, and cognitive processes, with effects on synaptic plasticity and the extracellular matrix, via interactions with co-expressed trophic/peptide modulators. These ideas now require experimental investigation.

[1] Sudhof TC (2017) Molecular neuroscience in the 21st Century: A personal perspective. *Neuron* 96, 536-41

[2] Paul A *et al.* (2017) Transcriptional architecture of synaptic communication delineates GABAergic neuron identity. *Cell* 171, 522-39

[3] Ma S *et al.* (2017) Distribution, physiology and pharmacology of relaxin-3/RXFP3 systems in brain. *Br J Pharmacol* 174, 1034-48

[4] Sabetghadam A *et al.* (2018) Melanin-concentrating hormone (MCH) and orexin systems in nucleus incertus: dual innervation, bidirectional effects on neuron activity, and differential influences on arousal and feeding. *Neuropharmacology*, in press

The role of α9-nAChRs in pain, stress and affective behaviour

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α9-nAChRs have been touted as a novel drug target to treat chronic pain despite no definitive mechanism being described. Furthermore, the precise physiological role of this receptor and any potential adverse effects resulting from the inhibition of this receptor are not known. We aimed to investigate the role that α9-nAChRs play in chronic pain and stress, and the impact they have on behaviour. Mice with a germline knockout (KO) of the α9-nAChR were compared to their WT counterparts in neuropathic (chronic constriction injury) and inflammatory (Complete Freud's Adjuvant) models of pain. Nerve histology after injury showed no pathological difference between the WT mice and the α9-nAChR KO mice. Pain states were measured including temperature sensitivity, mechanical allodynia and mechanical hyperalgesia. Nociceptive thresholds between the genotypes were comparable, as were the pain states in the inflammatory and neuropathic pain models, except for mechanical hyperalgesia, which was attenuated in α9-nAChR KO mice. From these studies we questioned whether there may be an altered stress phenotype of the KO mice. After restraint stress, α9-nAChR KO mice exhibited blunted stress-induced arousal and increased anxiety-like behaviours. Corticosterone levels were dysregulated in stressed α9-nAChR KO mice. The importance of a9-nAChRs in chronic pain appears to be minimal, while, it seems that the receptors play an important regulatory role in stress pathways and affective behaviour. This raises the concern that there may be potential adverse effects of pharmacologically blocking α9-nAChRs.

Cellular and circuit mechanisms underlying processing of binocular visual information in visual cortex

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The binocular region of primary visual cortex plays a critical role in processing visual information received from the eyes. To better understand how binocular information is integrated in binocular visual cortex here we combine optogenetic and electrophysiological methods to identify binocular and monocular neurons *in vitro* and *in vivo* and characterize their active, passive and morphological properties. We have identified two distinct populations of layer 2/3 pyramidal neurons in binocular visual cortex, one that receives long-range monosynaptic excitatory input from the contralateral visual cortex (binocular neurons) and one that does not (monocular neurons). In addition, we find that input from the contralateral visual cortex strongly excites a subset of fast-spiking, putative parvalbumin positive (PV) interneurons, activating feed-forward inhibition that could drive sub-linear synaptic integration in layer 2/3 pyramidal neurons. While we found no differences in passive and morphological properties of binocular and monocular layer 2/3 pyramidal neurons, the active properties of binocular neurons were significantly different from monocular neurons. Specifically, the slope of the input/output (f/I) curve generated during somatic current injection was lower in binocular layer 2/3 pyramidal neurons, leading to reduced action potential firing. These data suggest that binocular layer 2/3 pyramidal neurons are intrinsically less excitable than monocular neurons. This difference indicates that binocular layer 2/3 pyramidal neurons may have different cellular integration rules from monocular neurons during synaptic integration. Using a morphologically realistic active model of layer 2/3 pyramidal neurons, we demonstrate that differences in axonal potassium channels likely underlie the difference in f/I curves of binocular and monocular neurons. Consistent with this idea we found that low concentrations of 4-AP (300 μM), which block D-type potassium channels, increased the slope and maximum firing rate of binocular but not

monocular neurons. In conclusion, we provide evidence that distinct populations of both excitatory and inhibitory neurons are involved in processing binocular visual input in binocular visual cortex. Furthermore, we show that these different neuronal populations have different active properties. These findings provide insight into the cellular and circuit mechanisms used by the cortex to process binocular visual information.

Distinct properties of binocular inputs in the mouse primary visual cortex

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Binocular neurons in primary visual cortex (V1) receive visual information from both eyes and represent the cortical substrate for binocular vision. Given that the majority of retinal projections (>95%) cross the midline, we surmised that V1 binocular neurons receive input from the ipsilateral eye primarily through an indirect callosal projection that originates from contralateral V1. To test this hypothesis we performed whole-cell recordings from layer 2/3 pyramidal cells in the binocular region of V1 in urethane-anaesthetised C57BL6/J mice. Whole-field retinal illumination was delivered to either eye individually or to both eyes together via a set of light-emitting diode (LED) "goggles". Excitatory postsynaptic potentials (EPSPs) evoked by stimulating the ipsilateral eye were markedly delayed relative to those evoked by stimulating the contralateral eye (mean difference in response onset latency=10.8±1.5ms; $p<0.0001$), consistent with the notion that input from the ipsilateral eye arises via a synaptic pathway that is distinct from the contralateral eye. This synaptic delay was modulated by the strength of retinal stimulation, with EPSP onset latency becoming shorter with increasing strength of retinal illumination (n=10). Ipsilateral eye evoked EPSPs, however, were delayed relative to their contralateral eye evoked EPSPs at all retinal illumination levels tested. Moreover, the rise time of EPSPs evoked by ipsilateral eye stimulation were significantly longer than those generated during stimulation of the contralateral eye (mean difference=7.93±1.8ms; $p<0.0001$), possibly reflecting the asynchronous and more polysynaptic nature of this input. To directly test the role of callosal inputs from the contralateral V1 to the ipsilateral eye response, tetrodotoxin (TTX) was injected into the contralateral V1 while recording the local field potential in V1 in both hemispheres. TTX injections abolished responses from either eye in the injected hemisphere and significantly reduced responses to the ipsilateral eye in the opposite hemisphere (39±14% reduction, n=3 mice). Taken together, our results suggest that the visual input from the ipsilateral eye to binocular V1 contains a significant callosal projection from the contralateral V1. Experiments are currently underway to quantify the contribution of this callosal component to cortical binocular processing using optogenetics.

