id #11502

Inhibition of heparan sulfate biosynthesis as a novel substrate reduction therapy for a paediatric-onset dementia

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Sanfilippo syndrome (Mucopolysaccharidosis (MPS) types IIIA-D) is caused by the lack of a specific lysosomal enzyme involved in the degradation of heparan sulfate (HS). Consequently, partially degraded HS fragments accumulate, presenting as a severe neurodevelopmental disorder. No treatment is available at present, and patients generally live until their mid-teens. We have examined the ability of a small molecule drug to modulate HS biosynthesis as a potential therapy for Sanfilippo syndrome using cultured human MPS IIIA fibroblasts and novel *Drosophila* knockdown models of MPS IIIA and IIIC. Ubiquitous knockdown MPS IIIC flies exhibit significant HS accumulation as measured by tandem mass spectrometry, progressive motor deficits in climbing assays and a shortened life span compared to wild-type controls. Oral delivery of the compound in newly hatched ubiquitous knockdown flies resulted in a significant decrease in HS storage compared to those fed with the parent compound control. Ongoing studies are examining the effect of earlier treatment initiation (i.e. larval stages). These findings indicate that this compound may be beneficial in slowing the accumulation of HS in Sanfilippo syndrome.

id #11513

LRP1 inhibits myelination and remyelination in the adult central nervous system

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Oligodendrocyte progenitor cells (OPCs) are immature brain cells that proliferate and produce new myelinating oligodendrocytes throughout life. When oligodendrocytes are lost to multiple sclerosis (MS), OPCs generate larger numbers of new oligodendrocytes, but signals within their environment limit or impede this natural repair process. We report that the low-density lipoprotein receptor related protein 1 (LRP1), a large endocytic cell surface

receptor and member of the LDL receptor family, is a negative regulator of oligodendrocyte generation in the healthy and demyelinated mouse brain. LRP1 is highly expressed in multiple brain cell types, including OPCs, but is down-regulated as the cells mature. By using a conditional gene deletion approach, to delete *Lrp1* from OPCs in early adulthood, we found that OPCs lacking *Lrp1* were able to proliferate more rapidly (p<0.01), and produce a larger number of new oligodendrocytes (p<0.0001). As the deletion of *Lrp1* from OPCs *in vitro* did not affect OPC proliferation, but was associated with reduced oligodendrocyte differentiation, it is likely that LRP1 directly suppresses differentiation and has a secondary effect on proliferation *in vivo*. Cuprizone delivery to adult mice induces demyelination of the corpus callosum, and in mice that lacked *Lrp1*, lesion size was found to be smaller, suggesting that LRP1 naturally blocks OPC maturation and oligodendrocyte production, and that suppressing LRP1 can enhance the ability of OPCs to repair demyelinated lesions.

id #11521

Dragonfly target-detection neuron ignores high-contrast distractors.

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Animals that hunt in swarms require the ability to 'lock on' to individual instances of prey in order to achieve high capture success. For example, dragonflies are excellent aerial predators that do not suffer from a reduction of success when hunting in swarms. We previously identified a visual neuron in the dragonfly (*Hemicordulia tau),* termed 'Centrifugal Small Target Motion Detector 1' (CSTMD1) that exhibits a 'winner-takes-all' selective attention. We recorded intracellularly from CSTMD1 *in vivo*, whilst presenting a pair of differentially, frequency-tagged, moving targets. We showed that in over 75% of trials, CSTMD1's spiking activity is modulated at the frequency of the selected target. A wavelet analysis of this neuronal response allows for the identification of which target is selected (from the pair) over the stimulus duration. We find that on a trial-by-trial basis CSTMD1 either 'locks on' to one target, or occasionally switches between targets.

When a preceding 'primer' target is presented before the pair, it can be 'locked on' to, with the abruptly appearing distracter ignored. We show that this even occurs when the primer target is at a lower contrast than the distracter target. Thus this 'selective attention' mechanism is more complex than the traditionally modelled winner-takes-all system. The ability to 'lock on' to a target and ignore distracters of transiently higher salience in favour of continuous tracking may underlie the dragonfly's ability to successfully hunt amidst cluttered backgrounds and amongst large swarms.

id #11552

Identifying early molecular and vascular changes in young adult zebrafish brains due to mutations in early-onset, familial Alzheimer's disease-related genes

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Alzheimer's disease (AD) develops silently over decades. To delay or prevent the onset of AD, we must understand the initial molecular stresses/changes that drive the disease. However, we cannot easily investigate these changes in molecular detail in humans as access to asymptomatic brain material is limited.

SORTILIN-RELATED RECEPTOR 1 (SORL1) encodes a multi-domain receptor protein genetically associated with both early-onset and late-onset AD (EOAD and LOAD respectively). SORL1 plays a role in the trafficking of the AMYLOID β A4 PRECURSOR PROTEIN (APP) which is cleaved proteolytically to form one of the pathological hallmarks of AD, AMYLOID BETA peptide. However, the other functions of SORL1 are less well understood. Therefore, we used zebrafish as a genetic model to investigate the effects of AD-like mutations in *SORL1* on the young adult brain transcriptome and proteome. This has not been done previously in an animal model. The dominant, EOAD-like W1818* mutation in endogenous zebrafish *sorl1* has subtle effects on heterozygous brains including on energy metabolism, on pathways involving cytochromes, and on protein synthesis. To determine if these effects are due to loss-of-function, we have also generated a putative null mutation, R122Pfs, for comparison.

The vascular hypothesis of AD is a popular theory of AD pathogenesis and posits that changes in vascular function lead to neurodegeneration. We are using transgenic zebrafish with GFP-labelled vasculature to analyse whether heterozygosity for an EOAD-like mutation in the zebrafish *presenilin 1* gene changes the brain vascular network structure.

id #11554

Mechanosensors underlying flight by feel on dragonfly wings

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Dragonflies are acrobatic insects that rely on flight for hunting, mating and transportation. During flight their wings undergo large, periodic deformation on each flapping cycle. This aeroelastic response is determined by the interaction of inertial and aerodynamic loads as well as the detailed architectural and material characteristics of the wings. Flying insects must detect the associated mechanical and aerodynamic forces acting on their wings in order to maximise performance. This detection occurs via sensory structures embedded within the cuticle of wing veins, such as campaniform or trichoid sensilla. Information encoded by these sensilla may be used to monitor instantaneous wing loads, unstable aerodynamic flows and control wing stroke kinematics.

Here we combine Scanning Electron Microscopy (SEM) and electrophysiological techniques to describe the distribution and function of campaniform and trichoid sensilla on the

dragonfly wing. The sensory information perceived by each sensillum is largely dependent on its position on the wing, and its own mechanical structure. By capturing the position of sensilla across the dragonfly wing we can predict how aeroelastic loads are monitored during flight. In addition, we can combine morphological measurements from SEM micrographs with electrophysiological recordings from sensory neurons to investigate sensor function and tuning. These data are informative for electrophysiological and modelling studies of dragonfly flight.

id #11555

ULTRASOUND MEDIATED PIEZOELECTRIC STIMULATION OF HUMAN NEURAL STEM CELLS

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In vivo bioelectricity is important for normal development and function of human tissues, and wound healing. We and others have demonstrated that exogenously delivered electrical stimulation in vitro can be used for a biomimetic approach to cell culture[1,2]. However, traditional electrical stimulation methods rely on undesirable tethering and insertion of potentially fouling electrodes into cell cultures. Here, we report the use of piezoelectric nanoparticles with ultrasound stimulation to generate small local potentials in a manner reminiscent of traditional electrical stimulation. We have applied the platform to stimulate human neural stem cell cultures resulting in increased cell signalling and activity. Our results highlight the potential of the approach to wirelessly stimulate cells, with prospective applications including modelling neural tissue development and disease, and electroceuticals for therapeutics.

1. Stewart et al. Tissue Engineering: Part C. 21(4):1-9.
2. Tomaskovic-Crook et al. Advanced Healthcare Materials. In press

id #11574

Investigating Alzheimer's disease through mutating the *psen1* gene in zebrafish

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Alzheimer's disease (AD) is a progressive neurodegenerative brain disease that can be classified into sporadic and familial forms. Familial Alzheimer's disease (fAD) is autosomal and dominantly inherited, which allows for generation of genetic animal models. The fAD-

causative mutations occur in a small number of genes. Most of the mutations have been identified in *PSEN1*, which produces PSEN1 protein that functions to cleave APP in production of amyloid β peptide. However, the role of PSEN1 in fAD pathology remains unclear. To investigate the effects of PSEN1 in AD, I have generated fAD-like *psen1* mutations in zebrafish through application of CRISR-Cas9 technology. 6 indel mutations were isolated in *psen1* near the site of a frameshift mutation that, in humans, causes the skin disease, Acne Inversa. Two of these 6 mutations are frame shifts causing premature translation termination. They can be considered as models of the Acne Inversa mutation. Four of the 6 mutations preserve the reading frame but are predicted to change the protein's hydrophilicity and structure in its third lumenal loop domain. Thus, they resemble fAD-causative mutations. A RNA-seq analysis comparing the brain gene expression of fAD-like mutants with wild type and Acne Inversa-like mutants would help differentiate fAD-specific patterns of gene regulation from the patterns due to other types of mutations and inform us regarding the specific roles of PSEN1 in fAD.

id #11587

A NEWLY CHARACTERISED NEURODEVELOPMENTAL DISORDER IS CAUSED BY MUTATIONS IN SYNAPTOTAGMIN-1 THAT ALTER SYNAPTIC VESICLE DYNAMICS

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Presynaptic proteins that coordinate and sustain neurotransmission are increasingly being implicated in the aetiology of neurodevelopmental disorders. Synaptotagmin-1 (SYT1) is an essential synaptic vesicle protein that acts as the Ca^{2+}-sensor for fast, synchronous neurotransmitter release, with auxiliary roles in other aspects of synaptic physiology. We have recently characterised the first known cases of human mutations in SYT1, where several recurrent *de novo* mutations were identified in individuals who exhibit a phenotypic spectrum of shared symptoms including motor delay, intellectual disability, movement disorders and behavioural abnormalities. All human mutations cluster within the C2B domain, a Ca^{2+}-binding region crucial for SYT1 function, and all variants localise to synaptic vesicles and express similarly to the WT protein, with one exception ($p<0.05$, n=3-8). We therefore anticipated that these mutations may perturb the fusogenic capacity of SYT1. To investigate this, we expressed mutant rat SYT1 tagged with pHluorin (a pH-sensitive fluorescent protein that reports synaptic vesicle cycling) in cultured mouse hippocampal neurons. Live cell imaging revealed that SYT1 mutants slow the rate of evoked exocytosis in a dominant-negative manner ($p<0.05$, n=5-7), and notably this slowing was less pronounced for the variant associated with a less severe clinical presentation. Additionally, exocytic efficiency could be rescued by increasing extracellular Ca^{2+} concentration ($p<0.05$, n=7-8). These findings demonstrate that mutations in SYT1 disrupt neurotransmitter release causing a distinct neurodevelopmental disorder. Furthermore, we provide proof of principle that neurotransmission can be normalised, thereby suggesting potential therapeutic avenues for this syndrome.

id #11601

A model for the origin and development of orientation selectivity

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Aims: Orientation selectivity, a key property of the visual cortex, is hypothesised to originate from the spatial offset between on- and off-centre subcortical pathways converging onto cortical neurons. However, how these subcortical inputs spatially segregate during development remains poorly understood, since on- and off-centre pathways are intermingled. Our aim was to describe a developmental computational model of an orientation-selective cortical neuron in which this segregation arises via Hebbian plasticity of geniculocortical synapses.
Methods: The model consists of an array of on- and off- subcortical channels, each converging onto two networks of cortical neurons: one excitatory and the other inhibitory. The inhibitory network then converges onto the excitatory network. Neuronal responses are simulated with a drifting sinusoidal grating over all orientations. To prevent synaptic strengthening beyond physiologically plausible limits, we have also incorporated intracortical inhibition in the model driven by the same geniculocortical input as the target cortical neuron.
Results: The model yields several key findings: 1) Hebbian maturation of geniculocortical synapses is sufficient to functionally segregate on-centre and off-centre inputs to V1; 2) This segregation produces orientation-selective neurons with characteristics that mirror empirical cortical time courses, orientation tuning curves, and orientation preference maps; and 3) Orientation preference maps of cortical neurons are determined even before development: they are derived from the spatial arrangement of retinal on- and off-centre neurons, rather than the cortex.
Conclusions: The origin of orientation selectivity and the mechanism of visual system maturation can be explained by geniculocortical synaptic changes according to the rules of Hebbian plasticity.

id #11604

EVIDENCE THAT HETEROMERS OF DOPAMINE AND GHRELIN RECEPTORS IN THE SPINAL CORD CONTROL COLORECTAL FUNCTION

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Agonists of ghrelin receptors (GhrR) and of dopamine 2 receptors (DRD2) both act in the spinal cord to stimulate colorectal propulsion and defecation. However, we have found that there is no ghrelin in the spinal cord. Here we present data indicating that effects through

spinal GhrR receptors are mediated by dopamine acting at DRD2 that is coupled to GhrR. In rats, we evoked defecation by stimulating central pathways by water avoidance stress. The responses were inhibited both by the DRD2 antagonist, sulpiride, or the GhrR antagonist, YIL781. The effect of the DRD2 agonist, quinpirole, applied directly to the defecation centres, was antagonized by YIL781. Neurons expressing both DRD2 and GhrR were found in the defecation centres. In cells transfected with DRD2, dopamine receptor agonists inhibited adenylyl cyclase but had no effect on Ca^{2+}. GhrR agonists had no effect. In cells transfected with DRD2 plus GhrR, agonists of either receptor caused increased cytoplasmic Ca^{2+}. The effects were antagonized by the GhrR antagonist, YIL781, in both cases. We conclude that the involvement of spinal GhrR in colorectal control in the absence of ghrelin is explained by dopamine being the transmitter that acts at DRD2/ GhrR heteromeric receptors.

id #11617

RECONSTRUCTING THREE-DIMENSIONAL TRAJECTORIES OF FREELY FLYING HONEYBEES IN A SEMI-OUTDOOR ENVIRONMENT

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So far, few studies have investigated the collision avoidance behaviour of a group of insects flying in close proximity in an outdoor environment. This is mainly because of the technical difficulties involved in detecting and tracking individual insects in three dimensions, as well the challenge of matching views of corresponding insects in stereo images (the so-called 'Correspondence Problem'). In this study, we describe the methods we have used to simultaneously track and reconstruct the trajectories and body orientations of a large number of bees, flying in a 'bee cloud', using just two cameras configured as a stereo pair. We compare two methods for 'correspondence matching', to determine which one provides more accurate results. The accuracy of the preferred method is verified with the aid of a 'ground truth' control experiment. Using these methods, two separate bee clouds were filmed using high-speed video cameras, and the data were analysed to reconstruct the three dimensional trajectories, including the head and tail positions, and body orientations, of a total of 438 bees. The methods that we have developed and investigated not only enable accurate reconstruction of the flight trajectories, but also help resolve ambiguities of identity that occur when a bee is not simultaneously visible to both cameras, or when one bee is temporarily occluded by another bee in a camera view. Our preliminary analysis of the flight trajectories is beginning to provide us with insights into the collision avoidance strategies exercised by large groups of bees flying in close proximity.

id #11624

Neural Correlates of Novel Object Recognition in the medial Prefrontal Cortex and Hippocampus

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In a given environment, novel object recognition (NOR) describes the ability to distinguish a new object or stimulus from the other previously encountered one. Recognition memory is fundamental for animals and human beings to understand their 'space' and guide appropriate behaviour. Several brain regions have been implicated in this process with two key ones being the hippocampus (HPC) and the medial prefrontal cortex (mPFC). However, the dynamics of how these regions interact via neural oscillation, single-unit activity and synchrony shifts in NOR are not known. In our study, electrophysiological recordings were simultaneously acquired from the HPC and mPFC in freely-moving rats undergoing a NOR task. As expected, our results revealed that animals showed an exploratory preference for novel objects. Local field potential theta(2-6Hz) power was enhanced during NOR in both the HPC and mPFC. Comparing their coupled theta-oscillations, revealed an elevated correlation, providing evidence for the involvement of these two regions in NOR. Single units recorded from the mPFC were classified into pyramidal neurons (PNs) and interneurons (INs). PNs showed an overall increasing firing rate in object exploration compared with the resting state. Moreover, we found familiar or novel object-responsive INs which only enhanced the firing rate in specific object exploration states. Phase difference and the Granger Causality analysis between these two regions suggested that the HPC leads the mPFC during the recognition phase of novel objects. Together, our findings show that the mPFC and its synchronized activity pattern with the HPC play a functional role in novel object recognition.

id #11630

Pattern electroretinography reveals the spatial resolving power and contrast sensitivity of insect compound eyes

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Many animals rely on vision for everyday tasks such as navigation, foraging, finding conspecifics and avoiding predators. The visual capabilities of an animal are typically characterised by their spatial resolving power and contrast sensitivity. One technique that can provide information of those characteristics simultaneously is pattern electroretinography (PERG), which has been used mainly in vertebrates. Here, we performed PERG to compare the spatial vision of compound eyes between diurnal/nocturnal ants and between walking/flying insects. We found that spatial resolving power was 0.60 cycles per degree (cpd) in the diurnal-crepuscular *Myrmecia tarsata*, while it was 0.57 cpd in the nocturnal *M. midas*. This variation is explained by difference in ommatidial facet diameters, which are significantly larger in *M. midas*. The spatial resolving power was 0.54 cpd in the flying *Apis mellifera*, although their facet size was comparable to the pedestrian *M. tarsata*. This may be because the larger rhabdoms in *M. tarsata* provide greater spatial resolving power than *A. mellifera* by improving the light capture. The contrast sensitivity reached a maximum of 15.5 at 0.1 cpd in *M. tarsata*, 21.2 at 0.05 cpd in *M. midas* and 16.9 at 0.05 cpd in *A. mellifera*. Their contrast sensitivity functions were comparable. The high contrast sensitivity would be important under low luminance conditions and/or for discriminating objects of low contrast

as well as for a flight in *A. mellifera*. I will discuss the application of PERG for assessing the spatial vision in compound eyes and compare our results with anatomical and behavioural estimates.

id #11641

The Effect of a Six-month Exercise Intervention on Executive Function: Results from The Intense Physical Activity and Cognition (IPAC) study

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Background: There is a paucity of research that systematically assesses the role of physical activity intensity and cardiorespiratory fitness, and their relationship with Executive Function (EF) in older adults. To address this limitation, we examined the effect of a systematically manipulated exercise intervention on EF.

Method: Ninety-nine participants (age=69.10 ± 5.2; *n*=54 female) were randomized into either a high-intensity exercise, moderate-intensity exercise, or inactive control group. Those in an intervention group participated in six-months of cycle-based exercise twice weekly, at 50-minutes per session. All participants underwent neuropsychological testing and fitness assessment at baseline and post-intervention. EF was measured comprehensively, including measures of each subdomain: Shifting, Updating, Inhibition, Verbal-Generativity, and Non-verbal Reasoning. Fitness was measured by analysis of peak aerobic capacity; VO_{2peak}.

Results: There were no differences pre- to post-intervention across groups for any of the EF subdomains. Changes in cardiorespiratory fitness from pre- to post-intervention differed across groups (*F*=7.36, *p*=.001; high-intensity>moderate intensity and control group). Thus, we conducted follow-up analyses of the associations between individual fitness change scores and individual EF change scores in those randomized to an exercise group. Change in fitness was positively associated with change in Verbal-Generativity from pre- to post-intervention (*B*=.51, *p*=.03).

Conclusion: We did not find evidence that six-months of aerobic exercise improves EF in older adults. It remains possible that changes in cardiorespiratory fitness may be associated with changes in Verbal-Generativity. Further research on individual variability in exercise-

induced cognitive change is vital in establishing physical activity as a protective factor against age-associated cognitive decline.

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2. Frost, Natalie et al. "Higher Cardiorespiratory Fitness Is Associated With Better Verbal Generativity in Community-Dwelling Older Adults." Journal of Aging and Physical Activity (2019): 1–8. Web.

id #11649

NG2+ cells are vulnerable at breaches of the blood brain barrier during secondary degeneration following neurotrauma

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Neurotrauma is accompanied by blood brain barrier disruption both adjacent to and remote from the injury, associated with increased neuroinflammation and damage. NG2+ cells are an integral component of the blood brain barrier, and encompass both pericytes and oligodendrocyte precursor cells. However it is not yet known if oxidative damage to NG2+ cells occurs to a greater degree at sites of blood brain barrier breach than sites where the barrier is intact. Here we use the partial optic nerve transection model of secondary degeneration in adult female PVG rats and semi-quantify 8-hydroxy deoxyguanosine (8OHdG) immunoreactivity as an indicator of oxidative damage to DNA in NG2+ cells and glial fibrillary acidic protein (GFAP)+ astrocytes, together with Immunoglobulin G (IgG) immunoreactivity as an indicator of blood brain barrier breach. 8OHdG immunoreactivity was increased 1 day after injury in both NG2+ cells and GFAP+ astrocytes surrounding blood vessels, in optic nerve vulnerable to secondary degeneration ($p \leq 0.001$). However, only in NG2+ cells surrounding vessels, was 8OHdG immunoreactivity higher at sites of blood brain barrier breach than where the barrier was intact ($p \leq 0.01$). Ethynyldeoxyuridine delivered in vivo to label proliferating cells was used to demonstrate that the percentage of proliferating NG2+ cells around RECA+ blood vessels was increased after injury ($p \leq 0.05$), whereas the percentage of proliferating RECA+ endothelial cells did not increase in response to injury. Thus, NG2+ glia may be particularly vulnerable to oxidative damage at sites of blood brain barrier breach, associated with a proliferative response.

id #11654

Heterozygous loss of function of *IQSEC2/Iqsec2* leads to increased activated Arf6 and severe neurocognitive seizure phenotype in females.

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Clinical presentations of mutations in the *IQSEC2* gene on the X-chromosome initially implicated to cause non-syndromic intellectual disability (ID) in males have expanded to include early onset seizures in males and females. The molecular pathogenesis is not well understood, nor the mechanisms driving disease expression in heterozygous females.Utilising a CRISPR/Cas9 edited *Iqsec2* KO mouse model we demonstrate loss of *Iqsec2*mRNA expression and Iqsec2 protein within the brain of founder and progeny mice. Male (52%) and female (46%) *Iqsec2* KO mice present with frequent and recurrent seizures. Focusing on *Iqsec2*KO heterozygous female mice, we demonstrate increased hyperactivity, altered anxiety and fear responses, decreased social interactions, delayed learning capacity and decreased memory retention/novel recognition; recapitulating psychiatric issues, autistic-like features and cognitive deficits present in female patients with loss-of-function *IQSEC2*variants. Despite Iqsec2 acting to activate Arf6 substrate, we demonstrate that mice modelling the loss of Iqsec2 function present with increased levels of activated Arf6. We contend that loss of Iqsec2 function leads to altered regulation of activated Arf6 mediated responses to synaptic signalling and immature synaptic networks. Wehighlight the importance of IQSEC2 function for females by reporting a novel nonsense variant c.566C>A, p.(S189*) in an elderly female patient with profound ID, generalised seizures and behavioural disturbances. Our human and mouse data reaffirm *IQSEC2* as a disease gene with an unexpected X-chromosome heterozygous female-phenotype. The Iqsec2 mouse model recapitulates the phenotypes observed in human patients despite the differences in the IQSEC2/Iqsec2 gene X-chromosome inactivation between the species.

id #11668

Microglia in HIV pain-associated synaptic degeneration

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HIV patients with chronic pain develop synaptic degeneration in the spinal cord dorsal horn, but the patients without the pain disorder do not show this neuropathology, indicating a

pathogenic contribution of the synaptic degeneration to the development of HIV-associated pain. However, the mechanism underlying the synaptic degeneration is unclear. We report here that HIV-1 gp120, a neurotoxic protein that is specifically associated with the manifestation of pain in HIV patients, induces synapse loss via microglia. Further studies elucidate that gp120 activates microglia by stimulating Wnt/β-catenin-regulated fractalkine in neuron. The results demonstrate a critical role of microglia in the pathogenesis of HIV-associated synaptic degeneration in the spinal pain neural circuit.

id #11684

ULTRASTRUCTURAL EVALUATION OF SYNAPTOGENESIS INDUCED BY ELECTROCONVULSIVE SHOCK IN THE RAT BRAIN

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It's been known that mood disorders are related to neurochemical changes; yet, the relationship between neuroplasticity and depression has been uncharted territory. Neuroplasticity changes underlie improvement in or recovery from depression, stroke, and possibly certain neurodegenarative diseases. Neuroplasticity changes have been proposed as a mechanism of action of Electro-Convulsive therapy (ECT). Electroconvulsive shock (ECS) has been shown to induce nerve cell proliferation in the hippocampus. The present study is designed to determine the role of ECS in synaptogenesis in different regions of rat brain. Constant current, at low frequency and brief-pulse ECS (charge = 10mC) was given to the adult male Wistar rats through gel-coated earclip electrodes. Sham-stimulated animals received the same treatment with no current passed were considered as control. After 10 days of ECS administration, the animals were perfused. Prefrontal cortex, hippocampus and amygdala regions of the rat brain were dissected out and processed for ultrastructural study. Newly formed neurons were noted in the prefrontal cortex, whereas CA1 region of the hippocampus showed axo-dendritic synapses with more number of type-I synaptic vesicles than type-II vesicles. Axo-somatic synapses were noticed more in CA1 region compared to CA3. CA3 region showed few newly formed axo-dendritic synapses with less number of synaptic vesicles. Many immature axo-dendritic synapses having electron dense synaptic clefts with or without synaptic vesicles were also found in CA1 region. Axo-dendritic synapses with type-I vesicles were less in amygdala region. Our results establishes at low frequency and brief pulse ECS induces synaptogenesis in the rat brain.

id #11708

Bilateral regulation of histone deacetylase activity and gene expression is associated with an intermediate form long-term potentiation *in vivo*.

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Long-term potentiation (LTP) is a synaptic plasticity mechanism critical to long-term memory. LTP induced *in vivo* is associated with rapid upregulation and subsequent downregulation of gene expression. This temporal shift in gene expression is predicted to be partly mediated by histone deacetylases (HDACs), epigenetic inhibitors of gene expression. Further, pharmacological inhibition of HDACs has previously been shown to enhance LTP persistence *in vitro* and been proposed as a therapeutic intervention to alleviate memory impairments. To explore the contribution of HDACs to the persistence of LTP, we examined HDAC1 and HDAC2 activity over a 24 h period following unilateral LTP induction *in vivo* in the rat dentate gyrus. We found changes in HDAC1 and HDAC2 activity in both the stimulated and unstimulated hemispheres, with the largest increase in activity occurring 20 min post-HFS. During these timepoints of heighted activity, Chromatin immunoprecipition showed that HDAC1 and HDAC2 are enriched at distinct sets of genes in both hemispheres. Further, we found the HDAC inhibitor Trichostatin A enhanced LTP2 an intermediate, protein synthesis dependent form which has not previously associated with alterations in transcription. The inhibitor had no effect on the persistence of LTP3. This suggests that mechanisms which have previously been attributed to long-term plasticity may instead only be involved in the intermediate stages of LTP maintenance.

id #11727

Active microRNA and binding partners in the degenerating retina

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Purpose: MicroRNA (miRNA) have been identified as playing an critical role in the pathogenesis of neurodegenerative diseases, including Age-Related Macular Degeneration (AMD). To identify active miRNA involved in retinal degeneration and the mRNA targets that they regulate, we have performed high-throughput sequencing of miRNA and mRNA isolated by crosslinking immunoprecipitation (HITS-CLIP) with argonaute 2 (Ago2) in a model of retinal degenerations.

Methods: C57BL/6J mice were exposed to 5 days of 100k lux photo-oxidative damage (PD). Immunoprecipitation was performed against Ago2 protein on isolated retinas, and the Ago:miRNA and Ago:miRNA:mRNA factions separated. Both miRNA and mRNA were extracted and cDNA libraries prepared. Samples were sequenced on an Illumina HiSeq 2500 and differential expression analysis performed using R.

Results: Sequencing analysis of the Ago:miRNA:mRNA complexes revealed a subset of mRNA that were differentially expressed in mice which had been exposed to photo-oxidative damage ($P<0.05$, n=4). Among the Ago-bound miRNA, miR-124-3p was the most highly expressed in both dim-reared and PD retinas constituting the majority of the proportion of miRNA sequenced (~80%). Gene ontology and gene set enrichment analysis of Ago-bound miRNA and mRNA revealed significant roles in the modulation of miRNA in regulating biological processes such as phototransduction, inflammation, apoptosis, and complement following retinal degeneration.

Conclusions: A subset of miRNA and mRNA targets were differentially regulated as a consequence of retinal degeneration. Characterisation of these miRNA/mRNA interactions in the context of the degenerating retina may provide an important insight into the active role these may play in many neurodegenerative diseases including AMD.

id #11728

ULTRASTRUCTURAL EVALUATION OF SYNAPTOGENESIS INDUCED BY ELECTROCONVULSIVE SHOCK IN THE RAT BRAIN

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It's been known that mood disorders are related to neurochemical changes; yet, the relationship between neuroplasticity and depression has been uncharted territory. Neuroplasticity changes underlie improvement in or recovery from depression, stroke, and possibly certain neurodegenarative diseases. Neuroplasticity changes have been proposed as a mechanism of action of Electro-Convulsive therapy (ECT). Electroconvulsive shock (ECS) has been shown to induce nerve cell proliferation in the hippocampus. The present study is designed to determine the role of ECS in synaptogenesis in different regions of rat brain. Constant current, at low frequency and brief-pulse ECS (charge = 10mC) was given to the adult male Wistar rats through gel-coated ear clip electrodes. Sham-stimulated animals received the same treatment with no current passed were considered as control. After 10 days of ECS administration, the animals were perfused. Prefrontal cortex, hippocampus and amygdala regions of the rat brain were dissected out and processed for ultrastructural study. Newly formed neurons were noted in the prefrontal cortex, whereas CA1 region of the hippocampus showed axo-dendritic synapses with more number of type-I synaptic vesicles than type-II vesicles. Axo-somatic synapses were noticed more in CA1 region compared to CA3. CA3 region showed few newly formed axo-dendritic synapses with less number of synaptic vesicles. Many immature axo-dendritic synapses having electron dense synaptic clefts with or without synaptic vesicles were also found in CA1 region. Axo-dendritic synapses with type-I vesicles were less in amygdala region. Our results establishes at low frequency and brief pulse ECS induces synaptogenesis in the rat brain.

id #11729

Extracellular matrix remodelling regulates adult hippocampal neurogenesis and improves spatial learning

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The adult hippocampus harbors distinct populations of quiescent neural precursor cells (NPCs) that drive the production of new neurons. However, molecular mechanisms that drive the activation of quiescent NPCs remain largely elusive. To identify such mechanisms, we purified hippocampal NPCs and used RNA-seq to disclose their molecular identity. Gene ontology analysis revealed preferential enrichment of genes associated with extracellular matrix (ECM) and identified several receptors known to bind ECM that are selectively expressed in the NPCs. Although ECM has been implicated in neurogenesis, the role of ECM mediated juxtracrine pathway has not been thoroughly investigated in the neurogenic niche. Here, using clonal neurosphere assay, we reveal a previously unknown size-dependent, dual and distinctly opposite role of a select ECM component in regulating NPC activity. Furthermore, we demonstrate that intrahippocampal injections disrupting ECM microstructure activated NPCs and significantly increased the production of newborn neurons (BrdU$^+$DCX$^+$ cells). To investigate the functional impact of this pathway on hippocampus-dependent cognitive behaviour, we used the active place avoidance task, and found that ECM remodelling accelerates spatial learning in young mice. Collectively, our findings identify a novel niche-mediated juxtracrine mechanism that directly regulates the activity of hippocampal NPCs and modulates hippocampus-dependent behaviour. Given that hippocampal neurogenesis is implicated in various neurological and mental disorders, further studies exploiting this ECM-mediated pathway may inform the development of novel pharmacotherapies that enhance neurogenesis and improve behaviour.

id #11731

TRANSNEURONAL TRACING OF COLONIC-RELATED AFFERENT CIRCUITS IN MICE USING HERPES SIMPLEX VIRUS 1 STRAIN H129 EXPRESSING EGFP

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The central circuits of spinal afferent pathways innervating pelvic visceral organs remain to be characterised in the mouse, despite the depth of knowledge on peripheral properties. To

address this, we used anterograde transneuronal tracing with a strain of herpes simplex virus 1 expressing EGFP (HSV-H129-EGFP). We injected HSV-H129-EGFP into the distal colon wall and neuronal expression of EGFP was tracked over time (24-120 hours; N=4/time) within thoracolumbar (TL; T10-L1) and lumbosacral (LS; L6-S1) dorsal root ganglia (DRG), spinal cord and brain. HSV-H129-EGFP was evident in LS DRG neurons 48 hours after colonic injection and in TL DRG after 72 hours. By 72-96 hours, EGFP labelled neurons were prevalent in the spinal cord, including LS dorsal horn laminae (LI-LV), dorsal grey commissure and sacral parasympathetic nucleus, and within the TL dorsal horn lamina I, the intermediolateral nucleus and around the central canal. After 96 hours, EGFP neurons were also evident within the caudal medulla, specifically within the dorsal motor vagal complex, reticular nuclei, nucleus raphe magnus and caudal locus coeruleus. After 120 hours, EGFP labelling had spread more rostral into the pontine locus coeruleus, Barrington's nucleus, lateral parabrachial nucleus and pontine reticular formation. Dense labelling was also evident in the hypothalamic paraventricular nucleus, whilst sparse labelling was observed in the midbrain periaqueductal gray and thalamic nuclei (intermediodorsal and paraventricular) and medial-frontal cortex. These data provide the first description of the spinal cord and brain circuits relevant to the central neural control of colonic autonomic and sensory function in the mouse.

id #11745

The effect of low-intensity ultrasound on tau pathology in a transgenic mouse model and on the transport mechanisms across the blood-brain barrier

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Objectives:

A major obstacle in developing effective treatments for neurological disorders is the blood-brain barrier (BBB) that restricts access of therapeutic agents to the brain. Low-intensity ultrasound (US) in conjunction with intravenously-injected microbubbles is an emerging therapeutic strategy to transiently open the BBB to overcome this hurdle.

1. While the potential of US as a drug delivery system[1] and reducing amyloid pathology in the absence of therapeutic agents[2] is firmly established, the main objective of this study was to assess the effect of US on K3 mice that have a tau-dependent motor and memory deficit.
2. For a mechanistic understanding of US, we separately elucidated its effect on paracellular and transcytotic transport modes across the BBB for various cargoes.

Methods:

1. To assess the effect of ultrasound on pathological tau, we performed repeated scanning ultrasound (SUS) treatments of K3 mice for 15 weeks and conducted histological, biochemical and behavioural analysis.

2. To understand the effect of US on transport mechanisms, we assessed the transport of differently-sized dextrans following US in mice lacking the vesicle-forming protein caveolin, compared to wild-type mice.

Results:

1. SUS treatment regime significantly reduced neuronal tau pathology by facilitating autophagy and improved associated behaviours.
2. Larger sized cargoes partially utilised caveolae-mediated transcytosis following US.

Conclusions:

Repeated SUS treatments reduce pathological tau in transgenic mice and ameliorate associated motor and memory functions via an induction of autophagy in neurons[3].

Additionally, transcytosis was shown to play a major role in transporting larger cargoes following US, providing a mechanism of action for increased BBB permeability.

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id #11747

Discrimination of Features in Natural Scenes driven by Selective Attention

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Aerial predators, such as the dragonfly, must determine the position and movement of their prey, even when embedded in natural scenes. This task is likely supported by neurons in in the dragonfly optic lobes that are tuned to moving targets of less than a few degrees. These Small Target Motion Detector (STMD) neurons are also tuned to target velocity, facilitating their response to targets that move along continuous trajectories. Some STMDs even competitively select one single target, when presented with a pair of targets as if the distracter does not exist.

Here we describe STMD intracellular responses when presented with many potential distracters within cluttered environments. We vary target contrast and background scenes and assay across both target and background velocities. We find that background motion affects

neuronal responses indirectly, via the competitive selection of background features, resulting in robust discrimination only when the target velocity is matched to or greater than that of the background. We also find that target discriminability is dependent on background direction, with backgrounds moving in the anti-preferred direction for a neuron resulting in the least performance degradation.

id #11748

Investigation of mismatch responses in rats: towards a new outcome measure for preclinical schizrenia research

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Reduction in the size of mismatch negativity (MMN), an auditory prediction error signal, is one of the most highly replicated neurophysiological findings in schizophrenia. The ability of MMN to be elicited without the subject attending to stimuli makes it an excellent candidate for back-translation into animal models. To offer new, and potentially more translatable outcome measure options for preclinical schizophrenia research, we have been focused on developing a rodent model of MMN. It has been demonstrated that rat brains are certainly capable of generating a human-like prediction error signal, both by surface-level and local field potential recordings. The next step for the field, however, is to find an animal model system in which these responses are reduced, similar to what is seen in schizophrenia. We have demonstrated that acute exposure to MK-801, a glutamate N-methyl-D-aspartate receptor (NMDAr) antagonist, reduces prediction error responses in rats. To examine whether endogenous prediction error responses can be altered, we examined these in two risk factor-based rat models of schizophrenia: in rats exposed to early or late Maternal Immune Activation (MIA), and, in a cumulative risk factor model, rats exposed to MIA alone, Adolescent Cannabinoid Exposure (ACE) alone, or both in combination. These developmental manipulations did not result in schizophrenia-related reductions in the prediction error response. Future directions will focus on probing the neurobiology and neurocircuitry of the prediction error response and on continuing the search for a model demonstrating endogenous impairments, to be used as a tool for drug discovery research.

id #11753

Gastrointestinal dysmotility in the C57BL/6 NL3^{R451C} mouse model of autism

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Gastrointestinal dysfunction affects up to 90% of individuals with autism. Multiple gene mutations identified in autism alter neuronal signalling in the brain. The R451C missense mutation was found in siblings with autism and affects the functioning of a synaptic protein, Neuroligin-3 (NL3). It is well established that GABAergic signalling in brain slices is altered in NL3^{R451C} mice. We recently reported GABA-mediated colonic dysmotility in NL3^{R451C} mice bred on a mixed background strain, however it is unknown whether these changes persist on a C57/Bl6 background. Here we report that C57/Bl6 R451C (B6NL3) mice have faster gut transit using *in vivo* X-ray imaging. *Ex vivo* video imaging experiments revealed these mice also had fewer colonic short contractions in the presence of the GABAA receptor antagonist gabazine. Because Nitric Oxide is the major inhibitory neurotransmitter in the enteric nervous system we also examined intestinal motility in the presence of NOLA (Nitric Oxide Synthase inhibitor). B6NL3 mice had significantly longer colonic migrating motor complexes (CMMCs) compared to WT and in the small intestine, 7 out of 17 (41%) B6NL3 mice showed persistent propagating contractile complexes versus 1 out of 15 (6%) WT mice in the presence of NOLA. Our results suggest that B6NL3 mice are more resistant to the effects of NOLA and more sensitive to Gabazine. In summary, we show that B6NL3 mice have altered *in vivo* and *in vitro* motility in both small intestine and colon. These findings extend our understanding of how the enteric neurons are affected in autism.

id #11759

Altered gastrointestinal motility in a mouse model of cigarette smoking-induced Chronic Obstructive Pulmonary Disease.

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Cigarette smoking (CS) is a major cause of Chronic Obstructive Pulmonary Disease (COPD). Gastrointestinal (GI) dysfunction reduces quality of life for COPD patients. However, the underlying mechanisms and precise effects of CS on gut contractility are not fully characterised. We examined gut anatomy, colonic motility, and enteric neurons to understand the relationship between CS, COPD and GI dysfunction. Male BALB/c mice (7 weeks old) were exposed to room air (Sham) or CS (9 cigarettes/day, 5 days/week) for 2 and 6 months. Video imaging was used to construct high resolution spatiotemporal maps of motor patterns *ex vivo* to examine colonic migrating motor complexes (CMMCs). Wholemount immunofluorescence with the pan-neuronal marker (Hu) and nitric oxide synthase (NOS) was performed to quantify myenteric neurons. CS reduced body weights and increased colon length to body weight ratio. 2 months CS led to more CMMCs (CS:13.2±0.6; SS:7.8±0.6CMMCs/15min, p<0.0001), reduced resting colonic diameter (CS:3.6±0.1mm; Sham:4.1±0.2mm, p=0.01) and faster transit (CS:3.2±0.2mm/s; Sham:2.3±0.2mm/s, p=0.0024). A 10-day cessation after CS for 2 months reversed the changes in CMMC frequency, however had no effect on reduced colonic diameter. Moreover, 2 months CS had no effects on enteric neuron numbers, whereas there was a significant reduction in NOS neurons at 6 months (CS:35±2.5%; Sham:42±0.8%, p=0.039). This is the first report of increased colonic motility in CS-induced COPD. The changes are not due to altered neuron numbers in 2 months CS. Prolonged exposure to CS has greater effects on enteric neurons. Further research is necessary to identify the exact causes of dysmotility.

id #11761

Investigation of axonal excitability in preclinical mouse models of acute oxaliplatin-induced neurotoxicity

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Neurotoxicity is the most frequent and debilitating adverse effect of the chemotherapeutic agent oxaliplatin, however, the mechanisms are not well understood. This study utilised nerve excitability techniques to investigate the neurotoxic effects of acute oxaliplatin treatment in a mouse model. Mature C57BL/6 mice (>20 weeks old) were injected with a single bolus dose of 10mg/kg oxaliplatin (prepared in 5% dextrose) intraperitoneally, or a single dose of 15mg/kg intramuscularly at the base of the tail. In both models, mice injected with dextrose were used as controls. Nerve excitability testing was performed on the motor and sensory axons of the caudal nerve at different time points following the injection using the TROND protocol. Mice treated with oxaliplatin intramuscularly displayed an increase in rheobase, a fanning out of the depolarising threshold electrotonus (increased TEd (peak) and TEd 10-20ms) and an upward shift in the recovery cycle causing a reduced superexcitability and an increased subexcitability. Furthermore, a train of delayed repetitive discharges following the compound action potential waveform were detected. These changes were observed as early as 30 minutes post-injection and were sustained for up to 4 days. Conversely, there were no changes in motor axon excitability in mice injected with oxaliplatin intraperitoneally, and in sensory axons in either injection model. Our results show that a local, but not systemic, injection of oxaliplatin in mice induces changes in motor axonal excitability suggestive of slowed sodium channel inactivation, similar to changes observed in the clinical setting in oxaliplatin-treated patients.

id #11770

Changes in caecal neuroimmune interactions in the Neuroligin-3^{R451C} mouse model of autism

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Many rare mutations implicated in autism are involved in synaptic cell-adhesion pathways including the well-studied neuroligin-3 R451C missense mutation. Individuals with this mutation experience gastrointestinal dysfunction, a common comorbidity affecting up to 90% of individuals with autism. Gut microbial communities produce neuro-active molecules that modulate the central and enteric nervous systems. Little is known about caecal neuro-immune and microbial interactions even though it is involved in generating immune responses and

acts as a repository of intestinal microorganisms. Using immunofluorescence in wholemount preparations from wild-type and NL3^{R451C} mutant mice, we quantified the total number of myenteric neurons (stained for the pan-neuronal marker Hu). Using frozen cross sections of caecal immune patch, we examined IBa1-expressing immune cells. We also assessed caecal microbial community composition using Illumina deep sequencing. NL3^{R451C} mice have significantly reduced caecal weight compared to wild-type (0.54±0.01g in NL3^{R451C}, n=36 and 0.65±0.02g in WT, n=38; p=0.0001). NL3^{R451C} caecal myenteric ganglia were 53% larger (WT: 0.623±0.05µm; NL3^{R451C}: 1.34±0.02µm, n=5; p=0.0079) and had increased Hu+ neurons per ganglion (NL3: 15 ±1 and WT: 11±1; n=5; p=0.005; 10 ganglia/sample). 3D image analysis revealed that Iba1-expressing immune cells in the caecal patch had a smaller volume in NL3 mice (WT: 977±76.9µm^3; NL3: 649±108.3µm^3, n=5; p=0.039). Moreover, distinctly different caecal microbial community structures (66% difference in composition) were observed in NL3^{R451C} compared to wild-type. These results suggest that the NL3^{R451C} mutation alters the bi-directional communication between caecal myenteric neurons and the microbial community.

id #11789

Examining supplementary motor area-primary motor cortex connectivity using a novel transcranial magnetic stimulation protocol: Application in tremor-dominant Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disease resulting in progressive motor dysfunctions, including tremor. The pathophysiology of tremor-dominant PD remains poorly understood. The current project has three objectives: First, to examine test re-test reliability of supplementary motor area-primary motor cortex (SMA-M1) connectivity measured using dual-coil transcranial magnetic stimulation (TMS) in younger and older adults; second, to examine whether SMA-M1 connectivity is altered in people with tremor-dominant PD compared to age- and sex-matched controls; and third, to examine whether the strength of SMA-M1 connectivity is associated with tremor severity in people with PD.

