id #8626

AN EXAMINATION OF THE CELLULAR AND INFLAMMATORY RESPONSE IN RATS AFTER SPINAL CORD INJURY, AND THE EFFECTS OF AGE AND SURVIVAL TIME.

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Spinal cord injury (SCI) results in tissue loss and functional impairment and exhibits limited repair. The inflammatory response plays a significant role in the progression of the secondary injury phase of SCI. The exact mechanisms of this have yet to be elucidated so this is an area of great interest in SCI research. There appears to be a trend for a better recovery in younger patients compared to adults which is also reported for animal studies, however the reasons for this are still largely unknown. This study aims to fill in some of the gaps in our knowledge of paediatric SCI and how it differs from that in mature subjects using a contusion injury model in adult (9wk), juvenile (P35) & infant (P7) Sprague-Dawley rats. One cohort was compared behaviourally and histologically post-injury (n=108), while a second cohort (n=97) was assessed using flow cytometry on the injured tissue homogenate and multiplex cytokine ELIZA on the tissue supernatant. The results of this study showed that there were significantly different injury patterns and alterations to locomotor functional recovery in infant animals compared to their mature counterparts. There was a decreased and significantly different inflammatory response between developing and mature spinal cords, with the infant response appearing more balanced and potentially more beneficial to injury resolution. If we can manipulate the adult responses to resemble the infants this may be of great therapeutic potential for patients of all ages, however more exploration into the mechanisms behind these observed differences is required.

1. Sutherland, TC., Mathews, KJ., Mao, Y., Nguyen, T. and Gorrie, CA (2017) Differences in the Cellular Response to Acute Spinal Cord Injury between Developing and Mature Rats Highlights the Potential Significance of the Inflammatory Response. Frontiers in Cellular Neuroscience 10(310).

id #9011

A proposed novel mechanism for neurodegeneration in Parkinson’s disease

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Our discovery that neurotoxic superoxide dismutase-1 (SOD1) proteinopathy in *SOD1*-associated familial amyotrophic lateral sclerosis (fALS) is recapitulated in idiopathic Parkinson’s disease suggests that these two phenotypically-distinct disorders share an etiological pathway, and tractable therapeutic target(s). The absence of *SOD1* mutations in Parkinson’s disease indicates *SOD1* mutations are not the sole cause of SOD1 protein misfolding occasioning oligomerization and toxicity, and reinforces the importance of non-genetic factors, such as protein metallation and post-translational modification in determining SOD1 stability and function. Therapies aimed at modulating protein aggregation in neurodegenerative disease have met with limited success to date, however increasing our understanding of initial protein misfolding events may lead to the development of therapies which target biomolecular events upstream of protein deposition. Treatments that modulate SOD1 copper binding, a key factor in preventing initial misfolding events, have yielded remarkable improvements in motor function and cell loss in multiple animal models of both *SOD1*-fALS and Parkinson’s disease, resulting in phase 1 clinical trials. These data indicate targeted supplementation of neuronal copper levels constitutes a beneficial therapeutic strategy in both Parkinson’s disease and *SOD1*-associated fALS, and that its efficacy lies in the restoration of physiological structure and function of copper-dependent proteins such as SOD1.

id #9208

Neuroprotective potential of phosphatidylcholine analog soya-lecithin against ICV-STZ induced cognitive dysfunction: Targeting cholinergic dysfunction, neuroinflammation, and neurotransmission alterations

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Background: Soya-lecithin (phosphatidylcholine analog) is used in the treatment of cognitive dysfunction, rofecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor and berberine, an isoquinoline alkaloid having antioxidant like effect.

Aim: To investigate the neuroprotective potential of soya-lecithin along with rofecoxib and berberine in ICV-STZ induced cognitive dysfunction.

Materials & methods: Animals received single bilateral ICV injections of STZ (3 mg/kg). Drugs galantamine (2 mg/kg), soya-lecithin (100, 200 & 400 mg/kg), berberine (50 & 100 mg/kg), rofecoxib (10 & 20 mg/kg) and their combination was administered for a period of 21 days. Various neurobehavioral parameters, followed by biochemical (oxidative stress markers), AChEs level, molecular (TNF-α level), mitochondrial respiratory enzyme complexes (I-IV), neurotransmitter levels and histopathological (H&E staining) evaluations.

Results: ICV-STZ administration significantly ($p<0.05$) impaired spatial learning and memory performance in MWM task, increased oxidative stress, AChEs level, neuroinflammation, altered neurotransmitter levels, causes mitochondrial dysfunction and histopathological alterations as compared to the sham group. Chronic treatment with soya-lecithin (100, 200 & 400 mg/kg), berberine (50 & 100 mg/kg) and rofecoxib (10 & 20 mg/kg) alone for 21 days significantly ($p<0.05$) improved cognitive performance as compared to the ICV-STZ treated animals. Further, combination treatment of soya-lecithin (100 & 200 mg/kg) with berberine (50 & 100 mg/kg) and rofecoxib (10 & 20 mg/kg) for 21 days significantly ($p<0.05$) modulated their neuroprotective potential as compared to their effect *per se*.

Conclusion: Soya-lecithin in combination with rofecoxib and berberine improves various alterations against ICV-STZ induced cognitive dysfunction in rats via anti-oxidant and anti-inflammatory mechanism.

1. Bruni, A., Toffano, G., 1982. Lysophosphatidylserine, a short-lived intermediate with plasma membrane regulatory properties. Pharmacological Research Communications 14, 469-484.

2. Singh, A., Kumar, A., 2015. Microglial Inhibitory Mechanism of Coenzyme Q10 Against Aβ(142) Induced Cognitive Dysfunctions: Possible Behavioral, Biochemical, Cellular, and Histopathological Alterations. Frontiers in Pharmacol. 6, 1-14.
3. Zhang, Z., Li, X., Li, F., An, L., 2016. Berberine alleviates postoperative cognitive dysfunction by suppressing neuroinflammation in aged mice. International Immunopharmacology. 38, 426–433.
4. Xu, Y., Yan, J., Zhou, P., Li, J., Gao, H., Xia, Y., Wang, Q., 2012. Neurotransmitter receptors and cognitive dysfunction in Alzheimer's disease and Parkinson's disease. Prog. Neurobiol. 97(1), 1–13.
5. Saxena G., Patro, I.K., Nath, C., 2011. ICV STZ induced impairment in memory and neuronal mitochondrial function:A protective role of nicotinic receptor. Behav. Brain Res. 224, 50-57.
6. Min, S.S., Quan, H.Y., Ma, J., Han, J.-S., Jeon, B.H., Seol, G.H., 2009. Chronic brain inflammation impairs two forms of long-term potentiation in the rat hippocampal CA1 area. Neurosci. Lett. 456, 20-24.
7. Kumar, A., Singh, A., (2015). A review on mitochondrial restorative mechanism of antioxidants in Alzheimer's disease and other neurological conditions. Frontiers in Pharmacology. 6(206), 1-13.
8. Kumar, A., Singh, A., Ekavali (2015). A review on Alzheimer's disease pathophysiology and its management: an update. Pharmacological Reports 67, 195-203.
9. Kalonia, H., Kumar, P., Kumar, A., Nehru, B., 2010. Protective effect of rofecoxib and nimesulide against intra-striatal quinolinic acid-induced behavioral, oxidative stress and mitochondrial dysfunctions in rats. NeuroToxicology. 31, 195–203.
10. Patel, S., Roelke, C.T., Rademacher, D.J., Hillard, C.J., 2005. Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling, Eur. J. Neurosci. 21, 1057–1069.

id #9209

CEREBROVASCULAR EXPRESSION OF GABA SIGNALLING COMPONENTS IN THE HUMAN MIDDLE TEMPORAL GYRUS

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Vascular changes and their mechanisms are involved in neurodegeneration which contributes to several brain disorders. The inhibitory neurotransmitter γ-aminobutyric acid (GABA) can regulate changes in blood flow through neurovascular coupling, but the underlying mechanisms are unclear. The GABA signalling system is structurally heterogeneous, comprising multiple subtypes of GABA receptors and transporters. The effect of GABA on the cerebral vasculature may be relevant to cerebrovascular dysfunction in diseases involving the GABAergic system, including Alzheimer's disease and other dementias. However, the expression of specific GABA receptors and transporters on the human cerebral vasculature has never been demonstrated. In this study, we investigated the cerebrovascular expression of GABA signalling components in the post-mortem human middle temporal gyrus (MTG). Fluorescence immunohistochemistry and confocal imaging were utilised to detect the cerebrovascular expression of GABA signalling components in post-mortem MTG sections. We report, for the first time, the endothelial expression of the alpha 2, alpha 3, alpha 5, beta 1-3, gamma 3 and epsilon GABAA receptor (GABAAR) subunits, as well as the GABA transporters, in the human MTG cerebral vasculature. Some GABAAR subunits, including beta 1 and beta 2, were expressed at low levels. While the alpha 1 subunits were not expressed on the vasculature, in contrast with their widespread incorporation into neuronal GABAARs. In summary, GABAARs may be expressed with a unique subunit composition on the cerebral vasculature, and may potentially play a role in cerebral blood flow regulation in health and disease.

id #9210

CHANGED FRONTAL POLE GENE EXPRESSION SUGGEST ALTERED INTERPLAY BETWEEN NEUROTRANSMITTER, DEVELOPMENTAL AND INFLAMMATORY PATHWAYS IN SCHIZOPHRENIA

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Schizophrenia (Sz) likely occurs because of changes in CNS gene expression caused by an inherited genome and environmental factors changing epigenetic mechanisms. To identify changes in cortical gene expression in Sz, we compared mRNA levels in the frontal pole (Brodmann's area (BA) 10), the dorsolateral prefrontal cortex (BA 9) and cingulate cortex (BA 33) from 15 subjects with Sz and 15 controls using the Affymetrix™ Human Exon 1.0 ST Array. Differences in mRNA levels (±≥ 20%; $p < 0.01$) were identified (JMP Genomics 5.1) and used to predict pathways likely affected by changed in gene expression using Ingenuity Pathway Analysis. Compared to controls, there was significant variation in mRNA levels in BA 10 ($n = 566$), BA 9 ($n = 65$) and in BA 33 ($n = 40$) in Sz with an over-representation of genes with changed expression involved in inflammation and development in BA 10, cell morphology in BA 9 and amino acid metabolism and small molecule biochemistry in BA 33. We identified 94 genes with altered levels of expression in BA 10 from subjects with Sz that formed an interactome of proven direct gene x gene interactions that was enriched for genes in inflammatory, developmental, oestrogen, serotonergic, cholinergic and NRG1 regulated pathways; all shown to be important in the aetiology of Sz. Our data showing extensive changes in gene expression BA 10 in Sz is significant as BA 10 influences cortical function in at least 13 other cortical regions and Sz involves abnormal functioning in many cortical regions.

id #9212

Ameliorative effects of A1 receptor agonist on depressive-like behavior through Glutamatergic pathway modulation in a Pentylenetetrazole rat model of epilepsy

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Depression is the most significant cause of suicide among neuropsychiatry illnesses. Major depression further affects the quality of life in an individual with epilepsy. The treatment of depression in an epileptic patient could be very challenging because of drug selection or the fact that some anti-epileptic drugs are known to cause depression. It has been shown that in addition to the known involvement of the serotonergic pathway in depression, the glutamatergic system is also involved in the evolution of the disease. This study assessed if induction of epilepsy in rats will cause depressive-like behavior, alter the concentrations of metabotropic receptor 5 (mGluR5), glutamate transport protein (GLAST), glutamate synthetase

(GS) and brain derived neurotrophic factor (BDNF). Also, the ameliorative effect of treatment with 2-Chloro-N6-cyclopentyladenosine (CCPA) on neurobehavioral and neurochemistry in epilepsy was also investigated. Epilepsy was induced in rats by injecting Pentylenetetrazole at 35 mg/kg every other day. At kindle, rats were subjected to sucrose preference test (SPT) and forced swim test (FST) and decapitated 4 hours after. Using hippocampal tissues, BDNF concentration was determined with ELISA while mGluR5 and GS protein expression were measured using western blot. GLAST expression in amygdala tissue was also determined using flow cytometry. Our result showed that CCPA ameliorates the depressive-like behavior seen in the rats, increased the hippocampal concentration of BDNF and expression of mGluR5, GS and amygdala GLAST respectively. Our study concluded that CCPA works effectively to treat depression associated with epilepsy.

id #9213

Exploratory study of Risperidone on Reelin DNA methylation in patients diagnosed with first episode psychosis

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Background: Reelin, a neurotrophic gene that is important for neuroplasticity, is found to be down-regulated in schizophrenia by DNA methylation - a stable repressive epigenetic DNA mark. However, little is known about the effects of antipsychotic drugs on reelin DNA methylation. Antipsychotic drugs are given to patients as part of the medication regime to improve psychotic symptoms. This study aims to investigate the effects of antipsychotic drugs and reelin DNA methylation in first episode psychosis (FEP) patients over a period of 12 months.

Materials and Methods: 11 FEP patients and 20 healthy controls were recruited for this study, before and after risperidone treatment. DNA extracted from the blood lymphocytes were processed using bisulfite conversion, DNA amplification and pyrosequencing. Reelin DNA methylation of the 5 CpG sites (-131bp to -111bp) of exon 1 in the reelin promoter region were measured and analysed.

Results: CpG site 4, 6 and 7 show an increase trend in Reelin DNA methylation in FEP patients between 0 month and 12 months after risperidone treatment whereas CpG5 and 8 remain about the same during this period. Comparably to healthy controls, reelin DNA methylation generally fluctuates at basal level between the two-time points. A potential synergistic effect is found in patients who took risperidone and antidepressant concurrently by reducing the DNA methylation of various CpG sites. CpG7 increase in methylation is most obvious in the male population of the FEP patients, while decrease in reelin DNA methylation is found in the female population of FEP at CpG5.

id #9214

Evaluation of Two Structurally Novel G Protein Biased Agonists with Improved Analgesic and Side Effect Profiles

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Opioid analgesics commonly used for the treatment of chronic pain, such as morphine and fentanyl, have been shown to induce their analgesic effects via G-protein signalling pathways, while the β-arrestin pathway has been associated with side effects such as tolerance and respiratory depression. Therefore, developing agonists that selectively active the G protein pathway provides a new avenue for the development of safer more effective opioid analgesics. Here we show the structurally novel Salvinorin A analogues Kurkinorin (bias = 0.57) and Kurkinol (bias=0.14) have reduced β-arrestin recruitment (ED_{50}= 14 nM, ED_{50}= 140 nM) while inhibiting cAMP production (ED_{50}=1.2 nM, ED_{50}=0.03 nM) and inducing inwardly rectifying potassium channels (ED_{50}= 285.7 nM, ED_{50}= 45.5 nM), resulting in a bias for the G protein pathway. We further show that this bias correlates to potent analgesic effects with reduced tolerance, inhibition of small intestinal transit, motor coordination impairment, and lower abuse liability compared to morphine using preclinical animal behavioural models. These results highlight the potential biased agonists hold for the development of non-addictive analgesics for the treatment of chronic pain.

id #9217

Orientation maps in the primary visual cortex of an Australian marsupial, the Tammar Wallaby *Macropus eugenii.*

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In placental mammals of the carnivora and primate orders, orientation selective cells in the primary visual cortex (V1) are organised into structured orientation columns, where cells with the same preference cluster together into vertical cylinders. These columns are organised into two-dimensional orientation maps, where columns with different orientation preferences are arranged sequentially and radially around a pinwheel centre. Some placental species (rodents and lagomorphs) have a random distribution of orientation selective cells. We do not know yet why some mammals have structured pinwheel maps while others have randomly arranged maps. To enhance our understanding of the generality of orientation map formation, we studied orientation maps in a marsupial mammal, the Tammar wallaby *(Macropus eugenii)*. We used a combination of intrinsic optical imaging (OI) and multi-channel electrophysiology methods to examine the functional organization of the wallaby cortex. We found that wallaby orientation maps contain patches of iso-orientation domains that are biased towards vertical and horizontal preferences. In some cortical regions, the iso-orientation domains are arranged radially around a pinwheel centre but this is not as uniform across the map as in placental mammals. Assessment of receptive field (RF) characteristics revealed robust orientation and direction selectivities, which corresponded strongly with the OI maps.

id #9218

Aminoacyl-tRNA synthetases in Schwann cells regulate the peripheral nerve degeneration after nerve injury.

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Aminoacyl-tRNA synthetases (AminoARSs) are key enzymes which attach specific amino acids to tRNA during protein synthesis process. Recent studies have focused on not only the canonical functions of AminoARSs in protein synthesis but also the non-canonical functions of AminoARSs in several cells such as inflammation, proliferation, apoptosis and so on. For example, with exogenous human lysyl-tRNA synthetase (KARS) treatment, TNF-α is secreted in Raw264.7 cell line which is originated from macrophages. It is shown that KARS is related to proinflammatory response. Human glutaminyl-tRNA synthetase (QARS) binds with apoptosis signal-regulating kinase 1 (ASK1) and then the apoptosis is suppressed through the inhibition of the activity of ASK1 in 293 cells. However, there is still unknown about non-canonical functions of AminoARSs in Schwann cells, which are an essential cell type in the peripheral nervous system (PNS). Their roles in PNS are i) to support the peripheral axon, ii) to form a myelin sheath around the axon and iii) to regulate nerve degeneration (Wallerian degeneration, WD). Thus, to examine whether AminoARSs show non-canonical functions in Schwann cells during WD, we screened 20 AminoARSs after sciatic nerve axotomy. Among them, three were up-regulated and one was down-regulated in Schwann cells, but not in axons during WD. Using pharmacological and genetic modulation to inhibit AminoARSs expression, we confirmed that AminoARSs extensively regulate WD, including myelin fragmentation, axon degradation, Schwann cell de-differentiation and Schwann cell proliferation in Schwann cells. These results suggest that AminoARSs play important roles for Schwann cells in response to peripheral nerve injury.

id #9238

Plasma 25-hydroxyvitamin D is not associated with either cognitive function or academic performance in adolescents

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Several studies have reported that vitamin D deficiency (VD) in adults is associated with poor cognition, but data on such association in adolescents is limited. We investigated the association between VD and cognitive function or academic achievement among adolescents. Students (N=1370) were selected from public middle schools in Kuwait using stratified multistage cluster random sampling. Plasma 25-hydroxy VD (25-OH-D) was measured by LC-MS/MS. Age-adjusted standard score (ASC) calculated from Raven's Standard Progressive Matrices was used for cognitive function, whereas, school examination results extracted from the school records were used for academic performance. Data on various covariates were collected through self-administered questionnaire (parents) and face-to-face interviews (students). 25-OH-D was weakly correlated positively with ASC (r=0.06; p=0.038). Univariate linear regression analysis showed association between 25-OH-D categories and ASC after adjusting for gender but adjusting for parental education nullified this association. Multivariate analysis showed no association between 25-OH-D and ASC after adjusting for potential confounders whether 25-OH-D was fitted as a continuous (p=0.725), categorized by acceptable cutoff points (p=0.475) or as quartiles (p=0.881). 25-OH-D was not associated with academic performance. We conclude that 25-OH-D is not associated with either cognitive function or academic performance in adolescents.

id #9243

MAGNETIC RESONANCE IMAGING OF THE BRAIN OF A SIRENIAN, *DUGONG DUGON*

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Sirenians (dugong, manatees) are a group of aquatic herbivorous mammals of the superorder Afrotheria. The brains of West Indian manatees show evidence of trigeminal and facial motor specialization for grazing on sea-grasses, but no MRI studies have analyzed the brain of the Indo-Pacific dugong. We have used magnetic resonance imaging to study the brain of a dugong, which died in captivity. The brain was fixed in formaldehyde and scanned using high resolution T1w and T2w protocols in a pre-clinical 9.4-T Bruker BioSpec 94/20 Avance III MRI system (*Bruker*, Ettlingen, Germany). Quantitative findings were compared with data on the brain of the manatee and 49 other therians (neurosciencelibrary.org). The dugong brain is smaller than that of the manatee (240 ml vs 346 ml) but with a similarly lissencephalic cerebrum (gyrification index of 1.02 vs 1.11). Subcortical white matter is remarkably large in both sirenians (61.7 ml or 26% of brain volume in dugong, 52.8 ml or 15% of brain volume in manatee), which is much greater than most therians of that brain size. Like other marine mammals, the hippocampal formation is small in both sirenians. The dugong has similar trigeminal and facial motor specialization to the manatee. The spinal trigeminal tract in the dugong brain is even larger than in the manatee (13.1 mm^2 vs 5.9 mm^2) and the facial motor nucleus and facial nerve are large in both. Sirenians exhibit a suite of neurological specializations that have probably been stable in the group since the Eocene.

id #9244

Identification of a shared pathway to neuronal death in post-mortem Parkinson's disease and amyotrophic lateral sclerosis

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Shared molecular pathologies are emerging within respective vulnerable neuronal populations in Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), suggesting common mechanisms of neurodegeneration may be present. We investigated regional alterations to the antioxidant metalloprotein superoxide dismutase 1 (SOD1), and copper transport proteins, in post-mortem PD and ALS tissues.

We analysed SOD1 protein aggregation, enzymatic function and metallation in degenerating and non-degenerating brain and spinal cord tissues from post-mortem PD (n=9), familial (f; n=5) and sporadic (s; n=10) ALS patients, and age-matched controls (n=10). SOD1 proteinopathy and neuronal loss were characterized using immunohistochemistry. Protein levels of SOD1, CCS, and Ctr1 were quantified using immunoblotting, metal levels (Cu, Zn, Fe, Ca, Cd) quantified using inductively-coupled plasma mass spectrometry, glutathione quantified using a modified Tietze enzyme-recycling assay and SOD1 activity quantified using a WST-Xanthine Oxidase assay. SOD1 metallation was assessed using size-exclusion-chromatography coupled with native isoelectric focussing and PIXE.

Dysfunctional wild-type SOD1 proteinopathy was selectively accumulated in degenerating PD brain, and closely resembled mutant SOD1 proteinopathy in fALS ventral spinal cord. SOD1 deposition was associated with neuronal loss and α-synuclein deposition in PD, and with copper and glutathione depletion in PD and ALS. Changes in SOD1 structure and antioxidant activity indicated SOD1 is copper-deficient in vulnerable PD and ALS regions.

Copper supplementation via CuATSM is shown to improve motor symptomology and slow neurodegeneration in multiple animal models of PD and ALS. Our data identify a specific cellular pathway linked to neuronal loss in both disorders, which CuATSM may modify for clinical benefit.

id #9245

MTHFR deficiency and low dietary folate interact to promote the accumulation of phosphorylated amyloid protein precursor and tau.

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Deregulation of amyloid protein precursor (APP) and tau plays a critical role in the neurodegenerative cascade of Alzheimer's disease (AD). Significantly, common functional polymorphisms in the methylenetetrahydrofolate reductase (*Mthfr*) gene are risk factors for late-onset AD. Reduced MTHFR activity can synergise with dietary folate deficiency to induce alterations in folate and homocysteine metabolism. Here, using MTHFR knockout models, we investigated how genetic and diet-induced disturbances in folate metabolism influence the phosphorylation of tau at the AD-like PHF-1 phosphoepitope, and of APP at the Thr-668 site, which influences amyloidogenic processing. Western blot analyses were performed in regional brain homogenates prepared from 5-week-old wild-type and knockout *Mthfr* mice fed a normal folate diet, and from 22-month-old wild-type and *Mthfr*$^{+/-}$ mice that had been fed for 6 months a normal or low folate diet prior to sacrifice. In young mice, severe MTHFR deficiency markedly increased p-Tau (PHF-1) and pThr668-APP levels in the hippocampus and cortex. Significantly, this was recapitulated by prolonged dietary folate deficiency in old wild-type and *Mthfr*$^{+/-}$ mice. The incremental tau and APP phosphorylation mediated by severe folate deficiency correlated with enhanced accumulation of demethylated protein phosphatase 2A (PP2A) and activation of glycogen synthase kinase-3β (GSK-3)β. Our findings identify a novel link between genetic- or diet-induced folate deficiency, deregulation of PP2A, GSK-3β, and accumulation of phosphorylated APP and tau in the mouse brain. Deregulation of APP and tau provides a compelling mechanism for explaining how *Mthfr* polymorphisms may interact with dietary folate deficiency to increase the risk for sporadic AD.

1. Hoffman, A., et al., Methylenetetrahydrofolate Reductase Deficiency Deregulates Regional Brain Amyloid-beta Protein Precursor Expression and Phosphorylation Levels. J Alzheimers Dis, 2018. 64(1): p. 223-237.
2. Sontag, J.M., et al., Altered protein phosphatase 2A methylation and Tau phosphorylation in the young and aged brain of methylenetetrahydrofolate reductase (MTHFR) deficient mice. Front Aging Neurosci, 2014. 6: p. 214.

id #9246

Assessment of long-term functional deficits and axonal damage in a mouse model of peripheral nerve injury

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Animal models of peripheral nerve injury (PNI) are often used to model peripheral neuropathy. However, most animal models of PNI are short term, and evidence related to axonal damage in chronic PNI is limited. In this study, we performed a longitudinal assessment of functional deficits and axonal damage over a 6 month period in a chronic constriction injury (CCI) mouse model. CCI was performed on 7 week old C57BL/6 male mice by tying loose ligatures on the left sciatic nerve approximately 1 cm proximal to site of trifurcation. Sham animals which had their sciatic nerves exposed but not constricted were used as controls. Animals were thereafter tracked over a span of 5 months for changes in pain behaviour (von-frey), hindlimb strength (grip test) and downstream axonal excitability (threshold tracking). Transmission electron microscopy was used to assess distal demyelination 6-months post-surgery. Nerve-injured mice had chronic ipsilateral pain hypersensitivity over the course of the testing period and also scored consistently low on the hind limb grip test. Electrophysiology showed an overall reduced state of axonal excitability, a relatively hyperpolarised resting membrane potential and transient conduction block distal to site of injury. Electron microscopy showed evidence of demyelination in distal nerve segments, which is consistent with the pattern of axonal excitability. The results show that mice with PNI had chronic and debilitating comorbidities associated with their injury, which may be associated by a demyelinating pathology. It follows that this animal model may be relevant in understanding and managing nerve injury in humans.

id #9249

The Expression of Bradykinin and its Receptors in Spinal Cord Ischemia-Reperfusion Injury Rat Model

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Objective: To investigate the expression of bradykinin(BK) and its receptors(Bradykinin receptors 1 and 2, B1R and B2R) in spinal cord ischemia-reperfusion injury (SCII) in rat model.

Methods: Sprague-Dawley (SD) rats was subjected to 1h of infra-renal abdominal aorta occlusion and reperfused for 3h to 5d to induce SCII. The concentration of BK in serum was detected by enzyme linked immunosorbent assay(ELISA). In situ expression of BK receptors was evaluated by immunochemistry and their mRNA level was evaluated by Real time quantitative-PCR (RTq-PCR).

Results:, The concentration of BK in serum was increased following SCII. Both of the BK receptors were detected in normal and injured spinal cord. And the mRNAs of B1R and B2R were up-regulated after SCII.

Conclusion: This study provides the first evidence of the expression of BK and its receptors in SCII in rat model, and suggests that BK and its receptors may have some physiological or pathological significance in SCII.

id #9252

In vivo simultaneous germline inactivation of multiple genes in mouse through CRISPR/Cas9-mediated base editing

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In vivo genetic mutations has become a powerful tool for dissecting gene function; however, multi-gene interaction and compensatory mechanisms involving can make findings from single mutations at best difficult to interpret, and at worst, misleading. Hence, it is necessary to establish an efficient way to disrupt multiple genes simultaneously. The CRISPR/Cas9-mediated base editing disrupts gene function by converting a protein coding sequence into a stop codon; this is referred to as CRISPR-stop. Its application in generating germline mutations in zygotes has not been well explored yet. Here, we firstly performed a proof-of-principle test by disrupting *Atoh1*, a gene critical for auditory hair cell generation. Next, we individually mutated *vGlut3*, *Otoferlin* and *Prestin*, three genes needed for normal hearing function. Finally, we successfully disrupted *vGlut3*, *Otoferlin* and *Prestin* simultaneously. Our results show that CRISPR-stop can efficiently generate single or triple homozygous F0 mice mutants, bypassing laborious mouse breeding. We believe that CRISPR-stop is a powerful method that will pave the way for high-throughput screening mouse developmental and functional genes, matching the efficiency of methods available for model organisms, such as *Drosophila.*

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id #9253

AN EXAMINATION OF THE CELLULAR AND INFLAMMATORY RESPONSE IN RATS AFTER SPINAL CORD INJURY, AND THE EFFECTS OF AGE AND SURVIVAL TIME.

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Spinal cord injury (SCI) results in tissue loss and functional impairment and exhibits limited repair. The inflammatory response plays a significant role in the progression of the secondary injury phase of SCI. The exact mechanisms of this have yet to be elucidated so this is an area of great interest in SCI research. There appears to be a trend for a better recovery in younger patients compared to adults which is also reported for animal studies, however the reasons for this are still largely unknown. This study aims to fill in some of the gaps in our knowledge of paediatric SCI and how it differs from that in mature subjects using a contusion injury model in adult (9wk), juvenile (P35) & infant (P7) Sprague-Dawley rats. One cohort was compared behaviourally and histologically post-injury (n=108), while a second cohort (n=97) was assessed using flow cytometry on the injured tissue homogenate and multiplex cytokine ELIZA on the tissue supernatant. The results of this study showed that there were significantly different injury patterns and alterations to locomotor functional recovery in infant animals compared to their mature counterparts. There was a decreased and significantly different inflammatory response between developing and mature spinal cords, with the infant response appearing more balanced and potentially more beneficial to injury resolution. If we can manipulate the adult responses to resemble the infants this may be of great therapeutic potential for patients of all ages, however more exploration into the mechanisms behind these observed differences is required.

id #9255

EARLY CELL DEATH IN ADULT HIPPOCAMPAL NEUROGENESIS IS FERROPTOTIC AND RESCUED BY SELENIUM

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The control of cell death is a key mechanism in the regulation of adult hippocampal neurogenesis. The stem cells in the hippocampus continuously divide to generate a large pool of proliferating progenitor cells. This population of cells however, undergoes a major culling at the progenitor cell stage. Apoptotic elimination of progenitor cells is critically involved in controlling the number of newborn neurons in the adult hippocampus. However, the very low number of apoptotic newborn cells compared to the total number of cells lost, suggests that elimination also occurs by non-apoptotic means.Ferroptosis is a recently identified non-apoptotic, caspase-3-independent form of cell death, characterised by the iron-dependent accumulation of lethal reactive oxygen species (ROS) and lipid peroxidation products. We hypothesised that ferroptotic cell death might be involved in controlling the number of surviving hippocampal progenitor cells. At the core of the ferroptotic pathway is the selenium-containing protein Gpx4. We therefore examined whether selenium-mediated perturbations could decrease ferroptosis and thus increase in adult neurogenesis. Our results reveal that selenium reduces the levels of intracellular ROS and subsequently decreases lipid peroxidation, a hallmark of ferroptotic cell death, specifically in the Nestin-positive progenitor cells. Importantly, this results in an increase in neural progenitor cell survival and neuronal-lineage differentiation *in vivo* in the hippocampus of both young adult and aged mice. This process is restricted to the hippocampal stem cell niche and we suggest that it may beinvolved in hippocampal progenitor cell maintenance in the context of the activity-dependent regulation of adult neurogenesis.

id #9256

ASK1 inhibition; A potential strategy for the prevention of acquired hearing loss.

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Introduction:

Aminoglycoside antibiotics and platinum based chemotherapeutics induce the death of cochlear hair cells. However, the process by which hair cells die remains uncertain.

The JNK pathway has been implicated as a mediator of hair cell death, but upstream apoptotic regulators such as Apoptotic Signal Regulating Kinase 1 (ASK1) have not yet been investigated in the inner ear

We hypothesise that ASK1 deficiency will prevent sensory cell death and protect against ototoxic drug-induced hearing loss.

Specifically, this study uses *in vitro* explants to (1) Investigate the effect of ASK1 deficiency on resistance to aminoglycoside-induced sensory cell death, (2) Investigate the efficacy of an ASK1 inhibitor in promoting sensory hair cell survival, and (3) assess the efficacy of aminoglycoside antibiotics when co-administered with the ASK1 inhibitor.

Post-natal day 3 cochlear explants were cultured in neurobasal medium containing neomycin (400 micromolar - 1 millimolar) or DMSO for 24- 72 hours. Hair cell counts were performed on mid turn segments. MCRI Animal Ethics approval number A844.

Results:

In vivo, ASK1 deficiency has no effect on development of the ear and hearing. *In vitro*, ASK1 deficiency attenuates neomycin induced hair cell death. The ASK1 inhibitor produces similar rates of hair cell survival when compared with ASK1 Knock Out hair cells.

Conclusion:

This data suggests that ASK1 is a promising target for the prevention of drug induced hearing loss. *In vivo* studies are currently underway.

id #9259

Granule neuron precursor cell proliferation is regulated by NFIX and intersectin 1 during postnatal cerebellar development

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Cerebellar granule neurons are the most numerous neuronal subtype in the central nervous system. Within the developing cerebellum, these neurons are derived from a population of progenitor cells found within the external granule layer of the cerebellar anlage, namely the cerebellar granule neuron precursors (GNPs). The timely proliferation and differentiation of these precursor cells, which, in rodents occurs predominantly in the postnatal period, is tightly controlled to ensure the normal morphogenesis of the cerebellum. Despite this, our understanding of the factors mediating how GNP differentiation is controlled remains limited. Here, we reveal that the transcription factor nuclear factor I X (NFIX) plays an important role in this process. Mice lacking *Nfix*exhibit reduced numbers of GNPs during early postnatal development, but elevated numbers of these cells at postnatal day 15. Moreover, *Nfix*$^{-/-}$GNPs exhibit increased proliferation when cultured *in vitro*, suggestive of a role for NFIX in promoting GNP differentiation. At a mechanistic level, profiling analyses using both ChIP-seq and RNA-seq identified the actin-associated factor *intersectin 1*as a downstream target of NFIX during cerebellar development. In support of this, mice lacking *intersectin 1*also displayed delayed GNP differentiation. Collectively, these findings highlight a key role for NFIX and intersectin 1 in the regulation of cerebellar development.

id #9265

Intrinsic mutant HTT-mediated defects in oligodendroglia cause myelination deficits and behavioural abnormalities in Huntington disease

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White matter atrophy and myelin abnormalities appear to be significant features in Huntington disease (HD). Although white matter atrophy appears very early in the disease course, the molecular factors that underline myelination deficits in HD are poorly understood. We hypothesize that intrinsic mutant huntingtin (mHTT)-mediated effects in oligodendroglia contribute to myelin deficits and behavioural manifestations in HD. To test this hypothesis, we crossed the BACHD mouse model of HD, which expresses full-length human mHTT and mimics many of the behavioural and neuropathological features of the human condition, to NG2-Cre mice in order to inactivate mHTT specifically in oligodendroglia. Electron microscopy was used to analyze myelin fibers of the corpus callosum (CC) at 1 and 12 months of age and a battery of behavioral tests was performed to evaluate motor and psychiatric-like phenotypes in mice. To gain insights into the molecular mechanisms underlying the oligodendrocyte dysfunction observed in HD mice, we performed RNA-seq and ChIP-seq analysis on the CC of mice at 1 month of age. We show that selective inactivation of mHTT from oligodendroglia rescues the deficits in thickness and compactness of myelin sheaths and improves certain aspects of behavioural dysfunction in the HD mice. Our results also show that mHTT promotes increased PRC2 binding and thereby potential histone modification and transcriptional dysregulation. Together, our findings suggest that epigenetic mechanisms are involved in intrinsic oligodendroglia dysfunction in HD and contribute to myelin abnormalities and certain behavioural manifestations of the disease.

id #9266

ALTERATIONS IN GLUTAMATE RECEPTOR AND TRANSPORTER EXPRESSION IN THE HIPPOCAMPUS OF AN IN VIVO ALZHEIMERS DISEASE MOUSE MODEL

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Alzheimer's Disease (AD) is the leading type of dementia worldwide, and with an increasing burden due to an aging population combined with a lack of any foreseeable cure, it warrants a large driving force of research. Glutamate is the main excitatory neurotransmitter in the brain and plays an essential role in the function and health of neurons and neuronal excitability. Previous studies have shown alterations in expression of glutamatergic signalling components in AD. This study aimed to characterise changes in specific glutamate receptors and transporters 30 days post hippocampal beta-amyloid ($A\beta_{1\text{-}42}$) stereotactic injection of a mouse model of AD using immunohistochemistry and confocal microscopy. We report significant decreases in density of glutamate receptor subunits GluA1, GluN2A and the vesicular glutamate transporter VGluT1 in the CA1 region of the hippocampus in the AD mice compared to controls, notably in the stratum oriens and stratum radiatum. These changes are in line with findings observed in the human AD hippocampus. Glutamate receptor subunits GluA2, GluN1 and transporter VGluT2 showed no changes in expression. These findings indicate that the expression of the glutamatergic receptors and transporters show brain region and layer specific changes in AD, suggesting complex activation mechanisms and expression changes during neuropathology.

id #9267

Using Different EEG Connectivity Methods to Measure Neurological Characteristics in Young People with Autism

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Over the last two decades, increasing numbers of EEG-based research reports have consistently demonstrated reduced global coherence in children and adolescents with ASD. Although coherence has been the primary method used to measure neural connectivity in this population, some recent literature has criticised coherence because of its limited information about non-linear relationships, plus the presence of potentially-confounding volume conduction. These criticisms challenge the reported results to date, leading to the exploration of other functional and effective multivariate connectivity measures. Some of these are time-consuming and may eliminate necessary data in an effort to increase precision. Therefore, in order to test the relative effectiveness of the most common connectivity methods used in ASD research, six different EEG connectivity methods were used during resting eyes-open and eyes-closed conditions in 41 male participants diagnosed with ASD, aged 6 to 17 years. By comparing basic and complex multivariate connectivity methods, a more accurate model of how different cortical regions communicate in the young ASD population will be developed, leading to greater validity and reliability in these data and more informed conclusions regarding the neurological behaviour of this population.

id #9268

Adenosine receptor-based strategies for cochlear rescue from injury

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Sensorineural hearing loss (SNHL) can result from exposure to excessive noise, ototoxic drugs, infection or progression with age. SNHL is associated with cochlear injury, including loss of sensory hair cells and primary auditory neurons. We have previously identified the adenosine signalling pathways as important regulators of cellular responses to injury in cochlear tissues and in particular pathways activated by the A_1 adenosine receptor (A_1R). Activation of the A_1R appears to be a promising strategy for the treatment of acute noise-induced cochlear injury and other forms of SNHL such as from cytotoxic drugs, including cisplatin and aminoglycosides. As an alternative to the use of exogenous A_1R agonists, we have recently identified a new and potent otoprotective paradigm based on increasing A_1R responsiveness to endogenous adenosine by

inhibiting the molecular complex (RGS4-neurabin) that regulates A_1R signalling. Regulators of G protein Signalling (RGS) are a family of proteins that inhibit signal transduction pathways initiated by G protein-coupled receptors (GPCR) by enhancing GPCR deactivation and receptor desensitisation. Here, we demonstrate that intratympanic administration of a small molecule RGS4 inhibitor, 24 and 48 hours after traumatic noise exposure, improves the survival of sensorineural tissues and attenuates noise-induced hearing loss. This research represents a novel paradigm for the treatment of various forms of SNHL based on regulation of GPCR.

id #9269

Modulating ivermectin sensitivity at glutamate-gated chloride channels of *H. contortus* and study of inhibitory postsynaptic currents mediated by α and αβ HcoGluCl receptors

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Glutamate-gated chloride channel receptors (GluClRs) are important therapeutic targets for controlling parasitic pest species in agriculture wherein the choice of drug is Ivermectin (IVM). However, the continuous use of IVM has led to emergence of resistance in pest species.

