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Stress vulnerability, stress resilience, and PTSD

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Post-traumatic Stress Disorder (PTSD) follows an exposure to a traumatic event, but only in about 15-20% of the exposed individuals. These proportions indicate that the trauma is not sufficient to induce PTSD and that it must interact with pre-existing risk factors if to lead to PTSD. Exposure to childhood adversities is considered in humans to be a risk factor and we could demonstrate that exposure to adversities in juvenility (the developmental period homologous to human childhood) indeed leads to increased susceptibility to developing PTSD symptoms later on. However, even when combining juvenile and adulthood stress, not all individuals are affected. Diagnosis of psychopathologies in humans is based on diagnosis of individuals. Many animal models, however, analyse averaged group effects, compromising their translational power. To address that limitation, we have developed a novel ‘Behavioural Profiling’ approach allowing differentiating between exposed-affected and exposed-unaffected individuals. Identifying the affected individuals enables focusing on in brain mechanisms that are associated with developing psychopathology. Furthermore, the behavioural profiling analysis also enables examining those animals that were exposed to the trauma but did not develop symptoms. By this, it also enables research into the neurobiology of stress resilience. Indeed, employing this analysis we could identify epigenetic and gene expression alterations specific to resilient individuals. The findings pave the way to consider novel approaches for treating trauma-related psychopathologies, such as PTSD.

2.1

Mind over matter: The anterior cingulate cortex’s role in driving action towards difficult but worthwhile goals

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An animal’s ability to assess the value of their behaviours to minimise energy use while maximizing goal achievement is critical to its survival. The anterior cingulate cortex (ACC) has been previously shown to play a critical role in this behavioural optimisation process, especially when animals are faced with effortful behaviours. Using single-unit in vivo electrophysiology and T-maze paradigms in rat, we demonstrate that ACC neurons encode the effort-discounted value of behavioural options when faced with multiple choices that vary in effort. Using tilting shuttle box paradigms, we further reveal how the ACC monitors the effort-discounted value of multiple behaviours in non-choice scenarios. Using Principal Component Analysis to characterise the activity of ACC neurons we show that across the population, individual ACC neurons exhibit a diverse range of responses to specific levels of effort and reward associated with behaviours. Specifically, slope direction (uphill/downhill) could explain 44% of the variance in population activity while differences in the tilt angle the shuttle box, or amount of effort, explained 42%. In aggregate, ACC neural populations represent the most valuable behavioural option available to a rat taking into account the effort required to reach the goal. Here we summarise these published findings and share our most recent work, which focuses on how the values assigned to behaviours by the ACC may in turn drive effortful behaviours. To this end we have developed a novel weight lifting task for rodents which is not only physically challenging but also allows us to probe persistence behaviours in the face of increasing effort and energy expenditure.
Optogenetic stimulation of the anterior thalamic nuclei ameliorates impaired spatial memory in rats with mammillothalamic tract lesions

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The mammillothalamic tract (MTT) provides a unidirectional pathway from the mammillary bodies to the anterior thalamic nuclei (ATN). MTT lesions produce severe spatial memory impairments and negatively impact other memory-related structures that receive ATN efferents. We tested if optogenetic theta stimulation of the ATN would ameliorate the memory deficits produced by MTT lesions. Without stimulation, rats with MTT lesions exhibited impaired performance in a radial-arm maze task (RAM). When making correct arm choices, sham rats (N=9) exhibited peak theta (4-12 Hz) power at 8.5 Hz in the ATN; MTT-lesioned rats (N=11) exhibited peak theta above 9 Hz. Spatial memory was improved in MTT-lesioned rats given a regular pattern of theta-burst stimulation (TBS) at 8.5 Hz using blue light (465 nm) in the opsin-transduced ATN (LV-CaMKIIa-ChR2 (H134R)-mCherry) during RAM performance, whereas control stimulation (orange, 620 nm) had no effect (blue vs orange: Cohen’s d = 1.71 (95%CI, 0.75,2.64) and 2.0 (95%CI, 0.94,3.03) for two separate replications). No light stimulation effects were found in sham rats given the opsin construct or in two MTT-lesioned rats given LV-CaMKIIa-mCherry (i.e. no opsin construct). Blue light using an irregular pattern of closed-loop 8.5 Hz TBS, but derived from the rat’s own hippocampal theta rhythm, had no effect. Viral vector histology awaits completion, but memory function appears to be improved by regular optogenetic TBS of the ATN after MTT injury.

Neuroplasticity is enhanced by ketamine in patients with major depression

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In the search for novel treatments for depression, ketamine is unparalleled in its rapid acting and long-lasting antidepressant properties. There is strong evidence that synaptic plasticity mechanisms such as LTP may underlie ketamine’s antidepressant properties. Visual-LTP is a validated index of LTP-driven changes to visually evoked potentials (VEPs). Contrastingly, predictive coding indexes functional plasticity using the roving mismatch negativity (rMNN) and posits efficient information processing requires the brain to predict future sensory input. Prediction errors occur with unexpected input, causing the brain to update its internal model. The current study investigated the effect of ketamine on two non-invasive measures of neuroplasticity – visual-LTP and predictive coding – in humans with major depression. In a double-blind, active placebo-controlled crossover trial, EEG was recorded 3-4 hours following a 0.44mg/kg intravenous dose of ketamine or placebo (1.7ng/mL remifentanil). The Montgomery-Asberg depression rating scale showed 70% of participants experienced ≥50% reduction in depression symptoms within 24 hours of receiving ketamine. Ketamine selectively increased P2 VEP potentiation following high-frequency stimulation, indicating enhanced visual-LTP (t(136)=3.94, p=0.001). Ketamine also significantly increased the rMNN (F(1,232)=56.10, p<0.001) and P3 evoked-response (F(1,232)=26.28, p=0.003). Dynamic causal modelling showed greater modulation of the connection from primary auditory cortex to inferior temporal gyrus was correlated with greater antidepressant response (r(56)=-0.578, p=0.001). These results indicate that enhanced neuroplasticity, exemplified by increased visual-LTP and greater sensitivity to prediction errors, may underlie the antidepressant properties of ketamine.
2.4

**Discrimination difficulty, cognitive burden, and reversal impairments in a maternal immune activation model of schizophrenia risk**

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Reversal-learning impairments in the maternal immune activation (MIA) model of schizophrenia risk have been interpreted as being indicative of deficits in reinforcement learning. Here we sought to assess the specific role of cognitive burden in discrimination learning and reversal performance in this model. Control and MIA rats were trained on a visual discrimination task in which responses on either a left or right lever were rewarded depending on the location of a cue light at the beginning of the trial. Groups of MIA and control rats differed in the difficulty of the discrimination rule they initially learned (pressing the lever on the same side vs the opposite side of the cue light). Once the discrimination was learned, performance was tested across four reversals. Across all phases of the experiment, rats in Group Same performed better than rats in Group Opposite. There was no difference in performance of control and MIA rats during acquisition or baseline, but MIA rats displayed impaired performance across reversals, with performance decrements manifesting later in reversals after the new discrimination rule had been learnt. Across reversals, MIA rats also made more perseverative errors than control rats. These results are consistent with others that have shown reversal learning impairments in MIA rats. The results further suggest that impaired behavioural flexibility in the MIA model is not due to a deficit in reinforcement learning, but due to an impaired ability to organize information gained from experience into an accurate and stable representation of the current task requirements.

3

**Space, time and fear: Survival decisions along defensive circuits**

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For the purpose of survival, agents use space and time as cues to engage reactive and strategic defensive actions. These time-varying decisions reflect different survival problems that are evoked by external and internal milieu and, which in turn, conscious emotions such as fear and anxiety, emerge. Here, I will discuss a framework according to which fear and anxiety can be mapped along a spatiotemporal dimension of predatory imminence which is associated with structurally different decision-making problems and solutions and maps onto a canonical defense circuit. At the sharpest end, proximal threat is answered by a limited repertoire of reflexive and myopic actions. Abstract or distal threats allow for a wider range of options that engage internal milieu and afford deeper processing, including prospection, replay, planning and controlled actions. This suggests that proximal and distal threat engage distinct defense circuits that work in harmony where the aim is to produce the best survival decision.
4.1

Understanding the behavioural, molecular and neuronal-network effects of SSRI antidepressant drugs

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Social behaviour is controlled by a complex and interconnected network of neuronal ensembles, including brain serotonergic systems. Dysregulation of these networks is hypothesized to underlie the social deficits observed in autism spectrum disorders and social anxiety disorder. Selective serotonin reuptake inhibitor (SSRI) drugs, which are a first-line treatment for anxiety disorders and are prescribed for some individuals with autism, alter social approach-avoidance behaviours in preclinical models. While several brain regions are thought to be involved in mediating this effect, the functional connectivity between these regions and the impact of serotonergic drugs on these networks remain unclear. We tested the involvement of 70 regions of interest (ROIs) in 24 juvenile male BALB/c mice, which display high baseline anxiety and low sociability. Mice were exposed to the SSRI fluoxetine either chronically (18 mg/kg/day for 12 days, in drinking water) or acutely (18 mg/kg, i.p.), or to vehicle control conditions (0.9% sterile saline, i.p.), prior to being exposed to the three-chambered sociability test. Brain tissue was collected 90 minutes after the onset of the sociability test and prepared for immunohistochemical staining for the protein product of the immediate early gene c-fos (c-Fos). Graph-theory-based network analysis of c-Fos immunoreactivity in the 70 ROIs was used to characterise the functional connectivity between subcortical and cortical regions implicated in social approach-avoidance behaviour and identify changes in patterns of connectivity following acute and chronic antidepressant treatment. These results will help identify key components in the circuits underlying approach-avoidance behaviours and advance our understanding of the mechanisms of action of serotonergic antidepressant drugs.

4.2

Cortical mechanisms of impaired cognitive control in anxiety

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Anxiety increases vigilance to the environment, an adaptive response in threatening situations. Recently, we described anxious hypervigilance as biased feedforward signalling in a temporo-frontal cortical network, which could be normalized by acute anxiolytic treatment. This was shown with passive auditory oddball stimulation, leaving us only to speculate as to its basis for impaired cognitive control in anxiety. To examine this link, healthy participants performed a stop-signal inhibition task, which requires aborting an initiated action when an infrequent (oddball) stimulus occurs. Anxious arousal was manipulated by exposure to threat of unpredictable electric shocks. In three experiments, we found poorer inhibitory control during threat compared to safe periods as evidenced by slower stop-signal reaction times (SSRTs) in the former. Whole-head magnetoencephalography was recorded with a 306-channel magnetometer in 18 participants to compare stop-signal elicited changes in beta (14-30Hz) oscillatory power under threat versus safe conditions. Volumetric mapping of beta power was carried out with adaptive beamformer analyses to isolate correlates of inhibitory control in the 150-300ms interval between stop-signal presentation and estimated SSRTs. Notably, action inhibition was marked by stop-signal induced beta activity in the left inferior frontal gyrus (IFG) rather than the right IFG, the latter being more commonly observed with fMRI. Right IFG showed increases in beta irrespective of whether an action was successfully inhibited, a pattern consistent with its role in stimulus-driven reorienting to infrequent events. Moreover, compared to safe periods, greater right temporo-frontal cortical beta responses during threat were correlated with larger threat-related deficits in inhibitory control. We conclude that anxiety weakens inhibitory control indirectly, by enhancing stimulus-driven attention to potential threat signals. Targeting these cortical mechanisms may be useful in testing novel anxiolytics.
4.3

From rat to human to rat: a parallel-circuit homology-based model of anxiety, goal conflict, and stopping

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Hippocampal rhythmical slow activity (5-12Hz) in rats provides the only model of human clinical anxiolytic action with no false positives or false negatives. This is the foundation of a theory of anxiety in which the hippocampus is the core of a Behavioural Inhibition System that detects goal conflict and outputs increased inhibition, attention, and arousal. In humans, goal conflict elicits anxiolytic-sensitive 5-12Hz rhythmicity in the right frontal EEG in the stop signal task – from a source that appears close to areas known to control stopping. (Stopping itself is not sensitive to anxiolytics.) Intracranial recording in rats in the stop signal task shows increased orbital frontal (homologous to human right inferior frontal gyrus) power and increased hippocampal-orbital frontal-subthalamic coherence at 5Hz. The data, combined across paradigms and species, are currently consistent with a model that has proposed parallel act (fast), action (slow) and goal (even slower) circuits that each control prevention of pre-potent behaviours that are no longer appropriate but under different conditions of time-pressure for the prevented behaviour. Only the slowest (goal-related) inhibition of behaviour appears to be involved in anxiety.

4.4

Neuropeptide modulation of anxiety neurocircuitry and behaviour

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Corticotropin-releasing factor (CRF) is a neuropeptide tightly linked to adaptive and maladaptive stress responses. The role of CRF-type 1 receptors (CRF1) have been well characterized in preclinical models for mediating neural and behavioural correlates of stress-related disorders such as depression, anxiety and substance dependence. However the role of the CRF-type 2 (CRF2) receptor has been less clear, with inconsistent findings among studies involving pharmacological and genetic manipulations. Here we will present our recent findings in two rat models of heightened anxiety – amphetamine withdrawal and early-life social isolation. Our microdialysis experiments show that CRF1 and CRF2 receptor activation in the serotonergic cell body region of the dorsal raphe have opposing effects, with CRF2 receptor activity increasing terminal serotonin release throughout the limbic system. Furthermore, rats exhibiting heightened anxiety show greater stress-induced c-fos activation of CRF neurons, heightened CRF2-mediated serotoninergic release in limbic terminal regions, accompanied by elevated levels of CRF2 receptors in discrete regions of the dorsal raphe. In concordance with a role for CRF2 receptors in anxiety, antagonism of CRF2 receptors both within the dorsal raphe and globally in the brain via intra-ventricular administration, ameliorate anxiety-like behaviours in pre-stressed rats. Using a novel nanotechnology delivery system, we also show that systemic administration of peptide CRF2 receptor antagonists reach target neurons in the brain and selectively reduce anxiety-like behaviours. Together our findings suggest an important role for the neural CRF2 receptor in mediating anxiety states, and highlight the feasibility of targeting these receptors in the treatment of stress-related mental illness.

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5.1

The parabrachial nucleus regulates initial intake of ethanol, sucrose and other solutions

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Oxytocin receptor-expressing neurons in the parabrachial nucleus (Oxtr<sup>BN</sup>) suppress water intake, but not food or highly caloric liquids such as Ensure. This suggests that Oxtr<sup>BN</sup> neurons may differentiate solutions based on their caloric content and/or palatability; however, the effect of these neurons on intake of other solutions has not been investigated. In this study, we examined whether Oxtr<sup>BN</sup> neurons regulated consumption of caloric solutions (ethanol, sucrose, Ensure), and non-caloric solutions (saccharin, salt). Oxtr<sup>cre</sup> mice were injected with the Cre-dependent stimulatory DREADD, hM<sub>3</sub>D<sub>q</sub>, into the parabrachial nucleus, then tested in a two-bottle choice of water vs another solution (Ensure, ethanol, sucrose, saccharin or salt) at different concentrations. Mice had access to fluids for 4 h/day. When Oxtr<sup>cre</sup> neurons were activated by injecting the designer drug CNO (3 mg/kg ip), we observed a significant decrease in rapid (15-minute) intake of all solutions (P<0.05). There was also significantly decreased intake of low palatable, non-caloric solutions at 2 hours in a concentration-related manner (P<0.05), but not of highly caloric and/or palatable solutions, suggesting the major effect of Oxtr<sup>BN</sup> neurons is on initial rapid fluid consumption. We also assessed expression of Fos in different subdivisions of the parabrachial nucleus after intake of different solutions. We observed increased Fos in the dorsolateral PBN following intake of all fluids, in the external lateral PBN following intake of caloric solutions, and in the central lateral PBN following intake of sweet-tasting fluids, suggesting differential activation of PBN subdivisions depending upon fluid properties. This study reveals a key but complex role of the parabrachial nucleus in regulating fluid intake.