SMA-M1 connectivity was measured using dual-coil TMS in younger and older adults without PD (N = 60) in two identical sessions separated by ~7 days. We have begun to investigate SMA-M1 connectivity and tremor severity in people with tremor-dominant PD.

Test re-test reliability of SMA-M1 connectivity was moderate-to-good in younger and older adults without PD. There was no significant difference in the strength of SMA-M1 connectivity between younger and older adults without PD. Based on preliminary results, we hypothesise that people with tremor-dominant PD will show reduced SMA-M1 connectivity

when compared to age- and sex-matched controls, and the strength of SMA-M1 connectivity will be negatively associated with tremor severity in tremor-dominant PD.

The current findings suggest that SMA-M1 connectivity can be reliably measured using dual-coil TMS. This has important implications for the use of dual-coil TMS to investigate SMA-M1 connectivity in tremor-dominant PD, as well as increase our understanding of the pathophysiology of other movement disorders.

id #11797

Cognitive deficits in the CNTNAP2$^{-/-}$ mice model of autism: How altered circuit maturation affects adult memory.

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Autism Spectrum Disorder (ASD) comprises deficits in cognitive domains, but no treatment are available to alleviate such impairments. The absence of therapeutic strategy is likely due to a lack of knowledge regarding the precise mechanisms underlying these cognitive defects. Here, we aimed to dissect how circuit maturation contributes to hippocampus-dependent memory impairments associated with ASD in the CNTNAP2$^{-/-}$ mouse model.

We investigated the involvement of an identified risk gene: the neurokinin 3 receptor (NK3R) whose function remains unknown. We found a specific pattern of expression of NK3R during development in the hippocampus. We determined 1/ the morphological and physiological properties of the developing Parvalbumin interneurons (PV-INs) in the hippocampus of CNTNAP2$^{-/-}$ mice and 2/ whether challenging NK3R activity during development could rescue deficits.

We found a drastic reduction of the complexity and length of the dendrites in PV-INs, normalised by a decrease in NK3R activity during early postnatal stages. PV-INs exhibited increased excitability in CNTNAP2$^{-/-}$ compared to WT, revealed by a hyperpolarized threshold and shorter action potential; both alterations were normalised by decreasing NK3R activity in CNTNAP2$^{-/-}$ mice. Future studies will assess alterations of declarative memory and working memory, two major cognitive features of ASD. Together, the present work identifies alterations of the PV-INs in autistic mice during a critical window of maturation.

id #11817

Longitudinal omics approach to investigate the microbiome-gut-brain-axis in R6/1 mice, a transgenic mouse model of Huntington's disease

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Huntington's Disease (HD) is a progressive neurodegenerative disorder caused by a trinucleotide repeat expansion in the huntingtin (HTT) gene and the resultant protein is ubiquitously expressed throughout the brain and periphery tissues. The gastrointestinal microbiome has recently been implicated in various aspects of brain function, cognition and behavior, in health and disease. We have previously described the first evidence of gut dysbiosis in the R6/1 mouse model of HD prior to overt motor symptoms at 12 weeks of age [1]. Therefore, in this study, we performed longitudinal shotgun sequencing on faecal samples at time point 4, 6, 8, 10 and 12 weeks of age to probe the gut microbiome trajectory of the R6/1 mice. In addition, we hypothesized that gut dysbiosis will alter plasma metabolite profile, hence, we performed targeted HILIC LC-MS/MS metabolomics on the plasma of R6/1 mice at 12 weeks of age. The R6/1 mice microbiome displayed a more volatile trajectory across all timepoints when compared to their wild-type littermates. We also confirmed and extended our previous findings of gut dysbiosis in R6/1 mice at 12 weeks of age, specifically, we obtained HD-microbiome signature at species-level-resolution. The circulating metabolites profile of R6/1 mice, particularly some of the known microbial-derived metabolites, were altered when compared to their wild-type-counterparts. Together, our findings suggest that changes in gut microbiome composition could partially contribute to the altered plasma metabolome in the R6/1 mice which could be play a part in HD-related symptoms.

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id #11831

Sphingosine kinase 2 protects oligodendrocytes and is required for remyelination.

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Restoration of neurological function in multiple sclerosis (MS) and other demyelinating conditions requires new therapeutics that promote oligodendrocyte survival and remyelination. Sphingosine 1-phosphate (S1P) is an essential lipid metabolite that signals through five G-protein coupled receptors. Previous studies suggest that the MS drug Fingolimod, an S1P receptor agonist, promotes oligodendrocyte differentiation and survival, however the role of endogenous ligand S1P in oligodendrocyte survival and myelination has not been established. In the central nervous system, S1P is synthesized primarily by sphingosine kinase 2 (SphK2). We therefore investigated whether SphK2 is required for protection of oligodendrocytes in the cuprizone mouse model of acute demyelination, and for spontaneous remyelination following cuprizone withdrawal, comparing SphK2 knockout (SphK2-/-) with wild-type (WT) mice. Myelin and oligodendrocyte markers were significantly reduced by cuprizone treatment in corpus callosum (CC), cerebral cortex, and hippocampus of both WT and SphK2-/- mice. However, MBP and MOG were 35.5% (p <

0.0001) and 51.4% (p < 0.01) lower, respectively, in CC of cuprizone-treated SphK2-/-compared to cuprizone-treated WT mice. Mature oligodendrocyte numbers did not differ between untreated WT and SphK2-/- mice, but were 58.0% lower in WT and 84.9% lower in SphK2-/- mice with cuprizone treatment (p < 0.05, SphK2-/- vs WT), indicating more extensive oligodendrocyte loss in SphK2-/- mice. Following cuprizone withdrawal, extensive remyelination was observed in WT but not SphK2-/- mice. These results demonstrate the importance of SphK2 for oligodendrocyte stress resistance and spontaneous remyelination. Future work will determine whether SphK2 promotes remyelination through autocrine stimulus of oligodendrocyte S1P receptors.

id #11925

Optical Control of Neuronal Behaviour with Organic Electronic Devices

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Extracellular stimulation of nervous tissue has been utilised therapeutically in treatments for various neurological conditions including Parkinson's disease, epilepsy, and dystonia. Established and emerging prosthetic technologies such as cochlear implants and retinal visual prostheses are also dependent upon this approach. In contrast to entirely electrical devices, light-based devices can be wireless, minimally invasive, and offer high spatial and temporal resolution. Furthermore, organic electronic devices can be fabricated at nanometre thicknesses, are highly flexible and can conform to curved surfaces of the human body. While these devices show vast potential, much work remains to be done to elucidate the mechanisms of photostimulation at the single-cell level. We have fabricated organic electronic photostimulation devices that are biocompatible under tissue culture conditions, and characterised their interaction with cultured neurons. We present devices which are capable of sustaining both primary mouse and primary foetal human neuronal cultures. We also present data demonstrating both photostimulation and photoinhibition at the level of single neurons. These results reveal the influence of different design factors on the latency, efficacy, and persistence of optical photostimulation and photoinhibition with organic electronic devices.

id #11928

Age Differences in the Processing of Emotional Information: A Meta-Analysis investigating Face versus Non-Face Pictures as Potential Moderators

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Although a large number of empirical studies have found support for the age-related positivity effect, it remains unclear whether this effect is due to increased processing of

positive or decreased processing of negative information. Additionally, previous meta-analyses on age differences in emotional information processing did not address the role of stimulus type (face vs. non-face) as a potential moderator. In consideration of these issues, the present meta-analysis investigated the role of stimulus and valence type in the age-related positivity effect. We included 1561 younger (mean age = 21.97 years) and 1543 older adult samples (mean age = 71.78 years) from 51 data sets, of which 13 came from experiments that used face stimuli and 38 from experiments that used non-face stimuli. Overall, our findings indicated a significant positivity effect (Hedge's g = .285). However, processing positive versus negative stimuli, compared to neutral stimuli, did not differ significantly across age groups. Regarding stimulus type, both younger and older adults showed a bias against negative face and towards negative non-face stimuli compared to neutral stimuli. In contrast, the processing of positive compared to neutral stimuli did not depend on stimulus type. Additional moderator analyses revealed that the magnitude of the effect size for processing positive and negative information varied as a function of paradigm type (attention, recall, or recognition memory). In conclusion, the present meta-analysis found evidence of an overall age-related positivity effect consistent with the literature, and provides new insight into the conditions under which a positivity effect may emerge.

id #11932

A dual role for the UNC-13 M domain in Ca^{2+}- triggered neurotransmitter release

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Synaptic vesicle (SV) priming and fusion require the Munc13 family of proteins, several of which (e.g. Munc13-1, Munc13-2, and ubMunc13-2) have been shown to be essential in regulating short-term synaptic plasticity. However, the underlying molecular mechanisms remain unclear. The nematode C. elegans expresses two UNC-13 isoforms, UNC-13L and UNC-13S (also called UNC-13MR). Here we report a novel dual function of the N-terminal M domain in C. elegans UNC-13MR, a Munc13-2 ortholog. Deleting the M domain in UNC-13MR led to a significant increase in tonic and evoked neurotransmitter release, as well as the size of the readily releasable vesicle pool, revealing an inhibitory function of the M domain in SV priming and fusion. The inhibitory effects of the M domain were eliminated in the absence of the C1 and C2B domains. This suggests that the M domain inhibits the C1-C2B module during synaptic transmission. Interestingly, we found that the M domain directly promoted SV fusion when fused to the MUNC2C fragment, which has been shown to be the minimal region required for priming and fusion. These findings reveal that the M domain regulates synaptic transmission via dual modes. However, it is still unclear how it switches between these modes under physiological conditions.

id #11955

Effects of mutations in the GABA-A receptor α3 subunit that underlie epilepsy, autism, anxiety, facial deformities and intellectual disabilities

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Type-A GABA receptors (GABAARs) are ubiquitously present in the brain and mediate most fast inhibitory neurotransmission. The α3 GABAAR subunit is mainly found at inhibitory synapses of the reticular thalamic nucleus, amygdala and basal forebrain. Mutations in this subunit are linked to neurological disorders including epilepsy, autism, anxiety, facial deformities and intellectual disabilities. This study aimed to understand the functional characteristics of wild-type (WT) α3-containing GABAARs, as well as the effect of four disease-linked α3 mutations (T166M, Q242L, T336M, Y474C). We employed a neuron-HEK293 cell co-culture assay to isolate the effect of these mutations on the inhibitory post-synaptic currents (IPSCs) mediated by pure populations of α3-containing GABAARs. IPSCs were measured by whole cell recording. Our results show that the WT α3-containing receptors mediate slower IPSCs compared to α1-containing GABAARs. The mutations T166M and Y474C prolong the decay time of the IPSCs, whereas T336M reduces the decay time, while Q242L does not seem to alter the function of the receptor. These results not only elucidate the properties of IPSCs mediated by α3-containing GABAARs, but also provide new insights into the pathomechanisms of disease mutations in this subunit.

id #11958

Region specific trends of amyloid deposition in APP/PS1 mice following mid-life environmental enrichment.

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Environmentally enriched (EE) housing conditions are associated with enhanced sensory, motor and cognitive stimulation in animals. In mouse models of Alzheimer's disease in particular, EE can lead to increased performance on memory tasks, increased expression of synaptic proteins and changes in amyloid beta (Abeta) deposition. However, the effects of EE on plaque load have been inconclusive so far, with studies reporting an increase, decrease, or no change in plaque load. We hypothesise that these differential findings might be partly attributable to regional differences in susceptibility to Abeta deposition. Using Thioflavin - S stained coronal brain sections of APP/PS1 mice, we first analysed Abeta deposition in 6, 12 and 18-22 months (m) old animals across 3 brain regions: prefrontal cortex (PFC), somatosensory cortex (SS2) and primary motor cortex (M1). Overall, we found a significant ($p < 0.001$) increase in plaque load between 6m and 12m in all regions, driven by increasing

plaque number rather than increasing plaque size. Plaque deposition plateaued in M1 and SS2 between 12m and 18-22m, however in the PFC region plaque deposition continued in the 18-22m brains ($p < 0.05$). We then used the same analysis for mice housed in EE or standard housing from 6m to 12m and found that at 12m the EE brains had significantly ($p < 0.001$) fewer plaques in the M1 and SS2, but not PFC region. These findings suggest that the PFC region might be selectively more susceptible to deposition of Abeta and less responsive to the effects of EE.

id #11976

Effects of Repetitive Transcranial Magnetic Stimulation on Motivation in a Mouse Model

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Changes within the dopaminergic system induced by repetitive transcranial magnetic stimulation (rTMS) may be a mechanism of rTMS' therapeutic effects. However, dopamine-related behavioural effects of rTMS have not been widely investigated. We recently showed that ephrin-A2A5-/- mice completed significantly fewer trials in a visual task than wildtype mice, and that concurrent low-intensity (LI-) rTMS during the task could partially rescue the abnormal behaviour [Poh et al. 2018, eNeuro, vol. 5]. Here, we investigated whether the behavioural differences in ephrin-A2A5-/- mice is due to abnormal motivation, a dopamine-modulated behaviour, and whether LI-rTMS would increase motivation. Groups of food-restricted ephrin-A2A5-/- and wildtype mice underwent 14 daily sessions of progressive ratio tasks which progressively increases work requirements for a sucrose reward. Mice received either sham or LI-rTMS during the first 10 minutes. Ephrin-A2A5-/- mice that received LI-rTMS responded less per session than sham comparisons, but this was not significant (M=230±21 vs. M=201±18 responses, F (1,5) = 15.6, p=.16). However, ephrin-A2A5-/- mice, surprisingly, responded significantly more than wildtype mice (M=215±15 vs. M=139±15, F(13, 20), p =.002). These results suggest that concurrent stimulation does not influence motivation in a progressive ratio task, refuting our treatment hypothesis. However, we demonstrate that ephrin-A2A5-/- mice have abnormal motivation and reward-related behaviour making them a useful model in which to investigate the effects of rTMS on these behaviours. The different outcome compared to our previous study [Poh et al] may reflect the timing of LI-rTMS delivery in relation to the training period and the reward structure of the task.

1. Poh, EZ, Harvey, AR, Makowiecki, K, & Rodger, J 2018, 'Online LI-rTMS during a Visual Learning Task : differential Impacts on Visual Circuit and Behavioural Plasticity in Adult Ephrin-A2A5 Mice', eNeuro, vol. 5, no. 1.

id #11977

Chronic white matter disruption following paediatric traumatic brain injury (pTBI) in mice

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Traumatic brain injury (TBI) is particularly prevalent in the paediatric population (age 0-4 years). This is also an age when the brain is particularly vulnerable to insult, in part due to the ongoing development of new white matter (WM) tracts and resulting brain networks. The aim of this study was to investigate WM changes longitudinally after pTBI, by examining the expression of several important genes associated with myelin structure and function. Three week old male mice were subjected to unilateral experimental TBI (controlled cortical impact model) at two severity of injuries (mild or severe) compared to sham age-matched controls. At 1 (sub-acute) and 8 (chronic) weeks post-injury, both ipsilateral and contralateral WM were collected. A fully automated QIAcube robot was used for RNA extraction, and qPCR used to quantify 9 genes of interest. Relative gene expression was compared using 2-way ANOVA to measure the effect of injury severity and time post injury. Our preliminary data show increased expression levels of *SOX10* and *Olig1* in the ipsilateral WM from severe TBI group at 8 weeks compared to mild and sham groups, and increased *MBP* chronically following injury in the contralateral hemisphere. Collectively our data suggest ongoing bilateral myelin disruption in WM tracts, both focally in the ipsilateral hemisphere as well as extending into remote contralateral regions.

id #11983

IN VIVO IMAGING OF CORTICAL AXON DEGENERATION AFTER LASER INDUCED INJURY

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Despite the widespread occurrence of axon degeneration in the injured and diseased nervous system, the mechanisms of the degenerative process remain incompletely understood. In particular, the factors that regulate how individual axons degenerate within their native environment in the mammalian brain are unknown. Longitudinal imaging of over 120 individually injured cortical axons in the mouse cerebral cortex revealed a threshold length

below which injured axons undergo a rapid-onset form of Wallerian degeneration (RO-WD). RO-WD consistently starts 10 times earlier and is executed 4 times slower than classic Wallerian degeneration (WD) described in other regions of the nervous system. Unlike classic WD, RO-WD is dependent on synaptic density, but is independent of axon complexity. Finally, we provide both pharmacological and genetic evidence that a Nicotinamide Adenine Dinucleotide (NAD$^+$)-dependent pathway controls cortical axon RO-WD independent of transcription in the damaged neurons. Thus, our data redefine the therapeutic window for intervention to maintain neurological function in injured cortical neurons and support the use of *in vivo* optical imaging to gain unique insights into the mechanisms of axon degeneration in the brain.

id #12001

Maternal antibiotic consumption during pregnancy affects offspring ENS development

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Several antibiotics including amoxicillin and penicillin are considered safe for use during pregnancy. Though antibiotic treatment is often necessary, recent studies have linked maternal antibiotics with increased infant risk of developing several diseases including gastroenteritis. The Enteric Nervous System (ENS) is a network of neurons and glia within the gut that underlies vital digestive functions. ENS development commences during embryogenesis, and recent reports suggest possible ENS and microbiota interaction *in utero*. However the impact of maternal antibiotics on foetal microbiota and ENS development remains unclear. Here, we investigated the effects of maternal antibiotics during pregnancy on offspring ENS. Female mice were mated with male mice, and the morning that a copulatory plug is found, is designated embryonic day (E0.5). Female mice were orally administered antibiotics (vancomycin, 500μg/L or penicillin G, 712.5μg/L) or water throughout gestation (E0.5 – postnatal day, P0), or amoxicillin (200μg/L) between E12.5-P0. The ENS of P0 colon was assessed immunohistochemically. Enteric neurons were stained for the pan-neuronal marker, Hu, and distinct subtype markers: neuronal nitric oxide synthase (nNOS) and calbindin. Enteric glia or progenitors were stained for the marker, Sox10. We found that the numbers of Hu+ and Sox10+ cells were significantly reduced (p=0.037; 0.002), while the proportions of nNOS+ and calbindin+ neuronal subtypes significantly increased (p<0.0001; <0.0001) in the colons of P0 pups from dams given antibiotics compared to controls. This applies to all treatment regimes. Overall we show that exposure to antibiotics during pregnancy affects the developing ENS of the infant and thus gastrointestinal functions.

id #12006

Exploiting naturally occurring mutations to unravel the neurodevelopmental functions of the deubiquitylating enzyme USP9X.

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The X-chromosome gene *USP9X* encodes a deubiquitylating enzyme that we associated with neurodevelopmental disorders (NDDs) primarily in females. *USP9X* escapes X-inactivation, and in females *de novo* heterozygous copy number loss or truncating mutations cause haploinsufficiency culminating in a recognisable syndrome with intellectual disability (ID), signature brain and congenital abnormalities. In contrast, the involvement of *USP9X* in male NDDs remained tentative. We discovered and interrogated the pathogenicity of 44 male-ascertained *USP9X* variants associated with NDDs. Twelve missense variants were classified as pathogenic using clinically recommended guidelines, and *in silico* and phenotypical features align additional variants of unknown significance with pathogenicity. We define a characteristic phenotype of the CNS (white matter disturbances, thin corpus callosum and widened ventricles), and global delay with significant alteration of speech, language, movement and behaviour. We used patient derived cell lines to show variants disrupted the ability of USP9X to regulate a specific subset of substrates which regulate neurodevelopmental signalling pathways, including TGFβ, mTOR and Wnt. We show these same substrates and pathways are disrupted in *Usp9x* brain-specific knockout mice, and drive defects in neural stem cell function, and axon growth. Furthermore we resolve the presence of cortical malformations, and learning and memory defects which are hallmarks of the human phenotype. Our data thus align *USP9X* variants with a distinctive neurodevelopmental syndrome in males and identify plausible mechanisms of pathogenesis centred on disrupted neurodevelopmental signalling and cortical development and function.

id #12011

The effect of low-intensity repetitive Transcranial Magnetic Stimulation delivered using standard and accelerated stimulation protocols on brain and behaviour in an animal model of depression.

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Daily, repetitive transcranial magnetic stimulation (rTMS) at 10 Hz is widely used clinically for treatment-resistant depression. The related time burden for patients is a major disadvantage of current clinical rTMS protocols. Emerging, clinical research suggests accelerated protocols (rTMS sessions delivered 3 or more times throughout the day as opposed to once-daily) may provide the same outcomes more rapidly. Here we characterise neurobiological and behavioural changes in a rat model of depression after standard or accelerated low-intensity rTMS (LI-rTMS). Depression was induced in rats (n=5 per group) using a chronic restraint stress paradigm, followed by rTMS daily for 4 weeks (standard) or three times daily for 2 weeks (accelerated). Multiple MRI techniques were used to detect functional, chemical, and structural changes in the brain, and Forced Swim Test (FST) and Elevated Plus Maze (EPM) quantified depression-like behaviours. Rats were tested at baseline, after restraint and midway through rTMS treatment. After restraint, there was a significant decrease in hippocampal volume, changes within several resting-state networks, and decrease in sensorimotor cortical glutamate and glutamine. In addition, FST showed a significant increase in learned-helplessness and EPM showed a significant decrease in exploration of centre and open arms (p<0.05). Depression-like behaviours responded to accelerated, but not standard LI-rTMS (p<0.05 compared to sham) in FST, and exploration increased (but p>0.05) in EPM. Our findings characterise changes in the rat brain that are associated with depression-like behaviours induced by restraint, and provide evidence to support the use of accelerated rTMS protocols to treat depression in human patients.

id #12024

Enhanced sensory coding in mouse vibrissal and visual cortex through TRPA1

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Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel, which is broadly expressed throughout the body. We recently demonstrated the expression of TRPA1 in rodent cortex, and its functional activation by agonists *in vitro*. In order to understand the contribution of TRPA1 to sensory processing, here we performed *in vivo* loose-patch recording and 2-photon calcium imaging from two sensory areas in mice: (i) the primary vibrissal somatosensory cortex (vS1) and (ii) the primary visual cortex (V1). We

characterised the neuronal response function to vibrotactile stimuli (6 different amplitudes) and visual stimuli (gratings drifting in 16 directions) under local infusion of artificial cerebrospinal fluid (aCSF), TRPA1 agonist (AITC) or antagonist (HC-030031). In vS1, local activation of TRPA1 by its agonist AITC significantly increased the spontaneous ongoing activity of cortical neurons, their evoked response to vibrissal stimulation, and their response range consistent with a positive gain modulation. TRPA1 inhibition with HC-030031 reversed these modulations to below initial control gains. The gain modulations were absent in TRPA1 Knockout (KO) mice. In V1, TRPA1 activation increased the gain of direction selectivity similarly to the gain modulations observed in vS1 cortex. Multivariate pattern analysis revealed that TRPA1 activation increased and its suppression reduced coding efficacy in V1 population activity. Overall, our findings suggest a physiological role for TRPA1 in the processing of sensory signals in the mammalian cortex.

id #12027

Bioengineering a cell transplantation therapy to repair the injured spinal cord

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Around 300 incidents of traumatic spinal cord injury (SCI) occur in Australia each year, resulting in permanent paralysis with loss of motor, sensory and autonomic function. Some 15,000 Australians are living with chronic SCI and with modern health care, people living with SCI have near-normal life expectancies. Consequently the socio-economic impact of the injury is very high. In 2009, an Access Economics report calculated the annual burden of SCI for Australia to be over $2 billion; costs in 2019 are likely to be much higher. Olfactory ensheathing cell transplantation has been demonstrated by various teams around the world to be effective in partially repairing acute and chronic SCI, yet the results have been variable. We have adopted a translational approach in which we have identified and solved key bottlenecks in the cell transplantation therapy. In particular, we have used our deep fundamental knowledge of the biology of the olfactory system to improve the cell purification and identification process in order to generate high quality, consistent cell preparations. We have also invented a new technology to produce nerve bridges of various dimensions suitable for neural repair. The transplantation of the high-quality nerve bridges into the injured spinal cord of rodents results in significantly improved cell survival and integration, with excellent axon regeneration over the injury site. We now anticipate progressing to a Phase I human clinical trial in 2020. This project demonstrates that a focussed translational bioengineering research program can fast-track development of a cell transplantation therapy.

id #12067

Thalamic Shape and Cognitive Performance in Amnestic Mild Cognitive Impairment

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Objective: This study aimed to investigate thalamic shape alterations and their relationships with various episodic memory impairments in subjects with amnestic mild cognitive impairment (aMCI).

Methods: We compared volumes and morphological alterations of the thalamus between aMCI subjects and healthy controls. In addition, we investigated the correlation between thalamic deformations and various memory impairments in aMCI subjects using a comprehensive neuropsychological battery.

Results: The normalized left thalamic volumes of the aMCI group were significantly smaller than those of the healthy control group ($p < 0.0001$). aMCI subjects exhibited significant thalamic deformations in the left thalamic dorso-medial and antero-medial areas compared with healthy individuals. CERAD-K Word List Memory scores were significantly correlated with the left dorso-medial areas in aMCI subjects. There were no significant correlations between verbal fluency, Boston naming test, constructional praxis, Word List Recognition, and Visuospatial Recall scores and thalamic shape in aMCI subjects. Verbal delayed recall scores were also significantly correlated with the left dorso-medial areas in the aMCI group.

Conclusion: Structural alterations in the thalamic deformations in the left dorso-medial and antero-medial areas might be core underlying neurobiological mechanisms of thalamic dysfunction related to Word List Memory and delayed verbal recall in individuals with aMCI.

id #12106

Influence of Maternal High Zinc Diet on the Development of Autism-associated Behaviours in Shank3-Knockout Mice

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Autism Spectrum Disorders (ASDs) are characterised by deficits in social interactions and repetitive behaviours. Many ASD-associated mutations occur in the Shank family of synaptic proteins resulting in weakened synapse function. Zinc deficiency is a risk factor in ASD, and low zinc levels have been found in autistic children. Zinc supplementation enhances Shank protein stability and recruitment to glutamatergic synapses, enhancing synapse function. Shank deletion and zinc deficiency results in presentation of ASD-associated behaviours in mice. We therefore aimed to determine whether dietary zinc supplementation during pregnancy and lactation could prevent the development of ASD-associated behaviours in Shank3-knockout (KO) offspring. Social interaction (three-chamber test), repetitive grooming (cylinder test), and anxiety (light-dark emergence test) were assessed in three, nine, and 16 week old Shank3-wildtype and Shank3-KO mice born from mothers fed control or supplemented zinc diet. Supplemented maternal zinc diet normalised sociability and social novelty recognition in juvenile, adolescent and adult Shank3-KO mice. Anxiety behaviours, such as increased latency to enter bright arena and decreased time spent in bright arena were also prevented by supplemented maternal dietary

zinc. Contrastingly, repetitive grooming was not prevented in adolescence but only in adulthood by maternal zinc supplementation. Our data show that zinc supplementation from the beginning of brain development can result in behavioural benefits persisting into adulthood in Shank3-KO mice, and that maternal zinc supplementation has the potential to prevent ASD-associated deficits in Shank3-KO ASD mice.

id #12117

Brain Insulin Resistance in Alzheimer's disease: Targeting phosphoinositide 3-kinase (PI3K)/Akt/GSK-3β pathway

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ICV-STZ was used for the model of sporadic Alzheimer's disease being established. Adult male Wistar rats (48) weighing 200-300 g were used. Animals were randomly divided into 8 groups comprising 6 animals in each group as follows:

Protocol lasted for 21 days, sacrificing animals on 22nd day followed by isolation of serum and dissection of cortex and hippocampus.

Behavioral studies like Morris water maze was done for assessing spatial memory, novel object recognition for associative memory and actophotometer was performed for locomotor activity.

Biochemical estimations for antioxidant activity or oxidative stress such as reduced glutathione estimation, superoxide dismutase assay, catalase assay, glutathione peroxidase assay, myeloperoxidase assay, glutathione S-transferase assay, lipid peroxidation assay, and protein carbonylation assay were performed in the homogenates of cortex and hippocampus of the brain. For nitrosative stress, nitrite estimation was done. Protein concentrations were determined by the biuret method. Cholinergic activity was evaluated by acetylcholinesterase assay to assess the cholinergic dysfunction which is one of the core pathologies of dementia and AD.

Inflammatory cytokines like TNF-α, IL-6 was determined by ELISA method to evaluate the neuroinflammation which is aggravated by insulin resistance. C-reactive protein, a marker of neuroinflammation and neurodegeneration was also determined by ELISA.

Mitochondrial dysfunction was evaluated estimating mitochondrial enzyme complex-I, II, III, IV depicting picture of viable and non-viable neuronal cells.

Histopathology was done by H&E staining to find out apoptotic cells, neuroinflammation, and neurodegeneration.

Molecular technique like RT-PCR for IRS-1, PI3-K, AKT, GSK 3-β and BDNF was performed for gene expression analysis.

id #12120

Mapping wired and wireless neural connectivity to investigate behavioural and pathological states in *C. elegans*

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Can mapping neural connectivity tell us how the brain works? A major goal of global brain research initiatives is the expensive and labour-intensive mapping of neural networks. Much of this focuses on mapping synaptic connections between neurons. However, a considerable amount of neuronal communication occurs via neuromodulators such as neuropeptides and monoamines, which can act outside synapses. Neuromodulator-dependent signalling clearly drives important behaviours. During my postdoc at the Laboratory of Molecular Biology, Cambridge, I explored the functions of neuropeptide networks and circuits in *C. elegans*, using automated behavioural tracking, optogenetics, microfluidics-based calcium-imaging, and high-content phenotyping. We showed that locomotor and sensory sensitisation during behavioural arousal in response to aversive touch occurs in a two-step process of neuropeptide signalling: afferent neuropeptides first convey mechanosensory information from sensory neurons to central interneurons, and these neuroendocrine centres then release efferent signals to convey behavioural state information to the periphery. Importantly, these peripheral neurons include pain-sensing cells, or nociceptors, that become sensitised in response to repeated stimulation. Future work could exploit this system as a model to understand the principles through which neuropeptide signalling networks interact with synaptic connections to control behavioural states - or to trigger pathological states such as nociceptor sensitisation, a key factor in chronic pain. This work presents the exciting possibility of using the worm as a fully-described prototype for multilayer neuronal connections in bigger brains, generating a uniquely powerful experimental system for studies of learning, memory, behaviour and neuropathology.

id #12126

Cholesterol-related gene is associated with longitudinal cognitive decline in an Australian cohort of Parkinson's disease patients

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Non-motor symptoms are debilitating features of Parkinson's disease (PD). Although patients with PD (PwP) experience greater incidence of cognitive decline than healthy people, the underlying cause is unknown. Genetic variability may explain susceptibility to cognitive impairment and help identification of individuals at risk. While a relationship between cholesterol-related genes and Alzheimer's disease is well established, there are limited longitudinal studies examining these genetic variants within cognitive decline in PwP. This study investigated the relationships between various genetic variants, PD disease risk and cognitive decline in an Australian PD cohort. Participants were assessed at the Perron Institute's Movement Disorders Clinic (200 PD, 100 control), and were genotyped for single-nucleotide polymorphisms in *APOE, CYP46A1* (rs754203), *ABCA1* (rs2230806)*, and *CLU* (rs11136000) by Sanger Sequencing. Cognitive function was assessed using the MMSE and ACE-R in a subset of PwP, at baseline and 60-months (n=50). The relationships between these variants, PD risk (Pearson's chi-square test of contingencies) and percentage of cognitive decline over 60-months (Kruskal-Wallis ANOVA and generalized linear models) were investigated. The *ABCA1* variant was significantly associated with PD risk ($p<.05$). Additionally, the relationship between *ABCA1* and cognitive decline (assessed by MMSE) approached significance ($p=.062$). This finding was explored using more comprehensive assessment (ACE-R), revealing significant differences between *ABCA1* and cognitive decline over time ($p=.022$). Finally, multivariate models revealed associations between variants and progression to cognitive impairment, while controlling for co-variates. This longitudinal study aids in assessing the genetic contribution to cognitive phenotypic variability within PD.

id #12131

Rapid, unbiased identification of protein inclusion components from patient post-mortem brain tissue using targeted-biotinylation and mass spectrometry.

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Many neurodegenerative diseases are characterised by the formation of insoluble protein inclusions in the brains of affected patients [1]. The composition of these aggregates from patient post-mortem tissue has provided invaluable insight into the mechanisms that lead to disease however, the insolubility of aggregate components limits the use of standard antibody-based approaches frequently used to study protein-protein interactions. A recently developed proximity-ligation method enables identification of insoluble interactomes from fixed, post-mortem tissue [2].

Here, we apply Biotinylation by Antibody Recognition (BAR) followed by mass spectrometry to identify the composition of phospho-Tau aggregates found in Progressive supranuclear palsy (PSP) patients. BAR is a recently developed method, by which a primary antibody recognises the target of interest in fixed samples. A secondary antibody, conjugated to horseradish peroxidase, recognises the primary antibody and facilitates the rapid deposition of biotin onto proteins within the vicinity of the antibody complex [2]. Biotinylated proteins are subsequently identified following reverse cross-linking, streptavidin-conjugated bead pull-down and mass spectrometry.

Using BAR in fixed, post-mortem brain tissue from PSP patients, we identified several known aggregate components found in Tauopathies. Our data also identified confidently

assigned phosphorylation sites on aggregated Tau. Together these data validate our approach for rapidly revealing the aggregate components of Tauopathies whilst also identifying many novel components that may provide valuable insight into disease mechanisms upon careful validation. Overall, this methodology should be broadly applicable for rapid and unbiased identification of aggregate components directly from the post-mortem tissue of patients with neurodegenerative diseases.

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id #12141

ADAPTIVE MYELINATION REGULATED BY CHANGES TO OLIGODENDROCYTE SURVIVAL

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Myelin addition in response to neural activity (adaptive myelination) facilitates learning and regulates neural plasticity. However, it is unclear how the myelin-forming cells (oligodendrocytes, OLs) are added to our nervous system in response to neural activity and their longevity as we age.

We investigated the production of OLs in the murine prefrontal cortex (PFC) and corpus callosum (CC) following periods of sensory enrichment, sensory deprivation (social isolation) or regular housing during juvenile development (P21-P35; n=5-6/condition). Through combining Double-S phase BrdU labelling and stereology together with mathematical modelling, we measured oligodendrocyte precursor cell proliferation rates and changes in total OL numbers, and their growth for each housing group compared to integrated OL numbers at the end of the exposure period. This multi-disciplinary approach enables us to estimate OL death– a readout not achieved with any other technique to date.

Counterintuitively, both enrichment and isolation increase daily OL production relative to regular-housed controls in the PFC (p=0.0008 and p=0.0010, respectively). Survival rates, however, vastly differed between housing groups and only in the enriched condition was production greater than death (p<0.0001), enabling retention of OLs. In contrast, preliminary CC data reveals cell number is unaffected, suggesting changes in circuit activity evoke regionally-specific changes to OL development.

Together, our data reveal that production of new OLs is uncoupled from successful cellular integration, and ultimately the level of survival dictates total oligodendrocyte number in a circuit- or region-specific manner. Targeting survival of newly-generated oligodendrocytes may have important implications for promoting myelination and neural plasticity.

id #12144

Does APOE genotype moderate the relationship between physical activity and brain health and dementia risk?

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Physical activity (PA) has been consistently associated with improvements in cognitive performance and reduced risk of cognitive decline and Alzheimer's disease (AD). Nevertheless, available data is inconclusive with respect to how genetic risk for AD, defined as apolipoprotein (APOE) ε4 allele carriage, modulates the relationship between PA and markers associated with AD. To date, most studies claim that the protective effect of PA predominantly manifests among ε4 carriers. On the contrary, some studies reveal that PA-induced benefits to the brain exist only in ε4 non-carriers. However, to date, no systematic analysis of this topic has been undertaken. To address this gap in the literature, we have carried out a systematic review (including cross-sectional and longitudinal studies, and randomised controlled trials) to evaluate the moderating effect of APOE ε4 carriage on the relationship between PA and AD markers (such as cognition, tau and amyloid-beta burden, grey matter volumes and white matter integrity). In conclusion, PA exerts beneficial effects on both ε4 carriers and non-carriers, although it might do so via different mechanisms and in different populations. Greater engagement in PA impacts brain structure to a greater extent among ε4 carriers. On the other hand, AD conversion is reduced with increased PA mainly (but not only) in ε4 non-carriers, especially when the age range is higher. The mechanisms underlying this effect require further investigation to identify whether genotype effects are due to biological effects of genetic carriage, or are a consequence of phenotypic traits (such as increased risk of cognitive decline).

id #12145

USING SERIAL BLOCK FACE SCANNING ELECTRON MICROSCOPY TO INVESTIGATE LONG-TERM EFFECTS OF BINGE ALCOHOL EXPOSURE

DURING DEVELOPMENT ON MATURE BRAIN STRUCTURE IN THE RAT: PRELIMINARY FINDINGS.

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Animal studies have shown that some brain regions exhibit permanent neuronal loss that is accompanied by functional deficits, following binge-like exposure to alcohol during development. This study investigated ultrastructural aspects of spiny dendrites in cingulate cortex layer 2 neurons, cerebellar Purkinje neurons and CA1 pyramidal neurons. Tissue from adult Long-Evans rats, exposed to either alcohol (E) or a sham intubation (IC) on postnatal day 6 were perfused and brain tissue was processed for serial block-face scanning electron microscopy (SBF-SEM). Preliminary data found the total number of dendritic spines per µm of CA1 pyramidal cell dendrite in the Schaffer collateral zone, was significantly less in E (1.70 ± 0.09) (mean ± s.d.) than IC animals (3.57 ± 0.29) (p=0.0001). There were significantly less spine-synapses in E than IC but dendritic shaft synapse number was not altered [F (3, 24) = 61.34, p<0.0001]. There were no significant differences in the spine number on the spiny dendrites of Purkinje neurons or layer 2 cingulate cortex neurons. Our results indicate that a single binge drinking episode during late pregnancy may result in long-term structural changes that are location dependent. Importantly, such changes may underlie functional deficits due to the disruption of the network structure. Three-dimensional reconstruction using SBF-SEM data, is a relatively new and efficient method that can be used to assess how brain synaptic connectivity is altered following developmental cell loss.

id #12147

Patterns of conversion and reversion between cognitive states (normal, MCI and dementia) in Parkinson's disease: a systematic review and meta-analysis

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We aimed to systematically review and meta-analyse conversion rates from normal cognition to Mild Cognitive Impairment (MCI) and dementia in Parkinson's disease (PD) patients; reversion rates in patients with MCI (i.e. PD-MCI) were also investigated. Electronic searches of PsycINFO, Medline and EBSCOhost were conducted in January 2018, with 1833 articles identified after duplicate removal. Articles were included if they assessed conversion/reversion in PD patients between normal cognition, PD-MCI and PD dementia (PD-D). In total, 39 articles met the inclusion criteria, representing 4011 patients (mean age range 58–75; 61% male). Within three years, in those with PD and normal cognition, 25% (95%CI 20–30%) converted to PD-MCI and 2% (95%CI 1–7%) converted to dementia. Of those with PD-MCI, 20% (95%CI 13–30%) converted to dementia while 28% (95%CI 20–37%) reverted back to a state of normal cognitive function. The conversion rates to MCI and

dementia were higher, and reversion rates lower, when follow-up was ≥3 years. When International Parkinson and Movement Disorder Society (MDS) criteria were used to diagnose MCI, Level I criteria were associated with a greater reversion estimate from PD-MCI to normal cognitive function. These findings summarise the trajectory of cognitive impairment in PD and highlight that MCI is common in this patient group. Understanding cognitive trajectories in PD patients is important for patient care in terms of prognosis, as well as for identifying windows for intervention for cognitive symptoms. As the number of PD patients increases with an ageing population, this information can inform future policy and planning.

id #12149

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.

id #12159

Studying the role of Kv7.2 and Kv7.3 ion channels in the mouse enteric nervous system with the assistance of computational modelling

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The neurons in the enteric nervous system (ENS) of the gut regulate gastrointestinal functions. These neurons have differing firing patterns attributable to interactions between various membrane ion channels. Therefore, enteric neurons with distinct ion channel expression are largely responsible for colonic motility patterns, but it is unclear which ion channel interactions produce different firing properties, and their effects on colonic motility. Here, we studied the involvement of Kv7.2 and Kv7.3 channels in enteric neuronal firing and colonic motility using a multipronged approach including immunohistochemistry, functional studies, and computational modelling. Immunohistochemical analysis showed that virtually all enteric neurons express Kv7.2 (99.03%) and Kv7.3 channel (98.51%). Computational modelling using a compartmental model of enteric neurons revealed that Kv7.2 and Kv7.3 channels limit firing to the start of an imposed depolarization. Functional analysis of colonic motility in vitro showed that blocking Kv7.2/Kv7.3 channels using a specific antagonist ML252 (0.1 μM) did not alter the frequency of contractions initiated in proximal colon (oral contractions) but reduces contractions initiated in distal colon (anal contractions) (n=9, p=0.016). Activating Kv7.2/Kv7.3 channels with an agonist ML213 (0.03 μM) decreased both oral and anal contractions (n=7, p=0.008 for both), as well as contraction propagation length (p=0.013). In combination, ML252 (0.1 μM) and ML213 (0.03 μM) only decreased anal contractions (n=8, p=0.008). A higher concentration of ML213 (0.3 μM) abolished colonic contractions (n=8, p=0.016) without changing colonic diameter. These findings demonstrate that Kv7.2/Kv7.3 channels on enteric neurons are important for the regulation of colonic activities and motor complexes.

id #12160

Regional iron distribution and soluble ferroprotein profiles in the healthy human brain

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Iron is essential for brain development and health where its redox properties are used for a number of neurological processes. However, iron is also a major driver of oxidative stress if not properly controlled. Iron distribution in the brain is highly compartmentalised and regulated by a number of proteins and small biomolecules. Here, we examine heterogeneity in regional iron levels in 10 anatomical structures from seven *post mortem* human brains with no apparent neuropathology. Putamen contained the highest levels, and most case-to-case variability, of iron compared with the other nine regions examined. Partitioning of iron between cytosolic and membrane-bound iron was generally consistent in each region, with a slightly higher proportion (55%) in the 'insoluble' phase. Using the Allen Human Brain Atlas, we examined patterns between iron levels and transcriptomic expression of primary iron regulatory proteins, and used quantitative size exclusion chromatography-inductively coupled plasma-mass spectrometry to assess regional differences in the molecular masses to which iron predominantly binds. Of the total soluble iron bound to proteins, approximately

60% was associated with ferritin, equating to approximately 25% of total tissue iron essentially in storage.

id #12164

Characterisation of Forkhead Protein (Foxp1) expressing cells in the Marmoset Retina

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Purpose: The retinal ganglion cells (RGC) serving high acuity (central) vision named midget and parasol cells are well characterized. In contrast, the number and types of ganglion cell serving the peripheral visual field are not well understood. The present study aims to use molecular markers to identify and characterize low-density ganglion cells in the retina of the common marmoset (*Callithrix jacchus*). **Methods:** Four post-mortem marmoset eyes were fixed in 4% paraformaldehyde. Retinal quadrants were processed with antibodies against the transcription factor Forkhead Protein (Foxp1) and the ganglion cell marker RNA binding protein with multiple splicing (RBPMS). Other retinal whole quadrants were immuno-labelled with Foxp1 antibodies and labelled somas were intracellular injected with the lipophilic dye DiI (n=12 cells). **Results:** Foxp1-cells included ganglion cells and amacrine cells. Foxp1-positive RGCs make up an average of 1.61% (329/16537 cells) of the total ganglion cell population in peripheral retina. Within an eccentricity range of 2 to 12mm, Foxp1 ganglion cells show a peak density of 110 cells/mm^2 at 2.2 mm which falls to less than 20 cells/mm^2 at around 6.5mm. The injected cells include two types of wide-field ganglion cells i.e. broad thorny (n=8) and tufted cells (n=2). In addition two displaced amacrine cells were injected. The dendritic field diameters range from 240µm to 360µm for the two cell types. **Conclusion:** Foxp1 transcription factor is expressed in a subset of low-density RGCs in the periphery of the marmoset retina.

id #12166

Cognitive Load During Multitasking Can Be Accurately Assessed Based on a Single Channel EEG Recording

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Multitasking related mental workload can be estimated based on multichannel dry electroenchephalography (EEG) by using correlation or principal component analysis (PCA) methods (Lim et al. 2018). However, it is not known whether cognitive load can be assessed by using only one EEG channel. We hypothesize that this is possible and investigate validity of this hypothesis by estimating cognitive workload based on single channel wireless EEG.

