Symposium 21 – Opioids revisited: new developments and opportunities for opioid pharmacology

Theme: Neuronal, glial and cellular mechanisms

21.01. Mechanisms of μ-opioid receptor desensitisation and tolerance

Dr Chris Bailey - *University of Bath, UK*

Although ~40% of current drugs act through G protein-coupled receptors (GPCRs), either directly or indirectly, very few are GPCR agonists. A primary reason for this is agonist-induced desensitization of GPCRs, which leads to progressive loss of receptor function and drug tolerance. One currently used class of GPCR agonists is the mu-opioid receptor (MOPr), with agonists used clinically as analgesics and abused recreationally for their euphoric and rewarding properties. Tolerance to MOPr agonists compromises their long-term use as analgesics and contributes to the health and societal dangers of opioid addiction. Classically, desensitization of G protein-coupled receptors (GPCRs) is caused by phosphorylation of the receptor by G protein-coupled receptor kinases (GRKs) and subsequent recruitment of arrestin.

However, recent studies have shown various forms of functional selectivity of MOPr desensitization. First, different agonists can induce MOPr desensitization and tolerance by different intracellular mechanisms. Further, the rate and extent of MOPr desensitization depends on the receptor's cellular and subcellular localization.

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21.02. Ligand bias at the μ-opioid receptor

Professor Eamonn Kelly - *University of Bristol, UK*

Ligand bias refers to the ability of different agonists acting at a particular receptor type to activate distinct signalling pathways downstream of the receptor. This property is thought to be due to the ability of the agonists to stabilise distinct active conformations of the receptor. Accordingly it has been proposed that biased agonists at the mu opioid receptor (MOPr) might be able to activate neuronal signalling leading to therapeutically desirable effects (analgesia) but not signalling leading to undesirable effects (e.g. tolerance and dependence). Previously we investigated bias between G protein- and arrestin-dependent signalling for a range of MOPr agonists (McPherson et al, 2010, Mol Pharmacol 78:756-66) and identified endomorphin-2 as an arrestin-biased agonist (Rivero et al, 2012, Mol Pharmacol 82:178-88). More recently others have identified G protein-biased agonists (DeWire et al, 2013, J Pharmacol Exp Ther 344:708-17; Manglik et al, 2016, Nature 537:185-190). However to date it is still not fully clear which type of bias is really preferable for MOPr agonists as medicines; I will discuss some of the issues surrounding this. I will also discuss some more recent techniques to study ligand bias which we have been using, including Molecular Dynamics simulations and Phosphoproteomics/Bioinformatics. It is hoped that a combination of these approaches along with rigorous in vivo testing will allow us to determine the type of bias that is desirable, and hence develop appropriate ligands for the MOPr that will become more effective medicines for the future.

This work has been supported by the BBSRC and MRC.

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21.03. Biased ligand signalling for kappa opioid receptor agonists and antagonists

Professor Charles Chavkin - *University of Washington, USA*

When kappa opioid receptors are activated by pharmacological administration of selective agonists, humans report feelings of dysphoria and cognitive disruption. The endogenous dynorphins are also kappa opioid agonists that are released during stress exposure to mediate anxiety-like and aversive behaviors in rodents. These properties suggest that kappa opioid antagonists may have therapeutic potential by promoting stress-resilience in vulnerable individuals, and preclinical studies support the hypothesis that kappa antagonists may useful adjunct in the treatment of depression, anxiety disorders and drug addiction. Advancing these concepts has stimulated research in kappa opioid receptor signal transduction events, and new insights have revealed a complex pharmacology. Kappa receptor activation by efficacious agonists results in both G-protein signaling through G?? regulation of ion

channels and GRK/arrestin dependent signaling through p38 MAPK pathways. Kappa opioid receptor inactivation by antagonists can result from conventional competitive inhibition or from noncompetitive receptor inhibition through c-Jun Kinase activation mechanisms. Recent studies describing the signaling mechanisms responsible for functionally selective kappa agonism and antagonism will be presented.

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21.04. Circuit dynamics of in vivo dynorphin release in the nucleus accumbens shell

Dr Ream Al-Hasani - *Washington University School of Medicine, USA*

The nucleus accumbens (NAc) and the dynorphinergic system are widely implicated in motivated behaviors. Prior studies have shown that activation of the dynorphin-kappa opioid receptor (KOR) system leads to aversive, dysphoria-like behavior in both human and animal models (Shippenberg et al., 2007). However, the mechanisms and role of endogenous dynorphin in the regulation of KOR-mediated negative affective behaviors are unresolved. We used an optogenetic approach to demonstrate that stimulation of dynorphinergic cells in the ventral nucleus accumbens shell (vNAcSh) elicits robust aversive behavior and photostimulation of dorsal NAcSh dynorphin (dNAcSh) cells induces a place preference and is positively reinforcing. Both are dependent on kappa opioid receptor (KOR) activation. To follow these findings, we are investigating how KOR is able to mediate these opposing behaviors in two distinct regions of the NAcSh. We are using an opto-microdialysis approach which combines optogenetics with microdialysis for use in awake, freely moving mice. This system allows quantification of neuropeptide release while directly modulating cell-type specific neuronal firing in the NAcSh. Samples were analysed using liquid chromatography-mass spectrometry (LC-MS) detection. We have identified that the amount of dynorphin and met-enkephalin released during optogenetic stimulation is equal in the dNAcSh and vNAcSh. Interestingly, release of leu-enkephalin and dopamine is only detectable following photostimulation in the dNAcSh release. To understand the circuitry driving these opposing behaviors and distinct neuropeptide release profiles, we are mapping the projections to and from discrete regions with the dyn-reporter mouse and using multiple viral tracing approaches. Understanding the regional specificity by which NAc dynorphinerigic cells regulate preference and aversion provides insight into motivated behaviors that are dysregulated in stress, reward and psychiatric disease. Shippenberg, T.S., Zapata, A., and Chefer, V.I. (2007). Dynorphin and the pathophysiology of drug addiction.

Pharmacol. Ther. 116, 306–321.Supported by NIDA K99/R00 DA038725 (R.A), R01DA033396 (M.R.B).

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Symposium 22 – Information integration across the senses

Theme: Sensory and motor systems

22.01. The pain matrix ‘reloaded’: a multimodal saliency-detection system for the body and the peripersonal space

Professor Giandomenico Iannetti - *UCL, UK*

Neuroimaging and neurophysiological studies in humans have shown that transient nociceptive stimuli causing pain elicit responses in an extensive network of cortical structures. This network, often referred to as the “pain matrix”, has been assumed to specifically reflect nociceptive processing, and extensively used in the past 30 years to gain knowledge about the cortical mechanisms underlying nociception and pain perception in humans.

In the first part of this talk I will provide evidence that, in contrast with this dominant view, these brain responses are not specific for the perception of pain. These results indicate that it is incorrect to refer to these responses as originating from a “pain matrix”, and question the appropriateness of relying on them to infer that an individual is in pain, or to build models of where and how nociceptive input is processed in the human brain to generate painful percepts.

Instead, I will suggest that the largest part of these brain responses reflect a basic mechanism through which the individual detects, reorients attention and reacts to behaviourally-relevant sensory events, regardless of the sensory modality conveying this information.

In the second part of this talk I will provide evidence that these brain responses are sensitive to changes in the location of environmental threats with respect to the body, and are related to the execution of defensive movements aimed to protect the body from threats in the sensory environment.

I will finally show that the dependence of such responses on the position of threatening stimuli in space supports the existence of a part of space surrounding the body (a "defensive" peripersonal space, DPPS) representing a safety margin advantageous for survival.