Mechanisms of drug-resistance in glutamate-gated chloride channels of *H. contortus*

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Glutamate-gated chloride channels (GluClRs) are neurotransmitter-gated receptors found at neuronal and neuromuscular inhibitory synapses of invertebrates. GluClRs are members of the pentameric ligand-gated receptor family that includes the inhibitory γ-aminobutyric acid and excitatory acetylcholine gated receptors. Many invertebrates that are commercially important pest species in agriculture, aquiculture, veterinary and human health are becoming resistant to drugs that target their GluClRs, such as ivermectin (IVM). One of these species is the nematode, *H. contortus*, which is an agricultural endoparasite that infects ruminant animals, such as sheep, cattle and goats. The molecular-level mechanisms that render GluClRs resistant to IVM are unknown. In this investigation, we used the GluClR of *H. contortus* as a model to explore two possible mechanisms of drug resistance, (a) missense point mutations to GluClRs that have been identified in other drug-resistant pest species and (b) alterations to GluClR subunit composition. To achieve this, we used a co-culture of cortical neurons and HEK293 cells transfected with GluClRs of *H. contortus* to record inhibitory post-synaptic currents (IPSCs) for the first time, using patch-clamp electrophysiology. Together with single receptor current recordings, we have discovered that IVM-resistant receptors are those that activate for brief periods and mediate IPSCs that decay rapidly. This fundamental observation applies to mutated and wild-type heteromeric GluClRs, and thus represents a universal mechanism whereby invertebrate pests can become resistant to drugs.

TRPA1 modulates evoked neuronal responses in mouse somatosensory (barrel) cortex

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Transient Receptor Potential Ankyrin 1 (TRPA1), a non-selective cation channel, is broadly expressed throughout the body including the nervous system. We recently demonstrated the expression of TRPA1 in rodent cortex through immunostaining, and its functional activation in cortical slices. In order to better understand the contribution of TRPA1 to sensory processing, here we use the rodent vibrissal primary somatosensory (vS1) cortex and quantify how TRPA1 activation modulates the representation of the sensory inputs. We performed *in vivo* juxtacelluar recordings from the mouse vS1 cortex under local infusion of aCSF, TRPA1 agonist (AITC) or antagonist (HC-030031) while applying brief vibrotactile stimuli to the whiskers at 6 different intensities (0-200 µm). Under aCSF infusion, neurons exhibited a sigmoidal input-output function with an increase in evoked response with stimulus velocity, which is typical of vS1 neurons. Application of AITC and HC-030031 produced significant modulations both in the baseline firing rate and the evoked response of vS1 neurons. AITC increased the baseline activity of neurons (n=16), the maximum evoked responses and the overall response range. TRPA1 activation thus resulted in a gain modulation which improved sensory detection of the vS1 neurons. After replacing AITC by HC-030031, the response functions returned to their original values. The AITC and HC-030031 modulations were absent in the TRPA1 Knockout (KO) mice (7 neurons). In 4 recording sessions in wild-type mice, we introduced HC-030031 in the absence of any prior activation of TRPA1. Here HC-030031 produced a significant reduction in both the maximum evoked response and the response range (n=6 neurons). These results confirm the earlier *in vitro* experiments by demonstrating the presence of TRPA1 and their functional activation in cortical neurons. The results also suggest that TRPA1 has a baseline level of activation in cortex and may thus contribute to sensory processing under normal physiological conditions.

The completeness of context learning impacts hippocampal neural ensembles during memory formation but not retrieval

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In a contextual fear conditioning paradigm, animals require a minimum period of time in the context prior to shock to display substantial conditioned fear at test. This period of time is thought to be necessary for animals to form an integrated representation of the context, which then comes into association with the shock. According to this model, longer placement shock intervals support the generation of more complete contextual representations. Yet, the neural underpinnings of this increasing completeness have yet to be systematically investigated. Here we examined the effect of PSI on memory completeness by using immediate-early gene expression as an indicator of hippocampal context memory related activity. In the first study we examined how the duration of initial context exposure influences hippocampal neural activity after learning. In the second study we investigated how PSI alters the rate and magnitude of hippocampal pattern-completion based memory retrieval. The results showed that hippocampal neural activity corresponded with the duration of the current session, rather the level of contextual experience or the strength of responding. These findings will be discussed in terms of the nature of IEGs and theoretical models of hippocampal memory formation and retrieval.