14 channel EEG recordings (sampling rate=128 Hz) of 40 subjects performing the Simultaneous Capacity Test (Acquired from an open access database, Lim et al. 2018) were analysed using oblique visibility graph (OVG) methods. Each multitasking test was labeled to have either low or high cognitive load. Three graph features: average degree, clustering coefficient (*CC*), and average shortest distance (*L*), from EEG recordings were forwarded into a support vector machine to conduct the cognitive load classification. Finally, the channel with the highest accuracy was selected as the optimal channel by compared with PCA method.

Based on the present results, F8 channel was found optimal for estimation of cognitive load. *CC* during low cognitive load were significantly lower than those associated with high cognitive load ($p<0.01$). Conversely, the *L* values related to low cognitive load were slightly higher than those related to high cognitive load. By using graph features the accuracy of identifying high mental load was 85%, which is higher than 69% of PCA. The present results indicate that mental workload associated with multitasking can be accurately evaluated based on single optimally selected EEG channel.

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id #12167

Long term high-fat diet aggravates anxiety, depression and cognitive dysfunction in pR5 mice

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Alzheimer's disease (AD) is highly associated with type 2 diabetes mellitus (T2DM) and it has been postulated as "type 3 diabetes". Dietary fat intake is associated with obesity which is a driving force for T2DM. To investigate the influence of diet induced obesity on AD phenotypes, we fed high fat diet (HFD) to pR5 mice expressing P301L mutant human tau. pR5 and C57BL/6 (WT) mice were randomly allocated to a standard diet (STD) or HFD for 30 weeks starting at 8-weeks of age. The bodyweight, food intake and fasting glucose levels were measured every one or two weeks.A comprehensive behavioral test battery was performed to assess anxiety, depression and cognitive dysfunction. Also,glucose and insulin tolerance tests were performed after 30 weeks of HFD before the animals were humanely culled.pR5 mice fed with HFD were vulnerable to diet induced obesity compared to C57BL/6 (WT) mice especially with increasing age.Also, pR5 mice on HFD showed aggravated hyperglycemia, glucose intolerance and insulin resistance after 30 weeks of HFD compared to WT STD, WT HFD and pR5 STD groups. Furthermore, long term HFD caused anxiety-like behavior in pR5 and WT mice which was not observed in the STD groups, and significantly aggravated depression-like behavior and impaired cognitive function in pR5 mice. These results indicate that HFD induced obesity was associated with mood and cognitive dysfunction, and derangements in glucose homeostasis in WT mice, which were augmented in pR5 mice.

id #12168

Relationship between plasma Aβ and structural brain alterations: A systematic review

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In the present systematic review we investigated the relation between plasma Aβ and brain alterations examined with structural MRI technique. Pubmed, Scopus, Ovid, and EBSCo were searched from January, 2014 till February, 2019. Different combinations of keywords regarding plasma Aβ and structural brain alterations in patients with AD and dementia were undertaken. Additional search was performed through a hand-search of the reference lists of included articles. Two independent researchers assessed and reviewed eligible articles for risk

of bias according to the modified Newcastle-Ottawa Scale. Five articles were identified to meet the inclusion criteria. All eligible studies were case-control studies. Some evidence was found for the association between plasma Aβ42 and atrophy of the precuneus and amygdala. In addition some evidence was found for the association between Aβ40 and atrophy of the (1) whole brain, (2) entorhinal volume/thickness, (3) precuneus and amygdala; and moderate evidence for the association between Aβ40 and atrophy of the hippocampus. However, inconclusive evidence exists regarding the direction of change of Aβ40 levels and hippocampal volume. Very few studies were available. Despite this limitation, there is some evidence that plasma Aβ 42 is associated with precuneus and amygdala. Also, some to moderate evidence showed an association of Aβ 40 with whole brain, entorhinal volume/thickness, precuneus and amygdala. However, inconclusive evidence exists regarding the direction of change of Aβ40 levels and its relation with hippocampal volume. More studies are required to fill this gap in order to be able to draw a conclusive message.

id #12169

Brain atrophy and its relationship with brain amyloid deposition, tau levels, and cerebral glucose metabolism in individuals at risk of cognitive decline

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There are mixed evidence on the relationship between Aβ and brain volumes in elderly subjects with or without cognitive impairment. The present review summarizes the available evidence on brain atrophy on MRI and its relation with PET evidence of amyloid deposition, tau levels, and cerebral glucose metabolism. PubMed and Scopus databases were search from 1 January 2018 to 22nd May, 2019. The following search terms were keyed in varying combinations with "brain atrophy" and "cognitive function" to identify relevant articles: MRI" and "magnetic resonance imaging " and "PET" and "positron emission tomography". Reference lists of included articles were hand-searched for additional literature. Eligible articles were assessed on risk of bias and reviewed by two independent researchers. The search query yielded 27 articles meeting the inclusion criteria. There were 16 amyloid PET studies (8 were case control), 4 FDG-PET studies (3 were case control), and 7 tau PET studies (4 were case control). Increased evidence showed that decreased volumes of gray matter, hippocampus, amygdala, temporal and increased ventricular volumes are associated with increased Pittsburgh Compound B uptake. Decreased metabolism is associated with GM atrophy; and increased tau uptake is associated with decreased GM volume. This study

demonstrates that the combination of structural MRI for evidence of brain atrophy with PET may help provide potentially useful multimodal-based biomarkers for early identification of individuals who may be at risk of AD, prediction of converters from normal to MCI and from MCI to AD, and therapeutic interventions.

id #12170

The role of ON/OFF sub-regions of visual cortical simple cells in sharpening orientation selectivity.

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The mechanism underlying the sharp orientation selectivity in the primary visual (striate) cortex has been highly debated. One of the factors that is said to underlie the orientation selectivity of a simple cell is the interaction between its various sub-regions. In this project, we probed the contribution of ON and OFF sub-regions of receptive fields to the orientation selectivity of neurons.In anaesthetised cats, we recorded the response of layer 4 simple cells to light and dark bars of different orientations. We related the orientation selectivity (circular variance, CV) of simple cells to the relative contributions of the ON and OFF sub-regions to the response. The latter was quantified using a Bimodality index (BI; value of 1 meaning ON and OFF sub-regions firing with equal strength). We hypothesised that if ON and OFF inputs to neurons contributed to sharpening of the orientation selectivity, neurons that showed a higher BI would show sharper orientation selectivity.We found that there was no significant relationship between the bimodality index and the circular variance (n=47; ρ=0.1783; p>0.2306).Our results suggest that the ON and OFF sub-regions of a neuron do not play a significant role in sharpening of orientation selectivity, though one cannot exclude the possibility that they may contribute significantly to orientation selectivity in some cells. Other mechanisms such as sub-cortical orientation biases and intracortical inhibition are likely to play more prominent roles in the generation of sharp orientation selectivity in the striate cortex.

id #12171

The transcription factor 'Olig2' recruits chromatin remodelling complexes to regulate proliferation and myelination in oligodendrocytes.

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Myelination of the CNS occurs throughout life. Self-renewing oligodendrocyte lineage precursors (OLPs) residing in the CNS are responsible for this long-term myelination, whereby they exit cell cycle and differentiate into myelinating oligodendrocytes (OLs). The transcription factor 'Olig2' is expressed throughout the OL lineage and has a multifaceted role at different stages of OL development. It has been shown to have a critical role in both specification of OLPs and differentiation into OLs. We and others have demonstrated that Olig2 can alter its protein binding partners in a stage-specific manner which results in changes to the fate of the cell. Interestingly, Olig2 has been shown to interact with 2 classes of chromatin remodelling complexes, the BAF and NuRD complex. These complexes are involved in organising and restructuring nucleosomes, facilitating the silencing and activation of genes. Does Olig2 interact with other chromatin remodelling complexes? We aimed to address this question using mass spectrometry. By immuno-precipitating Olig2 from OLs, we showed that Olig2 interacts with four chromatin remodelling complexes – the BAF and NuRD complexes (previously shown to regulate OP differentiation) and the ISWI and INO80 complexes. Conditional deletion of Ino80 from OPs resulted in reduced cell proliferation. Further analysis revealed that this deficit was due a slower cell cycle rate. Interestingly, Ino80 ablation didn't affect OP differentiation. These data indicates that Olig2 regulates the nucleosome landscape through recruiting chromatin remodelling complexes. Recruitment of Ino80 is necessary for correct OP proliferation whereas recruitment of BAF and NuRD later in the lineage drives myelination.

id #12178

Categorical and invariant visual object capabilities of the superior colliculus – a threat detection centre of the brain.

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The superior colliculus (SC) is a sensory integration hub in the dorsal brainstem where multimodal information is combined and, depending on the saliency of the competing sensory inputs, appropriate motor commands and supportive autonomic changes initiated. In rodents, the SC is indispensable for initiating behavioural responses to stereotypical visual stimuli that resemble approaching objects, such as looming (an expanding overhead black circle). In pilot experiments, we found that presentation of overhead looming stimuli drove acute surges in blood pressure in telemetered conscious rats. Surprisingly, equivalent autonomic responses could be driven by more complex visual stimuli (e.g. snakes) but not by control stimulations. We hypothesized that the encoding capabilities of the SC might extend beyond detection of stereotypical approach conventionally attributed to the region, and that complex naturalistic shapes could be detected by SC circuits based on a saliency-map of behaviourally relevant cues important for survival (from appetitive to threatening). To investigate this idea, we made extracellular single-unit recordings from a population of SC neurons in anaesthetised rats and used machine-learning based approaches to decode the visual cues presented, clustering stimuli according to the similarity of population responses. We report categorical and invariant visual object capabilities of SC microcircuits that differ by subregion and are several orders of magnitude more complex than previously recognised.

Furthermore, the addition of granular noise caused a progressive, level-dependant attenuation of these responses. Our data suggest that the SC is capable of nuanced object recognition, suggesting mechanisms through which complex visual cues can initiate defence manoeuvres.

id #12179

A Novel role of Extracellular Ferritin in Ferroptosis: Implications for Neurodegenerative Diseases

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Ferroptosis is an iron-dependent form of regulated cell death that has been implicated in various neurodegenerative diseases. Ferroptosis involves the degradation of cytosolic ferritin, the iron storage protein of cells. Ferritin is also present in cerebrospinal fluid (CSF), where it has been shown to be associated with Alzheimer's disease progression, however, the function of extracellular ferritin is not known. Here, by using our established *in vitro* neuronal model of ferroptosis, we report that extracellular ferritin can act both to promote and inhibit ferroptosis in neurons, which depends on its isoform and the extent of iron loading. L-ferritin did not have an effect on ferroptosis, whereas, endocytosis of iron-poor H-ferritin protected neurons against ferroptosis and lipid peroxidation. Circulating ferritin species may modify the vulnerability of cells and tissues to ferroptosis. These findings bring a new perspective into iron neurobiology and pathophysiology in neurodegenerative diseases.

id #12183

Cellular Changes in the Substantia Nigra and Subthalamic Nucleus in Parkinson Disease pathology and Deep Brain Stimulation Treatment

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Parkinson's Disease (PD) is a progressive neurodegenerative disorder pathologically hallmarked by the loss of dopamine neurons in the substantia nigra (SN) and expression of alpha- synuclein-positive Lewy bodies and neurites. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is used to treat the motor symptoms, although the precise mechanism of therapeutic action is currently unknown and little research exists regarding STN cellular changes in PD. This study investigated cellular changes in the SN and STN in paraffin-embedded brain tissue from patients with PD, PD with STN DBS (STN-DBS) and age-matched controls. Brain sections were stained with markers for neurons (TH and NeuN), glia (GFAP and Iba1) and pathology- specific (alpha-synuclein and NLRP3) antibodies. Changes were assessed using quantitative and semi-quantitative microscopy techniques. This study confirms previous findings showing significant neuronal loss ($p<0.001$) and an increase in neuroinflammation ($p<0.018$) in the SN in PD patients compared to controls. STN-DBS did not alter neuronal loss or neuroinflammation in the SN ($p>0.639$). Remarkably, the STN showed no significant

changes in these markers in PD, despite equivalent alpha-synuclein pathology in this region (STN versus SN p>0.432). This research sheds light upon cellular changes in the STN in human PD, an understanding previously limited. The presence of alpha-synuclein in the STN in the absence of neuronal loss and neuroinflammation adds support to the neuroprotective theory of Lewy body formation. Further, this study illustrates that the mechanism whereby DBS exerts its therapeutic effects is complex and likely to work independently of the cellular changes examined.

id #12184

Sex dependent effects on pre-frontal cortex development and executive function following a mild traumatic brain injury in adolescence

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Adolescents are more likely to develop chronic symptoms such as impulsivity and difficulty concentrating following a mild traumatic brain injury (mTBI) than adults. This may be due to disruption of pre-frontal cortex (PFC) development. Dopaminergic neurons grow into the PFC until early adulthood, allowing maturation of executive functions including attention, motivation and impulse control. The effects of mTBI in mid-adolescence on male and female Sprague Dawley rats on executive function in adulthood (12 weeks) were examined via the 5-choice serial reaction task. Animals learn to respond to a stimulus (light) in one of five potential locations for a reward. Difficulty was increased by decreasing stimulus time. Animals were injured in mid-adolescence (p35: n=12-16 per group) via weight drop (100g from 0.75m) and injury confirmed by a significant increase in righting time. Previous mTBI in females led to significantly higher omissions and decreased accuracy in 5-CSRT when task difficulty was high (stimulus duration 1s), whilst males had significantly increased premature response rate and preservation when task difficulty was low (stimulus duration 4s). Levels of TH, a reflection of dopaminergic innervation, were no different in either sex post-TBI in the PFC, but a significant increase in the limbic system (nucleus accumbens) was seen in males only chronically post-TBI, suggesting an imbalance which could drive impulsive behaviour. Subtle deficits in executive function were noted in adulthood post-mTBI in adolescence, with different domains affected depending on sex, which may relate to sex specific alterations in the development of the PFC and related structures.

id #12185

Activation of Dopamine D2 receptors in the CNS reduces thermogenesis elicited by activation of the lateral habenula

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The lateral habenula (LHb) plays an important role in the behavioral response to adverse environmental situations. Physiological responses to adverse situations includes emotional

hyperthermia induced by brown adipose tissue (BAT) thermogenesis. Our laboratory has elucidated the role of the LHb in physiological thermoregulatory changes, demonstrating that the LHb can mediate emotional hyperthermia by eliciting BAT thermogenesis and cutaneous vasoconstriction, responses mediated via the medullary raphé. We also recently showed that BAT thermogenesis, elicited by activation of the LHb, is abolished by blockade of GABAergic transmission in the ventral tegmental area (VTA). Thus, suggesting the dopamine system contributes to LHb-elicited thermogenesis. The present study investigated whether activation of dopamine D2 receptors attenuates LHb-elicited BAT thermogenesis. We measured BAT temperature and sympathetic nerve activity in anesthetized Sprague-Dawley rats. Activation of LHb neurons, with bicuculline (1nmol in 100nl/site), increased BAT nerve activity by 74±6% and BAT temperature by 1.5±0.3ºC. Administration of the D2 receptor agonist Quinpirole (25µg/kg, i.v.) reversed this LHb-elicited response (n=6, p<0.01). However, BAT thermogenesis elicited by stimulation of the medullary raphe was unaffected (n=6, p>0.05). The results suggest that the LHb, via its inhibition of the VTA dopamine system, mediates emotional hyperthermia via activation of GABAergic inhibitory inputs to VTA dopamine D2 receptors located in the pathway from the LHb to the medullary raphé.

id #12186

THE IMPACT OF CHRONIC FLUOXETINE TREATMENT IN ADOLESCENCE OR ADULTHOOD ON FEAR LEARNING, PARVALBUMIN NEURONS, AND PERINEURONAL NETS

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Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, are commonly prescribed for anxiety. Fluoxetine may reduce the heightened fear seen in anxiety disorders because this adjunct markedly impairs context fear learning in adult non-human animals. This effect is associated with altered expression of extracellular matrix structures called perineuronal nets (PNNs) in the basolateral amygdala (BLA) and hippocampus, two brain regions which regulate fear. PNNs are essential for neurodevelopment, they preferentially surround mature parvalbumin (PV) neurons, and they regulate plasticity. We investigated the effect of fluoxetine exposure during adolescence or adulthood on fear learning and PNNs in the BLA and the CA1 hippocampal region. Adolescent (30 days old) or adult male rats received fluoxetine (~10mg/kg/day) in their drinking water, or water only, for two weeks before context fear conditioning or neural analyses. The behavioural results indicated that fluoxetine differentially affected adolescents and adults; relative to controls, this adjunct reduced context fear in adults, replicating past research, but not in adolescents. In contrast, fluoxetine had similar effects on PNNs across age. Adults had more PV neurons surrounded by a PNN in the BLA and CA1, but fluoxetine increased the number of these neurons at both ages. Contrary to previous reports, fluoxetine did not shift the percentage of PNNs toward non-PV cells in either the BLA or CA1 in the adults, and a similar result was found in adolescents. These findings demonstrate that fluoxetine differentially affects fear learning in adolescent and adult animals, but this adjunct may not have age-specific effects on PNNs.

id #12188

Expansions of a pentameric intronic ATTTC repeat in *STARD7* lead to familial adult myoclonic epilepsy linked to chromosome 2

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Familial Adult Myoclonic Epilepsy (FAME) is characterised by cortical myoclonic tremor usually from the second decade of life and overt myoclonic or generalised tonic-clonic seizures. Four independent loci have been implicated in FAME on chromosomes (chr) 2, 3, 5 and 8; affecting over 100 families internationally. Using bespoke whole genome sequencing analyses targeting non-coding regions and repeat primed PCR, we show that chr2-linked FAME (FAME2) is caused by an expansion of an ATTTC pentamer within the first intron of *STARD7. The ATTTC expansions segregated in 158/158 individuals typically affected by FAME from 22 pedigrees including 16 previously reported families recruited worldwide.* Consistent with other repeat expansion disorders, we observed anticipation with symptom onset advancing by 5-7 years per generation in a large family from Australia and New Zealand (of European descent) with over 50 affected individuals. RNA sequencing from patient derived fibroblasts showed no accumulation of the AUUUU or AUUUC repeat sequences and *STARD7* gene expression was not affected. These data, in combination with other genes bearing similar mutations that we have implicated in FAME, suggest ATTTC expansions may cause this disorder, irrespective of the genomic locus involved.

id #12190

Adult vitamin D deficiency disrupts spatial memory and perineuronal nets in the hippocampus

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Vitamin D deficiency is a global public health burden, affecting millions of people worldwide. Low serum vitamin D levels are associated with many neuropsychiatric diseases, such as schizophrenia, in which impaired hippocampal function has been reported as a central issue. Vitamin D may have a specific role in the hippocampus, since neurons in the hippocampus express the vitamin D receptor (VDR). In this study we examined spatial

memory impairment in adult vitamin D-deficient BALB/c mice and its underlying mechanism by measuring perineuronal nets and GABAergic interneuron density in the hippocampus. We also examined structural connectivity using 16.4 T MRI scans. Adult male BALB/c mice were fed a control or vitamin D deficient diet for 20 weeks. We showed that AVD-deficient BALB/c mice took significantly longer to learn to avoid the shock zone than control mice over 5 days. All mice performed similarly on the last day of training, demonstrating that AVD deficiency produced a delay in learning. There was a significant reduction in the number of cells that immunostained positive for perineuronal nets and neural nitric oxide synthase in all subfields of the hippocampus. The structural connectivity changes were specific to the hippocampal network. Therefore, we can show that adult vitamin D deficiency is associated with impaired spatial learning and altered hippocampal function in mice. We were able to show that chronic vitamin D supplementation prevented the decline in spatial learning, showing that vitamin D supplementation for a prolonged duration may be required to restore hippocampal-dependent function.

id #12192

ISOFLURANE ANAESTHESIA FACILITATES SHORT-TERM AVOIDANCE MEMORY ON A SPATIAL LEARNING TASK IN MICE

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Postoperative cognitive dysfunction (POCD) is a significant clinical problem, with an increased risk of cognitive decline for up to one year after surgery. Recently, inhalation anaesthetics have been proposed as a main contributor to POCD. To investigate the role of anaesthetics on POCD, we tested young adult BALB/c mice that received isoflurane anaesthesia (operated mice) or controls (non-operated mice) for short-term avoidance memory using the one-day active place avoidance task. We found that mice which received saline-injections into the retrosplenial cortex whilst under the influence of isoflurane, a widely used inhalation anaesthetic, had significantly ($p<0.05$) longer latencies to first entry to the shock zone and received fewer shocks, compared with non-operated controls. Mice were tested 14 days post-surgery to assess memory. Operated mice had significantly ($p<0.05$) longer latencies to first entry to the shock zone and received fewer shocks, compared with non-operated controls. Reversal learning abilities were also assessed. We found that operated mice received significantly ($p<0.05$) fewer shocks compared to the non-operated controls when the shock zone was reversed 180 degrees. These behavioural results suggest that isoflurane facilitates avoidance learning and memory, contrary to our hypothesis. We propose that anaesthetic agents, such as isoflurane, may affect a component of the extracellular matrix called perineuronal nets (PNNs), which has recently emerged as a critical participant of the synaptic machinery, and we are currently exploring this possibility. Further understanding of the role of inhalation anaesthetics on synaptic reorganisation may help elucidate the mechanism by which anaesthetic agents contribute to POCD.

id #12199

Environment is a significant parameter in the switch between defensive behavioural responses

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Selecting and switching between defensive behavioural responses to a threat is of great importance for survival. In experimental situations, using Pavlovian learning paradigms, rodents typically display freezing or flight behaviour as a result of associative learning. This project aims to determine whether the amygdala is involved in the switch between freezing and fleeing as a response to the conditioned stimulus. Rats were fear conditioned in a traditional fear conditioning chamber and the fear memory was then tested in both small and large environments with access to shelter. In this behavioural paradigm, local field potentials in the basolateral amygdala (BLA), medial prefrontal cortex (mPFC) and ventral hippocampus (VH) were recorded, extracting delta (2-4 HZ), low theta (4-8 HZ), high theta (8-12 HZ), beta (12-30 HZ) and gamma (30-100 HZ) oscillations. The results demonstrate that rats do not show high levels of freezing in the large environment, spending significantly more time in the shelter, compared to the fear test in the small environment. Moreover, the power in both delta and low theta oscillation bands in the BLA and mPFC increased in the large environment during fear memory retrieval. Our results suggest that the environment plays a crucial role in the switch between fear behaviour responses. This switch in BLA activity could possibly be dependent on the VH and mPFC as theta oscillations are often hippocampus driven.

id #12205

Improving brain autophagy to counteract Alzheimer's disease.

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Autophagy is an evolutionarily conserved degradative pathway that eliminates damaged organelles and protein aggregates and recycles them to produce intracellular nutrients. This process is mis-regulated in various neurodegenerative diseases characterized by the accumulation of protein aggregates such as amyloid-β in Alzheimer's disease (AD). Autophagy is regulated by the nutrient sensor mTOR, which can directly suppress autophagy in response to nutrient excess. On the other hand, autophagy is stimulated by nutrient starvation (e.g. caloric restriction or protein restriction), a condition which necessitates cells to mobilize intracellular nutrients. Thus, improving brain autophagy by targeting mTOR may help prevent amyloid deposition in the AD brain.

We recently showed that pharmacological mTOR inhibition was able to alter autophagy pathways in mouse brain. Then, we subjected an AD mouse model which developed brain amyloid plaques to an isocaloric low protein/high carbohydrate diet for 4 months. Our first results showed that mice fed the low-protein diet are metabolically healthier than mutant control mice. Autophagy regulation and amyloid pathogenesis in the brain are currently being analysed.

This project may provide novel approaches by which to control protein clearance dysfunction observed in neurodegenerative disorders.

id #12208

CHARACTERISATION OF GANGLION CELL TYPES IN HUMAN RETINA EXPRESSING THE TRANSCRIPTION FACTOR SATB2

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Purpose: Retinal ganglion cells are the output neurons of the retina, sending visual information to different targets in the brain. There are at least 17 types of ganglion cell in primate retina but only a few classic types (midget and parasol cells) are well understood. The aim of the present study is to use molecular markers to identify and characterize non-classic ganglion cells in human retina. **Method:**Three *post mortem* human donor retinas (age range 29 to 52 years) obtained from the Lions NSW Eyebank were immersion fixed in 2% paraformaldehyde. Pieces from two retinas were used for single cell injection with the lipophilic dye DiI following immuno-labelling with antibodies against Special AT-rich binding protein 2 (Satb2). One retina was processed for standard double label immunohistochemistry with antibodies against Satb2 and the ganglion cell marker RBPMS. **Results:** All Satb2 positive cells located in the ganglion cell layer (n = 423 cells) were also labelled for RBPMS and thus are ganglion cells. Satb2 ganglion cells make up on average 0.8% of the ganglion cell population in central and 1.5% in peripheral retina. The peak density of Satb2 ganglion cells is 145 cells/mm^{2}at ~1 mm eccentricity, the density decreases to less than 10 cells/mm^2 at >13 mm eccentricity. All intracellularly injected Satb2 positive cells (n=11) were classified as large sparse OFF with dendritic fields ranging from 410 to 656 mm in diameter. **Conclusion:** The transcription factor Satb2 is expressed by a single type of non-classic ganglion cell in human retina.

id #12211

Autophagy clears Tau aggregates in a prion-like model of Tauopathy

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Pathological amyloid aggregation of the Tau protein is a hallmark of several neurodegenerative diseases including Alzheimer's disease. In Alzheimer's disease, Tau pathology spreads trans-neuronally throughout the brain. It is thought that prion-like

mechanisms are at least in part responsible for the amplification of Tau pathology in the brain. In this study, we have used brain lysate from a rTg4510 mouse model of Tauopathy to seed the aggregation of the repeat domain of Tau in HEK293 cells. Then, using traditional cell-cloning methods we isolated cell clones that faithfully propagate several unique aggregate morphologies. In this model, we found that pharmacologically inhibiting autophagy—a cellular house-keeping mechanism—caused a marked accumulation of tau aggregates of various sizes. These data support the idea that autophagic/lysosomal dysfunction plays a key role in the pathogenesis of Alzheimer's disease.

id #12213

BILATERAL INTEGRATION OF SENSORY INOFMRATION IN THE MOUSE WHISKER SYSTEM

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To generate a coherent percept of the external world, the brain needs to combine and integrate sensory signals processed in the left and right hemispheres. Currently, little is known about the neuronal mechanisms underlying bilateral integration. Here, we investigate bilateral integration of somatosensory inputs in mouse primary vibrissal (vS1) cortex under urethane anaesthesia, and during a sensory integration task. For the behavioural task, the mice were trained to compare the stimuli between the two whisker pads during head-fixation. In Experiment 1 (Go/No-Go), mice were trained to respond to uni-lateral (Go) but not to bilateral stimulation (No-Go) of whiskers. In Experiment 2 (2-Alternative-Forced-Choice), mice were trained to choose one of two reward spouts that corresponded to the stronger stimulus. Mice learned to perform the task in both behavioural paradigms. During the behaviour, we visualised vS1 neuronal activity by two-photon imaging using the genetically encoded calcium indicator GCAMP6f. Under anaesthesia, vS1 neurons were predominantly responsive to contra-lateral and bilateral whisker stimulation with little modulation by the ipsi-lateral stimulation. This response profile changed during the Go/No-Go paradigm, where vS1 neurons were responsive to both ipsi- and contra-lateral stimulation (Go trials), with minimal response to bilateral stimulation (No-Go trials). Our results thus showed the neuronal correlates of the sensory decision in the vS1 cortex. Future experiments could investigate how interactions with higher order areas, such as the premotor cortex, may underlie this finding.

id #12214

Frequency tagging in the mouse vibrissal cortex

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Frequency tagging has been used to study how attention affects the neural representation of sensory information measured with EEG in humans. Neuronal-scale frequency tagging studies are expected to provide a bridge to this rich body of literature. The rodent vibrissal cortex is an attractive system in which to conduct such studies due to its tractability and compatibility with genetic methods. In Experiment 1, we applied steady-state pulsatile deflections (10-s epochs) to the whiskers of urethane-anaesthetised mice at four frequencies and three amplitudes while recording extracellular activity from vibrissal somatosensory (vS1) cortex. Frequency modulation was apparent at all stimulus amplitudes in 122 units. In Experiment 2, we applied the same stimulus set to awake head-fixed mice and were able to extract the frequency tags from single units and local field potential. In Experiment 3, we applied frequency modulated stimulation in a head-fixed behavioural paradigm designed to test for spatial attention in mice. In this paradigm, the mouse was required to detect vibrissal stimulation on the left or right whisker pad by licking a central spout. On every trial mice were cued by a continuous green LED to either the left or right whisker pad likely (90% of trials) to be stimulated. Licking within a brief (0.5-1 s) window of opportunity after the target earned the mouse a reward. The valid cues improved the detection rate compared to invalid cues. Results show the potential application of frequency tagging in rodents and how the vS1 neuronal representation of stimuli changes with cue.

id #12215

State-dependence of gamma-band spiking activity in 'blue-OFF' cells in marmoset Lateral Geniculate Nucleus.

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Purpose: We previously reported that neurons in the intercalated (koniocellular, K) layers of the lateral geniculate nucleus (LGN) show brain state-related variability in spike rate (Cheong SK et. al., P.N.A.S. 2011). Koniocellular cells include those processing short wavelength (S) cone increments ('blue-ON') or S-cone decrements ('blue-OFF'). Here we asked whether higher-order statistics of spiking activity in these koniocellular cells also varies with brain state.

Methods: Extracellular spike activity of K cells in the LGN classified as 'blue-OFF' (n=11) or 'blue-ON' (n=8) were recorded in Sufentanil-anaesthetised marmosets (*Callithrix*

jacchus). Spike activity was recorded while animal viewed a uniform grey background near 50 Cd / m^2. Autocorrelograms of spiking activity were constructed using standard methods.

Results: In 'blue-OFF' cells the autocorrelograms showed a peak at average 20.4 ± 5.3 ms (52 ± 17 Hz) n = 11). Separating epochs of low and high firing rate showed that the amplitude of the peak was higher during periods of high maintained spike rate (0.43 ± 0.19) than during periods of low spike rate (0.016 ± 0.005, p = 0.005, Wilcoxon paired rank sum test). The peak was much weaker in blue-ON (amplitude < 0.03) and was absent from responses of magnocellular (M) and parvocellular (P) cells.

Conclusion: Selective emergence of gamma frequency activity in 'blue-OFF' cells is a new asymmetry between 'blue-ON' and 'blue-OFF' pathways. Gamma-band oscillations in cat LGN (Neuenschwander and Singer, Nature, 1996) and mouse LGN (Saleem et al., Current Biology, 2017) are more widespread.

id #12216

Dysregulation of the neuronal epigenome occurs prior to pathology-onset and alters with progressive amyloidosis.

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Epigenetic machinery is at the interface between our genes and the environment and is well placed to explain some of the heritability gap of sporadic Alzheimer's disease (AD). The dysfunction and death of neurons underlie the symptoms of AD, yet few studies focus on neuronal epigenetic alterations in AD. We examined H3K4me3 and H3K27ac histone modifications (ChIP-seq) in neurons from 3, 6 and 12-month-old wild-type and APP/PS1 mice, representing pre-pathology, pathology-onset and pathology-rich time-points (n=5/genotype/timepoint). H3K27ac and H3K4me3 marking at promoters was increased in APP/PS1 versus wild-type neurons at the pre-pathology time-point. Enhancers and super-enhancers were also differentially enriched for H3K27ac marking between APP/PS1 and wild-type neurons at both pre-pathology and pathology-onset time-points. Interestingly, promoters and enhancers followed a similar pattern of enrichment for H3K4me3 and H3K27ac across the time-course of amyloidosis, whereas super-enhancers exhibited a different pattern of H3K27ac enrichment over time. We observed a partial recapitulation of a pre-pathology/juvenile-like histone landscape in both aged wild-type and APP/PS1 neurons (>23% of differentially H3K4me3 and H3K27ac marked sites were shared). To validate our findings, we compared our data to human neuronal single-cell RNA-seq data (Mathys et al., 2019, *Nature*). Over 80% of the transcripts that were differentially expressed between neurons from human AD cases and controls exhibited differential promoter enrichment for H3K4me3 between wild-type and APP/PS1 neurons at the pathology-rich time-point. Our

data provides a unique insight into the epigenetic dysregulation occurring in neurons in a milieu of amyloidosis.

id #12219

Vagus nerve stimulation induces cellular plasticity in NTS neurons

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The nucleus of the solitary tract (NTS) receives the majority of visceral sensory information that travels in the vagus nerve and is thus key for physiological homeostasis. Electrical vagus nerve stimulation (VNS) is an adjunct therapy for several conditions, including drug-resistant epilepsy, but its central mechanisms are unknown. We investigated cellular and synaptic plasticity within the NTS following long-term VNS. Adult Sprague-Dawley rats received either VNS or sham stimulation for 4 weeks. Brainstem slices were taken for *in vitro* whole-cell electrophysiology. VNS increased the input resistance, decreased rheobase, and depolarized the resting membrane potential of NTS neurons. Fast-inactivating potassium currents were reduced, contributing to a reduction in potassium mediated inhibition of action potential throughput. EPSCs evoked by stimulation of sensory afferent input were unchanged in amplitude, and spontaneous EPSCs were unchanged in frequency and amplitude, suggesting no evidence for synaptic plasticity. Together, these data demonstrate that chronic VNS induces postsynaptic cellular plasticity within the NTS via modulation of voltage-gated ion channels. This plasticity is likely a homeostatic mechanism to maintain gain of physiological reflexes in the face of increased afferent activity, and may contribute to the mechanism of VNS.

id #12227

Investigation of polysialic acid expression in response to immune signalling in the nervous system

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Polysialic acid (polySia) is a long homopolymer of α2,8-linked sialic acids that is expressed in high abundance in the embryonic brain and remains restricted to only some regions of the adult nervous system that are associated with neuroplasticity.The endotoxin lipopolysaccharide (LPS) and morphine-3-glucuronide (M3G), a metabolite of morphine, cause immune signalling in the central nervous system.We revealed in 2D *in vitro* cultures that polySia is up-regulated in neuronal-phenotype cells when exposed to LPS and M3G, which was similar to the induction of polySia expression following neuronal differentiation via neurite-growth factor (NGF).Under these conditions, migration and oxidative stress states were altered in the neuronal-phenotype cells when polySia was enzymatically removed.PolySia was not expressed on glial-phenotype cells grown on 2D but was abundant in a 3D *in vitro* brain model of cell culture that we developed.Using this novel 3D culture system for brain cells, we revealed that LPS and M3G stimulation caused a profound decrease of polySia in glial cells; whereas in neuronal-like cells,polySia increased.PolySia was also measured in a chronic constriction injury (CCI) mouse model of pain with greatly increased central immune signalling, showing an overall reduction of polySia in dorsal root ganglia and spinal cord tissues in response to pain.Our findings suggest that polySia is differentially regulated in neuronal and glial cells under induced immune signalling.We also observed that polySia expression in a 3D cell culture model of immune signalling following exposure to LPS or M3G more accurately reflects expression levels in a mouse model of heightened central immune signalling.

id #12228

Reduced neuronal activation of attention and cognitive control mechanisms in amnestic mild cognitive impairment (aMCI) compared to healthy controls

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Mild cognitive impairment (MCI), conceptualised as the prodromal phase of dementia, causes a decline in cognition, affecting ~30% of Australians aged >70 years. People with MCI who have a memory deficit (amnestic MCI; aMCI) have an increased risk of Alzheimer's disease (AD); the most common cause of dementia. The aim of this study was to characterise the real-time neuronal activity in people with aMCI whilst they engage in a cognitive task in order to elucidate the early changes in brain function associated with increased AD risk. Fifty-eight people with aMCI ($n = 42$) and healthy age, gender, and education-matched controls (HCs; $n = 16$) completed an auditory equiprobable Go/NoGo task whilst EEG was recorded. EOG-corrected averaged event-related potentials (ERPs) for

correct Go and NoGo trials for each group were submitted to four temporal principal components analyses (PCAs). Four components were assessed for Go (N1-1, P2, P3b, and SW) and NoGo (N1-1, P2, P3a, Late Positivity; LP) from each PCA. Go N1-1, P3b, and SW, and NoGo N1-1, P2, and LP had significant ($p < .05$) topographic x group interactions; individuals with aMCI had lower component amplitudes than HCs. Results demonstrate that compared to HCs, people with aMCI have reduced neuronal activation when encoding attentional information in the auditory cortex (Go/NoGo N1-1), disengaging from both sensory (NoGo P2) and cognitive processing (NoGo LP), and initiating motor response selection (Go P3b) and evaluation (Go SW). This study furthers our understanding of the brain function changes in early-stage AD preceding major cognitive decline.

id #12229

Spatially mapping brain metabolism in a mouse model of Huntington's disease

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Background: Huntington disease (HD) is a neurodegenerative disease, whose key pathological signature is the formation of intracellular inclusions[1]. However, the exact role of inclusions in driving HD pathology remains to be clearly understood. Our lab has previously shown that the formation of huntingtin inclusions in cell culture models correlates with the cells becoming functionally quiescent and undergoing a slow death by necrosis [2].

Hypothesis: We hypothesize that neuronal dysfunction *in vivo* is driven by an induced quiescent state as huntingtin inclusions form and that this happens far earlier than cell death.

Aim: Our goal is to assess the extent to which neurons *in vivo* are metabolically quiescent and how this relates to the presence of inclusions in a transgenic mouse model of HD.

Methods: We have conducted a metabolic flux analysis of neuronal membrane lipids by feeding wild-type and R6/1, a mouse model of HD, with deuterated water to track cell turnover. The left hemisphere of the brain was reserved for determining the spatial distribution and the abundance of the brain metabolites using MALDI-TOF imaging mass spectrometry (MALDI-IMS). The right hemisphere was dissected and the frontal cortex, striatum, and hippocampus reserved for Liquid chromatography-Mass spectrometry analysis.

Results: Our preliminary data points towards a change in deuterium incorporation rate in neuronal membrane lipids in different brain regions of WT and HD mice, in particular, the pyramidal neuronal cell layer of the hippocampus, an area that has been found to have a dense population of inclusions using EM48-immunohistochemistry directed to inclusions.

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DeepSlice: A Deep Neural Network for Fully Automatic Alignment of Histological Sections

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CONFIDENTIAL

An almost ubiquitous component of laboratory neuroscience research is mapping the locations of neurons based on their function, genetic profile or connectivity. An obvious but fundamental aspect of this task is accurately documenting the locations of neurons of interest. In thousands of laboratories across the world, this is done in a subjective and time-consuming process in which histological sections are compared to 2- or 3-dimensional reference atlases, such as the Allen Mouse Brain Atlas, and sections are 'aligned' to the region of the atlas that most closely resembles their tissue. This task is slow and error-prone, even for experts, with accurate alignment taking several minutes per section. Here we present DeepSlice, a convolutional neural network developed to register histological images of the mouse brain to the Allen Common Coordinate Framework. DeepSlice analyzes histological sections obtained using diverse imaging modalities (block-face confocal, fluorescent immunohistochemistry, NISSL, bright-field *in situ* hybridization) and aligns them to the Allen Brain Atlas with accuracy comparable to human experts but orders of magnitude faster. Our system requires no user input, can identify imperfections in cutting angle or image rotation, and is computationally efficient, requiring no specialized hardware. Recent technological innovations have revolutionalised the potential scope of neuroscience research, but the process of orienting yourself in the brain remains essentially unchanged for at least 30 years: DeepSlice represents a one-button image alignment tool that requires no specialist expertise and can process thousands of images per minute on a middle-of-the-range laptop, and may therefore unblock this longstanding bottleneck.

Comparative transcriptomic analysis of forebrain connectivity across mammalian evolution

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The cerebral cortex is exclusively present in mammals but not in other vertebrates. Despite all mammals sharing a similar connectome, only eutherians evolved a corpus callosum, while in monotremes and marsupials interhemispheric connections course via the anterior commissure. As very little is known about the genetic underpinnings of these brain wiring differences, we compared the developmental transcriptome of cortical neurons in mice and the marsupial fat-tailed dunnart. First, we sequenced all genes expressed at stages equivalent

to infragranular and supragranular layer neurogenesis, and then compared gene expression in cortical neurons based on their projection targets in both species. Interestingly, we found that an earlier onset of gene expression associated with mature neuronal processes in dunnarts than mice at equivalent developmental stages. Moreover, RNA sequencing in neurons isolated via FACS after stereotaxic retrograde tracer injections revealed that transcriptomic profiles of cortical neurons associated more strongly with the initial medial/lateral direction of axon growth than with the identity of their targets. For example, differences in the direction of initial commissural axon growth between mice (which grow medially) and dunnarts (which grow laterally) were more important for gene clustering. Therefore, in dunnarts commissural genes clustered more closely with genes expressed by laterally projecting subcerebral neurons than with genes expressed by intracortical neurons. Additional differences in gene expression of cortical projection neurons between species were also noted. This rich transcriptomic resource of multiple developmental stages across species highlights genes that might have driven the evolution of different wiring phenotypes in mammals.

id #12233

EXERCISE ALTERS BINOCULAR RIVALRY DYNAMICS IN HEALTHY YOUNG ADULTS

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In amblyopic rodents exposed to increased physical exercise, improvement in visual function is associated with reduced release of gamma aminobutyric acid (GABA) in primary visual cortex. Here, we investigated whether mild-moderate exercise in healthy, young human adults with normal vision and ocular health (n=20, aged 20-34 years) produces effects consistent with a reduction in visual cortical inhibition. Using a visual perceptual task that is thought to be mediated, at least in part, by GABAergic inhibition (binocular rivalry), we demonstrate that median percept duration decreased (repeated-measures ANOVA main effect of exercise: $F(1,19)=6.78$, $p=0.02$) in the first 9 minutes after mild-moderate exercise (alternating periods of 10 mins cycling, 10 mins rest for 2 hours' duration aiming to keep heart rate at 60-70% of maximum heart rate for age). There was a concomitant change in ocular dominance, where the percentage of time spent in the dominant eye percept decreased after exercise (paired t-test: $t(19)=3.13$, $p=0.006$). Our findings demonstrate that exercise alters binocular rivalry dynamics in healthy human observers in a direction consistent with reduced GABAergic inhibition.

id #12234

Reprogramming Human Dental Pulp Stem Cells into Neural Stem Cells

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Dental pulp stem cells (DPSC), harvested from the pulp tissue of wisdom teeth, are multipotent stem cells with neurogenic potential. They exert positive effects in the injured/diseased brain via multiple molecular and cellular mechanisms, and are potential candidates for cell-based therapies for treatment of neurological diseases, such as stroke. In the adult brain, neural stem cells (NSC) residing in specific neurogenic niches have a role in repair, however they are limited. Cellular reprogramming has been used to convert more accessible cell types into NSC. DPSC may be a suitable cell type from which to obtain NSC, due to their neural crest origin. The aim of this study is to investigate the ability to reprogram DPSC into NSC. Several transcription factors (OCT4, SOX2, HES1) were selected and overexpressed in human DPSC via lentiviral transduction. Cells were cultured under neural conditions, and analysed for expression of neural markers, self-renewal, and multilineage differentiation. *In vivo* analysis was performed using a developmental avian model. DPSC overexpressing OCT4 underwent morphological changes, displaying a neuronal-like phenotype. Multiple neural conditions were tested. A three-step neural reprogramming method utilising β-mercaptoethanol was most promising showing increased expression of neural markers used to characterise NSC. Furthermore, i*n vivo* analysis showed a neuronal phenotype, and transcriptomic data will further characterise these cells. Human DPSC are easily accessible and possess neurogenic properties, which may be enhanced if modified into NSC. An efficient and reliable reprogramming method could provide an alternative source of NSC for use in cell-based therapies.

id #12241

Heparan sulfate proteoglycans as markers of human neuronal phenotypes

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Human stem cells are routinely used as *in vitro* models to study human neurogenesis [1]. How these processes are regulated has potential application for brain damage therapy, neurogenesis modelling and screening for drug neurotoxicity. Fir therapeutic efficacy, the identification of novel cell markers of human neurons to develop more efficient characterisation of neuronal populations is needed. We have previously reported the heparan sulfate proteoglycan (HSPG) glypicans (GPCs) as markers of lineage specific human neural stem cells (hNSCs) and mediators of hNSC plasticity [2]. Further examination of GPCs was

undertaken in neurons differentiated from hNSCs in the presence of two neurogenic growth factors reported to bind to heparan sulfate (HS). hNSC cultures were augmented with the common neuron culture supplement, brain-derived neurotrophic factor (BDNF), and platelet-derived growth factor-B (PDGF-B). Long-term (40 – 60 days) differentiated neurons demonstrated differences in HSPG localisation, suggesting potentially different functions within neurons. Differentiation of hNSCs to neurons in the presence of BDNF or PDGF-B generated phenotypically different neurons, along with differences in heterogeneity and neuron subtype marker expression. Untreated neuronal cultures with no growth factor supplementation were found to be highly variable, supporting the use of neuroregulatory growth factors for guided neuron differentiation. These results further indicate GPCs as human neuronal proteins, providing potentially novel markers to characterise neuron populations in terms of maturity, heterogeneity and subtypic differentiation.