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22.02. Multiple stages of multisensory perception: evidence from local cortical oscillations and functional connectivity

Dr Julian Keil - *Charité – Universitätsmedizin Berlin, Germany*

Multisensory processing requires the concerted activity of distinct cortical areas. At any given moment, we receive input from multiple different sensory systems, and this complex information needs to be processed and integrated. Local cortical oscillations and functional connectivity between distant cortical areas have been implicated as key mechanisms underlying multisensory processing. Evidence is now emerging which indicates that different aspects of multisensory processing are reflected in oscillatory neural activity of distinct frequencies. This talk will review the recent literature on the mechanisms underlying multisensory processing, focusing on neural oscillations. Based on recent findings, a model will be derived to integrate findings on bottom-up driven multisensory integration, the influence of top-down information on multisensory integration, and the role of predictions for the formation of multisensory perception. In this talk, the idea that cortical oscillations in different frequency bands are instrumental to distinct but complementary processing modes will be discussed. These modes act in parallel and are essential for multisensory perception.

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22.03. Auditory-visual integration in auditory cortex facilitates auditory scene analysis

Dr Jennifer Bizley - *UCL, UK*

Over the past decade there has been a paradigm shift in how we view early sensory cortex: we now know that multisensory interactions are abundant at the earliest stages of sensory processing. Despite physiological and anatomical evidence in support of crossmodal integration in sensory cortex, the role that such early integration plays in perception is much less clear. Exactly how and where crossmodal signals are linked ("bound") to form coherent perceptual constructs is also unknown. In this talk I will argue that one role for integrating visual information into auditory cortex is to support multisensory binding, and that audio-visual binding can enhance auditory scene analysis – i.e. the ability to separate an auditory scene into its component sources. I will present behavioural evidence that visual information can help listeners to separate competing sounds in a mixture. Extracellular recordings in auditory cortex demonstrate that when a visual stimulus is temporally coherent with one sound in a mixture, the neural representation of that sound is enhanced. I will discuss these data and their implications for our understanding multisensory interactions in sensory cortex.

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22.04. See what you hear - how the brain forms a representation across the senses

Professor Uta Noppeney - *University of Birmingham, UK*

To form a coherent percept of the environment the brain needs to integrate sensory signals from a common source and segregate those from different sources. Human observers have been shown to integrate sensory signals in line with Bayesian Causal Inference by taking into account the uncertainty about the world's causal structure. Combining Bayesian modeling, multivariate decoding and EEG/fMRI we show that the brain integrates sensory signals in line with Bayesian Causal Inference by encoding multiple perceptual estimates along the cortical hierarchy. Only at the top of the hierarchy, in anterior intraparietal sulcus, at about 300-500 ms the uncertainty about the world's causal structure is taken into account and sensory signals are combined weighted by their sensory

reliabilities and task-relevance as predicted by Bayesian Causal Inference. The intraparietal sulcus arbitrates between signal integration and segregation to guide behavioural choices and motor responses.

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Symposium 23 – The APOE paradox – Pathway to Alzheimer's disease

Theme: Neurodegenerative disorders and ageing

23.01. APOE4 from man to mouse

Dr Sarah King - *University of Sussex, UK*

Carrying the E4 variant of the Apolipoprotein E (APOE) gene is the greatest risk factor for sporadic Alzheimer's disease aside from age. Along with the increased Alzheimer's risk carrying one or two copies of E4 associates with cognitive impairments in older adulthood. Paradoxically in younger adults, E4 can be associated with cognitive benefits. This talk will investigate performance and functional imaging data for cognitive tasks in young and middle aged participants (APOE3 (control) vs APOE4 carriers) and how these might relate to suggested hypotheses of APOE4 function across the lifespan, e.g. antagonistic pleiotropy or accelerated ageing. Irrespective of differences in cognition, we repeatedly see differential recruitment of brain areas during performance between genotype: e.g. in young adults, during a rapid visual information processing task (RVIP) we see differential activation of frontal and parietal activity in APOE3 homozygotes and APOE4 carriers respectively. In other tasks (e.g. prospective memory and covert attention) we see genotype differences in performance and concurrent neural activity between young and mid-age participants. Understanding how APOE impacts functional brain activation and cognition across the lifespan will enable us to predict at what stage APOE-targeted therapies are most likely to beneficial in preventing or reversing age-related cognitive decline and Alzheimer's disease. As well as human studies we are using targeted replacement mice, carrying the human APOE genes to determine the tipping point between the beneficial and deleterious effects of APOE4.

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23.02. APOE4 across the ages: what changes when? MRI signatures of brain function in humans

Dr Sana Suri - *University of Oxford, UK*

The apolipoprotein E (APOE) gene has three alleles (ε 2, ε 3, and ε 4) that differently influence lifetime risk for developing late-onset Alzheimer's disease (AD). The ε 4 allele is the best-established genetic risk factor for AD, whereas the ε 2 allele may be protective. Give its close association with a risk for AD much of the APOE research has, understandably, focused on ε 4, with the putative protective role of ε 2 receiving little attention (1). Magnetic resonance imaging (MRI) studies have found that ε 4 influences brain function decades before potential cognitive decline, but that its effects may vary across the lifespan. Thus, while younger ε 4 carriers show greater hippocampal activation during memory tasks than ε 3 homozygotes, this pattern appears to be reversed in older ages (2). Differences between ε 4 carriers and ε 3 homozygotes have typically been attributed to risk for AD, however recent MRI studies reporting similarities in brain activity in ε 2 and ε 4 carriers seem to question this interpretation (3). Explaining this paradox would not only further our understanding of the complexities of ε 4, but also lend valuable insights into why ε 2 carriers lead relatively long and healthy lives. With a focus on multi-modal MRI techniques examining brain structure, function and vascular health, this session will review how APOE-mediated risk and protection for AD are represented within the brain across a wide age-range, and highlight some of the challenges of reproducibility as they relate to neuroimaging studies of APOE.

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23.03. Using APOE targeted replacement mice to probe APOE4 function

Professor Daniel Michaelson - *Tel Aviv University, Israel*

Alzheimer's disease (AD) can not be treated effectively. Since AD is heterogeneous a possible novel approach to this problem is to focus on sub populations of AD patients which share common genetic risk factors. Apolipoprotein E4 (apoE4) is the most prevalent genetic risk factor for AD. More than half of the AD patients express apoE4 and it increases the risk for AD by advancing the age of onset by 7 to 9 years per allele copy. ApoE4 is thus a promising AD therapeutic target.

We will first review the suggested mechanisms of action of apoE4 with particular emphasis on animal model translational approaches to counteract its pathological effects. These studies can be divided into approaches which focus on the apoE4 molecule and try to either remove it from the brain or to reverse its' structural pathological properties, and to downstream approaches which are directed at reversing biochemical processes specifically triggered by apoE4. The former include immunotherapy which show, in analogy to previous amyloid- β studies, that key pathological effects of apoE4 can be counteracted by peripheral injections of specific anti-apoE4 mAbs (1). The studies directed at reversal of structural pathological properties of apoE4 focus on the observation that apoE4 is hypolipidated and reveal that the apoE4 driven brain patrhological effects in apoE4 expressing mice and the associated cognitive deficits can be counteracted by treatments which reverse the hypolipidation of apoE4(2). The down stream biochemical approach showed that key behavioral and brain pathological effects of apoE4 in mice can be reversed by counteracting the effects of apoE4 on distinct signaling pathways, such as VEGF.

In conclusion, several novel apoE4 related therapeutic approaches have been identified. Further studies are required for assessing the relative impact and possible complementarity of these apoE4 directed translational approaches.

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2. Boehm-Cagan,A. Bar, R.., Liraz,O., Bielicki,J.K., Johansson, J.O., and Michaelson, D.M. (2016)ABCA1 agonistr reverses the apoE4 – driven cognitive and brain pathology. J Alzheimer's Dis 54 (3), 1219-1233

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23.04. Structural and cellular studies to elucidate the mechanisms of APOE isoform action and provide targets for therapy

Professor Louise Serpell - *University of Sussex, UK*

Alzheimer's disease is characterised pathologically by the deposition of Amyloid-beta in extracellular amyloid plaques and tau in neurofibrillary tangles in the cell bodies of neurons. Hereditary forms of Alzheimer's disease have been linked to the over production of a wild type or variant form of the Amyloid-beta peptide. However, other genes have been identified that are linked to disease. For example, ApoE genotype is a major risk factor for late onset Alzheimer's disease, whereby being ApoE4 homozygous leads to an increased risk of developing Alzheimer's. Our research focuses on exploring the roles of Amyloid-beta, tau and the ApoE genotype on the downstream neurodegeneration central to Alzheimer's disease pathology. In order to elucidate mechanisms that lie at the centre of the disease progression, we have explored the interplay between these three key players. Here we report new insights into the role of Amyloid-beta in cellular toxicity, its downstream effects on tau and the interplay with the ApoE proteins.