We explored possible mechanisms of IVM resistance in pest species by measuring the glutamate and IVM sensitivity for different isoforms of GluClRs. We used a) TEVC and b) heterosynapses to measure the inhibitory postsynaptic currents (IPSCs) of GluClRs subunits from *Haemonchus contortus* (HcoGluClR).

The α HcoGluClRs and αβ HcoGluClRs had similar EC_{50}s to glutamate, being between 20-30 μM. In contrast, the β subunits exhibit an EC_{50} of 300 μM. α HcoGluClRs with an EC_{50} value of 20 nM for IVM, followed by αβ HCoGluClRs with EC_{50} of 130-200 nM. The β HCoGluClRs were insensitive to IVM (EC_{50} > 10 μM).

We also studied IPSCs mediated by the two isoforms in a cortical neuron-HEK 293 co-culture assay. The decay time constant was faster for heteromeric receptors (αβ:15 ms) than for homomeric receptors (α:40 ms). IVM application prolonged the decay times for both the isoforms wherein increasing the decay time of the α by 2.5 fold to 100 ms and that of αβ to 70 ms.

Our data from TEVC and IPSCs suggests that a significant determinant of IVM sensitivity at GluClRs is the subunit composition. This implies that an organism can increase resistance to IVM without losing glutamate sensitivity by upregulating the expression of an IVM-insensitive subunit to produce heteromeric receptors.

id #9270

TEMPORAL CHANGES IN AUTOPHAGY FOLLOW SEEDING OF ALPHA- SYNUCLEIN PRE-FORMED FIBRILS IN HUMAN NEURAL CELLS

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Parkinson's disease (PD) is a progressively debilitating neurodegenerative disorder that forms intracellular Lewy inclusion pathologies (LP) in the brain. The most prominent and well-studied component of LP is α-synuclein, which is thought to propagate through PD brain in a prion-like manner. The misfolded pathological α-synuclein can be taken up by a neuron and trigger the conversion of normal endogenous α-synuclein to the misfolded pathological form. The pathological fibrilar forms of α-synuclein have been widely used to demonstrate propagation in cell and animal models of PD. As a result, there is now much interest from a therapeutic perspective in understanding the mechanism regulating uptake and turnover of α-synuclein protein in neurons.

In this study, we have investigated the relationship between α-synuclein and autophagy in human neural cell lines following treatment with α-synuclein pre-formed fibrils (PFFs). We robustly found that the number of α-synuclein aggregates peaked at 4-6 days post treatment, and this was associated with a block in autophagy. After this time, autophagy markers returned towards normal and the number of α-synuclein aggregates declined. Meanwhile, the PFF-induced aggregation of α-synuclein was significantly inhibited by AMPK agonists. These results provide further evidence of the dynamic relationship between autophagy and α- synuclein in primary human neurons and provide a model that can be further interrogated to understand the mechanistic nature of this relationship.

id #9271

THETA BURST STIMULATION TO THE DORSOLATERAL PREFRONTAL CORTEX IN FIBROMYALGIA SYNDROME: PRELIMINARY FINDINGS OF A RANDOMISED-CONTROLLED TRIAL

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Fibromyalgia syndrome (FMS) is a highly prevalent chronic disorder. There are no known mechanism specific treatments for FMS, with available treatment options often ineffective. A crucial role for central sensitization has been identified in the pathogenesis and maintenance of FMS. A promising new treatment option to target central sensitisation in FMS is Theta Burst Stimulation (TBS), a non-invasive technique involving the patterned application of brief magnetic pulses to change the electrical activity within cortical networks. Here we present preliminary results of a Phase II, randomised, double-blind, placebo-controlled superiority trial of TBS to the dorsolateral prefrontal cortex (DLPFC) in FMS. Patients undergo 32 sessions of TBS to the left DLPFC over four weeks and are assessed at baseline, end of treatment, and at 1 month follow-up. Outcome assessments include questionnaires and concurrent transcranial magnetic stimulation-electroencephalogram recording (TMS-EEG). In a preliminary analysis of 17 participants, we observed a trend towards reduced pain function from baseline to the end of treatment in the active group that was not observed in the sham treatment group. In an EEG power analysis, we observed a significant reduction in somatosensory gamma power at end of treatment compared to baseline in the active group compared to sham. Together, these preliminary results suggest a 4 week left DLPFC TBS

treatment to have a trending impact on FMS symptoms as well a significant effect on brain function. Further analysis is needed to explore connectivity and cross-frequency coupling; analyses most likely to inform how pain is integrated in the brain.

id #9272

The molecular specification of commissural and subcerebral projection neurons in the neocortex is conserved in Therian mammals

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The correct formation of neocortical circuits is essential for sensory-motor and cognitive functions, and requires accurate regulation of gene expression by transcription factors. Among these, SATB2 and BCL11B (CTIP2) have been previously shown to regulate callosal and subcerebral projections, respectively. In *Satb2* knock-out mice, BCL11B is overexpressed and commissural axons fail to project medially to the corpus callosum, instead projecting laterally towards subcerebral targets via the internal capsule or towards the contralateral hemisphere via the anterior commissure. Strikingly, this resembles the marsupial phenotype, where there is no corpus callosum and the anterior commissure is the main neocortical interhemispheric tract, raising questions about the roles of SATB2 and BCL11B in specifying neuronal projection fate throughout evolution. To address this, we investigated SATB2 and BCL11B expression and function in the Australian marsupial fat-tailed dunnart during neocortical development. Surprisingly, despite the different commissural strategy, we found that, not only are the expression patterns of SATB2 and BCL11B comparable in dunnart and mouse throughout development, but also the generation, migration, and projection targets of SATB2- and BCL11B - positive neurons are broadly conserved; SATB2 was found to specify a commissural fate, while BCL11B regulates the formation of subcerebral projections. *In vivo* experiments were performed to manipulate SATB2 or BCL11B expression in mice and dunnarts, demonstrating a conservation of neuronal phenotypes controlled by these transcription factors. Collectively, these results reveal a conserved transcriptional network controlling commissural and subcerebral projection fate in Therian mammals, which likely predates the evolution of the corpus callosum.

id #9273

Pharmacological blockade of TrkB receptors enhances hippocampal neurogenesis and regulates mood-related behaviour in a mouse model of depression.

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A leading hypothesis for the aetiology of depression involves impairment in adult hippocampal neurogenesis via stress, leading to depressed mood. To examine if increasing the production of new neurons alleviates depression-related behaviours, pharmacological agents that regulate the activity of neural precursor cells (NPCs) and enhance neuron production, are required. We have discovered that blockade of TrkB signalling using ANA-12, a TrkB-specific antagonist, results in the activation of a subpopulation of NPCs. Importantly, we found that *in vivo* administration of ANA-12 over 7 days leads to a significant increase in hippocampal neurogenesis with 65% more $BrdU^{+}Dcx^{+}$ cells. Therefore, to determine whether the ANA-12-mediated increase in neurogenesis ameliorates mood-related deficits associated with depression, we utilised exogenous corticosterone (CORT) administration as a model of depression. We show that chronic corticosterone treatment for 4 weeks induced depressive-like behaviours including increased anxiety and anhedonia in adult C57Bl/6 mice. Interestingly, a 3-week treatment with ANA-12 reversed the CORT-mediated anhedonia in the sucrose preference test. However, this treatment exacerbated anxiety-like behaviour in the novelty suppressed feeding test and in the open field test. Notably, chronic CORT treatment impaired neurogenesis which was ameliorated by the ANA-12 treatment. These results suggest that TrkB signalling may differentially regulate the brain circuits involved in anxiety versus anhedonia in a neurogenesis-dependent or independent fashion. Based on our findings, we propose that increasing hippocampal neurogenesis during chronic stress may negatively impact anxiety levels.

id #9274

Secreted amyloid precursor protein alpha regulates AMPA receptor synthesis and trafficking

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Secreted amyloid precursor protein-α (sAPPα) mediates neuroprotection, neurogenesis and long-term potentiation (LTP), however, the underlying molecular mechanisms employed by sAPPα are not well elucidated. Since LTP is dependent on synthesis and regulated trafficking of AMPA glutamate receptors (AMPARs) we hypothesized that sAPPα may harness these mechanisms to promote synaptic plasticity. Using FUNCAT-PLA to specifically label newly synthesized proteins in primary hippocampal neurons, we discovered that sAPPα (1 nM, 2 h), increased GluA1 synthesis three-fold ($p < 0.0001$), while GluA2 synthesis was unaffected. Moreover, there was a significant increase in the surface expression of pre-existing GluA1-containing AMPARs ($p = 0.0003$) but, curiously, there was no difference in the surface expression of the *de novo* synthesized GluA1 at the time-point investigated. These data suggest that enhanced synthesis and trafficking of GluA1-containing AMPARs, by increasing the pool of available AMPARs, may contribute to the LTP-enhancing properties of sAPPα.

id #9275

Cholinergic signalling from basal forebrain regulates adult hippocampal neurogenesis via Chrm4.

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The adult hippocampus harbors distinct populations of quiescent and active neural precursor cells (NPCs) that drive the production of new neurons which play pivotal roles in the regulation of learning, memory and mood. However, molecular mechanisms that govern the activation and those that maintain quiescence of NPCs remain largely elusive. Previous work from our laboratory have identified and purified hippocampal NPCs using a novel cell-sorting protocol and have examined their molecular identity using RNA-seq. Amongst others, RNA-seq revealed selective and preferential expression of the cholinergic receptor muscarinic 4 (Chrm4) in the hippocampal NPCs. To examine its role in directly regulating NPC activity, we conducted neurosphere assay at clonal density and show that treatment with muscarine, a selective agonist of muscarinic acetylcholine receptors, led to activation of NPCs resulting in over two-fold increase in the number of neurospheres. Using pharmacological agents that selectively stimulate or inhibit chrm4 activity, we further demonstrate chrm4 receptor as the key receptor mediating effects of muscarine. To investigate whether cholinergic signalling regulates NPC activity and neurogenesis *in vivo*, we conducted basal forebrain-selective lesion of cholinergic neurons. We found a significant impairment in the production of new neurons ($BrdU^+DCX^+$ cells) in lesioned compared to control mice. Collectively, these data suggest that cholinergic signalling from the basal forebrain may directly regulate adult hippocampal neurogenesis via chrm4. Given that the hippocampus and basal forebrain are key brain regions implicated in dementia and aging, further evidence supporting the significance of chrm4 may inform development of a novel pharmaceutical approach.

id #9276

Effects of daily low-intensity 10 Hz repetitive transcranial magnetic stimulation on rodent resting-state network: A longitudinal resting-state fMRI study.

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Brain regions with coherent spontaneous fluctuations in activity in a task-free setting form organised networks called the resting-state networks (RSNs). Compared to healthy individuals, people with neuropsychiatric disorders have been identified with RSN dysregulation. Several lines of evidence suggest that RSN plasticity is sensitive to repetitive transcranial magnetic stimulation (rTMS), a novel non-invasive neuromodulation technique. However, the mechanisms underlying its therapeutic effects remain poorly understood. Information about the degree to which RSNs can be modulated by rTMS may prove helpful in the development of treatment options. In this study, we investigated the effects of low-intensity rTMS (LI-rTMS) on rodent RSNs using resting-state functional MRI (rs-fMRI). Daily 10 min sessions of 10 Hz LI-rTMS were delivered over the right brain hemisphere of nine lightly-restrained Sprague-Dawley rats using a circular stimulation coil over a period of two weeks. Weekly rs-fMRI data were acquired at 9.4 Tesla to determine the cumulative effects of LI-rTMS. We also investigated the duration of these effects by performing two imaging sessions seven days and 20 days after stimulation was ceased. We used independent component analysis and a regression approach to uncover changes in the RSNs. LI-rTMS induced a significant increase in functional connectivity following 14 days of stimulation. Functional connectivity decreased seven days after stimulation was ceased and following another 13 days of no stimulation, a further decrease in functional connectivity was noted. Therefore, the long-lasting changes decreased slowly over time, which is consistent with rTMS treatment regimes recommending regular “top up” treatments in patients with depression.

id #9277

Single receptor recordings reveal mechanisms of drug-resistance in glutamate-gated chloride channels of *H. contortus*

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id #9278

Single receptor recordings reveal mechanisms of drug-resistance in glutamate-gated chloride channels of *H. contortus*

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Glutamate-gated chloride channels (GluClRs) are neurotransmitter-gated receptors found at neuronal and neuromuscular inhibitory synapses of invertebrates.

Many invertebrates that are important pest species in agriculture, aquiculture, and in veterinary and human health are becoming resistant to drugs that target their GluClRs, such as ivermectin (IVM). One of these species is the nematode*, H. contortus,* which is an agricultural endoparasite that infects ruminant animals, such as sheep, cattle and goats.

The molecular-level mechanisms that render GluClRs resistant to IVM and similar drugs are unknown.

In this investigation, we used the GluClR of *H. contortus* as a model to explore two possible mechanisms of drug resistance, (a) point mutations to GluClRs and (b) alterations to GluClR subunit composition.

We used single receptor current recordings to show that IVM-resistant receptors are those that activate for brief periods. This fundamental observation applies to mutated and wild-type heteromeric GluClRs, and thus represents a universal mechanism whereby invertebrate pests can become resistant to drugs.

Our recordings also demonstrate that IVM-induced changes to single receptor activations require several minutes to stabilize, suggesting that IVM accumulates in cell membranes. This was confirmed in whole cells using a fluorescent analogue of IVM and microscopy. Overall, the data demonstrate a mechanism of IVM resistance that is determined by the intrinsic activation properties of the receptors, and that the dominant pathway for IVM to GluClR binding sites is via interactions with the cell membrane.

id #9279

Biased allele expression and loss of hypoxia response in a zebrafish model of familial Alzheimer's disease

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Alzheimer's disease (AD) is the most prevalent form of dementia and is characterised by loss of brain energy production (hypometabolism). Age is the main risk factor for AD but the reason for this is unclear. Brain expression of the master transcriptional regulator of hypoxia, HIF1, increases with age and hypoxia appears to coordinate many AD phenomena such as increased oxidative stress and upregulated expression of the genes involved in familial Alzheimer's disease (fAD), leading to increased production of amyloidβ peptide. *PRESENILIN 1* (*PSEN1*) is the major locus for mutations causing fAD and the PSEN1 protein interacts with the HIF1 component, HIF1α. Zebrafish are a versatile model organism for genetic analyses of acute hypoxic responses so we introduced a fAD-like mutation into its *psen1* gene and analysed the effects on HIF1-controlled gene expression with age. We observed age- and hypoxia-dependent bias favouring expression of the fAD-like mutant allele of *psen1*. The fAD-like mutant allele appeared to accelerate brain aging in terms of changes in basal, normoxic, Hif1-responsive gene expression and an eventual shift of brains into an unexpected state where Hif1- responsive genes show "inverted" expression responses to hypoxia. Measurement of allele-specific expression in post-mortem brains of three human *PSEN1* fAD mutation carriers did not show allelic expression bias but revealed greatly reduced expression of both mutant and wild type alleles relative to cognitively normal age-matched controls. Our results are consistent with a view that the pathological brain molecular state of AD is an inevitable, but individualised, consequence of aging.

id #9280

Hypoxia or nicotine- which is worse on the young brain? From neurotransmitters, growth factors, to apoptosis and microglia.

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Introduction and Aim: A respiratory hypoxic environment and cigarette smoke exposure around babies have long lasting effects on the brain such as decreased neuroprotection, decreased IQ, and long-term increased addictive behaviours (cigarette smoke exposure). Extensive studies of the effects of such exposures to the developing brain on the expression of neurotransmitters, receptors, growth factors, markers of apoptosis and microglia in the brain have been undertaken in our laboratory over the past decade and will be presented herein.

Methods: Our two brain tissue datasets are from infants who died suddenly and unexpectedly, and from piglet models of intermittent hypercapnic hypoxia (IHH) and postnatal nicotine exposure. Brain tissue was subjected to immunohistochemistry for apoptotic markers (caspase-3 & TUNEL), NMDA receptor 1, brain derived neurotrophic factor (BDNF) and its receptor TrkB, serotonin receptor 1A (5HT1A), pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor PAC1, orexin, nicotinic acetylcholine receptors (nAChRs) and microglia. Staining was quantified and compared between exposures to control (non-exposures).

Results and Conclusion: Across the studies, the IHH exposure tended to induce greater expressional changes than nicotine, and many changes equated between the piglet models with the infant findings when stratified for hypoxia related conditions of prone sleeping, bedsharing, and cigarette exposure, including the syndrome of Sudden Infant death (SIDS). These changes predominated in the brainstem medulla.

id #9281

Investigating ZBTB18 missense variants in brain development and disease.

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During mammalian foetal development, the activities of DNA-binding transcription factors are critical for the guidance of gene regulatory programmes essential for the expansion and maturation of brain cells. Transcriptional activators such as the proneural bHLH proteins NEUROG2 and NEUROD initiate neuronal differentiation programmes, while the zinc finger repressor ZBTB18 (also known as RP58 or ZNF238) governs the fine-tuning of gene expression in neurons. ZBTB18 is involved in guiding production, maturation, and integration of neurons into the functional circuits of the brain required to establish suitable neuronal function. Disruptions to *ZBTB18* are linked with abnormal brain development and disease in humans. Currently, seven missense variants associated with abnormal brain development have been identified in ZBTB18; with the majority lying within the Zinc Finger domain[1], responsible for DNA-binding. Among a cohort of 60,706 individuals with no dramatic brain developmental phenotypes, 203 missense variants were identified; with the majority of variants occurring outside of the Zinc Finger domain [2]. Here we investigate the impact of ZBTB18 missense variants identified in this cohort of individuals with no dramatic brain developmental phenotypes. We found that several missense variants map to essential residues critical for DNA-binding; with some exhibiting altered transcriptional regulatory activity of ZBTB18, *in vitro*. Our evidence indicates that some missense variants in ZBTB18 have an impact on gene expression regulation during brain development. Thus, it is reasonably anticipated that this work will eventually facilitate more timely diagnosis of genetic risk factors for human brain disorder -where possible allowing for early intervention and treatment.

id #9282

NFIX-mediated inhibition of neuroblast branching regulates migration within the adult mouse ventricular-subventricular zone.

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Understanding the migration of new-born neurons within the brain presents a major challenge in contemporary biology. Neuronal migration is widespread within the within the ventricular-subventricular zone (V-SVZ) and the subgranular zone of dentate gyrus of the adult rodent brain produce neuroblasts that migrate to the regulate key brain functions including innate olfactory responses, learning and memory. Critically, our understanding of the factors mediating neuroblast migration remains limited. The transcription factor Nuclear factor I X (NFIX) has previously been implicated in embryonic cortical development. Here, we employed conditional ablation of Nfix from the adult mouse brain, and demonstrated that the removal of this gene from either neural stem and progenitor cells, or neuroblasts, within the V-SVZ culminated in neuroblast migration defects. Mechanistically, we identified aberrant neuroblast branching, due in part to increased expression of the guanylyl cyclase natriuretic peptide receptor 2 (Npr2), as a factor contributing to abnormal migration in Nfix-deficient adult mice. Collectively, these data provide new insights into how neuroblast migration is regulated at a transcriptional level within the adult brain.

id #9284

Lithium reverses mechanical allodynia through a mu opioid receptor-dependent mechanism

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Lithium is used to treat bipolar disorders and painful cluster headache. Several research groups reported functional interactions between lithium and the opioid system. In particular, lithium affects morphine-induced analgesia and reduces morphine tolerance and dependence. In addition, lithium attenuates thermal hyperalgesia and mechanical allodynia in neuropathic rats via a naloxone-sensitive mechanism, suggesting a mu opioid receptor (MOR)-dependent effect.

By using a combination of genetic, pharmacological, behavioural and biochemical tools, we investigated the link between lithium analgesia and the endogenous opioid system in the mouse cuff model of neuropathic pain.

We show that acute injection of lithium alleviates neuropathic pain in a mouse model of sciatic nerve chronic constriction and demonstrate that the MOR is necessary for lithium analgesia. This confirms and extends data reported by other groups on rat neuropathic models. Notably, our results suggest that lithium analgesia involves the upregulation of beta-endorphin synthesis in the CNS. This would explain, at least in part, the MOR-dependent nature of the analgesic properties of lithium.

Reference: Weinsanto I & al. Lithium reverses mechanical allodynia through a mu opioid-dependent mechanism. Mol Pain. 2018 Jan-Dec;14:1744806917754142. doi:10.1177/1744806917754142. Epub 2018 Jan 21. PubMed PMID: 29353538

id #9285

Improving recovery after stroke through stabilisation of astrocytes to retain the neurovascular unit and reduce glial scar expansion into the non-damaged brain.

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A major challenge for recovery after stroke lies in overcoming the endogenous response to injury, where changes within the neurovascular unit affect even the non-damaged brain[1]. To circumvent this, we investigated the effects of delayed Rho/kinase inhibition after stroke on astrocyte reactivity, neurovascular uncoupling, and protein signalling relative to functional recovery. Following middle cerebral artery stroke in rats (Endothlin-1, 60pmol) separate groups (n=9/group) received either the ROCK inhibitor Fasudil (10mg/kg i.p. daily) or vehicle (saline) 3 days post-stroke and recovered to 28 days for histology. A smaller cohort (n=5/group) were recovered to 14 days for Mass Spectrometry using stable isotope dimethyl labelling. Functional outcomes were assessed in all groups using a neurological deficit score and cylinder test. Fasudil significantly reduced reactive astrocyte transition and glial scar expansion into the non-damaged brain, retained astrocyte coupling with blood vessels and recovered lost motor functions after stroke. Over 1800 common proteins were detected in all samples (Sham/Vehicle/Fasudil) and Heat Map analysis showed that Fasudil differentially altered proteins that were regulated in response to stroke, including proteins associated with astrogliosis, oxidative stress, actin cytoskeleton organization, cellular metabolism and synaptic transmission. Using Ingenuity Pathway Analysis (IPA) we identified 2 common signalling networks that were each linked to 5 astrocytic proteins that were improved by Fasudil: Tuberous Sclerosis 2 (TSC2) and Activating transcription factor 3 (ATF3). Collectively, these results show that astrocyte transition after stroke can be contained to support recovery, and novel signalling networks associated with this effect provide new targets for therapeutic intervention.

1. 1. Abeysinghe HCS, Phillips EL, Cheng HC, Beart PM, Roulston CL (2016) Modulating Astrocyte Transition after Stroke to Promote Brain Rescue and Functional Recovery: Emerging Targets Include Rho Kinase. International Journal of Molecular Sciences 17:288-305.

id #9286

Stable Isotope-Labelled Morphine to Study *in vivo* Central and Peripheral Morphine Glucuronidation and Brain Transport in Tolerant Mice

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Chronic treatments can have an important impact on metabolic enzymes leading to physiological adaptations. Morphine metabolism in the liver has been extensively studied but morphine metabolic processes in the central nervous system are poorly characterized. Long-term morphine treatment is limited by the development of tolerance, resulting in a decrease of its analgesic effect. Whether or not morphine analgesic tolerance affects *in vivo* brain morphine metabolism and blood-brain barrier (BBB) permeability remains a pending question.

Our aim was to characterize the *in vivo* metabolism and BBB permeability of morphine after long-term treatment at both central and peripheral levels. Mice were injected with morphine or saline solution for 8 consecutive days to induce morphine analgesic tolerance. On the ninth day, both groups received a final injection of d3-morphine (morphine bearing three ^{2}H). LC-MS/MS was used to quantify morphine, its metabolite morphine-3-glucuronide (M3G) and their respective d3-labelled counterparts in blood, urine, brain and liver samples.

We found no significant differences in morphine CNS uptake and metabolism between control and tolerant mice. This suggests that morphine analgesic tolerance is not linked to an increase of morphine glucuronidation into M3G or an alteration of BBB permeability.

Reference : Weinsanto I & al. Stable Isotope-Labelled Morphine to Study in vivo Central and Peripheral Morphine Glucuronidation and Brain Transport in Tolerant Mice. Br J Pharmacol. 2018 Jul 26. doi: 10.1111/bph.14454.

id #9288

Revisiting Serotonin Signalling in Axon Guidance

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Axon guidance is crucial to the development of the neuronal circuitry of the brain. Axon guidance is the process by which the leading tip of an axon, the growth cone, navigates the complex environment of the developing brain to form accurate connections with its target cell. Understanding the molecules that control growth cone motility and the molecular mechanisms that enable neurons to form precise connections is essential to prevent the onset of neurological defects. Although dysregulation of the serotonin (5-HT) signalling pathway has been implicated in many neurodevelopmental disorders, the exact role of serotonin in axon guidance is unclear. Evidence from invertebrates suggests that serotonin might regulate growth cone behaviour. However, whether this is true in vertebrate neurons has not been demonstrated. We therefore sought to elucidate the role of serotonin in instructing growth cones of embryonic (E16.5-E18.5) rat dorsal root ganglion neurons. We used the growth cone turning assay and pharmacology to decipher the signalling pathways by which serotonin regulates growth cone motility. Our data reveal that serotonin can cause attraction and repulsion of growth cones, and this is a dose dependent phenomenon. We have demonstrated that activation of the 5-HT2 receptor promotes growth cone attraction (+7.25° ± 1.076, n=43) whereas activation of the 5-HT1 receptor elicits repulsion (-8.28° ± 1.572, n=20). We confirm that serotonin-mediated growth cone attraction is calcium-dependent and rely on calcium signals from intracellular stores. These data now allow us to further study the role of serotonin in axon guidance and circuit development in vivo.

id #9289

Reducing excessive D2R-DISC1 complex formation prevents synaptic spine lesion through NPY system in striatal neurons

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Background: The excessive formation of dopamine D2 receptor (D2R)-DISC1 complex induced by D2R hyperactivity contributes to psychotic-symptoms. Psychosis has been considered as a disorder of disconnection resulted from impaired neuronal communication. Previous studies suggest that Neuropeptide Y (NPY) is involved in the action of antipsychotic effects, which protects neuronal connection in the striatum. However, the mechanism is still largely unknown. This study aims to investigate that the excessive D2R-DISC1 complex formation influences neurite growth and synaptic connectivity through NPY system in striatal neurons.

Methods: (1) Primary striatal neurons from postnatal 0 to 3 days of mice were cultured; (2) After 7 days of culturing, striatal neurons were treated with either D2R agonist (quinpirole) or quinpirole and D2R-DISC1 interfering peptide; (3) Fluorescence resonance energy transfer (FRET) was applied to examine D2R-DISC1 complex formation; (4) Western blot was used to quantify protein expression.

Results: This study showed that (1) Hyper-activation of D2R caused neurite impairment and decreased synaptic spine density in striatal neurons; (2) D2R-DISC1 interfering peptide protected neurite growth and synaptic spine density through upregulating synaptic proteins and interneuron NPY expression; (3) D2R was found on the mitochondria of striatal neurons.

Conclusions: The D2R-DISC1 complex formation induced by quinpirole decreased interneuron NPY expression and neurite length of striatal neurons. D2R-DISC1 interfering peptide protected neuronal morphology and synaptic connection via NPY system. Thus, the D2R-DISC1 complex might be a novel therapeutic target for treating neurite deficits in patients suffering psychosis.

id #9290

Platelets and platelet derived microparticles express P2X7 and activation of this receptor releases free mitochondria

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The acute inflammatory lesion of relapsing multiple sclerosis (MS) is associated with an increased platelet-derived CD41+/CD62P+ microparticles count in the circulation of patients compared to controls. In various infective and autoimmune conditions, these platelet microparticles contain proinflammatory mediators but the stimulus for MP release is unclear. We demonstrated that platelets and microparticles expressed the purinergic receptor P2X7 by flow cytometry, which was confirmed by Western blotting analysis and cryo-electron microscopy (cryo-EM). We then studied the release of microparticles from gel-filtered platelets incubated in Tyrodes medium with agonists for the P2X7 receptor (ATP, and BzATP), the P2Y1 agonist (ADP and MRS2365), and thrombin. All agonists released large numbers of microparticles in a rank order thrombin > P2Y1 > P2X7. However P2X7 agonists released a subpopulation of microparticles in the large size range 200-600nm and which accumulated MitoTracker dye specific for mitochondria. Further, Cryo-EM revealed that BzATP /ATP treated platelets released free mitochondria. In contrast, thrombin-treated platelets released fewer mitochondria, most of which were enclosed in a vesicular membrane, while P2Y1 agonists released almost no free mitochondria. Agonists for P2X7 also released free mitochondria from platelets suspended in plasma (platelet rich plasma) and flow cytometry identified numbers of these organelles between 0.13 and 3.8×10^6/mL plasma in normal donors.

Conclusion: Platelets and microparticles express P2X7 and activation of this receptor releases plasma microparticles, a subpopulation of which are free mitochondria. Free mitochondria in the circulation of MS patients may exert inflammatory effects on vascular endothelium and permeabilise the blood brain barrier.

id #9291

Excessive Sugar Consumption Changes Neural Circuitry and Function. Implications for Addiction and Obesity.

Selena Bartlett, Kate Beecher, Masroor Shariff, Syed Ali, Arnauld Belmer

A compelling body of evidence suggests that excessive sugar consumption plays a role in the worldwide obesity epidemic, typified by the modern western diet. Our lab recently demonstrated that excess sugar consumption leads to maladaptive changes in the mesolimbic reward pathway of the brain. In a series of studies, we found that long-term sucrose consumption changes the brain circuitry and activity in a manner that mimics the changes we found following long-term ethanol consumption. For example, changes in the expression of nicotinic receptors, reductions in consumption using nicotinic receptor compounds, decreased total dendritic length of Nucleus accumbens (NAc) shell of medium spiny neurons (MSNs). We also found that the restructuring of these neurons resulted primarily from reduced distal dendritic complexity. Conversely, we observed increased spine densities at the distal branch orders of NAc shell MSNs from long-term sucrose consuming rats. More recently, we have shown that long-term ethanol consumption elicits profound deficits in neurogenesis and neuronal fate specification in the dorsal hippocampus that are entirely reversed by a 2-week chronic treatment with the 5-HT_{1A} partial agonist tandospirone (3 mg/kg/day). We have begun to examine whether sucrose consumption leads to similar deficits in neurogenesis. It is clear that certain types of sugar/high fat foods beverages have a profound influence on adult brain function and development.

1. Belmer A, Patkar OL, Lanoue V, Bartlett SE. (2018) 5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol. Sci Rep. 2018 Feb 1;8(1):2099. doi: 10.1038/s41598-018-20504-z.

id #9292

Synapses under the nanoscope

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A number of new developments in single molecule imaging has allowed us to image and track individual synaptic vesicles within the confinement of the presynapse. Moreover, we have also designed new ways of imaging molecules involved in vesicular exocytosis in live neurons and within live synapses of small organisms. In this talk I will be presenting our latest results in the nanoscale imaging of the functioning synapse.

id #9294

The Marmoset Brain Architecture Project: An open-access resource for visualisation and analysis of cortico-cortical connections

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Understanding the principles of connectivity in the cerebral cortex will enable informed models of the neural processes underlying perception, action and cognition. Here we describe a web-implemented public resource which gives access to the world's most comprehensive data on the cortico-cortical connectivity of any primate species. The data are presented in formats which enable either area-based or parcellation-free analyses.

The connectome is based on the results of over 140 monosynaptic retrograde tracer injections into marmoset cerebral cortex, available on the http://marmosetbrain.org website. The results from multiple animals were mapped into a 3d template based on the stereotaxic atlas of Paxinos et al., (2012), using a computerised registration procedure that is guided by expert delineation of cortical area boundaries.

The web site allows one to establish a spatial location of each labelled cell to a specific cortical area (Majka et al., 2016, J Comp Neurol 524:2161-2181), and to determine its location relative to the granular cell layer of the cortex. To make the connectome readily accessible for exploration and visualization, an online analytical framework was developed (http://analysis.marmosetbrain.org) which enables a graphical (web browser-based) or a programmatic access to the data. All data are licensed under a Creative Commons Attribution-ShareAlike 4.0License.