Brain state dependent changes in spatial-temporal dynamics in the somatosensory cortex

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Information processing in the sensory cortex is not solely determined by sensory input, but also the behavioural state of the animal (i.e. quiet wakefulness versus awake-behaving). The animal's behavioural state is known to affect the dynamics of population activity (e.g. the degree of synchrony) which in turn determine the cortical information processing capacity. Here, we use two-photon calcium imaging in awake mice performing a sensory detection task and quantify the spatiotemporal dynamics of activity in populations of Layer 2/3 neurons across different behavioural/cortical states. We injected GCamp6f into the vibrissal primary somatosensory cortex of mice (n=4) and allowed 2-4 weeks for the expression of GCamp6f during which we habituated the mice to head-fixation and trained them in a whisker vibration detection task. The imaging commenced thereafter whereby the same neurons (n=550) were imaged across different states, defined based on the behaviour of the animal (quiet wakefulness and awake-behaving), as well as their performance on the task (hit and missed trials). As the mice switched from quiet wakefulness to active states, both neuronal and behavioural input-output curves shifted towards lower stimulus intensities, revealing enhanced detection sensitivity. Responses to low-intensity stimulus were higher for hit trials compared to missed trials. Changes in calcium fluorescence before stimulus onset were also predictive of the animal's behaviour (hit versus miss). We quantified spatiotemporal dynamics of activity among nearby and distant neurons by characterising spike triggered probability density functions. Behavioural state influenced neuronal synchronicity, with more alert states exhibiting lower synchrony during spontaneous periods and higher synchrony for evoked responses. Across all behavioural states, nearby cells showed higher levels of correlation in activity compared to distant neurons. The drop in correlation with distance was modulated by behavioural state. Mutual information analyses revealed that neurons with stronger correlation to the network conveyed more information about the stimuli in their evoked response, and more so in active states. The change of sensory coding regimes in a state-dependant manner may help animals to adaptively route relevant information through the brain for survival.

Cell-specific epigenetic modification of the CACNA1B gene controls calcium channel function in nociceptors

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Voltage-gated calcium (Ca_V) channels control transmission of noxious stimuli at nociceptor terminals in dorsal horn spinal cord. Ca_V2 are the dominant source of calcium to trigger transmitter release at synapses and of these, $Ca_V2.2$ is the principle target of drugs and neurotransmitters that activate G-protein coupled receptors to down regulate nociception. Nociceptors express multiple isoforms of $Ca_V2.2$ generated by cell-specific processing of pre-mRNAs. Two alternate forms of $Ca_V2.2$, a and b, are generated by mutually exclusive splicing of e37a and e37b. Both isoforms are found in nociceptors that express TRPV1, whereas most neurons only express $Ca_V2.2b$. We have shown that $Ca_V2.2a$ is more sensitive to inhibition by mu-opioid receptor activation and its presence augments the analgesic actions of morphine to thermal stimuli. Here, we study the mechanism that determines exon selection in nociceptors. The multi-zinc finger DNA binding protein CCCTC-binding factor (CTCF) is a master regulator of gene expression that may also promote exon recognition of weak splice junctions. Based on publicly available ChIP-seq data from several cell lines, we found that CTCF binds *Cacna1b* e37a locus. By electrophoretic mobility shift assay, we confirmed that CTCF binds a 60 bp region of e37a, but not e37b. Using the dorsal root ganglia (DRG)-derived F11 cell line we found that: 1) CTCF binds e37a, but not e37b *in vivo*; 2) CTCF overexpression increases, while CTCF siRNA knockdown decreases e37a inclusion; 3) inhibition of gDNA methylation leads to a decrease in 5mC of e37a locus, increased CTCF binding to e37a locus, and increased abundance of $Ca_V2.2a$-mRNAs; 4) siRNA knockdown of DNA methyltransferase DNMT3a, but not DNMT1 or DNMT3b, promotes e37a inclusion; 5) active DNA demethylating enzymes TET1 and TET2, but not TET3, when overexpressed, increase e37a inclusion. *In vivo*, we found that *Cacna1b* e37a locus is hypo-methylated in TRPV1-expressing compared to TRPV1-negative neurons in DRG, and that only TRPV1-lineage neurons express $Ca_V2.2a$-mRNAs. We are currently investigating how exon selection is altered in neuropathic pain states. Collectively, we show that cell specific epigenetic factors change alternative splicing exon selection in *Cacna1b* gene, thereby regulating transmission of nociceptive information in the primary afferent pain pathway.

Balanced prefrontal activity controls the expression of emotional memories

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The amygdala, the main brain structure involved in encoding emotional memories, forms extensive connections with the hippocampus (HPC) and the medial prefrontal cortex (mPFC) to control extinction learning. In rodents, the prelimbic (PL) and infralimbic (IL) prefrontal cortices play distinct roles in fear learning and extinction, whereas the hippocampus is crucially involved in regulating the contextual information in which extinction occurs. Using optogenetic approaches *in vitro* as well as chemogenetic manipulation *in vivo*, we have identified the synaptic projection that regulates fear expression following extinction, which is also responsible for the relapse of fear. Moreover, recordings of local field potentials and single unit activity in the PL, IL and HPC has revealed a dynamic activity pattern to mediate the context-dependent expression of fear after extinction. Together, the findings illustrate the importance of the mPFC in controlling emotional memories, via synaptic connections with the HPC and the amygdala.