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id #12284

TROPOMYOSIN Tpm3.1 and Tpm4.2 ISOFORMS IMPACT THE MORPHOLOGY OF MOUSE PRIMARY HIPPOCAMPAL NEURONS AND SHOW FUNCTIONAL DIFFERENCES IN THE SYNAPSE REGULATION

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Tropomyosin (Tpm) has been regarded as the master regulator of actin dynamics in the cytoskeleton. Tpm isoforms found in neurons are encoded by the *TPM1*, *TPM3* and *TPM4* gene and they are differentially expressed, both temporally and spatially. The actin cytoskeleton is the predominant cytoskeletal structure in the postsynaptic compartment of excitatory synapses which are formed at the distal site of dendritic spines. *TPM3* and *TPM4* gene products have been found to segregate to the postsynaptic region of central nervous system synapses. Here, we show that neither Tpm3.1 nor Tpm4.2 overexpression affects the basal activity or spine morphology, while long-term potentiation (LTP) is augmented when overexpressing Tpm3.1. Knock-out (KO) of Tpm4.2 reduces basal synaptic activity without changing spine morphology. Tpm3.1 and Tpm4.2 isoform dynamics at the postsynaptic compartment was further investigated via Fluorescent Recovery After Photobleaching (FRAP) experiments of the dendritic spines. We have also performed a detailed morphometric analysis of mouse primary hippocampal neurons, isolated from wild type and Tpm4.2 KO mice. The Tpm4.2 KO neurons showed greater dendritic complexity when compared to wild-type neurons, as well as the increased axonal length. Overall, the effect of Tpm3.1 and Tpm4.2 isoforms on neuron morphology and function of dendritic spines provides an evidence for their potential regulation of different actin filament populations at the post-synaptic compartment.

Isolation, enrichment and characterization of olfactory ensheathing glia from the olfactory mucosa

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Transplantation of olfactory ensheathing cells (OECs) to promote repair after spinal cord injury has been identified as a safe and promising cell-based therapy. Primary olfactory mucosal cultures are heterogeneous, mainly comprised of OECs and olfactory nerve fibroblasts (ONFs). However, the anatomical region dissected, and the culturing method can significantly influence the final cell populations resulting in variable cell proportions between different primary cultures. In order to obtain robust reparative clinical outcomes, accurate estimation of the proportions of OECs and ONFs should be obtained prior to transplantation.

Using a line of fluorescent reporter transgenic mice, S100β-DsRed mice, in which glial cells express the fluorescent protein DsRed and fluorescence activated cell sorting, we have purified populations of OECs to assess their behaviour. We can now establish and characterize predefined proportions of OECs to ONFs in cell preparations prior to transplantation.

Our protocol is a reproducible and short method (<2 weeks) to generate highly pure populations of OECs (93.9%) and ONFs (98%) that can be used for co-culture and transplantation in animal models of injury with reduced experimental variation. Use of purified populations will also make it possible to examine the cellular interactions and the ability of OECs to promote neuronal regeneration.

Proteomic study explains a possible molecular mechanism of the novel neuromodulatory activity of a sterol in primary hippocampal neuron

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Potentiating neuritogenesis through pharmacological intervention might hold therapeutic promise in neurodegenerative disorders and acute brain injury, where neurons suffer from extensive damage of network connectivity. Our effort was dedicated to exploring such novel

compounds from natural products or their synthetic analogs that have potentials of neurite outgrowth. Here, we investigated the novel neuritogenic potentials of a sterol and the change in cellular proteome to gain insight into the underlying mechanism of its neurotrophic activity in primary hippocampal neurons. Morphometric analysis showed that sterol promoted early neuronal differentiation, dendritic arborization and axonal maturation. Proteomic and bioinformatic analysis revealed that sterol induced upregulation of several proteins, including those associated with neuronal differentiation and development. Immunocytochemical data further indicates that sterol-treated neurons showed overexpression of Hnrnpa2b1 and Map1b, validating their proteomic profiles. In addition, a protein-protein interaction network analysis identified TrkA as a central component connecting most of the upregulated proteins. The neurite outgrowth effect of sterol was suppressed by TrkA inhibitor, GW441756, verifying TrkA-dependent activity of sterol, which further supports the connection of TrkA with the upregulated proteins. Also, *in silico* analysis revealed that sterol interacts with the NGF-binding domain of TrkA through Thr325 and Phe327. Collectively, our findings provide the evidence that sterol promotes neuronal development via upregulating TrkA-signaling proteins and suggest that sterol could be a novel therapeutic lead in the prevention and treatment of neurodegenerative disorders as well as acute brain injury.

id #12335

Functional Connectivity In The Ageing Human Maternal Brain, And The Neuroprotective Effects Of Motherhood.

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The maternal brain undergoes structural and functional plasticity during the transition to motherhood. Although, we have a growing understanding of the human maternal brain's response to infant stimuli, very little is known about functional plasticity outside of this infant-centric context, and whether these changes persist beyond the postpartum period, into late-life.

Functional connectivity measures the temporal correlation in blood oxygenation level dependent signal between brain regions. Resting state functional connectivity (rs-FC) indicates the intrinsic connectivity of the brain when it is not engaged in a specific task. We correlated rs-FC of 82 brain regions with number of children (1-6 children) for 221 elderly women (73.8±3.52 years), to examine the relationship between rs-FC and parity (number of children) in the ageing maternal brain

We show widespread decreasing functional connectivity with increasing number of children. Regions with the highest degree (number of connections that correlated with number of children) include the prefrontal cortex, inferior parietal lobe, right temporal pole, and left thalamus. This network overlaps substantially with regions implicated in early motherhood, suggesting the functional adaptations of motherhood persist into older age, potentially permanently. Our results also show decreased connectivity between, but not within networks, and decreased connectivity from the prefrontal cortex. Both of these patterns are shown in the opposite direction in studies of age-related decline, suggesting motherhood confers a neuroprotective effect on the ageing brain.

Our results suggest that the neural adaptation to motherhood is both long-lasting, and potentially beneficial for the function of the maternal brain in late-life.

id #12336

DOPAMINE NEURON ACTIVATION IN THE VTA INFLUENCES THERMOREGULATORY FACTORS AND EMOTIONAL HYPERTHERMIA IN THE RAT

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Body temperature typically increases when aversive situations are encountered. This is known as emotional hyperthermia, which involves heat production in brown adipose tissue (BAT). We previously reported that activation of dopamine D_2 receptors attenuates emotional hyperthermia, suggesting activation of the dopamine system has inhibitory effects on this response. The ventral tegmental area (VTA) is the start of the mesolimbic dopamine system, which regulates emotional function. The present study investigated whether activation of dopamine-synthesizing neurons in the VTA attenuates emotional hyperthermia. We used male transgenic rats that express Cre recombinase selectively in dopamine neurons, with the dopamine transporter promoter; and incorporated the designer receptor exclusively activated by designer drugs system (DREADD). Adeno-associated viral vectors, with a floxed-excitatory DREADD receptor gene, were injected bilaterally into the VTA. BAT and body temperature were measured with pre-implanted thermistors, and the resident-intruder model was used to elicit emotional hyperthermia. A male intruder rat, in a small cage, was introduced to the resident rat's cage 1 hour after administration of vehicle or DREADD agonists (clozapine-N-oxide or C21)(2mg/kg, i.p.). In the vehicle group, this introduction increased the BAT temperature of the resident rat by 1.2±0.2°C (n=8), and 1.1±0.2°C for body (n=6) (P<0.05). This response was significantly attenuated in the DREADD agonist treated group. Interestingly, activation of VTA dopamine neurons themselves increased basal BAT and body temperatures. The reduced emotional hyperthermia may, therefore, be due to this increased baseline level. These results suggest that the VTA dopamine system has an excitatory, rather than inhibitory, influence on thermoregulatory outputs.

id #12337

A RESEARCH PROGRAM TO DISCOVER THE EARLY BRAIN MOLECULAR CHANGES/STRESSES THAT DRIVE ALZHEIMER'S DISEASE THROUGH COMPARATIVE 'OMICS ANALYSES OF GENOME-EDITED ZEBRAFISH

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To delay or prevent Alzheimer's disease (AD) we first must understand the molecular mechanisms underlying how and why it begins. AD is thought to take decades to develop but our molecular understanding of AD from humans is built almost entirely on analyses of deceased brains where the initiating stresses may be absent or masked by later-stage pathological processes and compensatory responses. The currently favoured transgenic rodent models (based on ectopic expression and/or multiple mutations in genes causing early onset familial AD, EOfAD) have been selected because they mimic somewhat the histopathology thought to define AD. However, the most detailed form of molecular analysis currently available (transcriptome analysis) shows these models bear little relationship to the human disease (Hargis & Blalock, 2017, *Behav Brain Res* 322:311). We posit that, in the absence of an AD hypothesis with reliable predictive power, the most objective approach to genetic modelling (making fewest assumptions) is to copy the genetic state of EOfAD as closely as possible – i.e. heterozygosity for a single mutation in an EOfAD gene. Mouse knock-in models were generated 15+ years ago but never analysed using modern 'omics technologies. Six years ago the ADGL began a program of introducing EOfAD-like (and other) mutations into the zebrafish genome. By comparing transcriptomes and proteomes in young adult (pre-pathology) brains from different EOfAD-like mutants (and non-EOfAD-like mutants) we aim to identify the "signature" molecular changes-in-common that define the stresses eventually leading to AD. Our first comparative analyses support that cellular energy stress may initiate EOfAD.

id #12352

Heritability of pruritus – A survey in inbred mouse strains

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Pruritus, commonly known as itch, is a debilitating, though not life-threatening symptom of many skin diseases and in other fields of medicine. Especially in its chronic form, which affects up to 27% of the general population worldwide, it has a severe impact on patient's quality of life. However, molecular mechanisms of acute and, above all, chronic itch including receptors and signaling pathways are largely unknown which is a major challenge for the treatment of pruritus. The difference in pruritus severity in ethnical groups and between the sexes imply that genetic factors mediate variable sensitivity to itch in human. To test this hypothesis and to find causative genes responsible for variable itch sensation, we quantified the acute scratch behavior of male and female mice using an automated and bias-free magnet-based recording technology. In total 21 inbred mouse strains from 6 phylogenetically distant families were measured after intradermal injection of 10 itch-inducing stimuli (pruritogens). We found that the overall effect of the genetic strain background on the trait values is large, with several strains being highly sensitive and several strains showing almost complete resistance to some or all pruritogens. These phenotypic differences were then correlated with the genetic variation by computational haplotype mapping, thereby identifying genes that are likely associated with the phenotypic differences. Altogether these findings may pave the way for the development of novel anti-pruritus therapies.

id #12356

Neuroprotective potential of doxycycline in combination with gallic acid against Thiamethoxam induced neurotoxicity in Zebrafish

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In the current investigation, we surveyed the local farmers regarding the pesticide which is excessively used in crops and found that Thiamethoxam to be used extensively. Thiamethoxam is a systemic insecticide, absorbed quickly by plants and transported to all of its parts, including pollen, where it acts to deter insect feeding. The compound gets in the way of information transfer between nerve cells by interfering with nicotinic acetylcholine receptors in the central nervous system, and eventually paralyzes the muscles of the insects. It is also very toxic to aquatic life, whereas till date there is no antidote for its toxicity and treatment is only symptomatic. In this study, we have exposed Zebrafish to different doses (below LD50) of the toxin to study its effects on the neuronal system, for 96 hrs (acute toxicity study). In the treatment group, fishes were given treatment with doxycycline in combination with gallic acid with simultaneous exposure to thiamethoxam for the same time period. During the exposure, behavioral studies like effect on shoaling, bottom-dwelling, buoyancy, T-maze test for memory function, novel diving tank test, etc., were carried out at different time intervals for 96 hrs. After 96 hrs, the fishes were humanely euthanized and oxidative stress studies (SOD, Catalase, GSH, AchE) were carried out. It was observed that Thiamethoxam significantly affected the neuronal system as observed by behavioral parameters, also there were increased levels of oxidative stress which were significantly reversed by treatment with doxycycline and gallic acid when given in combination.

id #12362

Interrogating the association of diabetes with developmental cognitive impairments using an in vitro model of human neuronal development

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The association between the dysglycaemia of diabetes and disordered human neural ontogeny resulting in functional deficits has been established for some time. Children who develop type 1 diabetes are observed to have developmental abnormalities in cognition, mental health, brain morphology and brain biochemistry. Exposure to dysglycaemic conditions during pregnancy may also have ongoing impacts on cognition of the developing infant, including lower IQ, increase incidence of autism spectrum disorder and attention deficit hyperactivity disorder. In this study, we used cortical neurospheres (NSPs) derived from human embryonic stem cells (hESC) as a cellular model system to examine possible mechanisms underlying the long-term detrimental consequences of dysglycaemia in the developing brain. NSPs were transiently exposed to different combinations of glucose and insulin concentrations to mimic diabetic-like conditions. Mass spectrometry proteomics followed by IPA software analysis were then performed on the various NSP treatment groups to identify candidate proteins and pathways that are potentially dysregulated in neurons, upon exposure to altered glucose and/or insulin concentrations. Preliminary findings highlight observed changes in canonical pathways, including axonal guidance signalling, gap junction signalling and protein ubiquitination, which are critical for normal brain development. To further validate the proteomic data, reverse phase protein array has been applied. This tool allows a large number of western blots to run in a highly sensitive and high throughput manner.

These findings are significant for revealing initial key events underlying cognitive impairments associated with dysglycaemic conditions during early brain development.

id #12396

SOD1 dysfunction and disease mechanisms in postmortem ALS tissues

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Mutations in superoxide dismutase 1 (SOD1) are linked to familial amyotrophic lateral sclerosis (fALS). Misfolded variants of the mutated protein are proposed to underlie neuronal loss, with toxicity attributed to a loss of antioxidant function and a yet-uncharacterised neurotoxic gain of function. Data from fALS murine models suggest genetics are not the sole contributor to SOD1 dysfunction. We have hypothesised that non-genetic factors, such as mismetallation and atypical post-translational modifications, also modify SOD1 structure and function and can result in protein misfolding and toxicity. We observe misfolded wildtype

SOD1 associated with neuronal death in sporadic ALS (sALS), and also in idiopathic Parkinson's disease, but changes to the protein upstream from insoluble SOD1 deposits are scarcely investigated in human tissues. We investigated SOD1 protein levels, conformation and enzymatic activity in fALS cases, sALS cases and age-matched healthy controls. Results demonstrated increased SOD1 levels, but marked reductions in protein activity and altered protein surface charge consistent with structural changes, within the ventral spinal cord in fALS and sALS. These changes were strongly correlated with neuronal loss in this degenerating region of the CNS. Quantification of spinal cord Cu levels demonstrated significantly reduced cytosolic Cu levels in ventral spinal cord in fALS and sALS but no loss of the copper chaperone for SOD1. Cu changes were correlated with SOD1 activity and neuronal loss in these tissues. Our data demonstrate upstream changes in soluble misfolded SOD1 consistent with protein mis-metalation irrespective of *SOD1* gene status restricted to the degenerating ventral spinal cord.

id #12436

Investigation of structural compensation following oligomeric Aβ induced dendritic spine loss

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Alzheimer's disease, the most common form of dementia is a dual protein pathology resulting in progressive loss of synapses, neurons and eventual cognitive decline. One of the pathogenic peptides, Amyloid beta (Aβ) tends to form soluble oligomers which gradually aggregate into insoluble amyloid plaques. We have used soluble Aβ oligomers to induce dendritic spine loss in primary hippocampal neurons overexpressing Homer1c and tdTomato, used as a synaptic and structural marker, respectively, and investigated the spatio-temporal dynamics of structural compensation in surviving spines. Significant spine loss was achieved in cultured hippocampal neurons following Aβ treatment for both 3 and 5 hours treatment at 2.5uM. We observed an overall increase in the size of Homer1c clusters in surviving spines only after 5 hours of Aβ treatment. This preliminary *in vitro* data supports the hypothesis that spine loss during early, asymptomatic Alzheimer's pathogenesis is structurally compensated. However, it is possible that neurons lose this ability of compensation with progressive spine loss and impairment in synaptic plasticity.

id #12472

PP1 inhibition is involved in lead-induced neurotoxicity in cultured rat embryonic hippocampal cells

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Both lead (Pb) and okadaic acid (OA) are known to cause excitotoxicity. OA is a potent inhibitor of serine/threonine protein phosphatases, PP1 and PP2A. We investigated the effects

of Pb and/or OA on the expression of phosphatases (PP1, PP2A), the NMDA receptor subunits (NR1, NR2B), and molecules involved in NMDA signaling (CREB, pCREB) and synaptogenesis (PSD-95, synaptophysin) in cultured rat embryonic hippocampal cells. After 9 days in culture, primary embryonic hippocampal cells from Wistar rats were treated with Pb (0.97 µm; 20 µg/dL), OA (1nM) or Pb+OA for 72 hours. Expression of the above molecules in the culture lysate was determined by Western blot, and in neurons and astrocytes by immunocytochemistry. Compared to control cultures, the expression of PP1 was decreased by either Pb or OA (on average by 46-52%; $p<0.05$); the effect was similar in both neurons and astrocytes. The expression of NR1, on the other hand, was increased by either Pb or OA (on average by 37-40%; $p<0.05$). Expression of PP2A and NR2B were largely unaffected by either treatment. Expression of CREB was significantly decreased by both Pb or OA (on average by 50-63%), but its phosphorylation at serine-133 was not significantly affected. Expression of PSD-95 in neurons was also significantly decreased by both Pb or OA. These results suggest that Pb and OA produce excitotoxicity and the subsequent neurotoxicity by common mechanism(s) which involves inhibition of PP1 expression and overexpression of the NMDA receptor during early embryonic brain development.

id #12478

Subunit-specific Augmentation of AMPA Receptor Ubiquitination by Phorbol Ester

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Excitatory neurotransmission relies on the precise targeting of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors to the neuronal plasma membrane. Activity-dependent ubiquitination of AMPA receptor (AMPAR) subunits sorts internalised receptors to late endosomes for degradation, which ultimately determines the number of AMPARs on neuronal membrane (1, 2). Our recent study has demonstrated a functional cross-talk between the phosphorylation and ubiquitination of the GluA1 subunit in mammalian central neurons (3). However, the existence of such a cross modulation for the GluA2 subunit and its underlying molecular mechanism remain unknown. Here, we mapped the major GluA2 ubiquitination sites following bicuculline treatment to Lys-870 and Lys-882 in its C-terminal tail. Interestingly, bicuculline-induced ubiquitination was markedly enhanced by the phospho-mimetic GluA2 S880E mutant. Consistent to this, pharmacological activation of protein kinase C (PKC) by phorbol ester, which mediates the phosphorylation of GluA2 at Ser-880, augmented bicuculline-induced ubiquitination of GluA2 in cultured neurons. This effect was specific for the GluA2 subunit because phorbol ester did not alter the level of GluA1 ubiquitination. However, phorbol ester-induced enhancement of GluA2 ubiquitination was independent on Ser-880 phosphorylation. Collectively, these data provide the first demonstration of subunit-specific modulation of AMPAR ubiquitination by the PKC-dependent signalling pathway in mammalian central neurons.

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id #12483

Translational consequences of premature neurodegeneration in dystrophic nerves from the *mdx* mouse model of Duchenne Muscular Dystrophy

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In the childhood disease Duchenne Muscular Dystrophy (DMD) and the dystrophic *mdx* mouse and dystrophic dog models of DMD, repeated bouts of intrinsic skeletal muscle necrosis result in progressive altered and denervated neuromuscular junctions (NMJs). Based on our previous studies quantifying proteins associated with neurodegeneration in normal nerves from ageing C57Bl/6J mice that showed age-related increases for Tau5 and other proteins by 18 months (M), we hypothesize that the ongoing intrinsic myonecrosis and NMJ denervation in *mdx* mice will result in premature progressive neurodegeneration in the dystrophic peripheral nerves. To test whether early neurodegeneration (increased neuronal proteins) occurs in dystrophic peripheral nerves of *mdx* mice peripheral nerves (sciatic and radial) were sampled from classic *mdx* and normal C57Bl/10Scsn wildtype (WT) male mice aged 13, 15 and 18M (n=8-10/group), and snap frozen for immunoblotting to quantify levels of many neuronal proteins. Dystrophic *mdx* nerves (compared with WT) had significantly increased levels of S100 and Tau5 proteins by 13M (9M earlier than in normal ageing nerves), confirming our hypothesis; with further increased protein levels for S100 and Tau5 by 15 and 18M indicating progressive neurodegeneration.These novel observations of premature progressive neurodegeneration for dystrophic *mdx* nerves (probably irreversible) provide new insight into loss of function of dystrophic muscles; and a valuable new readout in long-term preclinical trials to monitor benefits of therapies designed to protect *mdx* muscles from myonecrosis; plus, present potential neuronal targets for drugs in clinical therapies to help maintain function of DMD muscles.

id #12491

Deciphering the fate of integrated pericytes in mouse motor cortex

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Finding new ways to encourage brain self-repair after injury as a realistic and effective therapy for stroke is a major goal of modern neuroscience. A subset of pericytes may hold the key to these efforts 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 around the site of pericyte (not sham) injections. In summary, preliminary results from this study demonstrated 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.

id #12508

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

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Optogenetics is a technique 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 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. The novel object recognition and novel object alteration behavioural tests demonstrated that the implant does not affect the animals' normal behaviour and cognition. Brain sections were immunolabelled for Iba-1 and GFAP to examine the level of damage and inflammation from the device. There were no significant differences in the expression of inflammatory markers in the hippocampus between both groups. This device provides an accurate and advanced optical control of hippocampal neurons, offering solutions to study complex animal behaviours.

id #12516

The correlation between individual differences of interoceptive accuracy and salience network connectivity in older adults.

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Background Interoceptive accuracy describes the ability to consciously perceive the physical condition of the entire body such as one's heartbeat. Chong et al. (2017)[1] reported that interoceptive accuracy is correlated with especially insular cortex and orbitofrontal cortex in salience network in younger adults. It is reported that interoceptive accuracy is decreasing with aging. However, the correlation between salience network connectivity and interoceptive accuracy in older adults is still unknown.

Methods 27 older adults (mean age 77.29 years, SD = 6.24, 19 women) were scanned T1-waighted structural images (3D MPRAGE) and functional T2*-weighted images (resting-state fMRI), and investigated heartbeat counting task and neuropsychological test after MRI scanning. Using CONN functional connectivity toolbox, we analyzed the correlation between interoceptive accuracy and ROI-to-ROI within salience network with age, gender and MMSE as covariates.

Results ROI-to-ROI analysis showed that left rostral prefrontal cortex as a seed was positively correlated to right insular cortex (IC), right orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC). Moreover, left anterior insular cortex as a seed was negatively correlated to both lateral intra-calcarine cortex, supra-calcarine cortex, and visual medial cortex.

Discussion   This study showed that the correlation between greater interoceptive accuracy and greater activity of left rostral prefrontal cortex and left insular cortex remains in older adults. The findings suggested that left rostral prefrontal cortex performs positive feedback in salience network of IC, OFC, and ACC and left insular cortex perform negative feedback to visional exteroception to remain interoceptive accuracy.

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id #12529

Nanoscale imaging of TDP43 in primary cortical neurons with expansion microscopy

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Expansion Microscopy is a recently developed imaging method that enables nanoscale resolution of biological samples using a conventional microscope. Here we employ this protocol to visualise the localized pattern of RNA binding protein (RBP) expression in primary cortical neurons, *in vitro*. We have found that TDP43, an RBP known to play an important role in alternative splicing, localizes to the post-synaptic compartment in an activity-dependent manner, suggesting a potential role for TDP-43 in RNA localization. These findings demonstrate the utility of expansion microscopy to reveal new activity-dependent processes in neurons that, using standard approaches, would otherwise remain hidden from view.

id #12540

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^{PBN}) suppress water intake, but not food or highly caloric liquids such as Ensure, suggesting Oxtr^{PBN} neurons may differentiate solutions by their caloric content and/or palatability; however, their effect on intake of other solutions has not been investigated. Here, we examined whether Oxtr^{PBN} neurons regulated consumption of caloric solutions (ethanol, sucrose, Ensure), and non-caloric solutions (saccharin, salt). $Oxtr^{Cre}$ mice were injected with the Cre-dependent stimulatory DREADD, hM$_3$Dq, 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[PBN] neurons were activated by injecting the designer drug, CNO (3 mg/kg ip), we observed a significant decrease in 15-minute intake of all solutions ($P<0.05$). There was also significantly decreased intake of low palatable, non-caloric solutions at 2 hours, but not of highly caloric and/or palatable solutions, suggesting the major effect of Oxtr[PBN] 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 and 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.

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id #12606

Cross-streams through the Thalamus to Relay Vibrissal Information

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Ventral posteromedial thalamic nucleus (VPM) relay different mouse vibrissal information through different pathways. There may be the subcortical pathways though the VPM neuclei between lemniscal pathway and multi-extralemniscal pathway to surport the multi-whiskers characteristics of the barrel columns in primary somatosensory cortex (S1), except the intracortically trans-columnar processes. The cross-streams between those two parallel thalamic pathways maybe exist to explain this phenomenon. While the global patterns of VPM projections are well-known, the complete morphology of individual VPM neurons had never been investigated, and the inputs originating from SP5i to VPM remain to be investigated further. Using sparsely and brightly viral strategy to label VPM neurons, and whole-brain reconstruction of optical images at 1-μm resolution, we reconstructed the complete morphology of individual VPM neurons. Also using dual-viral strategy, we identified the projections of SP5i in VPM. We found that the new type in VPMvl mainly project to barrel columns in S1 and secondary somatosensory cortex (S2), typical VPMvl to septa region extending to barrel columns and S2, and the typical VPMc to barrels. And the new type are similar to the VPMc ones in branches and length of dentrites and axons, projections in S1 and bouton density in layers, while different from those of the tipical VPMvl ones. Also, VPMdm neurons are sufficiently innervated by SP5i. Therefore, there are new anatomical pathways for the cross-streams of vibrissal sensory that the signals are conveyed from multi-whiskers VPMvl to barrel columns, and from multi-whiskers SP5i nuclei through VPMdm to barrel columns.

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id #12624

A novel weight lifting task for investigating effort and persistence in rats

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Here we present a novel effort-based task for laboratory rats: the weight lifting task (WLT). Studies of effort expenditure in rodents have typically involved climbing barriers within T-mazes or operant lever pressing paradigms. These task designs have been successful for neuropharmacological and neurophysiological investigations, but both tasks involve simple action patterns prone to automatization. Furthermore, high climbing barriers present risk of injury to animals and/or tethered recording equipment. In the WLT, a rat is placed in a large arena and tasked with pulling a rope 30 cm to trigger food delivery at a nearby spout; weights can be added to the rope in 45 g increments to increase the intensity of effort. As compared to lever pressing and barrier jumping, 30 cm of rope pulling is a multi-step action sequence requiring sustained effort. The actions are carried out on the single plane of the arena floor, making it safer for the animal and more suitable for tethered equipment and video tracking. A microcontroller and associated sensors enable precise timestamping of specific behaviours to synchronize with electrophysiological recordings. We validated the task across five cohorts of rats (total n=35) and report consistent behavioural metrics. The WLT is well-suited for neuropharmacological and/or *in vivo* neurophysiological investigations surrounding effortful behaviours, particularly when wanting to probe different aspects of effort expenditure (intensity vs. duration).

id #12638

Altered gut microbiome in an Australian cohort of Parkinson's disease, and the influence of bacterial endotoxin in an α-synuclein over-expressing mouse model.

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The interaction between the gut microbiota and alpha-synuclein (αSyn) aggregation in Parkinson's disease (PD) is receiving increasing attention. The objective of this study was to investigate gut microbiota, and effects of an inflammatory lipopolysaccharide (LPS) trigger in a human αSyn over-expressing mouse model of PD (Thy1-αSyn). Stool samples from patients with confirmed PD and Thy1-αSyn mice were analyzed using 16S ribosomal RNA sequencing. Compared to healthy controls, the relative abundance of mucin-degrading Verrucomicrobiae and LPS-producing Gammaproteobacteria were greater in PD patients. In mice, the abundance of Gammaproteobacteria was negligible in both Thy1-αSyn and wild-type (WT) animals, while Verrucomicrobiae were reduced in Thy1-αSyn mice. The effect of LPS on intestinal barrier function was investigated *in vivo* via administration of LPS in drinking water to Thy1-αSyn mice. LPS administration in Thy1-αSyn mice resulted in the emergence of early motor manifestations at 10 weeks, compared to untreated mice who were still asymptomatic at this age. This study reaffirms that an altered microbiome exists in patients with PD, and supports the notion of a proinflammatory gut microbiome environment as a trigger for PD pathogenesis.

id #12639

Variation in bacterial sensing peptidoglycan recognition proteins modulates age-of-onset in patients with idiopathic Parkinson's disease

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Parkinson's disease (PD) is an incurable, debilitating neurodegenerative disorder. PD is increasingly viewed as an autoimmune condition arising from complex interactions between genetic and environmental factors causing chronic inflammation, oxidative stress and neuronal damage. Research is focussing on the gut as the starting point for chronic inflammation, as many PD patients exhibit a pro-inflammatory gut microbiome (dysbiosis) and intestinal inflammation, with many reporting gastrointestinal complaints years before diagnosis. Although various environmental factors (pesticides, herbicides and heavy metals) are associated with PD risk, exposure is not always causative. As these factors can cause dysbiosis and intestinal inflammation, this study investigated genetic variants in genes responsible for binding of bacterial metabolites and subsequent pro-inflammatory cascades: toll-like receptors (TLR) and CD14 (lipopolysaccharide receptors) and peptidoglycan recognition proteins (PGLYRP) (peptidoglycan receptors). This study utilised DNA samples

from an Australian cohort of PD ($n = 200$) and age-matched healthy controls ($n = 100$) to analyse the following genetic variants: TLR1 (rs4833095), TLR2 (rs3804099), TLR4 (rs7873784), CD14 (rs2569190), PGLYRP2 (rs892145) and PGLYRP4 (rs10888557). Chi squared analysis revealed no significant association between candidate variants and PD risk. Within-PD analysis indicated that mean age of PD onset was significantly different between PGLYRP4 genotypes ($p<0.05$). Multivariate regression analysis confirmed that compared to heterozygotes, PGLYRP4 CC genotype is associated with 4.3-year earlier onset of disease ($p=0.028$), after controlling for toxin exposure and other cofounding variables. These results demonstrate genetic variation in bacterial receptors may modulate age-of-onset in idiopathic PD, however future studies should investigate more co-variates in a larger cohort.

id #12683

Unexpected promoter activity of protein cording sequence in bicistronic constructs confer constitutive gene expression of second gene in bicistronic inducible gene expression vector.

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Cre-inducible gene expression systems such as lox-STOP-lox cassette is widely used for overexpression of transgenes. Multiple proteins are able to be over-expressed under the transcriptional control of a single promoter by self-cleaving 2A peptide and lox-STOP-lox cassette. Using lox-STOP-lox and P2A peptide, we generated a Cre-responsive bicistronic vector, pCAG-lox-GFP-STOP-lox-rtTA-P2A-mCherry, expressing nucleus targeted GFP in the absence of Cre, and expressing the reverse tetracycline-controlled transactivator (rtTA) plus membrane targeted mCherry after Cre-mediated recombination. Unexpectedly, upon transfection into cells, $> 7\%$ of transfected cells misexpressed mCherry without Cre recombination, whereas rtTA was expressed in a strictly Cre-dependent manner. When the order of mCherry and rtTA coding sequences was swapped, mCherry expression was tightly regulated but rtTA expression became leaky (i.e., the second gene is leaky). Changing or removing promoter did not prevent the leaky expression. In silico analysis revealed multiple putative transcription factor binding sites in both mCherry and rtTA CDS. Putting another lox-STOP-lox cassette after 2A sequence dramatically reduced the leaky expression. This study indicates that CDS located 3' region of bicistronic vector with inducible expression construct is leaky because CDS located at the 5' region behave as a promoter. Our data suggest that bicistronic vectors incorporating inducible protein expression cassette must be used with considering small expression of CDS located at 3' region.

id #12725

Investigating changes in androgen and progesterone receptor expression in the aetiology of PNA-induced polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a female endocrine disorder that is associated with androgen excess. It is hypothesised that high levels of this typically male hormone in specific windows of development may cause changes in gene expression of the progesterone receptor (PR) in the arcuate nucleus, which may drive impaired negative feedback of progesterone in the gonadal axis in PCOS. However, the impact of androgen excess on PR expression, and whether the androgen excess is involved in the modification of neural circuitry in the developing brain, is not known.

To address these knowledge gaps, we first investigated the effect of androgen on the expression of steroid hormone receptors in the arcuate nucleus at different developmental times using prenatal androgen exposed (PNA) female mice. Quantitative PCR showed a significant decrease of androgen receptor (AR) mRNA expression in PNA mice at postnatal day 60 compared to vehicle controls. No significant difference was observed in PR mRNA at any stages. Experiments investigating the PR and AR mRNA expression in the arcuate nucleus by RNAscope is ongoing.

Second, we tested the direct action of androgen on arcuat γ-aminobutyric acid (GABA) neuron development, as these cells are known to exhibit clear morphological and gene expression changes following PNA treatment. Using VGaT-ires-Cre/tdTomato mice, we are investigating the effect of androgen on GABA neuron morphology and axonal and dendritic development.

Together, these experiments will lead to clearer understanding of the impact of androgen actions in the developing female brain, in both the initiation and maintenance of PCOS.

id #12745

Early Patterns of Excitation-induced Neuronal Translation are Regulated Independently of mRNA Levels and MicroRNA

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Experience-dependent changes to neural circuitry are shaped by spatially and temporally coordinated patterns of activity-associated mRNA translation. Although mRNA translation in the neuron is known to be distinctly complex relative to other cell types, the post-transcriptional mechanisms which fine-tune this process remain obscure. In the current study, we employed ribosome profiling to investigate transcriptome-wide profiles of mRNA translation in neuronally differentiated neuroblast cultures at multiple time-points after whole cell depolarisation, and assessed the contribution of post-transcriptional regulatory mechanisms using mRNA-Seq and small RNA-Seq. Immediately after depolarisation, many genes with known function at the synapse displayed a surge in translational activity without corresponding changes in mRNA levels. At later time-points, however, mRNA translation

became synchronised with underlying mRNA levels, implying there are different layers of post-transcriptional regulation which are temporally separated but become coordinated over time. Analysis of microRNA (miRNA) and novel tRNA-derived small RNA fragments (tRFs) revealed these molecules were subject to peak changes in expression when mRNA translation was decoupled from mRNA levels, however they exhibited strongest association with mRNA stability. Translational fluctuations were found to be more highly influenced by intrinsic mRNA properties, such as mRNA length, GC content, secondary structure and the expression of RNA binding protein motifs. Our findings highlight that mRNA translational status and abundance are transiently regulated independently after neuronal excitation, with contributions from both small RNAs and inherent transcript features.

id #12753

REGULATION OF ADRENAL TH AND PNMT IN RESPONSE TO CHRONIC STRESS; DOES THE TYPE OF STRESSOR MATTER?

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The adrenal medulla plays a major role in stress response by producing and releasing catecholamines into the bloodstream. We aimed to compare the effects of different types of chronic stress including chronic unpredictable mild stress (CUMS), restraint stress (RS), chronic corticosterone (CORT) administration and maternal separation with early weaning (MSWEW) on the catecholamine synthetic capacity of the adrenal medulla. Specifically, we have assessed adrenal tyrosine hydroxylase (TH) protein and phosphorylation at three serine residues Ser19, Ser31 and Ser40 and phenylethanolamine N-methyltransferase (PNMT) protein in C57BL6 mice using western blot techniques. CUMS, in which only male mice were used, produced a decrease in the phosphorylation of Ser19, 31 & 40 of TH and PNMT protein levels, while RS, performed with both genders, increased phosphorylation of Ser31 & 40 of TH in female mice, with no changes seen in male mice. Chronic CORT administration, performed on both genders, had no effect on TH phosphorylation, but decreased adrenal PNMT protein levels, while MSWEW, in which only male mice were used, only resulted in decreased phosphorylation of Ser19 of TH. Thus, we have found that these different types of chronic stress did not significantly alter TH protein but produced distinct effects on site-specific TH phosphorylation and PNMT protein in the adrenal medulla, suggesting differential effects of each model and mice gender on the catecholamine synthetic capacity of the adrenal gland.

id #12769

Olfactory fear conditioning enhanced neuroplasticity and neurogenesis in the different brain regions of adults rat

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Odors have proven to be the most resilient trigger for memories of high emotional saliency. Fear associated olfactory memories pose a detrimental threat of potentially transforming into severe mental illness such as fear and anxiety related disorders. Many studies have deliberated on auditory, visual and general contextual fear memory processes; however, fewer studies have investigated mechanisms of olfactory fear memory. Here, we investigated the neurocircuitry of olfactory fear memory acquisition and consolidation. We used Pavlovian fear conditioning in adults rats to determine whether recollection of olfactory memories changes the proliferation of newborn neurons or leads to neuroplasticity of neurons in the olfactory pathway, amygdala and the hippocampus which are associated with fear memory. We used the plasticity marker CREB, PMAPK and EdU (5-ethynyl-2'-deoxyuridine) labelling to study neuroplasticity and neurogenesis of different brain regions after olfactory fear memory. Our results showed that the olfactory fear conditioning resulted in a significant increase in number of PMAPK positive neurons in the medial and cortical subnuclei of the amygdala and the piriform cortex. We also found that there was a significant increase in number of EdU positive neurons in the dentate gyrus of the hippocampus and the glomerular layer of the olfactory bulb 24 hours and 14 days post-conditioning. These findings contributed to the complete understanding of the neurocircuitry of olfactory fear conditioning and the role of neurogenesis in olfactory fear memory consolidation.

id #12772

Neuronal degeneration in the Marmoset Pulvinar following V1 lesions.

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Past research on damage to the primary visual cortex (V1) has highlighted the importance of the pathway linking retina, lateral geniculate nucleus (LGN) and V1 to conscious vision processing. However, there exists significant debate on the contribution of visual pathways through the thalamic pulvinar nuclei, which have substantial connections with visual areas.

We investigated anatomical changes in the pulvinar complex following V1 lesions, to better understand the potential contribution of these nuclei as alternative pathways.

Six marmoset brains (3 with unilateral adult V1 lesions, and 3 age-matched controls) were immunohistochemically stained for neuronal marker NeuN. Stained sections were analysed in Aperio Imagescope software for neuronal density and volume of the subdivisions of the pulvinar.

Varying degrees of volume loss were found in the lateral and inferior pulvinar divisions, which have known connections to visual areas. There was no volume loss in the medial pulvinar. Quantification of pulvinar neurons also showed reduced neuronal density compared to controls.

Loss of V1 causes clear neuronal degeneration to structures in the main pathway like the LGN. The pulvinar, unlike the LGN, also has connections to multiple visual areas besides V1. Despite the loss of V1 input, the subtle degeneration in the pulvinar suggests the bulk of neural circuitry connected to other areas remains intact. This lends support to the pulvinar's potential role in an alternative visual pathway.

id #12777

Spatially distinct patterns of RNA modification in neurons following fear extinction learning in mice

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RNA modification has recently emerged as an important aspect of RNA metabolism by promoting RNA localization, translation and/or RNA degradation in a variety of biological contexts. Previously, we found a role for the RNA modification, N6-methyladenosine (m^6A), in the formation of fear memory (Widagdo et al, Journal of Neuroscience, 2016). However, the molecular mechanisms associated with m^6A in memory formation beyond fear learning remain unknown. We therefore examined the subcellular localisation of m^6A-modified transcripts in the adult prefrontal cortex in response to fear extinction learning, and discovered a unique pattern of RNA methylation at the synapse that appear to be associated with RNA binding proteins known to be involved in RNA localisation. Together, these findings suggest a critical role for RNA methylation in regulating experience-dependent patterns of subcellular compartmentalization of RNA, which could have a significant impact on local translation events in response to fear extinction learning.

id #12781

Histological Characterisation of the Retrotrapezoid Nucleus in the Piglet and Effects of Hypercapnic Hypoxia

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Phox2b-expressing chemoreceptor neurons in the retrotrapezoid nucleus (RTN) are CO_2-responsive, and critical in mediating the central respiratory chemoreflex. They directly sense CO_2/H^+ and adjust ventilation to rapidly regulate CO_2 excretion and short-term acid-base balance. Chemoreception is a vital autonomic function, which may be impaired in Sudden Infant Death Syndrome (SIDS). However, despite decades of research, SIDS is a diagnosis of exclusion and its pathophysiology remains elusive. Human autopsy tissue is scarce, and as such piglets are often used as developmentally-appropriate animal models to mimic the cardiorespiratory vulnerabilities of human infants. We aimed to identify the RTN in a piglet model of SIDS using the anatomical (ventral to the facial motor nucleus) and histological ($Phox2b^+$, tyrosine hydroxylase$^-$) definitions of the RTN, using immunohistochemistry in archival formalin-fixed paraffin-embedded tissue. The location and neurochemical signature of the piglet RTN were found to be homologous to the rodent and primate RTN. In piglets exposed to a one-day SIDS-like paradigm of intermittent hypercapnic hypoxia (IHH), there was no statistically significant difference in the number of RTN neurons compared to controls ($p > 0.05$). However, this data was based on a snap-shot of the RTN, rather than serial counts along its full rostrocaudal extent, due to limited tissue availability. Further research, including prolonged exposure to IHH and assessment of activation status of RTN neurons, is necessary to clarify the consequences of IHH exposure on the developing piglet RTN.

id #12782

Investigating the Effects of Edaravone in an Animal Model of Frontotemporal Dementia

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Dementia is considered the second leading cause of death in Australia in which Alzheimer's disease (AD) and frontotemporal dementia (FTD) represent the most common forms. They are characterized by the accumulation of toxic amyloid plaques and neurofibrillary tangles of tau protein in certain brain regions which are responsible for memory and learning. Possible predisposing factors of dementia include genetic mutation and insulin resistance in which oxidative stress was confirmed to be a significant component of the underlying pathophysiology. This study will explore, for the first time, the effect of the antioxidant drug, Edaravone, to reduce oxidative stress on animal model (P301L mice) of frontotemporal dementia. Results showed improvement in memory and motor functions with a slight reduction in tau pathology in the hippocampus of these mice. There was a significant reduction in 4-HNE (reactive oxygen species) in the brain with a modest reduction of hyperphosphorylated tau in the amygdala. These results show that edaravone could be a potential drug candidate for improving the prognosis of dementia.

id #12790

OVER-EXPRESSION OF PROBDNF BY AAV VECTOR IN MUSCLE TRIGGERS DEPRESSION-LIKE BEHAVIOURS IN MICE.

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Major depression is a leading cause of morbidity and disease burden in the modern society. However, the mechanisms underlying its development remain unknown. In the last several years, our group have found that proBDNF, a precursor of brain derived neurotrophic factor (BDNF), and its receptor are highly upregulated in patients with major depression and in animal models of depression induced by chronic stress. We further found that blocking proBDNF in the central nervous system (CNS) and in the periphery can alleviate depression-like behaviours in rodents. Here we hypothesize that proBDNF may be a pathogenic factor triggering major depression.

In a pilot study, 24 mice were injected in the bilateral gluteus maximus muscle with AAV-proBDNF or AAV-EGFP. Two weeks after injection, all animals were subjected for behavioural tests. In a separate study, 36 mice were divided into three groups, one group of mice were also treated with proBDNF blocking agent following AAV-proBDNF injection. After behavioural test, the muscle and liver tissues were collected for proBDNF immunostaining. Mice injected with proBDNF triggered time-dependent decrease in immobility in the tail suspension test (TST) and forced swim test (FST), reduced sucrose consumption, and decreased in grooming time after sucrose spray. Treatment with proBDNF antagonist p75ECD-Fc can alleviate above depressive-like symptoms. Histological examination showed that proBDNF is expressed in the injected muscle and hepatocytes.