The availability of this resource the scientific community will likely provide a fertile ground for studies involving modelling and simulation of neural interactions in the cerebral cortex. Further work is necessary to include connections with other structures, such as the thalamus and claustrum.

id #9295

P75 NEUROTROPHIN RECEPTOR FUNCTION IN CORTICAL NEUROGENESIS

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During development, the p75 neurotrophin receptor ($p75^{NTR}$) is widely expressed in the nervous system where it regulates neuronal differentiation, migration and axonal outgrowth, and mediates the survival and death of newly born neurons. Activation of $p75^{NTR}$ by neurotrophin binding and its association with co-receptors can induce a variety of different downstream signalling pathways with functional outcomes being dependent on both timing and cellular context. To date, most studies investigating $p75^{NTR}$ function during development have focussed on the peripheral nervous system leaving its function in the developing brain largely unexplored. Here we show using MRI imaging and histological techniques that knockout of $p75^{NTR}$ in neural progenitors in a conditional *Nestin-Cre; $p75^{NTR}$ flox/flox* mouse results in reduced cortical thickness, and volume of the basal forebrain and striatum. Furthermore, there were significantly fewer cortical interneurons and cholinergic basal forebrain neurons following loss of $p75^{NTR}$ expression at embryonic day 10. This coincided with apoptosis of dividing pallidal progenitors during embryogenesis suggesting that $p75^{NTR}$ functions in the survival of these cells. Consistently, conditional knockout of $p75^{NTR}$ in the medial ganglionic eminences, the birthplace of the majority of cortical GABAergic interneurons, resulted in a significant reduction of parvalbumin-expressing interneurons in the adult animal and a reduction in thickness of deeper cortical layers. These results suggest that $p75^{NTR}$ is required for the generation of interneurons during early neurogenesis and regulates both cell-autonomous and non-autonomous aspects of cortical development.

id #9296

Meth

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id #9297

EFFECT OF AGEING ON H-REFLEX RESPONSE TO FATIGUE

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Injury as a result of falling is relatively common among older people. If the injury is severe, it can lead to complications resulting in morbidity, hospitalization and institutionalization. The risk of falling increases with fatigue and of particular importance is the ability to dorsiflex the foot through timely activation of the tibialis anterior (TA) muscle to ensure the foot clears the ground, or an obstacle, during the swing phase of walking. In this study we used electrical stimulation of the common peroneal nerve to assess the M-wave and H-reflex before, immediately after and four and eight minutes after a fatiguing maximal isometric contraction while subjects lay on a plinth in the prone position and maintained an isometric contraction of 10% MVC. We found that synaptic efficacy of muscle spindle primary endings in TA change significantly during fatigue. The main contributor to the tonicity of TA muscle, i.e., excitatory synapses of spindle primary endings on motoneurons that innervate TA muscle, lose their efficacy during fatigue only in the older individuals. Since TA muscle is the main dorsiflexor of the foot and that it needs to be active during the swing phase of stepping to prevent tripping, older individuals are more susceptible to falling especially when their muscles are fatigued.

id #9298

Neurite repair for severe mental illness

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Neurites are projections from the cell body of neurons, which allow neurons to connect to each other. Neurite deficits impair neural connectivity and alter brain function. Dopamine D2 receptor and NMDAR-NR2B (D2R/NR2B) regulate neurites, synaptogenesis and connectivity and are also major antipsychotic drug targets. The following methods were used in this project. 1) cell cultures; 2) Fluorescence Resonance Energy Transfer to examine signalling molecular interaction; 3) siRNA down regulation of targeted gene expression; 4) Nrg1-TM +/-, DISC1-FI, PCP mouse models; 5) clinical trials in 1st episode schizophrenia patients. Results are: 1) D2R and DISC1 interaction regulates neurite outgrowth which responds to anti-psychotic drug treatment; 2) D2R and NR2B show a direct reciprocal regulatory mechanism; 3) 3-D cell culture showed that Nrg1-FI induced neurite lesion could be repaired by electrical stimulation; 4) neural adhesion molecule-PSA responsible for neural plasticity is significantly reduced in first episode schizophrenia patients; 5) identification of altered microbiome species in the first episode schizophrenia patients. From above studies, we conclude that D2R plays important role in the regulation of neural connectivity and plasticity. D2R directly interacts with DISC1 at a specific site. When there was D2R hyperactivity, D2R-DISC1 forms dimerization disrupting intracellular molecular trafficking and causing neurite withdraw. When D2R is down regulated by siRNA, NR2B specific phosphorylation sites were activated. Altered gut microbiota in patients can lead to their metabolites changes including short chain fatty acids. A supplementation of specific short chain fatty acid is capable of repair neurites in vitro.

id #9299

Myelin and nodal plasticity synchronises action potential conduction in the adult mouse brain

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Changes in brain activity, brought about through learning, novel sensory experiences or direct neuronal stimulation, increases oligodendrocyte addition and myelination within the activated regions. Yet, how adaptive myelination in adulthood influences action potential conduction is unclear. To investigate this, adult (P83) *Plp-CreER^{T2} :: Tau$_m$GFP* transgenic mice received tamoxifen to fluorescently label mature oligodendrocytes. From P90, neuronal activity was increased by delivering 3 minutes of non-invasive, low-intensity repetitive transcranial magnetic stimulation (rTMS; 120mT rodent coil) for 14 consecutive days, with control mice receiving sham-stimulation. While, the gross myelinating morphology of pre-existing oligodendrocytes (*Plp-CreER^{T2} :: Tau$_m$GFP*) was unaltered by rTMS treatment, the average length of nodes of Ranvier was reduced by ~30% in the cortex, and by ~10% in the CC. This narrowing of the nodes appears to be the result of altered myelin ultrastructure, as TEM analysis revealed a ~6% reduction in the average g-ratio of axons within the CC after rTMS. Furthermore, the myelin and nodal changes induced by rTMS were functionally associated with a ~20% reduction in the average conduction velocity of the compound action potential (CAP) and a ~30% increase in the amplitude of the myelinated axon peak. These data are the first to demonstrate that increased neuronal activity is detected by already mature oligodendrocytes, that then respond by subtly altering internode ultrastructure, to promote action potential synchrony, for neurons that are trained to fire in response to a common stimulus.

id #9300

TYRO3 IS A KEY REGULATOR OF MYELIN THICKNESS IN THE CENTRAL NERVOUS SYSTEM

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The disease multiple sclerosis involves an autoimmune attack on myelin sheaths within the central nervous system (CNS). Major physical deficits arise in MS patients due to an inability to repair damaged myelin following a demyelinating event, leaving axons exposed to neurodegeneration. Tyro3, Axl and Mertk (TAM) are a family of tyrosine kinase receptors which have been implicated in demyelination, remyelination and MS susceptibility. We have previously found that the Tyro3 receptor is involved in developmental myelination and the regulation of myelin thickness, at least in the optic nerve and rostral region of the corpus callosum (CC) of adult mice. This study verifies and extends on these previous findings via a comprehensive analysis of axonal ensheathment and myelin thickness in the CC of unchallenged mice, during demyelination and during remyelination. We show that the absence of Tyro3 correlates to significantly thinner myelin sheath compared with wild-type controls in both unchallenged mice and during remyelination, particularly in larger caliber axons. The hypomyelinated phenotype observed in the Tyro3 deficient mice occurs in the absence of an influence upon OPC maturation, or the density of oligodendrocytes and microglia, suggesting the major effect of the Tyro3 receptor on myelination is upon myelin radial expansion rather than the regulation of glial populations. Finally, we demonstrate that the loss of Tyro3 results in fewer myelin lamellae which ensheath CNS axons, suggesting that Tyro3 is a fundamental component of the machinery which correlates myelin deposition to axonal diameter.

id #9301

Using EEG and MRI at Term Equivalent Age in Very Preterm Infants to Predict Visual and Cognitive Outcome

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Very preterm infants (VPTs; ≤30 weeks gestational age) are at risk of neurodevelopmental deficits including cognitive impairments and blindness. However, early and accurate prognostication is challenging. This study investigated whether quantitative electroencephalography (qEEG) and/or diffusion magnetic resonance imaging (dMRI) measures at term equivalent age (TEA) could predict visual function at 3 months corrected age (CA) and cognitive function at 12 months CA.

EEG and MRI were recorded at TEA for 79 VPTs without any major congenital or chromosomal abnormalities. qEEG measures (relative power and partial directed coherence [PDC]) were computed for delta (0.5-2Hz), theta (2-6 Hz), alpha (6-13 Hz), and beta (13-30 Hz) frequency bands at five parietal-occipital and seven fronto-central electrodes. dMRI measures (fractional anisotropy [FA] and mean diffusivity [MD]) of optic radiation and corpus callosum were computed. Visual function was assessed at 3 months CA using Neonatal Visual Assessment (Ricci). Cognitive function was assessed at 12 months CA using Bayley-III. Linear regression was used to determine whether qEEG and/or dMRI measures could predict visual and cognitive scores respectively.

Visual scores were significantly associated with relative delta power (negative), relative alpha and beta power (positive) and mean PDC in all four frequency bands (positive). Furthermore, dMRI measures were significantly associated with cognitive scores, especially mean FA (positive) and mean MD (negative).

These results suggest that in VPTs at TEA, qEEG and dMRI measures may inform visual function at 3 months old and cognitive function at 12 months old respectively. These measures could potentially help identify at-risk preterm infants.

id #9302

Heterozygosity for *NFIX* in mice models features of Malan syndrome

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Objective: To investigate the cause of macrocephaly in *Nuclear Factor One X* (*NFIX*) haploinsufficient Malan syndrome patients, as well as deficits in cortically-controlled behavior underlying intellectual disability displayed by these patients, using a rodent model system, the *Nfix* heterozygous mouse line.

Methods: Adult mice lacking one allele of *Nfix* (*Nfix*$^{+/-}$) and wildtype controls were used in this study. Volumetric magnetic resonance imaging (MRI) of *Nfix*$^{+/-}$ and wildtype mouse brains was used to calculate the volumes of 20 brain sub regions. Diffusion tensor MRI was used to perform tractography-based analyses of the corpus callosum, hippocampal commissure, and anterior commissure, as well as structural connectome mapping of the whole brain. Immunohistochemistry examined the neocortical cellular populations. Two behavioral assays were performed, including the active place avoidance task to assess spatial navigation and learning and memory function, and the 3-chambered sociability task to examine social behaviour.

Results: Adult *Nfix*$^{+/-}$ mice exhibit significantly increased brain volume (megalencephaly) compared to wildtypes, with the cerebral cortex showing the highest increase. Moreover, all three forebrain commissures, in particular the anterior commissure, revealed significantly reduced fractional anisotropy, axial and radial diffusivity, and tract density intensity. Structural connectome analyses revealed aberrant connectivity between many crucial brain regions. Finally, *Nfix*$^{+/-}$ mice exhibit behavioral deficits that model intellectual disability.

Conclusion: Collectively, these data provide a significant conceptual advance in our understanding of Malan syndrome by suggesting that megalencephaly underlies the macrocephalic phenotype of these patients, and that disrupted cortical connectivity may contribute to intellectual disability.

id #9303

Differential effects of chronic 17β-estradiol treatment on behaviours relevant to depression

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Background: Neurobiological sex differences play an important role in the pathophysiology of psychiatric disorders, such as major depressive disorder which affects women at a higher incidence than men. Research indicates that the most potent endogenous estrogen, 17β-estradiol, may have therapeutic potential in treating depression. This study aimed to investigate the effects of chronic 17β-estradiol on behaviours relevant to depression.

Methods: This study used adult female Sprague-Dawley rats that were ovariectomised and treated with chronic 17β-estradiol or vehicle, via a subcutaneous silastic implant. Rats were assessed in the forced swim test, saccharin preference test, novel object recognition memory test and for possible confounding behaviours, including locomotion and anxiety (open field test) and motivation and anxiety (novelty supressed feeding test).

Results: Treatment effects were verified using body and uterus weight, as well as serum concentrations of 17β-estradiol, progesterone and testosterone. Compared to ovariectomised rats, chronic 17β-estradiol treatment enhanced saccharin preference and novel object recognition performance, with results unlikely to be due to group differences in confounding behaviours. There were no group differences in passive or active coping behaviour when assayed using the forced swim test.

Conclusions: Taken together, these results support an antidepressant-like action of estrogens but highlight that the beneficial effects of chronic 17β-estradiol treatment are on specific depression-related symptoms, particularly anhedonia and memory.

id #9304

Deletion Of Trkb Neurotrophin Receptor In Neurons Leads To Functional Deficits After Myelin Injury In The CNS

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Despite the important roles of neurons in regulating normal CNS myelination, an intriguing but yet proved question is whether neuronal signals regulate CNS myelin repair and functional recovery after a demyelinating insult. To address this, we specifically deleted the BDNF receptor TrkB(tropomyosin receptor kinaseB) in adult cortical neurons via generating an inducible neuronal-specific TrkB conditional knockout mice(TrkB icKO, TrkB^{flfl}:Thy1-EGFP-CreER^{T2}). Brain demyelination is induced in the TrkB mutant mice using a murine cuprizone model of demyelination.

To investigate any functional change following neuronal TrkB deletion and in response to myelin injury, ledged beam test was carried out. We found that TrkB icKO mice displayed clear motor deficits as evident by significantly more foot slips compared to oil controls at both 4($p=0.0006$) and 5($p<0.0001$)weeks after deletion of neuronal TrkB.

To induce central demyelination, cuprizone-supplemented diet was administered to both TrkB icKO and oil control groups. We found that, during 1week recovery following a withdrawal of cuprizone diet, TrkBicKO mice showed significantly worse($p=0.0478$) motor performance compared to the

oil controls.

We are currently examining the extent of myelin pathology of those mice to determine if TrkB expression in adult neurons regulates the innate capacity of remyelination and whether this correlates with the aforementioned functional changes. Together, our current data suggest that removal of TrkB from neurons result in functional deficit and neuronal TrkB is potentially required for functional recovery after myelin injury, proposing a possibility that neurons could also be considered as therapeutic targets when searching for myelin repair strategies.

id #9305

Exposure to e-cigarette aerosols results in neurological changes in both *in vivo* and *in vitro* models

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Electronic cigarettes (e-cigarettes) are a relatively new phenomenon that are becoming increasingly popular in young people. There is a perception in pregnant women that e-cigarettes are a safer alternative to smoking tobacco cigarettes. However, that does not mean that e-cigarettes are completely safe. Little is known about how maternal exposure to e-cigarette aerosols affects the neurological development of offspring *in utero*.

The offspring from mice mothers exposed to e-cigarette aerosols were tested for cognitive changes (NOR and EPM test)and brains were examined for epigenetic changes (PCR array). Now, we have examined the effects of the tobacco flavoured e-liquid with and without 18mg nicotine on human neurons (SHSY5Y), murine microglia (BV2) as well as a neuron (SHSY5Y)/endothelial (HBEC) cell co-culture system. The aerosols were created using an e-cigarette device and the condensate was collected and added to the culture medium. The cells were tested for survival (MTT assay), mitochondrial function (JC10 assay) and the integrity and permeability of the HBEC monolayer in the co-culture model (FITC-Dextran and TEER).

The *in vivo* results showed significant changes in the offspring for short-term memory, hyperactivity, anxiety-like behaviour and epigenetic genes. In addition, it was found that the flavouring alone, and with nicotine had strong but differing effects at the cellular level. This indicates that maternal vaping should not be considered completely safe but the specific mechanisms have yet to be explained.

id #9306

DCC SIGNALING INITIATES FORMATION OF A GLIAL SUBSTRATE FOR CORPUS CALLOSUM DEVELOPMENT

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The corpus callosum is a large nerve tract that forms between the brain hemispheres and mediates interhemispheric communication. During brain development, axons of the corpus callosum require a substrate to cross the telencephalic midline. We recently demonstrated that midline zipper glia remodel the interhemispheric fissure, which initially separates the two brain hemispheres, to form a bridging substrate for callosal axon growth. Defects in interhemispheric remodelling are associated with human congenital absence of the corpus callosum, but few molecular cues have been identified that govern this process. Mutations in the transmembrane receptor, *Deleted in colorectal cancer* (*DCC*), cause corpus callosum agenesis in humans and mice. We, therefore, investigated whether DCC signalling is crucial for interhemispheric fissure remodelling. In mice we show that DCC and its ligands, NTN1 and DRAXIN, are expressed in midline zipper glia during interhemispheric remodelling. In *Dcc* or *Ntn1* knockout mice, midline zipper glia display aberrant morphologies and fail to migrate to the pial surface of the interhemispheric fissure or initiate interhemispheric fissure remodelling. Next, we assayed the biological performance of *DCC* mutations, which are found in humans with callosal agenesis. We found that all mutations studied were unable to mimic the wildtype receptor in eliciting changes in cell morphology and are, therefore, loss-of-function mutations. Collectively, our results suggest that genes involved in DCC signalling which regulate astroglial morphology or migration may be important for corpus callosum development, and mutations in these genes are likely to underlie congenital absence of the corpus callosum in human individuals.

id #9307

The beneficial effects of highly bioavailable curcumin preparations on chronic neuroinflammation in the rodent brain

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Objective: Chronic neuroinflammation is a promising therapeutic target in many neurodegenerative and age-related brain disorders. In this study, we investigated two highly bioavailable curcumin preparations, Longvida (LC) and Meriva (MC) for their anti-inflammatory properties, using the GFAP-IL6 mouse model. We hypothesized that the highly bioavailable curcumin, as a potent cytokine-suppressive anti-inflammatory drug, will decrease microglial and astroglial activation, both in terms of cell numbers and morphology.

Key methods: Both GFAP-IL6 and wild type mice were fed with LC and MC for 1 and 6 months, respectively. Brains were collected and processed for immunohistological staining for microglia (Iba-1 and TSPO) and astrocytes, followed by stereological and morphological analysis of the treated and control groups.

Findings: Short-term oral administration of MC leads to a dose-dependent reduction in neuroinflammatory markers in the cerebellum and the hippocampus. MC not only decreased the number of activated microglia and astroglia but also significantly changed the microglial morphology from activated to resting state. Long-term LC treatment also led to a significant decrease in the number of microglia in the hippocampus and cerebellum. However, long term feeding of LC did not result in significantly higher cell number reduction.

Conclusion: Both short- and long-term consumption of modified curcumin preparations significantly downregulated microglial activation GFAP-IL6 mice in the cerebellum and the hippocampus. Our results promising potential therapeutic applications of both Longvida and Meriva curcumin against brain inflammation, potentially reducing or even preventing chronic neuroinflammation and consequentially neurodegenerative diseases.

id #9308

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

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

id #9309

Mechanisms underlying the anterior expansion of the central nervous system

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The central nervous system (CNS) is a defining feature of bilaterally symmetric animals (bilateria), and can be grossly sub-divided into the brain and the nerve cord. A striking feature of the CNS is the anterior expansion of the brain relative to the nerve cord. This feature is evolutionarily conserved; evident in annelids, early arthropods and chordates, and becoming increasingly pronounced in vertebrates to reach its zenith in mammals, with the dramatic expansion of the telencephalon. However, the driving forces underlying this size-difference are not well understood. We are addressing this expansion in both *Drosophila* and mouse. We find that the brain, in both *Drosophila* and mouse, displays several distinguishing features that contribute to anterior CNS expansion. These include extended progenitor proliferation, more elaborate daughter cell proliferation and more rapid cell cycles. With regards to the genetic control of these features, enhanced brain proliferation is severely reduced by ectopic Hox gene expression, by either Hox misexpression or by loss of Polycomb Group (PcG) function. Interestingly, in PcG mutants, early CNS proliferation appears unaffected, whereas subsequently, brain proliferation is severely reduced. Hence, a conserved PcG-Hox program promotes the anterior expansion of the CNS. In addition, the profound differences in proliferation and in the underlying genetic mechanisms between brain and nerve cord lend support to the emerging concept of separate evolutionary origins of these two CNS regions.

id #9310

Radical revision of brain stem nomenclature

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Almost all current neuroanatomy textbooks have failed to recognize the impact of the findings of developmental gene expression on brain subdivision. For example, gene expression studies have revealed the details of the segmental nature of the hind brain (the isthmus plus 11 rhombomeres), but these discoveries have been ignored by textbooks. In the brain stem the most common mistakes are the incorrect placement of the pretectal area and the isthmus in the midbrain, and exaggeration of the size of the pons. The pretectal area, which contains the posterior commissure, has been shown to be the caudal segment of the diencephalon. The isthmus, containing the trochlear nucleus, does not belong to the midbrain, but instead forms the first segment of the hindbrain. The mushroom-like expansion of the human pons has tricked anatomists in to thinking that the pons extends from the midbrain to the inferior olive, whereas the pontine nuclei are found only in rhombomeres 3 and 4 in all mammals. It is time for a complete overhaul of brain stem nomenclature based on the reality of its segmental organisation.

id #9311

18-kDa translocator protein radioligand [^{18}F]GE-180 as a neuroinflammatory biomarker following spinal cord injury

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Currently, prognosis of spinal cord injury (SCI) in humans is based upon standardised physical examination of motor and sensory function, and magnetic resonance imaging. More recently, advanced imaging, particularly positron emission tomography (PET) has emerged as a new method for assessing metabolic and inflammatory biomarkers in common CNS disorders. PET techniques allow radioligands to track biochemical and physiological responses at the molecular level, and form the basis of new tools for the quantitative assessment of cell viability and neuroinflammation

in these contexts. The most extensively investigated and characterised biomarker for *in vivo* imaging of inflammation is the 18-kDa translocator protein (TSPO). [^{18}F]GE-180 is a third generation TSPO tracer which has been shown to bind selectively and with high affinity to TSPO expressed by activated glia. The present study investigates whether PET imaging of [^{18}F]GE-180 / TSPO binding affinity discriminates the time course of innate and adaptive inflammatory responses in a clinically relevant model of SCI. Sprague Dawley rats (♀; 200-300g) were subjected to a moderate-severe (200kD) contusive cord injury (T10) and Acute (24h, 48h), Sub-acute (Days 7, 14), and Intermediate (Days 21, 28) time points were assessed *in vivo* by dynamic PET imaging of [^{18}F]GE-180 tracer at the site of injury. Assessment up to and including 21 days post-surgery revealed significant increases in TSPO expression (kBq/cc) at the injury site in SCI animals compared to non-injured controls (P<0.05). PET imaging of the third generation TSPO ligand [^{18}F]GE-180 allows for an accurate, minimally non-invasive assessment of the neuroinflammatory following SCI.

id #9312

Maternal Vitamin D-deficiency and the epigenetic regulation of brain development

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Epigenetic events are critical for normal brain development. DNA and histone methylation dysregulation in the frontal cortex has been associated with autism[1]. We have shown that the absence of vitamin D in gestation impairs various aspects of brain development in rodents and increases the risk of autism in children[2]. Considering the substantial sex-dependent effects on the prevalence of autism the aim of this study was to identify the epigenetic effects of vitamin D deficiency on males and females, at different developmental stages.

To achieve this, we used a developmental vitamin D (DVD)-deficiency rat model. Using quantitative PCR we analysed the expression of several genes that code for epigenetic enzymes. Moreover, the expression of epigenetically modulated genes related to sex-specific behaviours, estrogen receptor alpha (ESR1) and estrogen receptor beta (ESR2) were assessed.

Our results show that DVD-deficiency alters expression of epigenetic modifiers such as JMJD3, DNMT3a, TET1 and EZH2 in a sex- and time-specific manner. In the frontal cortex at post-natal day 2 DVD-deficient males showed increased ESR2 expression.

These results suggest that DVD-deficiency may modulate estrogen signalling in the frontal cortex via epigenetic mechanisms which are age- and sex-specific. Especially, ESR2 is involved in the migration of cortical neurons in the developing brain[3]. Coupled with results from our group that show DVD-deficiency decreases aromatase expression and increases testosterone in perinatal male brain, these findings have implications for understanding the relationship between gestational vitamin D, brain sexual differentiation and autism.

1. Eshraghi, A.A., et al., Epigenetics and Autism Spectrum Disorder: Is There a Correlation? Front Cell Neurosci, 2018. 12: p. 78.
2. Vinkhuyzen, A.A.E., et al., Gestational vitamin D deficiency and autism-related traits: the Generation R Study. Mol Psychiatry, 2018. 23(2): p. 240-246.
3. Wang, L., et al., Estrogen receptor (ER)beta knockout mice reveal a role for ERbeta in migration of cortical neurons in the developing brain. Proc Natl Acad Sci U S A, 2003. 100(2): p. 703-8.

id #9313

Exosomal transmission of α-synuclein results in Parkinson's disease-like pathology and movement deficits in vivo.

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Recent evidence implicates the transmission of α-synuclein in the brain as being involved in the pathogenesis of Parkinson's disease (PD). However, little is known about the initial cellular events that result in the propagation of pathology associated with PD. Here we have identified a mechanistic pathway for the loading of α-synuclein into exosomes and observed the transmission of α-synuclein in both in vitro and in vivo models. To investigate the ability of exosomes containing α-synuclein to transmit disease we administered exosomes via nasal delivery, a system we have previously identified to deliver functional exosomes to the brain[1]. After delivery of exosomes containing α-synuclein to WT or M83 α-synuclein transgenic mice we observed Lewy body-like aggregates in the brain. Delivery of control exosomes did not result in brain aggregates, similarly, delivery of α-synuclein containing exosomes to α-synuclein knockout mice did not result in brain aggregates. Behavioural testing showed that both WT and M83 mice given exosomes containing α-synuclein had movement deficits in pole, beam and Rotarod testing, whereas animals given control exosomes or α-synuclein exosomes to knockout mice did not display any behavioural deficits.

In summary, we have identified a mechanistic pathway for the loading of α-synuclein into exosomes and show that these exosomes are able to propagate aggregated forms of the protein to the brains of rodents. These findings show how exosomes can transmit α-synuclein in the brain resulting in Lewy body-like aggregates and movement deficits that are found in PD.

1. Sterzenbach U, Putz U, Low L-H, Silke J, Tan S-S, Howitt J. Engineered exosomes as vehicles for biologically active proteins. Mol Therp. 2017, Jun 7;25(6):1269-1278.

id #9314

Early brain energy stress and the "inversion" into Alzheimer's disease

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Alzheimer's disease (AD) develops over decades but we cannot make detailed molecular analyses of the brains of people in the earliest stages of the disease. Low oxygen (hypoxia, that causes oxidative stress) drives increased expression of the amyloid beta (Abeta) peptide supporting that vascular deficiencies may drive early Abeta accumulation. HIF1alpha, a component of the HIF1 transcription factor that regulates gene expression

responses to hypoxia, is stabilised by low oxygen but also by deficient intracellular levels of ferric iron so that changes in gene expression due to hypoxia and low ferric iron appear similar. Stabilisation of HIf1alpha also requires direct interaction with the protein product of the PSEN1 gene that is the majority locus for mutations causing early onset, familial AD (fAD) and that probably plays a role in cellular iron import and recycling. The APP gene is another fAD mutation locus and encodes the precursor of the Abeta peptide. APP protein plays a role in export of iron from neurons. Thus fAD mutations in several loci may share effects on neuronal iron homeostasis, thereby affecting mitochondrial activity. An fAD-like mutation of PSEN1 in zebrafish causes an apparent hypoxia response in young adult brains but does this reflect low oxygen, low ferric iron, or some other stressor? Aged fish brains show “inverted” responses to hypoxia reminiscent of transcriptional inversions seen in comparisons of healthy control, mild cognitive impairment (MCI) and AD brains. These and other “inversion” phenomena support existence of a non-linear state-shift into the hypometabolic AD brain state.

id #9315

Investigations of the mechanisms of tinnitus: Dysfunctional sensory gating

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Tinnitus is a common phantom auditory perception that can result in severe stress and depression. However, the neural mechanisms are still unresolved which is hampering the search for a cure. Tinnitus is strongly associated with cochlear trauma, which evokes plasticity in the central auditory system, resulting in altered levels and patterns of spontaneous activity. It is has been proposed that tinnitus is generated from these alterations in neural activity in combination with dysfunctional sensory gating at the level of the auditory thalamus. This would allow the altered neural signals reaching the cortex, leading to perception. In our laboratory, we use animal models of cochlear trauma and tinnitus to investigate the underlying neural circuitry of sensory gating. Electrophysiological recordings in auditory thalamus in animals with and without tinnitus were combined with chronic and acute stimulation of prefrontal cortex, which is thought to be an element in sensory gating circuitry. Stimulation was achieved by focal electrodes or by repetitive transcranial magnetic stimulation. Our results demonstrate that prefrontal cortex stimulation can modulate the altered patterns of activity in auditory thalamus and that these effects are different between animals with and without tinnitus. Our data support the notion that sensory gating is involved in tinnitus generation and open up avenues for treatment.

id #9316

GABAergic changes in the somatosensory cortex of the stargazer mouse model of absence epilepsy

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Absence seizures are associated with hyperexcitation within the cortico-thalamocortical network, arising from an altered balance between excitation and inhibition. However, the precise cellular and molecular mechanisms underlying seizure generation are not well understood and appear to be multifactorial. Current antiepileptic drugs fail to suppress seizures in over 30% of patients. Hence there is a pressing need to understand how seizures arise in patients from different genetic backgrounds. Recent experimental evidence from our laboratory on the stargazer model of absence epilepsy suggests that changes in cortical inhibitory microcircuits, as a result of loss of excitation of feedforward inhibitory interneurons, may contribute to the generation of the hallmark spike-wave discharges. The aim of the current study was to examine how altered excitatory inputs to feed-forward parvalbumin-positive (PV^+) interneurons impacts GABAergic inhibition within the somatosensory cortex (SC). High-performance liquid chromatography revealed a 23.5% increase in GABA in epileptic stargazer SC. Conversely, immunogold-electron-microscopy revealed a global 20% reduction in GABA in inhibitory presynaptic terminals in stargazer SC, with a specific 18.5% reduction of GABA in PV^+ interneurons. Western blot and biochemical synaptic fractionation analyses of phasic $GABA_A$ receptor ($GABA_AR$ α1) revealed reductions of 26% in tissue and 30% in synaptic expression respectively, in epileptic SC. However, no statistically significant difference could be detected at PV^+ synapses using immunogold-electron-microscopy. These data suggest a reduction in phasic inhibition at feedforward GABAergic synapses but increase in extracellular GABA and thus potentially enhanced tonic inhibition in the stargazer SC. This could be important for designing future therapeutic strategies.

id #9317

Alzheimer’s disease risk factor gene, *PICALM*, regulates localisation of lysosomal enzymes

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Late onset Alzheimer’s disease (AD) is a major cause of dementia, which affects over 400,000 Australians. We and others have shown that autophagic and endo-lysosomal (lysosomal system) dysfunction is widespread in the AD brain, and genetic variation within the lysosomal system is associated with AD. This is important because the lysosomal system is required for clearing protein aggregates and other waste materials from the cell, and is required for healthy neurological function. We therefore sought to investigate the role of genes identified in AD genome-wide association studies in regulating lysosomal system function. We used CRISPR/Cas9 genome editing to abolish *PICALM* gene function in HeLa cells. We identified an enlargement of early endosomal size in *PICALM* KO cells compared with wild type control cell lines. To further investigate this phenomenon, we used Percoll gradient fractionation to isolate fractions from cell homogenates that were enriched for early endosomes. Mass spectrometry revealed increased abundance of lysosomal enzymes in these fractions in *PICALM* KO cells. Immunostaining in *PICALM* KO cells showed more lysosomal protease cathepsin D in the early endosomal compartment. Western blot analysis of lysosomal subcellular fractions revealed an increase in immature lysosomal enzymes upon *PICALM* deletion, showing inefficient proteolytic processing of these enzymes. This study shows that the AD risk factor gene, *PICALM*, is required for efficient lysosomal system activity. As such, genetic variation in *PICALM* could drive AD by negatively impacting lysosomal function.

id #9318

The Netrin/RGMa receptor Neogenin controls actin remodelling during dendritic spine maturation

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Aberrant dendritic spine morphogenesis has been associated with autism spectrum disorders, schizophrenia and intellectual disabilities. The frequent occurrence of disruptive *de novo* mutations in genes associated with the actin cytoskeleton and spine morphogenesis strongly implicates actin remodelling pathways in the aetiology of autism spectrum disorders. Dendritic spine maturation is dependent on the polymerization of a branched actin network within the spine head, mediated by the actin nucleation complex Arp2/3. Within the postsynaptic density (PSD) Arp2/3 activity is regulated through its interaction with the Wave Regulatory Complex (WRC). The depletion of the WRC subunit Cyfip1 results in spine loss and autism-like behaviour in mice. Currently, the molecular mechanism controlling the recruitment of the WRC to the PSD is unknown.

We have recently shown that netrin/RGMa guidance receptor Neogenin controls actin polymerization in cortical progenitors by directly binding Cyfip1. Loss of Neogenin or inhibition of its interaction with the WRC leads to dissociation of WRC-Arp2/3 from the cell membrane and disintegration of the actin cytoskeleton. Recently, truncating Neogenin mutations have been identified in autism patients. Here we test the hypothesis that Neogenin spatially-restricts branched actin remodelling within the PSD, thereby enabling spine maturation. We show that Neogenin is expressed in the spines of cultured hippocampal neurons and its depletion via RNA interference leads to decreased spine maturation. Furthermore, we show that the addition of the Neogenin ligand, RGMa, increases spine maturation. We therefore conclude that Neogenin is required for actin remodelling during spine maturation in primary hippocampal neurons.

id #9319

Spontaneous reconfiguration of waves in large-scale brain dynamics

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Traveling patterns of neuronal activity – *brain waves* – have been observed across a breadth of neuronal recordings, states of awareness, and species, but their emergence in the human brain lacks a firm understanding. Here, we analyze the complex nonlinear dynamics that emerge from modeling large-scale spontaneous neural activity on a whole-brain network derived from human tractography [1]. We find a rich array of three-dimensional wave patterns, including traveling waves, spiral waves, sources, and sinks. These patterns are metastable, such that system visits multiple spatiotemporal wave patterns in sequence. Transitions between metastable states correspond to reconfigurations of an underlying phase flow, characterized by complex nonlinear instabilities. These metastable dynamics accord with empirical data from multiple imaging modalities, including electrical waves in cortical tissue, the presence of sequential spatiotemporal patterns in resting state MEG data, and large-scale waves in human electrocorticography. By moving the study of functional networks from a static to an inherently dynamic frame, our work unifies apparently diverse phenomena across functional neuroimaging modalities and makes specific predictions for further experimentation.

1. Roberts JA, Gollo LL, Abeysuriya R, Roberts G, Mitchell PB, Woolrich MW, Breakspear M (2018). Metastable brain waves. bioRxiv: 347054. doi: https://doi.org/10.1101/347054

id #9320

Early brain energy stress and the "inversion" into Alzheimer's disease

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Alzheimer's disease (AD) develops over decades but we cannot make detailed molecular analyses of the brains of people in the earliest stages of the disease. Low oxygen (hypoxia, that causes oxidative stress) drives increased expression of the amyloid beta (Abeta) peptide supporting that vascular deficiencies may drive early Abeta accumulation. HIF1alpha, a component of the HIF1 transcription factor that regulates gene expression responses to hypoxia, is stabilised by low oxygen but also by deficient intracellular levels of ferrous iron so that changes in gene expression due to hypoxia and low ferrous iron appear similar. Stabilisation of HIF1alpha also requires direct interaction with the protein product of the *PSEN1* gene that is the majority locus for mutations causing early onset, familial AD (fAD) and that probably plays a role in cellular iron import and recycling. The *APP* gene is another fAD mutation locus and encodes the precursor of the Abeta peptide. APP protein plays a role in export of iron from neurons. Thus fAD mutations in several loci may share effects on neuronal iron homeostasis, thereby affecting mitochondrial activity. An fAD-like mutation of PSEN1 in zebrafish causes an apparent hypoxia response in young adult brains but does this reflect low oxygen, low ferrous iron, or some other stressor? Aged fish brains show “inverted” responses to hypoxia reminiscent of transcriptional inversions seen in comparisons of healthy control, mild cognitive impairment (MCI) and AD brains. These and other “inversion” phenomena support existence of a non-linear state-shift into the hypometabolic AD brain state.

id #9322

A ONE YEAR TIME COURSE ON THE RELATIONSHIP BETWEEN EXPERIMENTAL TRAUMATIC BRAIN INJURY AND NEURODEGENERATION: INJURY SEVERITY EFFECT ON FUNCTIONAL OUTCOMES AND ITS ASSOCIATED NEUROPATHOLOGY

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Traumatic brain injury (TBI) is a known risk factor for neurodegenerative diseases. However, the time-course of this relationship and whether it is dependent on the severity of the original impact is unknown. The current study investigated functional impairments and its associated neuropathology following mild (mTBI), repetitive mild (rmTBI) and moderate-severe TBI (msTBI) up to 12 months following Marmarou model of diffuse injury (n=10-14/group/timepoint). Anxiety measures at 1 month post-injury found that msTBI displayed a significant decrease in anxiety ($p<0.0001$), which returned to sham levels by 6 months. Depressive like behaviour in the forced swim test showed that only the rmTBI exhibited a

significant depressive behaviour at 6 months post injury ($p<0.05$) compared to shams, which had resolved by 12 months. For cognition, the Barnes maze showed that only msTBI had a significantly impaired recall memory at 1 month ($p<0.01$), learning deficits at 6 months ($p<0.01$) and impaired cognitive flexibility at 12 months ($p<0.01$) when compared to shams. Preliminary western analysis indicated msTBI had a significantly higher levels of pTDP-43 and oxidative stress at 6 months post injury in the lumbar spine while at 12 months, there was a significant reduction in neurofilament in the cervical spine, suggesting potential pathology progression.This study indicates that the nature/severity of the original insult determines the functional and neuropathological outcomes, which can develop over time. Nevertheless, msTBI was associated with the most persistent impairments post-injury and may therefore increase the risk for the later emergence of neurodegenerative pathology compared to other injury severity groups.

id #9324

Environmental enrichment triggers distinct patterns of oligodendrocyte production in the young adult brain

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Myelin, a critical central nervous system component, is now recognised as a dynamic and adaptive structure. Myelin plays an important role in maintaining synchronicity across key neural networks and alterations to the myelination pattern constitutes a form of 'plasticity'. Neural activity is known to influence the production of myelin-forming oligodendrocytes (OL) in adulthood, however the underpinning cellular and molecular mechanisms are unknown. We used Environmental Enrichment (EE) to provide non-invasive neural stimulation to adult mice and found that OLs were differentially triggered to respond, depending on location within the brain. Cortical OLs were stimulated to proliferate without subsequent differentiation, sub-cortical OLs to proliferate and differentiate, and subcortical white matter OLs to differentiate without prior proliferation. The enhanced OL differentiation in subcortical white matter tracts following EE accompanied improved motor coordination. We are using an inducible conditional knockout mouse to investigate neuronal TrkB as molecular mediator of this response and optical methods to assess cortical myelin formation. This study was the first to comprehensively study the cellular response of OLs to neural stimulation and our data indicate that this is triggered in a region-specific manner, which may lead to distinct functional outcomes.

id #9325

Actomyosin-II facilitates long-range retrograde transport of large cargoes by maintaining axonal radial contractility

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Tightly-regulated axonal transport is pivotal for neuronal development and survival. Despite the heavy trafficking, thin axons (diameter < 1 mm) are the most abundant type in the mammalian central nervous system (CNS). In contrast, the size of axonal cargoes is comparable to, or surprisingly bigger than some of the CNS axons themselves. This advocates for the existence of axonal radial contractility, which allows the transient expansion of axon calibre to facilitate the cargo passage. The underlying structural basis for the axonal radial contractility is the subcortical actomyosin network, which is organized into membrane-associated periodic cytoskeletal structures (MPS) that is dynamically regulated by none muscle myosin-II (NM-II). The understanding of how the dynamic cytoskeletal architecture coordinates the radial axonal contractility and cargo trafficking is therefore warranted. Here we used super-resolution live-imaging approaches to examine the correlation between the speed of axonal cargoes and their size, and found that the speed inversely correlates with the size of the cargo, which induces transient enlargement of axonal diameter. This transient change in axon diameter is mediated by NM-II, and short-term inhibition of axonal NM-II does not affect the periodicity of either F-actin or NM-II itself, but effectively decreases their colocalization and expands the diameter of the axon, leading to an increase in cargo mobility at the expense of overall trafficking efficacy. Whereas prolonged blebbistatin treatment caused the formation of focal axon swellings (FAS) and accumulation of axonal cargos. Collectively, actomyosin-II in axon shaft intensified the structural stability and thereby facilitate the long-range axonal trafficking.

id #9326

Microbiota-gut-brain axis influenced by beta-glucan dietary fiber intake for cognition

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id #9327

Microbiota-gut-brain axis influenced by beta-glucan dietary fiber intake for cognition

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Low-fiber and high-fat diet contributes to obesity, which is associated with cognitive decline. Dietary fiber intake is positively correlated with cognition reported in clinical study. This study examined effects of two types of soluble dietary fibres (beta-glucan) on gut microbiota, post-synaptic density,

and cognition in mice. Mice were fed 1-3,1-4 beta-glucan (oat bran) or 1-3,1-6 beta-glucan (mushroom) for 3 days (acute study) and 15 weeks (chronic study), mixed in high-fat (HF) or low-fat diet (LF). Gut microbiota were examined by 16S RNA sequencing in the acute study. Temporal order, novel object recognition, and Y-maze tests were performed in the chronic study. The synapse morphology was examined in the prefrontal cortex (PFC) and hippocampus by transmission electron microscopy (TEM). Acute treatment with 1-3,1-4 and 1-3,1-6 beta-glucan decreased the abundance of gut *Firmicutes* and increased *Bacterioides* in mice on both LF and HF diets. In the chronic study, both beta-glucans increased the temper-order memory in LF mice. Chronic HF diet impaired temper-order memory, novel object recognition memory, and spontaneous alteration in Y maze test. Chronic 1-3,1-4 beta-glucan improved these three cognition scores in HF mice, while 1-3,1-6 beta-glucan only improved the first two impairments in cognition. These two types of beta-glucan diets increased the post-synaptic density in the hippocampus and PFC of HF mice. Overall, these findings suggest that increasing the beta-glucan dietary fibre intake prevents alterations of gut microbiota and post-synaptic morphology in the brain regions for cognition. This maybe the neurobiological basis for improving altered cognition associated with obesity.

id #9328

PHOTOBIOMODULATION OF THE ABDOMEN PROTECTS THE BRAIN AGAINST PARKINSONIAN MPTP INSULT

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We have previously reported the phenomenon of remote photobiomodulation (PBM), whereby irradiation of a peripheral tissue with low-intensity red light (670nm) can provide protection of distal, life-critical organs such as the brain. For example, applying PBM (670nm, 50mW/cm^2, 180s/day) to the dorsum of mice during insult with the parkinsonian neurotoxin MPTP (50mg/kg over 24h) mitigated loss of functional dopaminergic neurons in the substantia nigra pars compacta (SNc) by 50% ($p<0.05$). Furthermore, pre-conditioning with remote PBM (90s/day) for 10 days prior to MPTP insult maintained SNc dopaminergic cell numbers ($p<0.05$) and neuronal activity in the caudate-putamen complex ($p<0.0001$) at healthy control levels.

