

id #11557

Accelerated 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 decreased 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 amyloid peptide. PRESENILIN 1 (PSEN1) is the majority locus for mutations causing fAD and the PSEN1 protein interacts with the HIF1 component, HIF1a. 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. 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 acute hypoxia. Intriguingly, these fAD-like mutant adult zebrafish also show an accelerated loss of spatial working memory with age. Our results are consistent with a view of AD as both dependent upon brain aging but manifesting as a distinct, pathological brain molecular state.

id #11562

Using DREADD technology to interrogate dysfunctional feed-forward inhibition within cortico-thalamo-cortical microcircuits to investigate absence seizure generation and altered behaviour

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Within the brain, feed-forward inhibition (FFI) regulates neuronal firing and prevents runaway excitation. FFI is mediated by fast spiking parvalbumin-expressing (PV+) inhibitory interneurons. In the cortico-thalamo-cortical (CTC) network, dysfunctional FFI has been implicated in absence seizure generation. We previously reported that defects in activation of PV+ interneurons in the stargazer model of absence epilepsy could disrupt CTC network oscillation contributing to seizure generation. In this study we used Designer Receptors Exclusively Activated by Designer Drug (DREADD) technology to silence/excite PV+ interneurons to investigate the impact of dysfunctional FFI. We first confirmed selective expression of DREADD receptors in PV+ interneurons via confocal fluorescence microscopy. Simultaneous EEG/video recordings and behavioural tests were performed before and after injecting Clozapine-N-Oxide (CNO) to silence Gi-DREADD or to excite Gq-DREADD. Silencing PV+ interneurons in CTC network using PV-Cre x Gi-DREADD animals generated bursts of oscillatory activity with the characteristics of spike-wave

discharges (SWDs) and also reduced ambulation. The mean duration and frequency of SWDs were 2.9 ± 0.3 sec and 5.5 ± 0.5 spikes/sec, respectively after silencing PV+ interneurons in CTC network. Bursts of SWDs were suppressed by administering the anti-epileptic drug ethosuximide (200mg/kg) i.p. However, CNO injection into non-DREADD controls showed neither epileptiform activity nor changes in behavioural parameters. Currently, we are testing the effect of exciting PV+ interneurons through Gq-DREADD after chemically inducing absence seizures to verify that activation of PV+ inhibitory interneurons suppresses SWDs. Understanding the functional roles of FFI microcircuits is important for identifying targets for future improved patient specific treatment strategies for absence epilepsy.

id #11576

A high density of dysmorphic neurons in the centre of dysplasias are the driver of epileptic seizures in tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem epileptic disorder characterised by cortical tubers, a brain malformation with disrupted cortical layering and abnormal cells termed dysmorphic neurons (DNs). Surgical resection is frequently required for seizure control and current practise is to resect the entire lesion, however, these invasive resections may be associated with adverse neurological outcomes. We have previously performed electrophysiological and MRI studies that identified the tuber centre as the source of seizures, suggesting that limiting resections to the centre may be favourable.

Here we report complete seizure elimination and favourable neurological outcomes in five children whom underwent centre-only tuber resections. Histopathological studies and unbiased stereology of the epileptogenic centre region identified an ~4 fold greater density of DNs compared to the biopsied tuber rim.

To investigate the pathogenic mechanisms underlying seizure generation we performed single nuclei RNA-sequencing on paired centre and rim tissue (n=12). Ongoing analysis of our dataset including >100,000 nuclei that represent 9 distinct cell types and includes a unique population of DNs with a characteristic and inherently dysregulated transcriptional profile.