Conclusion: Peripheral proBDNF is a likely pathogenic factor which triggers depression-like behavioural changes in mice. ProBDNF may be a therapeutic target for developing drugs for major depression.

id #12801

GABA$_A$ Receptors Are Well Preserved in the Hippocampus of Aged Mice

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Gamma-aminobutyric acid (GABA) is the dominant inhibitory neurotransmitter in the nervous system serving as the major effector of the neuronal excitation. Subunit composition of the GABA A receptors (GABAARs) is associated with the affinity of GABA binding and its downstream inhibitory actions. Fluctuations in subunit expression levels with increasing age have been demonstrated in animal and human studies. However, our knowledge regarding the age-related hippocampal GABAAR expression changes is limited and based on rat studies. Western blotting and fluorescence immunohistochemistry were performed on the mouse brain tissues from young (6 months) and old (21 months) age groups to detect and compare GABAAR α1, α2, α3, α5, β3 and γ2 subunit expression within the Cornu Ammonis

1 (CA1), CA2/3 and dentate gyrus (DG) regions of the hippocampus. GABAergic system is robust, with no significant age-related differences in GABAAR α1, α2, α3, α5, β3 and γ2 subunit expression level between the young and old age groups in any of the hippocampal regions examined. However, we detected a localized decrease in α2 subunit expression around the soma, proximal dendrites and in the axon initial segment of pyramidal cells in the CA1 and CA3 regions that is accompanied by a pronounced upregulation of the α2 subunit immunoreactivity in the neuropil of aged mice. GABAARs are well preserved in the mouse hippocampus during normal aging although GABAARs in the hippocampus are severely affected in age-related neurological disorders, including Alzheimer's disease.

id #12806

FUNCTIONAL CHARACTERIZATION OF *GRIN2A* MUTATIONS IN LANDAU-KLEFFNER SYNDROME

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Landau-Kleffner syndrome (LKS) is a rare epileptic encephalopathy. Mutations in *GRIN2A*, encoding the NMDA receptor GluN2A subunit, have been reported in 8-20% of individuals with LKS and related epilepsy aphasia spectrum disorders. Functional investigations of GluN2A missense mutations have demonstrated both loss and gain of function effects. Here we report the identification of two *GRIN2A* mutations (p.R518C and p.R518H) in the S1-M1 linker in individuals with LKS. The functional consequences of the identified missense mutations were investigated using: (i) an over-expression HEK293 cell model to study gene expression, protein expression and cell-surface localization and; (ii) artificial synapses and whole-cell patch clamping to study ion channel function. Despite previous reports linking the p.R518H variant to a gain of function (increased open-time and decreased closed-time durations) both mutants were found to have significantly reduced cell-surface trafficking. Electrophysiology studies recorded excitatory post-synaptic currents (EPSCs) in cells expressing wild-type receptors, but there was no evidence for EPSCs mediated by cells expressing the p.R518C or p.R518H mutants. Additionally, there was no evidence for whole-cell glutamate-activated currents in cells expressing these mutants. Our data suggest that in a cellular and synaptic context, both GluN2A-R518H and the novel GluN2A-R518C variant represent loss-of-function mutations. This study highlights that *GRIN2A* mutations lead to LKS through diverse pathophysiological mechanisms and that careful study of receptor function is an essential requirement for future treatments based on personalized medicines.

id #12807

MISSENSE MUTATIONS IN THE NMDAR GLUN2B SUBUNIT ALTER RECEPTOR LIGAND BINDING AND ION CHANNEL PROPERTIES

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Genetic and bioinformatic analyses have identified missense mutations in *GRIN2B* encoding the NMDA receptor GluN2B subunit in autism, intellectual disability, Lennox Gastaut and West Syndromes. Here, we investigated several such mutations using a hybrid 3D model of the human NMDAR and studied their consequences using electrophysiology. Selected GluN2B subunit mutants revealed reductions in glutamate potency, increased receptor desensitisation or ablation of voltage-dependent Mg^{2+} block. In addition, our NMDAR model provides new views of binding sites for Mg^{2+}, and for memantine which has been utilised as a personalised medicine in individuals with NMDAR gain-of-function mutations. Functional studies also revealed that the West syndrome NMDAR channel mutant $GluN2B^{V618G}$ unusually allowed Mg^{2+} permeation, whereas nearby $GluN2B^{N615I}$ reduced Ca^{2+} permeability. Importantly, these two mutants also differed in their response to memantine - either increasing ($GluN1\text{-}GluN2B^{N615I}$) or reducing ($GluN1\text{-}GluN2B^{V618G}$) memantine potency compared to wild-type GluN1-GluN2B in zero Mg^{2+} at -30 mV. This study highlights that *GRIN2B* mutations lead to different neurological disorders via diverse pathophysiological mechanisms and that careful study of NMDAR function is an essential requirement for future treatments based on personalized medicines.

id #12817

Neuroinflammation in the Human Cingulate Cortex in Huntington's disease.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disease in which patients presents with the loss of voluntary movement resulting in dance-like movements, behavioural and psychiatric symptoms along with cognitive decline. Significant evidence of neuroinflammatory changes in HD is evident in the literature. The cingulate cortex plays a vital role in learning, memory and emotion processing. Previous research in our laboratory suggests that the cingulate cortex is affected in HD, and mood symptoms in HD cases are linked with major cell loss in the cingulate cortex. Our study is the first to examine neuroinflammatory changes in the cingulate cortex in human HD post-mortem tissue. We

aimed to investigate whether there is significant neuroinflammation in the cingulate cortex of human HD case, with a particular focus on astrocytes and microglia. This was carried out by using immunohistochemistry to identify markers of inflammation in tissue sections of the cingulate cortex of HD cases and controls. Our preliminary data suggests that there is no significant difference in the staining intensity of microglia in HD. However we see qualitative changes in these microglia which suggests the need to further quantify morphological changes in microglia. Understanding the role of astrocytes and microglia in the human cingulate cortex and how they correlate to cell death and symptomology may help us understand the underlying mechanisms of cell death in HD. Understanding this will help us in the development of targeted therapies for HD.

id #12822

Roles for gliotransmitters in long-range metaplasticity at hippocampal synapses

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Long-term potentiation (LTP) is an activity-dependent long-lasting increase in the efficacy of synaptic transmission that is vital for learning and memory. LTP is regulated by past events, i.e. by *metaplasticity*, which refers to activity-dependent changes in neuronal state that orchestrate the magnitude of future synaptic change. Previously, we showed that high-frequency priming stimulation in the basilar dendritic stratum oriens (SO; 6x100 Hz) inhibits subsequent LTP (2 x TBS) elicited in the apical dendritic stratum radiatum (SR) of area CA1 of the hippocampus. This heterodendritic metaplasticity is independent of postsynaptic depolarization and action potential firing and may instead be mediated by activation of nearby glial cells such as astrocytes. Here we tested potential astrocytic mechanisms mediating this effect in acute hippocampal slices prepared from male Sprague-Dawley rats. Field excitatory postsynaptic potentials were recorded either extracellularly or via patch-clamped astrocytes in area CA1 following stimulation of Schaffer collateral fibres in SR.

Bath application of tumour necrosis factor-α (TNFα, 1.18 nM, 10 min) impaired LTP 20-30 min later, but this effect was blocked by clamping astrocytic Ca^{2+} at basal levels, In contrast, blocking putative extrasynaptic NMDA receptors with GluN2B but not GluN2A antagonists did block the priming effect. Our data point to a model whereby priming causes the release of gliotransmitters such as TNFα, ultimately leading to the release of glutamate from glial cells that binds to extrasynaptic GluN2B receptors on neurons to inhibit the later induction of LTP.

id #12825

EXPRESSION PROFILING OF *REST* AND *RCOR* GENES IN NEUROGENESIS USING 2D AND 3D HUMAN PLURIPOTENT STEM CELL MODELS

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Repressor element-1 silencing transcription factor (REST) is a transcriptional repressor of neuronal genes that forms a complex with the corepressor of REST1 (CoREST1), CoREST2 or CoREST3 (encoded by *RCOR1*, *RCOR2* and *RCOR3*, respectively). Emerging evidence suggests the CoREST family have the ability to target unique genes, in a REST-independent manner, in various neural and glial cell types at different stages of development. Research on *REST* and *RCOR* genes has been largely based on animal models and established cell lines. Accordingly, human stem cells offer an effective *in vitro* cell-based model towards defining the expression profile of *REST* and *RCOR* genes in neurogenesis and thus provide insight into their function during neurodevelopment. This study used 2D and 3D stem cell models to interrogate *REST* and *RCOR* gene expression levels during neural differentiation using RT-qPCR and Nanostring. Human pluripotent stem cells (hPSCs) were differentiated into glutamatergic cortical neurons and GABAergic ventral neurons using a small-molecule inhibitor driven approach, functional cortical induced neurons (iNs) via neurogenin-2 (*NGN2*) overexpression and 3D cerebral organoids matured for 9 months. In line with previously published findings, *REST*, *RCOR1* and *RCOR2* mRNA levels decreased with neuronal differentiation. However, *RCOR3* expression increased in cortical and ventral neurons, iNs and cerebral organoids. Further work is required to identify the function of CoREST3 in neurons. In summary, this study has defined an expression pattern for *REST* and *RCOR* genes in neurogenesis using 2D and 3D hPSC models.

id #12826

Two arms of the Unfolded Protein Response (UPR) are increased in the SIDS brainstem and cerebellum

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The unfolded protein response (UPR) has links to multiple neurodegenerative diseases, and has been implicated in a subset of infants who died suddenly and unexpectedly and diagnosed as Sudden Infant Death Syndrome (SIDS). Recent work from our laboratory showed an increase in phosphorylated protein kinase R (PKR)-like endoplasmic reticulum (ER) kinase (p-PERK) – the enzyme that governs one of the arms of the UPR – in the hypothalamus of SIDS infants ($p < 0.000$) (Hunt et al., 2015). We aimed to determine whether this effect was restricted to the hypothalamus or was more widespread in the brain. P-PERK and ATF6 (a 2nd arm of the UPR) were immunohistochemically stained and quantified in the brainstem pons, medulla and cerebellum of SIDS (n=27) compared to non-SIDS (n=12) infants. There was a

significant increase in p-PERK in the cuneate nucleus (p=0.035) and ATF6 in the inferior olivary nucleus (p=0.015) in SIDS. These results indicate that differing arms of the UPR are altered in brainstem medullary regions of SIDS infants known to control proprioception and balance co-ordination (ION).

id #12827

Exploring the relationship between metabolic indicators of neuroinflammation, excitatory neurotransmission, and ERP responses during a continuous performance test in mild cognitive impairment (MCI) due to AD.

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Neurodegenerative diseases such as Alzheimer's Disease (AD) and the prodromal phase, mild cognitive impairment (MCI), are characterised by accelerated age-inappropriate cognitive decline. Currently, there are no early interventional strategies for MCI and it is not known what causes some patients to progress to AD. Previously, it has been shown that deficits in executive function can be a strong predictor for MCI conversion to AD. Furthermore, deficits in executive function are also the strongest cognitive predictor of future functional impairment. It is well known that many aspects of executive function are mediated by monoamine neurotransmitters, such as dopamine, norepinephrine, and epinephrine. However, it is unknown what event-related potential (ERP) components underpinning executive function are affected by MCI-derived cognitive decline, and how they relate to metabolic indicators of inflammation and neurotransmission. This study will explore ERP component outcomes for people with MCI in comparison to cognitively normal people during the AX variant of a continuous performance test (CPT). Additionally, we will explore the relationship between ERP component amplitudes and cognitive deficits with peripheral neurotransmitters and inflammatory mediators. We predict that AX-CPT ERP components underpinning executive function (e.g., N2) will be attenuated in people with MCI compared to HCs, and that this relationship will be mediated by dysregulation of monoamine neurotransmitter levels and pro-inflammatory cytokines (e.g., TNF-alpha, IL-6, IL-1beta). Overall, this study aims to better characterise the neural substrates that underlie cognitive deficits in MCI.

id #12828

Secreted amyloid precursor protein-alpha enhances amyloid-beta protofibril degradation in primary murine astrocytes

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Secreted amyloid precursor protein-alpha (sAPPα) is a neurotrophic, neuroprotective and plasticity-enhancing peptide, which has been shown to protect neurons against amyloid-beta (Aβ) toxicity. Though extensively studied in neurons, the effects of sAPPα on astrocytes are currently unknown. Given previous findings that astrocytes were ineffective in their ability to degrade Aβ protofibrils, we used immunocytochemistry to investigate whether co-administration of 1 nM sAPPα to Aβ-insulted primary murine astrocytes could enhance astrocytic degradation of Aβ inclusions. While we found that co-administration of sAPPα had no effect on the number of viable cells (control: 67 ± 15; sAPPα: 62 ± 6; Aβ: 69 ± 10; sAPPα + Aβ: 66 ± 12, $F_{(1.256, 2.512)} = 0.2206$; $p = 0.7265$), we observed a significant reduction in the size (Aβ: 1172 pixels/cell ± 23.8; sAPPα + Aβ: 883.5 pixels/cell ± 51.0, $t_{(2)} = 4.413$, $p = 0.0477$), but not the number (Aβ: 2.9 inclusions/cell ± 0.1; sAPPα + Aβ: 2.8 inclusions/cell ± 0.4, $t_{(2)} = 0.3499$, $p = 0.7599$) or average fluorescence signal (Aβ: 46.7 ± 5.6; sAPPα + Aβ: 40.3 ± 7.3, $t_{(2)} = 0.6832$, $p = 0.5650$), of the Aβ inclusions. To our knowledge, these results constitute the first evidence that sAPPα may protect astrocytes as well as neurons from Aβ toxicity.

id #12834

GABA$_A$ Receptor Subunits are Expressed by the Human Cerebral Vasculature

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Objective: γ-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the mammalian brain. GABA signalling involves the binding of extracellular or synaptic GABA to ionotropic GABAA receptors (GABAARs) and metabotropic GABAB receptors. In addition to its role in neurotransmission, GABA has been shown to act directly on the cerebral vasculature to mediate functions including vasomotor activity, collagen remodelling and developmental angiogenesis. The molecular basis of these functions has not been

explored. In this study, we investigated the cerebrovascular expression of GABAARs in the post-mortem human middle temporal gyrus (MTG) and in primary human brain pericytes and human cerebral microvascular endothelial cells. **Methods:** Fluorescence immunohistochemistry and confocal imaging were utilised to detect protein expression in the capillaries of post-mortem middle temporal gyrus (MTG) sections and fixed vascular cells. RNA expression in cell cultures was confirmed with NanoString nCounter analysis and protein expression with Western blot. **Results:** We report, for the first time, the robust cerebrovascular expression of the beta3, gamma3 and epsilon GABAARs subunits in the human MTG vasculature and in cultured human cerebrovascular cells. Other GABAAR subunits were expressed at low levels. Some GABAAR subunits like alpha1 were not found to be expressed in the MTG vasculature, in contrast to their widespread incorporation into neuronal GABAARs. **Conclusion:** GABAARs are expressed on the cerebral vasculature, potentially underlying the GABA-mediated vascular functions demonstrated in previous studies. The functional assembly and pharmacological relevance of these putative vascular GABAAR subtypes remains to be determined.

id #12843

NOVEL INHIBITORY OPTOGENETIC APPROACHES TO UNDERSTAND NEURONAL CIRCUITS IN VIVO

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Background: The light-activated chloride channel, GtACR2, potently silences brainstem neuron activity. To increase our ability to use this tool to understand circuit dynamics *in vivo*, we developed three different adeno-associated viral (AAV) vectors designed to express GtACR2 in distinct neuronal compartments. These AAV vectors were injected into the pre-Botzinger complex (PreBotC), a brainstem region that contains the inspiratory central pattern generator (CPG) for breathing. Inhibition of the PreBotC leads to apnea, decreased heart rate (HR), and altered blood pressure (BP). **Methods:** Three weeks after the injection into Sprague Dawley rats, BP, HR, and diaphragm muscle activity (dEMG) were recorded under anesthesia. Photoinhibition was induced by laser stimulation delivered by optical fibres (50Hz, 30-60 s). **Results:** In rats with non-selective expression of GtACR2 (GtACR2-YFP; n=4), or predominant somatic expression (GtACR2-muGFP-Kv2.1; n=5), in the PreBotC, photoinhibition induced apnea that, in some cases lasted the entire duration of the stimulus, in addition to bradycardia. When GtACR2 was expressed predominantly in the axon/terminal compartment (GtACR2-HA-neurexin; n=4) photoinhibition induced only bradycardia, without apnea. **Conclusion:** We hypothesize that the inspiratory drive from PreBotC involves a direct projection from the nucleus, whilst the cardiac effect involves an interneuron synapse within the PreBotC. These new optogenetic inhibitory constructs provide promise as tools which enable understanding of circuit connections.

id #12848

Long term behaviour outcomes of maternal obesity and hypoxic-ischemic brain injury on rat offspring

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Maternal obesity is associated with pregnancy-related complications such as birth asphyxia and neurodevelopmental deficits. Neonatal hypoxia-ischemia (HI) causes delayed brain development and motor dysfunction. While both maternal obesity and HI can induce brain and behavioural deficits, the long term outcomes of both insults combined has not been examined previously.

Female Sprague Dawley rats were allocated to chow (n=8) or High Fat Diet (HFD; n=10) groups for 7 weeks followed by mating with chow-fed males. On postnatal day 7 (P7), pups underwent unilateral occlusion of the right common carotid artery (HI) or sham surgery followed by hypoxia (7.5% O2, 3 hours). Behavioural outcomes were assessed between 6-14 weeks and brains collected for histological analysis.

Before mating, HFD mothers were 12% heavier than chow (p<0.05, t-test). At weaning both male and female sham offspring of HFD mothers were heavier than chow (male chow vs HFD; 17.5% and female chow vs HFD; 18.4%, n=15-19, p<0.05, t-test) with no effect of HI. At 13 weeks male chow-HI were 9% lighter than chow sham (p<0.05, t-test, n=20, 20). When testing motor function, male and female HI rats demonstrated more foot fault errors than respective shams (p<0.05 Tukey's post-hoc) with no effect of HFD. No significant differences were found between groups of either sex in anxiety measures (elevated plus maze, open field test), 3 chamber social interaction test, novel object and novel place test.

These data suggest minimal detrimental effects of maternal obesity and HI brain injury on long-term behavioural outcomes in rat offspring in adulthood.

id #12849

Dopaminergic lesions of the dorsolateral striatum prevent habitual actions resulting from extended training and L-dopa exposure

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When an action is well learned it can be performed without consideration of its consequences. Such habitual actions also occur following exposure to psychostimulants, which is considered to model aspects of addiction. Given that L-dopa (levodopa) treatment for Parkinson's disease (PD) is associated with the development of addiction-like behaviours, habitual actions may also model this common side-effect. The aim of this study was to determine the impact of L-dopa and striatal dopamine denervation on habitual behaviour in rodents. Sham or 6-hydroxydopamine (6OH) lesions of the dorsolateral striatum (DLS) of rats were carried out prior to vehicle or L-dopa treatment (25 mg/kg for 6 days). Animals were then trained to press a lever for a pellet outcome. Following extended training, sham animals were insensitive to outcome devaluation and demonstrated habitual behaviour, whilst lesioned animals demonstrated goal-directed behaviour. Following moderate training, the sham-vehicle group were goal-directed, whilst the sham-L-dopa group demonstrated accelerated habitual behaviour. Importantly, when the DLS was lesioned, L-dopa exposed animals remained goal directed. This study demonstrates that 6OH striatal lesions prevent habitual actions that develop from extended training and from L-dopa exposure. Further, it suggests that L-dopa acts via the DLS to potentiate habits. These findings add to the existing literature that indicates that dopamine is important for the development of habits. There was no evidence that striatal dopamine denervation and L-dopa interacted to promote habits, suggesting that this is not a good model of the side effects of L-dopa treatment in PD.

id #12858

Reelin in the human infant hippocampus and changes in Sudden Infant Death Syndrome (SIDS)

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Morphological abnormalities have been reported in the SIDS hippocampus and thought to be due to abnormal neuronal migration. Reelin is an extracellular matrix protein known to be critically involved in neuronal migration and lamination during human development. Its expression has not been well studied in the human infant hippocampus nor within SIDS infants. This pilot study is aimed towards identifying reelin expression within the human infant hippocampus and to determine whether levels differ in those diagnosed as SIDS I (n=7), SIDS II (n=34) or non-SIDS ('controls' n=9). Immunohistochemical expression of positive reelin cells were quantified in the molecular layer (ML) and hippocampal fissure (HF) of the left hippocampal formation at the level of the lateral geniculate nucleus. As expected, the HF had 10 times higher reelin expression than the ML, and there was a positive correlation with post-conceptional age (PCA) ($R^2 \geq 0.5$). When comparing between the 3 diagnostic groups, we found no statistically significant difference even after correcting for PCA. Research is currently underway to determine expression in the other layers of the hippocampus.

id #12859

THE α7 AND β2 NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS IN THE INFANT HIPPOCAMPUS AND CHANGES ATTRIBUTED TO SUDDEN INFANT DEATH SYNDROME AND SMOKE EXPOSURE

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Background: Cigarette smoke exposure (CSE) during pregnancy and postpartum contributes to several adverse outcomes to the infant including an increased risk of sudden infant death syndrome (SIDS). The proposed mechanism is hypothesised to be via nicotine inducing its actions by binding to nicotinic acetylcholine receptors (nAChRs) in the brain. Two predominant nAChR subunits in the brain are α7 and β2. The hippocampus is of interest given increased apoptosis and structural abnormalities reported in SIDS. **Objective:** To compare neuronal expression of the α7 and β2 nAChR subunits in the hippocampus of non-SIDS vs SIDS infants (SIDS I and II), and according to CSE. **Methods:** Hippocampal sections, at the level of lateral geniculate nucleus, were immunohistochemically stained for α7 and β2. CA4, CA3, CA2, CA1 regions of the hippocampus and the subiculum were quantitatively assessed to determine the percent of neurons positive for each subunit. Expression was compared amongst (non-SIDS) (n=11), SIDS I (n=8) and SIDS II (n=36) and of these, by CSE of yes (n=19) vs no (n=27). **Results:** SIDS I had a statistically significant increase in α7 compared to non SIDS in the CA2 (p=0.05) and a trend to increase in the CA3 (p=0.06) region. There was no effect of CSE on the expression of both subunits. **Conclusions**: The preliminary results of this study suggest that the CA2 and CA3 regions of the SIDS hippocampus are at risk of fast desensitising currents due to the increase in the α7 nAChR subunit.

id #12862

Detection of autoantibodies against GABA$_A$Rα1 in patients with schizophrenia

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Recent studies have identified autoantibodies against synaptic molecules in patients with encephalitis. Autoantibodies against the *N*-Methyl-D-Aspartate receptor have been reported in patients with schizophrenia; however, autoantibodies against other molecules are yet to be identified. This study used a cell-based assay to examine serum samples from individuals with schizophrenia and healthy controls. The results showed that 5 (8.6%) of 57 patients with schizophrenia harbor autoantibodies against the α1 subunit of the γ-aminobutyric acid A receptor (GABA$_A$Rα1), which are currently not know to be linked to the pathology of this disease. Some patients showed markedly high antibody titers (i.e., 1:10,000–100,000). None of the heathy control subjects were positive for GABA$_A$Rα1 antibodies. Therefore, these

autoantibodies may form the basis of GABA-mediated pathology in a subgroup of patients with schizophrenia.

id #12871

Viral Approaches to Studying the Organisation of Viscerosensory Afferent Neurons

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The brain receives continuous sensory information from the internal organs, via the vagus nerve, to modulate neuroendocrine and autonomic function. Vagal afferent neurons first terminate in the brainstem at the nucleus of the solitary tract (NTS), where information from various organs is integrated. The organisation of this input, relative to specific NTS neurons, remains incompletely understood. We used viral tracing tools to study the projection patterns of stomach vagal afferents in the NTS and relate these to phenotypically identified NTS neurons. To test viral transduction efficacy, we anaesthetised adult male Sprague Dawley rats and injected different viruses into the serosal layer of the fundus of the stomach. AAVrg, with a CAG promoter, provided best transduction, with axons predominantly observed in the intermediate NTS, at the level of the area postrema, in the medial and dorsomedial subnuclei. We also observed axons in the area postrema and, surprisingly, the dorsal motor nucleus of the vagus – locus of parasympathetic preganglionic neurons. Stomach vagal afferents closely apposed both tyrosine hydroxylase-expressing, noradrenergic, neurons and aldosterone-sensitive neurons, but not GLP-1 expressing neurons. Using this approach, we have begun clarifying the neuroanatomical organisation of the NTS. We also highlight the potential for direct afferent/efferent reflex function in the parasympathetic system. The viral transduction also enables functional manipulation to better understand circuit dynamics.

id #12872

A NEW AXOTOMY MODEL IN ZEBRAFISH: IN VIVO STUDY OF THE MICROGLIAL RESPONSE TO PECTORAL FIN INJURY

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Peripheral injury of a motor nerve is a well-established experimental model in rodents. It has been used extensively to study the responses of central glial cells to a remote lesion that does not lead to any direct trauma of the central nervous system. There is no comparable model in zebrafish. Taking advantage of the suitability of performing in vivo long-term imaging in zebrafish, we have now characterized in real-time microglia-neuron interactions following injury of the zebrafish pectoral fin innervation. Specifically, we use transgenic zebrafish lines

expressing fluorescent proteins in motor neurons and microglia as well as fluorescent axonal tracers to label the neurons innervating the pectoral fin musculature. In the case of larvae, we perform a pectoral fin nerve avulsion, and in adult fish a nerve transection at the base of the fin, respectively. In larvae we then monitor the dynamics of the microglia response using confocal microscopy. We further characterize the changes of microglia using post-imaging analysis and 3D rendering. Our preliminary results show an increase in the number of microglia at the site where the cell bodies of the affected motor neurons are located within less than 4 days after the injury. The newly appearing microglia do not assume a macrophage morphology but stay in the immediate vicinity of the injured nerve cells for several hours. Taken together, zebrafish permits the in vivo study of microglial dynamics taking advantage of the transparency of the larvae, as well as genetic manipulation, thereby overcoming critical limitations of rodent axotomy models.

id #12873

The role of sympathetic nerves in modulation of guinea pig colon motility

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Objectives

The sympathetic nervous system acts on the gut to inhibit motility, however the effect of sympathetic activity manipulation in vivo on faecal output is unknown. In our pilot study, we examined the outcomes of surgical sympathectomy of the distal colon on faecal pellet output followed by in-vitro motility experiments.

Methods

Adult guinea pigs underwent a laparotomy under anaesthesia according to our institutional animal ethics guidelines. All para-vascular nerves associated with the inferior mesenteric artery (IMA), majority of which are sympathetic efferents, were sharply divided and the artery swabbed with aqueous phenol for further chemical ablation. Pre- and post-operatively the daily faecal pellet output (FPO) was recorded for 7 days. Two stress-tests were also performed on each animal pre- and post-operatively during which the FPO was recorded.

Control animals (n=4) and sympathectomy animals (n=2) were euthanized and motility characterised in vitro. Spatiotemporal maps and suction electrodes recorded motility and smooth muscle electrical activity during spontaneous emptying and artificial pellet propulsion. Ring electrodes were used to stimulate the para-vascular nerves of the IMA. Degeneration of sympathetic fibres in the colon was confirmed by immunohistochemistry for tyrosine hydroxylase.

Results

Electrical stimulation of the IMA nerves in the sympathectomy animals had no effect on pellet propulsion compared to controls, where stimulation stopped pellet propulsion. There

was a trend towards a higher FPO in the first 24 hours postoperatively compared to preoperative numbers.

Conclusion

Activation of sympathetic fibres in-vitro inhibits distal colon motility. Potential compensatory mechanisms in-vivo may counteract the effects of surgical sympathectomy.

id #12874

Set-Size Sensitisation in Visual Search

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In everyday life, our brains are constantly searching the visual environment. Though we understand some of the ways we influence visual search performance, much of the story remains unclear. We investigated whether the brain could improve its visual search performance, by adapting to a repeated set-size (number of objects (n.o)) across search tasks, as opposed to any other regularity that might aid visual search, such as a repeated orientation or configuration of objects. We had volunteers (f=4, m=5, x=1) complete three blocks of search tasks, comprised of five conditions of different set-sizes (9,16,25,36,49). Participants found the letter 'L' among many letter 'T's. The first, control, block and the third, test, block had equal frequencies of the set-sizes, but the second, training, block repeated one set-size (n.o=25) four times more than the others. We calculated the mean differences in search time for all five conditions, between the control and test blocks, to measure the improvement in search times following the training block. We found a statistically significant difference in mean search times between conditions (One-way ANOVA, $f(4) = 6.80$, $p < 0.001$). Specifically, we found that the search time for the set size with more repetitions was significantly different from the search times for the smallest (Student t-test, $t(9)= 3.84$, $p < 0.005$) and largest (Student t-test, $t(9)=4.29$, $p < 0.005$) set sizes. Our results suggest that visual search mechanisms may retain an implicit sensitivity to the set-size they have to search through, as a way of facilitating better search performance.

id #12875

COORDINATION OF SPONTANEOUS ELECTRICAL AND MECHANICAL ACTIVITY IN THE ISOLATED NON-PREGNANT MOUSE UTERUS.

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Precisely coordinated contractions of the non-pregnant uterus are critical for the success of many reproductive functions. The mechanisms underlying such activity, however, are incompletely understood. We therefore sought to further characterise spontaneous electrical and mechanical activity in the mouse uterus during the oestrus (post-ovulatory) phase of the oestrous cycle. Contractions of isolated, intact uteri from nulliparous female C57BL/6 mice were video captured to generate spatiotemporal maps, and myoelectrical activity simultaneously recorded using suction electrodes attached along the uterine horn ($n = 5$). All recorded activity persisted in the presence of the sodium channel blocker, TTX (0.6µM), and was abolished by the L-type calcium channel blocker, nifedipine (1µM); supporting a myogenic origin. Contractions typically initiated from the oviduct-end of the uterus and propagated toward the uterine body at a frequency of ~1-2cpm. This activity appeared to occur independently in adjacent uterine horns, with only ~12% events happening concurrently. Within each contractile event, bursts of electrical activity corresponded to individual phasic contractions of both the circular and longitudinal muscle. Such electrical bursts consisted of major and minor (superimposed) peaks generated at two different frequencies: ~5Hz and ~25Hz, respectively. Additional calcium imaging experiments ($n = 2$) revealed that during contractile events, calcium waves occurred simultaneously in both muscle layers. However, spontaneous myometrial calcium waves between contractile events were not detected. This is the first description of propagating bursts of electrical activity along the uterine horn. The intrinsic pacemaker mechanisms underlying such rhythmicity are critically dependent upon L-type calcium channels.

id #12876

Analysis of mRNA and protein expression changes in the lipopolysaccharide model of Parkinson's disease

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Parkinson's disease (PD) is a predominantly idiopathic neurodegenerative disease affecting about 1% of Australian population aged over 50. The lipopolysaccharide (LPS) model emulates PD progression as it is characterized by degeneration of dopaminergic neurons in the substantia nigra (SN), while the ventral tegmental area (VTA) is spared. We aimed to identify differences in gene and protein expression after acute inflammation had subsided, but before the onset of neurodegeneration. Sprague-Dawley (SD) rats (N=4) were injected intraperitoneally with LPS (2 mg/kg) or saline and after four weeks SN and VTA were collected. Analysis of mRNA expression was performed on a subset of PD associated genes. Genes differentially expressed in SN in response to LPS vs saline treatment include Brain acid soluble protein 1 (BASP1) [fold-change -2.89, p=0.021], synpatogyrin 3 (SYNGR3) [fold-change -2.70; p=0.015], transcription factor 7 like 2 (TCF7L2) [fold-change -2.63; p=0.013], calcium dependent secretion activator (CADPS) [fold-change -2.31; p=0.010] and vesicular monoamine transporter 2 (VMAT2) [fold-change -1.97; p=0.044]. Interestingly, none of these genes were differentially expressed in VTA in response to LPS vs saline treatment. The study for corroborating protein expression of aforementioned genes is ongoing. However, preliminary analysis in SD rats (N=7 for LPS and N=6 for saline treatments respectively) showed no significant differences for both VMAT2 [fold-change -

0.06; p=0.55] and SYNGR3 [fold change 0.12; p=0.09] in SN and VTA (VMAT2 [fold-change -0.06; p=0.46], SYNGR3 [fold change 0.14; p=0.09]) between the LPS vs saline treatment. Therefore, the data suggests that mRNA changes for VMAT2 and SYNGR3 are SN specific.

id #12878

INHIBITION OF VAGAL AFFERENT INPUT TO PHOX2-EXPRESSING NEURONS IN THE NUCLUES TRACTUS SOLITARIUS CAUSES A SUSTAINED DECREASE IN BODYWEIGHT GAIN

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The nucleus tractus solitarius (NTS) receives sensory afferent input from multiple visceral organs, in particular those arising from the gastrointestinal tract. Lesions of the commissural NTS (cNTS) decrease bodyweight, but the mechanism by which this occurs remains unknown. We have developed a novel chemogenetic approach, using the insect allatostatin peptide system, which enables selective inhibition of a defined cell group by one input. We used this approach in vagal afferent neurons so they would inhibit, rather than activate, specific cNTS neurons. First, we injected an adeno-associated virus vector which expresses Ast and a fluorophore (mCherry) bilaterally into the nodose ganglion of Sprague Dawley (SD) rats. Two weeks later, a lentivirus, expressing either AstR (Experimental group) or green fluorescent protein (GFP) (Control group) under the control of a phox2 promoter, was injected into the cNTS. Bodyweight was measured following each surgical procedure and for 31 days after cNTS injections. All rats displayed surgery-related bodyweight decreases that lasted for 2-3 days post each injection. Following cNTS injections, Experimental SD rats exhibited significantly reduced bodyweight gain from day 10 onwards compared to Control SD rats (Experimental group (n=12): -8 ± 4g, Control group (n=10): +16 ± 5g, Bonferroni multiple comparison test, P<0.05). This remained significantly reduced compared to controls until the end of the study. The results show active vagal afferent input to phox-2 expressing neurons in the cNTS is crucial for bodyweight homeostasis.

id #12882

TOWARDS A MICROGLIA MAP IN ZEBRAFISH

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Microglia are the innate immune cells of the central nervous system (CNS) parenchyma. They originate from myeloid progenitors during development and play a critical role in tissue surveillance and maintenance of CNS homeostasis. Ramified microglial cells extend their processes to monitor the surrounding microenvironment and are involved in the fine-tuning of synaptic circuits but can retract their processes under pathological conditions and become motile cells that are involved in the removal of cellular debris and pathogens. Very little is known about the mechanisms underlying the microglia population response. Microglia unlike neuroglia are not coupled through gap junctions. However, a co-ordinated microglia population response is of greatest significance for the resolution of brain pathologies. The underlying mechanisms are largely unknown.

Here we establish a map of microglial cells in 5 days post fertilisation (dpf) transgenic zebrafish brain, using the macrophage expressed gene 1 protein (mpeg1) as a marker. The zebrafish larva is an excellent model due to its whole-body transparency and the ease with which the zebrafish genome can be manipulated. In zebrafish, macrophages migrate into the CNS parenchyma within the first 3dpf and then begin to differentiate into ramified microglial cells. Our preliminary results show that ramified microglial cells are more prevalent than ameboid microglia in 5dpf larvae, and that there are no significant differences in the number of microglia between larvae. The stable established microglia map will be used as a backdrop to elucidate mechanisms influencing behaviour of the microglial cell population in the brain. Supported by ARC DP150104472

id #12885

Impaired fear memory in BDNF val66met rats with a met/met genotype is reversed by chronic exercise

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Brain-derived neurotrophic factor (BDNF) is involved in neuroplasticity and has been implicated in affective disorders and their treatment. Exercise has been shown to have beneficial effects in these illnesses and increases BDNF signalling. The common BDNF val66met polymorphism is associated with reduced activity-dependent BDNF release and increased risk for anxiety and PTSD. We investigated the effect of chronic exercise on fear memory in a novel BDNF val66met rat model, using the three genotypes val/val, val/met and met/met (n=9-13). After weaning at three weeks, the animals were held in running wheel cages (LaFayette, USA) or remained sedentary in standard IVC cages. Behavioural testing from 8 weeks of age included a 3-day fear conditioning protocol (Med Associates). The number of running-wheel revolutions was higher in female than in male rats, independent of genotype. Female rats also showed generally lower freezing scores than male rats. There were no genotype differences in freezing behaviour during the conditioning phase on day 1, however chronic exercise significantly increased freezing acquisition in all three genotypes. Extinction testing on day 2 revealed significantly lower freezing in response to initial cue exposure in met/met rats compared to the other genotypes (53.8 ± 6.5s in met/met vs. 80.3 ± 4.3s in val/val and 73.4 ± 5.6s in val/met), suggesting impaired fear memory. This

difference was not seen in exercise rats (77.3±4.4s vs. 72.9±6.0s and 77.9±6.1s, respectively), suggesting exercise rescued impaired fear memory in met/met rats. These data illustrate how deficient BDNF release in the brain affects fear memory and how chronic exercise reverses this genotype effect.

id #12887

Development of anion channelrhodopsin mutants for spectrally distinct optogenetic excitation of neurons

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Channelrhodopsins (ChRs) are important tools in neuroscience that can be used to investigate neuronal connectivity, synaptic release and functional effect of a specific neuronal type. One limitation of ChR is that all red-light activatable variants (such as Chrimson and ReaChR) can still be strongly activated by blue (470-480 nm). If such excitation can be eliminated, it would be possible to use two wavelengths of light to independently excite two populations of neurons with blue (470-480 nm) and red/near-red (590-650 nm) light. This previously has been achieved by fine-tuning expression level of red ChR or light stimulation pulse width and intensity. One alternative solution that is more generalisable is to co-express a blue inhibitory anion ChR (ACR) with red cation ChR to suppress the excitatory effects of red ChR to blue light. To achieve this suppression, the kinetics and efficiency needs to be closely matched to the red ChR. We have chosen vfChrimson as the red ChR and ZipACR as blue ACR as the starting candidate for this co-expression strategy. Kinetically, ZipACR turns off faster than vfChrimson in response to blue light which can lead to residue excitation by blue light. We used structure guided mutagenesis approach to generate ZipACR mutants that were subjected to electrophysiological screening. We identified mutants that has suitable kinetics without reduction in efficiency. Here we present our results of the ZipACR mutants and their characterisation. We believe the identified mutant can be used for future co-expression approach to achieve spectrally distinct excitation of neurons.

id #12889

A NOVEL SURGICAL TECHNIQUE TO INVESTIGATE THE TOPOGRAPHICAL DISTRIBUTION OF THORACOLUMBAR AND LUMBOSACRAL SPINAL INNERVATION TO THE MOUSE UTERUS

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Pelvic pain in women can manifest from many different conditions, including those associated with the uterus. Pain-generating stimuli are detected by the uterus via sensory

nerves that project from the thoracolumbar and lumbosacral spinal cord. However, the extent to which these spinal afferent pathways innervate the uterus is currently unknown. To address this, unilateral transections of dorsal root ganglia (DRG) at thoracolumbar T13-L1 (n=5) and bilateral lumbosacral L5-S1 (n=5) were performed *in vivo* on female C57BL/6 mice. The contralateral uterine horn and sham surgery animals (n=4) served as controls, respectively. From 9 days post-procedure, uteri were processed immunohistochemically for expression of the nociceptive neuropeptide, calcitonin gene-related peptide (CGRP), then imaged, and the density of CGRP-immunoreactivity quantified. Mean CGRP expression in uterine horns ipsilateral to T13-L1 transection [1.8±0.9%] was reduced compared to contralateral tissues [3.8±1.2%]. This represented a ~52%, 54% and 38% depletion in CGRP-immunoreactivity in the oviduct, mid, and body regions, respectively. Mean CGRP expression in uteri from animals subjected to L5-S1 transection [1.9±0.5%] was also reduced compared to sham [2.8±0.5%]. Regionally, CGRP-immunoreactivity was depleted by ~28% (oviduct), 37% (mid) and 32% (body). These data indicate that thoracolumbar spinal afferent nerves provide greater innervation to the mouse uterus than lumbosacral spinal afferents. Moreover, a topographical arrangement of spinal afferents exists within the uterus, where thoracolumbar inputs typically innervate the oviduct-mid region, while most lumbosacral nerves distribute in the uterine mid-body. Therefore, pain signals from the uterus may be transmitted via distinct populations of spinal afferents depending on the stimulus location.

id #12891

PHARMACOLOGICAL AND ELECTROPHYSIOLOGICAL ANALYSIS OF POLARISED ENTERIC NEURAL PATHWAYS ACTIVATED BY BALLOON DISTENSION IN THE GUINEA PIG COLON

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Objective: Propulsion of individual faecal pellets in the guinea pig colon involves excitatory and inhibitory enteric neural pathways. The final inhibitory motor neurons to the circular muscle may utilise multiple transmitters. We tested antagonists to the three putative transmitters ATP, NO and VIP on distension-evoked anal inhibition. Methods: Guinea pig distal colon segments were placed in an organ bath containing Krebs solution at 36.5°. A small tube with a flaccid 14 mm long balloon was placed in the lumen. The tube was connected to a syringe, allowing manual distensions to a 6 mm maximum diameter. Spatiotemporal maps of distensions, generated from video recordings, were combined with localized intraluminal force recordings and extracellular smooth muscle electrical recordings, oral and anal to the balloon. Findings: Balloon distensions of 10 seconds duration elicited increased oral intraluminal forces preceded by a barrage of action potentials concomitant with anal inhibition, characterized by a reduction of intraluminal pressure preceded by a fast hyperpolarisation followed by a sustained hyperpolarisation. Hexamethonium (100μM, n=3) blocked oral excitation but only reduced the magnitude of anal inhibition. In hyoscine (1μM, n=9), oral excitation was reduced, while anal inhibition persisted unchanged. MRS2500

(1μM n=8), blocked the initial faster compound IJP while L-NA (100μM, n=8) blocked the slower sustained hyperpolarisation. VIP antagonists (1μM, n=2) had little or no effect on the hyperpolarising responses. Conclusion: The descending inhibitory reflex activated by acute distension in guinea pig colon involves mostly ATP and NO neurotransmitters.

id #12902

Topographical distribution of RVLM C1 neurons that respond to cardiovascular versus metabolic stimuli

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Neurons controlling cardiovascular responses and glucose counterregulation are co-located within the rostral ventrolateral medulla (RVLM). Cardiovascular adrenergic (C1) RVLM neurons differ electrophysiologically from adrenergic RVLM neurons that regulate blood glucose. We hypothesized that C1 neurons responding to cardiovascular versus metabolic stimuli would also be topographically distributed within RVLM. We compared the distributions of RVLM C1 neurons activated by either cardiovascular or metabolic stimuli using Fos to identify activated neurons. We injected conscious male Sprague Dawley rats with insulin (10 U/kg), or 2-deoxyglucose (2DG, 400 mg/kg) to induce hypoglycaemia or hydralazine (HDZ, 10 mg/kg), nitroprusside (NP, 1 mg/ml) or diazoxide (DZX, 50 mg/kg) to induce hypotension. Rats were perfused with formaldehyde 90 or 120 min later. Coronal 30μm sections of medulla (1:4 series) from the spinomedullary junction to mid-facial nucleus were immunoperoxidase-stained to show Fos-immunoreactivity plus phenylethanolamine N-methyl transferase (PNMT)-immunoreactivity to identify C1 neurons. The 6 sections containing the 600μm caudal to the caudal pole of FN (i.e., sections FN0 to FN-5) were each divided into 3 equal segments (medial, middle and lateral) and the total numbers of PNMT-immunoreactive neurons with Fos-immunoreactive nuclei (Fos+PNMT neurons) were counted. In insulin- and 2DG-treated rats, the numbers of Fos+PNMT neurons were greater in the medial and middle thirds of the C1 cell column. In NP-, DZX- and HDZ-treated rats the numbers of Fos+PNMT neurons were greater in the middle and lateral two thirds of the C1 cell column. These results indicate that, within the rat RVLM, C1 neurons are topographically organized according to function.

id #12903

VNS improves extinction memory of a visual context and increases novel object recognition memory?