Functional connectivity of the local striatal network

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The striatum is the main input structure to the basal ganglia and plays a major role in integrating and transducing excitatory information from the cortex and thalamus. It is composed almost entirely of GABAergic spiny projection neurons (SPNs), which in dorsal areas can be subdivided into two types: those that project directly to the substantia nigra (dSPNs) and those that project indirectly via the globus pallidus (iSPNs). Since their first anatomical description, it has been known that SPNs, in addition to projecting out of the striatum, possess extensive local axon collaterals and make synaptic contacts among each other. Despite this, direct electrophysiological evidence of GABAergic synaptic transmission between identified SPNs has been elusive. Here, using new viral approaches based on transneuronal labelling of thousands of neurons, we demonstrate a large-scale, asymmetrical connectivity between iSPNs and dSPNs that exerts major influence over striatal function. We also explore the contribution of asymmetrical local connectivity in the context of reinforcement learning and goal-directed action.

Neuronal growth and dendritic spine dynamics are regulated by secreted amyloid precursor protein-alpha and its C-terminal domain

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Secreted amyloid precursor protein-alpha (sAPPα) is a protein with neuroprotective and neurotrophic properties that is critically involved in learning and memory. Dendritic spines are thought to be the physical locus of information storage in the brain and their loss is often amongst the first symptoms in neurodegenerative diseases such as Alzheimer's disease (AD). sAPPa has been shown to reverse this loss in AD mouse models, however its effects on non-diseased dendritic morphology is largely unknown. This study investigated the effects of sAPPa and two of its biologically active domains on dendritic branching as a measure of complexity, as well as spine type (thin, stubby, mushroom) and density in rat hippocampal neurons in primary culture. Cultures were prepared from P0-P1 Sprague Dawley rat pups and maintained for 21 DIV. To visualise dendritic spines, neurons were transduced with VSVg-pseudotyped lentivirus delivering the green fluorescent protein (GFP) gene under control of a neuron-specific promoter (synapsin) at 18 DIV. At 21 DIV, cultures were treated with sAPPa, its biologically active 3 amino acid domain RER or its 16 amino acid C-terminal domain (CTa16) (all 1 nM) for 2 or 24 h. Following treatment, GFP-expressing neurons were visualised using a Nikon C2 confocal microscope. Image analysis software (ImageJ, NeuronStudio) was used to measure dendritic complexity through Scholl analyses and to perform automated analysis of dendritic spine type and density in dendritic segments located 100 µm from the soma. Analysis (2-way ANOVA, Sidak's post-hoc test) revealed that sAPPa and CTa16, but not RER, significantly increased dendritic branching at distances of 100-125 um (sAPPa, $P<0.05$,) and 125-15 µm (CTa16, $p<0.05$) from the soma after 24 h. For spine density, sAPPa, but not CTa16 or RER, affected spine density and then only after 2 h of treatment. No effects were observed following 24 h of treatment with sAPPa, CTa16 or RER. At 2 h, sAPPa caused a reduction in spine density ($p<0.05$, student's t-test) that was confined to thin spines. This effect was not seen at 24 h for any treatment. These findings demonstrate that sAPPa and its C-terminal domain promote dendritic growth in non-diseased neurons, while sAPPa appears to modulate short-term spine dynamics.

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Synapsin controls synaptic vesicle mobility in hippocampal nerve terminals

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Synaptic vesicle (SV) recycling in nerve terminals is essential for maintaining synaptic transmission during periods of intense synaptic activity. Newly recycled SVs are relatively mobile but their mobility decreases as some SVs are integrated into the reserve pool which is highly immobile as vesicles are tethered to the actin cytoskeleton. Key regulatory proteins such as synapsin, are involved in the regulation of SV mobility in both recycling pool and reserve pool of SVs. There are 3 mammalian isoforms of synapsin (synapsin I, II, and III). Synapsin triple-knockout (TKO) mice develop epilepsy, and synapsin TKO neurons contain noticeably fewer vesicles at release sites and exhibit accelerated synaptic depression. Here, we have monitored the mobility of recycling pool of SVs in hippocampal neurons from the synapsin TKO mice and wildtype controls with super-resolution imaging technique called subdiffractional tracking of internalized molecules (sdTIM). Neurons were transfected with vesicle-associated membrane protein 2 (VAMP2) tagged with a pH sensitive GFP-based fluorescent protein (pHluorin). Transfected neurons were stimulated with high K+ buffer in the presence of anti-GFP Atto647N-nanobodies to allow internalization of nanobodies into recycling SVs. Unquenching of pHluorin upon fusion of SVs with the plasma membrane during stimulation/loading of nanobodies allows the identification of active presynapses. Tracking of recycling SVs containing Atto647N-nanobodies in presynapses provides information about the mobility of recycling pool of SVs. In control neurons, longer chase time after a single pulse of stimulation/loading of nanobodies, lower the SV mobility, suggesting that some of the SVs are slowly recruited to form an immobile population of SVs possibly via the action of synapsin. In TKO cells, this time-dependent decrease in mobility was not observed, which suggests that synapsin is indeed involved in the immobilization of recycling vesicles to the reserve pool.