5.2

Development of novel optogenetic approaches to manipulate neuronal GPCR signalling

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The G protein-coupled receptor (GPCR) sub-family comprises the largest class of cell-surface receptors in the human genome, including over 370 non-sensory GPCRs. More than 90% of these are expressed within the brain, contributing to a vast array of neuronal functions such as cognition and mood through the transduction of signals from neuromodulators. Until recently, neuronal GPCRs have been manipulated by either pharmacological or chemogenetic means. However, these methods have limited spatiotemporal precision and are often irreversible. This becomes problematic for experiments requiring precise, controlled modulation of rapid intracellular events. Consequently, there is a need for a mechanism through which to modulate GPCRs that allows for precise and reversible control of intracellular signalling both in vitro and in vivo. To address this issue, we have developed a new suite of optogenetic tools for the targeted manipulation of GPCRs in the brain. This technique offers unprecedented spatiotemporal resolution; genetic targeting for cell type specific expression; and non-invasive manipulation of cultured cells and tissues, as well as awake, behaving animals. To achieve this, modified regulatory proteins were fused to photosensory domains, allowing for controlled localization and inhibition of target GPCRs following blue light exposure. We have shown that the use of these tools allows for the precise manipulation of specific GPCR signalling cascades. This was demonstrated in cultured cells using live-cell imaging techniques to visualise the inhibition of defined downstream signalling pathways. The tools were further confirmed through modification of GPCR-dependent behaviours in the nematode, C. elegans. Finally, we validated the tools in the brain of awake, behaving fruit flies, achieving targeted manipulation of GPCR-associated learning and memory processes.
A balance of power: Neurovascular coupling, the influence of NO, 20-HETE, GABA and NPY

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Reduced baseline blood flow is a common observation in neurodegenerative disease. Although nNOS interneurons (nNOS-Ins) only represent ~2% of cortical GABAergic interneurons, they appear to have a critical role in neurovascular function. Loss of NO regulation has been linked to pathological changes in both normal aging and neurodegenerative disease. Mice bred to express channelrhodopsin in nNOS-INSs showed that mechanical stimulation of the whiskers elicited normal haemodynamic and multi-unit activation of whisker barrel cortex. Selective optogenetic activation of nNOS-INSs evoked a robust increase in hemodynamic activity, but with little change in measured evoked or baseline neural activity. Experiments showed that the same optogenetic stimulation in wild-type animals had no effect on cortical haemodynamics or neural activity. This important finding suggests that nNOS-INSs could be a population of cortical cells exclusively dedicated to the regulation of local blood flow. Using the results from the above experiments a numerical model was developed to investigate the haemodynamic response from both somatosensory cortex neurons and nNOS-INSs. Results showed excellent agreement with the somatosensory experiments and indicated the balance of both eNOS and nNOS versus the constrictive pathway from 20-HETE. Comparisons with both the interneuron and somatosensory experiments indicated that nNOS does not seem to play role in neurovascular coupling however for the case of interneuronal stimulation there exists a delicate balance between the GABA_A pathway (causing dilation) and the constrictive role of NPY mediating the smooth muscle cell voltage-gated calcium channel.

Movement-related cortical potentials paired with peripheral electrical stimulation improves voluntary activation in people with stroke

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Neuromodulatory interventions that alter corticomotor excitability have potential to enhance recovery following stroke. One such intervention, known as exciteBCI, pairs movement-related cortical potentials (MRCPs) with peripheral electrical stimulation. Previous studies have used transcranial magnetic stimulation (TMS) to demonstrate immediate effects of exciteBCI on corticomotor excitability, but TMS use in stroke is limited by safety precautions, tolerance, and the inability to generate a measurable response in some individuals. Thus, we were interested in evaluating the effect of exciteBCI on more feasible measures in people with stroke. This repeated-measures cross-over study was carried out to determine whether exciteBCI could immediately increase the primary outcome of maximal voluntary contraction (MVC) of the dorsiflexor muscles, and secondary outcomes of rate of force development of the dorsiflexor muscles, voluntary activation (VA) of the tibialis anterior, and electromyography amplitude. Participants with chronic stroke (n=15) completed two interventions, exciteBCI and sham, in a randomised order. During exciteBCI, 50 repetitions of visually-cued dorsiflexion were completed, while single pulses of electrical stimulation were delivered to the deep peroneal nerve. Each somatosensory volley was timed to arrive in the motor cortex at the peak negativity of the MRCP. Statistical analyses demonstrated no significant effect of exciteBCI on the primary outcome (MVC) (p=0.64) or on the combined primary and secondary outcomes (p=0.14). However, the multivariate analysis revealed that exciteBCI significantly increased VA of the tibialis anterior by 7% (95% confidence interval 1.3-12.7%). This research confirmed the neuromodulatory effects of exciteBCI and offers an alternative, feasible measure for future studies.
5.5
Adaptive constrained constructive optimisation for complex vascularisation processes in brain cortex

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The automatic construction of vessel networks in vascular territories acquires importance towards the analysis of the multi-scale circulatory cascade and the ultimate coupling between blood flow and cell function. A proper in-silico brain vascularisation model is key to understanding the pathological hemodynamic patterns linked with inefficient brain waste clearance, which can trigger cognitive impairments. This work extends the Constrained Constructive Optimisation (CCO) algorithm to tackle the automatic vascularisation of the brain cortex. The proposed aDaptive CCO (DCCO) involves the vascularisation of territories, adaptable optimisation criteria and multi-staged space-filling tasks, either from scratch or from an existing network of vessels. Hence, the vascular territory is defined as a partition of vascular and avascular domains allowing us to model complex vascular structures. In turn, the multi-staged space-filling tasks allow us to delineate a sequence of biological strategies during the vascularisation process by using different constraints, optimisation strategies and domain partitions stage-wisely, improving the consistency with the architectural hierarchy observed in anatomical structures. With these features, the algorithm aims at improving the anatomical characteristics in automatically generated networks of vascular segments. The capabilities of the DCCO algorithm are assessed in the left frontal gyrus of a human brain following the anatomical description reported in the literature.

6.1
Improving drug delivery to the inner ear using the sheep round window membrane as a model

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Disabling hearing loss affects over 5% of the world population. One of the challenges in treating hearing loss is the effective delivery of therapeutic compounds to the inner ear hearing organ, the cochlea. Intratympanic injection involves drug application onto the round window membrane (RWM), which separates the cochlea fluid and the middle ear. The selective RWM permeability is a limitation for the amount and molecular size of drugs that can be delivered. The aim of this study was to characterize the structural and molecular properties of the RWM, and to test the permeability of the membrane using a large animal model (sheep). Fresh temporal bones from 2-4 year-old female sheep were obtained. Morphological assessment of the sheep RWM niche demonstrated dimensions similar to humans: 2.14± 0.30mm (height), 2.30 ± 0.37mm (width) and 107.71±30.56μm cross-sectional thickness. Both fresh and fixed RWM displayed characteristic curvature and tight attachment to the bony capsule. We applied tracer molecules, methylene blue (MM = 319g/mol) and Evans blue (MM = 960g/mol) to the RWM niche and assessed diffusion across the sheep RWM ex vivo. Both tracer dyes exhibited gradual diffusion across the RWM over the first 15 minutes, albeit only a fraction (1.4% from EB and 8.4% for MB) were detected in the cochlea, suggesting similar permeability properties as the human RWM. We are currently testing various modalities aimed at increasing the RWM permeability and in situ methods to visualize the RWM such as MRI and OCT. Preliminary studies will be reported.
6.2

**Visualising population voltage responses of cortical layer 2/3 during sensory stimulation in awake mice**

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Synaptic connections between brain cortical areas are critical for the coordination of information that enables sensation and perception. Yet our understanding of the specificity and timing of these connections even during the awareness of a simple sensation is limited. Using a novel, fluorescent genetically encoded voltage indicator – voltage sensitive fluorescent protein (VSP) – specifically targeted to layer 2/3 of the mouse cortex, we aimed to understand how recruitment of cortical areal connections occurs in awake mice as they performed two distinct simple behaviours. We used fast, fluorescence imaging of layer 2/3 voltage signals, well suited to detect synaptic potentials, through a thinned skull window in pre-trained head-fixed awake mice as they received single or double brief (<20 ms) light stimulation to the paw or air puff to the whiskers. We standardised methods to process real time fluorescence changes to reveal voltage changes including correction for haemodynamic and movement artefacts using Matlab. Our results show fast, dynamic increases, or depolarisation, of layer 2/3 cortical neurons specifically initiated and localised within distinct areas of the sensory cortex during whisker puff and paw stimulation. We observed a similar time scale of the sensory voltage responses in the distinct areas (p<0.05), and characteristic paired pulse depression of the signals consistent with their synaptic nature, at inter-stimulus intervals of 110 ms (p<0.01) and 210 ms (p<0.05). These unique real time brain activity maps show how areal recruitment of synaptic activity connects distinct cortical areas critical for encoding behavioural perception and awareness of sensation.

6.3

**Functional connectivity of muscle synergies**

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Muscle synergies are proposed as the modular strategy of the central nervous system for movement control. Coherence is a measure of correlation between two signals in a determined frequency band. Corticomuscular coherence (CMC) assesses the relationship between cortical and muscle activity, and suggests cortical control of movement. Intermuscular coherence (IMC) assesses activity between muscle pairs, and reflects subcortical control. We measured coherence to examine the neural inputs that contribute to the generation and modulation of muscle synergies during an isometric contraction of the upper limb. Fourteen participants performed two different tasks while seated: a multidirectional task of isometric contractions on 26 force directions, and a coherence task of 50 repetitions per synergy extracted from the multidirectional task. We recorded upper limb EMG, EEG, and the force exerted on a handle. Synergies were extracted from both tasks and coherence values were calculated within the bandwidth between 3 - 50 Hz. A coherence significance threshold was calculated based on the shuffled original data. From multidirectional trials, on average 4.2 synergies were extracted. CMC was below threshold for the coherence task. Significant IMC occurred within the alpha band (~10 Hz). Two muscles with high contributions within a synergy showed high IMC, while IMC calculated between a high and a low contributor to a synergy was significantly lower. The lack of CMC is consistent with a task requiring activation of multiple muscles, a low precision force target, and high cognitive involvement. The alpha band IMC suggests the existence of a subcortical mechanism for the generation of muscle synergies. The differential level of IMC between contributors within a synergy may reflect the level of regulation required to achieve the motor task.
Deciphering the fate of integrated pericytes in mouse motor cortex

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A major goal of modern neuroscience is to find new ways to encourage brain self-repair after injury that provide realistic and effective therapy post-stroke. A subset of pericytes may hold the key as they possess multipotency to trans-differentiate into vascular and neuronal cells in the context of damaged tissue. The current study aims to understand the role of grafted pericytes within the mouse motor cortex. Mouse brain pericytes were cultured, characterised for the co-expression of platelet-derived growth factor receptor-Beta (PDGFR-β), neuronal glia (NG2) and lack of glial-fibrillary acidic protein (GFAP) and alpha-smooth muscle actin (α-SMA) and further enriched using PDGFR-β expression and fluorescence assisted cell sorting (FACS). Enriched pericytes were transduced with lentivirus expressing iRFP (40%) in order to identify the grafted pericytes (24 h and 4-days post-grafting). iRFP+-sorted pericytes (50,000 cells/μL) were successfully grafted into the mouse motor cortex. Subsequent analysis identified the iRFP+ expression amongst pericyte, neuronal, astrocytic and microglial markers to decipher the fate of the grafted iRFP+-pericytes. We observed several grafted iRFP+-PDGFR-β+αSMA pericytes along the vasculature. Grafting iRFP+-pericytes also lead to expression of hyper-ramified microglia and accumulation of neural stem cell specific SOX2 around the site of pericyte (not sham) injections. In summary, preliminary results from this study demonstrate successful transplantation of iRFP+-pericytes. The changes in the expression pattern of different markers as a result of grafted pericytes could help us to further investigate their therapeutic potential during the events of stroke.

The neurobiology of homeostasis

Z. KNIGHT

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Our research investigates the neural mechanisms that govern hunger and thirst. Nearly a century ago, lesioning studies suggested that these fundamental drives originate from subcortical structures such as hypothalamus that are specialized for monitoring internal state. However the structure and dynamics of the underlying neural circuits has been poorly defined. I will discuss our work using calcium imaging to observe the natural activity of some of the key cell types that control eating and drinking. We have discovered that these homeostatic neurons receive sensory information from the outside world, which they use to predict impending physiologic changes and adjust behaviour pre-emptively. I will discuss our work investigating how these homeostatic circuits integrate external sensory cues with internal signals arising from the body in order to generate and shape goal-directed behaviours.
8.1

PSA-NCAM mediated plasticity is reduced in the human Alzheimer’s disease entorhinal cortex

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Polysialylated neural cell adhesion molecule (PSA-NCAM) is a membrane bound glycoprotein widely expressed during nervous system development. While commonly described in the neurogenic niches of the adult human brain, there is limited evidence of its distribution in other brain regions. PSA-NCAM is an important regulator of cell–cell interactions and facilitates cell migration and plasticity. Recent evidence suggests these functions may be altered in Alzheimer’s disease (AD). In this study we provide a detailed description of the PSA-NCAM distribution throughout the adult human brain and quantitatively compare the staining load in cortical regions and sub-cortical structures between neurologically normal and AD cases. We show that PSA-NCAM immunoreactivity and cell number are decreased in the entorhinal cortex of AD cases and are inversely correlated with hyperphosphorylated tau load. Furthermore, using fluorescent double labelling we found that PSA-NCAM is expressed by interneurons in the entorhinal cortex. These results demonstrate that PSA-NCAM is widely expressed in the adult human brain and PSA-NCAM-mediated structural plasticity of interneurons is reduced in the AD entorhinal cortex, a region that is severely affected by disease pathology.

8.2

Regulation of plasticity through PSA-NCAM in development and adulthood

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During development of the brain, neuroblasts express polysialic acid neural cell adhesion molecule (PSA-NCAM). This molecule reduces cellular friction to provide smooth passage of cells en route from their place of birth to their destination, where they will integrate to form the cortical circuitry. PSA-NCAM is also important in mature neurons for enabling synapse remodelling. As neuroblasts mature they reduce their PSA-NCAM expression but the mechanisms underlying the process of down-regulation in mature and immature neurons is poorly understood. In this talk I will discuss how PSA-NCAM is influenced by the extracellular matrix, nitric oxide and insulin, each having a significant effect on cell migration. Furthermore, I will discuss our studies on the normal, Alzheimer’s and Parkinson’s disease affected human brain, which exhibit PSA-NCAM positive cells in many cortical regions. Intriguingly, in Alzheimer’s disease the entorhinal cortex PSA-NCAM load is inversely related to the tau load. These studies highlight the importance of PSA-NCAM activity during development, into adulthood and in neurological disease.
8.3

**Targeting adult-born neurons to regulate neural circuitry and anxiety-related behaviour**

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A mechanistic understanding of how emotions are represented in the brain and lead to physiological changes that impact behaviour has been a major quest in neuroscience. Adult neurogenesis, the production and integration of new neurons in the adult brain, has emerged as a key plasticity mechanism regulating the neural circuitry and a vital player in the modulation of cognition and emotion. However, regulation and precise roles of adult-born neurons in these processes are not fully understood. In this talk, I will summarize our findings on regulation of adult neurogenesis in the mouse brain, highlighting our recent discovery of the generation of functional, new neurons from a resident pool of neural precursor cells in the basolateral amygdala. I will also present our new data showing that genetic ablation of adult-born neurons during a critical window of neuronal maturation prevents chronic stress-induced anxiety, and I will discuss the electrophysiology and morphometric changes in these neurons that underpin stress-induced behavioural outcomes. Together, these findings provide evidence for an instructive role of adult-born neurons in regulating the local circuitry and behaviour during stress and suggest that interventions that target the properties of these neurons may prove useful for the treatment of anxiety-related disorders.