In conclusion, our results support a change in surgical practise to limit resection to the tuber centre and supports that DNs concentrated at the tuber centre are the seizure generators. Our

results provide the first description of the cellular composition of dysplastic lesions at single-cell level and will potentially enable development of therapies targeting the molecular cause of seizure generation by DNAs. These discoveries may offer broader insights into the pathogenesis of epilepsy.

id #11579

Repopulating microglia promote brain repair in an IL-6-dependent manner

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Cognitive dysfunction and reactive microglia are hallmarks of traumatic brain injury (TBI), yet whether these cells contribute to cognitive deficits and secondary inflammatory pathology remains poorly understood. Here we show that removal of microglia from the mouse brain has little impact on the outcome from TBI, but inducing the turnover of these cells through either pharmacologic or genetic approaches can yield a neuroprotective microglial phenotype that profoundly aids recovery. These repopulating microglia are critically dependent on IL-6 signaling and robustly support adult neurogenesis, specifically by augmenting the survival of newborn neurons that directly support cognitive function. We conclude that microglia in the mammalian brain can be manipulated to adopt a neuroprotective and pro-regenerative phenotype that can aid repair and alleviate the cognitive deficits arising from brain injury.

id #11593

Genetic and microstructural differences between gyri and sulci during gyrification in the cortical plate of fetal sheep

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Objective: Mechanisms driving gyrification of the brain remain elusive. Recent theories have focused on the cortical plate (CP) as a key region involved in gyrification. Here, we sought to identify changes in gene expression and microarchitecture in the CP during differentiation of gyri and sulci.

Methods: We used Laser Capture Microdissection to obtain cells from a gyrus and a sulcus in the fetal sheep brain during gyrification (gestational age, (GA) 90, term GA ~147). We then used RNA-Seq to determine differentially expressed genes, and MRI-derived Neurite Orientation Dispersion and Density Imaging (NODDI) to identify micro-structural differences between gyri and sulci at GA 98. We selected a subset of differentially expressed genes for validation before (GA 70), during (GA 90) and after gyrification (GA 110) using immunohistochemistry and fluorescent in situ hybridization. Cell density (DAPI-stained nuclei per area) and neurite outgrowth marker MAP2 immunoreactivity were also assessed during gyrification.

Key findings: We observed higher BDNF ($p < 0.0001$), CDK5 ($p < 0.0001$) and NeuroD6 immunoreactivity ($p < 0.001$), and lower HDAC5 ($p < 0.01$) and MeCP2 mRNA expression ($p < 0.001$), in gyri compared to sulci during gyrification, but not before. During gyrification, MAP2 immunoreactivity was higher ($p < 0.0001$), and cellular density was decreased ($p < 0.05$), in gyri compared to sulci, consistent with NODDI parameters.

Conclusion: These results demonstrate differential gene expression, together with higher MAP2-immunoreactivity and lower total cell density, between gyri and sulci in the CP during gyrification. This is also consistent with NODDI parameters. Therefore, neurite outgrowth may be a fundamental mechanism that drives the process of gyrification.

id #11599

iPSC-derived brain endothelial cells carrying *PSEN1* Alzheimer's mutation exhibit altered phenotype and permeability in response to focused ultrasound, with potential implications for amyloid clearance and drug delivery

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The blood-brain barrier (BBB) presents a barrier for circulating factors, but simultaneously challenges drug delivery. How the BBB is altered in Alzheimer's disease (AD), and how this affects brain pathology and drug delivery is not well understood. To investigate this, we derived brain endothelial cells (iBECs) from human induced pluripotent stem cells (hiPSCs) of several patients carrying the familial AD *PSEN1* mutation. We demonstrate that compared to isogenic *PSEN1* corrected and control iBECs, AD-iBECs exhibit altered tight and adherens junction protein expression including elevated claudin 5 and decreased VE-cadherin. AD-iBECs also displayed reduced trans-epithelial electrical resistance (TEER) compared to control iBECs, and revealed aberrant efflux properties. Furthermore, we applied focused ultrasound (FUS) that transiently opens the BBB by the interaction with (intravenously injected) microbubbles and achieves therapeutic effects in AD mouse models. In response to FUS, we found an altered permeability to 3-5 kDa dextran molecules and the amyloid- β (A β) peptide in AD-iBECs compared to control iBECs. AD-iBECs were initially more resistant to FUS-mediated cell displacement, but were subsequently slower to return to a normal state. Our studies present an important advance in human-derived *in vitro* models of the BBB as a valuable tool to understand its role and properties in a disease context, with possible implications for drug delivery and therapeutic treatment in AD.