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Symposium 24 – Epilepsy and precision medicine

Theme: Novel treatments and translational neuroscience

24.01. Epilepsy genetics: contributions to cause and management

Professor Sanjay Sisodiya - *UCL, UK*

Epilepsy is not one condition, but a diverse group of entities that share the common phenomenon of recurrent seizures. In many cases, there are additional co-morbidities. Seizures and co-morbidities carry important risks, including that of heightened premature mortality, making epilepsy a burdensome condition. Complete seizure control is a key aim of treatment, and is the only proven method to improve overall quality of life. There are many treatments in use. The best guide to the use of currently available treatments is knowledge of the cause of the epilepsy, or as a surrogate, the type or syndrome of epilepsy. For most cases today, the cause of an individual's epilepsy remains unknown, even in the presence of a proximal abnormality on brain imaging. Where a precise cause is known, there are sometimes targeted treatments available, or at least guidance on the best choice from available treatments.

Genetic studies in the epilepsies have advanced understanding of cause and disease biology significantly over the last few years, driven on by large collaborations and the application of massively-parallel sequencing. Many rare epilepsies have now been solved at a genetic level, with new knowledge promoting drug adjustments or repurposing with a view to more rational treatment. In some cases, this has led to dramatic progress, with seizure freedom being associated with significant improvements in cognitive performance and quality of life. For some types of epilepsy, in particular the epileptic encephalopathies, the emerging picture is of a large collection of individually-rare conditions driven by gene mutation. Networks of such genes are emerging, driving a new mechanistic understanding of brain function and its disruption.

Important issues remain. Sequencing often provides too many options. Next-generation genetics is often not joined by next-generation phenotyping. 'Precision medicine' remains supported only anecdotally. Most cases of epilepsy, especially the 'common', remain unexplained. Other important facets, e.g. cognitive decline, premature mortality, have yet to be studied at scale. Genetics may help, but is likely only to form part of a broader understanding.

Funding: Epilepsy Society, Wellcome Trust, European Commission

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24.02. Aberrant glutamatergic signalling in brain tumour related seizures: opportunities for precision medicine

Dr Mark Cunningham - *Newcastle University, UK*

Brain tumours present with seizures as a major symptom in 30% of all cases. Seizures complicate the overall management of patients with glioma and contribute to significant morbidity. Tumour associated seizures also demonstrate significant resistance to treatment with anti-epileptic drugs. Attempts to understand the mechanisms underlying epileptogenic tumours have involved the development of animal models. The validity of these animal models and how adequately they recapitulate the human condition has been questioned. For example, the current animal models are more likely to match the clinical course of high grade gliomas but not that of low grade gliomas, the latter being more likely to be epileptogenic. Moreover, studying seizures towards the end stage of the animal life is complicated by considerable welfare concerns about animals with large intracranial brain tumours. In addition to these welfare concerns, a scientific limitation is the lack of availability of 'true' animal models of low grade glioma. To circumvent this, we routinely receive samples of live human brain tissue obtained from patients with seizures and low grade gliomas undergoing neurosurgery. We examine neocortical tissue from around the tumour, in the so-called 'peritumoural' region, using in vitro electrophysiology. This region is important as it contains the interface between 'normal' neurons and invading glioma cells. Increasing evidence points to this region as the area from which seizures arise. As such, understanding the processes that occur in this area will provide a better insight into the mechanisms that underlie tumour associated epilepsy. We have been examining the contribution of aberrant glutamatergic signaling in the peritumoural region and I will present electrophysiological and pharmacological data from our studies that illustrates how the neurobiological basis of epileptogenic tumours can be used to precisely inform the management of seizures associated with low grade gliomas.

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24.03. Autoantibody-mediated forms of epilepsy

Dr Sarosh Irani - *University of Oxford, UK*

Autoimmune encephalopathies are an expanding group of potentially treatable syndromes. Each is defined by the antigenic target of the autoantibody and the associated clinical features, which typically include neuropsychiatric features in addition to seizures.

These syndromes are likely to be mediated by the autoantibodies which are directed against neuronal membrane proteins, most commonly the NMDA-receptor, the secreted neuronal protein leucine-rich glioma-inactivated 1 (LGI1), the GABAB-receptor and contactin-associated protein 2 (CASPR2). Correspondingly, the patients often respond well to interventions which reduce the levels of autoantibodies, including corticosteroids, plasma exchange, intravenous immunoglobulins, cyclophosphamide and/or rituximab. As early immunotherapies improve outcomes, the importance of accurate clinical recognition is paramount.

In this talk, I will focus on the various forms of epilepsy associated with these autoantibodies, the methods used to detect the autoantibodies, the localisations and characteristics of the seizure semiologies, their response to antiepileptic drugs and immunotherapies, and the long-term prognoses for these patients. In particular, I will focus on the recently described semiology of faciobrachial dystonic seizures in patients with LGI1-antibodies.This syndrome is clinically distinctive and shows a remarkably rapid response to immunotherapies whilst being relatively refractory to anti-epileptic drugs.

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24.04. Autonomic modulation as a therapy for epilepsy: effective and non-invasive approach for future treatment

Dr Yoko Nagai - *University of Sussex, UK*

Over a decade ago, Nagai and her colleagues (2004a) introduced biofeedback to modulate sympathetic activity (Galvanic Skin Response: GSR) in patients with drug resistant epilepsy. The first randomized controlled trial demonstrated a robust positive effect that 60% of patients in the active biofeedback therapy group experienced seizure reduction of more than 50%. The therapy was established based on a series of neuroscientific studies characterizing an inverse relationship between EEG indices of cortical neural excitability and peripheral sympathetic arousal [Nagai et al., 2004b, 2009]. An increase in sympathetic activity reduces cortical excitation. Nagai et al., (2004, c) also identified that ventromedial prefrontal cortex (VMPFC) activity is inversely correlated to the tonic level of GSR suggesting that this part of the brain is an important hub for modulation of sympathetic activity.

In the current study, we conducted a wider clinical trial with 40 patients with drug resistant temporal lobe epilepsy (N= 20 Therapy group, N = 20 Control). Neuroimaging (fMRI) was conducted to explore neural network changes before and after GSR biofeedback intervention. A month of therapy training, elicited a significant reduction in the patients' seizure frequency ($p<0.001$). In the active therapy group, 9/20 of patients showed a reduction in seizure frequency of over 50%. The average seizure reduction in the active therapy group was -42.99%, compared to an increase of +31.07% seizure increase in the control (treatment as usual) group ($p < 0.001$). Resting state functional neuroimaging revealed that the patients who reduced greater number of seizures after a month of therapy training weakened neural connectivity between VMPFC and amygdala suggesting increased resilience of patients to stress and anxiety induced seizures.

Our combined clinical trial and neuroimaging study demonstrates the potential of autonomic biofeedback as an effective technology-driven therapy that can be widely offered for patients with drug resistant epilepsy in the near future.