8.4

**Can injury stimulate striatal neurogenesis in the postnatal brain?**

L. H. THOMPSON

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Studies in the early 2000’s reported that significant neuronal loss through damage to brain areas adjacent to neurogenic niches could redirect the fate of stem cell derived neuroblasts to not only migrate to the damaged area but also to differentiate into regionally appropriate neuronal subtypes. For example ischemic injury to the striatum has been reported to result in the repopulation of medium spiny projection (MSP) neurons via neurogenic progenitors in the nearby subventricular zone (SVZ). Subsequent studies contradicted this finding, suggesting that although injury could redirect the migration of SVZ neuroblasts to the injury site, these cells maintained their intrinsic differentiation potential as olfactory bulb interneurons and could not ‘switch’ to a striatal projection phenotype. Our own work in this area aligns with this more negative finding. Recently we reported that the tail end of the developmental program for production of MSPs persists into the early postnatal phase. This lead us to hypothesise that injury-induced regeneration of striatal projection neurons is perhaps more likely during this early postnatal period, when proliferating SVZ precursors remain competent for production of MSPs. However, birth-dating studies showed that even during this period of active striatal neurogenesis, striatal injury does not lead to an SVZ response that repopulates the damaged striatum. These results suggest that the generation of projection neurons in the postnatal mammalian brain is tightly regulated and not as responsive to extrinsic signalling through injury as previously thought. While this is a sobering conclusion, recent advances in the direct in vivo reprogramming of neural cells to specific neuronal identities suggests there is still scope for developing endogenous repair-based approaches to brain injury.
8.5

**Neuroplasticity delayed acceptance: Controversial issues in the late 1970’s in contrast to contemporary clinical practice**

M. S. KAPLAN

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Within the last three decades, the brain’s ability to regenerate has become a well-established fact. We now know that environmental factors can actually stimulate the production of new neurons and division of new nerve cells in the adult brain (neuro-blasts) have been identified. The story of the revolution begins in 1971. Along with my Professor, I conducted research on adult rats and found that living in an enriched environment (e.g. with wheels, balls and toys) caused the development of new nerve cells in the brain (neurogenesis). We discovered that the number of neurons in a region of the brain could increase by up to 55% over a period of up to a year. We now know that every day, thousands of new neurons are added to the mammalian brain. The ability of brain cells to regenerate is now a well understood and an accepted fact. My career in stroke and rehabilitative medicine has allowed me to develop unique insights into how this wealth of neurogenesis research can be used to help real people.

9.1

**Increased synaptic plasticity in the inferior colliculus of rats following acoustic trauma**

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Long-term potentiation (LTP) of synaptic transmission in the dorsal cochlear nucleus (DCN) has been found to be strongly facilitated in tinnitus mice. However, the fact that ablation of the DCN could only prevent tinnitus development but not reduce chronic tinnitus suggests that chronic tinnitus is likely to be maintained at higher centres of the auditory pathways, such as the inferior colliculus (IC). Therefore, we investigated whether LTP was facilitated in the IC of rats at 5 months after exposing them to a tinnitus-causing acoustic trauma. In the first part of this study, the induction of LTP was compared between naïve rats (n = 4 - 5 per group) with or without the blockade of GABA receptor activity using picrotoxin (2.5 mg/kg, i.p). In the second part of this study, the induction of LTP was compared between sham (n = 4) and acoustic trauma-exposed (n = 6) rats. Under urethane anaesthesia, a craniotomy was performed to expose the IC. A recording electrode was inserted into the central nucleus of the IC and a stimulating electrode was placed on the lateral surface of the IC to stimulate the lateral lemniscal fibres. We found that LTP could only be induced in naïve rats when GABAergic neurotransmission was inhibited, while LTP was readily induced in acoustic trauma-exposed rats without the GABAergic inhibition, which suggests that acoustic trauma may cause long-term loss of inhibition in the IC. Future studies are needed to understand the mechanisms of LTP facilitation following acoustic trauma.
9.2

**Metabotropic Glutamate Receptors as a potential therapeutic target for the treatment of Spino-cerebellar Ataxia Type 1 (SCA1)**

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Spino-cerebellar ataxia type 1 (SCA1) is an incurable human neurodegenerative movement disorder caused by an unstable expanded CAG trinucleotide (Q) repeat (39-82Q) in the ataxin-1 gene. The disorder is characterized by progressive motor incoordination, disruption of cerebellar excitatory synapse morphology and degeneration of Purkinje neurons (PN), the main output neurons of the cerebellar cortex. Metabotropic glutamate receptor type 1 (mGluR1) are highly expressed in PNs and critical for motor coordination. Overactive mGluR1 signalling occurs in an SCA1 82Q mouse model and normalisation of this activity with an mGluR1-specific negative allosteric modulator acutely restores motor coordination in the disorder. To further explore the role of enhanced mGluR1 signalling during SCA1 progression we chronically enhanced mGluR1 signalling by administering an mGluR1-specific positive allosteric modulator to SCA1 mice. This treatment hastened SCA1 progression in the mice, manifested by decreased motor performance on an accelerating rotarod (two-way ANOVA, P < 0.05, F (1, 14) = 6.0), decreased precision of PN firing output (unpaired t-test, P < 0.05) and the synaptic morphology remained disrupted (P > 0.05, unpaired t-test) in comparison to vehicle treated SCA1 mice. In contrast, chronically decreasing mGluR1 signalling by administering an mGluR1-specific negative allosteric modulator significantly improved PN firing frequency and rescued the disrupted synaptic morphology (P < 0.05, unpaired t-test). Our results demonstrate that enhanced mGluR1 signalling is a driving force for SCA1 disease progression and that successful targeting of mGluR1 is a promising approach to treat SCA1.

9.3

**Vildagliptin and dementia risk in type 2 diabetes patients**

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Animal studies suggest that vildagliptin might exert a beneficial effect on cognitive function. The present study evaluated whether the use of vildagliptin in patients with type 2 diabetes mellitus might affect dementia risk. The database of Taiwan's National Health Insurance was used to enrol a propensity score-matched-pair cohort of ever and never users of vildagliptin from patients with newly diagnosed diabetes mellitus during 2002-2014. Patients alive on January 1, 2015 were followed up for dementia diagnosis until December 31, 2016. Adjusted hazard ratios were estimated for vildagliptin ever versus never users and for cumulative duration and cumulative dose of vildagliptin therapy being treated as a continuous variable. There were 40489 never users and 40489 ever users of vildagliptin, with respective numbers of incident dementia of 47 and 44. The overall adjusted hazard ratio was 0.825 (95% confidence interval: 0.498-1.367) for ever versus never users. When cumulative duration and cumulative dose were treated as continuous variables, the respective adjusted hazard ratios were 0.993 (0.966-1.020) and 1.000 (1.000-1.000). This study showed a neutral effect of vildagliptin on dementia risk.
9.4
Altered brain arginine metabolism with age in the APPswe/PSEN1de9 mouse model of Alzheimer’s disease

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Amyloid-beta (Ab) cleaved from amyloid precursor protein (APP) has been proposed to play a central and causative role in the aetiology of Alzheimer’s disease (AD). APP/PS1 transgenic mice display chronic Ab accumulation and deposition in the brain. L-arginine is a semi-essential amino acid with a number of bioactive metabolites, and altered arginine metabolism has been implicated in the pathogenesis and/or the development of AD. The present study systematically investigated how the brain arginine metabolic profiles changed in male APP/PS1 mice at 4, 9 and 17 months of age. High-performance liquid chromatography and mass spectrometry revealed significantly increased levels of glutamine, spermidine and/or spermine in the frontal cortex, hippocampus and parahippocampal region, but not cerebellum, mainly in 17-month old APP/PS1 mice when compared to age-matched wild-type mice. Notably, increased spermine was also found in the frontal cortex in 9-month old APP/PS1 mice relative to their age-matched wild-type controls. The results demonstrate that the changes in brain arginine metabolism in APP/PS1 mice are age-, region- and chemical-specific. Given the role of polyamines in modulating the aggregation and toxicity of Aβ oligomers, increased spermidine and spermine levels may be a neuroprotective mechanism in response to age-related Aβ deposition in the brain. Future research is required to better understand the functional significance of these changes.

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9.5
Immunophenotype marker changes demonstrate microglial dysfunction in the human Alzheimer’s disease middle temporal gyrus

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Microglia, the innate immune cells in the brain, become activated in response to damage and disease. Microglial activation has been implicated in Alzheimer’s disease (AD) and understanding changes in this activation is key to understanding disease pathogenesis. Microglial activation can be analysed in post-mortem human brain using microglial immunophenotypes – the cellular abundance of proteins that indicate function. We quantified microglial immunophenotype markers in human normal and AD cortex to identify changes in microglial function in AD. Fluorescent immunohistochemistry on control (n = 8) and AD (n = 8) middle temporal gyrus (MTG) co-labelling was carried out using one of eleven immunophenotype markers of interest (MOI) with regulatory protein and pan microglial marker, Iba1, per section. A novel automated quantification method was developed, allowing for the quantification of the MOI and Iba1 abundance in each microglia in the MTG. In both normal and AD cases, three Iba1-MOI populations were identified: Iba1low MOIhigh, Iba1high MOIhigh, and Iba1high MOIlow. The abundance of Iba1low MOIhigh microglia was significantly greater in AD (p < 0.05) and correlated with tau pathology load (r = 0.8). Furthermore, this Iba1low MOIhigh population expressed higher levels of eight immunophenotype MOIs than the associated Iba1high MOIhigh population (p < 0.05). Interestingly, the Iba1low population was best delineated by high L-ferritin immunoreactivity. The low expression of Iba1 and high expression of L-ferritin, a degenerating marker, suggests this Iba1low population is dysfunctional. The identification of this dysfunctional microglial population whose abundance correlated with AD pathology, supports the microglial dysfunction hypothesis of AD.
Poster 10.1

Between-hand coupling during response inhibition
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Response inhibition reflects the process of terminating inappropriate pre-planned or ongoing movements. When one hand is cued to stop after preparing a bimanual response (Partial trial) there is a substantial delay on the responding side. This delay is termed the interference effect and identifies a constraint that limits selective response inhibition. Gamma-aminobutyric acid (GABA)-mediated networks within primary motor cortex (M1) may have distinct roles during response inhibition. In this study we examined whether the interference effect is the consequence of between-hand “coupling” into a unitary response, and whether this is reflected in GABAergic intracortical inhibition within M1. Eighteen healthy right-handed participants performed a bimanual synchronous (easy) and asynchronous (hard) anticipatory response inhibition task. Electromyographic recordings were obtained from the first dorsal interosseous muscle bilaterally. Motor evoked potentials were elicited by single- and paired-pulse transcranial magnetic stimulation over right M1. As expected, performance was better and paradoxically response delays were worse, with the easier versus the harder version of the task. Although task difficulty did not modulate GABAergic intracortical inhibition, there was a trend for between-hand coupling to be associated with greater inhibitory tone and lower synaptic inhibition. The novel findings indicate that the interference effect is in part a consequence of between-hand coupling into a unitary response. The ability to respond independently with the two hands may rely on modulation of distinct inhibitory processes.

Poster 10.2

Neurovascular unit characterization in the Alzheimer’s disease middle temporal gyrus using human brain tissue microarrays
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Alzheimer’s disease (AD) is the most common neurodegenerative disorder. Currently, there is increasing evidence implicating the neurovascular unit (NVU) in AD pathogenesis. This project aims to characterise the NVU in AD post-mortem human brain tissue by examining NVU cells in the cerebral cortex. Immunohistochemistry was conducted on human brain tissue microarrays (TMAs) from the middle temporal gyrus, each containing at least 21 control and 21 AD cases. Antibodies to NVU components including astrocytes (GFAP), microglia (Iba-1 and HLA-DR), smooth muscle cells (αSMA) and pericytes (PDGFRβ) were used. The immunolabelled TMAs were imaged and the acquired images were densitometrically analysed. Parametric methods compared the NVU immunolabels for the AD and control cohorts, and correlation analyses compared the expression of NVU immunoreactivity with tau pathology. Findings revealed a significant increase in GFAP+ cell number and protein expression in the AD cohort. HLA-DR+ cell number and protein expression increased in the AD group, while Iba-1 immunoreactivity was unchanged between control and AD. GFAP and HLA-DR immunoreactivity correlated with human tau immunoreactivity. αSMA expression per vessel increased while the number of PDGFRβ+ vessels reduced in AD. αSMA and PDGFRβ+ did not correlate with tau. These findings suggest the presence of AD-related changes in non-neuronal cells associated with neuroinflammation and perivascular dysfunction. Our data suggest that glia changes in AD are tau-dependent, whereas vascular changes are tau-independent. Overall, this study supports further investigation of NVU cells to better understand AD pathogenesis and the development of targeted therapeutic intervention strategies.
Poster 10.3

Event-related neural oscillations in individuals with a family history of Alzheimer’s disease and ApoE-4 genotype

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People without dementia who have a family history of Alzheimer’s Disease (ADFH) and apolipoprotein E ε4 allele (ApoE-4) are more likely to exhibit memory-related cognitive decline. However, the potential neurophysiological mechanisms have not been investigated. The purpose of the present research was thus to explore the neural oscillations in ADFH individuals with ApoE-4 genotype when performing a cognitive task. One hundred and eight individuals with ADFH provided blood samples to assess ApoE genotype for dividing into ApoE-4 group and non-ApoE-4 group. All participants completed a visuospatial working memory task (spatial and nonspatial recognition paradigm) with simultaneous recording of electroencephalographic signals. ApoE-4 individuals (n=15) relative to non-ApoE-4 individuals (n=15) showed significantly lower accuracy rates (ARs). In addition, the non-ApoE-4 group showed significantly greater decreases in oscillatory power at alpha band (9-13 Hz) in the 4-item condition approximately 300-550ms following target onset (all qs<.05 FDR corrected) as compared to the ApoE-4 group, whereas such an effect was not found in the 2-item condition. These findings show that ADFH individuals with ApoE-4 show altered neural oscillatory activity when performing the higher cognitive load visuospatial working memory task, which could be a potential mechanism underlying observed neuropsychological impairment (e.g., worse ARs).