id #11613

Iron dyshomeostasis manifests in the periphery in preclinical Alzheimer's disease

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Mounting evidence indicates a disruption of iron homeostasis in the brain in Alzheimer's disease (AD). Interestingly, this disruption has also been observed in the periphery. The current study investigated whether iron homeostasis is dysregulated in preclinical AD,

characterised by neocortical amyloid- β load(NAL), by examining the iron regulating hormone, hepcidin. Serum hepcidin was measured employing an ELISA in cognitively normal participants from the KARVIAH cohort. Participants(n=100) were within the age range of 65-90years. To evaluate NAL, all participants underwent positron emission tomography, wherein participants with a standard uptake value ratio(SUVR) <1.35 were classified as low NAL (at no apparent risk to AD) and those with SUVR ≥ 1.35 were classified as high NAL. Serum hepcidin was significantly elevated in participants with high NAL compared to those with low NAL, with and without correcting for age, gender, *APOE* $\epsilon 4$ carriage($p < .05$). A receiver operating characteristic curve based on a logistic regression of age, gender and *APOE* $\epsilon 4$ carriage, the base model, distinguished high NAL from low NAL (area under the curve, AUC=.786), but was outperformed when serum hepcidin was added to the base model(AUC=.813), such that at 80% sensitivity, the specificity increased from 61% to 66% on adding hepcidin to the base model. Hepcidin suppresses the release of iron by binding to the iron transporter protein, ferroportin, resulting in intracellular iron accumulation, and therefore increased circulating hepcidin levels observed in the current study are consistent with tissue iron loading observed in AD. Observations from the current study reveal the presence of iron dyshomeostasis in preclinical AD.

id #11615

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

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Synaptic connections between brain cortical areas are critical for the coordination of information that enables sensation and perception. Yet our understanding of the specificity and timing of these connections even during the awareness of a simple sensation is limited.

Using a novel, fluorescent genetically encoded voltage indicator, VSFP (voltage sensitive fluorescent protein) specifically targeted to layer 2/3 of the mouse cortex, we aimed to understand how recruitment of cortical areal connections occurs in awake mice as they performed two distinct simple behaviours. We used fast, fluorescence imaging of layer 2/3 voltage signals, well suited to detect synaptic potentials, through a thinned skull window in pre-trained head-fixed awake mice as they received single or double brief (<20 ms) light stimulation to the paw or air puff to the whiskers. We standardized methods to process real time fluorescence changes to reveal voltage changes including correction for hemodynamic and movement artefacts using Matlab.

Our results show fast, dynamic increases, or depolarisation, of layer 2/3 cortical neurons specifically initiated and localized within distinct areas of the sensory cortex during whisker puff and paw stimulation. We observed a similar time scale of the sensory voltage responses in the distinct areas ($p \geq 0.05$), and characteristic paired pulse depression of the signals consistent with their synaptic nature, at inter-stimulus intervals of 110 ms ($p < 0.01$) and 210 ms ($p < 0.05$).

These unique real time brain activity maps show how areal recruitment of synaptic activity connects distinct cortical areas critical for encoding behavioural perception and awareness of sensation.

id #11625

GROWTH HORMONE IN EXPERIMENTAL STROKE: FROM MOTOR IMPROVEMENT TO NEUROGENESIS AND BEYOND

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Growth hormone has shown promising results in terms of promoting cognitive recovery after experimental stroke (Ong et al., 2018, *Stroke*), but the effects on motor function have not been considered. In this study, we evaluated the effects of peripheral administration of growth hormone at 48 hours post-stroke for 28 days on motor function and the underlying mechanisms. Experimental stroke was induced by photothrombotic occlusion targeting the motor and somatosensory cortices in adult mice. We found that growth hormone treatment significantly improved motor deficits and reduced brain tissue loss after stroke. Motor improvement was indexed as decreased foot-faults on grid walk task and reduced preference for spontaneous use of ipsilateral forelimb on cylinder task. Further immunohistochemical and protein analyses of the brain revealed that this motor improvement was associated with increased neurogenesis (BrdU+NeuN+ cells and DCX+ cells), synaptic plasticity (GluR1) and growth of cerebrovascular (BrdU+Lectin+ cells) within the peri-infarct area. Growth hormone treatment also significantly enhanced levels of pro-regenerative factors as well as activation of mTOR signalling. Our results are striking, supporting the effectiveness of growth hormone in facilitating the neurorestorative processes, leading to motor recovery after stroke.