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Vagal nerve stimulation (VNS) decreases fear expression and enhances memory extinction in an auditory conditioned paradigm. Here, we assessed whether the enhancement of memory extinction by VNS is specific to auditory stimuli or generalize to other contextual domains. We indexed VNS intensity to a characterized vagal reflex, the Hering-Breuer reflex (HBR), in conscious male Sprague Dawley rats. We found that conventional measures of VNS electrode patency, such as electrode resistance, are unreliable. Groups were then accessed via two behavioral tests; a 9 day inhibitory avoidance assay (IAA) and a novel object recognition test (ORT). In the IAA, rats were aversively conditioned to a dark compartment and re-exposed daily over 7 days with or without VNS (30 s at 20 Hz, 0.100 ms stimulus duration at 1.5X HBR threshold). VNS significantly improved extinction memory (n=12) compared to sham animals (n=9) by decreasing fear correlates; latency to enter the dark compartment, freezing and fear reinstatement. The next day animals were habituated to the ORT box and then immediately familiarized to two identical objects (A, A). 3 and 24 hr later memory was tested by replacing the familiar object with a novel one (A/C or B/A). VNS was administered to one group for 30 s at completion of the novel object presentation. Although preliminary, VNS appears to improve novel object recognition 24 hr later (n=2) when compared to shams (n=6). We conclude that VNS accelerates contextual memory extinction, suggesting this phenomenon generalizes across different memory processes.

id #12906

Models of Depression in Rodents - Not All Created Equal

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Major Depressive Disorder (MDD) represents one of the greatest health concerns of the 21st century. MDD is described as a psychiatric disorder causing persistent feelings of sadness and lack of interest in stimuli. Rodent models of chronic stress are employed to understand mechanisms of MDD pathogenesis and progression, including changes in neurotransmitter, endocrine and metabolic systems. With a variety of stress models in the literature, it can be difficult to determine which most effectively induces a depression phenotype. This study characterised and compared behavioural and neurobiological outcomes in 3 commonly employed rodent stress models: chronic unpredictable mild stress (CMS), restraint stress and social stress in male C57Bl/6 mice. Behavioural changes were assessed using the open field and sucrose preference tests (pre- and post-stress), with circulating biomarkers also assayed. Brains were then harvested and frontal cortex and hippocampus isolated into *RNAlate*r until molecular analysis. Results reveal that CMS and social stress (not restraint), induce significantly 'depressed' behavioural phenotypes. This was associated with elevated

circulating adrenaline in the former models, whereas leptin, ghrelin and resistin were comparably elevated across models. Neurobiological profiles also differed: CMS induced greater shifts in inflammatory, glucocorticoid/stress pathway genes in frontal cortex and hippocampus compared with social stress (with the latter inducing greater down-regulation of hippocampal genes). Summarising, different forms of chronic stress may induce similar depressive phenotypes, however unique regional neurobiological changes emerge. Despite these unique outcomes, molecular analyses support shifts in BDNF, MAO, GABA, glucocorticoid, NFkB/inflammatory pathways with CMS and social stress.

id #12908

NEUROCHEMICAL CHARACTERISATION OF SENSORY AND AUTONOMIC ENDINGS IN THE MOUSE BLADDER

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The urinary bladder is innervated by sympathetic and parasympathetic efferents, and spinal afferent neurons with cell bodies in the lumbosacral and thoracolumbar dorsal root ganglia (DRG) which project to the bladder via splanchnic-pelvic and lumbar-hypogastric nerves. The objective of this study was to characterize anatomically and immunohistochemically the endings of sensory and autonomic neurons in the wholemounts of the mouse bladder. We combined *ex vivo* anterograde tracing with biotinamide applied to nerve trunks of the vesical plexus, with immunohistochemical labelling of calcitonin gene-related peptide (CGRP), vesicular acetylcholine transporter (VAChT), and tyrosine hydroxylase (TH), which label spinal afferent, parasympathetic, and sympathetic axons in mouse bladder, respectively. In biotinamide-labelled nerves, $37.5 \pm 2.6\%$ (n=6) of nerve fibres were immunoreactive for CGRP, $36.7 \pm 13.7\%$ (n=3) for VAChT and $15 \pm 3.5\%$ (n=3) for TH. Using triple labelling immunohistochemistry for neurofilament 200, CGRP and/or substance-P and vesicular glutamate transporter 2, we investigated the sub-populations of sensory nerve fibres innervating the muscle and suburothelium. In control experiments, there was no co-localisation of *in vivo* dextran-biotin (injected at L5-S2 DRG) labelled sensory fibres in the bladder with neurochemical markers TH (292 axons, n=5) or VAChT (283 axons, n=5) in either the detrusor or suburothelium. Our findings revealed that different subtypes of sensory and autonomic nerve endings can be reliably identified by combining anterograde labelling *ex vivo* with immunohistochemical markers, although morphologically some of these types of endings were undistinguishable.

id #12912

Dynamic DNA structure states regulate fear extinction memory

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Here we explore the functional role of G-quadruplex, an alternative DNA structure traditionally associated with reduced transcriptional inhibition, in the formation of fear extinction memory. We have discovered that G-quadruplex is dynamic and regulated in response to fear and extinction learning, and that knockdown of the G-quadruplex helicase DEAH-Box Helicase 36 (DHX36) leads to impaired extinction. In contrast, knockdown of activation-induced cytidine deaminase (AID), which normally functions as a G-quadraplex reader, leads to enhanced fear extinction memory. Ongoing experiments aim to identify the genome-wide overlap between these two marks, and specify how AID and DHX36 may serve opposing roles to promote and resolve G-quadruplex DNA for the modulation of fear memory.

id #12913

Dynamic experience-dependent accumulation of unique Gas5 variants in the prefrontal cortex following fear extinction learning in mice

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Long noncoding RNAs have recently been implicated in RNA directed epigenetic regulation of gene expression, but their role in learning and memory remains relatively unexplored. We therefore performed RNA sequencing on synaptosome and nuclear fractions isolated from the prefrontal cortex of mice that had undergone fear extinction training. We have discovered that the lncRNA, Gas5, accumulates at the synapse in an experience-dependent manner. We next performed RT-qPCR on several Gas5 variants, and found differential expression in subcellular compartments in response to fear and extinction learning. To address the functional role of Gas5 variants during fear extinction, we utilized the CRISPR-Cas-inspired RNA targeting system (CIRTS) to knockdown Gas5 transcript. Our preliminary *in-vitro* test has shown that one of the CIRTS guide RNA targeting the last exon of Gas5 can reduce its expression by 80%. We are currently attempting knockdown of Gas5 *in-vivo* to determine whether a reduction of Gas5 at the synapse can influence fear extinction memory.

id #12915

Insights into small nucleolar RNA expression, processing and function in the mouse prefrontal cortex

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C/D box small nucleolar RNAs (snoRNAs) are 60-150nt RNA species which guide post-transcriptional modification of other RNAs (canonically, pre-ribosomal and small nuclear RNAs) with 2'-O-methylribose. While several C/D box snoRNAs are known to be brain-specific, little research has investigated their function in learning and memory. Using a ligation-based small RNA library preparation method, we sequenced C/D box snoRNAs from the mouse prefrontal cortex with unprecedented depth, and detected expression of over 150 C/D box snoRNAs, including a number of unannotated sequences. We found that many C/D

box snoRNAs are rapidly upregulated in response to fear extinction learning, suggesting a role for these small RNAs in fine-tuning the function of neuronal RNAs in response to neuronal activation. In addition, using a candidate-gene approach, we were able to confirm the presence of a snoRNA-guided 2'-O-methylation on the Htr2c messenger RNA, furthering the hypothesis that snoRNA-dependent methylation of non-canonical targets may be an important regulatory mechanism for RNA function. Ongoing work in our laboratory aims to profile learning-dependent snoRNA targets across the transcriptome, with a focus on detecting further interactions between snoRNAs and non-canonical targets including mRNAs, to provide new insight into the biogenesis, processing and function of these small noncoding RNAs in the brain and elucidate their role in learning and memory.

id #12916

SIMULTANEOUS VIDEO IMAGING AND ELECTROPHYSIOLOGICAL RECORDINGS REVEAL A NEW MECHANISM OF THE ENTERIC NERVOUS SYSTEM UNDERLYING BOTH PROPULSIVE AND NON PROPULSIVE MOVEMENTS IN THE MOUSE COLON

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Recently, we revealed a new mechanism in the enteric nervous system (ENS) whereby large populations of neurons fire in rhythmic bursts to generate rhythmic depolarisations in the smooth muscle in isolated full-length sheet preparations of colon (Spencer *et al.* 2018). Here, we determined whether the same rhythmic ENS/smooth muscle firing pattern is responsible for the propulsion of liquid content along tubular colonic preparations. Extracellular electrophysiological smooth muscle recordings were made from two independent recording sites while simultaneously video imaging the colonic wall movements. This allowed us to correlate the electrical activity in the smooth muscle (and indirectly the ENS) at the same time as propulsion of fluid content along the colon. Colonic motor activity was evoked either by transient intraluminal fluid, or an immobile metal rod. Fluid distension evoked neurogenic spike bursts which was associated with anterograde fluid propulsion. The metal rod also evoked ongoing neurogenic spike bursts, without propulsion. The coherence of action potentials or subthreshold cholinergic depolarisations between two recording sites was high during neurogenic spike bursts regardless of the intraluminal stimulus, or longitudinal electrode separation (1mm to 30mm) (n=9). Coherence was low in quiescent periods. Synchronised excitatory junction potentials in the smooth muscle over large spatial fields were abolished by hexamethonium (n=5). The findings show that the ENS can temporally synchronise the firing of enteric neurons (at ~2Hz) along the full length of the colon, independent of contractile velocity in both propulsive and non-propulsive motor events.

1. Spencer N et al. (2018) J. Neurosci 38(24):5507-5522

id #12917

Modelling of SCN2A encephalopathies using human stem cell-derived neurons

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Developmental and epileptic encephalopathies (DEE) are a group of rare but severe disorders characterized by seizures, behavioural abnormalities and neurodevelopmental delays. SCN2A encodes the pore-forming α subunit ($Na_v1.2$) of voltage-gated sodium channels mostly expressed in excitatory neurons. *De novo* mutations of SCN2A have increasingly been implicated in cases of DEE. While mammalian cell systems and transgenic animals have been used to model these genetic disorders, a model that is more relevant to human is required to provide insights into disease mechanisms in a human biology context and bridge the gap between basic research and clinical testing. In this study, we set out to investigate the feasibility of using patient-derived neurons to model DEE caused by two most recurrent *de novo* SCN2A mutations resulting in early- (<2 weeks) and late- (>3 months) onset DEE (R1882Q and R853Q, respectively). Patient-derived iPSC cell lines and their corresponding isogenic controls were differentiated into cortical excitatory neurons using the NGN2 overexpression protocol. Electrophysiological properties and expression profiles were assessed to identify any functional abnormalities. Our data show that R1882Q neurons display increased activity compared to the control despite no apparent difference in the expression of voltage-gated sodium channels, confirming its gain-of-function phenotype. In contrast, no difference in neuronal activity or sodium channel expression is observed in R853Q neurons, likely due to unknown compensatory mechanisms. Overall, our data demonstrate the validity of using iPSC-derived neurons to model genetic epilepsies and their potential for screening drug therapies.

id #12918

Ultrasensitive Detection of Three Neurological Biomarkers In Un-Diseased Dried Blood Spots

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Dried blood spots (DBS) have many advantages over traditional sample matrices such as cerebrospinal fluid (CSF), serum, plasma, and whole blood. DBS can be collected via a finger prick and stored at room temperature, eliminating the need for invasive procedures, extensive laboratory equipment, and trained medical professionals However, in a traditional ELISA, low abundance neuronal biomarkers are often only measurable in plasma or CSF, and may not be detectable in DBS. Here, we show the ultra sensitive Simoa ® Neuro 4-Plex Assay can quantitatively measure levels of three biomarkers associated with neuronal damage in normal, un-diseased DBS- glial fibrillary acidic protein (GFAP), neurofilament light protein (NfL) and tau. Samples were evaluated for GFAP, NfL and tau concentration, signal reproducibility, stability at room temperature, and signal specificity. 100% of DBS tested

were quantifiable (above LLOQ). Inter-spot measurements were reproducible, with an average 15% coefficient of variation between two blood spots from the same individual. DBS GFAP and tau readings were stable at room temperature for 8 days. GFAP, NfL and tau demonstrated signal specificity from 72-100%. The ability to detect biomarkers of a range of neorological disorders in DBS may facilitate studies of disease in remote communities where the logistics of sample collection and processing have historically made such studies difficult.

id #12921

An enhancer-derived RNA mediates the regulation of the immediate early gene Nr4a2 is necessary for fear extinction memory

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The immediate early gene *Nr4a2* gene has been shown to play a fundamental role in memory formation; however, the precise mechanisms by which it is activated in response to learning remain to be determined. We have discovered a long non-coding RNA (BB557941) that is dynamically expressed from a distal enhancer element upstream of the *Nr4a2* locus and mediates fear extinction learning-induced Nr4a2 mRNA expression. This enhancer-derived RNA (eRNA) physically interacts with the shuttle protein 14-3-3 in a learning-dependent manner, which then enables the recruitment of the histone acetyltransferase CBP to the *Nr4a2* promoter to drive its expression. Furthermore, knockdown of BB557941 in the infralimbic prefrontal cortex (ILPFC) blocks the learning-induced induction ofNr4a2 mRNA expression and impairs the formation of fear extinction memory. Taken together, our data reveal a critical role for activity-induced eRNA in the regulation of experience-dependent gene expression and add this recently discovered class of non-coding RNA to the growing list of RNA-mediated regulatory mechanisms that are required for memory formation.

id #12923

Age- and Sex-specific changes of GABA signalling components in the human hippocampus

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Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the nervous system. The GABA signalling system in the brain is comprised of GABA synthesizing enzymes, transporters, $GABA_A$ and $GABA_B$ receptors ($GABA_A R$ and $GABA_B R$). Alterations in the expression of signalling components have been observed throughout aging and between sexes in various animal models. The hippocampus is the memory centre of the brain and is often impaired during age-related disorders. It is composed of two main regions: the Cornu Ammonis (CA1-4) and the Dentate Gyrus (DG), which are interconnected with the Entorhinal Cortex (ECx). The age- and sex-specific changes of GABA signalling components in these regions of the human brain have not been studied. This study is the first to determine the effect of age and sex on the expression of GABA signalling components ($GABA_A R$

α1,2,3,5, β1-3, γ2, GABA_BR R1 and R2 and GABA synthesizing enzymes) in the ECx and DG, CA1 regions of the human hippocampus using Western blotting. The results indicate, there were no significant age-related changes for GABARs or GABA synthesizing enzymes. Furthermore, there were also no significant sex-specific differences for GABARs or GABA synthesizing enzymes in these brain regions. In conclusion, our results showed that the GABAergic system is robust to age-related changes and there were no sex-specific differences in GABAergic signalling components.

id #12929

Controlling neurosphere size: a method to reduce variation and improve predictability of neuronal generation.

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Methods to generate neurons from human embryonic stem cells (hESCs) generally include a neurosphere formation phase during which individual small fragments of neural progenitor colonies develop into floating spheroids. The variation in size of these fragments, and thus variation in final neurosphere size, is a likely source of downstream unpredictability of neuronal yield. Here we describe a method to generate neurospheres of a consistent, predictable size allowing more precise control of the microenvironment in which neuronal progenitors grow and differentiate. Colonies of hESC-derived neural progenitor cells were dissociated to a single-cell suspension and seeded in round-bottomed low-attachment 96-well plates at varying defined seeding densities. Cells were aggregated by either gravity alone or centrifugation, and neurospheres allowed to grow for 14 days with regular measurements of diameter undertaken. The size of neurospheres grown from dissociated cells showed less variability than those grown from colony fragments and final size correlated to initial seeding density. When aggregation was assisted by centrifugation, neurospheres grew to a larger size and at a faster rate. When replated and differentiated for 49 days, neurospheres generated from single-cell suspensions produced neurons that were indistinguishable from those grown using previous methods. This method enables tight control of the neurosphere microenvironment which is expected to lead to greater reproducibility of downstream neuronal generation and thus increase ability to perform high-throughput analysis with human neurons.

id #12930

Sex differences in c-Fos and EGR-1/Zif268 activity maps of rat sacral spinal cord following cystometry-induced micturition

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Storage and voiding by the lower urinary tract is precisely timed to occur with appropriate behaviors triggered by interoceptive (fluid balance or exteroceptive (social, defence, sexual) cues. A key part of the CNS control circuit in sacral spinal cord relays sensory input encoding bladder fullness to the brain is the effector for motor commands from Barrington's nucleus

that initiate voiding or scent marking. Immediate-early gene (IEG) activity mapping has been widely used to study the sacral LUT-related circuit under anesthesia but not in awake behaving rodents. We therefore used c-Fos activity mapping to study sacral spinal cord following cystometry-induced micturition in awake female and male rats. Following cystometry, c-Fos neurons in spinal cord segments L5-S2 were concentrated in sacral parasympathetic nucleus (SPN), dorsal horn laminae II-IV, and dorsal commissural nucleus (SDCom). Comparisons of cystometry and control groups revealed sex differences. A class of catecholamine neurons in SPN and SDCom neurons were more strongly activated by micturition in females. Furthermore, dorsal horn laminae II-IV was activated in females but showed evidence of descending visceral inhibitory controls in males. This was associated with proportionally greater activation of inhibitory interneurons (identified by Pax2) in males, noting that most c-Fos neurons were excitatory in both sexes. Activity mapping with EGR-1/Zif268 only detected activation of the dorsal horn in males and was mostly induced in dorsal horn neurons that did not express c-Fos. IEG mapping in awake rats can identify neurons in the LUT-related circuit in sacral spinal cord required for micturition.

id #12931

EFFECTS OF LACTATE ON GROUP III STRIATED MUSCLE AFFERENT NEURONS IN THE MOUSE

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Background: Ergoreceptors are sensory nerves that detect contractile activity in skeletal muscle by metabolites and/or mechanical activity. "CT3" muscle afferents in mouse abdominal muscles have nerve endings in connective tissue close to muscle sheets and are sensitive to a mix of metabolites containing ATP, H+ and lactate (Peterson et al, Neuroscience in press). While responses to ATP and H+ are well characterised, how lactate affects neurons is not as clear. **Methods:** Extracellular recordings were made ex vivo from nerve trunks entering isolated preparations of mouse abdominal muscles. **Results.** Superfusion with 15mM lactate solution (pH 7.4, osmotically balanced by reduced NaCl) evoked increased firing in 8 of 14 identified CT3 afferent units. Previous studies suggested that lactate acts by chelating divalent cations which affects ASIC3 responses to H+ ions (Immke and Mc Cleskey, 2001). A solution that mimicked chelation (by reducing $[Ca^{2+}]$ and $[Mg^{2+}]$) did not mimic 15mM lactate. Furthermore, compensating for chelation by increasing $[Ca^{2+}]$ and $[Mg^{2+}]$ in the 15mM lactate solution did not block the effects of lactate. Real-time PCR demonstrated the presence of mRNA for hydroxyl carboxylic acid receptor 1 (HCAR1) in dorsal root ganglia. HCAR1 immunoreactivity was detected in cell bodies of dorsal root ganglia neurons retrogradely labelled from mouse abdominal skeletal muscle. HCAR1 immunoreactivity was also visible in nerve fibres in abdominal muscles. **Conclusions:** These data are consistent with the proposal that lactate activates sensory afferent endings in skeletal

muscle via a distinct receptor which may correspond to the G-protein-coupled receptor, HCAR1.

1. Peterson, RA., Barry, CM., Wiklendt, L., & Brookes, SJH. 2019. Characterisation of one class of group III sensory neurons innervating abdominal muscles of the mouse. Neuroscience. In Press. Accepted 12 Sept 2019
2. Immke, D. & Mc Cleskey, E. 2001. Lactate enhances the acid-sensing Na+ channel on ischemia-sensing neurons. Nature neuroscience, 4, 869-870.

id #12932

ASCENDING INTERNEURONAL PATHWAYS IN MYENTERIC PLEXUS OF HUMAN COLON

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BACKGROUND: Availability of colonic tissue from elective surgery (with ethics permits) allows direct study of human enteric neuronal pathways. Ascending interneurons form an important component. **METHOD:** Specimens of human colon were dissected in sterile Krebs solution and a small bead coated with the dye, DiI, was applied to the myenteric plexus or smooth muscle layers. After 4 days in organ culture, the preparation was fixed, processed for immunohistochemical multiple labelling, photographed and mapped. **RESULTS:** DiI applied to the circular or longitudinal muscle layers filled ascending motor neurons; more than 95% were within 8mm. However, when applied to the myenteric plexus, DiI-labelled nerve cell bodies were located up to 43mm aborally; ascending interneuronal pathways are much longer than motor neurons. In the population of DiI-filled interneuronal cell bodies located 8-43mm aborally, over 90% of the neurons (n=6) were immunoreactive for either leu-enkephalin (ENK+) or calbindin (Calb+) or for both markers (ENK+/Calb+) but none was calretinin-immunoreactive. Substance P immunoreactivity was present in 21% of ascending interneurons but was restricted to a subset of ENK+ cells. **CONCLUSIONS:** Ascending interneurons in the human colon project up to 43mm; nearly 70% contain ENK immunoreactivity, and most of the rest contained calbindin, suggesting the existence of 2 or 3 classes of ascending interneurons. Fewer than 6% of descending interneurons contained ENK, thus ENK-containing varicosities in the myenteric plexus that surround nerve cell bodies are likely to reflect inputs from ascending interneuronal pathways.

id #12933

Electrophysiological characterisation of virally-labelled mouse spino-parabrachial projection neurons identifies distinct properties and suggests further subpopulations exist.

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Projection neurons of the spinal cord dorsal horn (PNs) have classically been viewed as the binary endpoint for spinal sensory signals. That is, once incoming sensory volleys have been modulated, PNs must be recruited for this information to reach nuclei in the midbrain and brainstem. To date there have been no reports addressing variability within PNs that project to the same brain region, nor a characterisation within mouse. PNs were retrograde labelled by viral injection (AAV9-mCherry) in the parabrachial nucleus, and patch clamp recordings subsequently made from spinal cord slices. Comparison of recordings from spino-parabrachial PNs (n=84), and unidentified neighbouring neurons (UNs, n=30) assessed differences within the two populations before addressing variability within the PN population. Comparison of PN and UN data identified differences in both synaptic and spiking properties. For example, the kinetics of average EPSC's were slower in PNs (half-width: 5.80±1.9ms vs 4.65±1.1ms*; decay time constant: 5.85 ± 2.1 vs 4.47 ± 1.1, p<0.001)* whereas EPSC amplitude and overall excitatory drive were similar for both populations. In addition, action potential discharge occurred at lower frequency in PNs (11.64±8.5Hz *vs* 16.8±9.1Hz, *p=0.009*), but repetitive spiking attenuated less than for UNs (peak attenuation: 0.76±0.2 *vs* 0.56±0.2*, p<0.05)*. Subsequent hierarchical cluster analyses of the PN population, using electrophysiological characteristics, suggests two main groups can be distinguished, with a third sub-group also likely. The results suggest that functionally distinct subpopulations of projection neurons exist, further adding to the already considerable heterogeneity of interneurons in this complex region.

id #12935

DNA double-strand breaks may be associated with the subcellular localization of RNA expression in response to fear extinction learning in mice

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Recently, it was discovered that DNA double strand breaks (DSB) can be induced by neural activity and are involved in regulating gene expression in response to learning. However, these early observations were based on a candidate gene approach and, to date, there has been no attempt examine this process genome-wide. Here we performed DSB-capture sequencing on DNA derived from neurons in the adult medial prefrontal cortex (mPFC) in response to fear extinction learning. In an effort to correlate DSBs and gene activation, we also performed RNA-sequencing on nuclear and synapse-enriched RNA from animals that had undergone a similar behavioural training protocol. We found 1069 genes that were differentially expressed at the synapse in response to extinction training, with about 10% exhibiting a strong correlation with DSBs in proximal regulatory regions. Functional classification analysis revealed gene clusters that tend to be involved in G-protein signalling, mitochondrial biogenesis and microtubule proteins related with intracellular trafficking. These findings suggest that experience-induced DSBs may play a critical role in regulating

the expression of genes localized to the synapse and involved in homeostatic plasticity, and this may be important for the formation of fear extinction memory.

id #12936

Anti-inflammatory effects of synthetic phospholipids

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Inflammation is an important biological defence mechanism however, chronic inflammation can be destructive as it may cause tissue damages. Phospholipid-based therapies are deemed to be safe and have shown anti-inflammatory properties and potential therapeutic efficacy in inflammatory conditions.

Our aim was to investigate the anti-inflammatory application of a pharmaceutical formulation (referred to as UTS-L; Au patent AU2019900939) containing 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), in the brain following systemic inflammation.

UTS-Liposomes were prepared and characterized for the size and zeta potential. Systemic inflammation was induced via repeated intraperitoneal administration of lipopolysaccharide (250 ug/Kg, 7 days) to male C57BL/6 mice. Control animals were injected with phosphate buffer saline. For UTS-L mice, UTS-L was intraperitoneally injected simultaneously with LPS for either 7 or 14 consecutive days. On days 14, mice were culled and the brain and other organs were harvested. The mRNA expression level of IL-6, IL-1β, and TNF-α, TLR-4, NOX4 and iNOS in Cortex, Hippocampus and Striatum were studied using rt-PCR.

The liposomes were 190.86 ± 6.95 nm with a polydispersity index of 0.28 ± 0.01 and zeta potential of -1.39 ± 0.06. LPS administration caused an increase in mRNA expression level of the mentioned markers in cortex and hippocampus, while treatment with UTS-L decreased their expression level ($P<0.05$ for TNF-α in cortex and IL-1β in hippocampus).

Our preliminary findings suggest that daily UTS-L administration in LPS induced mice can alleviate the brain inflammation by reducing inflammatory cytokines. Future studies will investigate the therapeutic efficacy of UTS-L in different models of inflammation.

id #12937

Micro-electrode array analysis of spinal cord pain circuit activity in-vitro

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Background: 'Gate control theory' has formed the basis for our view of spinal pain processing since its publication in 1965, highlighting the actions of inhibitory interneurons in suppressing sensory signals. Recently, a group of inhibitory interneurons identified by parvalbumin (PV) expression have been identified as one source of inhibitory 'gating' in this region. The implications of these connections for broader, macrocircuit activity remains to be determined as these studies used single cell patch clamp recordings combined with optogenetic activation. The current work aims to establish a model of rhythmic activity in acute spinal cord slices, monitored using micro-electrode arrays (MEAs), for subsequent use with optogenetic activation of PV neurons. **Methods:** Spinal cord slices were prepared from transgenic mice expressing channelrhodopsin-2 in PV neurons. Recordings were obtained from 60-channel MEAs under control conditions, before slices were stimulated with bath applied 4-aminopyridine (4-AP) to induce rhythmic activity. Recordings were analysed to detect spike and slow potential waveforms using amplitude thresholding. **Results:** 4-AP significantly increased the frequency of spiking (65.56 ± 21.35Hz to 151.71 ± 47.79Hz, $p < 0.05$) and slow potentials (0.41 ± 0.24Hz to 2.40 ± 0.30Hz, $p < 0.01$) from baseline. Blocking GABAergic signalling with bicuculline significantly decreased the frequency of slow potential (2.35 ± 0.69Hz to 0.58 ± 0.75Hz, $p < 0.01$) activity from 4AP levels back to baseline levels but had no significant effect on spiking frequency ($p = 0.17$). **Conclusion:** We have established a functional model of dorsal horn activity on MEAs using 4-AP stimulated rhythmic activity, providing a platform to investigate the role of PV neurons at the macrocircuit level utilising optogenetics.

id #12938

DNA modifications underlying sex differences in fear-related learning and memory

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There are significant sex differences in gene expression underlying fear-related learning and memory. However, beyond the influence of hormonal states, how sex differences in gene regulation contribute to the formation and maintenance of fear-related memories remains to be determined. We previously discovered that the DNA dioxygenase Ten-eleven translocation protein 3 (Tet3) mediates the accumulation of 5-hydroxymethycytosine (5hmC) and promotes the deposition of the histone modification H3R2^{me2s}, leading to a poised euchromatin structure and priming of fear extinction related gene expression in male mice. We have now discovered that the expression of Tet3 in the PLPFC of female mice increases after fear learning relative to male mice. Based on these observations, we next asked whether there are sex differences in 5hmC distribution in the medial prefrontal cortex prior to any learning, which may contribute to the observed sex difference in fear-related

memory. 5hmC profiling on DNA derived from the medial prefrontal cortex of naïve male and female mice revealed that males tend to have higher levels of 5hmC overall. However, there are significant sex differences in the accumulation of 5hmC on genes encoded within the X chromosome, some of which may escape X inactivation. These genes include, Ogt, Cdkl5, Mecp2, Csmd1, Atrx, and Ophn1. Notably, all are strongly implicated in cognitive function, with mutations having been associated with intellectual disabilities. Together, these preliminary findings suggest that Tet3-induced accumulation of 5hmC in PLPFC may be a critical mechanism underlying sex differences in fear-related learning and memory.

id #12941

Effects of bisacodyl on extrinsic sensory neurons to the bowel

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Backgound: Bisacodyl is a widely used stimulant laxative that became available in the 1950s. It is a pro-drug that is converted to an active form, bis-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM) in the body. It has local effects on colonic secretion, absorption and smooth muscle tone/contractility but how it triggers the coordinated motor activity of defecation is not clear. **Methods**: Effects of Bisacodyl and BHPM were studied on extrinsic sensory nerves to the large intestine using extracellular recording from full thickness ex vivo sheets of mouse colorectum. **Results**. Both Bisacodyl (50µM) and BHPM (10µM) caused a significant increase in multi-unit firing (from 1-12 minutes after application) in murine rectal nerves. Single unit discrimination showed that some rectal afferents were activated by Bisacodyl and BHPM while others were unaffected or even modestly inhibited. After the initial peak response, enhanced firing was observed for at least 60 minutes, even with prolonged washing. During this period, responses to further application of 50µM Bisacodyl or 10µM BHPM showed marked tachyphylaxis. Responses to both Bisacodyl and BHPM could be recorded from preparations in which the mucosa had been removed, indicating that deacetylation of Bisacodyl did not require mucosal epithelium, as previously suggested. There was a significant correlation between low threshold stretch-sensitivity and excitatory responses to Bisacodyl in rectal afferents, but no correlation with responses to capsaicin. **Conclusions:** Bisacodyl and BHPM both activate a subset of stretch-sensitive rectal extrinsic afferents; this may activate spinal visceral motor pathways which generate coordinated motor patterns of defaecation.

id #12944

Analysis of a novel Parkinson's disease-associated mutation

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Parkinson's disease (PD) is a multifactorial neurodegenerative disease of which only 5-10% of cases have currently been linked to monogenic heritable mutations. Given the low

percentage of identified heritable PD mutations we have recently screened 1725 patients with clinically diagnosed Parkinson's disease in order to identify novel PD-linked genes. Using whole exome sequencing we identified a novel, rare exonic mutation in one of the protein tyrosine phosphatase receptor (PTPR) genes, which segregated in a family with PD. In the brain PTPRs participate in signalling pathways involved in oligodendrocyte function, neurodevelopment, and neurotransmitter function. PTPRs are also activators of the Src family kinases in which they dephosphorylate the inhibitory tyrosine residue Y^{527}. The mutation we identified is located in the wedge domain, a region required for dimerisation, which subsequently leads to phosphatase inactivation. Thus, we hypothesised that this mutation will hinder dimerisation and lead to increased phosphatase activity. To test this, we developed mammalian expression constructs expressing wild-type and mutant coding sequences and investigated their function. We found that the mutant protein was recovered as two additional cleaved forms under basal conditions. Following metalloproteinase inhibition, the lower molecular weight cleaved form was not recovered confirming that enhanced sheddase activity was occurring in the mutant protein. We additionally investigated the phosphatase activity of the wild-type and mutant and discovered that the mutant had increased activity on the inhibitory Src family residue Y^{527}.

id #12956

Mapping the subnuclear organisation of somatostatinergic neurons in the nucleus of the solitary tract.

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Peripheral viscerosensory information is transmitted to the nucleus of the solitary tract (NTS), where it is integrated and relayed to other brain nuclei to generate autonomic reflexes. This relay of information is subjected to powerful modulation by networks of local interneurons within the NTS. Despite its importance, the anatomical organisation of NTS interneurons remains poorly understood, partly owing to a lack of reliable markers. We attempted to identify these neurons using a common inhibitory interneuron marker, somatostatin (SST). The distribution of SST-immunoreactivity in the NTS has been reported but with little detail on the neurochemical phenotype or distribution.

Using RNAscope *in-situ* hybridisation, we mapped the subnuclear distribution of SST neurons in the rat NTS and examined whether these neurons co-expressed GAD1 (glutamate decarboxylase 1) and/or VGLUT2 (vesicular glutamate transporter 2) mRNA.

The SST neurons within the NTS had a characteristic, differential distribution in specific subnuclei. Distinct populations were either excitatory or inhibitory, with SST GABA-ergic neurons in the lateral and parasolitary subnuclei, and SST glutamatergic neurons in the subpostremal and central subnuclei. Surprisingly, SST neurons expressing both GAD1 and VGLUT2 mRNA were also observed, predominantly within the medial NTS.

Whilst SST neurons constitute a major class of exclusively GABA-ergic inhibitory interneurons in the cortex, glutamatergic SST neurons occur in several brainstem nuclei. This study reveals that the SST neuronal population in the NTS is molecularly and anatomically heterogeneous. We conclude that this will underlie functional differences that are not restricted to local inhibition.

id #12958

Effect of intermittent fasting on brain monoaminergic neurotransmitters and anxiety-like behavior in rats

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It is well known that the contents of food, or the intake method, affect health and performance. In this study, we investigated the effects of intermittent fasting on levels of brain monoaminergic neurotransmitters and anxiety-like behavior in rats. Male Wistar rats were housed at a temperature of $23 \pm 1°C$, with 12-hour light and dark cycles. The rats were divided into two groups: intermittent fasting (IF) and control (Cont). While the Cont group had free access to food and water, the IF group were given food every other day. After 4 weeks, the rats underwent open field tests (OFT) to assess anxiety-like behavior; after a further 2 weeks, they were sacrificed for analysis of monoaminergic neurotransmitters. Cell bodies, and several projection areas, containing serotonin (5-HT), dopamine (DA) and noradrenaline (NA), were immediately removed from the brains after the rats were sacrificed. Levels of 5-HT, DA, and NA were analyzed using high-performance liquid chromatography. Body weight in the IF group was significantly lower than that in the Cont group. The IF group showed high levels of 5-HT in many brain areas such as the frontal cortex, hippocampus, and dorsal and median raphe. The IF group also showed high values of NA in the frontal cortex and locus coeruleus. The levels of DA were not different in any of the areas. Interestingly, no differences in OFT results were noted between the IF and Cont groups. Our study shows that IF can possibly positively impact brain monoaminergic neurotransmitters, without causing anxiety-like behavior.

id #12964

Effects of increased physical activity on brain monoamine levels in multistory enriched environment

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Numerous studies have shown that enriched environments (EE) could be effective for experimental rodents to improve some brain functions related to stress response and anxiolytic effect, and speculating that playfulness in EE might influence these beneficial effects. On the other hands, it is well known that increasing levels of physical activity could have the beneficial effects as well as EE. Taken together with these evidence, the question

arises: Which is effective for improvement of brain function between playing or physical activity? The aims of present study is to answer the question using multistory enriched environment (Multi-EE), which can increase physical activity in rats. We originally made Multi-EE, which are consisted by three stories. The male Wistar rats housed the Multi-EE or normal EE for 4weeks in group housing conditions (3 rats per cage). The rats housed in Multi-EE allow to access to the three stories freely by ladders. Daily physical activity were recorded using implantable accelerometer. Following 4 weeks, brain monoamine levels, which is involved with stress response and anxiolytic effect, were measured by HPLC in several brain regions. The Multi-EE significantly increased physical activity compared to normal EE. Furthermore, the Multi-EE housing were able to change the brain monoamine levels, such as serotonin and dopamine. The changing levels of these monoamine are known to have some beneficial effects for stress response and anxiety. Therefore, the results of present study suggest that increasing levels of physical activity by Multi-EE could influence some brain functions.

id #12970

Coordination of enteric viscerofugal neuron firing with smooth muscle voltage oscillations during the motor complex in mouse colon

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Enteric viscerofugal neurons project from the gut via extrinsic nerve trunks, forming reflex arcs with autonomic neurons that can regulate gut motility and secretion. Large populations of enteric neurons fire synchronously in rhythmic bursts at ~2Hz to generate rhythmic smooth muscle depolarisations in the murine colonic motor complex. Thus, viscerofugal neurons may be involved in this behaviour. Here, electrophysiological recordings were made from rectal nerve trunks in full-length flat sheet preparations of colon (n=3). An extracellular suction electrode was applied to the serosa within ~5mm of rectal nerve entry to detect motor complexes. To assist recordings, colorectal sensory innervation was reduced with bilateral surgical removal of L5-S1 DRG, allowing 7 days recovery before recordings. Consistent with sensory denervation, immunohistochemical analysis showed a significant reduction of CGRP in DRG-lesioned mice compared to controls (n=3). Consistent with viscerofugal neurons, rectal nerve recordings in DRG-lesioned preparations revealed ongoing firing that could be activated by the nicotinic agonist, DMPP, but not capsaicin (n=3). Ongoing motor complexes were detected every 2.6±0.6min, comprising voltage spikes and oscillations in smooth muscle at ~2Hz (n=3). Motor complexes were temporally associated with a transition from a relatively disorganised firing pattern in rectal nerves to discrete action potential bursts that were synchronized with muscle voltage oscillations at ~2Hz. This suggests the rhythmic ENS discharge pattern underlying motor complexes is transmitted by viscerofugal neurons out of the gut to extrinsic autonomic neurons. This raises the question whether motor complexes may initiate autonomic reflexes to modulate gut function in other regions.

INTERMUSCULAR, AND NOT CORTICOMUSCULAR, COHERENCE REFLECTS SYNERGY STRUCTURE DURING ISOMETRIC UPPER LIMB TASKS

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Complex EMG patterns of many muscles, during a range of tasks, can be expressed as activation of a few patterns of common co-activation known as muscle synergies (MSs). Consequently, MSs have been proposed as a mechanism for reducing motor command complexity. However, neural origins of MSs have not been identified. This study assesses coherence, a measure of common input within EMG and EEG signals, during synergy-tuned isometric upper limb tasks to further elucidate the source of neural drive producing MSs.

Fourteen healthy participants performed 3-D force matching tasks with a force instrumented handle, while recording EMG from 16 upper limb muscles and 32 channels of EEG. Participants first matched 26 targets in a sphere around the starting position, from which a number of MSs were calculated. Preferred directions for each synergy were determined from the level of activation of each synergy in the different force directions. In the second, synergy-tuned task, force targets were set in the preferred directions of the extracted MSs. From this second task, MSs and corticomuscular and intermuscular coherence (IMC) were calculated.

No corticomuscular coherence was observed in any condition. Above-chance IMC levels in the 10 Hz (alpha) range were found between muscles with high weights within individual synergies during both the force-ramp and hold phases of each trial. IMC levels between high-synergy-weight muscles were higher than IMC levels between a high and a low muscle synergy contributor.

Our coherence results suggest a subcortical origin of MSs under these task conditions.

High-fat Diet Induces 'Depressive-like' Behaviours in an Animal Model of Type 2 Diabetes: PUFA Supplementation as a Potential Intervention

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Bi-directional relationships are observed between major depressive disorder (MDD) and type 2 diabetes (T2D), each increasing disease risk and frequently co-existing; T2D patients are at a 2-fold risk of developing of comorbid depression. Chronic sub-clinical inflammation is implicated in the pathogenesis of each condition independently and may present a nexus to

explain this common comorbid relationship. Traditionally, stress is used to induce 'depressive-like' symptoms in animal models, however, elevated circulating pro-inflammatory factors and metabolic dysregulation are also demonstrated to result in altered behaviour; TLR signalling may be the mechanism by which this occurs. The current study used C57Bl/6 male mice to establish a model of T2D (1x75 mg/kg STZ + 22 wk 43% kcal high-fat diet). Open field tests (OFT) and sucrose preference tests (SPT) were performed to assess emotionality, anxiety and anhedonia throughout. At wk 15, dietary supplementation commenced with α-linoleic acid (ALA), an omega-3 polyunsaturated fatty acid with proposed antidepressant properties (*via* antagonistic interactions with TLR4). Both anhedonia and a depressive-like phenotype were observed in T2D mice at wk 14 (Line crossing: $P = 0.001$; Rearing: $P = 0.05$; Grooming/Rest: $P = 0.029$). T2D animals supplemented with ALA showed improvements in their anhedonic state despite no observed changes to their metabolic profile (body weight, glucose handling, insulin levels). Further, changes to dopamine and leptin were observed in T2D animals. This study provides confirmation that in the absence of a traditional stressor, metabolic dysregulation is sufficient to significantly alter behaviour. ALA supplementation may be of therapeutic interest.

id #13086

COMBINED VASOCONSTRICTORS GENERATE PERSISTENT MOUSE MODEL OF FOCAL CEREBRAL ISCHAEMIA.

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Necessary for understanding and developing treatments for neural injuries is the establishment of appropriate models. Focal ischaemia is commonly modelled in rats using the potent vasoconstrictor endothelin-1 (ET-1). However, ET-1 fails to reliably produce an ischaemic infarct with long-term behavioural deficit in mice. We suspect the poor translational fidelity of ET-1 is due to a high ratio of vasodilatory to vasoconstrictor endothelin receptors within the mouse brain. The lack of an ischaemic stroke mouse model hinders the investigation of stroke pathophysiology in transgenic models. We aimed to develop a reproducible mouse model of focal cerebral ischaemia (FCI) with persistent histological and behavioural deficit by co-administration of ET-1 together with vasodilatory endothelin receptor antagonists (RES-701-1 and BQ-788, targeting the ET-B1 and ET-B2 receptors respectively) and/or nitric oxide synthase inhibitor (L-NAME). Combined administration of vasoconstrictor compounds into the motor cortex produced focal infarcts, demonstrated by NeuN+ and DAPI+ cell loss, which was most pronounced and reproducible in animals administered the triple cocktail of ET-1, RES-701-1 and L-NAME. These cortical infarcts resulted in sustained motor deficits (cylinder and Ladder walk, tests of various gross motor skills) (n=12-13/group). We found the triple cocktail successfully generated focal cerebral infarct with associated functional deficit in mice. Establishing a reliable model of FCI in mice will advance knowledge of ischaemic stroke and facilitate further stroke therapies.

id #13126

Age-Related Differences in Alpha Oscillations at Varying Working Memory Loads

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Working memory (WM) is vulnerable to age-related decline in performance, particularly when WM demands are high. Alpha oscillations in visual brain regions are thought to support WM performance in young adults, and though alpha oscillations are known to decrease in power and frequency with age, it is unclear whether alpha activity supports WM in older adults. To test this, we examined age-related differences in visual alpha activity during WM in younger and older adults by recording electroencephalography (EEG) while they performed a modified Sternberg task under 1-, 3- and 5-letter load conditions. Our results indicated that alpha power decreased, and alpha frequency increased with load during encoding, but this was not age dependent. Regardless of age, the magnitude of alpha suppression during retention was larger in load-5 than in load-1 and load-3 trials. While alpha power during retention was lower than fixation in older, but not younger adults, the relative change from fixation was not significantly different between age groups. Further, individual differences in visual alpha power did not predict individual task performance within age groups, at any WM loads. Our results demonstrate that though alpha rhythm slows with age and decreases in power, both alpha power and frequency were modulated in a similar task- and load-dependent manner during WM performance in younger and older adults. However, these changes in alpha were not associated with task performance, suggesting that age-related changes in alpha rhythm are not associated with a decline in WM performance.

id #13128

Serine Racemase expression and its function in striatum and in striatal GABAergic interneuron.

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D-serine is synthesized by serine racemase (SR) and is an excitatory neuronal modulator which works as a co-agonist of N-methyl-D-aspartate receptor (NMDAR). Excitatory neurons in Hippocampus and forebrain have been explored mainly for the existence of them. Herein we silenced SR in GABAergic interneuron in mice (iSR-/-) and investigated primarily in striatum and their behavioral change.
Our iSR-/- had reduced SR almost only in striatum and had almost no D-serine reduction. SR positive cells in striatum showed strong co-localyzation with dopamine- and cyclic AMP-regulated neuronal phosphoprotein (DARPP32) in wild type mice. The mice carrying fluorescent transgenes driven by the promoters for either D1 or D2 dopamine receptors showed 65%:35% co-localyzation of SR with Dopamine D1:D2 receptor positive cells. These results indicate that GABAergic interneurons receiving dopaminergic inputs in striatum have

rich SR which is NMDA modulator.

In behavioral tests, iSR-/- mice showed a blunted response to the hedonic and stimulant effects of cocaine without anxious behaviors. Because both the cocaine effects and anxious behaviors have been shown in the constitutive SR-/- mice, this unilateral effect of silenced SR in iSR-/- mice suggests the striatal SR's role in the behaviors reacting to dopaminergic psychostimulants' effects.