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Symposium 25 – Environment and synaptic function

Theme: The neurobiology of stress

25.01. Slave to the rhythm - ultradian glucocorticoid rhythms regulate distinctive gene expression profiles in the brain and pituitary

Dr Becky Conway-Campbell - *University of Bristol, UK*

The endogenous glucocorticoids (GCs) cortisol and corticosterone are secreted from the adrenal glands in a characteristic pulsatile manner, establishing an ultradian pattern. We have demonstrated, both in cell models and in vivo, that ultradian GC exposure induces a functional output in individual target cells. The intracellular GC receptor (GR) is activated in distinct pulses and transmits this signal to the chromatin template, resulting in a 'gene pulsing' effect for transcriptional regulation of GC target genes. Notably, dysregulated GC pulse characteristics are reported in a wide variety of chronic pathophysiological conditions, including Cushing's Disease and Obstructive Sleep Apnea. Symptoms including cognitive and affective dysfunction are often reported in these patients, therefore we have assessed the effect of altering the endogenous ultradian GC pattern on transcriptional output in the hippocampus, a brain region involved in cognitive processing and affective state. RNA-Seq expression profiling of hippocampus from adrenalectomised rats replaced with pulsatile or constant corticosterone revealed specific pattern-dependent regulation of GC target genes. Furthermore, chronic treatment with synthetic GCs (sGCs) resulted in even greater dysregulation of the endogenous GC profile. sGCs such as dexamethasone and prednisolone are potent anti-inflammatory agents, but have well-documented adverse side effects including memory impairment. Therefore we have characterised the molecular, physiological and cognitive impairments arising from chronic sGC treatment. Notably, we report prolonged central and pituitary GR activation, disruption of circadian GR activity and GC target gene regulation, disruption of circadian activity and body temperature profiles, and impaired hippocampal-dependent memory consolidation in the sGC treated rats.

In conclusion, we present a role for the endogenous GC ultradian rhythm in maintaining optimal function of GC target tissues, including the brain and pituitary. Pathophysiological or pharmacological alteration to GR dynamics can therefore result in profound functional changes in target tissue function, adversely affecting circadian physiological processes and functional output of discrete brain regions including the hippocampus.

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25.02. Stress, glutamate receptor trafficking and synaptic plasticity

Dr Garry Whitehead - *University of Bristol, UK*

Environmental stressors can have profound effects on the brain, both good and bad. Acute exposure to these stressors can have beneficial outcomes on brain function in terms of learning and memory, whilst continued exposure to throughout the lifespan of an individual has been linked with the onset of various pathological disorders in the later years of life. In this talk I will present data showing how stress hormones can be both beneficial and detrimental to synaptic function. I will begin by showing how brief stress can enhance synaptic activity through changes in glutamate receptor composition at the synaptic membrane. I will then discuss how this occurs in stark contrast to the effects of prolonged stress, which induce synaptic weakening via a mechanism that requires the phosphorylation of the microtubule binding protein tau. Finally, I will introduce some of our most recent work investigating the optogenetic control of the endogenous cellular environment of neurons, and how we will use this approach to identify links between perturbations in cellular cycles and the onset of disease.

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25.03. Dopamine-mediated regulation of expression of fear memory

Dr Joung-Hun Kim - *Pohang University of Science and Technology, South Korea*

Amygdala inhibitory circuits are considered to play regulatory roles for threat-related memory, but the functional and physiological effects that each inhibitory module exerts remain poorly understood. For example, while intercalated cell masses of the amygdala (ITC) seem to be required for fear extinction, the synaptic plasticity at a specific input are not elucidated and its functional roles have not been explored for acquisition and/or expression of fear memory. We show that synapses at the dorsal ITC undergo long-term depression (LTD) only upon exposure to less-salient experience, but not to salient experience. LTD in the LA-ITC pathway,

depends on dopamine and DrD4 activity. Mechanistically, this type of LTD is likely to be formed via presynaptic mechanisms, which would involve an increase of GABA release from neighboring ITC neurons. Pharmacological, genetic and optogenetic manipulations reveal that this LTD limits less salient experiences from forming persistent memory. In further support of the idea that LTD has a preventive and discriminative role, we find that in mice exhibiting PTSD-like behaviors, LTD at the dorsal ITC is impaired. These findings indicate a novel role that an inhibitory circuit in the amygdala has, which serves to dampen and restrict the level of fear expression. Given the importance of GABAergic signaling and potential relevance to psychiatric disorders, we also provide tangible evidence for possible molecular and cellular mechanisms whereby synaptic plasticity arises and is maintained at the amygdala inhibitory circuit.

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25.04. Strategies for preventing in vivo hippocampal synaptic plasticity disruption by stressors

Professor Michael Rowan - *Trinity College, Dublin, Republic of Ireland*

Psychological and cellular stressors dramatically change our behavior and in extreme can trigger neurological and psychiatric illnesses. Excessive stress caused by inescapable aversive environments and neurotoxic insults have profound and sometimes similar effects on synaptic plasticity mechanisms in the brain. This presentation will review how such stressors change the direction of synaptic plasticity in the rodent hippocampus, such that long-term potentiation (LTP) is inhibited whereas LTD is facilitated in vivo.

The mechanisms underlying such profound changes in synaptic plasticity often include the engagement of shared stress pathways of the innate immune system. In particular, the elevation of the levels of certain pro-inflammatory cytokines following stressors may be critical. Amongst cytokines, ongoing research implicates elevated concentrations of interleukin 1ß (IL1ß) and tumor necrosis factor α (TNFα). For example, our recent investigations strongly implicate these mediators in the inhibition of LTP or facilitation of LTD by intracerebral injection of Alzheimer's disease amyloid ß protein (Aß) aggregates in anaesthetized rats, or by endogenously generated Aß in freely behaving transgenic rats that provide a very complete animal model of Alzheimer's disease amyloidosis. Thus, agents that decrease the production or directly neutralize these cytokines have rapid and reversible effects, as does inhibition of the inflammasome.

Cytokines can disrupt synaptic plasticity in many ways but our research has focused especially on the likely role of disrupted glutamate homeostasis. Thus agents preventing inappropriate activation of certain subtypes of NMDA and metabotropic glutamate receptors, and blood-based interventions that promote clearance of glutamate from the brain can abrogate deficits in LTP in vivo.

Based on accumulating evidence directly targeting cytokines or their downstream effectors, prevents synaptic plasticity disruption in a number of stress-related models of disease, including depression and Alzheimer's disease. New and ongoing clinical trials, informed by this area of research, will hopefully have significant therapeutic impact.

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Symposium 26 – Why neuroinformatics and computational modelling matters for neuroscience

Theme: Methods and techniques

26.01. Neuroinformatics tools for sharing and analysing data

Professor Leslie Smith - *University of Stirling, UK*

Neuroscience data arises from many types of experiment, and arrives in various formats. Some formats are open (for example, image formats), but many others are proprietary. Analysis tools that are to be shared must either be able to read the data directly (which implies an open format, or at least that the structural metadata for the file is known). Alternatively software that allows interrogation of files has to be supplied by the owners of the proprietary format. Open formats are clearly to be preferred: however, scientists wanting to analyse data cannot choose the format in which the data is provided. Often all that is available is either analysis software provided by the organisation that developed the system providing the data, or software (often DLLs) for file interrogation. Certainly the scientific community does all it can to encourage the uptake of open formats (e.g. [1]), but sometimes commercial interests supervene.

Openness in the analysis tools is equally important. Many tools are based on equations in papers (and published at the same time as the paper), but the precise implementation in code of an equation may make a difference to results. Being able to inspect the (well commented) code can allow analysts to determine exactly what the tool does. Often there are a number of different techniques available for analysis of specific types of data (e.g., for spike sorting of extracellular recordings [2]), and analysts would like to be able to compare the results of different techniques, or simply of single techniques with different parameters.

Neuroscience research is a worldwide co-operative venture. Organisations such as the INCF (https://www.incf.org) exist to encourage and enable data and analysis tool sharing. The Neuroscience Information Framework (https://neuinfo.org) supports sharing of data tools and other materials. Portals can be used for sharing data, and systems like Github (https://github.com) used for sharing open analysis techniques.

[1] Science as an open enterprise, Royal Society Science Policy Centre Report 02/12

[2] Mahmud, M., & Vassanelli, S. (2016). Processing and Analysis of Multichannel Extracellular Neuronal Signals: State-of-the-Art and Challenges. Frontiers in Neuroscience, 10(16), 708.