We sought to identify the optimal peripheral target for remote PBM-induced neuroprotection. In an n=1 pilot study, MPTP-injected macaques received remote PBM to either the lower leg or abdomen (180s/day). Remarkably, the monkey treated with remote PBM to the abdomen showed no clinical signs and 50% more midbrain dopaminergic cells than untreated monkeys. We have confirmed this observation in a larger cohort of MPTP-injected mice, finding that post-conditioning with daily PBM directed at the abdomen mitigates loss of functional dopaminergic neurons in the SNc by 50% ($p<0.05$). While the mechanisms remain unclear, we have recently shown that abdomen-targeted PBM can influence diversity of the gut microbiome.

These data indicate that abdomen-targeted PBM offers neuroprotection against MPTP insult, possibly through effects on the gut microbiome. We are now assessing whether remote PBM provides neuroprotection to transgenic models of Parkinson's disease, and further exploring the mechanisms underlying this phenomenon.

id #9329

Repetitive transcranial magnetic stimulation induces dendritic spine plasticity – lessons from 2 photon imaging in Thy1-GFPM mice.

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Objective Modulation of cortical plasticity with repetitive transcranial magnetic stimulation (rTMS) has become a popular method of neuromodulation in both clinical and non-clinical populations. Typically used as a treatment for depression, repeated exposure of the brain to complex stimulation patterns such as intermittent theta burst stimulation (iTBS) have been shown to induce long lasting effects. Somewhat surprisingly, the biological mechanisms underpinning this functional plasticity remain poorly understood.

Brief Method We use a custom built circular coil to deliver iTBS to the mouse brain and combine it with repetitive 2-photon imaging of Thy1-gfp mice through a cranial window overlying the sensory-motor cortex to directly observe circuitry changes in response to stimulation. The dendritic spines are located on Layer 5 pyramidal neurons in the sensori-motor cortex. Image analysis is via Image J and Matlab.

Key Findings A single session of iTBS decreases dendritic spine density for at least 2 days (-9% $p=0.001$), which trends back towards baseline by 7 days post stimulation ($p=0.2$). We are now investigating whether repetitive stimulation (delivered over 4 consecutive days) can potentiate this transient change in connectivity. In addition to dendritic spine density changes, we also report synaptic dynamics – how synaptic gains, losses and stability are affected by single or multiple rounds of iTBS.

Conclusion We present evidence for iTBS induced neuroplasticity of dendritic spines. These synaptic changes are indicative of altered input into these projection neurons and could correlate with altered motor output and transient changes in motor function.

id #9330

Reprogramming human iPSC into sensory neurons to study Rett syndrome

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There is a large and growing population of individuals diagnosed with neurodevelopmental disorders. Rett syndrome is one of the major neurodevelopmental disorders. Symptoms include difficulties in social interaction, communication and repetitive behaviours as well as altered touch and pain sensitivity. Sensory neurons in the peripheral nervous system respond to somatosensory input including touch and pain. Our studies are

focused on evaluating sensory neurons from control subjects for comparison neurons from subjects with Rett syndrome. To this end we adapted a previously published protocol (Chambers et al., 2012) to produce populations of sensory neurons from induced Pluripotent Stem Cell (iPSC) lines derived from healthy control and Rett syndrome individuals (Cheung et al., 2011). Our modification of the growth factor and media composition in the Chambers et al. protocol resulted in 85% of the cells exhibiting a repetitive spiking pattern at 5 weeks consistent with nociceptor-like neurons. In contrast, preliminary lentivirus-mediated NGN2 infections and addition of neurotrophin-3 may accelerate the formation of single spiking mechanoreceptor / proprioceptor cell populations with repetitive spiking nociceptor population in sensory neuron culture. Western blots for TRK family proteins and immunostaining for sensory neuron markers support these conclusions and FACS analysis is underway. Preliminary screening of Rett syndrome patient lines using the adapted protocol suggests TRKA and glutamate receptor 1 (GLUR1) are down regulated whereas gamma amino-butyric acid (GABA) receptor seems unchanged in western blot which indicates potential synaptic / morphological abnormalities. In future, electrophysiology and morphological analysis will be performed for more depth analysis.

id #9331

The role of cortical and striatal circuits in action sequence learning

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A shift in control from the medial to the lateral regions of the dorsal striatum occurs as behaviours become more habitual or skilled. These regions receive input from the prefrontal and motor cortices. This study determined the role of striatal and cortical sub-regions on the acquisition of a skilled sequence task. We have a developed a five-step sequential nose poke task in rats for measuring initiation, execution and termination of skilled action sequences. We found that lesions to the dorsolateral striatum (DLS) impaired acquisition of sequencing, however lesions to the posterior dorsomedial striatum (pDMS) enhanced learning of action sequences. These results support suggestions of parallel processing in the medial and lateral sub-regions, whereby the DLS supports rigid habit-like responding while the DMS promotes flexible response patterns, and we show that sequence learning occurs more rapidly with DMS loss of function. Furthermore, DLS lesions delayed sequence initiation without impacting on reward collection latency, suggesting a deficit in self-initiated actions rather than a motivational or motor impairment. In separate cohorts of rats, we examined the role of the prelimbic (PrL) and infralimbic (IL) cortices; and the primary (M1) and secondary (M2) motor cortex to determine how dissociable inputs into the pDMS and DLS influence sequence acquisition and expression. Using a novel heterogeneous sequencing task, these results shed light on the opposing roles of corticostriatal sub-regions on the acquisition of sequence learning and skilled motor actions.

id #9332

Proteinopathies in dogs and marsupials

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Molecular studies of Proteinopathies have typically used transgenic mouse models, and subsequently translated to human clinical trials. However, the success rate of these translational studies has been limited and unfortunately resulted in negative outcomes and some with adverse events. Here, we describe molecular mechanisms, neuropathology and clinical signs in natural higher mammalian models of Proteinopathies similar to those recognized in humans.

We show that ageing beagles displayed similar neuroimaging, neuroinflammatory, neuropathological features and cognitive deficits similar to human AD. More importantly, soluble oligomers enriched from aging dogs were neurotoxic to human cell lines and altered significantly the aggregation kinetic of human synesthetic beta amyloid. Importantly, a small scale pilot study using single-domain bispecific anti-Aβ/tau antibody led to complete reversal of memory deficits.

A study of kangaroo poisoned with phalaris grass known to synthesise toxic alkaloids, revealed the presence of ‘movement-disorder’-like neuropathology, including alpha-synucleinopathy and neuromelanopathy.

These studies provide strong impetus to studying proteinopathies in higher mammalian models with spontaneous and naturally occurring disorders.

id #9334

Vitamin D deficiency is associated with disrupted structural brain connectivity in patients with mild cognitive impairment

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Epidemiological evidence suggests that vitamin D deficiency may exacerbate adverse cognitive outcomes in the progression of Alzheimer’s disease and other dementias. Mild cognitive impairment (MCI) is prodromal for these neurocognitive disorders and neuroimaging studies in the elderly suggest that this cognitive impairment is associated with a reduction in hippocampal volume and white matter structural integrity. To test whether vitamin D is associated with neuroanatomical correlates of MCI, we analysed an existing structural and diffusion MRI dataset of elderly patients with MCI. Based on serum 25-OHD levels, patients were categorised into serum 25-OHD deficient (<12ng/mL, *n*=27) or not-deficient (>12ng/mL, *n*=29). We used Freesurfer 6.0 to parcellate the whole brain into 164 structures and segment the hippocampal subfields. Whole-brain structural connectomes were generated using probabilistic tractography with MRtrix. The network-based statistic (NBS) was used to identify subnetworks of connections that differed significantly between the groups. We found a significant reduction in total hippocampal volume in the serum 25-OHD deficient group. We also observed a deficit in the connections from 13 regions with the the centre of the disrupted network located in the right hippocampus. Our results demonstrate that low vitamin D is associated with reduced volumes of the hippocampus and connection deficits in elderly people with MCI, which may exacerbate neurocognitive outcomes. These findings bring us closer to an understanding of the acceleration of

neurodegeneration in MCI and how vitamin D may play a role in predicting, prolonging or one day even preventing the progression from MCI to Alzheimer's disease.

id #9335

Effects of noise exposure on young adults with normal audiometric hearing

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Results from rodent models suggest that noise exposure can cause loss of synapses between inner hair cells and auditory nerve fibers (cochlear synaptopathy) without affecting threshold sensitivity. However, in a series of studies involving adults aged 18 to 35 with normal audiograms and a wide range of lifetime noise exposures we have found no evidence for an effect of noise exposure on auditory brainstem response amplitude (including wave I), or envelope following response amplitude. We have also found no evidence for an effect of noise exposure on behavioral measures including frequency discrimination, intensity discrimination, interaural phase discrimination, modulation detection, sound localisation, musical harmony perception, and speech-in-noise identification. In a companion fMRI study, we have also found no evidence for noise-induced changes to the central auditory pathways. It seems likely that humans are less susceptible to noise-induced synaptopathy than rodents. Noise-induced synaptopathy may only be a significant cause of hearing deficits in humans with extreme exposures, and may always occur in combination with a high-frequency audiometric loss.

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id #9337

Nuclear factor one transcription factors regulate developmental enhancers critical for cell differentiation during brain development

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The generation of specific cell types in a timely and orderly manner is a prerequisite for the development of normal brain structure and function. This process is largely governed by sequence-specific transcription factors, which bind to their cognate motifs on DNA to modulate gene expression. One such family of transcription factors known to regulate important developmental processes is the Nuclear factor one (NFI) family of transcription factors. Our analyses of *cis*-regulatory elements that were previously identified by the Mouse ENCODE project suggest that these transcription factors play a critical role in distal enhancer regulation during the onset of neurogenesis in the developing cortex. Our ChIP-seq data of NFIA and NFIB binding supports these hypotheses. Ongoing analyses of the epigenome in *Nfi* knockout mouse models have identified potential distal enhancers that are mis-regulated in the absence of NFI. Importantly, gene ontology demonstrates that these potential enhancers are associated with genes regulating cell proliferation and differentiation, coinciding with the phenotype we observed via histological analyses. Our ongoing work seeks to demonstrate that the potential enhancers we identified indeed function as distal enhancers in the developing cortex, and to determine whether loss of NFI results in the mis-regulation of gene expression from their gene targets. To summarise, our work demonstrates that the NFI transcription factors may be key regulators of distal enhancers important for neurogenesis in the developing brain.

id #9338

LIMK1 depletion prevents deficits in an APP transgenic mouse model of Alzheimer’s disease

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Publish consent withheld

id #9340

Defining the role of transthyretin in regulating central nervous system myelination.

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Thyroid hormones are essential for normal brain development and in the regulation of oligodendrocyte maturation and subsequently, myelination. Insufficient populations of oligodendrocytes required to remyelinate axons affected by demyelinating diseases such as multiple sclerosis result in neurodegeneration and disease progression.

Transthyretin is a thyroid hormone distributor protein which facilitates the movement of thyroid hormones in the periphery and more specifically across the blood-cerebral spinal fluid barrier and in the cerebral spinal fluid. Interestingly, a hypermyelination phenotype has been described in the corpus callosum of transthyretin-null mice, suggesting a possible role for transthyretin in the regulation of myelination.

To investigate the role of transthyretin in central nervous system myelination, a cuprizone-induced model of demyelination was used. The neurotoxicant cuprizone was added to normal rodent chow (0.2 % w/w) of adult wild type and transthyretin-null mice for 6 weeks. After the removal

of cuprizone from their diet, spontaneous remyelination soon followed. To study rates of remyelination in the corpus callosum between mouse genotypes, tissue samples were collected at 3, 4 and 6 weeks after the removal of cuprizone from their diets. Tissue was prepared for electron microscopy and the myelin thickness was measured around remyelinated axons, expressed as a g-ratio.
Remyelination was faster in transthyretin-null mice compared to wild type mice, suggesting the lack of transthyretin had increased the rate of remyelination of the adult mouse corpus callosum following cuprizone-induced demyelinating injury. This result could to be an important step forward in devising potential therapies for combating demyelinating diseases such as multiple sclerosis.

id #9341

COGNITIVE CONTROL OF REWARD NEUROCIRCUITRY IN THE ACTIVITY-BASED ANOREXIA RAT MODEL

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Anorexia nervosa (AN) has the highest mortality rate of any psychiatric disease, yet available treatments are largely ineffective, in part due to a lack of insight into underlying neurobiological drivers. Functional neuroimaging in AN patients suggests that the interplay between underactive reward and overactive cognitive neurocircuits may underscore pathological body weight loss. Utilising the activity-based anorexia (ABA) rodent model, we have previously shown that chemogenetic activation of the ventral reward circuitry prevents and rescues precipitous body weight loss by increasing food intake. Here, we hypothesised that reducing activity in neurons of the prefrontal cortex with direct projections to ventral reward circuits would similarly improve body weight maintenance in ABA.

Female Sprague-Dawley rats (*N*=36) underwent bilateral stereotaxic injections of retrogradely-transporting Cre (AAV-pmSyn1-EBFP-Cre) into the nucleus accumbens (NAc) and coincident injections of inhibiting (AAV-hSyn-DIO-hM4D(Gi)-mCherry), activating (AAV-hSyn-DIO-hM3D(Gq)-mCherry) or control (AAV-hSyn-DIO-mCherry) DREADD viruses into the prefrontal cortex (PFC). During exposure to the ABA paradigm, which involves unhindered access to a running wheel and time-limited access to food, all rats were administered clozapine-n-oxide (CNO) daily (0.3-3 mg/kg i.p.) at the onset of the dark phase.

Contrary to our hypothesis, chemogenetic modulation of PFC-NAc projection neurons increased susceptibility to body weight loss (χ^1=6.33, *p*=0.012) by exacerbating running activity (*F*=10.16, *p*=0.009) without influencing food intake (*t*=0.47, *p*=0.65). Taken together, our data indicate that both ventral reward and prefrontal control circuits respectively impact on food intake and running activity, both essential elements of the ABA phenotype and the AN condition that contribute to pathological body weight loss.

id #9342

AMPA Receptor Ubiquitination-deficient Knock-in Mice Display Enhanced Spatial Learning

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AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPARs) are the principal receptors that mediate fast excitatory neurotransmission in the brain. Dynamic modulation of the number of AMPARs at synapses underlies activity-dependent regulation of synaptic strength, an essential process in synaptic plasticity, learning and memory. All AMPAR subunits, GluA1-4, undergo post-translational ubiquitination following ligand binding, which act as a signal that directs the intracellular sorting of AMPARs toward late endosomes for degradation. Previously, we have mapped the GluA1 and GluA2 binding sites to the lysine residues in their carboxyl-terminal domains. In order to understand the functional role of AMPAR ubiquitination in vivo, we generated the GluA1 K868R and GluA2 K870/882R knock in mice, where the major ubiquitination sites in the GluA1 and GluA2 subunits were mutated to arginine residues. These mice showed normal gross brain cytoarchitecture and bred normally. Behaviourally, these mice performed significantly better than their wild-type littermates in an active-place avoidance task, a hippocampal-dependent spatial learning and memory test in rodents. No obvious differences in locomotor activity or anxiety were observed in the open-field test and elevated plus maze task, respectively. These results support our overall hypothesis that stabilisation of AMPARs by inhibiting GluA1 and GluA2 ubiquitination, can enhance cognitive function *in vivo*.

id #9343

PARTICLE-MEDIATED GENE TRANSFECTION OF GANGLION CELLS IN HUMAN RETINA.

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Purpose: Organotypic culture of mammalian retinas is used to study the differentiation and function of retinal cell types in vitro. Here we apply particle-mediated gene transfection and organotypic culture to investigate ganglion cells in human retinas. **Methods:** *Post mortem* human donor eyes (n=9) were obtained within 15 hours after death from the Lions NSW Eye Bank at Sydney Eye Hospital with ethical approval. Ganglion cells were transfected with an expression plasmid for postsynaptic density 95 conjugated to green or yellow fluorescent protein using a gene gun. Retinas were then cultured for three days, fixed and then processed with immunohistochemical markers to reveal presynaptic partners. **Results:** Transfected retinas maintained their morphology and immunohistochemical expression of markers for amacrine, bipolar, ganglion and Müller cells. We were also able to distinguish the presynaptic terminals of bipolar and amacrine cell types and the postsynaptic densities of transfected ganglion cells. In total over 100 transfected ganglion cells were analyzed and at least eleven morphological types were distinguished. The morphology of transfected ganglion cells is comparable to that observed previously using different methods. **Conclusion:** Particle-mediated gene transfer provides an efficient means of targeting cells in *post mortem* human retina. This method opens new doors for genetic manipulation of organotypic cultures which may be suitable for delivering and testing viral vectors in gene therapies.

id #9344

Neuroprotective sphingosine 1-phoshate is essential for beta secretase activity and amyloid formation, but paradoxically declines during normal ageing and Alzheimer's disease

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Sphingosine 1-phosphate (S1P) is a potent vasculo- and neuro-protective signalling lipid that promotes neurotrophic growth factor expression and pre-synaptic acetylcholine and glutamate release. S1P is synthesized primarily by sphingosine kinase 2 (SphK2) in the brain. We recently demonstrated pronounced loss of S1P, and SphK2 activity, early in Alzheimer's disease (AD) pathogenesis. Using human hippocampal tissue samples from neuropathologically normal donors, we very recently showed that S1P levels decline with age in the hippocampus of females ($r = -0.5$, $P = 0.002$), leading us to speculate that loss of S1P sensitizes to AD development. To test whether SphK2 deficiency synergises with amyloid beta (Aβ) in promoting AD, SphK2 knockout (SphK2-/-) mice were crossed to the J20 mouse model of familial AD amyloidosis.

Suprisingly, SphK2 deficiency profoundly reduced Aβ content, plaque burden and reactive astrocyte immunoreactivity in J20 mice. Reduced Aβ burden could be attributed to loss of β-secretase activity, and was associated with significant improvements in hypersynchronous activity and cross-frequency coupling measured by hippocampal electroencephalography. Despite reduced amyloid burden, SphK2-deficient J20 mice exhibited severe hypomyelination in the hippocampus and cortex, and significant deficits in the Y-maze and social novelty memory tests, when compared to the J20 or SphK2-/- strains.

In summary, endogenous S1P, synthesized by SphK2, is reduced with ageing and AD pathogenesis, yet required for Aβ formation. However, memory deficits and myelin loss in J20 mice were exacerbated on a SphK2-/- background, indicating that age-dependent SphK2 depletion promotes neurodegeneration and urging caution in use of β-secretase inhibitors for AD.

id #9345

Regulation of NMDA Receptor Trafficking by SNX27 and CaMKII

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NMDA receptors (NMDARs) are ionotropic glutamate receptors that mediate the flux of calcium into the postsynaptic compartment that underpins multiple forms of synaptic plasticity, learning and memory. Mice lacking functional NMDARs exhibit no long-term potentiation (LTP) in the hippocampus and display impairment in spatial memory. The majority of synaptic NMDAR currents is mediated by the GluN2A-containing heteromeric NMDARs. Although the cytoplasmic C-terminal tail of GluN2A is crucial for function, the molecular mechanisms underlying activity-dependent trafficking of GluN2A remains elusive. Recently, we identified a member of the sorting nexin (SNX) family of proteins, SNX27, as a GluN2A C-terminal interacting partner. Mutations of *SNX27* gene is linked to intellectual disability. Mice lacking SNX27 display impairments in glutamatergic neurotransmission, LTP and deficits in learning and memory. Here we report that SNX27 plays an important role in regulating the basal and activity-dependent forward trafficking of GluN2A-containing NMDARs in primary hippocampal neurons. SNX27 directly binds to GluN2A C-terminal tail through its postsynaptic density 95/discs large/zona occludens (PDZ) domain. Interestingly, their interaction can be modulated by the phosphorylation of GluN2A Ser-1459 residue by the Ca^{2+}/calmodulin-dependent kinase II (CaMKII), which is enhanced by glycine stimulation that mimics LTP *in vitro*. Overexpression of GluN2A S1459A phosphorylation-deficient mutant reduces GluN2A surface expression and activity-dependent insertion of NMDARs, whereas GluN2A S1459D phospho-mimetic mutant enhances basal GluN2A surface expression and occludes the glycine-induced enrichment of GluN2A in hippocampal neurons. Altogether, our study provides the first molecular link between GluN2A, SNX27 and CaMKII in controlling NMDAR surface expression in mammalian central neurons.

id #9346

The convergent role of transcription factor NFI in brain development and glioma

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The nuclear factor I (NFI) transcription factors regulate glial differentiation during brain development. During brain development, disruption of NFI leads to a delay and a reduction of astrocytic differentiation, as well as a retention of progenitor cells. Recently, the NFI genes have also been implicated in glioma. We hypothesise that NFI genes function as tumour suppressors via regulation of glial differentiation.

To determine whether loss of *Nfi* contributes to tumour initiation and progression, we have generated inducible glioma mice with and without conditional deletion of *Nfi*. Histological analyses demonstrated an increase in the number and the size of tumours in mice with deletion of *Nfi*. At the cellular level, tumours lacking NFI expression displayed fewer differentiated cells, increased infiltration into the surrounding tissue, an increase in the number of giant multinucleated cells and increased angiogenesis. These findings indicate that loss of NFI exacerbates tumour aggressiveness. Thus, NFI genes are tumour suppressors in glioma.

To determine whether NFI indeed directly regulated the glial differentiation program in tumour cells, we induced NFI expression via *in vivo* electroporation in four different patient-derived glioblastoma (GBM) xenografts in mice. Compared to cells electroporated with control plasmid, NFI overexpression reduced proliferation and increased glial differentiation. Therefore, NFI genes regulate tumour differentiation.

This study provides new insights into understanding the conserved roles of NFI in inducing glial differentiation in brain development and glioma. Furthermore, our data demonstrated that activation of the NFI pathway can halt tumour growth and therefore might provide a novel therapeutic target

in glioblastoma.

id #9347

Predicting individual psychotic experiences on a continuum using machine learning

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Previous studies of psychosis using machine learning methods have primarily been concerned with binary classification of patients and healthy controls. The aim of this study was to use electroencephalographic (EEG) data and pattern recognition to predict individual psychotic experiences on a continuum between these two extremes in otherwise healthy people.

From responses evoked by an auditory oddball paradigm, behavioural measures, neural activity, and effective connectivity were extracted as potential contributing features. Optimal performance was achieved using spatiotemporal maps of neural activity in response to frequent sounds, with late-P50 and early-N100 time windows contributing most to higher schizotypy scores. Effective connectivity estimates, in particular top-down frontotemporal connections, were also predictive of psychotic symptoms.

As a proof-of-concept, these findings demonstrate that individual psychotic experiences in healthy people can be predicted from EEG data alone, whilst also supporting the idea of altered sensory responses and the dysconnection hypothesis in schizophrenia, as well as the notion that psychosis may exist on a continuum.

id #9348

DIFFUSION TENSOR IMAGING DETECTS VENTILATION_INDUCED BRAIN INJURY IN PRETERM LAMBS.

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Injurious mechanical ventilation causes white matter (WM) injury in preterm infants through inflammatory and haemodynamic pathways. We investigated whether magnetic resonance imaging (MRI) can detect WM brain injury resulting from mechanical ventilation 24 h after preterm delivery and determine the relative contribution of each pathway.

Fetuses at 124±2 days gestation (term ~148d) were instrumented and were injuriously ventilated for 15min with the umbilical cord intact (INJ; inflammatory pathway only), or occluded (INJ+UCO; inflammatory and haemodynamic pathway). Ventilation groups were compared with sham (Sham) and unoperated controls (Cont). Fetuses were placed back *in utero* for twenty-four hours whereupon lambs were delivered and underwent MRI using structural, diffusion tensor imaging (DTI) and magnetic resonance spectroscopy (MRS) techniques.

Absolute MRS concentrations of creatine and choline were decreased in INJ+UCO compared to Cont lambs (p=0.03, p=0.009, respectively). Axial diffusivities in the internal capsule and frontal WM were lower in INJ and INJ+UCO compared to Cont (p=0.05, p=0.04, respectively). Lambs in the INJ and INJ+UCO groups had lower mean diffusivities in the frontal WM compared to Cont group (p=0.04). DTI colour mapping revealed lower diffusivity in specific WM regions in the Sham, INJ, and INJ+UCO groups compared to the Cont group. INJ+UCO lambs more likely to exhibit lower WM diffusivity than INJ lambs.

DTI and MRS showed increased brain injury in injuriously ventilated lambs compared to controls. DTI colour mapping threshold approach provides evidence that the haemodynamic and inflammatory pathways have additive effects on the progression of brain injury compared to the inflammatory pathway alone.

id #9349

DOES THE TIME-COURSE & NATURE OF NEURODEGENERATIVE CHANGE IN THE RETINA PARALLEL THAT OCCURRING IN THE BRAIN OF SANFILIPPO SYNDROME?

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The rare, currently untreatable lysosomal storage disorder Sanfilippo syndrome is characterised by failure to reach neurodevelopmental milestones followed by rapid loss of neurocognitive ability and early death (median 18 years). Whilst brain has been the focus of most studies to date, the visual system is also impacted. Here, we sought to characterise temporal changes in the retina of Sanfilippo syndrome mice and determine whether the age-of-onset of disease lesions parallels that in brain. Histological analysis of retina revealed overall thinning by 22-weeks of age ($p<0.01$; c.f. unaffected), with the outer nuclear layer reduced in thickness/cell number by 12-weeks ($p<0.05$) and photoreceptor outer segments shortened by 6-weeks ($p<0.05$). Bipolar ($p<0.05$) and amacrine ($p<0.01$) cells were reduced in number by 22-weeks, with no loss of horizontal or retinal ganglion cells to 25-weeks. The age-of-onset of endo/lysosomal expansion (determined using LIMP2 staining), microgliosis (isolectin-B4 staining) and macrogliosis (astrocytes/Müller cells stained with GFAP) in retina occurred by 3- ($p<0.001$), 3- ($p<0.01$) and 12-weeks ($p<0.05$) respectively. In the brain, significant change in each of these three markers was noted by 3-weeks of age ($p<0.05$). Finally, heparan sulphate levels were significantly elevated in both retina and brain by 3-weeks of age ($p<0.0001$). In summary, disease lesions appear in Sanfilippo retina and brain near simultaneously, therefore non-invasive imaging of retinal lesions may provide a means of 'staging' brain disease. Further, whilst brain-directed therapies are currently in clinical trial, it is imperative that the early-emerging retinal pathology is also addressed, thereby optimising Sanfilippo patient quality of life.

id #9350

PREDICTING OUTCOMES FOLLOWING MILD TRAUMATIC BRAIN INJURY

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Post-Concussion Syndrome (PCS) is a complex condition where symptoms of concussion persist beyond the timeframe that they typically resolve. However, there is currently a lack of predictive measures that can be used to direct clinical care. Here, we assessed blood-based biomarkers, MRI and neuropsychological outcomes in a cohort of concussion patients (mTBI) at the time of presentation to Royal Perth Hospital Emergency Department (T0), and related these to outcomes at 28 days (n=36), and/or age matched healthy controls. The Repeatable Battery for the Assessment of Neuropsychological Status total score was significantly lower at T0 in patients that developed PCS, than in patients that recovered normally (t (34) = 2.8215; p = 0.008). Diffusion Tensor Imaging analyses using tract based spatial statistics in a subset of patients indicated that fractional anisotropy measures in the left inferior frontal occipital fasciculus (IFOF) were significantly lower in mTBI patients than controls (t (20.587) = -2.174; p = 0.042). This area of the brain is implicated in visual-spatial processing abilities. There was a statistically significant difference in the plasma concentration of GFAP amongst the three groups (ANOVA: F (2,60) = 12.903, p < 0.001), with a significant increase with mTBI relative to control (p < 0.001). The goal is to establish a predictive model of PCS based on a suite of outcome measures that can be used to identify patients at risk of poor outcome following concussion. The work is being developed as a broader nationwide collaboration to improve lives following traumatic brain injury.

id #9351

The role of long non-coding RNA in the development of dopamine systems: A convergent mechanism for schizophrenia

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Schizophrenia is a neurodevelopmental psychiatric disorder with unknown aetiology. PET studies have established that dopamine (DA) uptake and synthesis is increased in schizophrenia, and that this is present prior to diagnosis. This suggests that developmental DA dysfunctions may be core to schizophrenia neurobiology. A comprehensive understanding of the developing DA system may be crucial to understand the origins of this disorder. Long non-coding (Lnc) RNAs such as HOTAIRM1 have been implicated in schizophrenia. This study aimed to determine the role of HOTAIRM1 in the DA ontogeny. we knocked down HOTAIRM1 using RNAi, both *in vitro* in a human dopaminergic neuroblastoma, and also *in vivo* in mouse DA progenitors using *in utero* electroporation. We showed that knocking down HOTAIRM1 reduced the mature DA marker tyrosine hydroxylase (TH) and increased neurogenin 2, an immature DA neuron marker, *in vitro*, suggesting that the differentiation of DA neurons was delayed. We also observed a reduction in the vesicular monoamine transporter 2 (VMAT2) and monoamine oxidase, proteins for DA packaging and metabolism. Additionally, we showed that reducing HOTAIRM1 decreased the epigenetic enzymes DNMT3a and JMJD3. This indicates that HOTAIRM1 potentially modulates the DA ontogeny via these epigenetic mechanisms. *In vivo* we further showed that knockdown of HOTAIRM1 within embryonic mesencephalon reduced the TH and VMAT2. These findings are important since they link an existing schizophrenia risk factor (lncRNA) with disruption in a key neurotransmitter pathway implicated in this disorder. Understanding of the role of LncRNAs in DA development has implications for schizophrenia and beyond.

id #9352

Amygdala NPY circuits are critical for the development of obesity under chronic stress

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Neuropeptide Y (NPY) exerts powerful feeding related functions in the hypothalamus. However, NPY is also present in extra-hypothalamic nuclei, but their influence on energy homeostasis is far less understood. We have now uncovered a previously unknown feeding stimulatory pathway that is activated under conditions of stress in combination with calorie dense food with NPY neurons in the central amygdala (CeA) being responsible for an exacerbated response to a combined stress and high fat diet intervention. By viral directed NPY neuron specific NPY over-expression in the CeA we can mimic the obese phenotype seen in the stress/HFD model, which importantly is prevented by the selective ablation of *Npy* in the CeA. Using food intake and energy expenditure (EE) as readout we were also able to demonstrate that selective activation of CeA NPY neurons via DREADD's results in a robust increase in food intake and decrease in EE, which requires the presence of NPY. We were further able to demonstrate that it is the failure of insulin to control CeA NPY neurons under stress/HFD conditions that manifests the underlying mechanism for the observed exaggerated

development of obesity. Taken together our results demonstrate a novel role of CeA NPY neurons, which control both feeding behaviour and energy homeostasis through the coordinated activation of amygdala and hypothalamic pathways that is particularly important under conditions of stress with the surplus of caloric dense food.

id #9353

Selectivity of Temporal Electrical Receptive Fields in Rat Retinal Ganglion Cells to Electrical Stimulation

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Retinal prostheses (bionic eyes), which aim to return vision to the blind through electrical stimulation of retinal ganglion cells (RGCs) currently present the only available clinical treatment for certain retinal degenerative diseases. However, the image perceived by the patients is still of poor quality. This limitation partially stems from the inability to selectively stimulate specific RGCs with electrical stimulation. In most mammalian retinas there are 10-15 types of RGCs that have different morphological and physiological properties. Consequently, we hypothesise that different types of RGCs have different temporal electrical receptive fields (tERFs), which refers to the temporal and spatial characteristics of the electrical stimuli that optimally activate the cell. In this study, we used Gaussian white noise electrical stimulation to recover the tERF of rat RGCs through intracellular recordings. We were able to cluster the RGCs into different clusters based on the features of their tERFs. Interestingly, the tERF clusters correlated accurately with their morphological types. By stimulating RGCs with waveforms derived from their own tERF, as well as tERFs from other RGCs cells, we found that most RGCs preferentially respond to stimulation derived from their own tERF. Many RGCs show preferential responses to tERFs of other RGCs that are from the same morphological type. Our findings show that RGCs are selective to specific features of electrical stimuli. Hence we could improve the efficacy of retinal prostheses by developing stimulation strategies that target specific RGC types critical for extracting the attributes of a visual scene.

id #9354

Identifying the cell type mediating NMDA receptor hypofunction effects on behaviours relevant to schizophrenia and gamma oscillations

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N-methyl-D-aspartate receptor (NMDAr) antagonists can induce behavioural and gamma oscillatory disturbances in rodents analogous to those seen in patients with schizophrenia. The aim of the current study was to assess whether the cell-types that generate gamma oscillations, pyramidal cells and parvalbumin-positive (PV+) interneurons, mediate these effects.

Transgenic mice lacking the NMDAr from PV+ interneurons or forebrain pyramidal cells, and their wild-type littermates, were trained to perform the Trial-unique Nonmatching to Location (TUNL) test of working memory. They were then implanted with local field potential (LFP) recording electrodes in the medial prefrontal cortex (mPFC) and dorsal hippocampus (dHPC). Mice were administered either MK-801 or saline prior to testing: 1) locomotion, 2) prepulse inhibition, 3) TUNL performance and, 4) recording LFPs during the presentation of an auditory stimulus.

Deletion of the NMDAr from either cell type attenuated the MK-801-induced increase in ongoing gamma power. NMDAr deletion selectively on PV+ interneurons attenuated the MK-801-induced increase in locomotion and that from forebrain pyramidal cells blocked the increase in mPFC-dHPC gamma coherence. The effects of MK-801 on all other measures were unchanged by genotype.