Results in this study suggest that SR is synthesizing D-serine not as a glutamatergic co-transmitter but rather as an autocrine whereby the GABAergic interneurons control the excitability of their NMDA receptors by determining the availability of the co-agonist, D-serine.

id #13155

The role of neurogenesis and axon guidance molecules in the formation of the neocortical commissure in marsupials compared with eutherians

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A defining feature of all mammals is the organisation of their telencephalon into a six-layered neocortex, with conserved organisation of projection neurons. Over one hundred million years ago, placental and marsupial mammals diverged evolutionarily, demonstrating a striking anatomical difference in the main connection between left and right neocortical hemispheres. In marsupials, interhemispheric neocortical axons turn laterally, before crossing the midline alongside olfactory axons in the anterior commissure. Exclusively in placental mammals, this population of axons turns medially to form the corpus callosum. Using an Australian marsupial, the fat-tailed dunnart, and the placental mouse, we compare similarities and differences in brain development to elucidate evolutionary steps that led to the emergence of the corpus callosum. Here, we uncover key features of marsupial neocortical commissure formation, demonstrating conservation of a neocortical neurogenic gradient initiating rostro-laterally and proceeding medio-caudally, similar to mice. This neurogenic gradient also has a conserved relationship with the order of crossing of neocortical commissural axons in dunnarts, indicating that this mechanism preceded callosal evolution. However, our data show that cingulate axons do not pioneer the commissural route in dunnarts. An analysis of axon guidance molecule expression in the dunnart brain suggests that marsupial neocortical axons use more 'callosal-like' cues rather than those used by the anterior commissure. Crucially, we identify candidate spatiotemporal differences in the expression of some of these cues, such as an inverted expression, which may underlie medial (placental) versus lateral (marsupial) turning of neocortical commissural axons and the emergence of the corpus callosum.

id #13158

Asperuloside reduces food intake and body weight via downregulation of orexigenic hypothalamic signalling in a mouse model of metabolic syndrome.

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Controlling the urge to eat requires adequate integration of hypothalamic neuropeptides regulating energy balance processes with dopaminergic circuits in the mesolimbic pathway. Disruption of this signalling alters the hedonic control of reward-driven food intake contributing to obesity. Our study aimed to understand the mechanism of action of the novel anti-obesity compound, asperuloside (ASP), and whether ASP treatment could be used as safe weight loss therapy.

Mice were fed with a 45% high-fat diet (HFD) and concurrently treated with ASP for 12 weeks. ASP reduced significantly (10.50%, $p < 0.05$) final body weight of HFD mice compared to their control group. The effects of ASP reached significance after five weeks of treatment and were maintained throughout the experiment. Over the experimental period, the compound induced a significant daily energy intake reduction (12.8%, $p < 0.05$) in mice consuming HFD compared to their control group. When standardised by body weight, the visceral adipose mass was increased by the effect of HFD and significantly reduced by ASP treatment (35%, $p < 0.05$). Orexigenic hypothalamic genes including NPY and ARGP as well as essential receptors involved in the control of food intakes such as ghrelin, leptin and cannabinoid receptors, were significantly ($p < 0.05$) downregulated by ASP treatment. These results suggest that ASP might induce weight loss plausibly via downregulation of hypothalamic signalling and support ASP candidacy as a potential and safe drug for the treatment of obesity.

id #13159

Tonotopic mapping in a mouse model of autism spectrum disorder

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Autism Spectrum Disorders (ASD) are a set of developmental disorders defined by impaired learning, sensory disorders, communication difficulties, social deficits and stereotyped behaviours. The social and communication difficulties in ASD are thought to be due to the distorted processing of sounds, which in turn impairs language abilities. We hypothesise that auditory cortex circuitry develops incorrectly, resulting in abnormal neuronal connectivity and impaired ability to process sound. Here we used in vivo calcium imaging to examine tonotopic maps in the auditory cortex in the Shank3b knockout mouse model of ASD. At postnatal day 1 mice were transduced with the genetically encoded calcium sensor GCaMP6 via injection of an adeno-associated viral vector into the lateral ventricle. At 4-7 weeks mice were anesthetized with isoflurane and a craniotomy was created over the auditory cortex. Fluorescent increases, which reflect neuronal firing, in response to tones of varying frequencies (5-50 kHz, 90bD) delivered to the contralateral ear were recorded using widefield imaging at 5Hz (2x magnification). Our results suggest that there are differences in the representation of tones in the auditory cortex of ASD mice. Specifically, ASD mice showed enlarged primary auditory cortex area relative to littermate controls. In addition, the variability of tone frequency preferences was increased in ASD mice. These findings indicate more poorly regulated cortical representation of sound in this model of ASD, which may link to the human phenotypes of auditory hypersensitivity and language impairments.

id #13174

Disturbances in ionic composition of cerebrospinal fluid after experimental ischemic stroke

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'Early neurological deterioration' (END) occurs when an ischemic stroke patient deteriorates due to infarct expansion 24-48 hours after stroke. Our lab recently demonstrated an oedema-independent increase in pressure in the skull after experimental stroke and that increased intracranial pressure reduces blood flow to the damaged area. As no oedema or bleed is involved in END, cerebrospinal fluid (CSF) is likely the main driver of this phenomenon. Stroke-induced necrosis and excitotoxicity cause intracellular ions to be released into the interstitial fluid. It is possible that this alters the ionic composition of CSF and may contribute to the mechanisms driving increased pressure in the skull. We induced stroke in male, adult, Wistar Kyoto rats by transiently occluding the middle cerebral artery for three hours. Stroke was confirmed by haematoxylin and eosin staining. CSF was collected by lumbar puncture at two, four, and six hours post-reperfusion and pooled. CSF sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and chloride (Cl^-) was measured using a clinical chemistry analyser (ARCHITECT *c16000).* Differences between stroke and sham were determined using Student's t-test. Elevated Mg^{2+}($p = 0.002$), and lowered K^+ ($p = 0.019$) and Ca^{2+} ($p = 0.009$) was identified in stroke rats when compared to sham. Interestingly, there

was no significant difference in Cl$^-$ or Na$^+$ concentrations. These results indicate extensive disturbance to normal CSF composition. The role of this disturbance in stroke outcome and, in particular, in altered intracranial pressure after stroke remains to be established.

id #13176

NEUROPROTECTIVE POTENTIAL OF TINOSPORA CORDIFOLIA EXTRACT IN ATTENUATING PRENATAL VIBRATORY STRESS INDUCED COGNITIVE DEFICITS IN WISTAR RAT DAMS AND THEIR NEONATES

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In the present day, pregnant women are exposed to vibrations while travelling which affects brain of developing fetus. *Tinospora cordifolia* (TC) is one of the *medhya* drugs in *Ayurveda* claimed to improve mental ability. However, experimental evidence to prove its anti-stress effect on cognition is lacking in literature. In the present study, pregnant Wistar rats (dams) were divided into normal control (NC), vehicle control (VC), vibratory stress (3 hrs/day) (VS) and VS+TC treatment (6mg TC extract/kg b.w /day) groups (n=6/group). The pups born to corresponding dam groups were allocated into NC_Neo, VC_Neo, VS_Neo and VS+TC_Neo (n=6/group). After the treatment period, dams and pups [at postnatal day 45] were subjected to Morris water maze and passive avoidance tests to study their spatial learning and memory retention respectively. Results of spatial learning and avoidance memory in dams showed significant decrease ($p<0.05$; $p<0.001$) in ability of VS rats compared to same in NC and significant increase ($p<0.001$) in VS+TC compared to same in VS and not in NC. Spatial learning and avoidance memory in neonates, showed significant decrease ($p<0.001$) in its ability in VS_Neo compared to NC_Neo and significant increase ($p<0.001$) in VS+TC_Neo compared to same in VS_Neo and not in NC_Neo. Significant decrease in spatial learning ability observed in VS_Neo was correlated to same in VS dams and significant increase in avoidance memory was observed in VS+TC_Neo compared to VS+TC dams. In conclusion vibratory stress has detrimental effect on cognition in dams and their neonates which can be attenuated by TC treatment.

id #13178

Presynaptic opiate receptor expression of Rostro Ventromedial Medulla projection neurons in the dorsal horn of the rat spinal cord

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Nociceptive information from the periphery is first integrated in dorsal horn of the spinal cord (SCDH) & changes to signalling here is associated with the development and maintenance of persistent pain states. The rostral ventromedial medulla (RVM) sends descending projections directly synapse with neurons in the SCDH and is strongly involved in anti-nociceptive actions of opioids.When the soma of GABAergic RVM projection neurons are exposed to

opiate receptor (OR) agonists, twice as many respond to mu-OR agonists than to kappa OR agonists. In the SCDH, it's unclear what opiate receptors are functionally expressed on the presynaptic terminals of RVM descending fibres or how they might contribute to spinal opiate analgesia.We used optogentics to selectively stimulate descending RVM projections and record light-induced inhibitory postsynaptic currents (l-IPSCs) from neurons in the SCDH. The effect of mu-, and kappa- agonists (3 μM DAMGO and 0.3 μM U69593, respectively) on the l-IPSC amplitude and paired pulse ratio (PPR) demonstrates that both these agonists inhibit l-IPSCs and increase PPR in an equal proportion of the SCDH neurons. Our data suggest that in contrast to in the neuronal soma, the presynaptic terminals of descending RVM projection neurons respond more consistently to kappa opiate receptor agonists.Given that spinal dynorphin (the endogenous kappa agonist) is elevate when persistent pain states develop, and that the transition from acute to chronic pain requires the input of descending RVM to SCDH projections, data suggests that endogenous opiate modulation of descending inhibitory inputs by kappa opiate receptors may play a key role.

id #13179

Retro-TRAP: a novel way to characterize the connectivity of the nucleus accumbens shell

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The ventral subiculum (vSub) to the nucleus accumbens shell (NAc shell) projection is implicated in context-induced relapse to alcohol-seeking. Whilst this projection is glutamatergic, the complete neurochemical phenotype of these projection neurons is still unknown. Here we aim to characterize vSub→NAc shell projection neurons using retro-TRAP, retrograde tracing in combination with translating ribosome affinity purification.

First, we validated the viral expression and cre-dependent feature of pAAV-Ef1a-DO_DIO-TdTomato-EGFP-WPRE-pA (retro-GFP) and pAAV-FLEX-NBL10 (NBL-10) by injecting retro-GFP into the NAc shell and NBL10 into the vSub in vglut2-cre mice (n=4) and wild-type mice (n=3). Subsequently, we injected retro-GFP virus (NAc shell) and NBL-10 (vSub) into vglut2-cre mice (n=6), used immunoprecipitation to extract mRNA from vSub-NAc shell projection neurons and examined GFP mRNA enrichment and glial marker GFAP depletion in IP/Input sample with droplet digital PCR (ddPCR).

Our virus validation showed that retro-GFP and NBL-10 could specifically transduce vglut2-cre-positive vSub-NAc shell projection neurons. ddPCR results showed enrichment in GFP and depletion in GFAP in IP/Input sample. These results suggest that we have successfully applied retro-TRAP to extract translating mRNA in the projection from the vSub-NAc shell. In future studies, we will use RNA sequencing to completely characterize this projection pathway. Following this, we will functionally examine the role of identified molecules in relapse to alcohol-seeking in rats.

id #13180

Retroactive interference of a learned action-outcome association by exposure to a stimulus-outcome learning experience

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The current study used the protocol established to produce retroactive interference (RI) in contextual memory paradigms and applied it to an appetitive learning setting. Rats were first subjected to the learning of an operant conditioning, action-outcome (A-O) association and were tested in a probe (A-O probe) session the following day. Rats that experienced an interpolated, classical conditioning, stimulus-outcome (S-O) learning experience performed more poorly in the probe session than those that did not undergo this experience. The S-O learning experience had therefore retroactively interfered with the A-O task. The degree of interference also varied with the amount of time lag between the three (A-O, S-O and the AO probe) conditions. The current study is the first to show evidence of RI by an S-O learning experience on a recently learnt A-O association. These results are similar to those from hippocampal-dependent learning paradigms and indicate that interference of memory consolidation in an appetitive learning paradigm can be produced by an intervening task that is behaviourally distinct from the initial learning experience.

id #13182

Tropomyosin Tpm3.1 is integral in maintaining the axon initial segment structure and function

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The axon initial segment (AIS) is the proximal region of the axon and the site at which action potentials initiate. The AIS also serves as a vesicular filter and diffusion barrier, restricting the access of dendritic and lipid membrane proteins into the axon, helping to maintain neuronal polarity. Recent studies describe a specialised actin complex within the AIS comprising highly organised actin ring structures and patches of filamentous actin. In the

current study, we further investigated the properties of actin within the AIS, with a focus on Tropomyosin isoform Tpm3.1. Tpm3.1 is an actin associated protein involved in stabilising actin filaments by regulating the access of other actin-binding proteins. We found that a population of stable actin filaments in the AIS are decorated by Tpm3.1. Furthermore, Tpm3.1 appears in a periodic structure in the AIS alongside the actin ring structures. Disruption of Tpm3.1 in 9-10 DIV hippocampal neurons leads to perturbations in the AIS structure, vesicle filter, and clustering of voltage-gated sodium ion channels. Specifically, inhibition of Tpm3.1 using small molecule inhibitors diminished the accumulation of the AIS scaffolding protein ankyrin G by more than 40%, in a time- and dose-dependent manner. Conditional KO of Tpm3.1 *in vitro* also led to reductions in ankyrin G signal by approximately 25%. Furthermore, Tpm3.1 inhibition resulted in somatodendritic protein GluA1 entering the axon, as well as a 20% reduction in action potential firing frequency. These results suggest Tpm3.1 is a necessary component of the actin cytoskeleton complex that maintains AIS structure and function.

id #13183

IDENTIFYING THE GENES REGULATING miRNA DURING NEUROGENESIS USING COLLABORATIVE CROSS MOUSE MODEL

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Most pervasive and conventional health problems are caused due to distinct genetic aetiologies in the population. Often it is not just single mutation that leads to complex disorders rather multiple genetic variants. However, existing resources employing single mouse strain as a model organism for studying complex diseases have only limited information on interactions between genes, environments and other factors. Therefore, the use of Collaborative Cross (CC) mice as a genetic reference panel of recombinant inbred lines of mice, will generate comprehensive information on genetic and phenotypic diversity derived from over 1000 genetically diverse lines. In our study, we aim to identify the upstream regulators of microrna through CC mice in order to dissect the genetic architecture involved in neurogenesis. miRNAs have been associated with many neurodevelopmental pathways and studying miRNA biogenesis will provide leads to many abrupt neurodegenerative mechanisms. We will look at the pattern of neruogenesis in both adolescent and adult age groups as not much is known of the difference in neurogenic processes between them. We have studied the expression of three micrornas in 54 cc lines from adolescent and adult aged mice and will further look into the genetic machineries driving neurogenesis in both age groups. Our results confirm the expression of mir9, mir29a and mir107 in cc lines. QTL analysis identified the genes that cause change in miRNA expression across different cc lines. Finally, the functional analysis of the candidate gene will help understand the underlying miRNA biogenesis and related pathways involved in the neurogenesis.

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id #13185

Assessment of cognitive impairment after experimental stroke using a rodent touchscreen platform

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Numerous clinical studies have documented the high incidence of cognitive impairment after stroke. The cognitive domains that are commonly effected by stroke include memory, learning and executive functions. Here, we aimed to investigate post-stroke cognitive impairment in an experimental model of stroke by using a mouse touchscreen platform and understand the mechanisms behind these deficits. C57BL/6 male mice were subjected to photothrombotic occlusion at the left motor and somatosensory cortex or sham surgery. Motor function was evaluated using the cylinder and grid walk task. Cognitive performance were assessed using the mouse touchscreen platform starting from 1 week to 3 months post-stroke. Brains were collected for further analyses. As expected, mice exhibited limb use deficits after stroke. Strikingly, stroke significantly impaired associative memory and behavioural flexibility performances compared to sham and these deficits extended up to 3 months after stroke. Further protein and histology analyses revealed a persistent loss of neuronal structure and accumulation of amyloid beta at both the peri-infarct and hippocampus. Our results demonstrate that stroke induce impairment in different cognitive domains and these deficits persist long-term. Our results also suggest these cognitive deficits might be due to secondary neurodegeneration processes in areas remote from the primary infarct.

id #13186

Understanding the Impact of Missense ZBTB18 Variants in Brain Development and Disease

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The activities of DNA-binding transcription factors, such as the multi-zinc finger protein ZBTB18 (also known as RP58, ZNF238, or ZFP238), are essential to coordinate mammalian neurodevelopment, including the birth and radial migration of newborn neurons within the fetal brain. In humans, the majority of disease-associated missense mutations in ZBTB18 lie within the DNA-binding zinc finger domain and are associated with brain developmental disorder, yet the molecular mechanisms explaining their role in disease remain unclear. To address this, we developed *in silico* models of ZBTB18 bound to DNA, and discovered that the majority of such variants map to residues with significant sequence-specific DNA contact, while general population variants were predominantly mapped to residues with limited contributions to DNA binding by ZBTB18. We studied disease-associated variants mapping to residues with close (N461S) and limited (R495G) DNA contact and found that each bound DNA promiscuously, displayed altered transcriptional regulatory activity in vitro, and influenced the radial migration of newborn neurons in vivo in different ways. Furthermore, general population variants were found to influence DNA binding and transcriptional regulation. Taken together, our results suggest that altered transcriptional regulation could represent an important pathological mechanism for ZBTB18 missense mutations in brain development and disease.

id #13187

NOVEL STEM CELL LINES FOR MODELING FAMILIAR PARKINSON'S DISEASE

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The application of Mesenchymal Stem Cells (hMSCs) and human Induced Pluripotent Stem Cells (hiPSCs) as a source of dopamine neurons for both disease modelling and cell-replacement therapy in Parkinson's disease (PD) has increased. Thus, refinement of neural differentiation protocols has been critically important for the correct mimicking of neural development and thus, generation of appropriate cells types from stem cells. Here we have used hMSCs in order to target the LRRK2 gene-region associated with the most common familial form of PD, through a Ribonucleoprotein (RNP) Cas9-eGFP-NLS reporter system, and transdifferentiated them into a neural-like state, achieving neural-like precursors in 7 days. We have also utilized a reprogramed hiPSCs-line derived from a patient with alpha-synuclein triplication and differentiated these towards a ventral-dopaminergic fate *in vitro*. After transplantation in mice the human dopamine neurons survive and show robust alpha-

synuclein expression. These cell-lines will be interesting resources for human disease-modeling of PD, helping to shorten the translational gap between animal models in PD.

id #13191

A Stroke of Genius: Investigations into the Guanidinium-Head Group of Poly- Arginine R18 Peptide

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Stroke is a leading cause of morbidity and mortality worldwide, therefore clinically effective neuroprotective agents are needed to salvage vulnerable brain tissue. Research has demonstrated that poly-arginine peptides have potent neuroprotective properties in *in vitro* and *in vivo* stroke models, with poly-arginine-18 (R18) peptide identified as being highly efficacious. Arginine residues are known to enhance uptake and neuroprotection, thought to occur by way of the guanidinium head group, which is found exclusively on arginine. Therefore, this study aimed to investigate the role of the guanidinium head group in R18 cellular uptake, intracellular localisation and neuroprotective function. Comparative studies were performed using R18 and poly-ornithine-18 (O18), which is structurally similar and displays the same net positive charge as R18 but does not possess guanidinium head groups. Cellular uptake and localisation of FITC-conjugated R18 and O18 peptides was examined using confocal microscopy. Furthermore, neuroprotection was assessed in a neuronal glutamic acid excitotoxicity model, with fluorometric and colourmetric assays performed to examine calcium influx, calcium-mediated caspase and calpain activity, and cellular viability. Both peptides demonstrated uptake in cell types, with differing localisation. In the excitotoxicity model, R18 was highly neuroprotective and significantly reduced intracellular calcium influx, whereas O18 displayed no neuroprotection, and a nonsignificant effect in reducing calcium influx. Fluorometric assays revealed R18 attenuated calcium-mediated caspase-3, 7 and 9 and calpain activation at 6- and 24-hours post-injury, whereas O18 was ineffective. Overall, this study indicates that the guanidinium head group doesn't appear to induce peptide uptake but is a critical factor for neuroprotection.

id #13193

CARNOSIC ACID PROMOTES SURVIVAL OF DISSOCIATION-INDUCED HUMAN PLURIPOTENT STEM CELL AND INFLUENCES NEURONAL DIFFERENTIATION

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Dissociation-induced cell death of human induced pluripotent stem cell (hiPSC) is an insurmountable hurdle in cell culturing. Currently, a small number of compounds such as Y-27632 that is a ROCK inhibitor, or Nicotinamide (NMN) is studied to decrease hiPSCs mortality when dissociated, however, most of which may bring potential complications such as morphological changes or unpredictable differentiation under indefinite mechanisms. Carnosic acid (CA), an extraction from herb Rosmarinus, is reported to exert its role of anti-oxidant and anti-inflammatory compound in various diseases. Here we assay hiPSCs in terms of survival ability, cloning efficiency with treatment of CA after dissociation, as well as the effect of CA in hiPSCs proliferation and differentiation. RNA-sequence is conducted to obtain involved genes that mediate regulation. Our study demonstrates that CA enables to promote cell survival and increase efficiency in cloning formation after dissociation. Among all genes involved, we show 16 most associated genes that are significantly regulated in particular pathways which may provide clues to track down potential mechanism. Besides, CA induces cell proliferation whereas shows few effects on cell spontaneous differentiation. Long-term treatment with CA up to 2 weeks may partially attribute to cell differentiation into neuron by modulation of screened genes specifically based on RNA-sequence data. Reversly, further analysis shows that during procedure of neural progenitor cell (NPC) differentiation, CA barely functions as a promoter in transforming efficiency. Collectively, CA is an alternative approach in protecting hiPSCs from dissociation-induced apoptosis and in promoting cell growth, whereas induces neuron differentiation with little.

id #13194

Wnt-3a can exacerbate pro-inflammatory stimuli in astrocytes and microglia

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Currently, treatments for Parkinson's disease (PD) are mainly symptomatic without any slowing of dopaminergic neuron degeneration; one of the main hallmarks of the disease. An increasing number of studies have shown chronic inflammation as a critical mechanism underlying neurodegeneration. A potential way to promote an anti-inflammatory milieu and

counteract the loss of neurons in PD is through application of Wnt-3a, leading to inhibition of the destruction complex and stabilisation of ß-catenin in glial cells and subsequent reduction in inflammatory signalling. Therefore, we hypothesised that stabilising ß-catenin in microglia and astrocytes through the addition of exogenous Wnt-3a could lead to an anti-inflammatory phenotype of these immune cells, which is necessary to protect neurons. Interestingly, the addition of Wnt-3a was unable to protect against loss of dopaminergic and total neuron populations in the substantia nigra in the MPTP mice model of PD. Wnt-3a also exacerbated the MPTP-induced increases in microglia and astrocyte populations, suggesting a potential inflammatory effect of Wnt-3a. Mirroring these results, Wnt-3a potentiated the increases in pro-inflammatory cytokines TNF-α and IL-1ß induced by 24 hours of LPS stimulation in primary astrocytes and microglia, despite an observed increase in ß-catenin expression. Interestingly, we found no effect of Wnt-3a on GSK3ß expression. Together, these results suggest that Wnt-3a may exacerbate neuroinflammation and subsequently promote neurodegeneration, potentially via activation of pathways independent of ß-catenin. Investigation of different post-stimulation timepoints and other intra-cellular signalling molecules will reveal a more complete view of the different events occurring after the addition of Wnt-3a.

id #13195

Selective accumulation of 5-formylcytosine in neurons activated by extinction learning

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The DNA modification 5-methylcytosine (5mC) can be sequentially oxidised and converted to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) by the ten-eleven translocation (Tet) family of DNA dioxygenases. Previous research suggested that 5fC could be a stable epigenetic mark, and it has been shown to regulate gene expression. We examined the genome-wide distribution of 5fC in prefrontal neurons that have been activated by fear extinction learning, by using the 5fC-CET method at single base resolution. Overall, 14299 5fC sites were detected, with 1365 found to be unique to extinction positive neurons and most harbouring a "CTT" motif. 5fC enrichment was predominantly found within promoter regions and a GO term analysis revealed "synapse" to be the top functional category of genes containing the 5fC modification. We further compared the 5fC genome-wide distribution data with RNA-seq data derived from an independent cohort and detected a significant overlap between 5fC accumulation and extinction-specific differential expression of 141 genes. Taken together, these data indicate that 5fC accumulation has a distinct pattern within the neuronal genome, which may serve to regulate the expression of memory- related genes in response to fear extinction learning.

id #13197

MicroRNA Expression in the Diagnosis of Parkinson's Disease

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Current clinical tests for Parkinson's Disease (PD) provide insufficient diagnostic accuracy leading to an urgent need for biomarkers. MicroRNAs (miRNAs) are non-coding RNAs involved in post-transcriptional gene expression control and are easily measured within various biofluids. We aimed to identify plasma-derived miRNAs to serve as potential diagnostic and prognostic biomarkers of PD. 35 PD patients and 34 age and sex-matched controls were recruited. A subgroup of PD (n=26) were reassessed longitudinally after 4 years. Data on demographics, disease severity, cognition, sleep quality, and anxiety/depression levels were collected. Fasting blood was collected at baseline and at 4-years. RNA was isolated from EDTA-plasma (mirVANA PARIS™). Neuropathology-related miRNAs (n=187) were measured using custom-designed low-density TaqMan arrays. Data were normalized using R 3.4.3 (HTqPCR, normrank). Differentially expressed (DE) miRNAs were identified using the $\Delta\Delta Ct$ method and the Mann-Whitney-U test for significance *(Benjamini-Hochberg, p<0.05)*. 12 DE miRNAs were found in PD vs controls (*adjusted p<0.05*) [9 upregulated (fold change: 1.31–1.73), 3 downregulated (fold change: 0.68–0.82)]. In longitudinal assessment of PD, 13 miRNAs were DE (*adjusted p<0.05*) [6 upregulated (fold change: 1.30–1.80); 7 downregulated (fold change: 0.59–0.86)]. 1 miRNA was common between the analyses, although the direction of change was up in PD vs control and down in longitudinal PD. Our results suggest that specific groups of miRNAs are altered at different stages of PD and could serve as biomarkers of diagnosis and disease progression. Early PD diagnosis is crucial for the development of new curative and preventative treatments and improvement of patients' quality of life.

id #13199

Deferiprone as a novel therapeutic for major depressive disorder

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Major depressive disorder (MDD) is identified by a prolonged period of depressed mood. Individuals with a genetic predisposition of the serotonin transporter have an increased propensity for developing MDD and are more resistant to the therapeutic effects of antidepressants. One potential treatment option that is known to alter serotonin levels is Deferiprone. Deferiprone is primarily an iron chelator, and has actions on mechanisms known to be altered in individuals with MDD. Serotonin transporter knock-out (5-HTT KO) mice can be used to model the neuropathology associated with MDD. Therefore, the aim of the project is to investigate the therapeutic effects of Deferiprone on the 5-HTT KO mice model of MDD. To assess the therapeutic viability of DFP on depression-like behaviours, a battery of behavioural tests were conducted. These included the forced swim test (FST) and the novelty-suppressed feeding test (NSFT). It is established that acute delivery of Deferiprone

reduced immobility time of 5-HTT KO mice in the FST, suggesting improved stress coping abilities and an antidepressant-like effect. Furthermore, in the NSFT used to model anxio-depressive behaviours, acute Deferiprone also improved the performance of 5-HTT KO mice. Chronic treatment of Deferiprone resulted in no difference in behavioural performance in the FST. In conclusion, acute Deferiprone is effective in ameliorating the depressive-like behaviours of the 5-HTT KO model. These behavioural observations present a potential therapeutic for alleviating symptoms associated with FST and NSFT. Further investigation must now be focused on deciphering the molecular and neuroanatomical mechanisms underpinning the therapeutic actions of Deferiprone.

id #13200

Secreted amyloid precursor protein-alpha and its bioactive domains initiate a global increase in protein synthesis in rat hippocampus

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Secreted amyloid precursor protein-alpha (sAPPa) has been shown to increase the synthesis of specific plasticity-related proteins. We examined whether sAPPa and two of its bioactive domains (the three amino acid (aa) tripeptide RER and the C-terminal 16 aa (CTa16)) could initiate a global increase in protein synthesis in CA1 mini-slices micro-dissected from adult rat hippocampal slices. To measure global protein synthesis, we used the SUnSET (surface sensing of translation) and western blot techniques. We found that sAPPa (1 nM, 30 min) caused a 1.89 ± 0.16-fold increase in newly synthetized proteins compared to controls (n=6, p=0.0156). The increase was blocked by the protein synthesis inhibitor anisomycin (40 µM). Of particular note, the addition of the isolated bioactive domains RER (1 nM, 30 min) and CTa16 (1 nM, 30 min) also caused a significant increase in global protein synthesis (1.74 ± 0.14, p=0.0259 and 1.516 ± 0.14, p=0.0459, respectively). Addition of sAPPa in which the RER sequence was replaced with alanines (sAPPa-AAA, 1 nM, 30 min) failed to cause an increase in protein synthesis, suggesting that RER is required for sAPPa to initiate protein synthesis. Treatment with a related peptide lacking the CTa16 sequence (sAPPb) also failed to initiate new protein synthesis. These findings suggest that at least two of the functional regions of sAPPa exert their beneficial effects through a rapidly induced global increase in protein synthesis.

id #13201

Investigating the effects of OxR1 antagonism on goal-directed decision making

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Orexins are neuropeptides produced by several thousand neurons in the lateral hypothalamus. These neurons project widely through the central nervous system where they bind to regionally selective and largely non-overlapping receptors: OxR1 and OxR2. Orexins are well known as regulators of the sleep/wake cycle; however, recent investigations into orexinergic modulation of feeding or drug seeking behaviour suggest they also play a role in reward processing and decision making, in particular, through OxR1 receptors located in the VTA.

In this study, we sought to investigate the effects of OxR1 antagonism on goal-directed decision making using an operant probabilistic reversal learning task.

40 male C57/BL6 mice were dosed daily with an OxR1 antagonist (1-SORA-51, 45mg/kg) or vehicle (20% w/v TPGS) prior to performing a PRL task consisting of 5 days of probabilistic discrimination learning, followed by 5 days of reversal learning, both on and off drug in a crossover design.

Results. Compared to controls, animals took significantly longer to make decisions in the operant task on drug. We then characterized animal choices using reinforcement learning models consisting of separate learning rates for positive/negative reward prediction errors (RPE). Animals dosed with 1-SORA-51 show a substantial decrease in positive RPE learning rate compared to TPGS control, with no differences in the negative RPE learning rate.

This suggests that OxR1 antagonists do not suppress learning overall, but rather decrease the updating of reward values following positive RPEs selectively. As such, OxR1 antagonists may be of interest in models of abnormal reward processing.

id #13202

Efficacy of Deep Brain Stimulation in the Treatment of Parkinson's Disease; A Longitudinal Study

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Deep brain stimulation (DBS) is a safe and proven neurosurgical therapy used in the treatment of wide range of conditions including Parkinson's Disease (PD). It involves implantation of electrode in specific part of the brain that delivers electric stimulation. Electrophysiological recordings are made during the surgery to confirm accurate placement in SubThalamic Nucleus (STN), the target of choice to treat PD symptoms. Action potentials and field potentials are then studied from individual cells within these recordings. During this surgery, the clinician uses various interventions, limb manipulation, and brief discussion with the patient. We recorded and analysed the change in beta activity during these evoked responses and found that it varies between physical and mental tasks. We constructed 3D structure of electrodes using pre-operative MRI and post-operative CT scans to locate the exact position and trajectory of the electrodes and the volume of tissue activated by the electrode was calculated. Before surgery the clinical signs tested by the clinicians are quantified and documented for comparison with assessments obtained during and after surgery. We used accelerometers to quantify the movement. Spirography was evaluated with a graphics tablet and found to be an important measure for quantitative assessment of PD symptoms like tremor and dyskinesia. In summary, we have developed a quantitative assessment of motor symptoms before and after DBS surgery to objectively assess the efficacy of DBS in PD. This approach will help in patient selection and predict patient outcomes of DBS.

id #13203

ATP13A2: Characterization of a Novel Human iPS Cell Model of Parkinson's and Batten Disease

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 Mutations in *ATP13A2* lead to the development of two distinct neurological disorders, Kufor-Rakeb Syndrome, a juvenile parkinsonism and CLN12 Batten disease, a lysosomal storage disease. The underlying cause of Parkinson's Disease (PD) is currently unknown, though the accumulation of misfolded proteins suggests improper disposal of aggregate-prone proteins plays an important role in the pathogenesis of neurodegenerative disorders. PD and Batten mutations have been modeled in animal and cell models, though none fully recapitulate the pathology seen in human disease. Therefore, it is imperative to develop better models for disease study. The aim of this study is to establish new models for Parkinson's and Batten disease as assess pathological hallmarks.

We have established a novel line of iPSC cultures to provide accurate modeling of human neuronal cell biology. CRISPRi was implemented to inhibit the endogenous ATP13A2 locus through lentiviral transduction of guide RNAs into neurons, achieving a 99% knockdown of *ATP13A2*. Pathology was assayed using the Mitohealth HCS kit, immunocytochemistry and western blot for SNCA, LAMP1, and TFEB, and lysotracker and mitotracker assays. Knockdown of *ATP13A2* was coincident with upregulation of SNCA, impaired lysosomal trafficking, decreased mitochondrial health and cell viability. These findings establish an hiPSC model of ATP13A2 deficiency as viable tool for further research into the commonalities and differences between Parkinson's and Batten diseases.

id #13204

Exploring a Critical Period of Development Through the Manipulation of the Neurokinin Receptor 3.

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Critical periods of early postnatal development underlie the proper function of the mature brain, and alterations to neural activity in this time frame contribute to neurodevelopmental pathophysiology. We explored the developmental role of the neurokinin receptor 3, previously shown to regulate cholinergic cell firing in the adult mouse striatum. As of yet, the function of this receptor during the key steps of brain maturation has not been defined. We predicted that the interruption of this receptor in early postnatal stages would have detrimental effects upon the activity of striatal cholinergic interneurons and related behaviours. We identified an enhanced expression and function of the neurokinin receptor 3 between postnatal days 6 and 10 compared to adult mice. We found that chronic pharmacological manipulation of the receptor during this period altered the intrinsic properties and tonic firing of striatal cholinergic interneurons. Moreover, chronic blockade had repercussions upon the onset of movement, and social behaviour of juvenile mice. In summary, our results identify a crucial developmental time frame for the function of the neurokinin receptor 3 that critically influences the activity of striatal cholinergic interneurons and behaviour.

id #13205

Generation of specific sub-populations of dorsal root ganglia sensory neurons from human pluripotent stem cells

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There are a multitude of different dorsal root ganglia sensory neuron subtypes that each have specific roles in detecting and communicating stimuli, such as pain (i.e. nociceptors), touch (i.e. mechanoreceptors) and muscle pressure (i.e. proprioceptors). Dysfunction in certain classes of sensory neurons can lead to debilitating peripheral neuropathies. Current strategies to study and characterize peripheral neuropathies often involve the use of human pluripotent stem cells (hPSCs), which can be differentiated to generate mixed populations of sensory neurons. However, there is a need to develop homogenous cultures of the specific affected sensory neurons, which will allow the characterisation of neurons without the ensemble averaging observed in a mixed population of neurons. A promising avenue for generating specific sensory neuron subtypes (e.g. proprioceptor vs nociceptor) is by using directed differentiation, whereby the exogenous expression of lineage specific transcriptions factors is used to drive neuronal fate. This project aims to use a combination of small molecules and overexpression of key transcription factors, to generate cultures of specific sensory neuron subtypes. Preliminary work demonstrates that the combination of driving hPSCs to neural crest cells (sensory neuron progenitors), via the use of small molecules, and then transducing these neural crest cells with key transcription factors result an increase in the expression of sensory neuron markers. Preliminary data shows that exogenous expression of Neurogenin-2 and Runt-related transcription factor-3 in hPSC-derived neural crest cells results in an increase in markers specific to proprioceptors. Collectively these results suggest an efficient method to generate specific human sensory neurons.

id #13207

Lithium reduces brown adipose tissue (BAT) thermogenesis associated with emotional hyperthermia

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The mechanism of lithium's anti-mania action remains unknown. Lithium is known to reduce body temperature in experimental animals. This study determines whether lithium reduces brown adipose tissue (BAT) thermogenesis in conscious unrestrained rats, and whether the agent reduces increases in BAT thermogenesis induced by a caged intruder rat. We continuously recorded BAT and body temperature (chronically implanted thermistors) and behavioural activity (infrared motion detector) in conscious rats maintained singly in a home cage (22-26°C) for at least 1 day before experimentation. Lithium chloride (LiCl; 0.2, 20, 200mg/kg i.p.) or vehicle was administered (resident rat). In some animals, a caged intruder rat was introduced 30min after drug or vehicle administration, and the effects on recorded parameters were noted. Lithium (200mg/kg) reduced resting resident rat BAT temperature (from $37.9 \pm 0.29^{\circ}$C to $36.6 \pm 0.06^{\circ}$C, $P < 0.01$, $n = 3$) and body temperature ($37.7 \pm 0.29^{\circ}$C to $35.7 \pm 0.54^{\circ}$C, $P < 0.01$, $n = 3$). Lithium also inhibited intruder-elicited BAT thermogenesis and behavioural activity. Cade [1] noted quiescence in his guinea pigs after lithium treatment. Behavioural activity, as well as BAT and body temperature, was also reduced in our rats. Nausea, a common vagally-mediated side-effect of lithium treatment, is associated with a fall in body temperature, possibly due to reduced metabolic rate. We suggest that lithium, via a vagally-mediated action, reduces body temperature and metabolic rate as part of its nausea side-effect. This action may contribute to lithium's anti-mania effect.

1. Cade JF. Medical Journal of Australia. 1949;2(10):349-52.

id #13211

Innervation of Supraclavicular Brown Adipose Tissue: a cadaveric study

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Historically, Brown adipose tissue (BAT) was thought to be of little consequence in adult humans, as it was posited that the tissue was only present in significant amounts until adolescence was reached. Functional BAT was not identified in adult humans until 2007 with the use of fluorodeoxyglucose positron emission tomography imaging. In rodents it is well established that BAT is stimulated by the sympathetic nervous system with the interscapular BAT being innervated via branches of intercostal nerves (thoracic ventral rami). Whilst there is evidence to suggest that BAT is also innervated by the sympathetic nervous system in humans, no studies have identified the specific nerve branch that carries the sympathetic innervation to BAT. The aim of this study was to characterise the innervation of the supraclavicular BAT depot in humans. The posterior triangle region of the neck of five human cadaveric specimens were dissected in order to identify any peripheral nerve branches piercing and/or terminating in supraclavicular BAT. A branch of the cervical plexus terminating in a supraclavicular fat depot that has not been previously described was identified in all 5 specimens. This was typically an independent branch of the plexus, likely C3 ventral ramus, but in one specimen was a branch of the supraclavicular nerve. Immunohistological analysis revealed the nerve branch identified, and the adipose tissue, contained sympathetic fibres. Identification of this nerve may allow microneurography for testing the efficacy of thermogenic pharmacological interventions, or targeted electrical nerve stimulation for the means of weight loss therapies.

id #13212

Stimulatory, not Anxiogenic, Doses of Caffeine act Centrally to Activate Interscapular Brown Adipose Tissue Thermogenesis

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The sympathetic nervous system regulates interscapular Brown Adipose Tissue (iBAT), through activation of beta-3 adrenergic (β3) receptors on brown adipocytes. The neural pathway that controls the regulation of BAT thermogenesis has been established. This study

aimed to test the possibility that a central administration of caffeine is sufficient to activate BAT thermogenesis. Efficacy of caffeine delivered both peripherally (intravenous, IV administration) and centrally (intracerebroventricular, ICV administration) was compared in male rats. Thermogenesis was measured via thermocouples placed underneath the iBAT depot and rectally (core). Immunohistochemical analysis for cFOS expression was performed, as expression of Fos is indicative of neuronal activity. Core temperature did not significantly differ after administration of caffeine or saline following IV administration; however, they were significantly different after ICV administration. Changes in BAT temperature were highly significant for both routes of administration. Heart rate and mean arterial pressure were not significantly different following either route of treatment administration. The results indicate that IV and ICV administration of stimulant, but not anxiogenic, doses of caffeine (5-10 micrograms/kg, ICV; 5-10mg/kg, IV) increases BAT thermogenesis. This study has the potential to explain the mechanism by which BAT function is potentiated by caffeine. Via linking the cFos data and temperature data together we can see a neural pathway which is distinct from the control of the cardiovascular system. Thus, demonstrating that caffeine evoked thermogenesis has a clear central effect. Our results highlight a critical role of caffeine acting through this specific central pathway with no significant cardiovascular effect.

id #13213

The Interaction Between Oestrogen, Thermogenesis, Appetite and Body Weight in Male and Female Rodents.

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Brown Adipose Tissue (BAT) possesses the capacity to expend energy in a futile cycle, thus increasing basal metabolic rate. In animal models, estrogen has been implicated in the regulation of body weight and it is hypothesized that estrogen is acting by modulating BAT metabolism. A systematic search was performed, to identify research articles implementing in-vivo estrogen-related interventions and reporting outcome measures that provide direct or indirect measures of BAT metabolism. Meta-analyses were conducted where sufficient data were available. The final library of 67 articles were all in rodent models and provided mostly indirect measures of BAT metabolism. Results of this review found that estrogen's effects on body weight, in rats and possibly mice, are likely facilitated by both metabolic and appetitive mechanisms but are largely only found in ovariectomized models. This review identified a need to clarify the potential effects of estrogen on BAT metabolism in male animal models. To clarify this question gonad-intact male rats were administered exogenous estrogen, either peripherally or centrally, in order to establish the effects on BAT thermogenesis. Inter-scapular BAT temperature was monitored, as well as blood pressure and heart rate. Immunohistochemistry for cFOS was performed to ascertain which brain nuclei were implicated. This study found no significant effect of estrogen on BAT temperature among either peripherally or centrally administered rats, when compared with control groups. This

suggests a sexually dimorphic response to exogenous estrogen. Further work investigating the phenomenon in castrated male animals may have implications for transgender humans.

id #13215

Visual receptive field mapping of linear and nonlinear responses using temporal decomposition and iterative tomography

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Purpose: Visual neurons selectively respond to inputs within their receptive field with patterns of spiking activity ranging from the simple (in the parvocellular-midget pathway) to the complex (such as colour-, motion-, or orientation-selective cells in the koniocellular-projecting pathways). Mapping receptive fields for these non-standard cells presents a challenge due to their heterogeneity and non-linearity.

Methods: Spike-trains were recorded extracellularly in the LGN of Sufentanil-anesthetised marmosets (N = 6), and membrane potential was recorded intracellularly in whole-mount murine retina (N = 15), in response to flashing bar stimuli. In order to map receptive fields, responses were reduced to a small number of components using matrix factorisation techniques, yielding temporal profiles and a sinogram of corresponding spatial weights. Receptive fields were revealed using an inverse radon transform.

Results and conclusion: Receptive fields could be reconstructed for 42 LGN neurons and 15 retinal neurons. This matrix factorisation analysis allowed us to isolate regions of excitatory and inhibitory input to the cells corresponding to the classical centre and surround, even when recoding from many cells in parallel, and yielded clear receptive field maps for standard (magnocellular and parvocellular) and non-standard (color-coding and on/off-rectifying) neurons. In combination, these two techniques provide a rapid, flexible, and saleable method which unifies previously disparate approaches to studying sensory processing in the early visual system.

id #13220

Patch-seq protocol to analyze the electrophysiology, morphology and transcriptome of human iPSC-derived whole single neurons

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The brain constitutes a complex assembly of thousands of different types of neurons, which neuroscientists have been classifying based on their morphology and functional properties since the seminal drawings of Ramón y Cajal. Recent advances in genomics technologies have permitted the accurate analysis of single-cell transcriptomes. We have built upon this technical progress to combine single-cell transcriptome profiling with classical patch-clamp electrophysiology and morphological analysis of human neurons *in vitro*. This powerful method, referred to as Patch-seq, enables a thorough, multimodal characterization of single neurons and permits us to expose the links between neuronal function, morphology and gene expression (1). In this poster, we explain in detail the protocol we established to isolate whole single neurons from *in vitro* neuronal cultures for RNA amplification and transcriptome sequencing (2). We have validated this protocol for human neurons generated from patient fibroblasts with induced pluripotent stem cell (iPSC) reprogramming technologies. The procedure, however, can be applied to any kind of cell type *in vitro* with only slight modification of the cell collection protocol. Patch-seq represents a powerful tool to investigate the molecular basis of human neuronal diversity and elucidate the mechanisms underlying brain function and disease (3).