Influence of cocaine exposure on dendritic activity

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Cocaine exposure causes dramatic changes to the structure and functioning of individual neurons, leading to extensive remapping of brain connectivity. These changes alter the processing of synaptic input and overall brain functioning. Despite the prevalence and detrimental effects of cocaine use, the functional effects of cocaine exposure on the activity of cortical neurons during sensory input are poorly understood and are the focus of this study. Here, two-photon calcium imaging was performed on layer 2/3 pyramidal neuron tuft dendrites in the primary somatosensory cortex of urethane anaesthetized mice previously transfected with the genetic indicator GCaMP6f. Single exposure of cocaine (20 mg/kg i.p.) altered both spontaneous and sensory evoked dendritic activity leading to an average increase in spontaneous activity after 20-30 minutes of cocaine delivery. In a subset of tuft dendrites, the calcium response to forepaw sensory stimulation was increased in both amplitude and response probability during cocaine exposure. These results illustrate the cellular basis of altered sensory perception during cocaine exposure, providing invaluable insight into the effects of cocaine on brain functioning.

Opioids and dopamine regulate the intercalated cells of the amygdala

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The amygdala is a brain region involved in the fear response. It is made up of several nuclei including the GABAergic intercalated cells (Im) which regulate fear learning, in particular extinction. Within the Im, there is both strong expression of the mu-opioid receptor (MOR) and the opioid peptide met-enkephalin (met-enk), and the dopaminergic D1-receptor (D1) and dopaminergic terminals. This suggests that both opioids and dopamine regulate Im function but neither systems regulatory role is fully defined. This study aims to define the role of the opioid and dopaminergic systems within the Im. Using whole-cell patch-clamp electrophysiology in brain slices from male Sprague-Dawley rats (3-9 weeks) we have found that the two systems act similarly at the local Im-Im GABAergic synapse. Met-enk (10uM) through MOR and dopamine (30uM) through D1-receptors inhibit the Im-Im evoked inhibitory post-synaptic currents (eIPSCs, met-enk: 54.39 ± 5.03% from baseline n = 9; dopamine: 57.81 ± 5.34% from baseline n = 11). Whilst both cause a strong inhibition of the local synapse, neither induce a change in the paired-pulse ratio (control; 1.24 ± 0.12 vs met-enk: 1.31 ± 0.17 n = 6; control: 1.37 ± 0.13 dopamine vs 1.39 ± 0.20 n = 6). In contrast both cause a similar reduction in the weighted time decay constant of the eIPSC (control; 34.30 ± 4.80 ms vs met-enk: 17.80 ± 2.80 ms n = 5; control: 27.03 ± 1.73 ms vs dopamine: 19.42 ± 0.96 ms n = 5). However, on cell bodies of the Im, met-enk and dopamine have differential effects on potassium currents. Met-enk robustly induces an outward current at -60 mV (23.66 ± 3.04pA, n = 7) whilst dopamine is without effect (-1.05 ± 2.31pA, n = 7). Given that MOR signals via $G_{i/o}$ g-proteins and D1-receptors via G_s g-proteins the similarity of the extent and nature of their inhibition of Im-Im eIPSCs is surprising. The mechanisms of dopamine or opioid inhibition in the Im and how this is changed by fear learning will be determined.

Unravelling the neural circuits related to movement disorders: the pedunculopontine nucleus

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Parkinson's disease (PD) is a progressive neurological disorder that results from loss of dopaminergic neurons in the midbrain. Freezing of gait (FOG) and postural instability are often seen in patients with advanced PD. FOG is defined as a brief, episodic absence or marked reduction of forward progression of the feet despite the intention to walk. It is one of the most debilitating motor symptoms in patients with PD as it may lead to falls and a loss of independence. Deep brain stimulation of the pedunclopontine nucleus offers relief of FOG for some patients. The pathophysiology of FOG remains poorly understood but is associated with deficits in cognitive function and motor preparation. The neural circuits involved in motor preparation have only partially been unravelled and involve persistent activity between the motor thalamus and motor cortex. PPN projections to motor thalamic regions suggest a role for the PPN in motor preparation and may be an additional pathway for the relief of FOG. To determine the connectivity of PPN projection neurons to the motor thalamic regions, we express channelrhodopsin (Chr2) in PPN neurons using viral transduction. After confirming terminal expression of Chr2 in motor thalamic regions, whole-cell recording from motor thalamic neurons are made to test what portion of neurons receive PPN inputs and whether these inputs are glutamatergic, GABAergic, cholinergic or a combination of these three. A better understanding of PPN neural circuits in relationship to motor preparation may prove to be valuable in developing a less invasive therapy for movement disorders like PD.

Does high frequency sensory stimulation result in long-term potentiation?

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Long-term potentiation (LTP) is a form of synaptic plasticity thought to play an important role in learning and memory. It can be induced *in vivo* and *in vitro* in animal brains by invasively stimulating afferent pathways with high frequency (~100 Hz) electrical pulses (known as a tetanus). This results in increased potentials recorded in the region where the afferent pathway terminates, compared to pre-tetanization measures. A 2005 study by Teyler and colleagues described a non-invasive technique where high frequency visual stimulation (~9 Hz) resulted in specific increases in human visual evoked potentials (VEP) measured *in vivo* with electroencephalography. Similarities were drawn between the methods and results of LTP studies and the non-invasive paradigm. Evidence from subsequent studies suggested that LTP might underlie the potentiation effects observed in high frequency sensory stimulation studies. It has also been proposed that the technique may be useful for the study of plasticity in clinical populations. However, recent evidence has called into question the robustness of these LTP-like effects. Our own research examined differences between audiovisual versus visual-only high frequency stimulation on VEP in young and elderly adults. We will discuss our findings in the context of the early and more recent research that has utilized high frequency sensory stimulation. If the technique is to have any clinical use or benefit, important questions need to be addressed about replicability, individual differences, and other factors that may be involved in these LTP-like effects.