Poster 10.4

Immediate early gene Zif268 response is reduced in the extended hippocampal system by senescence, but partially improved by oral administration of a connexin hemichannel blocker

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Declining function across an extended hippocampal system contributes to ageing-related cognitive impairments. Here, we examined the integrity of this system using zif268 protein expression in late-life rats (31 months; N = 22). A sub-group of senescent rats also received oral administration of the connexin hemichannel blocker tonabersat for three months prior to sacrifice to evaluate whether hemichannel function influences zif268 protein expression in aged rats (daily, 0.4mg/kg or 0.8mg/kg; one group was given vehicle only). All rats explored a radial arm maze for three 7-minute trials and were sacrificed 90 minutes later. Compared to young adult rats (6 months; N=13; vehicle only), senescent rats showed substantially decreased expression of zif268 protein in hippocampus, prefrontal cortex (PFC) and retrosplenial granular b cortex (RSGb), although substantial reductions were already evident by late middle-age (15 months; N=10; vehicle only). Irrespective of dose, tonabersat in senescent rats increased zif268 protein expression in the anterior cingulate (PFC) and RSGb deep cell layers to levels found in the late middle-aged group. Reduced neuronal activity across the extended hippocampal system is evident in senescent rats, but marked reductions already occur by late middle age. Tonabersat in senescence mildly improves the level of zif268 expression in the PFC and RSGb. Further work on the effects of tonabersat at earlier points in life is warranted.
**Poster 10.5**

**Investigating the mechanisms driving circadian and sex differences in the stress axis**

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Activation of corticotropin-releasing hormone (CRH) neurons leads to the release of CRH peptide, activation of pituitary corticotrophs and the secretion of adrenal corticosteroids. Previous research has shown a robust circadian rhythm in the secretion of ACTH from the pituitary and corticosteroids from the adrenal with a peak occurring at the onset of the active period. It is also known that females show a larger change in corticosteroid levels across the 24-hour day compared with males. However, there have been very few studies investigating whether CRH peptide secretion follows a circadian rhythm and if there are sex differences. To study this, we used western blot to measure the CRH peptide levels in the median eminence at four time points across a 24-hour period in male and female mice. We observed a statistically significant difference in serum corticosterone level across a 24-hour day, with a peak occurring at zeitgeber time 12. However, we found no statistically significant differences in the CRH peptide level in the median eminence between the different time points. Within each time point, there were no statistically significant differences in the CRH peptide level between female and male mice. These results indicate that CRH peptide levels in the median eminence are constant throughout the day. This suggests that the circadian rhythm of corticosterone is likely caused by other factors. On-going experiments are investigating if changes CRH neuron excitability contribute to circadian and sex differences in the stress axis.

**Poster 10.6**

**Language changes in Alzheimer’s disease: A systematic review of verb processing**

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Alzheimer’s dementia (AD), the most prevalent dementia, results in a range of cognitive impairments. These often include language problems such as word-finding issues, but also higher-level communication problems including failure to provide context-relevant information in conversations. Research into the language of people with AD (pwAD) has mainly focused on nouns. Less attention has been given to verbs, though verbs impose greater semantic and syntactic processing demands and are more complex morphologically. This study aimed to review existing evidence regarding verb comprehension and production in pwAD. It is anticipated that this knowledge will improve the diagnosis and monitoring of progressive language problems in pwAD and help direct the focus of language-based interventions. A systematic literature search identified 70 studies examining verb comprehension and production in pwAD. Studies reported that pwAD were less accurate than controls in performing single-word tasks requiring either production or comprehension of verbs and were less accurate at naming pictures of actions than of objects. Participants also exhibited difficulty comprehending sentences featuring multiple verbs or verbs whose semantics allowed surrounding nouns to be transposed. Discourse studies showed mixed results as to whether pwAD used verbs more or less than controls, but indicated that their verbs were less semantically complex. Throughout these studies, verb impairments were consistently present in comprehension and production by pwAD. The need for further study of discourse stands out as one of several issues to be addressed in future research, as studying discourse presents a fuller picture of effects of language decline on communication than assessments focused on single words.
Awake craniotomy surgery allows maximal tumour resection while preserving critical cognitive functions by using neuropsychological tests for individualised brain mapping. The Pyramids and Palm Trees Test (PPTT) is currently used to map the Inferior Fronto-Occipital Fasciculus (IFOF) but has questionable validity, low image quality, varying item performance, and significant culture and education effects. This project aims to develop a new, higher quality test which is specifically designed for use in a surgical context. A review was conducted of previous research into awake craniotomy, the IFOF, the PPTT, and semantic memory testing. Measures of use for semantic memory test item quality were identified (including measuring association strength using Latent Semantic Analysis) and an analysis was conducted of PPTT items. A new test format (composite images) was designed which may be better suited to use in a surgical context. The new composite images format requires fewer and simpler instructions than the PPTT and allows measurement of more types of associations than the Feature Reality Test. The patient does not need to provide a verbal response and is only required to point at a single target image. Item selection for the new test is in progress. Improved IFOF mapping would reduce the risks of neurosurgery by enabling surgeons to precisely locate this critical tract, allowing them to maximally resect tumour tissue up to its boundaries while still preserving its important functions. The new composite images format may reduce the demands on patients during surgery, increasing the proportion of patients for whom this mapping can be completed. Meeting attendees will be invited to contribute to the validation of the new test.

The pineal gland synthesises and secretes the hormone melatonin and its precursor N-acetylserotonin (NAS), which are potent antioxidants that are also implicated in the sleep-wake cycle. Disturbances to melatonin's normally high night-time peak are apparent in various sleep disorders and neurodegenerative diseases. Sympathetic innervation of the melatonin pathway passes through the intermediolateral column of the spinal cord (IML) and superior cervical ganglia (SCG). Previous in vivo research in animals show it is possible to upregulate melatonin levels via invasive electrical stimulation of the SCG and/or the post-ganglionic sympathetic fibres that innervate the pineal gland. However, the possibility of modulating melatonin and NAS levels via non-invasive electrical stimulation has not yet been explored. This project investigates whether stimulation of specific dermatomes (T1 and C2/C3) that share anatomical connections with the IML and SCG respectively, are able to modulate the hormonal output of the pineal gland. Wistar rats (2-3 months) will receive either high or low frequency stimulation, sham, or no stimulation at the T1 or C2/C3 dermatomes. The voltammetric profiles of melatonin and NAS were characterised using fast-scan cyclic voltammetry in order to monitor their levels in vivo in real-time. This involved a novel approach of mathematically subtracting a problematic fouling substance from melatonin's waveform. However, the complexity of chronic electrode implantation into the pineal made this methodology infeasible. Instead, pinealectomy and assessment for melatonin and NAS content compared to controls via nanoflow liquid chromatography/mass spectrometry will be used. Findings from this research may open up a new neuromodulation treatment avenue for sleep-wake disorders and related neurological diseases.
Stroke-induced cognitive impairments are of significant concern, however mechanisms underpinning these impairments remain poorly understood. Recently, we established a stroke model resulting in delayed impairment in spatial memory. To further characterise cognitive impairments in our model and align our assessments with what is used clinically, we have employed the use of touchscreen-based behavioural testing systems. Young C57BL/6 mice were trained in operant touchscreen chambers to complete the trial-unique nonmatched-to-location (TUNL) task. Based on baseline performance animals were given either bilateral photothrombotic stroke or sham surgery targeting the prefrontal cortex (PFC). Upon recovery, post-stroke spatial working memory was assessed by varying both the degree of separation and delay within the TUNL trials. To access PFC-thalamic connectivity, the retrograde tracer cholera toxin B (CTb-488), was injected into the prelimbic region 7-weeks after surgery. Five-days post-injections brains were collected and processed histologically and immunohistochemically to assess infarct volume and connectonal maps, respectively. Two-way ANOVA and multiple comparisons revealed stroke animals took significantly longer and performed worse during reacquisition of the TUNL task, relative to shams. Moreover, all animals performed worse when delay duration or separation level was either increased or decreased, respectively, which was exacerbated in stroke animals. Preliminary CTb-positive cell counts indicates that stroke to the PFC induces a loss in connections between the PFC and thalamus. The current study is the first to show stroke-induced spatial working memory impairments as assessed using a clinically relevant TUNL task. Our findings contribute to a better understanding of the neural mechanisms underlying stroke-induced cognitive impairments by also assessing changes in connections between brain regions.

Schizophrenia is associated with deficits in decision making and learning. The anterior cingulate cortex (ACC) and the ventral tegmental area (VTA) have been linked to decision making and motivation respectively. Here we examined VTA and ACC activity in a maternal immune activation (MIA) rodent model of schizophrenia risk. A cost-benefit T-maze reversal task was performed by control and MIA rats whilst local field potentials (LFPs) were recorded from their ACC and VTA. The T-maze task offered a choice between a higher cost, higher reward (HCHR) arm and a lower cost, lower reward (LCLR) arm. Halfway through a recording session the cost/benefit location reversed. After reversal Control rats chose the LCLR choice, which corresponded to the previous session’s final HCHR choice, significantly more often than MIA rats (and chance) on the first trial of each session. Control rats also showed a decrease in HCHR choices as they approached the reversal, but an increase as they approached the end of the session. In contrast MIA rats displayed little change in percentage of HCHR choices. Post-reversal, MIA rats had significantly lower HCHR choices than controls. MIA and control rats also demonstrated different levels of low-beta and delta LFP activation in the VTA and the ACC in the decision-making part of the maze. These results suggest that the MIA rats are failing to effectively incorporate previous knowledge into their decision-making. They also demonstrate inflexibility during reversal learning. The LFP data suggest that the ACC and VTA may be involved in this deficit.
Neuromodulation of olfactory performances using high-frequency vagus and median nerve stimulation in healthy male adults

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Existing literature indicates invasive vagus nerve stimulation (VNS) in animal models using high-frequencies produces increased activity of the olfactory bulb, while median nerve stimulation (MNS) using low-frequencies affects vagal brainstem nuclei pathways in the cortex. However, no studies in humans have investigated the effects of high-frequency VNS and MNS on olfactory performance. Our studies examined the effects of non-invasive VNS and MNS on olfactory performance, with supplementary orbitofrontal cortex (OFC) recordings using near-infrared spectroscopy (NIRS). Healthy male adult participants performed a supra-threshold test (labelled magnitude scale; STT-LMS) for four food-related odorants, before and after MNS. Supra-threshold test (STT) using Vanillin and odour threshold test (OTT) were performed before and after VNS. Both VNS and MNS were performed at low frequency (10Hz), high frequency (80Hz) and placebo in three separate streams. Data was analysed using non-parametric statistical tests for STT and OTT and repeated-measures ANOVA for STT-LMS and NIRS. Only high-frequency VNS and MNS on olfactory performance, with increased contralateral OFC activation while MNS showed decreased detection in the STT-LMS with increased bilateral OFC activation. Our results indicate that VNS and MNS present opposite effects, with corresponding differences in neural activation of the OFC. Further exploration in the MNS and VNS neurocircuitry with olfactory performance is required to explore current opposing effects found in our studies, and help provide a non-invasive treatment option for patients with olfactory impairments (e.g., the olfactory impairments associated with Alzheimer’s and Parkinson’s disease).
Poster 10.13

Dual in situ hybridization and immunohistochemistry for quantitative analysis of house-keeping gene changes in normal and Alzheimer’s disease brain tissue

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In situ hybridization (ISH) techniques are a powerful tool that can be used to identify and localize gene expression in tissue samples. Combining ISH with immunohistochemistry (IHC) gives the cellular context of mRNA expression and spatial variation in expression patterns, which cannot be achieved with gene microarray or polymerase chain reaction. To study gene and protein expression on the same section we investigated the use of RNAscope® ISH in combination with fluorescent IHC on paraffin embedded human brain tissue from neurologically normal and Alzheimer’s disease cases. We utilized tissue microarray (TMA) sections of middle temporal gyrus (MTG) from 22 neurologically normal and 19 Alzheimer’s disease cases to study the expression of three housekeeping genes: ubiquitin C (UBC), peptidyl-prolyl cis-trans isomerase B (PPIB) and DNA-directed RNA polymerase II subunit RPB1 (POLR2A). Furthermore, we developed an automated analysis method for quantifying RNA ISH puncta across the total cell population and within neurons identified by NeuN+ immunoreactivity. Overall, we saw a significant decrease in both UBC and PPIB expression in Alzheimer’s disease MTG cores, both in total cell expression and neuronal-specific expression, compared to normal cases. No significant correlations were observed between RNA ISH signal and age at death, post-mortem delay or time a tissue block had been cored for TMA construction. Therefore, we conclude that this technique used together with our custom automated analysis provides a suitable platform to study changes in gene expression in diseased brain tissue with both cellular and anatomical context.

Poster 10.14

Maternal immune activation alters pre-pulse inhibition and hippocampal nitric oxide synthase in postnatal day 35 and 60 rat offspring

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Maternal immune activation (MIA) during mid-gestation increases the risk of schizophrenia in offspring during adolescence and early adulthood. Experimentally, a rat model of MIA can be induced by a single systemic administration of the synthetic cytokine inducer polyinosinic-polycytidilic acid at gestational day 15. Our recent research has demonstrated significantly increased immunoreactivity of neuronal nitric oxide synthase (nNOS) in the hippocampus in rat MIA offspring at postnatal day (PND) 2 and 35. It is unclear, however, whether this long-lasting change in nNOS during development is associated with functional deficits in MIA offspring. The present study, therefore, performed the pre-pulse inhibition test (PPI, a marker of schizophrenia) and measured total NOS activity in the sub-regions of the hippocampus at PND 35 (adolescent) and 60 (young adult) (n=8/group/sex/age). Interestingly, MIA offspring at both age points displayed significant PPI deficits and increased total NOS activity in the CA3 and dentate gyrus of the hippocampus when compared to their age- and sex-matched controls. In conjunction with increased nNOS immunoreactivity in the hippocampus of PND35 MIA offspring, the observed increases in total NOS activity may be largely due to nNOS up-regulation. Given its critical role in the central nervous system development and neurotransmission, altered NOS and its associated mechanisms may contribute to PPI deficits in MIA offspring.
Poster 10.15

**Behavioural deficits and hippocampal apoptosis in offspring with prenatal Di-(2-ethylhexyl) phthalate exposure**

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Phthalates are a group of chemicals that are used to make many household items. Epidemiological studies have indicated that early-life exposure to phthalates may affect neurodevelopment, however the underlying mechanisms remain unclear. The present study was therefore designed to investigate how maternal di-(2-ethylhexyl) phthalate (DEHP) exposure affected behavioural function in offspring and to explore the underlying mechanisms. Pregnant mice received oral gavage of DEHP at the doses of 0, 50 (low) and 200 (high) mg/kg/day from gestational day 0 to 18. Their offspring were weaned on postnatal day (PND) 21 and then tested in the Morris water maze task or decapitated for brain tissue collection on PND 28. The offspring with high dose prenatal DEHP exposure displayed poor performance during the place navigation and subsequent probe test relative to those received no or low dose DEHP prenatal exposure. Prenatal DEHP exposure induced apoptosis in the hippocampus as evidenced by the activation of caspase-3 and caspase-9, up-regulation of Bax and down-regulation of Bcl-2 at PND 28 in a dose-dependent manner. Moreover, there was a small reduction in hippocampal and plasma acetylcholinesterase in offspring with prenatal DEHP exposure. These findings provide the first evidence that prenatal DEHP exposure results in spatial learning and memory deficits and induces hippocampal apoptosis in PND28 offspring. Future research is required to explore other mechanisms underlying prenatal DEHP exposure induced behavioural deficits.