We conclude that hyperlocomotion induced by NMDAr antagonism is mediated by PV+ interneurons but not forebrain pyramidal cells and that the increase in gamma coherence is mediated by pyramidal cells but not PV+ interneurons. The increase in ongoing gamma power, on the other hand, can be mediated by either cell type. The various impairments in behaviours and gamma activity induced by NMDAr antagonists, therefore, likely involve several cellular substrates.

id #9355

Pathological alterations to the Golgi apparatus of neurons is an early feature of disease in TDP-43 ALS mice

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The Golgi apparatus is a dynamic organelle critical for protein post-translational modification and intracellular transport. Amyotrophic lateral sclerosis (ALS) is a motor neuron disease characterised by rapidly progressive neurodegeneration causing paralysis and death. Golgi apparatus fragmentation is found in the spinal cord motor neurons with the TDP-43 pathology that is characteristic of ALS, in patient autopsy samples. However, it is unknown how early Golgi fragmentation occurs and how it contributes to neurodegeneration. We therefore studied the transgenic rNLS TDP-43 mouse model of ALS, which express human TDP-43 (hTDP-43) with a defective nuclear localisation sequence (NLS) in neurons, prior to symptoms and throughout disease. Golgi morphology was examined by transmission electron microscopy and confocal microscopy. Also, we used quantitative proteomics to identify proteins of altered abundance in rNLS mouse cortex and spinal cord, and candidate proteins of interest were validated by immunoblotting and immunohistochemistry. Even prior to disease, rNLS mice exhibited dramatic fragmentation of the Golgi apparatus

into discrete stacks that were dispersed throughout the cytoplasm of neurons. This was particularly evident in motor neurons, and persisted throughout the disease course. Using proteomics, immunoblotting, and immunofluorescence, we detected increased levels of several Golgi apparatus-related proteins in the rNLS mice compared to controls, even prior to neurodegeneration. Golgi apparatus changes therefore represent an early mechanism in disease that may reflect a compensatory response to cytoplasmic TDP-43 accumulation. These studies suggest that Golgi apparatus fragmentation and trafficking defects may contribute to development of ALS.

id #9357

Short term estradiol treatment improves seizure outcomes but not cognitive measures in mouse models of congenital epilepsy and intellectual disability.

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Children with severe intellectual disability have an increased prevalence of seizures that are refractory to anti-convulsant medication. Emerging evidence indicates that neurosteroids may provide treatment options. Here we investigate short term, daily delivery of the neurosteroid, 17β-estradiol (E2; 40ng/g) during early postnatal life (day 3 to 10) in genetic mouse models of intellectual disability and seizures. The mice model two expansions of polyalanine tracts (PA) in the Aristaless-related homeobox gene (*ARX*), a transcription factor with critical roles in brain development. Daily observation of mice up to two months of age demonstrate that the frequency of seizures was significantly reduced in E2 treated mice (PA1; 34% reduction) compared to vehicle treated mice, providing reproducible outcomes from an alternate PA1 model (Olivetti *et al*, Sci Transl Med, 6(220), 2014). We expand these findings to the most frequent ARX mutation in patients; expansion of polyalanine tract 2. Across the peak of seizure onset (characterised from untreated PA animals), non-invasive video monitoring captured significant reductions in both seizure occurrence (PA2; 33% reduction) and severity in E2 treated mice. Despite improvements to the seizure phenotype, E2 treatment did not improve survival or cognitive outcomes at two months of age in either mouse model. For the first time, we report that cognitive deficits due to mutations in *Arx* are already present prior to seizure onset, and do not worsen with seizures. We contend that the pathways underpinning improvements to seizures following neurosteroid administration in early postnatal life are distinct to those involved in manifesting cognitive deficits.

id #9358

EXPLORING THE ORIGINS OF GABAERGIC DYSFUNCTION IN SCHIZOPHRENIA USING THE MATERNAL IMMUNE ACTIVATION MODEL UNCOVERS A ROLE FOR THE ARX GENE

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Schizophrenia is associated with GABAergic dysfunction and disrupted high frequency neural oscillations, particularly in the gamma frequency. Epidemiological data identifies infection during pregnancy as a risk factor for the child to develop schizophrenia and the rodent maternal immune activation (MIA) model recapitulates neurochemical and behavioural features of schizophrenia, including GABAergic disturbances. This study explored the prenatal origins of GABAergic dysfunction associated with schizophrenia using the MIA model.

Pregnant dams received the viral mimetic, poly-I:C (20mg/kg, i.p.) at gestational day 17. Hippocampal local field potentials were simultaneously recorded during pre-pulse inhibition (PPI) in adult mice. Foetal (GD18) and adult brains were collected for mRNA (96.96Biomark) and protein expression analysis. Gene expression findings were aligned with human genomic data from the Australian Schizophrenia Research Bank (ASRB) and the Psychiatric Genomics Consortium (PGC2) to identify commonalities between the mouse model and the human condition.

Acoustic-evoked gamma and theta power were reduced during the pre-pulse response in adult poly-I:C exposed mice, with impaired PPI. Poly-I:C exposure reduced expression of mRNA transcripts for GABAergic cell migration/specification, (Arx, Nkx2.1) in foetal brain and reduced protein levels of parvalbumin and somatostatin, in adult forebrain. From the ASRB data we identified a female patient with schizoaffective disorder with a missense mutation in the Arx gene. Using a hypothesis-driven approach, we found a nominal association of proximal single nucleotide polymorphisms within a 6.5Kb region surrounding the Arx gene and schizophrenia from the PGC2 data. These data suggest the Arx gene plays a role in the prenatal origins of schizophrenia.

id #9359

Studying the functional maps of the primate primary visual cortex using multi-scale calcium imaging

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Multiple overlapping functional maps have been reported in the primate visual cortex. However, it has been difficult to characterise these maps in the same animal at high spatial resolutions. To study the precise spatial relationships among functional maps, we conducted in vivo calcium imaging of neuronal activities in the primary visual cortex (V1) of the marmoset monkey (Callithrix jacchus). An AAV viral vector carrying the GCaMP6s calcium indicator (amplified with the Tet-Tre system without Dox) was injected into the V1 of two marmosets. In awake and (sufentanil) anaesthetised preparations, we imaged the calcium activities surrounding the injection sites at columnar resolution with wide-field one-photon imaging, and at

single-neuron resolution with two-photon imaging. A suite of visual stimuli was used to quantify the selectivities for the stimulus orientation, direction, spatial frequency and colour. In addition, bars of randomised orientations and positions were used to map the retinotopy of the imaged regions. We were able to visualise multiple pinwheel centres using wide-field imaging, and observed tight correlations with the preferred orientations of the individual neurons. We also observed domains with different preferred spatial frequencies that overlapped, but were organised orthogonally to the orientation domains. Furthermore, blob-like clusters were revealed by isoluminant colour stimuli. Clusters with preferences to dark and bright stimuli were also observed. Our results demonstrate the power of calcium imaging for characterising the fine details of the functional maps in the primate visual cortex.

id #9360

Tropomyosin overexpression promotes neurite outgrowth in mouse hippocampal neurons in a growth-inhibitory environment.

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Neurite outgrowth after injury in the central nervous system (CNS) is inhibited by the release and accumulation of growth-inhibitory molecules, leading to the failure of neuron regeneration. These CNS inhibitory cues have also been shown to attenuate neurite outgrowth extension. Neurite outgrowth in developing or regenerating neurons is supported by the actin cytoskeleton. Actin-associated proteins have fundamental roles in regulating actin dynamics, of which tropomyosin is regarded as a master regulator of actin filament stability and turnover. Here, we designed a microfluidic device that enables neurons to be plated adjacent to the inhibitory substrate Nogo-66 and extend neurites into the growth inhibitory region. We validated the device for its use in a neurite outgrowth inhibition assay. We optimized surface-coating with Nogo-66 and tested its coating stability and neuronal cell viability over 7 days *in vitro* (DIV). Using human Tpm3.1 (hTpm3.1)-overexpressing mouse hippocampal neurons, we investigated the potential of this tropomyosin isoform to overcome inhibitory effects of the substrate Nogo-66. Live imaging of neurite growth over 24-hours (between 4 and 5 DIV) shows a 2-fold greater ability of hTpm3.1-overexpressing neurons to extend neurites into and past the area coated with Nogo-66, compared to control neurons. We are now establishing whether the growth-promoting effect is isoform specific, using transient transfections of different Tpm isoforms and assaying neurons, derived from Tpm-knockout mice (including Tpm3.1 and Tpm4.2 knockouts). These experiments will be able to test whether Tpm3.1-overexpression can act as a potential strategy promoting neurite regeneration in an inhibitory environment after CNS injury.

id #9361

SEX-SPECIFIC EFFECTS OF STANDARD RODENT DIETS ON ESTRADIOL, HIPPOCAMPAL ESTROGEN RECEPTOR EXPRESSION AND SPATIAL MEMORY IN THE RAT

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Standard laboratory diets contain high levels of soy, and are therefore high in phytoestrogens. Isoflavones, a class of phytoestrogen found in soy, are structurally similar to 17β-estradiol and can modulate estrogen receptor (ER) α and β activity. However, it is unknown whether dietary isoflavones affect estrogen-signaling and estrogen-dependent memory processes. To investigate this, we maintained adult male and female Sprague-Dawley rats on either a diet marketed as phytoestrogen free (AIN-93G), or one of two standard rodent diets (Gordon's Premium Rat and Mouse Pellets or Specialty Feed's Irradiated Rat and Mouse Diet). We assessed the isoflavone content of each diet, peripheral estradiol, ERα and β mRNA expression in the hippocampus, and performance on a hippocampal-dependent spatial recognition task. LC-APCI-MS analysis revealed variations in the quantity of isoflavones across diets: Gordon's > Specialty > AIN-93G. The effect of diet differed in male and female rats: in male rats, high soy diets increased peripheral estradiol but did not produce detectable effects on ERα or β expression. In contrast, diet had little impact on plasma estradiol levels in female rats, but did impact on ERβ expression in the hippocampus. Furthermore, diet significantly influenced performance on the object place recognition task in both sexes, with recognition greatest in female rats receiving the Gordon's diet and lowest in male rats receiving the Specialty diet. Together, these data suggest that isoflavone variations in standard laboratory diets can influence both peripheral estrogen and estrogen signaling in the brain, and that this may have implications for hippocampal-dependent memory performance.

id #9362

Basal forebrain mediates motivational recruitment of attention by reward-associated cues

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The basal forebrain plays a crucial neuromodulatory role in the brain. In particular, its projections to the prefrontal cortex have been shown to be important in a wide variety of brain processes and functions, including attention, learning and memory, and decision-making. In the present study, we asked whether the basal forebrain is involved in recruitment of cognitive effort in response to reward related cues. This interaction between motivation and cognition is critically impacted in psychiatric conditions such as schizophrenia. Using the Designer Receptor Exclusively Activated by Designer Drug (DREADD) technique combined with our recently developed signaled probability sustained attention task, which explicitly assays the interaction between motivation and attention, we sought to determine the role of the basal forebrain in this interaction. Rats were stereotaxically injected in the basal forebrain with either hM4D (a virus that expresses receptors which silence neurons in the presence of the clozapine-N-oxide) or a control virus and tested in the SPSA. Behaviour of rats during baseline and under saline indicated control by reward probability. In the presence of CNO, differential accuracy of hM4(Gi) rats on high and low reward-probability trials was abolished. This result occurred despite spared ability of the reward-probability signals to differentially impact choice-response latencies and omissions. These results indicate that the basal forebrain is critical

for the motivational recruitment of attention in response to reward-related cues and are consistent with a role for basal forebrain in encoding and transmitting motivational salience of reward-related cues and readying prefrontal circuits for further attentional processing.

id #9363

Mitochondrial DNA Damage Induces a Premature Ageing Phenotype in Neurons

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id #9364

The $mGluR_{2/3}$ agonist LY379268 reverses NMDA receptor antagonist effects on cortical gamma oscillations and coherence, but not working memory impairment in mice

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Abnormalities in gamma oscillations may underlie cognitive impairment in schizophrenia. Antagonists of the N-methyl-D-aspartate receptor (NMDAr) can induce cognitive impairment and gamma oscillatory disturbances in rodents. The aim of the current study was to assess whether a mechanistic relationship exists between working memory and gamma impairments following NMDAr antagonism. It was hypothesised that both working memory and gamma disturbances induced by an NMDAr antagonist would be reversed by the metabotropic glutamate receptor type 2/3 ($mGluR_{2/3}$) agonist LY379268.

C57/Bl6 mice (n=11) were trained to perform the Trial-unique Nonmatching to Location (TUNL) test of working memory. They were then implanted with local field potential (LFP) recording electrodes in the medial prefrontal cortex (mPFC) and dorsal hippocampus (dHPC). Mice were administered either LY379268 (3mg/kg) or vehicle followed by the NMDAr antagonist MK-801 (0.3 or 1mg/kg) or vehicle prior to testing on the TUNL task or recording LFPs during the presentation of an auditory stimulus.

Treatment with LY379268 prevented the increases in ongoing gamma power and mPFC-dHPC gamma coherence induced by MK-801, but failed to improve the auditory-evoked gamma oscillatory deficit. In addition, LY379268 did not restore deficits in working memory on the TUNL task.

We conclude that NMDA receptor antagonism impairs working memory in mice, but that this is not reversed by stimulation of $mGluR_{2/3}$ receptors. Since elevations in ongoing gamma power and regional coherence caused by MK-801 were improved by LY379268, it appears unlikely that these oscillatory abnormalities are responsible for working memory impairment caused by NMDAr antagonism.

id #9365

Stage-specific removal of TrkB from the oligodendroglial lineage influences precursor cell-cycle

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It is well-established that brain-derived neurotrophic factor (BDNF) promotes myelin formation through its receptor TrkB, expressed by oligodendrocytes. However, a role for BDNF in the regulation of oligodendrocyte precursor cell (OPC) proliferation remains controversial. Reduction of BDNF in heterozygote BDNF-knockout mice during adult remyelination reduces OPC proliferation. In contrast, we have shown developmental deletion of TrkB from mature MBP-expressing oligodendrocytes results in OPC hyperproliferation that resolves by adulthood, and activation of TrkB in adult remyelination increases differentiated oligodendrocyte numbers. Here, we examine the effects of BDNF-TrkB on OPC proliferation by using Ki67 and double S-phase labelling with EdU and BrdU to assess the proliferative fractions and cell-cycle dynamics of OPCs during development (P6, P12 and P30) in mouse lines in which TrkB was deleted ($TrkB^{fl/fl}$) at time of oligodendrocyte specification (Olig2Cre) or when oligodendrocytes commence myelination (MBPCre). In the lumbar spinal cord, we found that while oligodendrocyte populations were unchanged across genotypes, there was a significant increase in both the proportion of proliferating cells ($Ki67^{+}PDGFR\alpha^{+}$, p=0.010) as well as cell-cycle length (p=0.013) of OPCs in the MBPCre line at P12, but not P30 compared to wildtype controls ($Cre^{-/-}$). This suggests that without TrkB in maturing oligodendrocytes, OPCs spent longer in the cell cycle and supports a regulatory role for BDNF-TrkB in OPC proliferation. Analysis of the optic nerve and corpus callosum are in progress.

id #9366

Visual stimulus specificity of local field potentials in the primate lateral geniculate nucleus (LGN)

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Purpose: The lateral geniculate nucleus (LGN) is the primary thalamic relay nucleus of the primate visual system. The primate LGN is organised into distinct layers which form parallel functional pathways for visual information. We investigated local field potential (LFPs) responses to cone-

isolating stimuli which selectively drive different parallel pathways in the LGN. Short-wave sensitive (S) cone-isolating stimuli activate many cells in the koniocellular layers, and so we wished to see if the LFPs caused by S cone-isolating stimuli were likewise segregated to the koniocellular layers.

Methods: Extracellular recordings of LFPs and isolated single unit responses to cone-isolating flashed spots and drifting gratings were made in Sufentanil-anesthetised marmosets (N = 6) using 32-channel electrode arrays. LFP responses were analysed using Morlet wavelet analysis, which permits fine-grained time-frequency resolution of transient events in the LFP.

Results and conclusion: The spatial resolution of the LFP proved not sufficient to reliably distinguish individual layers or input from individual eyes in primate LGN. However, LFP responses reveal consistent differences in the processing of visual inputs. Luminance decrements produce broadband increases in ongoing gamma (30-80 Hz) power that exceeded responses to luminance increments ($p < 0.001$). S-cone-isolating stimuli produce consistently weaker LFPs than ML-cone-isolating stimuli across all frequency bands ($p < 0.001$), with one striking exception: the offset of an S-cone decrement induces LFP oscillations as powerful as those evoked by ML-cone-isolating stimuli. This result is not predicted by the responses of any known population of cells in the LGN.

id #9368

Apnea-induced intermittent hypoxia causes cholinergic basal forebrain degeneration which predisposes to Alzheimer's disease

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Neuronal death, leading to overall brain atrophy, is one of the fundamental characteristics of Alzheimer's disease. Cholinergic neurons of the basal forebrain are particularly vulnerable in Alzheimer's disease, and the consequent cholinergic neurotransmitter decline affects other neurotransmitter systems. Epidemiological studies indicate that sleep-disordered breathing is a strong risk factor for Alzheimer's disease but the mechanisms remain unclear. Here we show that lesions of mouse cholinergic mesopontine tegmentum (cMPT) neurons, which control upper airway muscle tone during sleep, result in altered breathing, moderate hypoxia and mild cognitive impairment. The APP/PS1 mice with cMPT neuronal lesions display severe cognitive impairment, and exacerbation of the pathological features of Alzheimer's disease, including increased levels of amyloid-beta and inflammatory markers. Furthermore, the cMPT lesions cause selective degeneration of cholinergic basal forebrain (cBF) neurons, which are also characteristically lost in Alzheimer's disease. Study in p75 neurotrophin receptor (p75NTR) reveal that this cBF neuronal loss is mediated by p75NTR and can be prevented by restoring blood oxygen levels during sleep. These findings provide a mechanism by which sleep apnea and Alzheimer's disease could be causally linked through intermittent hypoxia-induced cBF degeneration.

id #9369

In vivo imaging of spontaneous calcium activity in the developing neocortex of the Australian marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*: Dasyuridae)

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In the embryonic and early postnatal brain of mammals, spontaneous neuronal activity plays key roles in the formation and refinement of cortical circuits. This activity is characterised by transient patterns of activity that engage spatially distinct regions in age- and area- dependent fashions. However, investigations of spontaneous activity in the prenatal neocortex have been hindered by a lack of *in vivo* experimental paradigms, as eutherian embryos cannot survive outside of the uterus. To overcome this, we studied developing joeys of the Australian marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*; Marsupialia: Dasyuridae), which are born at a much earlier stage of development compared to eutherians, allowing experimental access from early stages of cortical formation. We overexpressed the genetically-encoded calcium indicator GCaMP6s in layer 2/3 cortical neurons via in pouch electroporation, and imaged dunnart joeys *in vivo* using two-photon microscopy at developmental stages equivalent to intra-uterine humans and rodents. Here we show three distinct classes of large-scale neuronal activity in the early developing dunnart neocortex, including: a) travelling waves, b) synchronous events, and c) asynchronous scattered events. Importantly, these patterns of spontaneous activity resemble those of embryonic (*in vitro*) and postnatal rodents, suggesting evolutionarily conserved features of neocortical development. The combination of in pouch genetic manipulations with *in vivo* imaging during early cortical development highlights the versatility of dunnarts as experimental models of brain development. This approach will allow detailed investigations of the emergence of organised cortical activity, as well as the mechanisms of such activity in the formation of functional circuits.

id #9370

Expansion of a novel pentanucleotide repeat causes cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS)

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Repeat expansions cause over twenty neurogenetic disorders of major clinical significance which can present with heterogenous, overlapping clinical phenotypes. Discovery of novel expansions and diagnostic testing of known loci has proven extremely challenging due to the repeat sequences being refractory to standard molecular techniques. However, there have been recent advances, by us and others, to develop methods to specifically identify repeat expansions in short-read sequencing data.

To test the utility of our methods, we sought to determine the genetic basis of disease in a cohort of 35 individuals with a clinical diagnosis of cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS). Affected individuals demonstrate cerebellar impairment, bilateral vestibular hypofunction and a somatosensory deficit. While CANVAS is genetically heterogenous, the predominant mode of inheritance appears to be autosomal recessive. We performed whole genome sequencing in a subset of individuals and searched for expanded repeat sequences. Our analysis identified a novel intronic pentanucleotide repeat expansion on chromosome 4. The repeat is located within a gene encoding a polymerase

accessory protein not previously been associated with any human disorder. We developed specific PCR-based assays and demonstrated homozygous expansions in the locus accounted for 16 of the 21 families collected. Our results demonstrate the utility of newly-developed algorithms to identify novel (and known) disease causing expansions in NGS data. We anticipate additional novel loci underpinning CANVAS and many other neurological disorders will be revealed in the near future as these new bioinformatics tools are integrated into both research and diagnostic data analysis pipelines.

id #9371

Excessive sugar intake produces a robust neuroinflammatory response characterised by microglial activation in the rat cortex.

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Neuroinflammation is an important histological feature of neurodegenerative disorders including Alzheimer's disease and multiple sclerosis. Interestingly, addiction to common substances of abuse such as ethanol provokes a severe neuroinflammatory response, which may be an adaptive (anti-inflammatory) or maladaptive (pro-inflammatory) reaction to physiological stress. In our established rat model of sucrose addition, we now find that excessive sugar consumption for three months provokes an inflammatory response in rat brain, as demonstrated by small animal positron emission tomography (μPET) with [18F]DPA714, a tracer for the 18kDa microglial translocator protein (TSPO). To immunohistochemical examination of the same rats, we find a 25% increase in the number of IBA-1 expressing microglial cells in the rat cortex, and see evidence for hyper-ramification of the resident microglia in the sucrose-fed rats. Our results suggest that inflammatory changes in brain may be common to excessive alcohol or sugar intake.

id #9372

Localisation and Differentiation of Catecholaminergic and Neural Staining in the Kidney

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Neural control of the kidney is an integral component of water and solute homeostasis. While innervation of the renal pelvis and the renal blood vessels of the kidney is well established innervation of the tubules is a matter of some debate. We aimed to assess the location of catecholaminergic and neural markers within the kidney in different species.

Formalin fixed paraffin embedded or 4% paraformaldehyde fixed tissue was used from normal mice (C57Bl6), type 2 diabetic/obese mice (db/db & non-diabetic Dbh-/-), Schlager mice (hypertensive BPH/2J & normotensive BPN/3J), as well as rabbit and sheep. The effect of renal denervation in mice and sheep was also investigated. DAB and fluorescence immunohistochemistry was conducted for catecholaminergic/neural markers tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), the ubiquitin protein hydrolase protein gene product 9.5 (PGP 9.5), S100B, and neurofilament M.

Renal denervation in sheep (Medtronic Symplicity catheter) and mice (surgical/chemical denervation) reduced tubular TH staining. Staining for TH and DBH was seen around as well as within the tubules of mice (intracellular and extracellular/neural) and sheep. PGP 9.5 and neurofilament M were also found intracellularly as well as extracellular neural staining. S100B (mice) showed exclusively neural axonal staining.

These results suggest that care needs to be taken in renal neural staining in order to differentiate between neural and tubular catecholaminergic staining. It appears that some markers are also localised within tubules as well as axons. In our hands, the number of neural markers that clearly define axons alone in the kidney is limited to S100B.

id #9373

CHARACTERISATION OF GANGLION CELLS IN THE MACAQUE RETINA EXPRESSING THE TRANSCRIPTION FACTOR SATB2

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Purpose: Retinal ganglion cells (RGCs) 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 are well understood. The aim of the present study is to use molecular markers to identify and characterize non-classic ganglion cells in macaque retina. **Method:** Posterior eyecups from two macaque monkeys (*Macaca fascicularis*) were obtained immediately after death and then immersion fixed in 4% paraformaldehyde. The retinas were dissected, cut into pieces and pre-labelled with antibodies against Special AT-rich binding protein 2 (SATB2). Labelled cells were intracellularly injected with the lipophilic dye DiI to reveal their morphology (n= 29). Other retinal pieces were double labelled with SATB2 and the ganglion cell marker RBPMS or with SATB2 and antibodies against melanopsin. **Results:** SATB2 positive ganglion cells make up on average 2.8% of the ganglion cell population in the peripheral retina. In addition, a small population of amacrine cells were labelled which make up on average 8% of SATB2 labelled cells. Injected SATB2 positive cells had large dendritic fields and comprise at least three morphological types including large bistratified (n= 4, 14%), broad thorny (n= 2, 6%) and large sparse cells (n= 23, 80%). SATB2 cells did not include melanopsin-expressing ganglion cells. **Conclusion:** The transcription factor SATB2 is expressed by a small population of non-classic ganglion cells. Future studies will aim at measuring the spatial density of SATB2 cells across the retina.

id #9374

Enhanced Dopamine in the Prodrome of Schizophrenia; A Novel Animal Model

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Although there are many useful animal models of schizophrenia, none so far have replicated perhaps the strongest neurochemical finding in the clinical population: increased synthesis and release of dopamine (DA) in the dorsal striatum (DS). We developed an animal model - Enhanced Dopamine in the Prodrome of Schizophrenia ("EDiPS") – to understand this finding. We injected a viral vector coding for TH and GCH1 - critical DA-synthesis enzymes – into the pars compacta of P35 rats. This increases the capacity for DA synthesis in the DS. To confirm a schizophrenia-like phenotype, we assessed PPI and amphetamine (AMPH)-induced locomotion. We then performed triple-probe microdialysis and ^{1}H-MRS. For both techniques, the DS, nucleus accumbens (NAc) and pre-frontal cortex (PFC) were assessed at baseline, after 0.6mg/kg AMPH, and finally after KCl (for microdialysis only). Response to quinpirole was also assessed. EDiPS animals display deficient PPI, increased AMPH-induced hyperlocomotion, and normal quinpirole-induced locomotion. Microdialysis indicates increased DA turnover at baseline, selectively in the DS of EDiPS animals. EDiPS animals show increased AMPH-induced DA release in the DS and, to a lesser extent, the NAc. ^{1}H-MRS indicates that EDiPS show an altered glutamine and NAA response to AMPH in the DS. This model could be crucial in understanding the mechanism by which increased pre-synaptic DA synthesis in the DS might result in the expression of schizophrenia phenotypes. The changes in the glutamatergic system resulting from this dopaminergic manipulation suggest that the complex interactions between these neurotransmitters may be key to understanding the neuropathology of schizophrenia.

id #9375

Pre- and post-synaptic inhibitory 'gating' of a direct allodynia microcircuit in the spinal cord

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Chronic pain is a significant clinical problem, with our limited understanding of the neuronal microcircuits underlying sensory perception remaining a major obstacle to better treatments. Recent work has shown that disinhibition in the spinal cord allows tactile (touch) information to excite nociceptive (pain) circuits, whereas activation of inhibitory interneurons can 'rescue' mice from neuropathic pain states. Despite their important role, the connectivity of different classes of inhibitory interneuron in this region remains largely unknown. We have previously shown that inhibitory interneurons which express parvalbumin (PV+INs) form axo-axonic synapses onto the central terminals of low-threshold mechanoreceptive (LTMR) afferents and proposed that a loss of PV+IN mediated inhibition could contribute to allodynia. Here we combine genetic, anatomical, and electrophysiological approaches to delineate a critical microcircuit in which PV+INs gate LTMR input onto a population of lamina II interneurons, referred to as vertical cells, that in turn relay information to lamina I. We show that PV+INs in lamina IIi-III receive monosynaptic input from both hairy and glabrous skin-derived myelinated LTMR afferents, and are a source of axo-axonic synapses onto the central terminals of the same LTMR afferent populations. We also utilize an optogenetic approach to provide functional evidence that PV+INs mediate both GABAergic presynaptic inhibition of LTMR afferents and postsynaptic inhibition of vertical cells mediated by GABA and glycine. Together, these findings identify PV+INs as a potent source of inhibitory gating in a microcircuit that links tactile and nociceptive processing, representing a potential target for therapeutic intervention to alleviate allodynia.

id #9376

Habituation to looming stimuli in zebrafish larvae

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Habituation, a simple form of learning defined as a diminishment of an innate response to a frequently repeated stimulus, is critical to our everyday focus and attention, and the neural mechanisms underlying habituation remain unclear. Zebrafish larvae show habituation to visual and auditory stimuli, and provide an appealing platform from which to study habituation's circuit-level mechanisms. When presented with a looming stimulus that resembles an approaching predator, larvae respond with a rapid escape behaviour. In this project, I will first explore whether zebrafish larvae are capable of habituation to repetitive looms, and then use a SPIM microscope and calcium imaging to visualize neuronal activity and localize the regions associated in this learning behaviour. In our behavioural experiments, we use a screen below an arena with freely swimming larval zebrafish. Our results confirm that they habituate visual threats, as they gradually decrease the probability of startle responses during a train of looming stimuli. Neuronal functional imaging experiments with GCaMP6s zebrafish larvae are now helping us uncover the neural circuits and patterns of activity responsible for this habituation. These experiments involve the simultaneous observation of activity across thousands of neurons spanning the entire brain early and late in the habituation process. By identifying the patterns of activity that precede, coincide with, and follow habituation, we aim to gain circuit-level insights into how habituation occurs.

id #9377

Neuro-humoral feedback control of sound transduction in the cochlea

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Each of the ~4,000 cochlear inner hair cells is uniquely innervated by ~20 dedicated type I spiral ganglion afferent neurons (SGN), while a small subpopulation, the type II SGN (~ 5%), provide distributed sensory innervation of the adjacent three rows of outer hair cells (OHCs). The OHCs also receive cholinergic medial olivocochlear efferent fibre innervation from the brainstem that drives contralateral suppression, where loud sound presented to one ear invokes rapid suppression of hearing in the opposite ear. This is due to the efferent feedback inhibiting the outer hair cell – based 'cochlear amplifier'. It is likely that the type II fibres are the sensory driver for this feedback loop. In the cochlea, the type III intermediate filament peripherin is specific to type II SGN. Knockout of the peripherin gene disrupts the outer spiral bundle (type II SGN) innervation of the OHCs and contralateral suppression is absent (Froud et al. Nature Comm. 2014). We have resolved a tonotopic bias in this loss of type II innervation of the OHCs that matches to the disruption of contralateral suppression. We complement this with study of a $P2X_2$ receptor knockout mouse model, where absence of these ATP-gated ion channels prevents reversible loss of hearing sensitivity with moderately loud sustained noise. Thus, noise – induced release of ATP drives 'purinergic hearing adaptation' that complements the neural feedback control of hearing. Mice and humans with dysfunctional $P2X_2$ receptors are vulnerable to noise-induced hearing loss (Housley et al. PNAS 2013, Yan et al. PNAS 2013).

id #9378

Establishing brain actions of selective estrogen receptor modulators *in vivo*

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Schizophrenia is a leading cause of disability world-wide. Cognitive dysfunctions affect the majority of patients and appear early in the disease progression. Despite this, current medication fails to treat this symptom domain. Evidence, including rodent data from our laboratory, suggest loss of female sex hormone estrogen is a risk factor for cognitive deficits. Recent data from small-scale clinical studies across independent laboratories suggest that selective estrogen receptor modulators (SERMs), in particular raloxifene, can enhance cognition in schizophrenia patients. SERMs are estrogen receptor (ER) modulators that can either act as activators or inhibitors depending on the organ and available co-factors. However, it is yet to be established if 1) SERMs can traverse the blood-brain-barrier when delivered exogenously, and 2) what precise actions they have in the brain. Here, we demonstrate using liquid-chromatography mass-spectrometry that raloxifene and bazedoxifene, two FDA approved SERMs, can enter the brain of mice through intraperitoneal injection. Furthermore, using the estrogen response element – luciferase reporter mouse model, we have shown that both raloxifene and bazedoxifene can initiate ER-dependent transcriptional activities in mouse brain. Both SERMs were also able to recover ovariectomy-induced cognitive deficits in mice. Interestingly, preliminary ER transactivation assays in the M17 neuroblastoma cell line saw bazedoxifene acting as ERα agonist but an ERβ antagonist. Raloxifene was an antagonist for both nuclear estrogen receptors. This supports the notion that these SERMs are active in the brain. However, further comprehensive assessment of their pharmacological actions in various brain regions is required to elucidate their cognitive benefit.

id #9379

IN VITRO CHARACTERISATION OF DYSREGULATED MICRORNA BIOGENESIS MACHINERY ASSOCIATED WITH SCHIZOPHRENIA

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The microRNA (miRNA) biogenesis gene *DGCR8* has been identified as a schizophrenia candidate gene; haploinsufficiency of *DGCR8* is observed in 22q11.2 deletion syndrome, which is a large genetic risk factor for schizophrenia, and overexpression of the gene in the dorsolateral prefrontal cortex (DLPFC) and superior temporal gyrus (STG) has also been observed in the disease. To date, the role that *DGCR8* overexpression plays in the pathophysiology of the disorder has not been studied. We performed an *in vitro* overexpression of *DGCR8* in neuron-like differentiated SH-SY5Y cells, and explored the resulting transcriptional changes using small RNA and mRNA sequencing. This analysis identified 171 upregulated and 150 downregulated miRNAs, and 505 upregulated and 375 downregulated genes ($p < 0.05$, FDR < 0.05). The upregulated genes were significantly enriched for cell-cycle- and apoptosis-related gene ontologies and pathways, while downregulated genes were enriched for extracellular matrix- and synapse-specific, genes. Furthermore, dozens of schizophrenia-associated genes were downregulated, including *MIR137HG*, *GRIK4*, and *CACNG8*, suggesting *DGCR8* elevation may play a direct role in schizophrenia pathophysiology. Furthermore, we observed the transcription factor YY1 – a known regulator of *DGCR8* – to be downregulated at the protein level in post-mortem STG in schizophrenia. An *in vitro* knockdown of *YY1* identified a significant downregulation of *DGCR8*, as well as two other miRNA biogenesis genes *DROSHA* and *DICER1*. These results add further evidence that abnormal miRNA biogenesis in the DLPFC and STG may be involved in schizophrenia pathophysiology, and that the transcription factor YY1 is an important regulator of this process.

id #9380

Localisation and Differentiation of Catecholaminergic and Neural Staining in the Kidney

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Neural control of the kidney is an integral component of water and solute homeostasis. While innervation of the renal pelvis and the renal blood vessels of the kidney is well established innervation of the tubules is a matter of some debate. We aimed to assess the location of catecholaminergic and neural markers within the kidney in different species.

Formalin fixed paraffin embedded or 4% paraformaldehyde fixed tissue was used from normal mice (C57Bl6), type 2 diabetic/obese mice (db/db & non-diabetic Dbh-/-), Schlager mice (hypertensive BPH/2J & normotensive BPN/3J), as well as rabbit and sheep. The effect of renal denervation in mice and sheep was also investigated. DAB and fluorescence immunohistochemistry was conducted for catecholaminergic/neural markers tyrosine hydroxylase (TH), dopamine beta-hydroxylase (DBH), the ubiquitin protein hydrolase protein gene product 9.5 (PGP 9.5), S100B, and neurofilament M.

Renal denervation in sheep (Medtronic Symplicity catheter) and mice (surgical/chemical denervation) reduced tubular TH staining. Staining for TH and DBH was seen around as well as within the tubules of mice (intracellular and extracellular/neural) and sheep. PGP 9.5 and neurofilament M were also found intracellularly as well as extracellular neural staining. S100B (mice) showed exclusively neural axonal staining.

These results suggest that care needs to be taken in renal neural staining in order to differentiate between neural and tubular catecholaminergic staining. It appears that some markers are also localised within tubules as well as axons. In our hands, the number of neural markers that clearly define axons alone in the kidney is limited to S100B.

id #9381

The regional pattern of neurogenesis along the anterior-posterior axis of the human hippocampus differs to that in non-human models

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Hippocampal neurogenesis, particularly in the posterior hippocampus, contributes to memory formation and consolidation. Animal studies suggests adult neurogenesis occurs at different rates along the dorsoventral axis of the hippocampus; the dorsal hippocampus producing new neurons at a faster rate than the ventral hippocampus. To date no studies have investigated regional patterns of neurogenesis in the human hippocampus.