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26.02. Modelling plasticity in networks

Dr Claudia Clopath - *Imperial College London, UK*

We are broadly interested in the field of neuroscience, especially insofar as it addresses the questions of learning and memory. Learning is thought to change the connections between the neurons in the brain, a process called synaptic plasticity. Using mathematical and computational tools, we model synaptic plasticity across different time scales that reproduces experimental findings. We then study the role of synaptic plasticity, by constructing networks of artificial neurons with plastic synapses.

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26.03. Statistical long-term excitatory and inhibitory synaptic plasticity

Dr Tim Vogels - *University Oxford, UK*

Long-term modifications in neuronal connections are critical for reliable memory storage in the brain. However, pre- and postsynaptic components can make synapses highly unreliable. How synaptic plasticity modifies this variability is poorly understood. Here we introduce a theoretical framework in which long-term plasticity performs an optimisation of the postsynaptic response statistics constrained by physiological bounds. In this framework of statistical long-term synaptic plasticity the state of the synapse at the time of plasticity induction determines the ratio of pre- and postsynaptic changes. When applied to plasticity of excitatory synapses, our theory explains the observed diversity in expression loci of individual hippocampal and neocortical potentiation and depression experiments. Moreover, our theory predicts changes at inhibitory synapses that are bounded by the mean excitation, which suggests an efficient excitation-inhibition balance in the brain. Our results propose a principled view of the diversity in expression loci of long-term synaptic plasticity observed in a wide range of slice experiments and reveal a statistically optimal, excitation-inhibition balance in the intact brain.

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26.04. Linking network structure and function in the cerebellar cortex

Professor Angus Silver - *UCL, UK*

Understanding how the structure of biological systems influence their function is a core research theme that cuts across multiple scales. Linking structure to function is challenging because it typically involves inferring dynamic properties, involving physio-chemical processes, from static structural information. This requires both experimental approaches and mathematical modelling. In neuroscience, the relationship between brain structure and function is poorly understood at most spatial scales. In this presentation I will draw on our recent work examining how the structure of the input layer of the cerebellar cortex contributes to the

transmission and transformation of sensorimotor information as it flows through the cerebellar cortex. In particular, I will show how the ultrastructure of mossy fibre synapses influence their ability to signal rate coded information in a sustained manner. While at the circuit level, I will show how the evolutionary conserved feedforward network structure of the cerebellar input layer, which is characterized by a considerably larger number of granule cells (outputs) than mossy fibres (inputs), and by each granule cell receiving few synaptic inputs, is optimized for performing spatial decorrelation and pattern separation.

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Symposium 27 – Towards a causal understanding of motor learning in humans: a role for non-invasive brain stimulation

Theme: Sensory and motor systems

27.01. Combining non-invasive brain stimulation with magnetic resonance imaging and spectroscopy to probe motor learning

Dr Charlotte Stagg - *University of Oxford, UK*

Learning new motor skills such as playing the piano or riding a bike is of fundamental importance not only for healthy people but also in the recovery of function after a stroke. How we learn and retain these skills is therefore a major neuroscience question with clear implications for clinical research.

Neuroimaging methodologies such as Functional Magnetic Resonance Imaging (fMRI), as well as related techniques such as Magnetic Resonance Spectroscopy (MRS), have provided a wealth of knowledge as to which brain regions associated with learning, but cannot inform us if these regions are causally involved in that learning. To overcome this, non-invasive brain stimulation (NIBS) techniques, which are capable of transiently and reversibly modulate activity within specific regions of the human brain, are increasingly being used to study the neural correlates of learning and explore the extent and limitations of neuroplasticity.

Here I will discuss how NIBS, in combination with MR techniques, has been used to explore the mechanisms underlying neuroplasticity in humans, using motor learning as an exemplar.

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27.02. Using non-invasive brain stimulation to study the role of primary motor cortex in motor learning

Dr Sheena Waters - *UCL, UK*

What is the role of ipsilateral primary motor cortex in motor learning? One view supposes that ipsilateral activity suppresses contralateral motor cortex, and, accordingly, that inhibiting ipsilateral regions can improve motor learning. Alternatively, the ipsilateral motor cortex may play an active role. We approached this question by applying double-blind bihemispheric transcranial direct current stimulation (tDCS) over both contralateral and ipsilateral motor cortex in a between-group design during four days of unimanual explicit sequence training in human participants. Independently of whether the anode was placed over contralateral or ipsilateral primary motor cortex, bihemispheric tDCS yielded substantial performance gains relative to unihemispheric or sham stimulation. The performance advantage associated with bihemispheric tDCS appeared to be supported by plastic changes in both hemispheres. First, we found that behavioural advantages generalised strongly to the untrained hand, suggesting that bihemispheric tDCS strengthened effector-independent representations. Secondly, functional imaging during speed-matched execution of trained sequences conducted 48 h after training revealed sustained, polarity-independent increases of activity in both motor cortices of bihemispheric tDCS recipients relative to sham. Finally, we found significant increase of effector-independent sequence encoding in both hemispheres in the bihemispheric tDCS groups relative to sham, and this measure was significantly correlated with the degree of behavioural generalisation. These results suggest a cooperative (rather than competitive) interaction of the two motor cortices during skill learning and suggest that bihemispheric brain stimulation during unimanual skill learning may be more beneficial than unihemispheric stimulation simply because it harnesses plasticity in both hemispheres.

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27.03. Non-invasive brain stimulation to dissociate the roles of the cerebellum and motor cortex in motor learning

Dr Joseph Galea - *University of Birmingham, UK*

Visuomotor adaptation has revealed important principles regarding motor learning and memory (Krakauer, 2009). Although computational and behavioural studies have suggested that the acquisition and retention of a new visuomotor transformation are distinct processes, this dissociation had never been clearly shown. I will describe work in which we used transcranial direct current stimulation (tDCS) to show that cerebellar tDCS caused faster adaptation to a visuomotor transformation, as shown by a rapid reduction of movement errors. In contrast, M1 tDCS did not affect adaptation but resulted in a marked increase in retention of the newly learnt visuomotor transformation (Galea et al., 2011). These results support the view that visuomotor acquisition and retention are independent processes, and demonstrate that the cerebellum and primary motor cortex have distinct functional roles. Next, I will discuss recent work which investigates the underlying mechanism of cerebellar tDCS. Using magnetic resonance spectroscopy and resting state functional magnetic resonance imaging (Bachtiar et al., 2015), we show that in a subset of participants (30-40%) cerebellar tDCS caused a reduction in local GABA and an increase in connectivity between the cerebellum and parietal cortex. Despite these changes being correlated not only with each other but with the effect cerebellar tDCS has on visuomotor adaptation, we believe the results reflect an 'all-or-nothing' effect of cerebellar tDCS across individual participants.

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27.04. The offline brain: understanding the regulation of memory consolidation using non-invasive brain stimulation

Professor Edwin Robertson - *University of Glasgow, UK*

Our memories continue to be processed "off-line" following their formation. We have an increasingly sophisticated understanding of these off-line processes, which lead to the reorganization, enhancement and stabilization of memories. Yet, how these off-line mechanisms are controlled leading to some memories being enhanced over wakefulness, while for others this is delayed until sleep is poorly understood. The processing pathway that a motor skill memory follows may be determined by functional changes within motor circuits. We tested this idea using transcranial magnetic stimulation (TMS) to measure cortical excitability at various time points after participants had learnt tasks that either were or were not enhanced over wakefulness. We found that cortical excitability does not change after learning a motor skill that is subsequently enhanced. By contrast, there is a substantial but transient decrease in excitability after learning a motor skill that is not enhanced. Preventing the decrease in cortical excitability by applying TMS alters the fate of the motor skill memory leading to its enhancement. Together, these experiments suggest that a decrease in cortical excitability prevents improvements from developing over wakefulness, and so when this signal is abolished improvements are induced.