1. Ong, L. K., Chow, W. Z., TeBay, C., Kluge, M., Pietrogrande, G., Zalewska, K., Crock, P., Åberg, N. D., Bivard, A., Johnson, S. J., Walker, F. R., Nilsson, M., & Isgaard, J. (2018). Growth hormone improves cognitive function after experimental stroke. *Stroke*, 49(5), 1257-1266.

id #11643

Elucidating the functional role of novel DNA modifications in fear extinction memory formation

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DNA modification is known to regulate experience-dependent gene expression. However, beyond cytosine methylation and its oxidated derivatives, very little is known about the functional importance of chemical modifications on other nucleobases in the brain. Here we report that in adult mice trained in fear extinction, the DNA modification N6-methyl-2'-deoxyadenosine (m6dA) accumulates along with promoters and coding sequences in activated prefrontal cortical neurons. The deposition of m6dA is associated with increased genome-wide occupancy of the mammalian m6dA methyltransferase, N6amt1, and this correlates with extinction-induced gene expression. The accumulation of m6dA is associated with transcriptional activation at the brain-derived neurotrophic factor (Bdnf) P4 promoter, which is required for *Bdnf* exon IV messenger RNA expression and for the extinction of conditioned fear. These results expand the scope of DNA modifications in the adult brain and highlight changes in m6dA as an epigenetic mechanism associated with activity-induced gene expression and the formation of fear extinction memory.

id #11650

Impairment of macroautophagy in dopamine neurons has opposing effects on parkinsonian pathology and behaviour

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Objectives: Parkinson's disease (PD) is an incurable disease characterised by death of substantia nigra dopamine (DA) neurons and accumulation of the protein alpha-synuclein. Macroautophagy, the major process by which deleterious cellular contents are degraded, is implicated in both sporadic and genetic PD, and macroautophagy inducers are currently in PD clinical trials. However, little is known about the interaction between alpha-synuclein and macroautophagy *in vivo*.

Methods: We investigated the interaction between alpha-synuclein and macroautophagic failure in dopamine neurons in the ageing mammalian brain by generating novel transgenic mice. These mice feature the full-length human alpha-synuclein locus, and conditional deletion of the essential autophagy gene, *Atg7*, in dopamine neurons by means of floxed alleles and the DAT-IRES-cre construct. We confirmed our findings in a separate TH-IRES-cre line.

Results: We found that impaired macroautophagy in DA neurons caused region-specific DA neuron loss and protein aggregation, both key features of PD pathology. Despite these parkinsonian pathological changes, we found that age-related parkinsonian motor phenotypes associated with alpha-synuclein overexpression were improved when macroautophagy was impaired. We used fast-scanning cyclic voltammetry to investigate for changes in dopamine neurotransmission underlying this phenomenon, revealing that macroautophagy impairment in dopamine neurons increased evoked extracellular concentrations of dopamine, reduced dopamine uptake, and relieved paired-stimulus depression.

Conclusions: Our findings that macroautophagy regulates dopamine neurotransmission, thus improving behavioural phenotypes while worsening some pathological phenotypes, has direct implications for PD pathogenesis, drug discovery and clinical trials. Additionally, it has broader implications for regulation and diseases of dopamine homeostasis.

id #11657

Short term estradiol treatment reduces seizure severity but does not improve 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 refractory seizures. Neurosteroid treatment may improve seizure outcomes, but the mechanism remains unknown. Here we demonstrate that short term, daily delivery of the neurosteroid 17β-estradiol (40 ng/g) in early postnatal life significantly reduced the number and severity of seizures in mice modelling mutations in the *Aristaless*-related homeobox gene (*ARX*)

expanding the first (PA1) or second (PA2) polyalanine tract. Frequency of seizures on handling (n = 14/treatment/genotype) were significantly reduced in PA1 mice (34%) and more modestly in PA2 mice (15%). Spontaneous seizures were assessed at 7 weeks of age (n = 7/treatment/genotype) coinciding with peak seizure activity. Using the International League Against Epilepsy's classification system we demonstrate that treated PA1 mice no longer present with the most severe category of prolonged myoclonic seizures. Even more striking, the treated PA2 mice had a complete absence of any seizures during this analysis.