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Poster 10.16

**I trust you, my imagined friend**

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Mental simulation is the ability to flexibly imagine future events. This ability has been suggested to allow individuals to prepare and plan for the future. A myriad of studies has shown that mental simulation can heighten the perceived likelihood of imagined events, and increases trust towards out-group members (Miles & Crisp, 2013; Pham & Taylor, 1999). More recently, Gaesser and Schacter (2014) showed that mental simulation can also influence prosocial decision-making. They found that people were more willing to help a person in need after having imagined a future scenario in which they helped this person. The impact of mental simulation on willingness to help has been replicated in a number of subsequent studies (Gaesser et al., 2017a; Gaesser et al., 2017b; Gaesser et al., 2018; Gaesser et al., 2019). Although this prosocial simulation effect has been well demonstrated (e.g. Gaesser & Schacter, 2014), no study to date has assessed whether simulation of a prosocial event will subsequently induce a more positive evaluation of the simulated person (e.g. increase in trust). Here, using the aforementioned paradigm, we found that participants who had imagined a prosocial event involving a specific person expressed a greater degree of trust that the person would help them in the future, relative to a control task. Furthermore, we found this increase in trust was mediated by the participant’s willingness to help the simulated person. These findings are consistent with the notion that simulating prosocial interactions can increase trust in the imagined partner through activating the social norm of reciprocity. Thus, our findings show that mental simulation of a prosocial interaction not only increases intention to help but also trust in the imagined partner.
ABSTRACTS

Poster 10.17

Local field potential recordings in rat anterior cingulate cortex, anterior insula, and ventral tegmental area during a voluntary effort exertion task

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The purpose of this study was to investigate neural activity changes in the anterior cingulate cortex (ACC), the anterior insula (AI), and the ventral tegmental area (VTA) during a fatiguing effort exertion task. Sprague-Dawley rats (n=10) were trained on the progressive Weight Lifting Task, a task our lab has recently developed where a rat voluntarily pulls a rope for a sucrose solution reward. The rope is attached to an adjustable weight, with the weight increasing (from 0g to 225g, at a 45g interval) every time the subject completes 10 successful trials. During the task, local field potentials (LFPs) from ACC, AI, and VTA were recorded alongside behavioural data. Across 80 recording sessions, behavioural data showed that as the weight got heavier, the rate of pulling attempts decreased while time per trial increased, indicating probable fatigue. When average LFP power (0.5-45 Hz) was examined from the first, the middle, and the last 10 sec of each session, all three brain regions demonstrated significant decreases in broadband power across the session. When looking specifically at regional LFP power during the reward phase for each weight, mean LFP power in the ACC decreased significantly in the beta frequency range (15-35 Hz) while mean LFP power in the AI increased in the beta band range. VTA LFP power did not change significantly in this band. These preliminary results suggest that dynamic neural activity changes in the ACC, AI, and VTA may be relevant to the development of fatigue.

Poster 10.18

Evaluating the potential of G protein biased Mu Opioid Receptor agonists as a treatment for chronic pain

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Mu opioid receptor (MOR) agonists are potent and efficacious analgesics, however, side-effects such as tolerance, respiratory depression and constipation plague their use. MOR side effects are widely believed to be due to activation of β-arrestin signalling pathways, and signalling selectively via G-protein pathways are likely to have fewer side effects. With this in mind we evaluated two structurally novel Salvinorin A analogues with different G-protein bias factors. Kurkinorin (bias factor: 0.57) and the highly biased Kurkinol (bias factor 0.14) were effective at attenuating chemotherapy-induced neuropathic pain. Kurkinol was more potent at reducing mechanical (ED50=1.5 mg/kg) and cold allodynia (ED50 = 1.74 mg/kg) than morphine (mechanical ED50=9.2 mg/kg; cold ED50=9.03 mg/kg), whereas Kurkinorin was equipotent to morphine (mechanical ED50=9.2 mg/kg; cold ED50=14.82 mg/kg). The effects on gastrointestinal transit were measured by faecal accumulation and charcoal-meal transit in the small intestine. Kurkinol inhibited gastrointestinal transit in both assays, whereas Kurkinorin induced inhibition overall, but not in the small intestine. Respiratory depressive effects were evaluated using whole body plethysmography in unrestrained mice. Kurkinol induced significant decreases in all respiratory measures, including respiratory frequency, tidal and minute volumes. Whereas Kurkinorin showed significantly reduced respiratory-depressive effects. Our results show that while analgesic potency is correlated to G protein bias side-effects are not. The more balanced MOR agonist, Kurkinorin, displayed the best safety profile. Our data shows that Kurkinorin is effective at modulating pain with significant improvements in side effects, highlighting its therapeutic potential as a safer treatment for modulating pain.
Parkinson’s Disease (PD) is a neurodegenerative disease caused by the depletion of dopamine in the brain. Tremor, bradykinesia, rigidity and postural stability are the four major symptoms of PD. These symptoms cause adverse effects on mobility that in turn affect the daily life of PD sufferers. The severity of these symptoms are usually assessed by a clinical scoring system based on the Unified Parkinson’s Disease Rating Scale (UPDRS). However, UPDRS is subjective and relies on the visual observations of physicians rather than quantitative measurements. Researchers have built custom devices for PD characterisation, however, these devices have reliability issues and are non-standard measurements. Hence we utilised a commercially available off-the-shelf product called Leap Motion for non-contact assessment of PD. Custom made software in conjunction with Leap enabled acquisition of three dimensional positioning of the hand. Accordingly, PD patients’ bilateral hand tremor motion was captured using the system and UPDRS (including Part III motor subsection & resting tremor) were also scored. Here we present preliminary results of the relationships between the physician based UPDRS scores and various features extracted from the Leap recordings such as the tremor volume and frequency spectrum.

Electroencephalography (EEG) is widely used to investigate brain responses related to sensory and cognitive processes. Depending on the process of interest, experimental procedures vary from passive perception to active task-based performances. Successful EEG recording depends on task compliance by the participants. This compliance becomes more challenging with decreasing age, and can be problematic in preschool-aged children. As significant cognitive and social development occurs in this age group, researchers have tried to minimise experimental demand characteristics by focusing on non-active tasks. However, to study complex processes such as picture naming, reliance on non-active tasks is insufficient and active task cooperation from the children is required. The current study set out to investigate the feasibility of using a picture-naming task (98 words, presented across 7 blocks) in 3-year-old children, and provide data on participant compliance for study planning purposes. To facilitate the children’s participation, we developed the Space Protocol. This embeds the EEG experiment in a spaceship adventure that prepares the children for all the potentially difficult aspects of undergoing EEG recording (putting electrode cap on, sitting still, naming pictures, etc.), before starting the EEG experiment. Twenty-three 3-year-old English speaking children with no history of neurological disease or hearing problems were recruited ($M=3.4$ years). Twenty-two children completed the preparation phase and started the EEG recording. Fifteen of them completed the whole EEG experiment, four completed more than the half of the trials and two completed 2 blocks. This shows that active participation in task-based protocols is achievable in 3-year-old children.
Poster 10.21

Cerebrovascular expression of GABAergic signalling components in the hippocampus of healthy and Alzheimer’s disease brains

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Alzheimer’s disease (AD) is a neurodegenerative condition characterised by progressive decline in memory and cognition. The temporal lobe and particularly the hippocampus are vulnerable to neurodegeneration in AD. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. GABAergic signalling is significantly disrupted in AD, with the subunit composition of GABA_A receptors (GABA_AR) predominantly affected. GABA is capable of inducing vasodilation and modulating regional cerebral blood flow, although the mechanism behind this remains unclear. Vascular dysfunction has been implicated in AD pathogenesis. Vascular changes begin at an early stage in AD development, prior to extensive neurodegeneration. This study aimed to quantify GABA_AR density on the cerebral vasculature in the middle temporal gyrus (MTG), hippocampus and entorhinal cortex of healthy and AD human brains using free-floating fluorescent immunohistochemistry with confocal microscopy. We have determined that cerebrovascular GABA_ARs have unique subunit compositions, with the β3, e and γ3 subunits displaying the highest expression levels, and with the absence of the α1 subunit. There were no significant differences in α2, α3, β1, β2, β3 or γ2 subunit expression levels in the MTG between AD and healthy cases. We will next look at potential changes in vascular e and γ3 subunit expression in AD. This is the first evidence confirming the expression of GABA_AR subunits in the human cerebral vasculature, which will help to determine whether GABAergic changes contribute to cerebrovascular alterations in AD.

Poster 10.22

Partial eNOS deficiency promotes cognitive impairment in a murine model of Alzheimer’s disease

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A growing body of evidence implicates the role of cerebrovascular dysfunction in the pathogenesis of sporadic Alzheimer’s disease (AD). Nitric oxide (NO) derived from endothelial NO synthase (eNOS) is essential in maintaining normal cerebral blood flow. Mice with eNOS deficiency (eNOS−/−) have increased amyloid beta (Aβ) levels and tau phosphorylation in the brain and cognitive decline. APPswe/PSdE1 (APP/PS1) mice display age-related Aβ accumulation and memory deficits. In order to add an element of cerebrovascular dysfunction to this model, we crossed eNOS−/− mice with APP/PS1 to produce APP/PS1/eNOS−/− mice. To characterise this new model, we assessed the behavioural performance of male wild-type (WT), eNOS+/−, APP/PS1 and APP/PS1/eNOS−/− mice at 8 months of age. There were no significant genotype-related behavioural changes in the Y-maze and elevated plus maze. When tested in a working memory version of the water maze task, there were no significant genotype effects on swimming speed and path length to a visible platform. During the subsequent trials to a hidden platform, both APP/PS1 and APP/PS1/eNOS−/− mice generated markedly longer path length and greater thigmotaxis relative to the WT controls. Moreover, APP/PS1/eNOS−/− mice generated significant longer path length when compared to APP/PS1 mice, with no difference between groups in thigmotaxis. These preliminary results demonstrate that partial eNOS deficiency exacerbates spatial learning deficits in APP/PS1 mice. Neuropathological characterisation and functional elucidation in APP/PS1/eNOS−/− mice is underway to understand the role of cerebrovascular dysfunction in AD pathogenesis.

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Poster 10.23
Assessing the impact of comorbidities on axonal sprouting after stroke to the motor cortex
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Recovery from stroke is notoriously difficult to predict, with certain stroke comorbidities contributing strongly to poor neurological outcome. In particular, clinical studies have shown that diabetes increases the risk of ischemic stroke and is associated with poor recovery of function. The exact reasons why diabetics are predisposed to poor stroke recovery are not known and require investigation in order to translate treatments. Hyperuricaemia is associated with various metabolic dysfunctions including obesity, diabetes and poor vascular health. With stroke induced recovery being linked to an increase in axon sprouting we hypothesised that obese prediabetic mice that are mildly hyperuricemic (POUND (Lepr(db/)) and chronic hyperuricemic, Urahplat2/plat2 (PLT2), mice would have altered axon sprouting after stroke compared to wild-type (WT) controls. Focal stroke to the motor cortex was induced using the photothrombosis method in POUND, PLT2 or WT mice. To assess changes in axonal sprouting, the neuroanatomical tracer biotinylated dextran amine was injected into the premotor region 3-weeks after surgery. One-week post-injections brains were collected and processed histologically and immunohistochemically to assess infarct volume and connectional maps, respectively. Assessment of infarct volume revealed no difference in stroke size between POUND and WT controls, however, a significant increase in infarct volume was observed in PLT2 mice. Even though no difference in infarct volume was observed in the POUND mice, a significant increase in mortality was observed, which supports the literature showing poor recovery after stroke. Ongoing analysis is assessing the impact that these two comorbidities have on axonal sprouting and functional recovery after stroke. This information is vital for assessing the population as a whole and understanding how different comorbidities impact recovery of function and also potential treatment options.

Poster 10.24
Evaluation of locomotor and pain behaviours in the cuprizone toxin-induced demyelination model of multiple sclerosis
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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) affecting approximately 2.5 million people worldwide. The toxin-induced cuprizone model is commonly utilised to replicate disease progression and recovery in MS. Administration of 0.3% cuprizone in ground mouse chow produces consistent demyelination in the CNS that can be evaluated and quantified histologically. While some behavioural measures are utilised to model disease progression, it is still unclear which behaviour is the most robust measure to evaluate. Therefore, in this study, we aimed to assess a variety of behavioural measures including weight loss, open-field locomotor activity and time spent in the centre (anxiety measure) and the passive wire hang test to assess demyelination in both male and female C57BL6 mice. Mice were fed ground food with or without 0.3% cuprizone for 5 weeks. Both male and female mice administered 0.3% cuprizone exhibited a dramatic decrease in weight compared to healthy control mice. No change in distance travelled was observed in cuprizone-treated mice. However, cuprizone-treated male but not female mice spent less time in the central zone suggesting anxiogenic-like effects in males. No change was observed in ability to grip in the wire hang test. These results indicate that male mice appear to be more sensitive to cuprizone-induced anxiety. However, these tests may not be sensitive enough to detect other behavioural manifestations of cuprizone exposure. Establishing robust behavioural measures that reflect the extent of demyelination or, conversely remyelination, are essential to aid in the development of better pharmacotherapies for MS.
Poster 10.25

Miniaturized wireless optoelectronic subdermal implants: a novel device for the optogenetic stimulation of hippocampal neurons

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Optogenetics is a technology that allows fast and specific activation or inhibition of genetically engineered neurons by light stimulation. Traditional tools for in vivo optogenetics require physical tethering of the animal to an external light source, restricting the scope of potential experiments. Currently available wireless alternatives avoid some of these limitations but still encompass disadvantages such as the weight of the device, implantation damage, and probe stiffness. Here, we present a novel in vivo optogenetic system, developed by Neurolux, which provides a fully implantable optoelectronic subdermal device with ultraminiaturized LEDs that can be wirelessly controlled and specifically customized to target the mouse hippocampus. The wireless coverage of the device was tested confirming complete coverage of activation in all areas of the behavioural apparatus. The device was implanted by stereotaxic surgery using coordinates targeted to the hippocampus and optimized for comfortable skull constraint. Novel object recognition and novel object alteration behavioural tests were conducted on naive controls and animals implanted with the device. Brain sections (30μm thick) were immunolabelled for IBA-1 and GFAP to examine the level of damage and inflammation from the device. The behavioural tests demonstrated that the implant does not affect the animals’ normal behaviour and cognition. There were no significant differences in the expression of inflammatory markers in the hippocampus between both groups. This device provides accurate and advanced optical control of hippocampal neurons, offering solutions to study complex animal behaviours.