Quantal transmission at single central synapses displays large variation in size with subunit

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In their seminal work, Katz and colleagues established the quantal nature of synaptic transmission, whereby the basic unit of neurotransmission is the quantal event detected postsynaptically as a small all-or-none similar sized miniature postsynaptic potential or current (mini), in response to the neurotransmitter release from a single vesicle. However, the quantal nature of minis has never been justified by the study at single synapses, leaving the open question whether minis are identical in size and follow the principle of invariance. Here, we selectively study the quantal transmission from single active zone contained synapses using whole-cell recording and quantitative analysis. It was found that the amplitude of spontaneous and evoked miniature events from single synapses displayed large variation and could be estimated as integer multiples of a subunit. Our study revealed a large encoding scope of quantal synaptic transmission and the higher capacity of synaptic information processing than that the quantal theory implied.

Anatomy and physiology of the central extended amygdala

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Anxiety is a sustained state of apprehension to distal and potential threat, which can become extremely debilitating in disease states. Although anxiety disorders are highly prevalent, the underlying neuronal mechanisms remain largely unknown. Several lines of evidence suggest that the central nucleus of the amygdala (CeA), and the central sublenticular extended amygdala (SLEAc) form a crucial circuit node in anxiety. Using a combination of tract tracing, electrophysiology, and behavioral approaches, we have dissected the synaptic connections and functional role between the CeA and SLEAc in mice. Our monosynaptic retrograde tracing experiments revealed that the most substantial projections from the CeA to SLEAc originate from the lateral division of the CeA (CeL), with the majority of the retrogradely labelled cells in the CeL expressing somatostatin (SOM). Using channelrhodopsin-2-assisted circuit mapping and whole cell patch-clamp recording, we confirmed that CeL SOM+ neurons innervate the SLEAc, with a preference for local GABAergic neurons. CeL neurons are GABAergic, and whole-cell recordings from neurons in the SLEAc confirmed that CeL inputs evoked inhibitory responses. Using open field test and elevated plus maze we find that selective activation of CeL SOM+ neurons or CeL-SLEAc circuit was anxiogenic. Together, these results define a inhibitory circuit between CeL and SLEAc, and show that SOM+ neurons in the CeL mediate anxiety by inhibiting GABAergic neurons in the SLEAc.

The role of NEGR1 in regulation of synaptogenesis and neurotransmitter release

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The underlying mechanisms of neurodevelopmental and neurodegenerative brain disorders often lie in the dysregulation of synaptogenesis and neurotransmitter release controlling mechanisms. Patient case studies have shown that individuals with interstitial deletion in chromosome 1 containing the *NEGR1* gene, present with developmental co-ordination disorder and various learning disabilities. Mechanisms of these developmental disorders remain unknown. We demonstrate that overexpression of NEGR1 in cultured cortical neurons results in inhibited synapse formation on dendrites of these neurons. An increase in NEGR1 levels is accompanied by a decrease in the densities of filopodia and dendritic spines along dendrites of these neurons suggesting that post-synaptic NEGR1 regulates synaptogenesis by modulating the filopodium formation. Analysis of the efficiency of neurotransmitter release in synaptic boutons of cortical neurons overexpressing NEGR1 using a reporter of synaptic vesicle recycling VGLUT1-pHluorin showed that overexpression of NEGR1 results in a progressive decline in exocytosis and endocytosis of synaptic vesicles with repeated stimulation. Interestingly, a similar stimulation-dependent reduction in synaptic vesicle recycling was also found in cortical neurons transfected with NEGR1 siRNA. Altogether, our results indicate that NEGR1 is involved in regulation of the integration of neurons into the neuronal circuits both pre- and post-synaptically, suggesting that the developmental disorders associated with altered expression of NEGR1 are associated with altered synaptogenesis and neurotransmitter release in the brain.

Inheritable mutations of a1 $GABA_A$ receptor subunit linked to epilepsy affect cellular morphology and physiology of cortical neurons.

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Mutations within the $GABA_A$ receptors ($GABA_A$Rs) have been linked to heritable human epilepsies. Of the 19 subunits that form $GABA_A$Rs, a1 is the most common within the brain (around 40%) and it is important for the formation of functional receptors within inhibitory synapses. In this study, we decided to investigate 3 different epilepsy-causing mutations of the α1 subunit of $GABA_A$Rs: $\alpha1^{T265I}$, $\alpha1^{D192N}$ and $\alpha1^{A295D}$. Cultured cortical neurons were transfected with DNA of the wild-type α1 subunit with SepH-GFP (α1-SE) or one of the three mutations (α1-SE^{T265I}, α1-SE^{D192N}, α1-SE^{A295D}). Miniature inhibitory post-synaptic currents (mIPSCs) were recorded at DIV22-25, around 2 weeks after transfection, and analysed to study the effect that each mutation had on rise-time, decay-time, amplitude and frequency of mIPSCs. In agreement with previous studies, we found that transfecting α1-SE induced a faster decay-time, without affecting amplitude, rise-time and frequency of mIPSCs. α1-SE^{D192N} mutation did not show any changes in amplitude, decay-time, rise-time and frequency of mIPSCs. On the contrary, α1-SE^{A295D} mutation increased both the rise-time and decay-time of mIPSCs, without affecting amplitude and frequency. α1-SE^{T265I} mutation also increased the decay-time but it did not affect either amplitude or rise-time of mIPSCs. Interestingly, α1-SE^{T265I} decreased drastically the frequency of mIPSCs, with 50% of recorded cells not showing any mIPSC events over 10 minutes. We also studied the effects that these mutations may have on the synaptic and extra-synaptic pools of $GABA_A$Rs. Interestingly, we found that all mutants had impaired surface expression in neurons. Compared to α1-SE, significantly smaller number of $GABA_A$Rs containing α1-SE^{T265I} subunit were found in both synaptic and extrasynaptic regions, while α1-SE^{D192N} mutant caused depletion of extrasynaptic population of receptors without affecting the number of $GABA_A$Rs at synapse. The α1-SE^{A295D} mutant was strongly retained in the endoplasmic reticulum (ER). When expressed in the plasma membrane, however, this mutant produced a large population of highly mobile extrasynaptic $GABA_A$Rs. This study shows that inheritable mutations of $GABA_A$R α1 subunit linked to epilepsy affect neuronal inhibition not only through channel function, but also by altering the number of receptors found at the synaptic and extrasynaptic regions.