Behavioural tests demonstrate that despite the reduction in seizures, 17 β -estradiol treated mice showed no improvement in cognitive outcomes after peak seizure onset. For the first time, we show that cognitive deficits due to mutations in *Arx* are already present prior to seizure onset and do not worsen with seizures. ARX is a transcription factor and *Arx* PolyA mutant mice have de-regulated transcriptome profiles in the developing embryonic brain. We contend that neurosteroid administration in early postnatal life improves seizure outcomes due to changes in gene expression pathways that are distinct to the deregulated gene pathways involved in manifesting cognitive deficits.

id #11664

Antisense oligonucleotide-mediated exon skipping to treat spinocerebellar ataxia type 3

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Spinocerebellar ataxia type 3 (SCA3) is a devastating neurodegenerative disease, which is one of nine polyglutamine disorders. Although SCA3 is pathogenically heterogeneous, the main feature is progressive ataxia, which in turn affects speech, balance and gait of the affected individual. There is currently no cure, nor effective treatment strategy for affected individuals. SCA3 is caused by an expanded polyglutamine tract found in ataxin-3, resulting in conformational changes that lead to toxic gain of function. This expanded glutamine tract is located at the 5' end of the penultimate exon (exon 10) of *ATXN3*. This study aims to use antisense oligonucleotide (AO) mediated exon skipping to develop a therapeutic strategy for the treatment of SCA3.

Initial *in vitro* data using 2'-O-methyl AOs in patient cells show that it is possible to create an internally truncated protein, missing the toxic CAG repeat contained in *ATXN3* and still maintain normal function of the protein. Confirmatory data using the clinically relevant *phosphorodiamidate morpholino oligomer* (PMO) chemistry showed complementary positive results to 2'-O-methyl data. Additionally, significant down-regulation of both the mutant and wild-type protein was observed, allowing for a combination of benefits. However, PMO is widely considered to be a superior chemistry when compared to 2'-O-methyl, as they are chemically stable and have an excellent safety profile to date. Further data shows that PMO chemistry is longer lasting and significantly better tolerated by cells. Therefore, this study provides a possible therapeutic strategy to treat SCA3.

id #11665

Progressive changes to neural stem cell quiescence ensure lifelong neurogenesis.

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In most mammals, neural stem cells (NSCs) persist in the hippocampus where they continue to generate neurons (neurogenesis). The general pattern of hippocampal neurogenesis is that it occurs at high levels in young animals, rapidly declines and then is maintained at low levels throughout life. In mice, the rapid decline in neurogenesis in juveniles, is due to the self-consuming divisions of NSCs. However, the mechanisms that underpin the relative maintenance of NSCs and neurogenesis in older animals are unknown. Here, we demonstrate that with age, NSCs steadily acquire the capacity to return to quiescence – instead of differentiating. And secondly, NSCs that have never activated, progress into a deeper state of quiescence. Both of these processes are controlled by a common mechanism: the relative abundance of the transcription factor *Ascl1*, which in turn, is controlled by sonic hedgehog signalling and the activity of the ubiquitin ligase *Huwe1*. In the absence of these cellular and molecular changes, the NSC pool exhausts and neurogenesis ends prematurely. Together, these findings provide insights into how the regulation of NSC quiescence ensures lifelong neurogenesis.

id #11672

NEONATAL EXPOSURE TO THE ANTIBIOTIC VANCOMYCIN INDUCES LONG-TERM EFFECTS ON THE MICROBIOTA, ENTERIC NERVOUS SYSTEM AND HOST METABOLISM