Poster 10.26

Establishing an animal model of paediatric mild traumatic brain injury

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Traumatic brain injury (TBI) is a leading cause of hospital visits among children. Clinical studies have linked mild TBI with an increased risk of developing long-term disorders such as depression, anxiety, and cognitive dysfunction. Despite the prevalence and severity of mild TBI, there is not a comprehensive understanding of the underlying pathology, due in part to the heterogeneous nature of the injury. This study aims to establish an animal model to replicate single and repetitive mild traumatic brain injuries in paediatric (postnatal day 17) mice. To better replicate the clinical setting of mild TBI, the head was not restrained and an impact over the parietal lobe of an intact skull was induced using an electromagnetic impactor. Reactive astrogliosis and vascular thinning were assessed at 4 and 12-weeks in young brains post-impact using immunohistochemical and vascular-gel casting respectively followed by fluorescence microscopy and ImageJ analysis. Preliminary evidence indicates that a very mild impact is enough to trigger an increase in reactive astrogliosis. Previous published research has shown that sustained mild reactive astrogliosis leads to long-term behavioural consequences. Behavioural changes are being investigated at 4 and 12-weeks post-impact on the open field, novel object recognition and novel object location recognition tasks, and the elevated plus maze. By establishing a closed head injury model of TBI, we aim to better understand the pathophysiological mechanism associated with mild TBI-induced impairment with the hope of improving the overall clinical translation of potential treatments.
Poster 10.27

The gliopeptide ODN regulates tonic GABA<sub>A</sub> receptor currents and boosts functional recovery after stroke

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Following stroke, the survival of neurons and their ability to re-establish connections is critical to functional recovery. This is strongly influenced by the balance between neuronal excitation and inhibition. In the acute phase of experimental stroke, lethal hyperexcitability can be attenuated by positive allosteric modulation of GABA<sub>A</sub> receptors (GABA<sub>A</sub>R). Conversely, in the late phase, negative allosteric modulation of GABA<sub>A</sub>R can correct the sub-optimal excitability and improve motor recovery. Here, we hypothesized that octadecaneuropeptide (ODN), an endogenous allosteric modulator of the GABA<sub>A</sub>Rs that is synthesised by astrocytes, improves functional recovery after stroke. Focal stroke to the motor cortex was induced using the photothermogenesis method in aged (20-24 month old) female mice. As ODN does not cross the blood-brain-barrier, we combined ODN into a biopolymer hydrogel and injected this into the stroke cavity 5-days post-stroke. Local targeting of ODN to the peri-infarct cortex resulted in a significant improvement in motor functional recovery, when assessed from weeks 1-6 post-stroke compared to vehicle-treated stroke controls, on both the grid-walking and cylinder tasks. To assess a potential underlying mechanism for this, we performed immunohistochemistry for GFAP. Levels of GFAP fluorescence were lower following treatment with ODN, indicating a reduction in reactive astrogliosis. Patch-clamp electrophysiological recordings taken 14-days post-stroke showed an increase in tonic inhibitory currents that were normalised following treatment with ODN. We show that local hydrogel delivery of ODN from 5-days post-stroke dampens the stroke-induced elevation in tonic inhibitory currents and improves motor functional recovery. These data indicate that the gliopeptide ODN could be a novel target for regulating functional recovery after stroke.

Poster 10.28

3-dimensional structural analysis of the sheep round window membrane

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Intratympanic drug administration to the cochlea has potential for treatment of inner ear diseases. For drugs to reach the cochlea, they must diffuse through the round window membrane (RWM), separating cochlear fluid and middle ear spaces. However, RWM structural variations and molecular properties pose challenges to effective drug delivery. This study aimed to characterize the molecular composition and 3-dimensional RWM structure in the sheep cochlea, which is structurally similar to human. Fresh sheep cochlear tissues were obtained from 2-4 year old sheep. Cochleae were dissected and fixed in 4% paraformaldehyde for 48 hours, and decalcified in 8% EDTA for 6-8 weeks. For structural analysis by microCT, cochleae were stained with 1% Osmium tetroxide for 3 days and visualised using Skyscan 1272 (Bruker). For histological analysis, paraaffin-embedded cochleae were sectioned and stained with Hematoxylin /Eosin and Mason’s trichrome stainings. Whole-mounted RWM were labelled with Phalloidin conjugated with Alexa 488, Wheat Germ Agglutinin-Alexa 594 and Hoechst (UV) for confocal FV-1000 live cell imaging. MicroCT analysis revealed the RWM is convex and has different zones of thickness. Histology showed three layers, two epithelial and an intervening connective tissue layer. Epithelial cells on the middle ear side were rich in actin, and a vasculature and melanocytes were also identified. Interestingly, a ‘fibrous meshwork’ structure connected to the RWM was observed covering the cochlear aqueduct, which provides communication between cochlear perilymph and cerebrospinal fluid in the posterior cranial fossa. This structure may support the pressure-dependent movement of the RWM. Building a comprehensive map of the RWM will contribute to the better understanding of drug delivery to the cochlear.
Poster 10.29
Changes in dopamine signalling and responses to drugs affecting dopaminergic neurotransmission in striatal and ventral midbrain slices from DAT-KO rats
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Cessation of dopamine (DA) transmission largely depends on reuptake by the DA transporter (DAT). DAT expression/activity is reduced in several neurological disorders and after exposure to drugs of abuse (e.g. amphetamine). Here we characterized the behavioural, neurochemical, and electrophysiological effects of eliminating DAT activity in a novel DAT-knockout rat generated using CRISPR/Cas9. As expected, DAT-KO rats displayed no DAT immunoreactivity in the striatum, increased basal locomotor activity, and paradoxical calming by amphetamine. Fast-scan cyclic voltammetry in brain slices demonstrated a large decrease in clearance of electrically stimulated DA release in the dorsal striatum and to a lesser extent in the Substantia Nigra pars compacta (SNc). We showed that DA clearance becomes more dependent on diffusion and uptake-2 (organic cation transporter-3; OCT3). Decreasing slice perfusion rate or applying the OCT3 blocker corticosterone increased half-life of DA release in the DAT-KO striatum. Cocaine increased the amplitude of DA release and slowed its clearance in slices from wild-type (WT), but not DAT-KO rats. Basal extracellular DA concentration ([DA]out), measured with fast-scan controlled-adsorption voltammetry was higher in DAT-KO rats compared to WT littermates, and was enhanced by L-DOPA. Baseline firing frequency of SNc neurons and GABA_A-mediated inhibition were similar in DAT-KO and WT rats. However, D_2-mediated inhibition was blunted in DAT-KOs, likely due to downregulation of D_2 receptors previously reported in DAT-KO mice. These results validate our DAT-KO model and provide novel insights into the mechanism of DA uptake which form the basis for our future in vivo studies.

Poster 10.30
The relationship between cognitive function and expressive language in individuals with Parkinson’s disease
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Parkinson’s Disease (PD) is identified as the second-most common neurodegenerative disorder, with up to 90% of patients developing communication difficulties. While the speech motor characteristics of PD have been documented in depth, associated language changes remain a topic of controversy. However, recent research has attributed communication changes to both speech motor and cognitive factors. The aim of this preliminary study was to 1) identify markers to monitor language changes in people with PD, and 2) test whether a relationship exists between cognitive functioning and language scores. Language samples of 11 participants with PD and nine control participants were transcribed and examined for sample duration, number of utterances, mean length of utterance, lexical diversity, propositional idea density, speech rate, and repetitions/retracings. Results indicated that participants with PD used significantly fewer words per minute than controls (p < .05) and had a significantly greater language sample duration than controls (p < .05). There were no significant differences between the groups on the remaining measures. Correlations between language measures and cognitive function, as assessed by the Montreal Cognitive Assessment (MoCA), revealed a significant positive correlation between MoCA score and speech rate, and a significant negative correlation with sample duration and repetitions/retracings. That is, participants with lower MoCA scores used fewer words per minute, had a longer speaking duration, and evidenced a greater number of self-corrections in their speech. These preliminary analyses indicate that individuals with PD differ from controls in measures of time, rather than language content, and that cognitive function may influence these measures. Further analyses are ongoing.
Poster 10.31

Analysing emotional brain functional connectivity networks using Electroencephalography signals

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This study aimed to investigate the distributed nature of the EEG signals concerning emotions (Low-High Valence). Furthermore, to analyse and elucidate the association of different emotions with functionally specialised brain regions. This theme has presented great demand in health care such as for neurodegenerative patients who can experience the emotions but can’t express them, psychological and entertainment industries, hence a need to explore further. Dataset for Emotion Analyses using Physiological signals (DEAP) alongside various functional connectivity measures such as correlation and phase synchronisation was used for this study. To analyse difference between induced emotions with different subjects and frequency-bands, EEG data (N=32) were collected and divided into different frequency-bands: Delta (1-4Hz), Theta (4-8Hz), Alpha (8-13Hz), Beta (13-31Hz), and Gamma (31-60Hz) using Fourier and Morlet Wavelet transform. Repeated measure ANOVA and Tukey posthoc analysis identified a significant difference among subjects and frequency-bands with certain types of emotions. All frequency-bands were affected with emotion elicitation, especially in the lower bands (Delta, Theta and Alpha). The correlation and phase synchronisation among these lower frequency-bands with valence emotion yielded a significant induction in frontal and parietal regions. Moreover, similar results were observed with an increase of power in these frequency-bands and were found to be more sensitive towards emotions (Low valence). Independently for each frequency-band and different subject groups at all the electrode pairs, the main effect of emotions was found statistically significant at Pre-frontal, and Posterior couples of electrodes.

In conclusion, rather than restricting to a particular frequency-band, EEG based brain functional connectivity analysis reveals that the emotion patterns occur in all frequency-bands and different brain regions mostly at frontal, and posterior sites.

Poster 10.32

Neuroimmune interactions in the ageing brain

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High fat diet (HFD) and obesity are triggers that cause widespread and lasting brain inflammation and this effect is mediated by activation of microglia, major immune cells of the brain. This neuroinflammation leads to cognitive dysfunction as we age. Since ageing itself also promotes brain inflammation we hypothesized here that HFD and ageing in combination are more detrimental to cognition than either alone and that microglia play a role in this. We therefore aimed to test if we could improve cognitive dysfunction in the aged by strategically suppressing microglial hyper-activity. To this end, we used CRISPR/Cas9 genome editing to develop a Cx3cr1-Dtr transgenic Wistar rat with a diphtheria toxin receptor (Dtr) gene expressed in the promoter for the fractalkine receptor (Cx3cr1), highly expressed on microglia and monocytes. Upon administration of diphtheria toxin (DT), microglia and monocytes are transiently depleted in Cx3cr1-Dtr, but not wildtype rats. This allows us to specifically investigate the role of microglia/monocytes in normal and disease states including ageing- and HFD-related cognitive decline. To directly assess the role of microglia in ageing-related vulnerability to HFD, we fed aged (and young) wildtype and Cx3cr1-Dtr rats HFD for 8 weeks and assessed cognitive function with and without microglial ablation. Our data suggest microglia are important contributors to ageing-related cognitive vulnerability that may be a useful potential target for treatment against cognitive decline.
Stimulation of neurogenesis by a potential therapeutic protein

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Secreted amyloid precursor protein-alpha (sAPPα) is a neuroprotective protein and has been reported to stimulate neurogenesis in cultured rat cells. Adult neurogenesis is important for certain hippocampus-dependent memories but is impaired in neurodegenerative diseases. In this study, we determined in a neurosphere preparation from mouse pups if sAPPα could promote the formation of neurospheres, and if sAPPα increased neuronal differentiation in vitro. We found that, relative to control conditions, 0.01nM sAPPα applied for 7 days increased the size of neurospheres by 30% (p = 0.005). However, after treatment with sAPPα for 9 days in a differentiation protocol, the resulting cells exhibited no change in the ratio of neurons to astrocytes. Finally, we attempted to increase neurogenesis in vivo by intrahippocampal administration of a viral vector which overexpressed sAPPα (AAV9-HA-s APPα). Very preliminary results indicate that, in wild-type mice, over-expression of sAPPα results in an increase in the percentage of GFAP+ adult-born cells (from 6% to 16% of cells birth-dated with a BrdU analogue 8 weeks prior), and a reduction in the percentage of simultaneously birth-dated NeuN+ cells in the granule cell layer of the dentate gyrus. Future work includes determining whether overexpression of sAPPα can promote neurogenesis in a mouse model of Alzheimer’s disease to provide further support for developing sAPPα-based therapies for the treatment of neurological disorders in which neurogenesis is impaired or significant loss of neurons occurs.

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Levels of activity-dependent effects of Li+ on mitral cell activity

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Bipolar disorder (BD) is characterized by mood swings and abnormal brain network activity. BD is commonly treated with Li+. Li+ was clinically introduced in 1949 but its therapeutic mechanism of action remains unknown. Understanding how Li+ alters and interacts with neuronal activity may help account for how Li+ stabilizes brain networks that regulate emotion. To investigate the effect of Li+ on neuronal activity I made extracellular recordings of mitral cells (MC), the major output cells of the mouse olfactory bulb, in brain slices in vitro. I used electrical stimulation of the olfactory nerve (ON) to examine activity-dependence of Li+ effects. I stimulated the ON at 0.1 Hz for 10 min to compare synaptically-evoked and spontaneous MC activity; I then stimulated the ON at rodent resting breathing frequency (delta ~3Hz for 10 s) and recorded MC activity with 0.1 Hz stimulation for 20 minutes, with and without Li+ (1-2 mM). Li+ had multiple activity-dependent effects on MC activity. Firstly, Li+ was more effective on evoked than on spontaneous action potential bursts, with Li+ increasing variance and the number of action potentials in evoked but not in spontaneous bursts. Secondly, delta-stimulation amplified the effectiveness of Li+; delta-stimulation in Li+ (1 mM), but neither Li+ nor delta stimulation alone, increased the duration of both spontaneous and evoked bursts. Finally, ON stimulation history influenced subsequent Li+ effects; when preceded by delta- and 0.1Hz stimulation without Li+, subsequent (+20 min) delta-stimulation in Li+ (2mM) abolished both spontaneous and synaptically evoked action potential generation. These activity-dependent effects of Li+ on neuronal activity may reflect electrophysiological mechanisms of action through which Li+ can stabilize brain network activity regulating mood in BD.
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Energy status-dependent functional plasticity of the hypothalamic melanocortin system: Focus on glucose and ghrelin

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Of the central neural pathways responsible for co-ordinating responses to changes in energy balance, the melanocortin system of the arcuate nucleus (ARC) is critical. Comprised of orexigenic Neuropeptide Y (NPY)/Agouti-related peptide (AGRP) and anorexigenic Pro-opiomelanocortin (POMC)/cocaine-and-amphetamine regulated transcript (CART) expressing neurons, the neural circuits of this system are responsible for co-ordinating appropriate behavioural changes to perturbations in energy balance, signalled through nutrients, such as glucose and neuro-hormonal routes, such as leptin and ghrelin. Using an electrophysiological and behavioural approach we have investigated how aspects of these neural circuits adapt during negative (fasted) and positive (diet-induced obese; DIO) states of energy balance. Both NPY and POMC neurones show a remarkable energy-status-dependent plasticity in response to fasting and in DIO states. This presentation will describe fundamental changes in the functional operation of these neurones and associated circuits. Both neuronal subtypes shift neuronal excitability via adaptations in extrinsic excitatory and inhibitory synaptic transmission and shifts in expression of intrinsic postsynaptic active conductances regulating neuronal output. The effects of fasting and impact of DIO on the ability of POMC and NPY neurones to sense glucose will be revealed along with the mechanisms by which these neurones detect physiological changes in extracellular glucose. Similarly, how energy status impacts the sensitivity of NPY neurones to ghrelin, and the differential ionic mechanisms underlying the effects of ghrelin will be discussed.

13.1

Do drugs targeting the kappa opioid receptor hold the key to finding effective treatments for neuropathic pain?