Fat matters: Mapping phospholipid and phospholipid metabolite changes during memory acquisition

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Far from being mere static containers and scaffolds for proteins, phospholipid membranes are extremely complex and dynamic molecular structures which undergo constant change in fluidity, curvature and surface chemistry, mediated by protein domains such as BAR, and phospholipid modifying enzymes. Enzymatic phospholipid remodelling generates metabolites such as free fatty acids (FFAs) and lysophospholipids (LPLs) which have further structural and signalling roles. In neurons, these membrane remodelling processes are critical to maintaining cellular and synaptic structure, endocytosis and exocytosis during neurotransmission, memory and learning. We have used LCMS lipidomics to establish the molecular profile of five classes of phospholipids and five classes of sn-position identified lysophospholipids across the rat brain, to establish how phospholipids respond to memory acquisition during auditory fear conditioning (AFC). Our results show that the phospholipid profile varies across the brain, and importantly, "canonical" phospholipids with saturated *sn1* and unsaturated *sn2* fatty acyl chains only contribute to half of the observed phospholipid signal. Non-canonical (unsaturated 1-acyl and saturated 2-acyl) lysophospholipids, particularly lysophosphatidylserine (LPS), responded strongly to AFC in the amygdala and forebrain, an effect that was ablated by blocking long-term memory acquisition with the NMDA receptor antagonist CPP. This data supports findings in our laboratory showing that saturated FFAs generated by hitherto unsuspected "non-canonical" phospholipid processing are important in consolidation of long-term memory.

Fat matters: Free fatty acid changes correlate with long-term memory acquisition in the fear-conditioned rat brain

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Neurotransmission, synaptic plasticity and memory formation involves tightly regulated dynamic modulation of phospholipid membrane fluidity, curvature and surface chemistry in concert with protein/protein and protein/lipid interactions at the pre- and post-synapse. This process generates phospholipid metabolites such as free fatty acids (FFAs) which can affect membrane dynamics and act as lipid signalling molecules. Numerous studies have pointed to the involvement of the polyunsaturated FFA arachidonic acid, generated by phospholipase A2 on "canonical" phospholipids with saturated *sn1* and unsaturated *sn2* fatty acyl chains, in a wide range of neuronal processes. However, much less is known about the overall dynamics of FFAs during memory acquisition. Using auditory fear conditioned rats as a memory model, we employed targeted lipidomics to quantify the brain-wide distribution and response of FFAs to memory acquisition. Saturated (C14:0-C22:0) and unsaturated (predominantly C20:4) FFAs dramatically increased in the amygdala and forebrain, an effect sensitive to systemic injection with CPP, an NMDA receptor antagonist known to block memory consolidation. Our results show that brain-region-specific changes in the FFA profile immediately following fear conditioning correlate with subsequent long-term fear-memory acquisition, and that these changes are in large part driven by hitherto unsuspected "non-canonical" pathways leading to saturated FFAs.

Identification of a defined population of neurons directly involved in fear memory in lateral amygdala

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Memory formation is thought to occur via enhanced synaptic connectivity between populations of neurons in the brain. However, it has been difficult to localize and identify the neurons that are directly involved in the formation of any specific memory. We have utilised the *fos-tau-LacZ* (FTL) transgenic mouse to identify a small number of discrete populations of neurons in the forebrain which were specifically activated by pavlovian fear conditioning. These populations of neurons may form part of the network that encodes fear memory. In particular, a population of learning activated neurons was found within a discrete region of the lateral amygdala (LA), a key nucleus required for fear conditioning. We asked if these LA neurons were directly involved in fear memory by analysing the expression of a known molecular requirement for fear memory in LA, phosphorylated Extracellular Signal Regulated Kinase (pERK). Fear conditioning induced pERK exclusively in the same discrete region of LA where we found learning specific *FTL+* neurons and a substantial proportion of these neurons double-labelled for FTL. These *FTL+* neurons in LA are thus most likely directly involved in fear memory. To determine if synaptic changes occurred in *FTL+* neurons, we adopted a fluorescence-based method to identify and record from these neurons in brain slices. Patch-clamp recordings in LA after fear conditioning revealed the *FTL+* neurons had increases in frequency and amplitude of spontaneous postsynaptic currents compared with *FTL-* neighbouring neurons. Thus, synaptic change occurs preferentially in the *FTL+* neurons activated by fear learning. This work has thus identified a discrete neuronal ensemble within LA which is directly involved in, and forms part of the wider circuitry for fear memory. Future experiments would seek to identify the precise learning related changes within these neurons and the mapping of their connections into the wider circuitry of fear memory.