We took fresh-frozen hippocampus tissue from the anterior, middle and posterior hippocampus of young (18-41, n=12), aged (55-97, n=9) and Parkinson's disease dementia (61-90, n=10) cases and analysed the expression of neurogenesis-related genes using reverse transcriptase quantitative polymerase chain reaction. Using linear mixed modelling, we found expression of genes associated with maturation, but not proliferation, of new cells was lower in the aged, compared with the young group, with lowest expression in Parkinson's disease dementia. Expression of markers of cell proliferation (Ki67) and stem cells (glial fibrillary acidic protein, isoform delta) did not vary with hippocampal region in any group. In contrast, we identified significant regional variation in expression of markers for cellular maturation (doublecortin, immature neurons: p=0.001; eomesodermin, neuronal progenitors: p=0.007 and glial fibrillary acidic protein, stem cells and astrocytes: p=0.013). This study suggests that expression of genes coding for neuronal fate choice and maturation in the human hippocampus decreases with ageing and disease. Further they suggest that the regional pattern of human hippocampal neurogenesis differs to that in non-human species. This finding suggests the validity of animal models for the study of hippocampal neurogenesis in ageing and disease requires further consideration.

id #9382

MOUSE KNOCKOUT OF NUCLEAR FACTOR I GENES CAUSE CORTICAL MALFORMATIONS THAT PERSIST INTO ADULTHOOD

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The Nuclear factor I (NFI) family of transcription factors are required for the development of multiple organ systems. Using mouse knockout models, we previously demonstrated that family members NFIA, NFIB and NFIX are important for normal brain development. These proteins have overlapping biological functions in regulating the transition of progenitor cells from proliferation to differentiation and therefore result in overlapping neurodevelopmental defects in embryos. *Nfia* and *Nfib* knockout mice die at birth due to kidney and lung defects, respectively. To investigate whether the phenotypes observed in embryonic development persist into adulthood, we generated cortexspecific conditional knockout mouse models of *Nfia* and *Nfib*. These *Nfia*^{flox} or *Nfib*^{flox}; Emx1-Cre mice are viable and fertile. Their postnatal cortical development is delayed, but no major defects are observed. In adulthood, these mutants have enlarged brains due to increased volume of the cerebral cortex and, in particular, the cingulate cortex. Preliminary assessment revealed only a minor behavioural phenotype. These observed phenotypes are comparable to those in humans with a deletion or mutation of *NFI*. Hence, we now have a model to further study the aetiology and the functional defects in the human disorders.

id #9383

A novel neuronal signal that instructs oligodendrocyte development and de novo myelination

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Despite the reciprocal interaction between neurons and myelin membranes, the neuronal cues that instruct oligodendrocyte development and myelin wrapping around CNS axons are still unknown. Through selectively deleting TrkB in neurons, we found that neuronal TrkB is essential for the

initiation and extent of CNS myelination independent of axonal calibre *in vivo*, influencing nerve signal conduction along key white matter tracts. Interestingly, we found neuronal TrkB drives two distinct modes of myelination in the CNS. In the optic nerve, neuronal TrkB exerted modest and transient effect upon oligodendroglial survival during early postnatal development but a significant and prolonged influence in promoting axonal ensheathment throughout development and into adulthood. However, in the subcortical white matter tract, neuronal TrkB exerted significant multi-faceted influence on promoting the myelinating process ranging from oligodendroglial lineage progression through to myelin wrapping. Together, our results at the first time identify TrkB as a novel neuronal signal that instructs oligodendrocyte development and *de novo* myelination of different white matter tracts, indicating that there are distinct cellular mechanisms underpinning CNS myelination within different neural circuits.

id #9384

Early administration of umbilical cord blood cells increases inflammation and blood-brain barrier breakdown in injuriously ventilated preterm lambs.

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Ventilation in the delivery room can cause brain injury in preterm neonates. The main pathways of ventilation-induced brain injury (VIBI) are cerebral inflammation and haemodynamic instability. Umbilical cord blood cell (UCBC) therapy is suggested to be neuroprotective by targeting inflammation, but its interaction with mechanical ventilation has not been studied.

We aimed to investigate the effects of early UCBC administration on VIBI in preterm lambs. Fetal lambs (0.85 gestation) were exteriorised, ventilated with an injurious high tidal volume strategy for 15 min with placental circulation intact, then returned to the uterus. Lambs were randomised to controls (INJ; n=7) or UCBC-treated (80 million allogeneic UCBC i.v. 1 h post-ventilation; INJ+UCBC; n=7). At 24 h, lambs were delivered, underwent magnetic resonance imaging, then euthanised. Brains were collected to assess inflammation, vascular leakage, and white matter injury in the periventricular and subcortical white matter (PVWM; SCWM).

Ventilation and physiological parameters during injurious ventilation and blood gas measurements over 24 h were not different between groups. Compared to INJ, INJ+UCBC lambs had decreased claudin-1 and VEGFA, and increased angiogenin-1 mRNA expression in the SCWM. INJ+UCBC lambs also had an increased number of microglial aggregations and microglia density within aggregations in the SCWM (p=0.048; p=0.007) and a higher number of blood vessels with protein extravasation in the PVWM (p=0.004) compared to INJ.

Administration of UCBC 1 h after ventilation onset increased protein extravasation in the PVWM, and increased microgliosis and altered mRNA expression in the SCWM of preterm lambs. Early UCBC administration may be detrimental.

id #9385

Linear summation of cone inputs to LGN suppressed-by-contrast cells in marmosets.

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Purpose: Suppressed-by-contrast (SBC) cells are characterised by high baseline spike rate which is transiently suppressed by stimulus presentation (Solomon et al., J Nphys 2010). These cells show excitation to short wavelength (S) cone decrements and have also been classified as 'Blue-OFF' cells. SBC cells show cone opponency similar which is complementary to that of 'Blue-ON' cells. We here show that achromatic stimulus increments are better predicted by cone response summation than achromatic stimuli decrements in blue-OFF cells.

Methods: Extracellular spike activity of K cells in the LGN classified as 'blue-OFF' (n=19) or SBC (n=9) were recorded in Sufentanil-anaesthetised marmosets (*Callithrix jacchus*). Visual stimuli (achromatic and cone-isolating, uniform fields and gratings) were presented against a uniform grey background near 50 Cd / m^2.

Results: Cone opponent signature (S-, ML+) was present in 89% of cells (25/28). Euclidean distance between measured and predicted achromatic stimuli was significantly lower for cone increments (62.2 ± 29.4 SD, n = 71) than for cone decrements (100.5 ± 59.3, $p < 0.01$, Wilcoxon paired rank sum test). Correlation coefficient was positive but not significantly different for increments (0.49 ± 0.3) and decrements (0.38 ± 0.2, $p = 0.15$, paired rank sum test). R-squared was significantly higher for increments (0.34 ± 0.3) than decrements (0.18 ± 0.2, $p < 0.05$).

Conclusion: The SBC cells in the LGN show an imbalance in the way cone inputs are summed: increments are summed more linearly than decrements are.

id #9386

Generation of tau-specific antibodies for the treatment of Alzheimer's disease and enhancement of their delivery into the brain using focused scanning ultrasound

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Alzheimer's disease (AD) and related dementias are progressive neurodegenerative diseases for which new treatments are crucial. AD is characterized by the extracellular deposition of amyloid-β (Ab) as amyloid plaques and the intracellular deposition of tau as neurofibrillary tangles, with the latter directly correlating with dementia in AD patients. Reducing tau levels abrogates Aβ-mediated toxicity, making tau an attractive therapeutic target. We have generated novel antibodies highly specific for tau and in a series of *in vitro* assays, have demonstrated that these antibodies can engage extracellular tau, promote it's clearance through the activation of microglia and prevent tau from seeding pathology in neighbouring neurons. *In vivo*, however, the blood-brain barrier (BBB) limits the passage of molecules from the blood into the central nervous system and remains a formidable obstacle for neurological therapeutics, particularly for molecules greater than 400 Da such as antibodies. We have therefore combined the delivery of our tau-specific antibodies with microbubbles and focused scanning ultrasound (SUS), a non-invasive technique

which transiently opens the BBB to allow peripherally delivered molecules to enter the brain, in mice. Using this technique, we have demonstrated that SUS increases the amount of antibody delivered to the brain by 10-fold allowing for an enhanced therapeutic effect. Furthermore, internalisation of tau-specific antibodies by neurons, where tau is predominantly localised, is observed. Taken together, our results validate the use of tau-specific antibodies for reducing tau pathogenesis and highlights SUS as an effective tool for enhancing the delivery of antibodies to the brain.

id #9387

MULTIPLE TYPES OF GANGLION CELLS EXPRESS THE TRANSCRIPTION FACTOR SATB1 IN MARMOSET RETINA

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Purpose: The transcription factor Special AT-rich Sequence Binding Protein 1 (SATB1) has been shown to be expressed by ON-OFF direction-selective ganglion cells in the mouse retina (Peng *et al.,* 2017). The purpose of this study was to identify the retinal ganglion cells expressing SATB1 in the marmoset retina. **Methods:** Six retinas were obtained from five adult marmosets (*Callithrix jacchus*). The retinas were fixed in 4% paraformaldehyde for 1 hour and then incubated with rabbit antibodies against SATB1. SATB1-positive ganglion cell nuclei were targeted for intracellular injection with the lipophilic dye DiI. Ganglion cells were imaged with a Zeiss confocal microscope and classified according to dendritic field size, stratification and branch density. **Results:** A total of 66 ganglion cells was injected at eccentricities from 2 mm to 11 mm. Most cells were classified as wide-field ganglion cells and 11% (7/66) were classified as OFF-parasol ganglion cells. Almost half of the SATB1 labeled cells were thorny ganglion cells (32/66; 48%). Other ganglion cell types included recursive cells (9/66; 14%), tufted cells (9/66; 14%) and small-bistratified cells (6/66; 9%). **Conclusion:** SATB1 is expressed by multiple types of ganglion cells in marmoset retina. We have previously shown that antibodies against calretinin and FoxP2 can be used to label thorny and tufted cells. These markers in combination with SATB1, may be used to tease out and characterize recursive cells (a candidate for ON-OFF direction-selective ganglion cells) in primate retina.

1. Peng Y-R, Tran N, Krishnaswamy A, Kostadinov D, Martersteck E, Sanes J. 2017. Satb1 Regulates Contactin 5 to Pattern Dendrites of a Mammalian Retinal Ganglion Cell. Neuron 95:869–883.e6.

id #9388

Comparison of phospho-signalling in cultured neurons and synaptosomes reveals a strong correlation and identifies bassoon as a major signalling target.

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Many proteins involved in the regulation of neurotransmission have been genetically linked to neurological disease. There is limited understanding of the molecular mechanisms of neuronal activity, despite these mechanisms being important for the development of novel therapeutics. Practical barriers have prevented the combination of high value biochemical analyses with physiologically relevant experimental models. We have made an important step towards the long-term goal of aligning molecular and physiological knowledge of neurotransmission. We compared the global activity-dependent phosphorylation-based signalling in a well-studied, but somewhat artificial experimental model, i.e. synaptosomes, with signalling in cultured hippocampal neurons. Both models were stimulated with elevated KCl, resulting in the identification of 1,917 and 7,070 activity-dependent phosphorylation sites, respectively. Despite the shortcomings of synaptosomes, there was a close correlation with cultured hippocampal neurons, made possible by focusing on presynaptic-specific proteins. Of these, the active zone scaffold protein, bassoon, was the major target of phospho-signalling, both in the number of targeted phosphorylation sites and the magnitude of change within the entire data set. The profile of bassoon phospho-signalling suggests it is a hub of signal integration. Also, presynaptic scaffold proteins were more highly targeted than postsynaptic scaffold proteins and neurotransmitter receptors that are similarly exposed to calcium and activity-dependent signalling. Thus, we have given context to a well-studied experimental model by comparing it to a more physiologically relevant model and provided a large data resource for neuroscientists. The experimental barriers overcome in this work will lead to the analysis of models with higher value physiological properties.

id #9389

Perceptual change-of-mind decisions are sensitive to absolute evidence magnitude

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In order to navigate the world safely, we rely on our ability to make accurate perceptual judgements. However, errors do inevitably occur. In these situations, rapid ‘changes of mind’ are required to correct or abandon ongoing actions. Recently-developed computational models posit that perceptual judgements are made when evidence is accumulated to a given criterion, and changes of mind occur if evidence later favors a different response. Critically, current models diverge in their predictions regarding the effects of absolute evidence magnitude (i.e., the sum of evidence for opposing choices) on changes of mind. The current study therefore investigated whether absolute evidence magnitude influences change of mind likelihood and timing. Participants (n=30) indicated which of two flickering greyscale squares was brightest. Critically, following an initial decision, the stimuli remained on screen for a brief period (1s) during which participants could change their response. To investigate the effect of absolute evidence, the total luminance of the two squares was varied whilst the difference in luminance was held constant. Increases in absolute evidence were associated with faster, less accurate initial responses. However, high levels of absolute evidence were also associated with slower, less frequent changes of mind. The observed initial response dynamics can be explained by either the presence of activation-dependent noise in the

decision process or by lateral inhibition processes. However, the observed patterns of change-of-mind responses cannot be explained by current models, and instead, suggest that change of mind decisions are additionally influenced by evidence reliability.

id #9390

Metaplasticity: Activity-dependent shaping of future plasticity

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The nervous system is built to continuously adapt its cellular properties and connectivity in response to changing environmental stimuli and contingencies. This allows an organism to behave optimally within that changing environment. Unfettered plasticity, however, is counter-productive; equally, having insufficient plasticity capability at critical times might be catastrophic. In the case of synaptic plasticity phenomena such as long-term potentiation and long-term depression, the nervous system has resolved these potential issues through activity-dependent mechanisms that regulate the threshold, amplitude, duration and even direction of future synaptic change. Moreover, mechanisms exist that can regulate future plasticity either locally at specific synapses, or more widely on portions of a dendritic arbour, across all the synaptic contacts of a cell, or even across a network of cells. Together these mechanisms are referred to as “Metaplasticity”, i.e. the plasticity of synaptic plasticity. Metaplasticity-like effects have also been observed to be engaged by behavioural events, and to regulate future learning and memory, thus revealing more global regulations which are increasingly referred to as “Behavioural Metaplasticity”. In this talk, various metaplasticity phenomena and mechanisms will be reviewed, followed by a presentation of recent findings regarding a novel form of astrocyte-mediated metaplasticity that spreads across a network of neurons, and its possible aberrant engagement in a mouse model of Alzheimer’s disease.

id #9391

Selective optogenetic stimulation of vagal neural crest and placodal-dervied fibres differentially regulates cardiorespiratory and oesophageal physiology

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Axons within the vagus nerve are derived from neurons of either neural crest (Wnt1-expressing) or placodal (Phox2b-expressing) origin, terminate differentially in peripheral visceral end-organs and have associations with different brainstem processing nuclei. Here, we examined the hypothesis that neural crest and placodal-derived neurons differentially regulate cardiorespiratory and gastrointestinal physiology, using transgenic mice expressing channelrhodopsin-2 under the promoter of Wnt1 (Wnt1 x ChR2) or Phox2b (Phox2b x ChR2). Under isoflurane anaesthesia, carotid blood pressure (BP), heart rate (HR), diaphragm electromyography and oesophageal pressure were recorded during optogenetic stimulation of the left cervical vagus nerve. In Phox2b x ChR2 mice, low frequency (40 Hz and below) stimulation of the intact vagus nerve significantly reduced both BP and HR and increased oesophageal pressure while higher frequency stimulation (50 Hz and above) decreased respiratory rate (RR). Stimulation of the distal vagus nerve after vagotomy also decreased BP and HR and increased oesophageal pressure while proximal stimulation induced apnoea. In Wnt1 x ChR2 mice, only 20 Hz stimulation of the intact nerve decreased BP and HR without effect on respiration and oesophageal pressure. Stimulation of the proximal vagus nerve after vagotomy increased BP and RR. These findings indicate that neural crest and placodal vagal fibres have differential sensory and motor effects in cardiorespiratory and oesophageal physiology. The central organisation of the neural circuitry mediating these effects is under active investigation.

id #9392

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 widely accepted that Tau elicits prion-like properties that are at least partly responsible for the amplification of Tau pathology *in vivo*. In this study, we used brain lysate from an rTg4510 mouse model of Tauopathy to seed the aggregation of the repeat domain of Tau in HEK293 cells. We isolated cell clones that faithfully propagate several unique aggregate morphologies. In this model, we found that pharmacologically inhibiting autophagy (a catabolic cellular house-keeping process) caused a marked accumulation of Tau aggregates of various sizes. These data support the idea that autophagic dysfunction plays a key role in Tau-mediated Alzheimer’s disease pathogenesis.

id #9393

Investigating early causative mechanisms that lead to *GBA* associated Parkinson’s Disease using induced pluripotent stem cells.

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Parkinson's disease (PD) is the second-most common neurodegenerative disease that results in progressive motor dysfunctions caused by the loss of dopaminergic neurons in the substantia nigra. Heterozygous mutations in the glucocerebrosidase gene (*GBA*^{het}) are the most common genetic risk factor for PD, however, carriers present with an incomplete penetrance of the disease and varied age at onset (AAO) of PD, suggesting a combination of risk factors lead to increased susceptibility. Using a lentiviral direct conversion method we generated induced neurons (iNs) from *GBA*^{het} iPSCs and non-diseased iPSCs that express mature neuronal markers and produce spontaneous action potentials. Furthermore, we have shown that *GBA*-derived iNs present with disease specific phenotypes including reduced GCase activity and perturbed neuronal activity. To identify differentially expressed genes and uncover genetic variants that contribute to *GBA*-associated PD we performed RNA sequencing. Our analyses revealed highly significant upregulation of *ELAVL4*, a neuronal gene encoding an RNA-binding protein that preferentially binds to the 3' untranslated region of mRNA to regulate stability, translation and transport. Of interest, this gene is located within the *PARK10* locus, a loci associated with AAO of PD. Using RNA immunoprecipitation, we have shown that ELAVL4 binds to human *SNCA* transcripts to regulate its expression. We have utilized a technique known as assay for accessible transposase chromatin to identify *cis*-acting elements that may regulate *ELAVL4* in our GBA-iN. Altogether, our data suggests that *ELAVL4* may be a potential modifier gene in *GBA*-associated PD pathogenesis.

id #9394

TRH and TRH-like Peptides Participate in the Interaction of the Oxytocinergic and Serotonergic Systems within Male Rat Brain and Peripheral Tissues

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Aim: Oxytocin (OT) analogs and mimetics have exciting potential as therapeutics for an array of psychiatric illnesses including major depression, autism, social anxiety disorder, and Prader-Willi syndrome. Serotonin has recently been reported to participate in the anxiolytic effects of OT. TRH and TRH-like peptides, with the structure pGlu-X-Pro-NH_2, where "X" can be any amino acid residue, ameliorate the effects of depression, anxiety, epilepsy, neurodegeneration and aging, and are responsive to changes in serotonin levels. We hypothesize that these tripeptides participate in the interactions of OT with serotonin.

Materials and Methods: We ip injected young adult male Sprague-Dawley rats with carbetocin, a long-acting OT analog, with and without ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist, to assess the role of TRH and TRH-like peptides as mediators of the neurobiochemical, metabolic, and reproductive effects of OT and serotonin. Carbetocin is a functional selective Gq agonist which interacts exclusively with the oxytocin receptor, OTR.

Key findings: Two-way ANOVA revealed highly significant interactions between carbetocin and ritanserin in the release of TRH and TRH-like peptides within the hypothalamus, medulla oblongata, cerebellum, epididymis, prostate and adrenals.

Significance: These peptides contribute to the therapeutic potential of carbetocin which can signal via the oxytocin receptor and serotonergic system.

id #9395

Modelling of subtype-specific nociceptive phenotypes in dorsal root ganglia-derived 50B11cells

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The embryonic rat dorsal root ganglion (DRG) neuron-derived 50B11 cell line is a promising sensory neuron model expressing markers characteristic of c-fibre nociceptors. The cells might be suitable to "measure" pain in a bio-nano-chip interface. However, whether 50B11 cells represent functional nociceptor-like neurons and can detect pain subtypes has not been investigated. Here we show that in the presence of reduced forskolin (FSK) concentration, 50B11 cultures maintain a differentiated neuronal phenotype for 7 days, and in combination with NGF or GDNF are critical in establishing differentially functional responses to capsaicin and ATP respectively. Western blot analysis of nociceptive markers demonstrates that the 50B11 cells partially recapitulate the functional phenotypes of classical NGF-dependent (peptidergic) and GDNF-dependent (non-peptidergic) neuronal subtypes described in DRGs. Further, 50B11 cells differentiated with NGF/FSK, but not GDNF/FSK, show a sensitization response to acute prostaglandin E2 treatment. Finally, RNA-seq analysis confirms that differentiation with NGF/FSK and GDNF/FSK produces 2 distinct functional cell subtypes and highlights the presence and absence of critical nociceptor specific gene expression profiles. In addition, serum samples from CCI chronic pain injury rat model produced calcium responses in 50B11 cells in line with capsaicin and ATP stimulation, whereas sham rat serum did not produce this effect. This study shows that the 50B11 cell line can be modelled into two classes of functional nociceptor-like cells that can detect chronic pain and provides a detailed genetic analysis of the suitability of the cells as a high-throughput nociceptor model.

id #9396

Graph Entropy Analysis of Sleep EEG in Alzheimer's Disease

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BACKGROUND: Patients with Alzheimer's disease (AD) always have sleep disturbances (Ju et al., 2014). Existing research show that entropy methods from EEG signals of AD are different from those of healthy people (Deng et al., 2017, Simons et al., 2018). However, the entropy differences between Alzheimer's disease (AD) and adults with sleep disorders are still unclear. This paper employs a graph entropy (GE) method to analyse the EEG signals to test the hypothesis that the GE associated with sleep EEG of AD is lower than those of age-matched control subjects.

METHODS: Two channel (C3 and C4) EEG from full overnight PSG recordings from four AD patients (average MMSE: 23) and ten control subjects with suspected sleep-disordered breathing (AHI>11) are collected. Each record is segmented into 30-second epochs. Then a difference visibility graph is used to convert each epoch EEG signals into a graph. A Shannon entropy is calculated from each graph. Finally, all extract GE features are divided into AD and control groups for statistical analysis.

RESULTS: During the awake period, the GEs in AD subject is not always lower those of age-matched control. However, in sleep status, the GEs of AD patients are significant lower than those of age-matched control group ($p < 2.2e-16$).

SIGNIFICANCE: This finding suggests that the GE may be a robust biomarker for distinguishing AD from older adults with sleep apnea syndrome.

1. DENG, B., CAI, L., LI, S., WANG, R., YU, H., CHEN, Y. & WANG, J. 2017. Multivariate multi-scale weighted permutation entropy analysis of EEG complexity for Alzheimer's disease. Cognitive neurodynamics, 11, 217-231.
2. JU, Y.-E. S., LUCEY, B. P. & HOLTZMAN, D. M. 2014. Sleep and Alzheimer disease pathology—a bidirectional relationship. Nature reviews Neurology, 10, 115.
3. SIMONS, S., ESPINO, P. & ABÁSOLO, D. 2018. Fuzzy Entropy Analysis of the Electroencephalogram in Patients with Alzheimer's Disease: Is the Method Superior to Sample Entropy? Entropy, 20, 21.

id #9398

ENHANCING BRAIN DELIVERY OF TAU ANTIBODIES USING ANTIBODY ENGINEERING AND SCANNING ULTRASOUND

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Alzheimer's disease (AD) is an extremely costly neurodegenerative disorder characterised by extracellular amyloid plaques and intraneuronal neurofibrillary tangles. The main component of neurofibrillary tangles is the protein tau in a hyperphosphorylated state. Tau is an attractive therapeutic target due to its pathology being a strong correlate of cognitive decline in AD, however the blood brain barrier and neuronal membrane are formidable obstacles for therapeutics. We have previously increased the delivery of an anti-tau antibody, in a 29 kDa single chain variable fragment (scFv) format, into the brains of mice by combining antibody administration with microbubbles and non-invasive scanning focused ultrasound (SUS). Following on from this study, here we compare brain uptake of the anti-tau scFv, delivered in combination with SUS, to that of a larger 52 kDa fragment antigen binding (Fab) format and full-sized 156 kDa IgG antibody, to elucidate the importance of antibody size, and Fc-mediated receptor binding, for neuronal uptake and tau engagement. We show that SUS increases the brain concentration of all antibody formats, and that IgG delivery is significantly enhanced compared to the smaller formats, which are rapidly cleared from the blood through the renal system. Overall, this study shows that the antibody Fc region is critical for lasting therapeutic delivery into the brain, and that this should be considered for future engineered immunotherapeutics targeting proteins implicated in neurological disorders. Moreover, we have validated the use of scanning ultrasound to deliver IgG and smaller antibody formats across the blood brain barrier.

id #9399

Mitochondrial DNA Damage Induces a Premature Ageing Phenotype in Neurons

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Neuronal function heavily relies on the mitochondrial network for several aspects, such as ATP biosynthesis and calcium ion homeostasis. Therefore, mitochondrial health as well as distribution along axons and dendrites is essential for correct neuronal function. Disruptions to either of these attributes can cause neurodegenerative diseases affecting both the central and peripheral nervous system. A major component of mitochondrial health is the integrity of the mitochondrial genome (mtDNA). Damage to the mtDNA is frequent and accumulates during ageing because of its exposure to reactive oxygen species, high replication rate and the scarcity of mitochondrial repair mechanisms. We have established a model in the nematode *C. elegans* that allows the investigation of the consequences of specific and intrinsic mtDNA damage in individual neurons. Intrinsic mtDNA damage impacts neuronal functionality and causes morphological changes that are associated with ageing, including inappropriate neurite branching, splitting, and formation of extra-axonal projections harbouring injured mitochondria. These morphological phenotypes appear to precede functional deficits. Interestingly, contrary to other neurological conditions, intrinsic mtDNA damage does not result in an adjustment of mitochondrial distribution along the main neuronal process. This suggests that affected neurons are unable to detect the mtDNA integrity of individual organelles and reallocate them to the neuronal cell body for repair and turnover. The absence of a mtDNA damage surveillance mechanism appears to facilitate the premature transition to a functional and morphological phenotype resembling that of aged neurons.

id #9400

Non-Reinforced Auditory Stimulus Alters the Neural Representation of Contextual Fear Memory

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Context is hypothesised to influence a number of psychological disorders, namely post-traumatic stress disorder (PTSD). To better understand PTSD, research has sought to identify the molecular mechanisms involved in contextual fear memory formation. The amygdala, hippocampus and prefrontal cortex have been identified as key brain regions. To date, little research has investigated how minor changes to the contextual environment (where the fear memory is initially formed) can directly alter neural activity in the amygdala. Two procedures were used to create contextual fear memories in rats: standard contextual fear conditioning (CFC; five 1.0 mA foot-shocks) and unpaired fear conditioning (UFC; identical foot-shocks with five randomly presented non-reinforced auditory tones). As compared to context only (CO) controls, fear-related freezing to context was higher following both forms of conditioning (CFC $p > 0.0001$; UFC $p > 0.0001$). Despite this, the pattern of activity-regulated cytoskeleton-

associated protein (Arc), c-Fos and brain derived neurotropic factor (BDNF) expression differed. As compared to controls, CFC resulted in increased Arc (p > 0.01) and c-Fos (p >0.01) expression in lateral amygdala (LA) subregion LaDL. Alternatively, UFC resulted in total LA activation, with significant increases in subregions LaDL (Arc p > 0.05; c-Fos p > 0.01), LaVM (Arc p > 0.01; c-Fos p > 0.05) and, in c-Fos only, LaVL (p > 0.05). Interestingly, neither contextual fear conditioning protocol (with or without the non-reinforced auditory stimulus) altered BDNF expression in LA. Data demonstrates how non-contingently reinforced contextual alterations can substantially alter LA activity (Arc and c-Fos) following fear conditioning.

id #9401

Parallel S1P1 and S1P2 receptor signalling pathways synergise to maintain neurotrophic gene expression in human astrocytes

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Sphingosine 1-phosphate (S1P) is an essential lipid metabolite with potent vasculoprotective and neuroprotective properties. S1P signals through its own family of five G-protein coupled receptors, $S1P_1$-$S1P_5$, activating multiple intracellular pathways that regulate cell proliferation, differentiation, and survival. The multiple sclerosis drug Fingolimod is a highly potent S1P receptor agonist that causes peripheral lymphopenia. However, recent research has also established direct neuroprotective properties of Fingolimod in multiple neurodegenerative paradigms including Alzheimer's and Parkinson's disease. In this study, we show that endogenous S1P regulates the transcription of important neurotrophic factors in the human brain, including brain-derived neurotrophic factor (BDNF), leukaemia inhibitory factor (LIF), platelet-derived growth factor B (PDGFB), and heparin-binding EGF-like growth factor (HBEGF). These factors are up-regulated by S1P in astrocytes but not neurons, and S1P is a much more potent inducer than Fingolimod. Accordingly, in an *in vitro* model of neuronal excitotoxic cell death, S1P significantly attenuates apoptosis whilst Fingolimod does not. Specific antagonists of $S1P_1$ and $S1P_2$ both inhibited neurotrophic gene induction in response to S1P, indicating simultaneous activation of both receptors is required. Phosphoproteomic analysis, siRNA, and Western blotting showed that $S1P_2$ signals through Ga_{13}, RhoA, Jun and Yap to drive neurotrophic gene expression. Fingolimod does not activate S1P2, explaining why it does not promote significant neurotrophic gene expression in astrocytes. Supplementing Fingolimod with a constitutively active G_{13} boosts expression of neurotrophic factors. These results demonstrate that S1P utilises dual signalling pathways from independent receptors to drive maximal neurotrophic gene expression and protection against excitotoxic cell death.

id #9403

Learning under conditions of uncertainty and threat

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Regularity learning is the process of learning to recognise patterns in the environment based on the statistics of inter-stimulus contingencies. Violations of learned environmental regularities induce differential brain responses, known as prediction errors (PEs) or mismatch signals. The magnitude of these signals has been shown to be affected by environmental factors, including imminent threat and volatility. These factors have previously been studied separately, but it is not known if or how they interact. We investigated how PEs were affected by these two factors by inviting adult volunteers to undergo functional Magnetic Resonance Imaging (fMRI) while completing an auditory regularity-learning task involving a duration deviant oddball paradigm with either volatile or stable statistics, and under either threatening or safe conditions. Volatility was induced in half the blocks by unpredictably reversing the probabilities of the two tones, and threat was independently induced in certain blocks by providing text-based warnings of an imminent uncomfortable electric shock. Our preliminary results show that threat significantly increased participants' anxiety ratings and reduced their accuracy in the regularity learning task, without impacting confidence; whereas volatility reduced both accuracy and confidence. We found that the head of the caudate, the right inferior frontal gyrus and the left superior temporal gyrus were more active in threatening than safe conditions. The right posterior cingulate cortex was more active in volatile than stable conditions. These findings suggest that a frontotemporal-striatal network and the cingulate cortex, underpin learning impairments caused by imminent threat and environmental volatility respectively.

id #9404

Hypoxia-induced cleavage of the p75 neurotrophin receptor mediates apoptosis in PC12 cells

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Neurodegeneration of cholinergic basal forebrain neurons (cBFNs) in obstructive sleep apnoea may explain the cognitive decline observed in these patients. cBFNs express the p75 neurotrophin receptor ($p75^{NTR}$), a regulator of neuronal death that can undergo regulated intramembrane cleavage in response to hypoxia. By employing a wild-type, $p75^{NTR}$-expressing PC12 cell line and a PC12/sh-p75 cell line expressing a $p75^{NTR}$-silencing RNA, this study aimed to evaluate the effect of hypoxia on hypoxia inducible factor 1α (HIF-1α) activity and to investigate whether cleavage of $p75^{NTR}$ can mediate apoptosis in response to chronic hypoxia. PC12 cells were differentiated and exposed to hypoxia for 4 to 48 hours. Immunocytochemistry and qRT-PCR were used to measure cell death, HIF-1α stabilisation, nuclear translocation and transcriptional activity in hypoxia. Cleavage of $p75^{NTR}$ was also modulated. Downregulation of $p75^{NTR}$ interfered with the stabilisation, nuclear translocation and transcriptional activity of HIF-1α in hypoxia. Hypoxia promoted $p75^{NTR}$ cleavage in PC12 cells and reduced their viability compared to normoxic

controls, whereas PC12/sh-p75 cell viability was unaffected. Enhancing $p75^{NTR}$ cleavage had no effect on cell survival in normoxia but increased the proportion of apoptotic cells compared to vehicle control following exposure to 24 hours of hypoxia. Inhibiting $p75^{NTR}$ cleavage with α and γ-secretase inhibitors decreased the number of apoptotic cells to levels comparable to normoxic PC12 cells. Hypoxia-induced $p75^{NTR}$ cleavage can induce apoptosis in PC12 cells, suggesting a possible mechanism underlying cBFN degeneration in response to hypoxia

id #9405

INVESTIGATING THE FUNCTIONAL ROLE OF THE ACTIVITY-ASSOCIATED MIR1271-5P IN VITRO BY DEEP SEQUENCING

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MicroRNA (miRNA) are short, non-coding RNAs which coordinate spatiotemporal fine-tuning of gene expression through post-transcriptional regulation of mRNA stability and translation. This regulatory capacity is particularly evident in the context of neuronal excitation, wherein many miRNA are known to exhibit activity-dependent patterns of expression for dynamic modification of neuronal structural and functional properties. In recent work, we have identified progressive upregulation of the brain-enriched miR-1271-5p in neuronally differentiated SH-SY5Y cells in response to K^+ membrane depolarisation, suggesting an important role in excitation-associated gene expression. To extend these findings, we overexpressed miR-1271-5p in neuronally differentiated SH-SY5Y cells and performed RNA sequencing (RNA-Seq) and ribosome profiling (Ribo-Seq) to investigate and profile the effects of miR-1271-5p gain-of-function on neuronal mRNA abundance and translational status. We found that miR-1271-5p overexpression substantially remodelled the neuronal transcriptome, with 7,849 genes exhibiting significant differential expression ($p < 0.05$, BH fdr < 0.05). Gene-set enrichment analysis of these differentially regulated genes indicated a predominant overrepresentation of biological processes relating to cytoskeletal organisation and extracellular matrix interactions. At the translational level, we observed significant differential translation of 804 genes, with functional enrichment similar to findings at the RNA-Seq level. Analysis of computationally predicted miR-1271-5p targets (TargetScan v7.2) revealed these genes were more strongly regulated at the level of mRNA steady-state abundance, suggesting mRNA destabilisation is the predominant mechanism of miR-1271-5p function. These analyses implicate miR-1271-5p in the regulation of genes associated with cytoskeletal and extracellular matrix remodelling, and suggest it plays a crucial role in neuronal activity-dependent morphology.

id #9406

From cognition to psychosis: identifying translational tests of schizophrenia neurobiology

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Even though our knowledge of the neurobiology of schizophrenia has advanced considerably, drug development is almost at a standstill. This is in part because there exists a large disparity between the tests we use for 'psychotic symptoms' in animal models and the underlying neurobiology in people with schizophrenia. We are interested in the associative striatum (aSTR) because it may represent a common area involved in the psychotic and cognitive symptoms of schizophrenia. The aSTR is the site of the increased dopaminergic transmission underlying psychotic symptoms and is densely innervated by the higher cortical areas underlying many of the cognitive symptoms in schizophrenia. Goal-directed behaviour is impaired in schizophrenia and also highly dependent on aSTR function. Therefore, we are using the outcome-specific devaluation task (ODT) to test goal-directed action in humans and rodents. Studies assessing ODT performance in first-episode psychosis and those with chronic schizophrenia are underway. Our animal studies using this task are probing the role of underlying dopaminergic dysfunction. Our results from both humans and rodents demonstrate the ODT is highly translatable and is a sensitive measure of goal-directed action. Moreover, increased dopaminergic transmission during action-outcome learning in mice impairs goal-directed action but not reward valuation, reflecting findings from previous studies in people with schizophrenia. Our data suggest the ODT is a cognitive task sensitive to the underlying dopaminergic dysfunction associated with psychotic symptoms in those with schizophrenia. Thus, it may be a useful tool for studying the interactions between the cognitive deficits and psychotic symptoms observed in schizophrenia.

id #9407

Mutations in Paraplegin *SPG7* gene lead to impaired mitochondrial function and oxidative stress in patient-derived stem cells

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Hereditary spastic paraplegia (HSP) is a neurological disorder characterised by degeneration of long axons along the corticospinal tract, leading to lower limb weakness, spasticity and paralysis. To date, more than 75 disease-loci and 60 genes are known to cause HSP**.** Spastic paraplegia type 7 (*SPG7*) is the most common form of autosomal recessive HSP accounting up to 12% of all autosomal recessive HSP cases**.** The cellular mechanisms for the axonal degeneration in HSP are unknown.

Our innovative strategy is to use two patient-derived stem cell models, olfactory neurosphere-derived stem (ONS) cells and cortical neurons from induced pluripotent stem (iPS) cells to evaluate disease-specific cell function defects and identify potential drug treatment.

Our evaluation using patient-derived ONS cells from *SPG7* patients and healthy controls suggests that the disease mutations cause impairment in mitochondrial function such as altered mitochondrial morphology (decreased interconnectivity, reduced mass), reduction in ATP content, increased mitochondrial membrane potential, altered oxygen consumption rate, increased mitochondrial oxidative stress, increased permeability of the mitochondrial permeability transition pore and increased cellular oxidative stress. Axonal transport is ATP dependent and impaired axonal transport

in neurons precedes axonal degeneration. To evaluate if reduced ATP in SPG7 patient cells leads to the axonal degeneration observed in HSP patients, we have generated cortical neurons from patient-derived iPS cells. This understanding is essential to screen drugs that can rescue these disease-specific defects.