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Neuropathic pain is chronic pain that forms following nerve damage or injury and is common in people with spinal cord injury and those suffering from diabetes, multiple sclerosis, stroke, or as a result of chemotherapy treatment. Neuropathic pain sufferers have a poor quality of life, reduced ability to work, and are more likely to suffer from depression. Current treatments are ineffective, cause tolerance and have severe side effects including addiction. Our research has identified new molecules that bind to the kappa opioid receptor that are potent and effective in attenuating chemotherapy-induced neuropathic pain, a model of peripheral neuropathy. Surprisingly, unlike mu opioid drugs such as morphine, little tolerance was observed following repeated administration. Drugs targeting the kappa opioid receptor, have no abuse potential and do not show respiratory depressive effects making them desirable drug candidates for treating chronic pain, moreover, they have been found to be particularly effective in neuropathic pain models. Further investigations into signalling pathways activated by kappa opioid suggest that compounds that preferentially signal via G-proteins have fewer side effects. I will discuss our recent findings on a variety of kappa opioid receptor agonists regarding their ability to modulate neuropathic pain, signalling pathways and side effects and discuss strategies for developing safer, more effective treatments for neuropathic pain.
13.2

Targeting spinal adenosine signalling to treat neuropathic pain
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Chronic pain is a major global health burden that results in hypersensitivity to sensory input. Neuropathic pain, one of the most intense types of chronic pain, is caused by malfunction of the nervous system and involves persistent changes in signalling within pain pathways. We have shown that there is an increase in endogenous adenosine in the spinal cord in chronic pain states, which is accompanied by increased sensitivity of adenosine A1 receptors in the dorsal horn. These adaptations produce anti-nociceptive activity that can be further enhanced by positive allosteric modulation of the adenosine A1 receptor. In this talk, I will describe our work investigating the effects of allosteric modulation of the adenosine receptor on spinal circuit activity in neuropathic pain states. We have used patch-clamp electrophysiology to measure synaptic input into the spinal dorsal horn and changes in intrinsic activity of dorsal horn nociceptive neurons in both primate and rodent ex vivo spinal cord to understand the analgesic mechanism at a circuit level. These findings are supported by our in vivo data which show potential for the adenosine A1 receptor as a therapeutic target to reduce pathological pain.

13.3

Voltage maps from somatosensory cortex in awake behaving mice – use for mapping chronic pain transition in the brain
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Acute pain is a protection mechanism that keeps us from further harm, and usually resolves as the injury heals. We all recognise pain as a temporary hyper-response to a “normal” sensation, but for many people their pain persists; this chronic pain is highly debilitating, hard to treat and is widely agreed to arise in the brain. Here we show how “normal” sensation behaviour is mapped as electrical signals in the brain and discuss how these signals might be subverted to create chronic pain signals. By imaging the brain through a thinned skull window of awake mice expressing a genetically encoded voltage indicator (GEVI) in cortical layer 2/3 neurons we can successfully record the activity of the cortico-cortical broadcast network of the brain during awake behaviour. In mice receiving a simple 2 ms vibration to the paw or a 20 ms air puff to the whisker we observe spatially and temporally distinct voltage signalling maps that initiate in the forelimb somatosensory and barrel field (whisker) areas respectively. Sensation perceived at the whisker or paw then spreads as a fast, broadcast signal across multiple cortical regions exhibiting fast excitatory and slower inhibitory components. Future studies aim to determine how these sensation signals, or sensory voltage maps, are broadcast across the cortex during the transition from acute to chronic pain in a pain model that recapitulates many of the human aspects of chronic pain. By understanding the nature of this transition at the level of the activity of cortico-cortical circuits we can begin to understand and manipulate this circuitry and so prevent the debilitating and often irreversible transition to chronic pain perception.
13.4

New therapeutic approaches for neuropathic pain

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The treatment of chronic pain represents a major, significant current and future medical challenge due to shortcomings of current analgesics together with the largely unmet clinical need. Neuropathic pain, caused by dysfunction in the somatosensory nervous system, affects approximately one in five adults globally and one in three elderly, highlighting its significance in ageing populations. Current treatment options are limited. Along with antidepressant medications (e.g. amitriptyline and duloxetine), gabapentin and pregabalin are considered first-line treatments. With prescription-based drug dependence and the associated risks becoming more prevalent in society there is a clear need to develop new therapeutic strategies. Here a series of industry partnered case studies will be outlined highlighting novel approaches to treat neuropathic pain. We use a multidisciplinary neuroscience research approach in rodent models to investigate neuronal mechanisms underpinning the development, manifestation and maintenance of neuropathic pain with electrophysiology at the centre of the approach. Through fundamental knowledge of the functional reorganisation of the peripheral nervous system and centrally at the level of the dorsal horn, novel strategies have been identified and validated. The first two studies will describe the mechanism of action of two small molecules targeting active conductances upregulated in neuropathic pain. The second shows a reverse translational approach where a compound reported effective in patients but through unknown mechanisms will be described. Proof-of-principal studies will highlight the utility of chemogenetic approaches. Finally, the use of electrical stimulation devices has gained popularity in recent years although why they are effective is still unclear. Here we will provide insight into mechanisms that may be contributing to their effectiveness in treating neuropathic pain.

14.1

Large-scale brain network analysis of anhedonia in youth: phenotypic demarcation and context specificity

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Anhedonia can present in early life and is associated with potentially detrimental clinical outcomes. However, an integrative account using large-scale functional networks of anhedonia in youth is still lacking. Here we employed the Adolescent-Brain-Cognitive Development (ABCD) dataset, the largest neuroimaging dataset in adolescents (4,524 Children from the 1st Release) so far, to: a) map anhedonia onto large-scale resting state fMRI (rs-fMRI) network and task-based fMRI activation and b) probe the specificity of anhedonia findings by comparing to other clinical phenotypes. In rs-fMRI, children with anhedonia showed hypoconnectivity between a reward-related area (striatum) and the alertness-related (Cingulo-Opercular) networks. The connectivity was significantly weaker in anhedonia than in low-mood and ADHD, indicating its phenotypic specificity. Similarly, in task-based fMRI, children with anhedonia demonstrated hypoactivation during reward-anticipation in the striatum and areas of the Cingulo-Opercular networks. More specifically, we found context- and phenotype-specific double-dissociation: while children with anhedonia showed altered activation during reward-anticipation (but not working-memory), those with ADHD showed altered activation during working-memory (but not reward-anticipation). Accordingly, using the large ABCD dataset, we are able to demonstrate context- and phenotype-specificity of abnormalities in large-scale connectivity and task-based activation in children with anhedonia. Specifically, hypoconnectivity at rest and hypoactivation during reward-processing in children with anhedonia both suggest altered reward-arousal integration typically served by the cross-talk between the striatum and the Cingulo-Opercular networks. Our findings are important for using large-scale networks as a tool for a neural-based taxonomy of anhedonia and related psychopathology.
14.2

Increased local grey matter in the maternal brain at 4-6 weeks after childbirth

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Existing research suggests a possible brain rejuvenation effect after giving birth, and this effect seems to manifest already within the first couple of months. An apparently younger brain might be explained by enhanced cerebral tissue in various (aging-relevant) regions, both within cortex as well as subcortical structures. The aim of our study was to map the exact location of possible grey matter changes — presumably increases — between two time points: within 1-2 days of childbirth (early postpartum) and at 4-6 weeks after childbirth (late postpartum). For this purpose we analysed high-resolution T1-weighted brain image data of 14 healthy women in their mid-twenties to late thirties, shortly after giving birth, using voxel-based morphometry. When comparing the voxel-wise grey matter between early and late postpartum, there was no evidence of any significant grey matter decrease. In contrast, significant effects indicating grey matter increase were wide-spread across the brain, evident in both hemispheres and all four lobes, and involved both cortical and subcortical regions. The effects were most significant within postcentral and precentral gyrus, central and frontal operculum, inferior frontal gyrus, precuneus, middle occipital gyrus, as well as in subcortical structures. These findings may suggest a restoration of the brain after pregnancy. Alternatively, they may reflect the powerful impact of the hormonal milieu to reorganize the maternal brain, possibly equipping the mother with the mental capacities to meet the needs of her newborn.

14.3

Development of sex differences in brain structure characterized using machine learning

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While puberty has been shown to exert sex-specific changes in the brain, it remains less clear to what degree neuroanatomical sex differences are a result of such late developmental factors (e.g., puberty) or rather attributable to early developmental factors (e.g., before/around birth). Here, we employed a machine-learning approach on T1-weighted MRI scans from 347 healthy children and adolescents (162 boys, 185 girls) aged between 5 and 18 years to assess the impact of chronological age on the classification of the developing brain as “male” or “female”. This classification used a relevance-vector machine to determine the subject’s sex using a 10-fold cross validation. Importantly, this classifier yielded a continuous probabilistic estimate for being male/female rather than forcing a binary classification. The resulting probabilistic estimates were then used to calculate the effect size (Cohen’s d) of the estimated sex differences at different age groups using a sliding-window approach with a window length of three years (i.e., 5-7, 6-8, 7-9 years, etc.). Assessing the changes in sex differences over age revealed a very large effect size of d=1.2 at age 6, which increased even further from age 11 onward to an effect size of d=1.6 at age 17. This suggests an increase in the sexual differentiation of cerebral features during puberty (i.e., between age 11 and 17), however, considerable sex differences in the anatomy of the brain seem to exist already very early in life (i.e., at age 6). This latter observation seems to indicate that detectable sex differences later in life might be, at least partly, driven by factors during early development.
14.4

EEG correlates of task difficulty: Development of an objective measure of cognitive workload

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Human performance is highly dependent on effective and efficient allocation of brain resources during demanding tasks. Such tasks increase cognitive workload with a corresponding reduction of available brain resources for other tasks. If brain resources are depleted below a certain threshold, cognitive processing of further tasks can be delayed or impeded. Therefore, evaluating cognitive workload can have numerous advantages especially in neurological rehabilitation paradigms where efficient allocation of the brain resources is very important to maximize recovery. In neurological rehabilitation, a clinician prescribes a rehabilitation task to the patient; this task needs to be challenging enough to help the rehabilitation process, yet not too challenging to hinder the process. Currently, this challenge point is based on the patient’s feedback and the clinician’s observation. These measures do not give any information regarding brain activity. So, there is a pressing need for an objective measure of brain activity to assist clinicians in setting the optimal cognitive workload in real-time. In recent years the development of non-invasive electroencephalography (EEG) based techniques to evaluate cognitive workload have become of interest to researchers and many new techniques have been proposed. These EEG based measures of cognitive workload can enhance user-task interaction by providing information about one’s cognitive state during the task. This research aims to identify and optimize the correlates of brain activity using electroencephalogram (EEG). These correlates will help in devising a clinical tool to measure and identify the optimal cognitive workload during rehabilitation.

14.5

Effects of blueberry-derived phenolic acid metabolites on neuronal mitochondria degradation

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Mitochondrial dysfunction occurs before the pathological symptoms of neurodegenerative diseases. Selective degradation of dysfunctional mitochondria (mitophagy) is important for healthy neuronal function. Recently, it was shown that the polyphenol-derived gut metabolite, urolithin A, can promote mitophagy (Andreux et al., 2019). However, it is not known whether other types of polyphenol metabolites, such as phenolic acids, could exert a similar effect. As such, we investigated whether phenolic acid metabolites, at concentrations found in plasma following blueberry polyphenol consumption (Tang et al., 2018), could stimulate mitophagy in a human neuroblastoma cell line, SH-SY5Y. We showed, using the MTT reduction and Propidium iodide assays, that physiological concentrations of these metabolites are not cytotoxic to SH-SY5Y cells. Next, we showed that SH-SY5Y treated for 24 h with metabolite mixture exhibited upregulation of the mitophagy-associated proteins, LC3B and PINK1, but not SQSTM1. The metabolites also increased expression of TOMM40, a mitochondrial import protein. Interestingly, we observed the localisation of discrete puncta of LC3B to mitochondria, with the nuclei containing most of the LC3B in SH-SY5Y treated for 24 h with metabolites. Our work shows physiologically-relevant concentrations of phenolic acid metabolites, achievable from blueberry polyphenol intake, can regulate molecular aspects of neuronal mitophagy. Further quantification of LC3B co-localisation with mitochondria, and assessing the ratio of nuclear:cytosolic LC3B, will enable better understanding of how these metabolites mechanistically acts to improve neuronal mitophagic efficiency.
15.1

Histological architecture underlying brain-immune cell-cell interactions

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The brain is interactive with the immune system. Our studies using bone marrow chimeric mice revealed the sites for brain-immune cell-cell interactions under non-inflammatory conditions: choroid plexus (CP), leptomeninges, perivascular space and circumventricular organs. In addition, bone marrow-derived cells populate limited brain regions and exhibit ramified morphology with myeloid lineage differentiation. Most of these regions are adjacent to the attachments of CP in which the brain parenchyma is extremely thin and consists of astrocytic processes occupying the narrow channel between ependyma and pia. These processes express CX3CL1, a chemokreceptor for myeloid lineage cells. Therefore, the brain region adjacent to the attachments of CP provide the brain-immune interface in adult mice. In lipopolysaccharide (LPS)-induced endotoxemia, the signalling using IL-1β from macrophages and IL-1 receptors on stromal cells in the CP and leptomeninges was the immediate reaction. The reaction triggered cytokine-mediated interactions between CP stromal and epithelial cells using CCL2, CXCL1, CXCL2, etc. earlier than parenchymal cells. In the parenchyma, astrocytes responded earlier than microglia to the cytokine signals by using endfeet located in close apposition to the interface cells. Therefore, stimulated astrocytes produced other cytokines, resulting in changes in the brain microenvironment in adult mice. Our ongoing studies using mice at postnatal day (PD) 1 suggest that LPS-induced endotoxemia induces macrophages in the CP stroma and cephalic mesenchyme (CM) but not parenchymal microglia to produce IL-1β. Developmental stage of the brains in PD1 mice corresponds to that in human foetus around 22 weeks gestation. Given CM macrophages are the precursors of microglia, further studies should address to how macrophage of CP and CM induce inflammatory milieu in developing brain, which is relevant to the pathogenesis of encephalopathy of prematurity.

15.2

Early-life immune activation and suppression similarly disrupt neuroendocrine development and function long-term

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Early-life experiences shape our development, and adverse environment during this critical period is well-known to increase our susceptibility to diseases long-term. We have previously shown that even a subtle immune challenge early in life disrupts female neuroendocrine development and function, including increased stress responsivity, depleted ovarian follicle pool, earlier pubertal onset and reproductive senescence, as well as compromised reproductive behaviours and breeding success. We have seen similar neuroendocrine outcomes in neonatally overfed female rats that typically also exhibit increased central and peripheral inflammatory profile throughout life. We then investigated whether a suppression of the immune system early in life, rather than its activation, is likely to induce similar neuroendocrine alterations. We used a Cx3cr1-Dtr transgenic Wistar rat with a diphtheria toxin receptor (Dtr) gene inserted into the promoter for the fractalkine receptor (Cx3cr1), expressed on microglia and monocytes. We administered diphtheria toxin (DT) during the critical time-points within the development of hypothalamic connectivity (postnatal day (P7)) and immediately after its completion (P14). Our data show microglial and monocyte ablation on P7, but not P14, leads to a significant reduction in circulating luteinising hormone (LH), reduction in basal release of adrenocorticotropic hormone (ACTH) and impacts on the ovarian follicle reserve in adulthood. These data suggest that both activation and suppression of the immune system in early-life lead to long-term neuroendocrine changes impacting both the stress and reproductive axes. These findings also indicate a critical window that occurs during hypothalamic development and when this region is more vulnerable to neuroinflammatory challenges.
CNS to Immune and Back: Neuroendocrine regulatory pathways of inflammation underlying neuropsychopathology

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Fast growing preclinical and clinical evidence indicates a key role played by the immune system in the pathogenesis and pathophysiology of a range of neuropsychopathological conditions such as depression, psychosis, PTSD and neurocognitive dysfunction. Meanwhile, knowledge of the mechanistic pathways of immune activation leading to neuropsychiatric disease outcomes remains limited. We investigated intracellular cytokine expression in LPS-stimulated monocytes via ex vivo cellular stimulation and flow cytometry using agonists and antagonists of beta-adrenergic (“BARIC”), glucocorticoid and nicotinic acetylcholine receptors for the investigation of sympathoadrenal, hypothalamic adrenal and cholinergic pathways of inflammation regulation in cohorts of individuals with hypertension. The blood levels of inflammatory markers were assessed using immunoassay. Overall, dysregulation of inflammatory responses via neurohormonal mechanisms mediated or was related to depressive mood or depression-obesity comorbidity. Somatic (vs. cognitive) depressive symptoms were associated with both levels of obesity and bAR and GR-mediated inflammation regulation and mediated obesity-inflammation associations. A sex difference emerged such that BMI was more strongly associated with depressive symptomatology among women than men (β = 0.34, p < 0.01; versus β = 0.18, p > 0.1). Poorer BARIC predicted higher somatic symptoms in women (β = -0.23, p < 0.05), but not men (β = -0.09, p > 0.1). Higher cardiometabolic risk predicted elevated somatic symptoms in men (β = 0.26, p < 0.05), but not in women (β = -0.11, p > 0.1). Our findings highlight that heightened or dysregulated inflammatory processes underlie a range of mental disorders including subclinical depressive symptoms. In particular, comorbid symptoms or risk and sex differences are critical factors in evaluating the implications of the inflammatory biomarker findings in neuropsychiatric outcomes.