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id #13222

Chronic glucocorticoid-induced alterations in the functional properties of adult-born hippocampal neurons lead to anxiety-like behaviour

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Adult-born neurons in the hippocampus have been implicated in the regulation of mood-related behaviour; however, their precise roles in mediating behavioural responses to stress and antidepressants are still not fully understood. To investigate the role of adult-born neurons in the behavioural response to stress, we ablated doublecortin (DCX)-expressing immature neurons using transgenic mice expressing diphtheria toxin receptor (DTR) in DCX-expressing neurons (DCX^{DTR}). We showed that selective and transient ablation of DCX-expressing immature neurons in the mouse hippocampus exerted no effect on anxiety or hedonic behaviour under physiological conditions. However, in an animal model of depression induced by chronic exogeneous corticosterone (CORT) treatment, ablating immature neurons blocked CORT-induced anxiety but not anhedonia. Using *ex vivo* electrophysiology, we demonstrated selective alterations in both passive and active membrane properties of 4-week-old newly generated but not mature granule neurons in the CORT-treated mice compared to control mice. In addition, we observed CORT treatment increased dendritic complexity and impaired axonal innervation in the CA3 region of newborn neurons. Strikingly, a 3-week treatment with noradrenergic modulators including a norepinephrine reuptake inhibitor ameliorated anxiety and reversed the stressed-induced changes in the properties and integration of these adult-born neurons. Collectively, our results demonstrate that immature, adult-born hippocampal neurons play an instructive role in encoding stress and suggest that interventions that regulate the functional properties of these neurons may prove useful for treating anxiety in various neuropsychiatric conditions.

id #13224

The Er81 transcription factor drives the proliferation of interneurons derived from the medial ganglionic eminence

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The transcription factor Er81 is required for the specification, connectivity, and the fine tuning of interneuron activity (Hippenmeyer et al., 2005; Arber et al., 2000; Dehorter et al., 2015). It is also present in the medial ganglionic eminence (MGE), a transitory, developmental structure responsible for the birth and proliferation of striatal and cortical interneurons (Flames et al., 2007; Mi et al., 2018). Despite its presence in the MGE and its link to interneuron development, nothing is known about the role of Er81 during neurogenesis. In this study, we show that the ablation of Er81 significantly impairs the development of MGE-derived interneurons. We reveal a significant loss of cell density that is

likely a result of decreased proliferation. Furthermore, we reveal the presence of a compensation mechanism that partially restores circuit maturation. We also demonstrate that changing the expression of Er81 alters the neurochemical composition of developing interneurons, specifically proteins involved in neuronal migration. Lastly, we reveal a change in the gait profile of Er81 conditional knockout mice, along with deficits in the onset of locomotion. In summary, we present a novel role of Er81 during the neurogenesis of MGE interneurons, which directly impacts brain development and the behaviour of maturing animals.

id #13226

Molecular Control of Connection Formation in Striatal Interneurons

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Both parvalbumin-positive and cholinergic interneurons vitally control the output neurons of the striatum and basal ganglia function. Studies have shown that these two interneuron subtypes exhibit weak connectivity with one another that can be reinforced in response to network activity changes. However, the developmental regulation and role of the reciprocal coupling between parvalbumin and cholinergic interneurons is currently unknown. We hypothesise that molecular components govern the formation of this specific interneuron association. One candidate is the activity-dependent transcription factor Er81, which has been shown to regulate the formation of a monosynaptic connection between parvalbumin and cholinergic neurons in the spinal cord.

Using immunohistochemistry, we have found that Er81 is expressed at different levels in parvalbumin and cholinergic interneurons of the striatum. We also found that Er81 regulates interneuron morphology, and that the specific loss of Er81 changes cell connectivity. In addition, we have begun to investigate the role of Er81 in the physiological properties of these two cell types via in-vitro electrophysiology. Our preliminary results indicate that Er81, as a developmental molecular controller, is important for the formation of the parvalbumin-cholinergic interneuron coupling, and may be a key factor in ensuring appropriate neuronal network development and overall striatal function.

id #13227

Using optogenetics to unravel the pathways involved in the regulation of autonomic function by the central amygdala.

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The central nucleus of the amygdala (CeA) modulates cardiovascular function via interactions with the autonomic nervous system. Previous tract-tracing studies have demonstrated projections from the CeA to multiple brainstem autonomic areas, but their specific roles are not elucidated as the techniques used lack the ability to activate specific pathways. To address this issue we have examined the interaction between the CeA and the nucleus of the solitary tract (NTS), the site of synapse of viscerosensory information in the brainstem. Adeno-associated viruses (AAV) were bilaterally injected into the CeA of anesthetized, male Sprague-Dawley (SD) rats to induce the expression of channelrhodopsin-2 (ChR2) and a fluorophore (mCherry) under a constitutive promoter. After six weeks recovery, optical fibres were implanted in the NTS to stimulate the axon of neurons projecting from the CeA using 473nm laser. Fibres were stimulated at 20Hz, 50Hz frequencies and continuous stimulation and blood pressure was monitored. Continuous stimulation of CeA axons in the intermediate NTS induced a significant pressor response and bradycardia. Moreover, stimulation of these CeA axons during activation of peripheral baroreceptors, with a bolus systemic injection of phenylephrine, increased baroreflex sensitivity. Anatomical characterisation of the CeA-NTS pathway revealed that CeA axons predominantly terminate in the medial and commissural subnuclei of the NTS subregions, in agreement with previous studies, and also in the central, interstitial and gelatinous subnuclei. The results of this study demonstrate the function of a specific pathway emanating from the CeA to modulate cardiovascular homeostasis.

id #13228

The Relationships of the Metrics of Graph Theoretical Analysis of Resting-state Functional Connectivity to Memory Test Performance in Cognitively Normal and Mild Cognitive Impairment Individuals.

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Alzheimer's disease (AD) is a chronic degenerative brain disorder with an insidious, gradual onset and a progressive course, culminating in the loss of functional connectivity (FC) and atrophy within the brain. Amnestic Mild Cognitive Impairment (aMCI), the prodromal stage of AD is characterised by impairments in episodic memory, and delayed recall of visual and auditory information. Changes in resting-state (rs) FC are observable across networks when comparing aMCI populations with cognitively normal groups. Graph theory, an emerging mathematical tool provides a means to measure and visualise the connectivity between nodes or regions (ROIs) within the brain. Graph theoretical analysis of ROIs within functional neural networks allows us to determine various metrics, for instance, the degree of connectivity or 'betweenness' between ROIs within a network. This research assessed the relationship between ROI connectivity and performance on behavioural neuropsychological test results across aMCI (n = 11) and cognitively unimpaired (HC) (n = 11) participant groups. Our results revealed decreased rs-FC in aMCI compared to HC, specifically with changes in FC within regions of the brain. We also found amIC participants demonstrated increased activation in some ROIs associated with a worsening performance in neuropsychological testing. The rehabilitation of strengths and the prevention of cognitive deterioration are fundamental in preventing conversion from MCI into AD. The combination of graph theoretical analysis, clinical neuropsychological testing and structural and functional

MRI may provide us with the ability to identify areas of strength and weakness for rehabilitation, and longitudinal observation of functional connectivity.

id #13229

PDZ-mediated regulation of levels and interaction with scribble of the neuroprotective kinase p38g

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p38 MAP kinases are central components of signal transduction pathways. We have recently shown that p38g has a neuroprotective effect in Alzheimer's disease (AD). This project aims to investigate a potential regulatory mechanism of p38g levels, that could direct future research towards enhancement of this neuroprotective effect. p38g levels are dependent on its C-terminal PDZ interaction motif. Preliminary work has shown that p38g may interact with scribble homologue, a scaffolding protein containing four PDZ binding domains. This interaction may stabilize the protein post-translationally, increasing levels of active p38g. This project employed co-immunoprecipitation (co-IP) and immunocytofluorescence to determine whether these two proteins interact, and to identify whether the interaction is PDZ dependent. Co-IP demonstrated that p38g does interact with scribble. Preliminary immunocytofluorescence work indicates that the interaction is localized to post-synaptic densities of primary murine hippocampal neurons. This work demonstrates a novel interaction, which may regulate expression levels of the MAP kinase p38g.

id #13230

Passive induction of hypothermia inhibits delayed intracranial pressure elevation following ischaemic stroke in rats

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There is a transient increase in intracranial pressure (ICP) within the first 24h after ischaemic stroke. We have shown that this ICP rise occurs in rats and humans and that it may reduce blood flow and contribute to early neurological deterioration. We found that short-duration hypothermia prevents ICP elevation in rats, and this may be an important mechanism of its known neuroprotection. Clinical trials of long-duration hypothermia have been limited by

feasibility, and complications such as pneumonia, which might be avoided by short-duration cooling. My aim was to develop a clinically feasible gradual cooling model and optimise hypothermia to prevent ICP elevation 24h post-stroke in rats.

Transient middle cerebral artery occlusion (tMCAo) was induced for 2h in male, outbred Wistar rats (n=5/group) using a 3mm silicone-tipped intraluminal filament. Passive cooling was initiated 1h post-occlusion in a 34°C group for 2h and a 32.5°C group for 2.5h. A normothermia group served as the control. ICP was monitored pre-stroke and 20-24h post-stroke.

Baseline pre-stroke ICP was 3.52 ± 2.07 mmHg across all groups (n=15). ICP for the normothermia group was 8.51 ± 2.22 mmHg at 24h. ICP was significantly lower in the 32.5°C group (4.27 ± 1.94 mmHg, $p = 0.01$) but not in the 34°C group (9.29 ± 9.24 mmHg, $p = 0.86$) when compared with normothermia.

We have shown that clinically achievable passive gradual cooling to 32.5°C prevented ICP elevation 24 post-stroke in rats. This is the first step towards a feasible short-duration hypothermia paradigm for testing in clinical trials.

id #13231

Functional analysis of actin-associated protein Tpm3.1 segregation in mouse hippocampal neurons

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Since their discovery in mammalian non-muscle cells, tropomyosin (Tpm) proteins have been brought into focus for their importance as master regulators of cytoskeletal actin filament function. Their importance for regulation of cell function derives from their functional diversity. Cells express multiple isoforms of tropomyosin and their distribution varies in different tissues and cell compartments and changes during development. The significance of Tpms to neuronal structure and function, and their exceptionally dynamic capacities, warrants the question; how do neurons regulate Tpm protein populations across space and time? To begin addressing this, the molecular pathways modulating the transport of Tpms require elucidating. Tpm3.1 is the most well-characterized isoform at present in the literature regarding non-muscle cells and therefore a strong candidate for investigating the trafficking principles of Tpms. Tpm3.1 has an array of morphological roles; impacting axonal length, dendritic branching and growth cone size. We have also shown that Tpm3.1 is a key regulator in the maintenance of the axon initial segment (AIS); pharmacological and genetic disruption of Tpm3.1 leads to perturbations in AIS structure. Here, we present novel data, addressing potential mechanisms regarding Tpm3.1 trafficking. Live cell imaging from our lab has shown that Tpm3.1 protein associates with dynein-mediated retrogradely transported vesicles. Inhibition of dynein motor function stalls retrograde transport of Tpm3.1 positive

vesicles in cultured hippocampal neurons. We found no association of Tpm3.1 with anterogradely transported vesicles. Our data provide new insight into the intracellular segregation of a key regulator of the neuronal cytoskeleton.

id #13233

Adaptation of respiratory-related brain regions to long-term hypercapnia: focus on neuropeptides in the RTN

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Animal studies demonstrate an initial increase in ventilatory drive within hours, followed by a decrease in this response over the long-term (days-weeks), in response to long term hypercapnia. Little is known about whether changes in the central respiratory chemoreflex are involved. Here we investigated whether central respiratory chemoreceptor neurons in the retrotrapezoid nucleus (RTN), have a role in the mechanism of associated neuroplasticity. Methods: Adult male C57BL/6 mice (n=5/group) were used. We aimed (1) to determine if neuropeptide gene expression is altered in the RTN after long-term hypercapnia. This was achieved using qPCR to measure changes of neuropeptide mRNA expression in the RTN after short-term hypercapnia (6 or 8 hours, 5 or 8% CO_2) or long-term hypercapnia exposure (10 day, 5 or 8% CO_2), (2) in the mouse brainstem, to determine the distribution of preprogalanin and galanin receptor, using in situ hybridisation (ISH), (3) to investigate whether long-term hypercapnia causes changes to recruitment (detected by cFos) of respiratory related neural populations including the RTN, in vivo. We found that hypercapnia decreases neuropeptide expression in the RTN in the short-term and has the opposite effect over the long-term. Following long term hypercapnia, the number of RTN galanin neurons remains unchanged, and their responsiveness to acute chemoreflex is sustained; in contrast, we identified multiple respiratory related sites that exhibit blunted chemoreflex activation. GalR1 was distributed in 11% of preBötC and 30% of BötC glycinergic neurons. These results assign a potential role to galanin in the RTN, in chemoreflex adaptation to long-term hypercapnia.

id #13234

LncRNA Ptchd1-as: A novel high functioning ASD mouse model

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Autism Spectrum Disorder (ASD) is a prevalent neurodevelopmental disorder characterized by social communication deficits and the display of restrictive, repetitive behaviors. Early studies evaluating the contribution of copy number variants (CNVs) have implicated the *PTCHD1* locus in ASD development. Located at Xp22.11, it encompasses genes *PTCHD1, DDX53* and long non-coding RNA (lncRNA) *PTCHD1-AS*, and is regarded as a highly-penetrant risk locus in males, contributing to an estimated ~1% of ASD and intellectual

disability (ID) cases. Evaluating both published and unpublished data from individuals with a constellation of neurodevelopmental phenotypes to assess the correlation between ASD and variants disrupting this locus suggests that, in fact, it is the *PTCHD1-AS,* which is underlying the association with ASD. Given the high penetrance of ASD linked to *PTCHD1-AS* microdeletions, we have initiated a characterization and functional study to elucidate its role in the etiology and expression of autism in a developmental model. Guided closely by the human phenotype data, we are assaying for behavioral and cellular effects in Ptchd1-as mutant mice to look at the transcriptional and translational consequences of a deletion in the critical region associated with high functioning austim.

id #13235

INVESTIGATION OF THE ENDO-LYSOSOMAL NETWORK IN A *DROSOPHILA* MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) is the most common form of dementia, which affects memory, behaviour and daily functioning of affected patients with its pathological characteristics consist of amyloid-beta (Aβ) plaques and phosphorylated tau that accumulate in the brain. The mechanisms underlying the disease remain unknown and no effective treatments are available to stop the disease progression. Although the genetic contribution in AD patients is not fully understood, genome-wide association study (GWAS) have reported several loci associated with increased AD risk in genes is involved within the endolysosomal network (ELN). Nonetheless, the fundamental mechanism of AD disease progression in regards to ELN is not well defined. Therefore, this projects aims to identify and characterise the contribution of specific ELN genes to neuronal dysfunction in a *Drosophila* model for AD.

Method: The effect of co-expression of full length amyloid precursor protein (APP) and beta-site APP cleaving enzyme (BACE) has been investigated in *Drosophila melanogaster* to determine whether flies are able to model symptoms observed in AD patients. This APP+BACE model will ensure correct spatial localisation of APP and its proteolytic fragments within the ELN.

Results: Our findings show that co-expression of APP+BACE has caused retinal degeneration in *Drosophila* adult eye. In addition, expression of APP+BACE in glial cells has severe phenotype shown in climbing and lifespan assays.

Conclusion: Using this system, we can characterise the genetic and molecular contribution of ELN genes in APP+BACE toxicity.

id #13236

Can we predict anticonvulsant efficacy of diazepam based on pre-seizure EEG parameters?

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Benzodiazepines are the first line therapy for acute treatment of prolonged seizures, yet remain ineffective in almost half of patients with status epilepticus. Predicting the response to benzodiazepines could help guide treatment protocols. We recently reported to the society (ANS, 2017) that, in a mouse model of prolonged seizures, the efficacy of diazepam was enhanced after upregulation of the membrane K^+-Cl^- cotransporter, KCC2. We here address the observed variability in diazepam responses to see whether ictal EEG features could predict the diazepam effect. We analyzed the EEG patterns from mice with the most divergent diazepam responses (n=8). Seizures were induced by two small doses of kainic acid (KA, 5 mg/kg, *ip*. 60 mins apart) and EEG measured by pre-implanted cortical screws (UNSW ethics HREC#15/136B). A single dose of diazepam (DZ, 5 mg/kg, *ip*) was administered 60 mins after the 2nd KA dose. Spectral EEG characteristics were analysed as power spectral densities (PSD) and time-frequency spectrograms. Total spectral power in responders was reduced by an average factor of q=13.3 in DZ responders vs. q=0.34 (p<0.01) in non-responders. PSD-normalized theta power (4-8 Hz) in responders was larger than in non-responders whereas total gamma power (30-60 Hz) was not significantly different (p=0.011). Visual analysis of spectrograms, however, showed marked gamma band activity preceding individual EEG seizures in DZ responders whereas time-averaged gamma power could not predict the DZ response. Our preliminary data support a more detailed analysis of pre-seizure EEG characteristics to predict subsequent drug responses with the goal of optimizing therapeutic strategies.

id #13237

The role of activity-dependent phosphorylation in the localisation and presynaptic function of alpha-synuclein

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Repeated rounds of exocytosis and endocytosis of neurotransmitter-containing synaptic vesicles play a critical role in the maintenance of neurotransmission. Alpha-synuclein, a protein which has been extensively studied for its role in Parkinson's disease, is a known modulator of the synaptic vesicle cycle, however its exact function remains unclear. This study aimed to determine how activity-dependent phosphorylation modulates the trafficking and function of alpha-synuclein. Phosphoproteomic LC-mass spectrometry of primary

hippocampal neuron lysates (resting or depolarised with 60mM KCl) revealed an activity-dependent increase in phosphorylation at two of four sites examined in alpha-synuclein. These two activity-dependent sites were further investigated by transfecting WT and alpha-synuclein-/- hippocampal neuron cultures with phosphomutant variants of alpha-synuclein that mimic or abolish phosphorylation at these residues. Neurons were fixed, immunolabelled and imaged to analyse protein expression and localisation. The expression of most alpha-synuclein phosphomutants is comparable to wild-type alpha-synuclein. WT alpha-synuclein is highly enriched at nerve terminals at rest, and upon depolarisation, moves out of the presynapse before returning to the nerve terminal during recovery from stimulation. Interestingly, alpha-synuclein with a phosphomimetic mutation at one of these activity-dependent sites is de-enriched from nerve terminals even at rest, and neither phosphonull or phosphomimetic variants at this site undergo mobilisation upon depolarisation. The localisation of the synaptic vesicle protein marker synaptotagmin-1 was shown to be unaffected by different alpha-synuclein phosphomutants, indicating that alpha-synuclein phosphorylation is not impacting synaptic vesicle distribution. Further experiments are required to understand the effect alpha-synuclein phosphorylation on the synaptic vesicle cycle.

id #13238

Using Drosophila Melanogaster to uncover the disease-related mechanisms of Sanfilippo Syndrome (MPS IIIC)

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Sanfilippo Syndrome or Mucopolysaccharidosis (MPS) IIIC is an autosomal recessive lysosomal disease that is characterised by deficiency of the catabolic lysosomal enzyme heparan acetyl-CoA:alpha-glucosaminide N-acetyltransferase (HGSNAT). This leads to the accumulation of partially-degraded heparan sulfate in all cells however this predominantly affects the brain. Clinical symptoms include progressive loss of motor coordination and behavioural problems in children before eventual death. As the pathological mechanisms that lead to neuronal dysfunction in MPS IIIC remain undetermined, our aim is therefore to elucidate the specific molecular and cellular mechanisms that contribute to the neurological symptoms.

We have developed the first *Drosophila melanogaster* model for MPS IIIC that show phenotypes that resemble those observed in children. MPS IIIC flies show heparan sulfate

accumulation accompanied by the appearance of large numbers of LysoTracker-positive inclusions as well as increased GFP-tagged lysosomal-associated membrane protein (LAMP1), indicative of lysosomal dysfunction. Furthermore, MPS IIIC flies exhibit symptoms observed in MPS IIIC patients such as locomotor dysfunction and altered behavioural symptoms.

Having established the first *Drosophila melanogaster* that recapitulates disease-related mechanisms of MPS IIIC, we can now use this model to determine the contribution of different molecular and cellular pathways to symptom generation in MPS IIIC, information that will guide the development of novel targeted treatments.

id #13239

Bruton's Tyrosine Kinase regulates inflammasome activation in Parkinson's disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide. PD is characterised by a progressive loss of nigrostriatal dopaminergic neurons and the accumulation of α-synuclein aggregates in the form of Lewy-bodies. Chronic immune and inflammasome activation is believed to underpin PD pathology with neuroinflammation commencing in the disease process and still strongly evident in post-mortem analyses of PD patient brains. Persistent immune activation has thus been closely linked to disease progression based on a wealth of accumulating evidence in clinical studies and experimental models. Inhibition of the NLRP3 inflammasome has recently been shown to prevent α-synuclein pathology and dopaminergic neurodegeneration, making it one of the most promising therapeutic targets for PD. Herein, we demonstrate that Bruton's Tyrosine Kinase (BTK) is activated by pathological synuclein and triggers NLRP3 inflammasome activation in microglia. BTK is also activated in the nigrostriatal system of experimental PD models at the same timepoints as NLRP3 activation. Crucially, pharmacological inhibition of BTK signalling prevents inflammasome activation in primary microglia. Additionally, daily oral dosing with small molecule BTK inhibitor can effectively reduce NLRP3 inflammasome activation markers and neuropathology in pre-clinical models of PD. Interestingly, we found that BTK is also expressed within dopaminergic neurons of the nigrostriatal system and therefore may have a more direct role in the development of neuropathology than previously thought. Together, our results indicate that BTK could be a potential druggable therapeutic target to mitigate inflammasome activation and provide neuroprotection in PD.

id #13240

Risk Factors for Psychosis: Investigating Polygenic Risk and the Cumulative Effects of Child and Adult Stress

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Genetic and environmental factors contribute to an individual's risk for psychosis. This study investigated whether the association between molecular genetic risk for schizophrenia and diagnosis of psychotic disorder (including schizophrenia, schizoaffective disorder, or psychotic bipolar-I disorder) is moderated by stress in early- and adult-life, and cumulatively across the life-course. Participants were 113 clinical cases with ICD-10 diagnosis of psychotic disorder ($N = 53$ schizophrenia or schizoaffective disorder, $N = 60$ (psychotic) bipolar I disorder; age range = 18-60; $M = 40.8$, $SD = 11.9$), and 51 healthy controls (age range = 19 – 59; $M = 37.2$, $SD = 12.2$). Genetic risk for schizophrenia was derived from polygenic risk scores. Childhood maltreatment (before 18 years) was measured with the Childhood Trauma Questionnaire Short-Form (CTQ-SF), and adult life stress with an adapted Traumatic Events Questionnaire (TEQ; after age 18). Cumulative stress was defined as exposure to stress in both early- and adult-life. Logistic regression analyses demonstrated clinical status (cases vs. controls) was significantly associated with polygenic risk ($OR = 1.74$, $p = .004$, 95% CI [1.19, 2.54]), childhood maltreatment ($OR = 2.79$, $p = .005$, 95% CI [1.36, 5.73]), and cumulative stress ($OR = 5.2$, $p = .006$, 95% CI [1.61, 16.75]). No association was observed between adult stress and diagnosis, or moderation of genetic risk with any measure of stress exposure (child, adult, or cumulative). The reliability of these findings may be impacted by the relatively small sample size. Further investigations of these relationships are warranted in larger, longitudinal samples.

id #13242

A RARE, *DE NOVO* HOMER1 MUTATION CAUSES SYNAPTIC AND AXON GUIDANCE DEFECTS

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Many neurological disorders are thought to be conditions of altered neuronal connectivity. There is an increasing recognition of the importance of rare, *de novo* variants as both contributors to disease risk and as targets for investigation of the disrupted cellular mechanisms which lead to neuronal connectivity. Genomic studies have identified mutations in scaffolding proteins as key contributors underpinning these disorders. These proteins offer us valuable targets to interrogate the synaptic and developmental changes that contribute to the altered neuronal connectivity observed in these disorders. We found a recently described rare variant in the postsynaptic scaffolding protein, Homer1 (R297W), located in the coiled-coil (CC) domain resulted in altered axon guidance, spine density and morphology in neurons. Reduction of Homer1 expression converted turning responses of sensory neuron growth cones *in vitro* from attraction to repulsion in response to the calcium-dependent guidance cue, brain-derived neurotropic factor (BDNF). This effect was rescued by expression of wild-type Homer1, but not the R297W variant. Additionally, overexpression of R297W converted growth cone attraction to repulsion. Overexpression of R297W in hippocampal neurons *in vitro* reduced dendritic spine density while increasing the mean length and width of dendritic spines, suggesting a change in the capacity of these cells to

maintain stable post-synaptic connections. Our results suggest the Homer1 CC domain is necessary for normal synapse development and demonstrate that a rare, *de novo* Homer1 mutation can cause disruptions to the synaptic and developmental machinery which underpins neuronal connectivity.

id #13243

Analysis of epigenetic aging acceleration among women with cocaine use disorder: association with childhood physical neglect exposure

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Evidence suggests accelerated aging mechanisms in drug addiction and this effect may be potentiated by stressful experiences, particularly those that occurred early in life. Most studies have been investigating cellular senescence and premature aging by the increase rate of the telomere shortening in psychiatric patients. Recent reports suggest that DNA methylation (DNAm) in blood also accounts as an epigenetic clock with recognized value as a biological aging marker. However, it is unknown whether accelerated DNAm aging is also evident in the blood of patients with cocaine use disorder (CUD), in particular in association with early life stressful experiences. Methods: Genome-wide DNA methylation was interrogated in blood from 140 female patients with CUD. DNAm age and epigenetic aging acceleration were estimated using the Horvath method. Early life stress experiences were assessed by the Childhood Trauma Questionnaire (CTQ). Association between epigenetic aging acceleration and early life stress was assessed by linear regression and univariate general linear models with age, race, and blood cell type (CD4, CD8) included as co-variates. Results: Childhood physical neglect score was positively associated with DNAm aging acceleration (B = 0.226; t = 2.005; p = 0.047). No significant associations between childhood emotional neglect, sexual abuse, physical abuse or emotional abuse were evidenced with DNAm aging. Conclusions: Childhood physical neglect seems to increase the rate of epigenetic aging in female patients with CUD. This association may be implicated with clinical manifestations of premature aging in CUD.

id #13244

Increased intracranial pressure 18-hours post-stroke correlates with faster outflow of cerebrospinal fluid tracer into the spinal subarachnoid space

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Intracranial pressure (ICP) rise post-stroke limits blood supply to the ischaemic penumbra and worsens patient outcome. A transient rise in ICP occurs 24-hours after experimental ischaemic stroke that is independent of oedema. Our data shows that resistance to cerebrospinal fluid (CSF) outflow is increased 18-hours post-stroke and recent evidence suggests that clearance of spinal CSF is an important route of CSF outflow. This led us to believe that decreased CSF outflow along this pathway may contribute to ICP rise. At 18-hours post-stroke, we used a white-light imaging technique at the C7-T1 spinal level and intraventricular Evan's Blue infusion to compare CSF flow into the spinal subarachnoid space of rats following photothrombotic stroke or sham procedure. We then quantified Evan's Blue contrast, and the time taken to reach 50% maximum contrast as surrogate indicators of CSF flow. Prior to Evan's Blue infusion, 6/12 animals had elevated ICP (>5mmHg). Stroke rats with ICP rise (27.6±5.1 mins, n=6) reached 50% maximum contrast significantly faster than stroke rats with no ICP rise (48.6±4.5 mins, n=6) and sham rats (47.9±4 mins, n=8), $F_{(2,17)}=0.1$, $p<0.01$; one-way ANOVA). Further, we found that ICP rise at 18-hours post-stroke correlated with time taken to reach 50% maximum contrast, R=-0.6, $p<0.01$. Our data shows that CSF tracer movement into the spinal subarachnoid space from the cerebral ventricular system occurs faster in stroke rats with greater ICP rise. These findings indicate that this pathway of CSF flow is not disrupted post-stroke and perhaps partially compensates for disrupted cranial CSF outflow pathways.

id #13246

The RGMa-Neogenin pathway regulates actin remodelling during dendritic spine maturation

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Autism spectrum disorders (ASD) have been associated with aberrant dendritic spine morphogenesis. Disturbances in actin remodelling pathways caused by frequently occurring disruptive *de novo* mutations of ASD-associated genes implicate a role of the actin cytoskeleton and spine morphogenesis in the aetiology of ASD.

Within the spine head the polymerization of branched actin filaments, mediated by the actin nucleation complex Arp2/3, is required for maturation of dendritic spines. The activity of Arp2/3 at the postsynaptic density (PSD) is regulated through its interaction with the Wave Regulatory Complex (WRC). As a result, mice with a depletion of Cyfip1 (a WRC subunit) show spine loss and autism-like behaviour. Interestingly, truncating Neogenin mutations have been recently identified in autism patients. However, the underlying molecular mechanism that control WRC recruitment to the PSD remain unknown.

Here we test the hypothesis that netrin/RGMa guidance receptor, Neogenin, spatially-restricts branched actin remodelling within dendritic spines, thereby enabling spine maturation. In support of this, we show that in the developmental context Neogenin controls branched actin polymerization in dendritic spines through its interaction with the WRC in cultured hippocampal neurons. Neogenin depletion via RNA interference and the inhibition of the direct interaction between Neogenin and the WRC both result in decreased spine maturation. Our most recent data suggest that the Neogenin ligand RGMa is also involved in this process.

id #13247

Does secreted amyloid precursor protein alpha affect the global proteome of astrocytes?

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Secreted amyloid precursor protein alpha (sAPPa) is a neuroprotective, neurogenic, memory enhancing molecule produced in the human brain. The mechanisms of how sAPPa achieves these outcomes are yet to be elucidated[1], but it has potential as an Alzheimer's disease therapy. sAPPa is produced in our laboratory[2] and its activity has been validated in functional studies. We used Sequential Window Acquisition of all Theoretical Fragment Ion Spectra-Mass Spectrometry (SWATH-MS), to identify changes in protein abundances mediated by sAPPa in mouse primary astrocyte cultures with the goal of increasing our understanding of pathways affected by this molecule.

Methods: Astrocyte cultures were prepared from P2, C47/B6 mice pups and characterised by immunocytochemistry. After treatment with 1 nM sAPPaor PBS for 2 h, peptides were generated from cell lysates. To prepare a SWATH library, protein from each sample was pooled, fractionated by isoelectric focusing and analysed by shotgun proteomics. sAPPa (n = 4) and vehicle treated controls (n = 4) were then subjected to SWATH-MS. Spectral information of 4,600 proteins was used for quantification and MarkerView software was employed to perform statistical analysis.

Results: Five proteins were significantly down regulated and 21 proteins were up regulated (P < 0.001 & Log (fold change) = < -0.2 or > 0.2).

Conclusion: Preliminary results suggest treatment of astrocytes with 1 nM sAPPa for 2 h has a modest effect on the global protein profile. **Future direction:** Validation by western blotting or immunocytochemistry. A similar study measuring the effect of sAPPa on human cortical neurons is underway.

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id #13248

Serotonin leads the way: from a guidance cue perspective

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The neurotransmitter serotonin (5-HT) is synthesized during early development of the brain, even before many neuronal circuits become functional. This suggests that 5-HT might have a regulatory role in shaping neuronal circuitry prior to its function as a neurotransmitter in the developing and adult brain. In this study, we hypothesized that 5-HT acts as a novel guidance cue during axon guidance and circuit development. Using a growth cone motility assay and rodent sensory neurons, we found that 5-HT acts bimodally, by attracting (at 50uM) or repelling (at 100uM) growth cones *in vitro*. Using a range of pharmacological tools, we confirmed that attraction was mediated by the 5-HT2A receptor and repulsion by the 5-HT1B receptor. This novel motile behaviour suggests that two distinct signalling mechanisms are responsible. The first, through IP_3-mediated calcium release from the endoplasmic reticulum and the second, through adenylyl cyclase-mediated cAMP signalling. We confirmed these mechanisms using high resolution calcium and cAMP imaging of growth cones exposed to gradients of 5-HT. These experiments revealed an influx of cytosolic calcium during 50uM 5-HT exposure and decreases in cAMP during 100uM 5-HT exposure. Overall, this novel characterisation of growth cone motility in response to 5-HT gradients supports the hypothesis of 5-HT as a guidance molecule during early brain circuit formation. Unravelling how 5-HT signals influence the normal wiring of the brain will allow us to better understand the molecular mechanisms in neurological disorders, caused by aberrant 5-HT levels or disrupted 5-HT receptor expression during development.

id #13249

Dopamine regulation of the intercalated cells of the amygdala.

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Our fear behaviours are gated by the inhibitory amygdala nucleus called the intercalated cells (Im). The Im forms GABAergic connections to the central amygdala, the main amygdala output nucleus, to reduce central amygdala neuron activity. Fear extinction potentiates Im activity. Dopamine D1 receptor activation in the amygdala is anxiolytic and dopamine hyperpolarises Im neurons. Our experiments investigated dopamine regulation of Im-Im GABAergic synapses. Using patch-clamp electrophysiology in brain slices from Sprague-Dawley rats, we found dopamine inhibited Im-Im synapses by activating D1 receptors. Cocaine mimicked the dopamine inhibition, suggesting an endogenous dopamine effect. Dopamine inhibition occurred without changes in paired-pulse ratio but quickened IPSC decay kinetics, giving an apparent postsynaptic locus of action. However, dopamine reduced miniature IPSCs frequency but not amplitude. In the presence of dopamine, the low-affinity $GABA_A$ antagonist, TPMPA, inhibited Im-Im synapses more than in control (inhibition in control: $25.92\pm7.885\%$; in dopamine $43.00\pm5.831\%$; n=5; paired t-test; p=0.0305). This suggests dopamine may lower GABA concentration at the synapse and allow TPMPA to

compete better with GABA at the $GABA_A$ receptor. Changing GABA concentration synapses can alter decay kinetics by changing the complement of receptors that are singly or doubly bound by GABA which alters $GABA_A$ receptor desensitisation. Thus, one possible explanation for this data is dopamine lowers GABA concentration at the Im-Im synapse and as a result changes $GABA_A$ receptor desensitisation, thus decay kinetics as well as reducing the amplitude of the response. Dopamine regulation of the Im-Im synapse likely allows for excitation of the postsynaptic neuron.

id #13250

Application of the Bradford Hill model supports empirical evidence for a role of metal changes in Parkinson's disease aetiology

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Analysis of post-mortem Parkinson's disease brains demonstrates concomitant iron accumulation and copper deficiency in the degenerating substantia nigra (SN), however evidence for a causal relationship between changes in these metal levels and Parkinson's disease are difficult to establish in post-mortem tissues. Here we apply the Bradford Hill model of causality to determine whether metal changes are contributing factors to degeneration of the SN in Parkinson's disease. This model proposes nine criteria to establish a causal inference between two variables based on published evidence supporting and opposing each criterion. We conducted a systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. An electronic search of articles published up to August 2019 was conducted in PubMed, EMBASE, Central Register of Controlled Trials and Scopus, and 8437 resultant articles were screened for pre-defined inclusion and exclusion criteria restricted to human research. Study quality was quantified using our novel Quality Assessment Scale. Preliminary data from 37 articles reporting post-mortem analyses of brain metal levels demonstrated that four of six (strength, consistency, specificity, temporality, experiment and biological plausibility) selected Bradford Hill criteria support a causal relationship between iron accumulation and copper deficiency, and neuronal death in the SN in Parkinson's disease, while data for the remaining criteria is equivocal or opposes this association. These data demonstrate that the Bradford Hill model is a useful approach for assessing the strength of evidence for hypotheses generated from human-derived data, where opportunities for experimental modification are necessarily limited.

id #13251

Flinders Environmental Epigenetics through Life Study

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Childhood stress affects individuals throughout their lives, and also their children. The mechanism of this intergenerational transmission of stress has been suggested to be epigenetic. Whilst epigenetic inheritance of stress in humans has been the subject of numerous recent reviews and media articles, a mechanism in humans has not been clearly established. Only the gametes—ova and sperm—can transmit genetic information across generations. There is emerging evidence DNA methylation is altered in the sperm of individuals with child or adult trauma but samples are small and methods limited. We describe a unique cohort currently being collected, from which we will determine if childhood stress is associated with molecular changes in sperm. This collection is undertaken collaboratively between Flinders University and Flinders Fertility clinic. Our pilot cohort will collect sperm from 100 men, and phenotypic data from a series of questionnaires capturing a range of psychosocial and lifestyle measures, including early-life exposures, as well as proximal measures of stress, diet, and exercise from the men and their partners. Although we are recruiting through a fertility clinic our current collection is restricted to men with fertile sperm parameters. This innovative collection method will allow us to address a significant gap in the literature, capitalising on procedures in place for in-vitro fertilisation (IVF) (e.g. sperm collection and analysis). At the point of molecular analyses we will undertake whole genome methylation and small RNA profiling. Expected outcomes of this project include establishing a critical evidence-based foundation and resource for future research into epigenetic transmission.

id #13252

Low oxygen post conditioning improves cognition and reduces amyloid beta accumulation post-stroke in mice.

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Objectives: We have recently shown an association between post-stroke cognitive impairment and the build-up of neurotoxic proteins (e.g amyloid beta; Aβ). Recently published evidence from our group has shown low oxygen post conditioning (LOPC) to improve motor deficits and to limit secondary neurodegeneration after experimental

stroke. In this study, we aimed to 1. determine whether LOPC improves cognition and 2. investigate potential mechanisms of LOPC.

Method: Male, C57BL/6 mice underwent photothrombotic occlusion or sham surgery. At 72h post-stroke mice were allocated to: sham + atmospheric air, stroke + atmospheric air, stroke + LOPC (11% 8h/day) or stroke + LOPC (11% 24h/day). LOPC treatment continued for 14 days, during which time cognitive performance was assessed using a mouse touchscreen platform (paired associated learning task). At day 14 of treatment brains were collected for further analysis.

Key Findings: The sham and LOPC treated animals had significantly higher percentages of correct responses when compared with the stroke group (sham, $p < 0.05$; LOPC 8h, $p < 0.05$; LOPC 24h, $p < 0.01$) in the second week of testing. Western blot analysis of peri-infarct tissue revealed that relative to stroke animals, LOPC treated animals displayed lower total levels of Aβ (LOPC 8h, $p < 0.05$; LOPC 24h, $p < 0.001$).

Conclusions: We have demonstrated that LOPC limits post-stroke cognitive impairment (specifically learning and memory). We also showed that LOPC reduced Aβ accumulation. The results presented here support LOPC as an extremely promising therapy post-stroke and may have wider implications for other disorders that cause cognitive impairment.

id #13253

Comparing EEG Aperiodic Signal between Mood Disorders and Healthy Controls

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Publish consent withheld

id #13254

Determining the role of DNA methylation in the relationship between the ZNF804A gene and cognitive sub-phenotypes in psychosis individuals

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The Single Nucleotide Polymorphism (SNP) rs1344706, located within the Zinc Finger 804A gene (*ZNF804A*), is a replicated risk variant for both schizophrenia and bipolar disorder. Research also demonstrates a relationship between this *ZNF804A* variant and spared cognitive functioning in individuals with schizophrenia. DNA methylation is an epigenetic mechanism that influences gene expression, and may provide a mechanism for this association. This study investigated whether DNA methylation of *ZNF804A* acts as a mediator in the relationship between variations in rs1344706 and cognitive functioning in people diagnosed with schizophrenia (N= 44) or bipolar disorder (N= 51). DNA was extracted from whole blood, genotyping assayed with the PsychArray-24 BeachChip, and DNA methylation assayed with the HumanMethylation450KBeadChip. A comprehensive battery of cognitive assessments was used to classify patients into 'cognitive deficit' or 'cognitively spared' subtypes according to individual patterns of functioning on all tests. Age, sex, medication, cell counts and ethnic stratification were included as covariates. A logistic regression analysis failed to replicate previous associations between variations in rs1344706 and cognitive sub-phenotypes within the mixed group of schizophrenia or bipolar disorder cases, nor was DNA methylation associated to cognitive sub-phenotypes. A PROCESS model failed to show indirect relationships between rs1344706 and cognitive subtypes when considering DNA methylation as a mediator in this relationship. Our findings should be considered in light of limitations, such as low statistical power. Whether DNA methylation plays a mediating role in the relationship between variations in rs1344706 and cognitive sub-phenotypes in individuals with psychosis spectrum disorders remains to be determined.

id #13255

ENHANCING REGENERATION BY REGULATING GROWTH CONE CALCIUM SIGNALING IN ZEBRAFISH

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A major focus within neuroscience is to enhance the regenerative capacity of neurons in order to repair neural circuitry after injury or disease. Spatiotemporal calcium (Ca^{++}) signals, including receptor mediated Ca^{++} influx and store operated calcium (SOC) entry are crucial determinants of axon guidance during development of the CNS. We hypothesised that specific Ca^{++} signalling mechanisms in neurons that are important for axon guidance in development, could be "repurposed" to "steer" axons *in vivo*. Molecular tools that utilise optogenetics have allowed for the activation and transport of Ca^{++} from specific intra- and extracellular sources with improved precision and sensitivity. In this study, we used an *in vivo* optogenetic approach to manipulate SOC signals using OptoSTIM1 or an activity dependent Ca^{++} influx using a channelrhodopsin, oChief, cell autonomously in zebrafish motor neurons. Using Tol2 transgenesis, optogenetic constructs were specifically targeted to spinal motor neurons within the Gal4^{s1020}t/UAS:mCherry zebrafish line. Stimulation of OptoSTIM1 in growth cones caused either growth cone retraction or a change in the direction of growth depending on the specific site of stimulation. Significantly, these experiments are the first to describe that optogenetic molecules could be utilised to guide growth cones within an intact animal. In conclusion, these results provide evidence that we can utilise specific patterns of Ca^{++} signals to manipulate axon guidance giving us the possibility to use optogenetic tools to steer neurons through hostile regenerative environments *in vivo*.

id #13256

Opioid withdrawal-induced neuroadaptations within the amygdala

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Withdrawal from opioid drugs has long-lasting effects on neuronal function and synaptic transmission in opioid-sensitive neurons throughout the brain. These persistent changes are thought to drive drug seeking and relapse behaviours, even after protracted abstinence. Despite this, the mechanisms and circuits underlying the altered behaviours following opioid withdrawal are only partially understood, limiting the development of addiction treatments. Neural circuits within the amygdala mediate drug seeking and relapse behaviours, thus, we hypothesised that neuroadaptations would manifest in opioid-sensitive amygdala cells during opioid withdrawal. Under normal physiological conditions, glutamatergic transmission from principal neurons within the basolateral amygdala to GABAergic intercalated cells within the neighbouring main intercalated cell island is strongly inhibited by endogenous opioid peptides. Using patch-clamp electrophysiology in rat brain slices, we found that opioid withdrawal strongly reduced the ability of met-enkephalin, an opioid peptide, to inhibit this glutamatergic transmission (control inhibition = 25 ± 4 %, n = 12 vs. withdrawal inhibition = 14 ± 3 %, n = 12; unpaired Student's t-test, p = 0.043). Furthermore, we observed that the reduction in opioid inhibition was due to increased degradation of opioid peptides by the enkephalin-degrading peptidase, neprilysin. Our results highlight that opioid peptide activity is decreased during opioid withdrawal; a change that alters synaptic transmission between amygdala nuclei. As amygdala neural circuits are involved in the development of addiction behaviours, restoring endogenous opioid activity within the amygdala during withdrawal may

return synaptic transmission to normal, mitigate withdrawal-induced neuroadaptations and rescue the addiction behaviours exhibited following opioid withdrawal.

id #13257

Protein-protein interaction analysis to discover new modulators for astrocyte stress response and reactivity pathways

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Astrocytes provide essential neuroprotection in the central nervous system and regulate numerous homeostatic functions from metabolism and synaptic support to axon guidance and blood flow. Astrocytes respond to stress and injury becoming reactive, which is connected to many acute and chronic neurological diseases such as ischemia and Parkinson's disease. *Astrocyte shape/volume changes are initiators for intracellular metabolic and signaling pathways that reflect astrocyte functional changes during stress responses.* We have now identified a specific astrocyte responder complex that is able to detect astrocyte volume/ shape changes and developed a model system to identify and characterize functional adaptive protein networks and biological pathways for astrocyte stress changes. In this study, we performed a comprehensive proximity proteomic analysis in conditions where shape/volume changes are functional or disrupted. We utilized combined affinity-purification and proximity-dependent labelling mass spectrometry with significance analysis of interactome to identify statistically high confidence static and dynamic protein-protein interaction networks.

 Our results show that when a*strocyte shape/volume changes are functional,* our responder is tightly connected to fundamental signalling and proteostasis initiators and enriched for pathway initiators for astrocyte immune response triggering, metabolism of glutamate and glucose. However, the pathway clusters were differential when astrocyte shape/volume response was disrupted and enriched toward processes involved in ion homeostasis and protein hydrolysis. As a summary, we have discovered important pathway initiator proteins that can modulate astrocyte functions under stress conditions.