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Symposium 28 – Epigenetics, placenta and developmental programming: coordination of mother and offspring brain

Theme: Genetics and epigenetics

28.01. Prenatal glucocorticoids and the developing brain

Dr Mandy Drake - *Queen's Medical Research Institute, Edinburgh, UK*

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Exposure to an adverse environment in early life is associated with an increased risk of cardiometabolic and neurodevelopmental disease in later life. In this talk I will discuss work describing the effects of prenatal glucocorticoids and maternal nutrition on

offspring neurodevelopment and additionally present some of our recent work in preterm babies exploring the mechanisms underpinning later neurodevelopmental problems.

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28.02. Maternal protein restriction around conception increases foetal neuronal differentiation and is associated with adult memory deficits

Dr Sandrine Willaime-Morawek - *University of Southampton, UK*

Maternal malnutrition during pregnancy is detrimental to foetal development and increases the risk of many chronic diseases in later life i.e. increased risk of schizophrenia. Previous studies have shown maternal protein malnutrition during pregnancy and lactation compromises brain development in late gestation and after birth, affecting structural, biochemical and pathway dynamics with lasting consequences for motor and cognitive function. However, the importance of nutrition during embryogenesis for early brain development is unknown. We have previously shown maternal low protein diet confined to the preimplantation period (Emb-LPD) in mice is sufficient to induce cardiometabolic and behavioural abnormalities in adult offspring. Using the same diet model, female mice were fed different diets from conception to the end of pregnancy: normal protein diet (NPD), low protein diet (LPD) or embryonic LPD (Emb-LPD: LPD for 3.5 days, NPD thereafter). Foetal brains were analysed during gestation with in vivo analysis using FACS and immunofluorescence for neurogenesis markers, and in vitro techniques using the neurosphere assay. Follow up behavioural tests in the offspring were performed, including the short-term memory novel object recognition. We have shown that Emb-LPD and sustained LPD reduce neural stem and progenitor cell numbers through decreased proliferation in both ganglionic eminences and cortex of the foetal brain at E12.5, E14.5 & E17.5 ($p=0.001$). Moreover, Emb-LPD causes remaining neural stem cells to upregulate the neuronal differentiation rate in compensation beyond control levels during gestation, independently of sex ($p<0.001$). When analysing the adult offspring behaviour, the Emb-LPD males and females show a clear deficit in short-term memory ($p=0.00001$). Our data are the first to demonstrate clearly that poor maternal nutrition around conception has adverse effects on early brain development and is associated with adult memory deficits.

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28.03. Sexually dimorphic programming of the developing dopamine system, with consequences for adult behaviour, by a low protein diet restricted to gestation

Dr Gráinne McNamara - *Cardiff University, UK*

Prenatal development is a time point of heightened vulnerability to the external environment. A suboptimal in utero environment has been associated with an increased risk of various metabolic and psychiatric disorders in later life. Numerous studies demonstrate that the developing nervous system can be influenced by environmental factors, including maternal diet, during pregnancy. Specifically, a suboptimal maternal diet has been linked to altered dopaminergic function. This observation may explain the association between early adversity and an increased risk of psychiatric disorders that are associated with dopaminergic dysregulation, including schizophrenia and substance abuse disorders. We find that a low protein diet (LPD) during gestation alone is sufficient to induce changes in the dopaminergic system at E18.5. Moreover, these changes were sexually dimorphic, even prior to parturition. These were associated with increased expression, and decreased promotor methylation, of the tightly epigenetically regulated gene, Cdkn1c, which may contribute to the misprogramming of the dopaminergic system. Furthermore, a LPD restricted to gestation was associated with sexually dimorphic changes in behaviours, including activity levels and inhibition of a startle response. These are suggestive of an altered dopaminergic system and link the observed prenatal neurobiological changes to behavioural outcomes. Importantly, this sexually dimorphic response to a prenatal stressor may have relevance to the gender differences in the rate of occurrence of a number of neurological disorders in humans.

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28.04. Prenatal maternal depression and aberrant placental imprinting

Dr Anna Janssen - *Cardiff University, UK*

Maternal depression during pregnancy is associated with fetal growth restriction and adverse neurodevelopmental outcomes. Imprinted genes have a well-established role in fetal growth and have been directly implicated as mediators of maternal behaviours. Moreover, a number of imprinted genes regulate the placental endocrine lineage and may therefore affect placental signals that prime the maternal brain for pregnancy.

This study investigated whether placental expression of the imprinted gene PEG3 and the placental lactogen hPL was associated with maternal prenatal depression in three independent cohorts. In the Queen Charlotte's (N=40) and MBAM Cohorts (N=81) participants were recruited before elective c-section and symptoms of prenatal depression assessed using the Edinburgh Postnatal Depression Scale (EPDS), with higher scores indicating increasing symptoms of depression. A diagnosis of depression during pregnancy was recorded from Manchester Cohort participant's medical notes (n=75). Villous trophoblast tissue samples were analysed for gene expression.

There was a significant decrease in both placental PEG3 and hPL expression in association with maternal depression in all three cohorts (Janssen et al. 2016). In all cohorts, the association between PEG3 and maternal depression was significant in male but not female placentas.

These novel findings provide the first evidence that aberrant placental imprinting is a feature of prenatal depression, which may have important implications for long-term offspring outcomes.

Janssen, A.B. et al. (2016). Maternal prenatal depression is associated with decreased placental expression of the imprinted gene PEG3. Psychological Medicine p. 1-13.

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Symposium 29 – From channelopathies to synaptopathies

Theme: Neuronal, glial and cellular mechanisms

29.01. Inherited and acquired presynaptic channelopathies

Professor Dimitri Kullmann - *UCL, UK*

Several neurological diseases are caused by mutations of ion channels that normally reside in axons and presynaptic terminals. These include CaV2.1 calcium channels (associated with dominantly inherited forms of migraine and episodic ataxia), and BK and Kv1.1 potassium channels (associated with paroxysmal dyskinesia, epilepsy and another form of episodic ataxia). Cav2.1 channels are also the target of autoantibodies in Lambert-Eaton myasthenic syndrome, and other autoantibodies may exert indirect effects on potassium channels. Elucidating the disease mechanisms requires not only an understanding of where the normal ion channel is located, but also the effects of the mutation or antibody on ion channel function and ultimately on action potential initiation and propagation, and neurotransmitter release.

I shall summarise the neurological features of a range of presynaptic channelopathies, and focus on recent attempts by my laboratory and others to relate molecular defects in Kv1.1 to dysfunction of cerebellar and forebrain circuits.

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29.02. What can we learn from tetanus toxin?

Dr Kinga Bercsenyi - *King's College London, UK*

Tetanus neurotoxin (TeNT) is among the most poisonous substances on Earth and a major cause of neonatal death in non-vaccinated areas. There are approximately 300,000 cases reported worldwide each year, and the mortality rate is between 10-20%.

TeNT binds to the neuromuscular junction with an extremely high affinity, yet the nature of its receptor complex was poorly understood. We showed that the presence of nidogens (also known as entactins) at the NMJ is the main determinant for TeNT binding. Nidogens are extracellular matrix (ECM) proteins, which are taken up into the endosomal carriers containing tetanus toxin binding fragment (HCT) in motor neurons. Inhibition of the HCT-nidogen interaction using a peptide originating from nidogen-1 abolishes HCT binding on these cells. Furthermore, when preincubated with the peptide originating from nidogen-1, TeNT injection does not cause tetanic paralysis. Genetic ablation of nidogens prevents the binding of HCT to neurons and the intact NMJ and protects mice from TeNT induced spastic paralysis.

Our study demonstrated for the first time, that an ECM protein accumulates and presents a neurotropic pathogen to the presynapse. This finding follows recent reports showing that growth factors trigger downstream signalling more efficiently if they bind to certain ECM components – a new and rising concept in neuroscience.