Opioid disinhibition of the midbrain periaqueductal grey descending analgesic pathway

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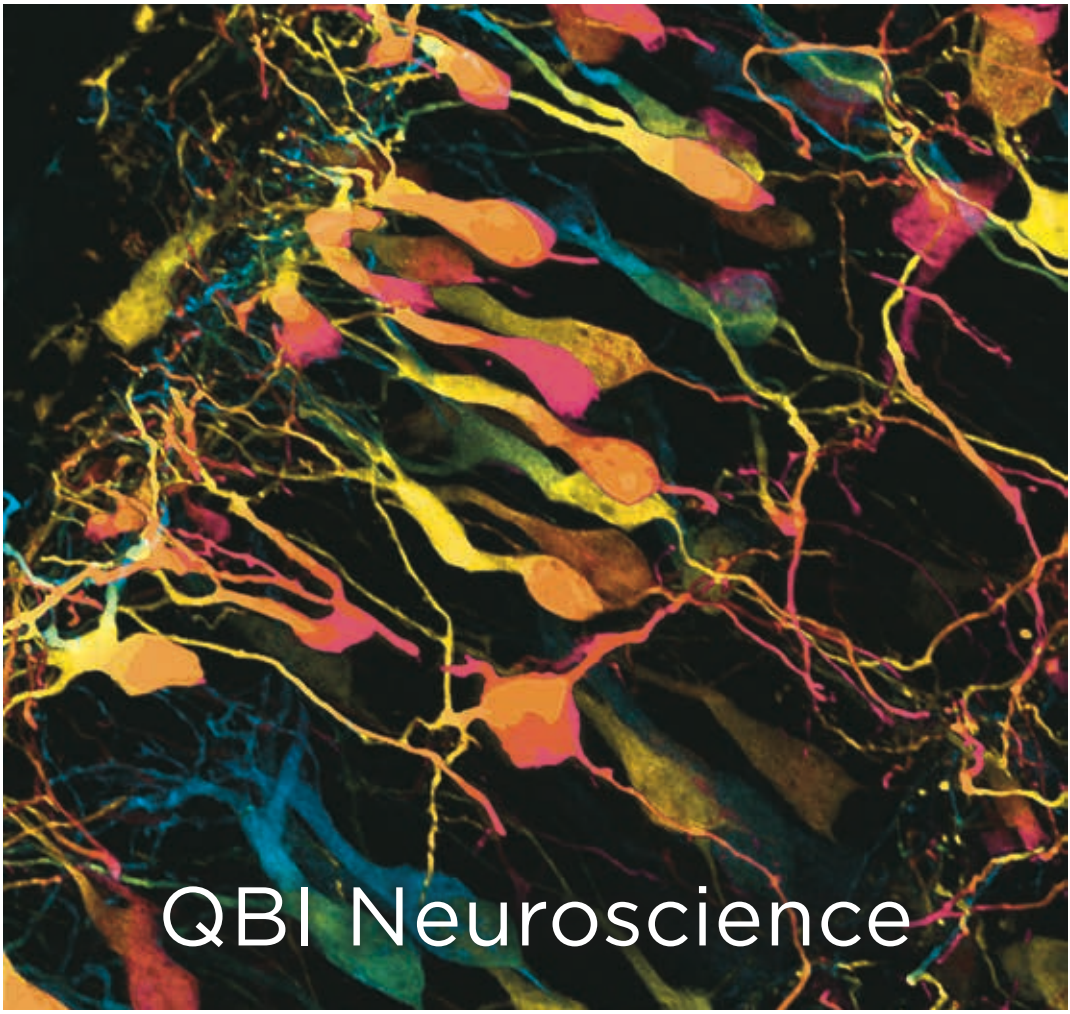
An important descending analgesic pathway projects from the midbrain periaqueductal grey (PAG) via the rostral ventral medulla (RVM) to inhibit ascending pain signals at the spinal level. Opioids are potent analgesics thought to activate this pathway by relieving the inhibition of PAG projection neurons by GABAergic interneurons. However evidence supporting this disinhibition hypothesis is indirect. Prior studies have not examined identified PAG projection neurons and the origin of the opioid sensitive GABAergic input is unknown. Our aim was to address this by directly investigating the effect of opioids on the input and activity of PAG projection neurons. To retrogradely label PAG projection neurons and gain optogenetic control of local inputs, we stereotaxically injected rhodamine-conjugated microspheres and AAV5-hSyn-ChR2(H134A)-eYFP into the RVM and PAG (respectively) of Sprague-Dawley rats. Performing whole-cell voltage-clamp recordings from rhodamine labelled cells in ex vivo PAG slices, we show that mu (DAMGO) and kappa (U69593) opioid receptor agonists reduce electrically evoked GABAergic currents (eIPSC) via a presynaptic mechanism. Optically evoked currents (oIPSC), which represent local inputs originating exclusively from within the PAG, were predominantly GABAergic. Interestingly, in contrast to eIPSCs, oIPSCs were more sensitive to DAMGO inhibition, whilst there was no difference in the U69593 effect. Further, the net effect of DAMGO was to unmask depolarising synaptic potentials in projection neurons, while hyperpolarising unlabelled neurons. Our findings offer direct support for the disinhibition hypothesis and indicate that whilst external inputs are both mu and kappa opioid receptor sensitive, local interneurons that directly innervate PAG projections cells are largely under mu opioid receptor control.



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