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id #9408

The Munc18-1 domain 3A loop controls Munc13-1 nanoscale organization during SNARE assembly

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The exocytosis of neurotransmitters from secretory vesicles forms the basis for neuronal communication and thus needs to be tightly regulated. The presynaptic proteins Munc13-1 and Munc18-1 are key regulators for the priming of vesicles prior to SNARE mediated release. However, it is not fully understood yet how Munc13-1 and Munc18-1 are organised at nanoscale level to perform this priming function. Is there a hierarchy in their action? Previous results have shown that Munc18-1 is organised in nanocluster and that secretagogue stimulation triggers the exit of Munc18-1 from the confinement of nanocluster. Importantly, a hinge loop in Munc18-1 domain 3a regulates SNARE assembly through opening Syntaxin-1a. Here, we apply single particle tracking Photoactivated Localization Microscopy (sptPALM) to analyse the nanoscale organisation of Munc13-1 and Munc18-1 in live neurosecretory cells engineered to knockout Munc18-1 and -2. Our results indicate that Munc18-1 hinge loop 3a also controls the confinement of Munc13-1 in nanodomains of the plasma membrane in response to stimulation. This suggests that Munc18-1 is a master regulator of priming and not only promotes the opening and engagement of Syntaxin1A in SNARE assembly but also controls Munc13-1 clustering on the plasma membrane.

id #9409

Increased oxidative stress and apoptotic cell death is closely correlated with reactive astrogliosis, altering their structural and functional properties in the aging retina

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Aging affects many structural and functional properties regulated by astrocytes such as synaptic plasticity, gap junction communication and water/ion metabolic balance. Cumulative oxidative stress alters glial phenotypic and gene expression initiating aberrant changes in cell viability, leading to dysfunction and impaired responses. In this study, we examined the retina as an ideal model of the CNS. We used a methodological combination of advanced technologies and high-end applications in multiplex immunohistochemistry, confocal laser microscopy, online emission fingerprinting and Imaris image processing, to provide well-defined qualitative and quantitative data analysis. Retinal whole-mounts were prepared from Wistar rats of four distinct age groups that included developing (postnatal day 0, 2, 5 and 12), young adult (3 months old), middle aged (9 months old), and aged (18, 22 and 31 months old). Our main findings show that aging leads to overall increases in oxidative stress (i.e. decreased NAD+/NADH ratio content, increased protein carbonyl formation and PARP enzymatic activity), as well increase in the number of apoptotic cell death among astrocytes and retinal ganglion cells. In our western blot and immunoreactivity data, we showed an age-related decline in drebrin, ezrin, Cx43, nestin, vimentin, pax2 and betaIII-tubulin, except for GFAP, in the retina. We further illustrated that astrocytic metabolic marker GS, KATII and AQP4 are highly expressed in the middle-aged. Our study shows that the NAD+/NADH ratio is necessary for maintenance of normal cellular function, and a direct link exists between oxidative stress, cell death and retinal aging.

id #9410

Rewiring of forebrain serotonergic inputs following chronic consumption of alcohol or sugar.

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Addiction is a chronic, relapsing brain disease that is characterized by compulsive drug seeking and use, despite harmful consequences people with substance use disorders usually have symptoms of depression and anxiety. Alterations in the serotonin (5-HT) system has been linked to various psychiatric disorders including anxiety, depression, compulsivity and addiction. However, the serotonin system is complex and serotonin can modulate the activity of both excitatory and inhibitory synapses at both a presynaptic and postsynaptic level. Recent studies have further shown that a subtype of serotonin neurons can store and co-release glutamate (GLU) via the vesicular glutamate transporter 3 (VGLUT3). Co-release of 5-HT and GLU in particular regions of the limbic system has been proposed to play a pivotal role in the development of certain pathologies like anxiety, depression and drug abuse. We therefore investigated the consequences of chronic substance abuse on the plasticity of the the different types of serotonin innervation in the limbic system, using a model of long-term binge-drinking of alcohol or sucrose in mice. We found that chronic exposure to alcohol or sugar similarly rewires the serotonin inputs innervating the limbic brain especially in regions involved in the regulation of emotional behaviours (extended amygdala) and the generation of new brain cells - neurogenesis (hippocampus), suggesting that emotional and neurogenic deficits following chronic substance abuse could be mediated by profound alterations in serotonin neuron plasticity.

id #9411

Defining microstructural components of the pelvic nerves to inform development of new neuromodulation devices for urological conditions

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Bioelectronic medicine is expanding into the visceral nervous system to develop new approaches for treating organ dysregulation and visceral pain. The pelvic splanchnic nerves have been proposed as sites suitable for lower urinary tract neuromodulation. These nerves are complex, containing preganglionic parasympathetic, sacral sensory and sympathetic postganglionic axons that are critical for function of urogenital organs and the lower bowel. We focused on the rodent equivalent of these nerves, the pelvic nerves. Our goal was to define the location of functional subclasses of axons within the pelvic nerves to determine principles of organisation that could direct the design of new electrical modulation devices and strategies. Major pelvic ganglia with attached pelvic, cavernous, accessory and hypogastric nerves were micro-dissected immediately post-mortem from young adult male Sprague-Dawley rats (6-8 weeks; n=14), fixed and processed for fluorescence immunohistochemistry as whole mounts. The pelvic nerves were composed of discrete fascicles of axons, which merged to connect with the major pelvic ganglia. High resolution confocal microscopy showed that sensory axons immunoreactive for calcitonin gene-related peptide were dispersed through the larger fascicles but aggregated within a discrete region of the smaller fascicles. After entering the pelvic ganglia, some of these fascicles projected directly into the cavernous nerve. Sympathetic axons immunolabelled with tyrosine hydroxylase were aggregated in the core of each fascicle, whereas myelinated sensory axons immunolabelled for neurofilament-M were limited to the periphery. Together these studies reveal distinct locations within the pelvic nerves of different axon classes, which may provide strategies for selectively modulating their function.

id #9412

PARATRIGEMINAL PROCESSING OF JUGULAR AFFERENTS IN THE CONSCIOUS PERCEPTION OF AIRWAYS IRRITATION

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The urge-to-cough is common in humans and is defined as the conscious perception of an airway irritation that precedes the motor act of coughing. It has not been studied in animals and therefore the specific airway afferents involved remain unknown. We have previously shown that nodose-derived vagal afferents project to the nucleus of the solitary tract while jugular-derived vagal afferents project to the paratrigeminal nucleus (Pa5) in the brainstem. The Pa5 is a likely candidate for processing afferents relating to the urge-to-cough given its connectivity with nociceptive brain circuits. We aimed to investigate the urge-to-cough in conscious guinea-pigs, using whole body plethysmography while quantifying changes in animal behaviour associated with airway irritation evoked by nebulized bradykinin (BK) or Adenosine 5'-Triphosphate (ATP), known to differentially activate vagal afferents. Similarly, to the urge-to-cough in humans, the time to first behaviour preceded the onset of cough for both BK (9.25 ± 2.2 vs 28.9 ±10min) and ATP (10±3.2 vs 35.2±1.3min); $p<0.0001$. We then assessed the effect of lesioning the Pa5 using substance P conjugated to saporin, since jugular ganglia neurons highly express the neuropeptide substance P. Pa5 lesions inhibited BK-evoked cough (7.2±2.8 vs. 0±0 coughs, 1mg/ml BK; $p=0.04$), which was accompanied by a 50% reduction in associated sensorimotor behaviours. Interestingly, Pa5 lesions had no effect on cough or behaviours evoked by the selective nodose afferent stimulus, ATP. These data implicate the jugular-Pa5 processing pathway in respiratory sensations associated with cough, which may lead to better treatment of perturbed respiratory sensations in disease.

id #9413

Effects of epilepsy-causing mutations found in GABA type-A receptor on inhibitory synapse

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The GABA type-A receptor (GABAAR) is a major genetic target for heritable human epilepsies. Here we examine the functional effects of epilepsy-causing mutations to the α1, γ2 and β3 subunits on inhibitory postsynaptic currents (IPSCs) mediated by synaptic GABAAR isoforms α1β2γ2L and α1β3γ2L. We employed a neuron-HEK293 cell hetero-synapse preparation to record IPSCs mediated by mutant-containing GABAARs in isolation from other GABAAR isoforms. We then expressed the mutant subunits in cortical neurons to evaluate the impact that epilepsy-causing mutations have on miniature IPSCs in neurons. Finally fluorescently labelled mutant subunits were transfected into cultured cortical neurons to investigate changes in neuronal morphology, synapse formation and GABAAR mobility using super-resolution fluorescence microscopy.

We found that IPSCs mediated by the mutant subunits induced IPSCs that differ from those mediated by *wild type* receptors in hetero-synapses and in cortical neurons. IPSCs produced by GABAARs with mutant subunits linked to febrile seizures also exhibited an increased temperature sensitivity. We successfully applied Vorinostat (SAHA) to rescue surface expression of the mutant subunits prone to retention in the endoplasmic reticulum. Super-resolution microscopy revealed changes the number of GABAARs found at synapse and in extra-synaptic regions. Dynamic exchange between synaptic and extra-synaptic regions was also altered. In addition, we observed mutation-specific changes to synaptic bouton size, synapse number and neurite branching. These results provide new insights into the mechanisms of epileptogenesis and suggest possible leads for improving treatments for patients harbouring these mutations.

id #9414

Molecular Control of the Neuronal Diversity in the Developing Striatum

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The medial ganglionic eminence (MGE) is an embryonic structure that generates the majority of interneurons of the striatum. Combinatorial interactions of transcription factors are thought to produce specific interneuron subtypes. Here, we dissected the molecular functions of the Er81 transcription factor in the developing striatal interneurons. We reveal that Er81 promotes cell identity by repressing genetic programs in the MGE. Er81-expressing cell subtypes display specific molecular, morphological and electrophysiological properties. The specific ablation of the Er81 gene expression in interneurons leads to deep changes in cell properties which strongly impacts on the function of the striatum and basal ganglia. This study highlights a critical modulator of cell specification, circuit assembly, and physiology of striatal interneurons. It represents the first

comprehensive analysis of Er81 transcription factor function in the developing brain and provides fundamental information to elucidate the principles of striatal wiring and activity.

id #9415

Heterodimerization of UNC-13/RIM regulates synaptic vesicle release probability but not priming

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UNC-13 proteins play an essential role in synaptic transmission by recruiting synaptic vesicles (SVs) to become available for release, which is termed SV priming. Here we show that the C2A domain of UNC-13L, like the corresponding domain in mammalian Munc13-1, displays two conserved binding modes: forming C2A/C2A homodimers, or forming a heterodimer with the zinc finger domain of UNC-10/RIM (C2A/RIM). Functional analysis revealed that UNC-13L's C2A promotes synaptic transmission by regulating a post-priming process. Stimulus-evoked neurotransmitter release but not SV priming, was impaired in unc-10 mutants deficient for C2A/RIM heterodimerization, leading to decreased release probability. Disrupting C2A/C2A homodimerization in UNC-13L-rescued animals had no effect on synaptic transmission, but fully restored the evoked release and the release probability of unc-10/RIM mutants deficient for C2A/RIM heterodimerization. C2A/RIM heterodimerization was not required for normal synaptic depression and recovery. Thus, our results support the model that C2A/RIM binding releases UNC-13L from an autoinhibitory homodimeric complex to become fusion-competent, and that the heterodimerization itself does not produce an additional role in synaptic transmission.

id #9416

Neuroprotective effects of an ellagitannin and a gut microbial-derived metabolite against Alzheimer's disease

id #9417

Neuroprotective effects of an ellagitannin and a gut microbial-derived metabolite against Alzheimer's disease

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Alzheimer's disease (AD) is the second leading cause of death in Australia. The AD brain is characterized by amyloid β (Aβ) protein deposits and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein. The absence of effective drug therapies for AD has resulted in alternate approaches such as nutrition-based lifestyle interventions for the prevention and treatment of AD. Punicalagin is the abundant type of polyphenol (ellagitannin) in pomegranates. Upon ingestion, punicalagin is hydrolyzed to ellagic acid (EA) and enters the circulation in small quantities via the small intestine. However, on entering the colon, EA is extensively metabolized by gut microbiota into smaller molecules known as urolithins. Urolithins are highly bioavailable and reach the brain through the blood-brain-barrier. Therefore, urolithins are the bioactive metabolites responsible for numerous health benefits exerted by punicalagins. The objective of the present study was to investigate the effect of punicalagin and urolithin A in combating the AD neurotoxic peptide, $A\beta_{(1-42)}$-, *in vitro*. The neuroprotective properties of the compounds on $A\beta_{(1-42)}$-induced neurotoxicity was measured using cultured human neuroblastoma BE (2)-M17 cells. After exposure to 20μM $A\beta_{(1-42)}$ for 72 h, the neuroblastoma cells exhibited a marked decrease of cell viability. Twenty four hours pre-treatment of punicalagin (5-40μM) as well as urolithin A (10-40μM) with 20μM $A\beta_{(1-42)}$ significantly protected the cells from $A\beta_{(1-42)}$-induced toxicity. The results indicate that both punicalagin and urolithin A inhibit $A\beta_{(1-42)}$-induced neuronal death but punicalagin was more effective. Further studies are underway to determine the underlying rescue mechanisms of the compounds in AD pathology.

id #9418

Microglial role in the neurovascular unit and changes during diabetic retinopathy.

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Objective: Blood flow in the retina is crucial for normal neuronal function. Vaso-dysregulation therefore plays a prominent part in diseases of the retina such as diabetic retinopathy. We investigated a novel role for microglia, the primary immune cells of the retina, in vascular regulation in the healthy and diabetic retina.

Findings: The microglial-vascular relationship was investigated using $Cx3cr1^{GFP/+}$ and $Cx3cr1^{GFP/GFP}$ transgenic mice, in which the microglia receptor Cx3cr1 is replaced with green fluorescent protein; as well as dark agouti rats, which were rendered diabetic by administration of Streptozotocin (55mg/kg).
Microglia were seen to contact neuronal synapses and the vasculature in the healthy retina (immunohistochemistry and transmission electron microscopy). In response to administered fractalkine, the sole ligand for Cx3cr1, blood vessels constricted in areas of microglial contact, a response

which was absent in animals deficient in Cx3cr1 (live cell imaging of retinal explants). Microglia were also observed to express a range of vaso-modulatory genes (RNA-Seq of FACS isolated microglia). After 4 weeks of diabetes, microglia increased contact with the capillary network and upregulated expression of the potent vasoconstrictor angiotensin. This occurred concurrent with increased constriction of fine capillaries and reduced retinal blood flow (*in vivo* retinal imaging). **Conclusion**: Microglia participate in the neurovascular unit, expressing a range of vasoactive genes and responding to fractalkine to alter blood vessel diameter in the retina. This novel finding increases knowledge of blood flow regulation in the central nervous system. In diabetes, and potentially other vascular diseases, dysregulation of this process may exacerbate pathology.

id #9420

Investigating the pattern of axonal injury in traumatic brain injury of varying intensity in a large animal model

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Traumatic brain injury (TBI) is the leading cause of death and disability in those aged less than 45. TBI induced axonal damage is a key cause of neurological impairment. To facilitate a greater understanding of its pathophysiology, modelling of TBI within gyrencephalic brains, which respond differently to shear strain than lissencephalic brains, is required. Consequently, this study characterised the distribution and degree of axonal injury following TBI at varying intensities (13 or 15 charge) in an ovine model, with confirmation of injury via alteration in blood pressure (BP) response. TBI was induced in anaethetised Merino wethers (n=3/charge), to the right temporal area between the zygomatic arch and coronoid process. Invasive BP was monitored for 4 hours post-injury, prior to formalin perfusion and brain removal. Axonal injury was assessed via staining with the amyloid precursor protein (APP). Injury led to an immediate significant decrease in BP compared to shams, as calculated relative to pre-injury baseline, that was similar in both injury groups (13=-10.9±1.5, 15=-10.7±1.3 vs sham=+2.0±0.65, $p<0.01$). BP returned to sham level within 10 minutes post-injury in both groups. In line with the similarity of the BP response, both injury groups had similar amounts of axonal injury. Moderate axonal injury was noted within the internal capsule and corona radiata, with minimal involvement of the corpus callosum. This pilot study indicates that moderate axonal injury is associated with an immediate drop in BP post-TBI. This contrasts with severe injury where BP increases and indicates a differing physiological response depending on injury severity.

id #9421

Differences in the orientation preferences of the spiking activity and the local field potentials recorded from the primary visual cortex of cats and macaques.

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Local field potentials (LFPs) and spiking activity of neurons, recorded from a single neuron or from a small collection of cells (Multiunit activity, or MUA) are commonly used measures to characterise neuronal responses. While the MUA, which is fairly localised, represents the outputs of neurons, the larger spatial scale signal of the LFP is said to reflect the local presynaptic, synaptic and dendritic activities of many neurons. We explored the relationship between LFP and MUA by using linear electrode arrays to record from the primary visual cortex of anaesthetised cats (n=2) and macaques (n=2) and calculated the preferred stimulus orientations of the LFP and MUA. We found that the preferred orientations of the two signals were uncorrelated (n= 64 sites; Spearman's rho= 0.17, p=0.18). Preferred orientations of the MUA varied systematically across the electrodes, but the LFP signal was tuned to a narrow range of preferred orientations. In macaques, we related the preferred orientation of the MUA and LFP to the radial orientation (line through receptive field and foveal centres). We found that the LFP was predominantly tuned to the radial orientation (n= 22 sites; χ^2= 8.18; df=3, p=0.04), but the MUA showed no such relationship (n=22; χ^2=3.09; df=3, p=0.38). The radial bias seen in the LFP reflects the radial bias reported sub-cortically in macaques, further suggesting that the LFP signal reflects the inputs to the site. The full range of orientation preferences observed in the MUA may then be elaborated from broad orientation biases established sub-cortically.

id #9422

The menopausal eye: loss of circulating estrogen does not alter the retina

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Hormonal withdrawal has been considered a risk factor for neurodegenerative diseases of the eye, such as glaucoma and age-related macular degeneration. In the case of menopause, the female sex hormones estrogen and progesterone are no longer produced by the ovaries and circulating levels of estrogen are severely reduced. The retinal tissues of the eye express estrogen receptors and it is assumed that the reduction of estrogen as seen in menopause has direct affects on the retina. This study will test the affect of estrogen deprivation on mammalian retina using genetically modified mice. The mice lack a key enzyme in the synthesis of estrogen (aromatase enzyme - Cyp19) and are unable to produce estrogen. Male and female mice (C57/Bl6 and aromatase knock-out (ArKo)) were aged to 10 months. At termination the eyes were collected, fixed, sectioned, stained and imaged. There were no differences detected between estrogen deprived mice (ArKo) in retinal morphology (H&E staining), oil deposition (Oil red labeling), retinal cell death (TUNEL labeling) and quantification of a retinal stress marker (GFAP expression). There were sex differences in GFAP expression, with higher levels of stress markers in female compared to male retinas. Our results demonstrate that chronic deprivation of estrogen does not alter the retina. These results suggest that the loss of estrogen that occurs during menopause does not affect the retina directly and rather alternative hormone changes (e.g. reductions in testosterone or progesterone) maybe responsible.

id #9423

A NOVEL METHOD TO ASSESS RECOVERY FOLLOWING STROKE: A BIOMECHANICAL AND NEUROLOGICAL OUTCOME APPROACH IN A LARGE ANIMAL MODEL

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Cerebral oedema and elevated intracranial pressure are the leading cause of death in the first week following stroke. Despite this, current treatments are limited and fail to target underlying pathophysiology, highlighting the need for novel treatments. When screening promising agents it is essential to use clinically-relevant large animal models to improve the likelihood of successful clinical translation. However, determining therapeutic benefit on long-term outcome in such models remains a significant hurdle. The current study sought to develop a biomechanical model of motor function to assess gait, in addition to developing a post-stroke outcome assessment scale in an ovine stroke model.

Merino sheep (6M;6F) were subject to 2hrs middle cerebral artery occlusion. Functional testing and neurological assessment was carried out 1,3,5,7,14,21 and 28 days following stroke. Gait was assessed using 3D motion capture and a blinded neurological assessment score was carried out to determine changes in appetite, postural reactions, circling behaviours and demeanour. Preliminary findings indicate that our method for motor assessment was able to consistently determine differences in gait post-stroke at all time points, with the most profound differences observed from days 1-7. Furthermore, our neurological assessment scale was able to reliably indicate differences in animal function as determined by aforementioned measures.

We have developed a robust and reproducible ovine functional assessment measure that can be used to quantify differences in gait and demeanor following stroke. This method will be used in future studies to determine the efficacy of the novel therapies targeting cerebral oedema and therefore outcome following stroke.

id #9424

Streamlined sensory motor communication through cortical reciprocal connectivity in a visually guided eye movement task.

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Cortical computation is distributed across multiple areas of the cortex by networks of reciprocal connectivity. However, how such connectivity contributes to the communication between the connected areas is not clear. In this study, we examine the communication between sensory and motor cortices. We develop an eye movement task in mice and combine it with optogenetic suppression and two-photon calcium imaging techniques. We identify a small region in the secondary motor cortex (MO_S) that controls eye movements and reciprocally connects with a rostrolateral part of the higher visual areas ($V_{RL/A/AL}$). These two regions encode both motor signals and visual information. However, the information flow between the regions depends on the direction of the connectivity: motor information is conveyed preferentially from the MO_S to the $V_{RL/A/AL}$, and sensory information is transferred primarily in the opposite direction. We propose that reciprocal connectivity streamlines information flow, enhancing the computational capacity of a distributed network.

id #9425

Phasic activity of amygdala neurons during concurrent Pavlovian and instrumental aversive learning

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Aversive reinforcement, such as delivery of footshock, can have two distinct consequences for learning and behavior. First, it supports learning about its environmental antecedents to imbue such stimuli with the ability to elicit conditioned responses (Pavlovian fear conditioning). Second, it supports learning about its behavioural antecedents and alters the probability that these behaviors will be emitted again in the future (punishment). The amygdala is essential to both fear and punishment but whether and how these might be differentially encoded in amygdala is unknown. We report a novel within-subjects task permitting concurrent assessment of these two different forms of learning in the same animals during the same sessions. We show that animals can concurrently learn both Pavlovian and instrumental aversive associations and that these exert contrasting control over reward behaviour. We then use a genetically-encoded calcium indicator expressed in CaMKII neurons to describe the profiles of amygdala neuron activity associated with various events and behaviours within this task.

id #9427

REVISITING THE BIMODAL CLASSIFICATION OF CELLS IN PRIMARY VISUAL CORTEX

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Neurons in the mammalian primary visual cortex (V1) are originally classified as either simple or complex based on their receptive field structures. Subsequently, studies have mainly used oriented drifting grating stimuli to classify V1 cells: simple cells are sensitive to spatial phase of the stimulus while complex cells are phase invariant. Here, we investigated whether the bimodal distribution of V1 phase sensitivity could be observed using other visual stimuli regularly employed in V1 studies. In the first study, we recorded the spike rate responses of cat V1 cells using pixelated Gaussian

white noise stimuli. The single-cell response was plotted as a function of the degree to which phase sensitivity was present in each noise sample. The width at half maximum of the responses (phase bandwidth) was used as a measure of phase sensitivity. In the second study, we examined the subthreshold responses of cells in mouse V1 using grating stimuli that are spatially stationary but modulate contrasts over time (contrast-reversing). The phase sensitivity of each cell was measured as response modulation ratios. In both cat and mouse V1, we observed a group of cells that showed mixed complex and simple receptive field characteristics and exhibit a broad range of spatial phase sensitivities. The phase sensitivities in each study showed clear unimodal distribution across V1 cell population. These findings challenge the current view of a bimodal division between simple and complex cells based on their phase sensitivity in the primary visual cortex.

id #9428

Poly-arginine peptides improve functional recovery and reduce neuroinflammation following traumatic brain injury

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Cationic arginine-rich peptides (CARPs) such as the poly-arginine R18 and R18D peptides have demonstrated neuroprotective efficacy in *in vitro* neuronal excitotoxic injury and *in vivo* rat models of stroke. These peptides have also demonstrated significantly reduced axonal injury and positive trends for functional recovery in a rat model of traumatic brain injury (TBI). Currently, there are no neuroprotective pharmacological treatments for TBI. Since poly-arginine peptides have demonstrated neuroprotective potential, both R18 and R18D were further characterised in a rat model of TBI. Using a weight-drop impact-acceleration apparatus, a closed-head TBI was induced in male rats. A dose-response study using R18D (100, 300, 1000 nmol/kg) administered intravenously at 30-min post-injury was conducted in Sprague-Dawley rats. A second study in Long-Evans rats examined R18 and R18D at a dose of 1000 nmol/kg administered intravenously at 30-min post-injury. In the Sprague-Dawley rat, doses of R18D at 100 and 1000 nmol/kg significantly reduced the extent of axonal injury in the corpus callosum, and demonstrated significant improvement in learning and memory outcome. In the Long-Evans rat, both R18 and R18D significantly reduced astrocytic activation, and reduced IL-6 levels in the brain following injury. R18D was particularly effective at improving sensorimotor function and generally produced more favourable functional outcomes than R18. Poly-arginine peptides have neuroprotective effects following TBI and warrant continued investigation as a novel therapeutic for TBI.

id #9430

Usp9x-null Mice Show Corpus Callosum Dysgenesis and Altered Behaviour

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Autism spectrum disorder (ASD) is a form of neurodevelopmental disorder that manifests in three major diagnostic features: deficits in social interactions, verbal and non-verbal communication, and restricted repetitive behaviour. Interestingly, the comorbidity of Intellectual Disability (ID) and ASD sits at around 60%. One of the genes that is implicated in ID and ASD is the deubiquitylating enzyme ubiquitin specific peptidase 9, X-linked (Usp9x). Using a conditional knock-out (cKO) mouse model, current study investigates the role of Usp9x in the pathophysiology associated with ID and ASD.

The thickness of the corpus callosum was measured from the rostral to caudal aspect of adult Usp9x cKO brain at the midsagittal line on coronal sections. Hematoxylin staining revealed corpus callosum dysgenesis of the cKO brain where axonal tracts failed to project across the midline. A significantly thinner corpus callosum was observed in the cKO brains.

Despite the unaltered overall cortical thickness, a significant decrease in the cellular density in the somatosensory and somatomotor cortices were observed in the cKO brains compared to controls. Anxiety traits were also observed in the cKO mice when observed in a home-cage setting for using the Phenomaster, elevated plus maze, and open field maze.

These findings show that the deletion of Usp9x in the cortex leads to corpus callosum dysgenesis and cortical dysplasia. Further experiments are warranted to characterise ASD-like behaviours in Usp9x KO mice and link them to the underlying mechanisms, which may help to identify novel therapeutic targets for ASD.

id #9431

Fyn and Tau under the nanoscope

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Deposits of amyloid-β (Aβ) and hyperphosphorylated Tau protein constitute the histological hallmark lesions of Alzheimer's disease (AD). The dendritic spines have been proposed to be one of the cellular structures involved in the initiation and progression of AD. The Src kinase Fyn has a critical role mediating the Aβ dendritic toxicity. Fyn activity is affected by oligomeric Aβ and can modulate Tau local translation into the cell body of neurons[1]. Conversely, Tau is required for efficiently targeting of Fyn to the dendritic compartment[2], suggesting a mutual effect of these two proteins of crucial importance for the development of AD. As signalling molecules such as Fyn are increasingly found to act within the confinement of nanoclusters, we wondered whether Fyn was compartmentalised in such clusters in dendrites and whether Tau affect such nanoscale organization.

We have used sptPALM (single particle tracking photo-activated localization microscopy) to assess the nanoscale distribution of Fyn-mEos in dendrites and examine the effect of Tau knockout and rescue.

We have discovered that Fyn is organised in nanoclusters often found in spines. Tau knockout and rescued expression affects Fyn nanoscale organisation. Understanding the molecular mechanisms involved in controlling the nanoscale dynamic organisation of these two crucial proteins during AD could open new strategies to fight against this devastating neurological disorder.

1. 1. Li C, Gotz J. Somatodendritic accumulation of Tau in Alzheimer's disease is promoted by Fyn-mediated local protein translation. EMBO J. 2017 Nov 2;36(21):3120-3138.
2. 2. Ittner LM, et al., Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell. 2010 Aug 6;142(3):387-97.

id #9434

test

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test

id #9436

PERIPHERAL MONITORING OF NEURODEGENERATION IN FRONTOTEMPORAL DEMENTIA AND HEALTHY CONTROLS USING CELL-FREE DNA METHYLATION

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Neurodegeneration occurs in a variety of human diseases including Frontotemporal Dementia (FTD). Currently, longitudinal neuroimaging represents the only way to assess neurodegeneration in-vivo, typically following symptom onset. Defining biomarkers of the presymptomatic disease will be important for future therapeutic trials. The development of blood tests to detect neurodegeneration would provide an economical screening method that could be applied earlier in the disease course and easily deployed at the population level. Cell-free DNA (cfDNA) derived from brain tissue holds great potential for the detection and monitoring of neurodegeneration through the blood. Within our lab we exploit the unique DNA methylation profiles of brain cells to create molecular diagnostic assays capable of detecting brain-derived cfDNA within peripheral blood plasma. Using longitudinal structural MRI, we have classified cases with Frontal Cortical (FC) and/ or Cerebellum (CRB) neurodegeneration. Using novel Next Generation Sequencing technology and Bioinformatic approaches we have completed the first proof-of-concept experiments for cfDNA analysis in a population of FTD and Healthy Controls. We found evidence of brain-derived cfDNA within 74% of samples analyzed (N=99). We were able to accurately differentiate cfDNA coming from the Cerebellum (CRB) compared to cfDNA derived from the Dorsolateral Prefrontal Cortex, establishing the first evidence of a peripheral biomarker with brain-region specificity. Interestingly, patients with greater CRB loss compared to FC loss by longitudinal imaging (CRB > FC) exhibited higher amounts of CRB-derived cfDNA then FC > CRB patients (longitudinal imaging). These studies provide important insights for future study design using a novel biomarker of neurodegeneration.

id #9438

Defining the role of transthyretin in regulating central nervous system myelination

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Thyroid hormones are essential for brain development and the regulation of oligodendrocyte maturation and subsequently, myelination. Insufficient populations of oligodendrocytes required to remyelinate axons affected by demyelinating diseases such as multiple sclerosis result in neurodegeneration and disease progression.

Transthyretin is a thyroid hormone distributor protein which facilitates movement of thyroid hormones in the periphery and more specifically across the blood-cerebral spinal fluid barrier and into cerebral spinal fluid. Interestingly, a hypermyelination phenotype was described in the corpus callosum of transthyretin-null mice, suggesting a possible role for transthyretin in regulating myelination. However, a role for transthyretin during remyelination is yet to be defined.

To investigate the role of transthyretin in central nervous system remyelination, a cuprizone-induced model of demyelination was used. The neurotoxicant cuprizone was added to normal rodent chow of adult wild type and transthyretin-null mice for 6 weeks. After the removal of cuprizone from their diet, spontaneous remyelination soon followed. To study rates of remyelination in the corpus callosum between mouse genotypes, tissue samples were collected at 3, 4 and 6 weeks after the removal of cuprizone from their diets. Tissue was prepared for electron microscopy and the myelin thickness was measured around remyelinated axons.

Remyelination was faster in transthyretin-null mice compared to wild type mice, suggesting the lack of transthyretin had increased the rate of remyelination of the adult mouse corpus callosum following cuprizone-induced demyelination. This result could to be an important step forward in devising potential therapies for combating demyelinating diseases such as multiple sclerosis.

id #9441

Sodium channel variants in febrile seizures: a prospective study of relationship to vaccination and outcome

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Febrile Seizures (FS) affect 2-5% of children and vaccination is a known risk factor. FS can be the first manifestation of epilepsies such as generalised epilepsy with febrile seizures plus (GEFS+) and of severe epileptic encephalopathies, particularly Dravet Syndrome. Over 80% of Dravet Syndrome cases are attributable to mutation in the sodium channel gene, *SCN1A*. Vaccination related FS thus raise the spectre of a poor outcome and has been inappropriately blamed as reason not to vaccinate. Common variants in *SCN1A* are also reported as risk alleles for FS. Here we report *SCN1A* sequencing in a prospective cohort recruited in Australian paediatric hospitals of children with first FS that was vaccine-proximate (VP-FS) (n=78), non-vaccine-proximate (NVP-FS) (n=92) and in children with no history of seizures (n=92). Molecular analysis was performed blind to clinical category. Sequence variants were compared across the three groups. We detected three pathogenic variants: two VP-FS cases (p.R568X and p.W932R), both of whom developed Dravet syndrome and one NVP-FS case (p.V947L). All three cases were under 12 months of age at the time of FS and all were generalised tonic-clonic seizures lasting more than 15 minutes. We also found enrichment of a reported FS risk allele, rs6432860-T, in all FS when compared to controls. We conclude that pathogenic *SCN1A* variants are infrequent amongst children with vaccine-related FS however, as evidence for the benefit of early treatment of Dravet syndrome accumulates, the case for *SCN1A* screening and early diagnosis in young children presenting with vaccine-proximate febrile seizures becomes stronger.

id #9447

The microtubule-stabilizing drug epothilone D resolves axonal degeneration following mild traumatic brain injury is age dependent

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Microtubule dynamics play an important role in the complicated development, structure, function and maintenance of the central nervous system. Microtubules are vulnerable to misalignment and dissolution in neurons and have been implicated in injury-induced glial responses following injury. However, there is no effective treatment for traumatic brain injury (TBI) to date. Thus, using a clinically relevant model of mild TBI in male Thy1-YFPH mice, we investigated the potential therapeutic effects of the brain-penetrant microtubule-stabilizing agent (MSA) epothilone D at 1 week and 4 weeks following a single mild lateral fluid percussion injury (LFPI) in young and adult mice. Our study found epothilone D did not alter cortical thickness, pyramidal neuronal number or size either in the young or adult brain. Moreover, no changes were observed in relation to astrogliosis in either age group following injury. Interestingly, epothilone D significantly resolved the injury related axonal degeneration in the external capsule by both 1 week and 4 weeks post-injury in the young mice but accentuated axonal degeneration in the external capsule by 4 weeks post-injury in the adult mice. Our finding may have important implication for MSA to be effective for manipulating neuroplasticity and the neuro-glia responses following brain injury, and the effect is age dependent, which suggests age-tailored MSA treatment following brain injury.

id #9448

Oligodendrocyte progenitor cell function is altered in transgenic mice carrying human mutations associated with amyloid accumulation and tau pathology

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In the central nervous system, myelin is produced by oligodendrocytes (OLs). Myelin loss is associated with healthy ageing but occurs more rapidly in people with Alzheimer's disease (AD). Oligodendrocyte progenitor cells (OPCs) proliferate and generate new oligodendrocytes in response to demyelinating events, and elevated OPC proliferation and differentiation have been reported in various AD transgenic mouse models in late stages of disease. In this study, we evaluate the membrane properties and behaviour of OPCs in the hippocampus and entorhinal cortex of transgenic mice in the early stages of developing amyloid (*APP* mutation; J20 mice) or tau (*MAPT* mutation; P301S mice) pathology. Whole cell patch clamp recording of OPCs from the CA1 of P30 and P100 control, *APP* and *MAPT* transgenic mice indicated that their membrane properties were unchanged, as was their response to 100μM kainate. However, P100 mice carrying the *APP* mutation responded more robustly to bath application of 100μM GABA. To assess whether this was associated with a change in OPC behaviour, we performed lineage tracing of OPCs in P60 *Pdgfra-CreER^{T2} :: Rosa26-YFP* transgenic mice, with and without the *APP* or *MAPT* transgenes. We found that OPC density was reduced in the hippocampus of *APP* mutants, however oligodendrogenesis was unchanged. By contrast, OPC density was normal in *MAPT* mutants, but newborn OL density increased in the hippocampus and entorhinal cortex, when compared with control littermates. Our results suggest that amyloid and tau pathology, characteristics of AD, differentially affect OPC function and behaviour in the early stages of disease.

id #9450

The poly-arginine-18 (R18) peptide is neuroprotective in a P7 rat model of perinatal hypoxic-ischaemic encephalopathy; a dose-response and therapeutic window study

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Introduction. Perinatal hypoxic-ischaemic encephalopathy (HIE) occurs in 1-3 per 1,000 live births and is the leading cause of infant mortality and morbidity (e.g. cerebral palsy and epilepsy), globally. Currently there are no clinically available neuroprotective pharmacotherapuetics to reduce brain injury and improve patient outcomes after HIE. Poly-arginine peptides (e.g. R18) have potent neuroprotective properties in *in vitro* and *in vivo* neuronal injury models. The aim of this study was to assess the efficacy of R18 in an animal model of HIE.

Methods. HIE model: ligation of common and external carotid arteries followed by hypoxia (92% N_2/8% O_2 for 2.5h). Peptide administered IP at 0, 0.5 or 1-hour post-hypoxia. Forty-eight hours after HIE, behavioural (righting reflex, negative geotactic response, and wire hang performance) and cerebral infarct volume assessments were performed. Fura-2 calcium kinetics was used to assess the ability of R18 to reduce excitotoxic intracellular calcium influx in primary cortical neuronal cultures.

Results. R18 administered 0, 0.5 or 1-hour after HIE reduced infarct volume by between 23.7% ($P < 0.01$) and 42.9% ($P < 0.001$). R18 also improved righting reflex ($P < 0.01$), negative geotactic reflex ($P < 0.01$), and/or rope hang time ($P = 0.02$) when administered after HIE. R18 also dose-dependently reduced excitotoxic neuronal calcium influx by up to 81% ($P = 0.0005$).

Conclusions. R18 reduces infarct volume and improves behavioural outcomes when administered up to 1 hour after HIE and mitigates excitotoxic neuronal calcium influx. These findings suggests R18 represents a promising and novel neuroprotective treatment for HIE.