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29.03. Ca2+ channels modulate dopamine-autoinhibition and vulnerability of dopaminergic neurons to Parkinson's disease trigger-factors

Professor Birgit Liss - *Ulm University, Germany, UK*

Dopamine releasing neurons within the Substantia nigra (SN DA) are particularly important, as their selective and progressive degeneration causes the major motor-symptoms of Parkinson's disease (PD). The causes for the differential vulnerability of DA neuron subpopulations to degenerative triggers, and for PD remain unclear. However, a variety of genetic (PARK-genes) and environmental disease triggers have been identified, that lead to mitochondrial dysfunction, elevated metabolic stress, and impaired neuronal Ca2+ homeostasis. The electrical activity of SN DA neurons itself also affects their vulnerability to degeneration and to PD-triggers. This activity is intrinsically generated and modulated by a complex interplay of distinct ion channels and receptors, and it is crucial for presynaptic and somatodendritic dopamine release. Voltage-gated Ca2+ channels (VGCCs), especially those of the Cav1.3 L-type, are from special interest in this context, as they not only modulate activity pattern and dopamine release of SN DA neurons, but they also generate an activity-related oscillatory Ca2+ burden that could trigger neurodegeneration and PD. Indeed, epidemiological studies indicate that L-type VGCC blockers of the dihydropyridine (DHP) class reduce the risk for PD by about 30%, and the DHP isradipine is currently in a phase III clinical trial for neuroprotective PD-therapy. However, studies addressing effects of isradipine in PD mouse-models lead to ambiguous results, and the physiological roles of distinct VGCCs for SN DA neuron function remain largely unclear. The functional activity of SN DA neurons is modulated by dopamine itself, in a negative feedback loop, by activation of GIRK2 K+ channels via dopamine autoreceptors of the D2-type (D2-AR). However, a variety of signaling molecules and pathways can modulate this dopamine-autoinhibition, including VGCCs and the Ca2+ mediated interaction of the neuronal calcium sensor NCS-1 with D2-ARs. These kind of feedback and feed-forward signaling networks can modulate activity pattern as well as vulnerability of SN DA neurons to degeneration in a complex manner, which will be discussed in this talk.

This work was supported by the Austrian FWF SFB-F44, the German DFG (LI 1754/1, CEMMA), and the Alfried Krupp foundation.

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29.04. Activity-dependent regulation of synaptic strength and cellular mechanisms of paroxysmal neurological disorders

Dr Kirill Volynski - *UCL, UK*

Some inherited cases of migraine, ataxia and epilepsy are due to mutations in neuronal K+, Na+, and Ca2+ ion channels. We investigate how these mutations affect Ca2+ signals in nerve terminals, and how they affect neurotransmitter release. Our aim is to establish how the disease-linked mutations change neurotransmission at the level of individual synapses, which is prerequisite for understanding of the abnormal neuronal network function in paroxysmal neurological disorders.

We have recently developed a set of new methods which, for the first time, allow us to study the relationship between Ca2+ entry and vesicular exocytosis, and to probe presynaptic ion channel function in individual small presynaptic terminals. This is based (i) on measuring, with fluorescence microscopy, rapid changes in the concentration of Ca2+ ions, as well as the rate at which small vesicles containing chemical neurotransmitters are discharged, and (ii) on using super resolution scanning ion conductance microscopy for nanoscale-targeted patch-clamp recordings in small presynaptic boutons. Using these methods we investigate how

different channels that mediate Ca2+ influx into the presynaptic terminal control the release of vesicles, how they influence synaptic plasticity, and how synapses are influenced by other modulatory neurotransmitters acting upon presynaptic terminals both in health and disease.

In this talk, I will present the data from an ongoing project focused on understanding of the role of activity dependent homeostatic compensation in Familial Hemiplegic Migraine type 1, which is caused by inherited mutations in presynaptic Ca2.1 voltage-gated Ca2+ channels (P/Q-type) that are the major triggers of neurotransmitter release in the brain.

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Symposium 30 – Bad pharma? Improving CNS drug discovery and development with live human CNS tissue

Theme: Novel treatments and translational neuroscience

30.01. CNS medicine discovery: starting and finishing with the patient in mind

Professor Ceri Davies - *Takeda Pharmaceuticals Ltd., Japan*

CNS drug discovery has evolved over the last 50 years from an era of serendipitous clinical observations through a molecular biological revolution and now to a more patient focused approach. In doing so the target validation and lead optimization process has shifted away from a reliance on rodent model systems to human assays and most recently to patient sample analyses; the ultimate goals being to identify with more confidence (1) molecular targets/pathways that are causal of human CNS disease, (2) therapeutic molecules that engage the human target in the appropriate way to treat disease and (3) biomarkers that can be used clinically to quantify the magnitude of target engagement and clinical efficacy. Patient sample "-omic" analysis and downstream bioinformatics analysis combined with functional analysis in biopsied human patient cells/tissues and patient induced pluripotent stem cells combined with advances in CNS penetrant large and small therapeutic molecules are revolutionizing medicine discovery for CNS disorders as evidenced by recent clinical successes in the treatment of Spinal

Muscular Atrophy using both intrathecal antisense oligonucleotide and intravenous adenoiviral mediated gene therapy approaches. In addition phenotypic screening in patient cells has enabled the identification of SMN gene splice modifiers and opened up the possibility of developing an orally administered medicine. A similar approach has also been used for ALS (amyotrophic lateral sclerosis - another spinomuscular disorder) and has led to the demonstration that retigabine can prevent motor neuronal death; an observation that has led to the initiation of a clinical trial to establish whether this effect is replicated in ALS patients and can lead to functional benefit for those suffering from this terminal disease. Further examples of advances in patient focused medicine discovery will be presented to illustrate how the drug discovery process is evolving from a humanized approach afforded by advances in molecular biology 30 years ago to a more patient-ized approach which is being realized by the development of ips cells and access to high quality patient biopsy and post mortem brain and spinal cord samples that have been well phenotyped clinically.

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30.02. Age dependent changes of synaptic composition in human cortical synapses

Dr Mariana Vargas-Caballero - *University of Southampton, UK*

Human cognitive abilities gradually decline with age; however, we still do not understand the changes in the brain that underpin this decline. In mice, ageing is associated with poorer performance in spatial memory tasks and decreased glutamatergic function in multiple brain areas.

Glutamate receptors of the NMDA subtype (NMDARs) are essential for many forms of synaptic plasticity, a molecular correlate of learning and memory, and consist of obligatory GluN1 and regulatory GluN2/3 subunits. Previous research has shown a correlation between synaptic GluN2B content and performance in memory tasks, and experiments in transgenic mice suggest a causal role of GluN2B recruitment at the synapse in regulating synaptic strength and memory storage in mice.

It is not known whether age-dependent changes in GluN2B synaptic composition also occur over the human lifespan and whether these explain cognitive decline. We analysed cortical tissue derived from neurosurgical cases in order to understand whether age dependent changes in NMDAR synaptic composition occur in humans. We obtained patch-clamp recordings from 120 adult neurons from temporal cortical tissue resected during neurosurgery. By analysing inputs to pyramidal Layer II-III with whole-cell voltage

clamp we measured NMDA/AMPA ratios prior to and following pharmacological block of GluN2B subtype NMDARs using a specific GluN2B subunit blocker, Ro-256981.

We have obtained data from 14 cases spanning 20 to 70 years of age, and we observe a stronger effect of Ro-256981 in recordings from younger neurons. Our findings indicate that a significant fraction of GluN2B containing NMDA receptors exists in synapses from young humans in temporal cortex and that this synaptic component declines with age. Our findings are relevant for understanding the effects of drugs targeting GluN2B in young vs older humans and suggest that analysis of synaptic localisation of proteins will be helpful in understanding the causal factors underpinning changes in human mental functions with age.

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30.03. Investigating the correspondence between rodent models of epilepsy and human brain tissue from children with drug resistant epilepsy

Professor Gavin Woodhall - *Aston University, UK*

The wide variety of animal models of the epilepsies have provided a great deal of information on the causes and consequences of epileptic seizures over the last several decades, however, the choice of model is based on a host of factors including the syndrome to be modelled, experimental goals, reproducibility and brain region of interest, to name but a few.