Neuroimmune interactions in the ageing brain

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Microglia are the brain’s resident immune cells. In young healthy adults, they perform various important immunoregulatory functions including phagocytosing pathogens after infection and dead cells after injury. In ageing, microglia proliferate, hypo-ramify, and are hyper-responsive to challenge. This microgliosis and microglial priming may account for some of the aging-associated decline in cognitive function and vulnerability to diseases of aging like Alzheimer’s. Alternatively, microglial priming may be an adaptive mechanism to combat cognitive decline-related damage in the ageing brain. To assess this, we gave ageing and young rats 3 days high-fat diet (HFD) and tested them in contextual or cued fear conditioning tests of memory for aversive experience. We found that acute HFD alone in young rats did not impair ability to remember the aversive context. Likewise, ageing per se did not impair memory. However, aged, HFD-fed rats showed a substantial impairment in fear memory relative to the other groups. This impairment was linked with microgliosis in the amygdala and was independent of microglial changes in the hippocampus. To directly assess the role of microglia in this ageing-related vulnerability to HFD, we next employed Cx3cr1-Dtr rats to specifically ablate microglia in aged HFD-fed rats. In this case, we fed aged (and young) rats HFD for 8 weeks and assessed cognitive function with and without microglial ablation, showing microglia are important contributors to ageing-related cognitive vulnerability that may be a useful potential target for treatment against cognitive decline.
16.1
Maternal immune activation in rats produces a subjective internal state that is analogous to human psychosis

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A key problem with animal models of psychosis lies with the animal’s inability to self-report its own internal state. Here we used the drug discrimination paradigm, an assay that can provide an objective measure of an animal’s subjective internal state, to assess whether the subjective internal state experienced by rats that model a specific aetiological schizophrenia risk factor (maternal immune activation; MIA) is analogous to that experienced in a model of human psychosis. MIA and control rats were trained to discriminate 7.5 mg/kg ketamine from saline in a two-lever operant chamber. Once discriminative control was established, dose-effect determinations were made, and control procedures were conducted to rule out potential confounds resulting from MIA treatment. The results showed a deficit in paired pulse inhibition and impaired drug discrimination of ketamine in MIA rats, with the difference in discrimination between MIA and control particular to the psychotomimetic dose range (3-10 mg/kg). These results were not due to: 1) a deficit in rat’s ability to learn or perform the drug discrimination task, as MIA animals readily acquired morphine discrimination; 2) a decreased sensitivity to ketamine, as MIA rats were actually more sensitive at psychotomimetic doses to ketamine-evoked hyperlocomotion; or 3) a general decrease in sensitivity to internal states, as these animals were as sensitive to the satiety cue as their control counterparts. Overall these data suggest that the subjective state of MIA rats is similar to that experienced in a ketamine model of psychosis and that the drug-discrimination paradigm may be a useful tool for understanding the neurophysiological underpinnings of human psychosis in animal models.

16.2
MRI-Derived estimated brain-age and cognitive decline in Parkinson’s disease

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Relevance Voxel Machine (RVoxM) regression can quantify a stereotypical trajectory of structural brain change that occurs in normal ageing. Recent RVoxM work in Alzheimer’s suggests an acceleration in brain ageing, however Parkinson’s disease (PD) has not been similarly examined. To characterise MRI-derived structural age-related brain health, 212 PD patients (45-86yrs; M=70yrs; 96 PD-N, 85 PD-MCI, 31 PDD at study entry) received cognitive assessments and T1-weighted MRI at least once over a 10-year period (571 participant sessions; 227 PD-N, 171 PD-MCI, 50 PDD). Global cognitive ability was derived by an aggregate z-score. A non-spatial RVoxM model, trained on scans from 101 healthy non-PD control participants (19-87yrs; M=55yrs; 10-fold cross validation; correlation=0.90, RMSE=9.7), was applied to the PD cohort and used to estimate age at each MRI. The difference between estimated age relative to chronological age is referred to as the age-difference score, which was assessed across cognitive abilities using Bayesian regression models. Compared to the PD-N group, mean age-difference scores of the PD-MCI group was 3.9 years (95% uncertainty interval [2.3,5.4]) greater and PDD group was 6.2 years [3.6,8.7] greater, accounting for sex and education. The difference between the PD-MCI and PDD groups was 2.3 years [-0.05,4.8]. PD symptom duration (0.33yrs [0.2,0.5]) and global cognitive ability (0.01yrs [-0.02,-0.01]), but not education, sex, motor impairment or levodopa equivalent dose, were associated with age-difference score, suggesting that brain structure in PD is different than normal ageing.
The link between mindfulness, immune function and memory

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While studies have shown that mindfulness meditation can improve pro-inflammatory immune profiles and cognition, the link between improved cognitive function and pro-inflammatory immune profiles remains unclear. The existing studies have predominantly assessed populations with chronic illness (i.e. cancer), which makes it challenging to generalize the findings to the healthy population. The present study aimed to examine 1) the effect of mindfulness on biomarkers of immune system and memory; and 2) the correlations between these immunological measures and memory in the healthy population. Participants (n=26) undertook a 6-week mindfulness training which was delivered via videoconferencing. Delayed and immediate memory recall was assessed utilising the computerised cognitive battery. CD4, CD8, CD69, Interleukin-6 (IL-6), C-reactive protein (CRP), and cortisol were examined with blood assays. There were significant improvements in immediate and long delay recall following mindfulness training, whereas immunological measures remained relatively stable and the only significant change was found in CD69 (Z=-4.2, P<=0.001). Nevertheless, the change observed in CD69 was not significantly correlated with any of memory measures. In contrast, baseline CD4 and CD8 were significantly correlated with memory recall and predicted memory performance following mindfulness. Our results suggest that a brief mindfulness training improves memory but has a minimum effect on immunological measures in the healthy population. We also report an association between memory recall and immunological measures in our healthy population, similar to that reported by clinical studies.

Can cerebellar transcranial direct current stimulation influence motor learning in healthy adults?

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The excitability of the cerebellum, a structure crucial for error-based learning, can be altered with cerebellar transcranial direct current stimulation (ctDCS) to transiently influence motor performance. It is unclear if repeated stimulation results in long-lasting changes in performance and thus influences motor learning. This study examined the effect of repeated anodal ctDCS on learning a split-belt treadmill task in healthy adults. In a randomised double-blinded, parallel design, 30 healthy adults received either anodal or sham ctDCS during three consecutive sessions of split-belt treadmill training. Each session involved walking on a split-belt treadmill in three phases based on whether the belts moved together or at different speeds: baseline, adaptation and de-adaptation. Motor performance was measured based on the number of strides taken to achieve steady-state performance using 3-D gait analysis data. Data from each intervention session and a follow-up one week later was analysed using a linear mixed model. Group analysis revealed no significant difference in the strides to steady-state (p=0.19) during the adaptation phase. There was a significant difference between the groups during the de-adaptation phase (p = 0.03) indicating anodal ctDCS prolonged the retention of the adapted pattern. Therefore, repeated anodal ctDCS did not influence how quickly healthy adults learnt a split-belt treadmill task, but supported the maintenance of learnt pattern for longer following the intervention. This effect may be useful for extending the benefits of motor training beyond the intervention. Future research should validate the findings in other types of task training and sporting and clinical populations.
Evidence of widespread metabolite abnormalities in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Assessment with whole-brain magnetic resonance spectroscopy

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Previous neuroimaging studies have detected markers of neuroinflammation in patients with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Magnetic Resonance Spectroscopy (MRS) is suitable for measuring brain metabolites linked to inflammation, but has only been applied to discrete regions of interest in ME/CFS. We extended the MRS analysis of ME/CFS by capturing multi-voxel information across the entire brain. Additionally, we tested whether MRS-derived brain temperature is elevated in ME/CFS patients. Fifteen women with ME/CFS and 15 age- and gender-matched healthy controls completed fatigue and mood symptom questionnaires and whole-brain echo-planar spectroscopic imaging (EPSI). Choline (CHO), myo-inositol (MI), lactate (LAC), and N-acetylaspartate (NAA) were quantified in 47 regions, expressed as ratios over creatine (CR), and compared between patients with ME/CFS and controls using independent-samples t-tests. Brain temperature was similarly tested between groups. Significant between-group differences were detected in several regions, most notably elevated CHO/CR in the left anterior cingulate (p < 0.001). Metabolite ratios in seven regions were correlated with fatigue (p < 0.05). ME/CFS patients had increased temperature in the right insula, putamen, frontal cortex, thalamus, and the cerebellum (all p < 0.05), which was not attributable to increased body temperature or differences in cerebral perfusion. Brain temperature increases converged with elevated LAC/CR in the right insula, right thalamus, and cerebellum (all p < 0.05). We report metabolite and temperature abnormalities in ME/CFS patients in widely distributed regions. Our findings may indicate that ME/CFS involves neuroinflammation.

Kiss1 neurons in the arcuate nucleus of the hypothalamus are a hub for metabolic, temperature and neuroendocrine outcomes

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Circulating humoral signals that are sensed in the nervous system can instate coordinated behavioural and physiological outcomes. Such adaptive state changes likely evolved to promote fitness. For example, the rise of ovarian oestrogen not only triggers the release of gonadotropins, but is also associated with changes in body temperature, body weight, and daily activity patterns – all of which likely optimize reproductive success. Oestrogen-sensitive Kiss1 neurons in the arcuate hypothalamus are well positioned to translate sex steroid status into multiple outcomes via axonal projections to numerous brain regions and cell types. We use functional circuit mapping techniques in mice to resolve the projections and signalling molecules from Kiss1 neurons that correspond to distinct steroid hormone-dependent state changes.
Hunger-sensing Agouti-related peptide (Agrp) neurons in the hypothalamic arcuate nucleus are fundamental to survival. They increase food intake during energy deficit and also facilitate adaptive behaviours, such as increasing motivation. To assess the role of metabolic sensing in Agrp neurons and the effects on motivation, we studied mice lacking carnitine acetyltransferase (Crat) in Agrp neurons. Electrophysiological studies showed Crat in Agrp neurons is required for appropriate neuronal glucose-sensing. Two-bottle choice tests showed that Crat in Agrp neurons is important for sensing the caloric value of sweet solutions; fasting increases sucrose consumption in WT more than in KO mice. During fasting, WT mice will still consume sucrose spiked with quinine to consume calories as required, whereas KO mice do not. Progressive ratio operant conditioning studies showed that WT mice, as compared to KO mice, are more motivated to obtain sucrose reward pellets in the fasted state but not the fed state. These results highlight that Crat in Agrp neurons regulates motivation in response to calorie deficit during the fast, rather than taste alone. To examine the brain substrates underlying these differences, we developed a whole animal PET/CT f18DOPA scan method to estimate dopamine activity of the dorsal and ventral striatum. Importantly, the dorsal striatum mediates the effects of calorie, and not taste, reward processing. Our results showed lower f18DOPA uptake in the dorsal striatum of KO mice in response to reward stimulus compared to WT mice and no differences in ventral striatum f18DOPA uptake. These studies highlight that Crat in Agrp neurons is crucial for the caloric assessment of sugar solutions and may link the detection of energy deficit with increased dopamine signaling in the dorsal striatum to ultimately impact food reward and motivation.

Synaptic modulation of viscerosensory signals within the brainstem

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Viscerosensory information is conveyed to the brainstem’s nucleus of the solitary tract (NTS) where it initiates neuroendocrine, behavioural and autonomic reflex responses that ensure optimal internal organ function. Autonomic reflexes are dynamic. For example, the baroreceptor reflex, which rapidly modulates blood pressure, is re-set to different operating sensitivity and gain during stress. It is not known how this flexibility in reflex processing is achieved. We hypothesize that viscerosensory signals entering the brain at the NTS are modulated by efferent activity from other brain regions (hypothalamus) and the intrinsic inhibitory network within NTS. We have used a combination of optogenetic tools and slice electrophysiology to define the neural circuits and mechanisms that modulate viscerosensory signals within the NTS. We find hypothalamic input to the NTS to be exclusively excitatory, AMPA receptor mediated and these efferents facilitate viscerosensory throughputs at second order NTS neurons. Hypothalamic efferent input also drives local inhibitory neurons within NTS. We defined the role of somatostatin (SST) neurons within NTS. Here we show that 65% of SST-expressing (SST) NTS neurons also express GAD67 and most SST neurons (57%) received direct input from solitary tract afferents, indicating that they form part of a feed forward circuit where all recorded SST-negative NTS neurons received SST input. SST neurons utilized both GABAergic and glycinergic systems to effectively gate viscerosensory signal throughputs within the NTS. These results indicate synaptic modulation of viscerosensory signals occurs via excitation or inhibition of second order NTS neurons directly, with the potential to gate viscerosensory input to powerfully alter autonomic reflex function and other behaviours.
Stress leaves a lasting impression on an organism and reshapes future responses. However, the influence of past experience and stress hormones on the activity of neural stress circuits remains undefined. Hypothalamic corticotropin-releasing hormone (CRH) neurons orchestrate behavioural and endocrine responses to stress and are themselves highly sensitive to corticosteroid (CORT) stress hormones. Using in vivo optical recordings, we find that CRH neurons are tonically active and their excitability can be rapidly modulated, revealing distinct patterns of activity at rest, during, and after stress (loud white noise). Interestingly, CRH neuron activity was incredibly adaptive. Habituation of CRH neuron responses to sequential presentations of the white noise stress were observed as early as 30 minutes and lasted at least 24 hours. This adaptation was dependent on stress familiarity as CRH responses failed to habituate against sequential presentations of unfamiliar threats. Following changes in stress hormone milieu, the kinetics of CRH neuron activation to acute stress and the ability to habituate to repeated stress remained unchanged. Instead, CORT was found to preferentially inhibit tonic CRH neuron activity during absence of stress stimuli. This research demonstrates the temporal and adaptive dynamics of CRH neuron activity and highlights the lasting neural imprints from a stress experience that may promote appropriate adaptations to stress.