1. Edwards et al., 2018. Assessment of therapeutic windoe for poly-arginine-18D (R18D) in a P7 rat model of perinatal hypoxic-ischaemic encephalopathy. Journal of Neuroscience Research.
2. Edwards et al., 2018. Poly-arginine R18 and R18D (D-enantiomer) peptides reduce infarct volume and improves behavioural outcomes following perinatal hypoxic-ischaemic encephalopathy in the P7 rat. Molecular Brain
3. Edwards et al., 2017. Modification to the Rice-Vannucci perinatal hypoxic-ischaemic encephalopathy model in the P7 rat improves the reliability of cerebral infarct development after 48 hours. Journal of Neuroscience Methods.

id #9451

Ablation of tau causes an olfactory deficit in a murine model of Parkinson's disease

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Parkinson's disease is diagnosed upon the presentation of motor symptoms, resulting from substantial degeneration of dopaminergic neurons in the midbrain. Prior to diagnosis, there is a lengthy prodromal stage in which non-motor symptoms, including olfactory deficits (hyposmia), develop. There is limited information about non-motor impairments and there is a need for directed research into these early pathogenic cellular pathways that precede extensive dopaminergic death in the midbrain. The protein tau has been identified as a genetic risk factor in the development of sporadic PD. Tau knockout mice have been reported as an age-dependent model of PD, and this study has demonstrated that they develop motor deficits at 15-months-old. We have shown that at 7-month-old tau knockout mice present with an overt hyposmic phenotype. This olfactory deficit correlates with an accumulation of α-synuclein, as well as autophagic impairment, in the olfactory bulb. This pathological feature becomes apparent in the striatum and substantia nigra of 15-month-old tau knockout mice, suggesting the potential for a spread of disease. Initial primary cell culture experiments have demonstrated that ablation of tau results in the release of α-synuclein enriched exosomes, providing a potential mechanism for disease spread. These alterations in α-synuclein level as well as a marked autophagy impairment in the tau knockout primary cells recapitulate results seen in the animal model. These data implicate a pathological role for tau in early Parkinson's disease.

id #9452

Auditory responses in zebrafish : investigating neurologic and behavioural sensory phenotypes of wildtype and fmr1 zebrafish

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The auditory systems of developing wildtype zebrafish have been investigated previously, but their hearing performance and auditory circuitry have yet to be described in detail in terms of frequency responses, sensitivity, and the brain regions and circuits carrying out auditory processing. This is due, in part, to the imprecise stimulus delivery systems that past researchers have utilised, and to the traditional barrier of observing large populations of individual neurons in vivo. Using selective plane illumination microscopy (SPIM) and imaging of genetically encoded calcium indicators during the direct delivery of auditory stimuli to the liquid media, we found that the neuronal responses of 6dpf larval zebrafish were consistently present for stimuli ranging from 100Hz to 2500Hz, with the initial analysis showing the presence of tonotopy in the brain. The results demonstrate that larval zebrafish auditory processing is more complex than previously reported, as are auditory processing regions and integration pathways. Our findings support previous accounts that larval zebrafish is a relevant model to study auditory function, signal processing and integration pathways. Additionally, with these detailed results on auditory processing, we are now able to accurately investigate auditory neural processing differences of populations that have atypical sensory experiences such as autism. We are currently implementing the above assay to study auditory processing in autism models of zebrafish, looking at differences within brain-wide, region-by-region and regional neural connectivity to determine if there is a auditory phenotype specific to an autism model of zebrafish.

1. Ausderau, Sideris, Little, Furlong, Bulluck, & Baranek, 2016
2. Baxendale, & Whitfield, 2016
3. Bretschneider, et al., 2012
4. Burgess & Granato, 2007
5. Jain et al., 2018
6. Bhandiwad, Zeddies, Raible, Rubel & Sisneros, 2013
7. Marsden & Grenato, 2015
8. Rotschafer & Cramer, 2017
9. Tabor et al., 2018
10. Vanwalleghem, Heap, & Scott, 2017

11. Sinclair, Oranje, Razak, Siegel, Schmid, 2016
12. Zeddies & Fay, 2005

id #9453

Neuroprotective sphingosine 1-phoshate is essential for beta secretase activity and amyloid formation, but paradoxically declines during normal ageing and Alzheimer's disease.

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Sphingosine 1-phosphate (S1P) is a potent vasculo- and neuro-protective signalling lipid that promotes neurotrophic growth factor expression and pre-synaptic acetylcholine and glutamate release. S1P is synthesized primarily by sphingosine kinase 2 (SphK2) in the brain. We recently demonstrated pronounced loss of S1P, and SphK2 activity, early in Alzheimer's disease (AD) pathogenesis. Using human hippocampal tissue samples from neuropathologically normal donors, we very recently showed that S1P levels decline with age in the hippocampus of females ($r = -0.5$, $P = 0.002$), leading us to speculate that loss of S1P sensitizes to AD development. To test whether SphK2 deficiency synergises with amyloid beta (Aβ) in promoting AD, SphK2 knockout (SphK2-/-) mice were crossed to the J20 mouse model of familial AD amyloidosis.

Suprisingly, SphK2 deficiency profoundly reduced Aβ content, plaque burden and reactive astrocyte immunoreactivity in J20 mice. Reduced Aβ burden could be attributed to loss of β-secretase activity, and was associated with significant improvements in hypersynchronous activity and cross-frequency coupling measured by hippocampal electroencephalography. Despite reduced amyloid burden, SphK2-deficient J20 mice exhibited severe hypomyelination in the hippocampus and cortex, and significant deficits in the Y-maze and social novelty memory tests, when compared to the J20 or SphK2-/- strains.

In summary, endogenous S1P, synthesized by SphK2, is reduced with ageing and AD pathogenesis, yet required for Aβ formation. However, memory deficits and myelin loss in J20 mice were exacerbated on a SphK2-/- background, indicating that age-dependent SphK2 depletion promotes neurodegeneration and urging caution in use of β-secretase inhibitors for AD.

id #9454

Nicotinamide mononucleotide (NMN) ameliorates chemotherapy-induced cognitive impairment

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The improvement in survival rates of cancer patients has rapidly increased due to the development of chemotherapeutic treatment regimes. While the success of these compounds has resulted in an increased number of cancer survivors, recent research has documented that cancer survivors are at an increased risk of serious health conditions. These include metabolic disorders, cardiotoxicity, infertility and neurocognitive deficits. One of the most prevailing side effect is chemotherapy-induced cognitive impairment (CICI), which is the deterioration of cognitive function after cessation of chemotherapy, for which currently there are no available treatments. SIRT1, a member of the sirtuin family and a NAD^+-dependent protein deacetylase, has been associated with neuroprotection and the regulation of various neurodegenerative disorders. Supplementation with NAD+ precursor, such as nicotinamide mononucleotide (NMN), can enhance sirtuin activity, and has been shown to effectively ameliorate cellular pathologies and cognitive impairments which resemble the cellular processes of CICI. As a result, we investigated the role of NMN to prevent or ameliorate CICI. Rats were treated with doxorubicin, a clinically relevant chemotherapy which causes a range of chronic health disorders including cognitive deficits. Rats treated with doxorubicin showed evidence of impaired spatial memory and object recognition. Rats pre-treated with NMN drinking water were protected against these cognitive impairments. This data provides preliminary evidence that supplementation with NAD+ precursors may alleviate CICI. Current investigations of the molecular mechanisms involved in this protection include changes in vasculature and cytokine modulation.

id #9455

The microtubule-stabilizing drug epothilone D shows age-dependent resolvation of mild traumatic brain injury induced axonal degeneration

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Microtubule dynamics play an important role in the complex development, structure, function and maintenance of the central nervous system. Microtubules are vulnerable to misalignment and dissolution in neurons following traumatic brain injury (TBI) and have been implicated in injury-induced glial responses. To date, there is no effective treatment for TBI. Thus, using a clinically relevant model of mild TBI in male Thy1-YFPH mice, we investigated the potential therapeutic effects of the brain-penetrant microtubule-stabilizing agent (MSA) epothilone D at 1 week and 4 weeks following a single mild lateral fluid percussion brain injury in young and adult mice. Our study found that epothilone D did not alter cortical thickness or pyramidal neuronal number or size in either the young or adult brain. Moreover, no changes were observed in relation to astrogliosis in either age group following injury. However, epothilone D significantly resolved injury-induced axonal degeneration in the external capsule at 1 week and 4 weeks post-injury in young mice, but accentuated axonal degeneration in the external capsule by 4 weeks post-injury in adult mice. Our findings indicate that MSAs may have the potential to manipulate injury-induced neural responses following mild TBI in an age dependent manner, implicating their use in age-tailored therapeutic interventions for the treatment of brain injury.

id #9456

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id #9457

Hyper-innervation, macrophage infiltration and nociceptor sensitisation in a model of vulvodynia

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Objective

Vulvodynia is a common pain disorder and a leading cause of dyspareunia. The key pathophysiological features are vaginal hyper-innervation and nociceptor sensitisation. No treatments target this pathophysiology. It has been shown that macrophages sensitise nociceptor neurons and promote axonal sprouting via cytokine and exosomal signalling. Therefore, this study aims to characterise macrophage infiltration and expression of neuron growth associated protein-43 (GAP-43) in a new mouse model of vaginal hyperinnervation, and establish a robust protocol to assess nociceptive signalling.

Methods

Mild chronic inflammation was induced using microinjection of CFA in the distal vagina of C57Bl/6 mice. Control mice received saline. Immuno-labelling identified GAP-43+ axons and M2-polarised macrophages (F4/80+CD206+) in vaginal sections. In naïve mice, sensory signalling was assessed by quantifying cFOS-immunoreactive dorsal horn neurons under baseline conditions and following a vaginal distension protocol.

Key findings

Abundance of GAP-43+ fibres was unchanged at 7d and 28d in mice that received CFA. CD206+ macrophages were increased at 28d but not 7d. In naïve mice, vaginal distension increased cFOS+ neurons in the S1 dorsal horn by 30%.

Conclusion

Interestingly, vaginal hyper-innervation was not associated with increased GAP-43+ axons, in contrast to CFA models of arthritis, indicating different mechanisms may be involved. Increased abundance of M2-polarised anti-inflammatory macrophages 28 days following CFA suggests resolution of inflammation in the presence of hyper-innervation. The increased number of activated cFOS+ neurons in the spinal cord demonstrates a robust read-out in response to stimulation and allows us to investigate increases in sensitivity in response to inflammation and hyper-innervation.

id #9458

The impact of pharmacological and molecular HCN4 channel block on seizure susceptibility.

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Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels are encoded by four genes (HCN1-4) and are considered good potential anticonvulsant targets due to their pacemaker properties.

This study explores the impact of both pharmacological and molecular HCN4 channel block on seizure susceptibility.

EC18, a compound with a ~6-fold increased selectivity for HCN4 over HCN1 and HCN2 channels, reduced seizure susceptibility in two proconvulsant assays in vivo, as well as reduced neuronal network excitability in culture.

The conditional knockout of HCN4 channels in adult mice was also sufficient to significantly reduce seizure susceptibility in proconvulsant tests with minimal toxicological effects.

Together these results suggest that HCN4 channels are important mediators of neuronal network excitability and therefore may be good targets for anti-seizure drugs.

id #9460

Regulation of synaptic function by actin-associated tropomyosins

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Without the dynamic actin cytoskeleton, neurons would not be able to form or maintain synapses. These processes depend on a variety of actin-associated proteins, but of particular importance is the tropomyosin family, which regulates access of other proteins to actin. Two isoforms, Tpm3.1 and Tpm4.2 have been found to be enriched in the postsynaptic compartment, and are thought to stabilize actin. Therefore, we hypothesized that they can affect dendritic spine formation and synaptic function.

Whole-cell patch clamp recordings in cultured hippocampal neurons overexpressing Tpm3.1 showed no difference in amplitude or frequency of mEPSCs compared to controls. Dendritic spine density was also unaffected. Conversely, hippocampal fEPSPs recorded in acute slices from male Tpm3.1 overexpressing mutant mice (n = 16) showed more persistent long-term potentiation than controls (n = 15), showing that Tpm3.1 overexpression improves synaptic plasticity ($p = 0.04$; RM-ANOVA).

Knockout of Tpm4.2 in hippocampal cultures did not change dendritic spine morphology, but caused reduced mEPSC amplitude ($p = 0.001$) and

frequency (p = 0.002) compared to (unpaired t-test, Tpm4.2 n = 16, WT n = 17). This suggests that Tpm4.2 is necessary for normal synaptic transmission.
Together, these data demonstrate a role of tropomyosins in maintaining and modulating synapse function.

id #9461

Medial amygdala arginine vasopressin neurons regulate the trade-off between reproductive and defensive behaviors.

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Aversion to environmental cues of predators is an integral part of defensive behaviors in many prey animals. It enhances survival and probability of future reproduction. At the same time, animals cannot be maximally defended because imperatives of defense usually tradeoff with behaviors required for sexual reproduction like display of dominance and production of sexual pheromones. Here we approach this tradeoff through the lens of arginine vasopressin neurons within posterodorsal medial amygdala of mice. This neuronal population is known to be involved in sexual behaviors like approach to sexually salient cues. We show that chemogenetic partial ablation of this neuronal population increases aversion to predator odors. Moreover, overexpression of arginine vasopressin within this population is sufficient to reduce aversion to predator odors. The loss of fear to the predator odor occurs in parallel with increased recruitment of arginine vasopressin neurons within posterodorsal medial amygdala. These observations suggest that arginine vasopressin neurons in extended medial amygdala are proximate locus for reduction in innate fear during reproductive events.

id #9462

Multi-modal investigation in a mouse model reveals behavioural, cellular and neuroimaging changes reflective of human major depressive disorders

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Major depressive disorder (MDD) is a heterogeneous and increasingly prevalent mental health condition that remains poorly understood, diagnosed and treated. Improved neurobiological understanding of structural and functional brain changes is essential for developing disease biomarkers, objective diagnosis tools and targeted, effective treatments. Here, we utilised exogenous corticosterone (CORT) treatment in mice to model depression/anxiety and characterised accompanying brain changes using a multi-modal approach including *in vivo* 9.4T magnetic resonance imaging (MRI), cellular and behavioural analysis. 4 week treatment with CORT led to increased anxiety in the novelty suppressed feeding test which correlated with a decline in doublecortin-expressing immature neurons, a marker of neurogenesis in the hippocampus. MRI-based volumetric changes were observed in key brain regions associated with MDD, including the somatosensory cortex which persisted and extended to the insula and amygdala, following 12 weeks of CORT treatment.Notably, a significant increase in the resting state functional connectivity within the default mode network (DMN) was detected in mice treated with CORT for 12 weeks. This DMN hyperactivity correlated with a decrease in sucrose consumption in the sucrose preference test (SPT), an indicator of anhedonia. Furthermore, reduced connectivity between regions within the DMN and the insula cortex were also observed. Overall, we report a number of structural and functional changes in chronic corticosterone-treated mice that parallel those observed in human depressive disorders. Together, these findings support the utility of this preclinical model and multimodal investigations in understanding the neurobiological mechanisms of depression.

id #9463

Large scale tissue slice simulations of cortical spreading depression

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Neuronal activity evokes a localised increase in cerebral blood flow in a response known as neurovascular coupling (NVC), achieved through communication via the neurovascular unit (NVU). Dysfunctional NVC can lead to pathologies such as cortical spreading depression (CSD), a slow moving wave of neuronal depolarisation and high extracellular potassium levels. CSD is associated with several neurological disorders such as migraine, stroke, and traumatic brain injury.

Our research group has developed a large scale numerical model able to simulate NVC in a vascularised cortical tissue slice, with an optional cerebral curvature mapping that can simulate the highly folded nature of the human cortex. "In silico" experiments are performed which are impossible in the wet-lab, providing an experimentally validated test-bed for a variety of neurological phenomena.

For a flat surface corresponding to a smooth murine cortex the model can simulate propagating waves of high extracellular potassium travelling radially outwards from a stimulated area at 6.7 mm/min. The high potassium concentration induces a corresponding wave of vasoconstriction (with decreased blood flow) then slight vasodilation, achieved through communication via the NVU. This behaviour is seen in multiple murine experimental results. Nutrient supply is severely reduced during the wave causing possible cellular damage.

For a strongly curved surface corresponding more to a human cortex we instead observe extracellular potassium travelling as smaller wave segments and the CSD wave is less able to spread far throughout the cortex. These results may provide some insight into the differences seen between human and murine experiments.

id #9464

An exercise ‘sweet spot’ reverses cognitive deficits of ageing by growth hormone-induced neurogenesis

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Hippocampal function is critical for spatial and contextual learning, and its decline with age contributes to cognitive impairment and eventually dementia. Physical exercise can significantly improve hippocampal function in both ageing humans and mice, however, the amount of exercise and the mechanism by which it mediates this improvement remain largely unknown. Here, we show that exercise can reverse learning deficits in aged (24 month-old) mice but, surprisingly, only when it occurs for a specific period of time: longer or shorter periods proving ineffective. This exercise 'sweet spot' corresponded to a spike in growth hormone (GH) levels in the blood and pituitary gland, and blocking GH receptor activation by infusing a competitive antagonist, G118R, into the brain abrogated the cognitive improvement. Moreover, this positive effect of exercise could be mimicked in sedentary aged mice by raising GH levels through treatment with the GH-releasing hormone (GHrH) receptor agonist JI-38. The 'sweet spot' for improved spatial learning also corresponded to a peak in neural precursor activity and neurogenesis in the hippocampus. Depletion of newborn neurons following exercise abolished the cognitive improvement, demonstrating the cognitive improvement was dependent on this process. Further, we demonstrate that GH enhances neurogenesis by directly activating the neurogenic precursors, indicating that the beneficial effect of exercise on cognition is mediated through the direct stimulation of neurogenesis by transiently raised levels of GH. Thus, GH levels provide a marker for detecting the exercise 'sweet spot' as well as a potential therapeutic agent for improving hippocampal-dependent cognition in ageing humans.

id #9465

EFFECT OF REPERFUSION ON THE ASSOCIATIONS BETWEEN SELECTED OUTCOME MEASURES FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION IN MICE.

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Pre-clinical stroke studies model the pathophysiology of clinical stroke where a range of parameters are measured to assess the severity of outcome. However, post-stroke pathology is complex and variable, and associations between specific outcome parameters are difficult to identify. Better understanding of relationships between post-stroke parameters could thus provide valuable knowledge to assist translation of pre-clinical findings into the clinic. To date, a large-scale analysis of data from multiple experimental protocols controlled for covariates has not taken place. Here, we performed retrospective analyses on data from 726 C57Bl/6 mice (6-26 week-old) subjected to intraluminal filament-induced middle cerebral artery occlusion (MCAO) to explore evidence for associations between parameters. Analyses were performed with R. When analysed as two separate experiments, infarct and edema volumes were significantly correlated in mice that received 1h MCAO+23h reperfusion ($r=0.6$, $p<0.0001$, $n=214$) but not in mice subjected to MCAO without reperfusion ($r=-0.2$, $p>0.05$, $n=51$). This relationship remains when rank of the ratio of edema and infarct between the two groups were compared ($p<0.01$). Multiple regression showed that edema was significantly associated with the timing of infarct assessment ($\beta=22.8$, $p<0.01$), age ($\beta=0.8$, $p=0.05$), female mice ($\beta=-32.1$, $p=0.02$) and infarct ($\beta=0.4$, $p<0.01$). The type of experiment (early reperfusion at 1h vs ischemia for >24h) did not remain in the regression analysis. Large-scale analysis of animal experiments provides insight into relationships between variables not available when experiments are analysed in isolation. We suggest that analyses of pre-clinical research data are performed similar to those for human studies where possible.

id #9466

Genetic and cellular characterisation of brain malformation using patient-derived brain tissues

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The development of cerebral cortex involves multiple steps of neuronal proliferation, migration, and differentiation. Interruption to these processes during development can cause congenital brain malformation including focal cortical dysplasia (FCD). Characterised by focal disruption of cortical layering and the presence of dysmorphic neurons, FCD is a leading cause of drug-resistant childhood epilepsy that often requires surgical intervention for seizure control. However, the genetic basis and pathogenesis of FCD remain poorly understood. Here, we utilise cutting-edge genomic technologies to comprehensively study a unique resource of patient-derived, resected brain tissues collected over an eight year period. Genetic analysis using deep targeted panel sequencing revealed low allele frequency, brain-specific somatic mutations in 8 out of 23 FCD cases. Further investigation in one case showed a 'mutation gradient', in which the highest level of mutation load was observed in the brain region with the strongest epileptic discharge and the most severe histopathology. To understand FCD at the transcriptomic level, we used single-nuclei RNA-seq to analyse fresh resected dysplastic (n=4) and normal (n=2) brain tissue. Preliminary analysis identified novel cell populations unique to the dysplastic tissue, suggesting that these cell populations may represent a marker for FCD and potentially the site of epileptogenicity. Our results provide the first description of the cellular composition of dysplastic lesions at single cell level and offer insights into the genetic basis of FCD and pathomechanisms underlying the associated epilepsy. A better understanding of pathogenesis in FCD will be highly relevant to the 1% of the population affected by epilepsy.

id #9467

A postsynaptic pathway of TDP-43-mediated pathology in ALS

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Amyotrophic Lateral Sclerosis (ALS) is characterised by the vulnerability of the motor system. Circuit dysfunction within the motor cortex is likely to be an early disease event, occurring prior to clinical symptoms, however why the motor cortex is vulnerable and the mechanisms driving changes to this network are unknown. We have found that TDP-43, the protein that is most frequently found in cytoplasmic aggregates in ALS, is involved in maintaining neuronal synapses in mouse models of ALS - regulating the number and maturation of dendritic spines. Spine changes occur well before symptom onset in the motor cortex, but not the somatosensory cortex, indicating that this is one of the earliest pathological changes associated with TDP-43. We advanced these finding through the application of 2-photon live imaging to reveal that the motor cortex exhibits higher turnover rates of dendritic spines, that is compromised in the presence of mutant TDP-43. The action of misprocessed TDP-43 at the spine may be due to its role in activity-dependent RNA translation. We have identified that mutant TDP-43 drives increased expression of TDP-43 in the cytoplasm and causes alterations in the composition and localisation of AMPA receptor proteins specifically at the spine head. We have identified that the motor cortex may be specifically vulnerable to TDP-43-mediated dendritic spine deficits. Outcomes of this study identify mechanisms that may drive ALS, and also provide a greater understanding of the vulnerability of the motor cortex, informing therapeutic development and subsequent clinical trial design for all future therapeutic interventions.

id #9468

Ubiquitination Regulates the Proteasomal Degradation and Nuclear Translocation of the Fat Mass and Obesity-Associated (FTO) Protein

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ABSTRACT

Single nucleotide polymorphisms revealed by genome-wide association studies have associated the fat mass and obesity-associated (*FTO)* gene and obesity phenotypes. FTO protein has been associated with cellular nutrient sensing through the mTORC1 pathway, a key regulator of cell growth and mRNA translation, although the precise mechanism is unknown. At cellular levels, FTO is mainly found in the nucleus and known to function as α-ketoglutarate-dependent dioxygenase which catalyzes the demethylation of N6-methyladenosine modification of RNA. To a less extent, there had been evidence showing FTO presence outside the nucleus, although its cytoplasmic function is largely uncharacterized. Moreover, the regulation of FTO at post-translational level has never been reported. Given the significant role of post-translational modifications may exert on a protein function, here, we investigated the ubiquitination process of FTO. We demonstrate that FTO is targeted by ubiquitination on lysine-216, which regulates its degradation rate. Knock-in mutation of this residue from lysine to arginine (K216R) in HeLa cells by CRISPR-Cas9 system revealed enhanced stability of the ubiquitination-deficient FTO. Furthermore, the cells also exhibited enhanced phosphorylation levels of S6K1. Under amino acid starvation, phosphorylation of S6K1 which is slightly decreased in the control cells appeared to be massively down-regulated in the FTO-K216R cells. In addition, we demonstrate nucleus translocation of wild-type FTO under starvation which is blocked by K216R mutation. Our current working model suggests that ubiquitination is important in controlling FTO level and cytoplasm-to-nucleus translocation, which may function in fine-tuning the mTORC1 pathway in amino acid sensing mechanism.

id #9469

PICK1 Regulates Presynaptic Vesicle Recycling in Primary Hippocampal Neurons

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Neurotransmitters are packaged inside synaptic vesicles that are located at the presynaptic nerve terminals. In response to an action potential, synaptic vesicles fuse with the plasma membrane (exocytosis), releasing neurotransmitters into the synaptic cleft. Following exocytosis, these vesicle membranes are retrieved through endocytosis and refilled with neurotransmitters for subsequent release. This recycling process is crucial to replenish the finite number of vesicles available in order to maintain synaptic transmission. The Protein Interacting with C-Kinase 1 (PICK1) is a lipid-binding protein that plays an important role in regulating the vesicular trafficking of postsynaptic neurotransmitter receptors and transporters. However, PICK1 is also expressed in the presynaptic terminals where its function is unknown. In this study, we manipulated the expression of PICK1 proteins in primary hippocampal neurons and assessed the efficiency of synaptic vesicle recycling using live-cell imaging technique to monitor the trafficking of a resident synaptic vesicle protein, synaptophysin, that is tagged with a pH-sensitive green fluorescent protein (pHluorin) in the luminal domain. We found that shRNA-mediated knockdown of PICK1 altered the kinetics of synaptic vesicle recycling. In addition, loss of PICK1 function leads to an increased stranding and mislocalisation of synaptophysin on the plasma membrane. Taken together, our data reveals a role for PICK1 as a novel regulator of presynaptic vesicle recycling in mammalian central neurons.

id #9470

Neuroprotection by Amla fruit extract against an in vivo & in vitro ischemic stroke via Downregulating intrinsic apoptosis pathways.

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Background: Cell death following ischemia brain injury leads to life threatening problem to patients. B Cell Lymphoma (Bcl-2) protein plays an important role in regulation of the apoptotic pathway.

Objectives: The aim of this study was to investigate the neuroprotective effects of Amla fruit extract on neuronal apoptosis and Caspase-3 & Bcl-2 protein level following in vivo & in vitro ischemic stroke.

Methods: A rat model of middle cerebral artery occlusion (MCAO) was constructed to induce ischemic stroke. Rats were orally administered Amla fruit extract post induction of ischemia. Oxygen-glucose deprivation (OGD) model was used to mimic an ischemic milieu in vitro in PC12 cells. Brain damage due to cerebral ischemia was assessed by Cellular morphology, and DNA fragmentation. These changes were further assessed by analyzing the changes of the oxidative stress marker malondialdehyde (MDA), GSH, Flow cytometry and the expression of Bcl-2, Bax and cleaved caspase 3 proteins under in vitro and/or in vivo ischemic conditions.

Results: The results showed that Amla fruit extract significantly decreased the apoptotic cells following ischemic stroke. Meanwhile, treatment with amla fruit extract retained serum GSH levels and led to a lower MDA level. Amla fruit extract significantly ameliorated Bcl-2 and cleaved caspase-3

protein expression following ischemic stroke and the number of TUNEL positive cells in the ipsilateral cerebral cortex and striatum region was significantly reduced following an *in vivo* ischemic conditions.

Conclusion: These results revealed that amla extract has a therapeutic effect on ischemic stroke by inducing anti-apoptotic mechanisms

id #9471

DECREASED SIGNALLING OF EPHA4 IMPROVES FUNCTIONAL PERFORMANCE AND MOTOR NEURON SURVIVAL IN THE SOD1G93A ALS MOUSE MODEL

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Amyotrophic lateral sclerosis (ALS) is an untreatable, progressive, neurodegenerative disease specifically affecting motor neurons. Recently, the tyrosine kinase receptor EphA4 was directly implicated in ALS disease progression. We report that a long-lived mutated form of the EphA4 antagonist EphA4-Fc (mutEphA4-Fc), which blocks EphA4 binding to its ligands and inhibits its function, significantly improved functional performance in $SOD1^{G93A}$ALS model mice, as assessed by rotarod and hind-limb grip strength tests. Further, heterozygous motor neuron-specific *EphA4*gene deletion in $SOD1^{G93A}$mice promoted significant improvement in functional performance during the disease course and a delay in disease onset relative to control mice. Importantly, mice in the heterozygous deletion group showed significantly improved survival of motor neurons and architecture of endplates of neuromuscular junctions compared with control and homozygous *EphA4*-deletion groups. Our novel results show that EphA4 signalling directly regulates motor neuron survival and that mutEphA4-Fc is a promising therapeutic candidate to slow disease progression in ALS

id #9472

EPHA4 REGULATES HIPPOCAMPAL NEURAL PRECURSOR PROLIFERATION IN THE ADULT MOUSE BRAIN BY D-SERINE MODULATION OF NMDAR SIGNALLING

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The hippocampal dentate gyrus is a major region of the adult rodent brain where neurogenesis occurs throughout life. The EphA4 receptor, which regulates neurogenesis and boundary formation in the developing brain, is also expressed in the adult dentate gyrus but whether it regulates adult hippocampal neurogenesis is not known. Here, we show that, in the adult mouse brain, EphA4 inhibits hippocampal precursor cell proliferation but does not affect precursor differentiation or survival. Genetic deletion or pharmacological inhibition of EphA4 significantly increased hippocampal precursor proliferation in vivoand in vitro, by blocking EphA4 forward signalling. EphA4 was expressed by mature hippocampal dentate gyrus neurons but not neural precursor cells, and an EphA4 antagonist, EphA4-Fc, did not activate clonal cultures of precursors until they were co-cultured with non-precursor cells, indicating an indirect effect of EphA4 on the regulation of precursor activity. Supplementation with D-serine blocked the increased precursor proliferation induced by EphA4 inhibition, whereas blocking the interaction between D-serine and N-methyl-D-aspartate receptors (NMDARs) promoted precursor activity, even at the clonal level. Collectively, these findings demonstrate that EphA4 indirectly regulates adult hippocampal precursor proliferation and thus plays a role in neurogenesis via D-serine-regulated NMDAR signalling

id #9473

PHYSICAL EXERCISE ALTERS THE STRUCTURE AND FUNCTIONAL CONNECTIVITY OF THE HIPPOCAMPUS IN THE AGED MURINE BRAIN

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Objective: Advancing age is often associated with decreased learning ability. The hippocampus is a brain region that is critically involved in different types of learning including spatial navigation and its function has been shown to be regulated by the production of new neurons. With ageing, hippocampal function and neuronal production decline, but both can be reversed with optimal exercise. However, little is known about the underlying neural mechanisms elicited by exercise, especially in relation to the circuitry within the hippocampus. The aim of this study is to determine the underlying neural mechanisms elicited by exercise.

Methods: 24-month-old C57BL/6 mice underwent voluntary running on a wheel and in vivo T2, DTI, and resting functional MRI was performed using a 9.4T MRI scanner to compare the changes in structure and functional circuitry between exercised animals and sedentary controls.

Key findings: Following exercise, mice displayed significant changes in both structure and functional connectivity when compared to controls. Structure changes are characterised by increased volume and decreased mean diffusivity value in the hippocampus formation; changes consistent with increased cell density. Functional changes are characterised by an increase in the strength of connection in the hippocampus. These changes were positively correlated with cognitive performance.

Conclusion: This study demonstrates that physical exercise can elicit changes in the hippocampus both structurally and functionally. This study also established the key connectivity components within the hippocampus that are required for successful spatial learning. This provides, in part, a mechanistic explanation for the beneficial effect of exercise.

id #9477

Dscam2 suppresses synaptic strength via an endosome-dependent mechanism

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Regulation of synaptic strength is critical for nervous system function. Although mechanisms for determining synaptic strength, such as the probability of neurotransmitter release, are well documented there remains much to learn with respect the molecules that regulate this process. Our recent work has focused on describing the consequences of genetic lesions in *Drosophila melanogaster* Dscam2 on synaptic morphological and physiological parameters. Dscam2 is a transmembrane protein that mediates homophilic interactions and induces repulsion or adhesion between neurons during development. It is alternatively spliced and produces two isoforms that are biochemically distinct. Cell type-specific expression of Dscam2 isoforms is crucial for maintaining axon terminal size, dendritic morphology and synaptic numbers. We wondered whether Dscam2 might also be used to regulate physiological parameters such as neurotransmission. To investigate this, we performed a combination of morphological and electrophysiological assays at the *Drosophila* neuromuscular junction (NMJ). Results from our analyses demonstrate that loss of Dscam2 specifically results in increased synaptic strength, revealing a novel role for Dscam2 in regulating neurotransmission. Through genetic interaction studies, we identify an endosomal signalling pathway that is linked to Dscam2's role at the NMJ. These findings demonstrate how a developmental cue can be re-used for regulating the output of synapses once they have formed and provide insight into the upstream signalling factors that determine synaptic strength.

id #9478

Endothelial NOX4 oxidase exacerbates motor dysfunction after ischemic stroke

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Oxidative stress plays a fundamental role in the pathogenesis of ischemic stroke. NOX1 and NOX2 oxidases have been shown to negatively impact stroke outcome; however, the role of NOX4 oxidase is less clear. We tested the hypothesis that overexpression of NOX4 oxidase in endothelial cells would worsen outcomes after ischemic stroke.

We studied male C57Bl6 wild type (WT; n=30) mice and mice overexpressing NOX4 oxidase in endothelial cells ($NOX4_{endo}$-Tg; n=25) aged 3-4 months (young adult) or 12-14 months (middle-age). Thrombotic stroke was targeted to the primary motor cortex. Outcomes (motor function, infarct volume, blood-brain barrier (BBB) permeability and NOX4 mRNA expression) were assessed at 7 days post-stroke.

In cerebral arteries from naïve animals, NOX4 mRNA in $NOX4_{endo}$-Tg mice was 17-fold greater than in WT animals ($P<0.05$). Following stroke, NOX4 mRNA expression in cerebral arteries was reduced by ~50% in both strains. Forelimb asymmetry was increased in young adult $NOX4_{endo}$-Tg mice, indicative of poorer outcome (WT, 20.0±3.1% vs. $NOX4_{endo}$-Tg, 32.4±4.9%; $P<0.05$). Similar to young adult mice, middle-aged mice displayed worsened forelimb asymmetry; however, the magnitude of impairment was greater (WT, 24.7±7.3% vs. $NOX4_{endo}$-Tg, 54.11±8.7%; $P<0.05$). Poorer functional outcomes were independent of infarct volume at both ages. BBB permeability was increased in middle middle-aged $NOX4_{endo}$-Tg mice after stroke.

The findings of the present study suggest that endothelial NOX4 oxidase plays a detrimental role in functional outcomes after ischemic stroke. As functional recovery is of utmost clinical importance, selective blockers that target endothelial NOX4 oxidase may be a novel therapeutic strategy.

id #9479

Vitamin D therapy for ischemic stroke

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Vitamin D (VitD) deficiency is endemic in humans and increases the risk for cardiovascular events, including stroke. VitD exerts a protective effect on the cardiovascular system. However, the effectiveness of VitD as a therapy for ischemic stroke is unclear.

We aimed to determine whether VitD deficiency and/or post-stroke supplementation with VitD affect stroke outcomes.

Young adult (4 months; n=72) or middle-aged (12-14 months; n=11) male C57Bl6 mice were placed on either a VitD sufficient (2200 IU/day) or VitD deficient (0 IU) diet. After 8 weeks on the diet, thrombotic stroke was induced in the primary motor cortex. Young adult mice received 1,25-OH_2 $VitD_3$ (active form; 1.4 mg/kg per dose i.p.) or vehicle at 30 min and days 1, 3, 5 and 7 post-stroke. Motor function, infarct volume, markers of inflammation and neurogenesis were assessed at day 7.

VitD deficiency exacerbated motor dysfunction ($p<0.05$), with the magnitude of impairment being greater in middle-aged VitD deficient mice ($p<0.05$). Infarct volume did not differ between groups. VitD deficiency was associated with increased Iba-1-positive microglia, loss of infarct containment by reactive astrocytes and a reduction in doublecortin-positive staining. Post-stroke supplementation with $VitD_3$ mitigated the motor impairment by ~50% ($p<0.05$) but did not affect infarct volume.

These data suggest that VitD deficiency worsens post-stroke functional outcomes via increasing brain inflammation and reducing recovery mechanisms. Administration of exogenous VitD can attenuate poorer functional outcomes and thus, may represent a direction for acute stroke therapy.

id #9482

Developing a high-throughput method for testing potential therapeutics in a human-based model of ischaemic stroke

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id #9483

Effects of prenatal hyperhomocysteinemia on rat brain development during the postnatal period

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Increased levels of maternal homocysteine (hyperhomocysteinemia, HHC) during pregnancy is associated with complications in fetal development. However, little is known about the effects of maternal HHC on subsequent fetal brain development. Using our model of prenatal HHC induced by daily administration of methionine (0.6 mg/kg) to Wistar rats from 4th day of pregnancy until pups' delivery we have evaluated the influence of prenatal HHC on the parameters of oxidative stress and apoptosis in the brain of developing pups and their brain structure. In the brain of the offspring subjected to prenatal HHC we observed increased neuroinflammation and changes in cell composition of the parietal cortex and hippocampus in different periods of postnatal development. Newborn pups subjected to prenatal HHC also had increased oxidative modifications of brain proteins and DNA. Analysis of the apoptotic markers revealed activation of caspase-3 and increase of neuroregulin NRG1 both in the brain of rat fetuses (E20) and in the early postembryonic period of the pups subjected to prenatal HHC. At later stages of postembryonic development of the pups subjected to prenatal HHC we observed a reduction in the total number of neurons and activation of glia in the parietal cortex and hippocampus. Glial activation was accompanied by an increase in IL-1beta content in the parietal cortex. The data suggest that homocysteine-induced oxidative stress and neuronal apoptosis during embryogenesis cause early developmental impairments of brain structure and maturation, which might induce neuroinflammation and other long-term neurological disorders. Supported by Russian Foundation for Basic Research (18-015-00099).

id #9485

Effects of prenatal hyperhomocysteinemia on rat brain development during the postnatal period

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id #9486

Effects of prenatal hyperhomocysteinemia on rat brain development during the postnatal period

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