The use of human tissue to investigate aspects of neuronal network function in epilepsy provides many challenges and opportunities, but also comes from a range of 'models' depending on the type of epilepsy, drug history of the patient and a host of other factors. Given these potentially confounding aspects of the approaches to investigation of epilepsy, we have recently begun to compare the characteristics of acute and chronic animal models with data obtained from human tissue studies, with a view to asking how closely network activity in human tissue in vitro represents activity in vivo, and whether there exists useful correspondence between animal models and the 'gold standard' of human epileptic tissue.

Using a variety of commonly used and novel antiepileptic drugs (AEDs) we have assessed their effects in a simple acute model (low [Mg2+]o), a chronic status-epilepticus based model (the RISE pilocarpine model) and human tissue obtained from a cohort of paediatric patients undergoing resections in order to treat intractable seizures. In brief, LFP and whole-cell voltage clamp recordings were made in brain slices made using different models, prior to and following application of a number of AEDs. Our studies show significant similarities between the responses of chronic models and human tissue, validating the use of both animal models and human tissue as tools to explore mechanistic aspects of epilepsy, and suggesting common factors which may be important in understanding this complex neurological disorder. Funded by Birmingham Children's Hospital Research Charity.

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30.04. Experimental models of cortical rhythms in live human brain tissue: translational biomarkers for CNS drug development

Dr Mark Cunningham - *University of Newcastle, UK*

Disorders of the human brain place a significant burden on society and economies on a global scale. In particular, psychiatric conditions such as schizophrenia pose a worldwide healthcare need. There is a major unmet need for effective treatments despite increased knowledge about the potential mechanisms that underlie the condition. Moreover, recent high profile failures of new drugs in clinical trials for the treatment of schizophrenia has discouraged the pharmaceutical industry sector and resulted in withdrawal of investment and research in this complex and challenging arena. The failure of translation of biological effects from preclinical animal models to humans remains a major barrier to the development of new and effective medicines for CNS disorders, particularly in psychiatry. One reason for this failure may be that human cortical microcircuits are likely to be more complex and exhibit different physiology and pharmacology to rodent neuronal circuits. As such, performing research in rodent systems has significant limitations and to reduce the risk of failure in the clinic it would be highly preferable to perform basic research in adult human brain tissue to eliminate species difference confounds and validate the efficacy of medicines in assays that are directly derived from the target organ that they are intended to treat. In this context, I will present data that demonstrates our use of live human neocortical tissue to examine a dynamic signature of human cortical function – the gamma rhythm. In vitro human brain slice preparations can be used to reproduce this translational spatiotemporal pattern and allow sufficient access and manipulation

to probe its network, cellular and synaptic origins. Using this platform, we have assessed the impact of a novel pharmacological treatment for schizophrenia.

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Symposium 31 – Long-term effects of early life activation of the hypothalamic pituitary adrenal (HPA) axis: a comparative approach

Theme: The neurobiology of stress

31.01. Epigenetic and behavioural outcomes associated with adverse caregiving

Dr Tania Roth - *University of Delaware, USA*

Epigenetics research continues to provide insight into a biological basis of gene-environment interactions and developmental trajectories. Epigenetic alterations have emerged as biomarkers for measuring the impact of stress and as important mechanisms by which adversity could interact with DNA to affect physical and mental health outcomes. We have designed a rodent model to better understand the capacity of early-life adversity to cause epigenetic alterations in the brain and their relevance to behavioral outcomes. This model employs resource scarcity (i.e., insufficient nesting materials) to elicit adverse caregiving conditions (including maltreatment) toward rodent neonates. We have observed sexually-dimorphic epigenetic alterations throughout brain regions known to be profoundly affected by child abuse and neglect, including the prefrontal cortex, amygdala, and hippocampus. In this talk I will highlight some of these data as well as present more recent data from our laboratory regarding the impact of our maltreatment regimen on several realms of behavior and whether manipulating chromatin structure impacts these behaviors. Results will be discussed in the framework of mechanisms and targets for interventions in early-life stress.

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31.02. Is glucocorticoid programming by early-life stress adaptive or maladaptive? Insights from birds

Dr Pralle Kriengwatana - *University of St Andrews, UK*

Glucocorticoids play a key role in coordinating an individual's response to environmental stressors. For instance, they support the shift from homeostasis into an "emergency life history stage" where processes that increase the individual's ability to cope with the stressor are prioritised over other processes (e.g. growth, somatic repair, and reproduction). Although activation of the stress response clearly has adaptive value, whether glucocorticoid programming via chronic/repeated activation of the stress response by early-life environments could also be considered adaptive is widely debated. Studies in birds may offer several insights into this debate that complement studies in rodents. For example, the abundant literature on avian ecology may critically inform interpretations of whether a phenotypic changes caused by early-life stress is adaptive or maladaptive. Many birds also have longer lifespans, and early-life stress may alter the life history strategies of longer- and shorter-lived animals differently. I will present evidence in birds of glucocorticoid programming by early-life postnatal stress and the resulting phenotypic changes that could be considered adaptive, such as enhanced learning and cognition, greater immune responses and body fat accumulation.

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31.03. Early life adversity and programming of the physiological stress response

Dr Karen Spencer - *University of St Andrews, UK*

Prolonged exposure to stress during development can have long-term detrimental effects on health and wellbeing in a wide range of species. However, a recent view suggests that these negative effects occur due to a mis-match between early life and later adult conditions. Indeed, the possibility exists that adverse experiences during early life can program an individual to cope better in later stressful environments. Here we utilise an avian model, the Japanese quail, to elucidate the long-term neuroendocrine effects of both pre- and post-natal exposure to 'stress'. I will present data from a series of experiments where we manipulated the in ovo concentration of corticosterone via direct injection into the yolk and post-natal access to food, creating an unpredictable feeding environment. In adulthood we determined the acute glucocorticoid response to a standardised stressor and quantified the mRNA levels of fundamental components of the hypothalamic pituitary adrenal (HPA) axis, which regulates the response to stress. Pre-

natal exposure to corticosterone had pleiotropic effects on the HPA axis. 'Stressed' birds exhibited an attenuated corticosterone response to acute stress. This was facilitated via increased expression of glucocorticoid (GR) and mineralocorticoid (MR) receptors in key regions of the HPA axis and significantly increased 11β-HSD type 1 in both the hippocampus and hypothalamus compared to pre-natal controls. There were no effects of post-natal stress on neuroendocrine parameters. These data show that pre-natal stress is the major driver in programming neuroendocrine traits at all levels, in this case creating a more 'efficient' stress response. This programming acts via long-term alterations in several aspects of the regulation of the HPA axis. Our data also suggest that adverse conditions during this developmental period can create a phenotype that may be better able to cope in stressful conditions in later life, lending support for the environmental matching hypothesis.

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31.04. Resilience to developmental stress exposure in serotonin-transporter deficient female mice

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Exposure to prenatal stress has been shown to have a profound impact on emotion regulation in adulthood (Alonso et al. 1991; van den Hove and Jakob et al. 2011; de Souza et al. 2013), while the underlying molecular mechanisms remain somewhat diffuse. In recent years, epigenetic programming (Weaver et al. 2004; Schraut et al. 2014) and changes in serotonin (5-HT) system function were pin-pointed as possible key mechanisms in the mediation of these effects (Marquez et al. 2013; van den Hove et al. 2014).

To elucidate the role of 5-HT in early life programming, we used various gene-by-environment (GxE) designs in mouse lines with altered 5-HT system function. In one of our most recent studies we exposed a cohort of wild-type C57/BL6 dams, which were impregnated by heterozygous serotonin transporter (5-Htt)-deficient C57/BL6 males, to restraint stress from embryonic day 13 to 17. Following birth, animals were allowed to grow up under normal conditions.

Subsequent behavioural analysis in the female offspring revealed several genotype-, stress- as well as GxE-specific effects, e.g. at the level of sociability / social anxiety. Follow-up molecular analysis revealed furthermore, amongst other candidates, a cluster of myelin-associated genes to be regulated in a GxE dependent fashion. Moreover, these genes were differentially affected in animals resilient or vulnerable to developmental stress exposure.

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