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Early-life antibiotic exposure is reported to increase susceptibility to diseases later in life including gastrointestinal (GI) and metabolic disorders, but little is known about antibiotic-induced disease mechanisms. Vancomycin is given as a prophylactic treatment to pre-term and low birth weight infants. We have shown that neonatal exposure to vancomycin disrupts the developing microbiota and nervous system of the gut (enteric nervous system, ENS), and reduces body weights in mouse pups. Here, we examine long-term effects of neonatal vancomycin treatment. Mice were given a single daily oral dose of vancomycin or water from birth (postnatal day, P0) to P10 and then left to grow up with standard animal husbandry procedures. At 6-weeks of age, mice underwent MiniSpec NMR analysis of body composition and whole-body metabolic assessment using indirect calorimetry. The animals were culled, and their colons were removed for further examination. Six-week-old mice given neonatal vancomycin had enlarged caeca ($P < 0.0001$), which is indicative of microbiota perturbation, and decreased faecal dry weight ($P < 0.05$), which only manifested at 6 weeks of age. They sustained an increased proportion of calbindin-immunoreactive enteric neurons ($P < 0.05$), a decrease in mucosal serotonin+ cells ($P < 0.05$) and lower body weights ($P < 0.01$). Male mice treated with neonatal vancomycin had a higher fat mass and lower lean mass

($P < 0.05$) compared to their water-fed controls, but vancomycin-treated mice consumed more food than controls. Overall, we show that neonatal exposure to antibiotics induced long-lasting effects on microbiota, ENS, host appetite and metabolism via a mechanism that involves mucosal serotonin.

id #11680

Super-resolving the nanoscale dynamics of Botulinum Neurotoxin Type-A intoxication journey

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Understanding how neurons in the human brain exchange and process information to generate thoughts and memories remains one of the great challenges of modern neurobiology. Neuronal communication is encoded by neurotransmitters stored in synaptic vesicles (SVs) that undergo Ca^{2+} -dependent fusion with the presynaptic plasma membrane (PM) upon stimulation, after which the SVs are rapidly reformed through endocytosis. Neurotoxins, such as Botulinum Neurotoxin Type-A (BoNT/A), use this endocytic pathway to internalize into nerve terminals as part of their intoxication strategy to incapacitate nerve-muscle communication. Our understanding of the molecular steps of intoxication has been hampered by the reliance on high concentrations of neurotoxins, far exceeding the pathogenic doses. Here, we used single-molecule techniques to investigate the dynamics of BoNT/A molecules at the PM (Universal Point Accumulation Imaging in Nanoscale Topography; Giannone *et al.*, 2013 *Methods in Molecular Biology*), in recycling SVs and upon retrograde trafficking (subdiffraction Tracking of Internalized Molecules; Joensuu *et al.*, 2016 *JCB*; Joensuu & Martinez-Marmol *et al.*, 2017 *Nature Protocols*). Both techniques allowed us to track picomolar-concentrations of Atto467N-BoNT/A molecules with 30-40nm localization precision. Genetic inactivation of the BoNT/A ganglioside (GT1b) and co-receptor SV2 binding sites affected the PM binding of the toxins. Internalization and the axonal retrograde transport was strongly inhibited by these mutations, suggesting that both receptors are involved in endocytosis of BoNT/A. Defective internalization of the mutants was confirmed with electron microscopy. Single-molecule imaging uncovers a dynamic view of the nanoscale organization of the intoxication cascade from PM binding to entry, sorting and retrograde trafficking.

id #11682

Paradoxical effects of chronic exercise on anxiety-like behaviour in rats: sex differences and role of the BDNF val66met polymorphism

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Brain-derived neurotrophic factor (BDNF) is involved in neuroplasticity and has been implicated in affective disorders and their treatment. Exercise has been shown to have beneficial effects in these illnesses and increases BDNF signalling in the brain. The common BDNF val66met polymorphism is associated with reduced BDNF release and increases risk for anxiety and depression. We investigated the effect of chronic exercise on anxiety-like behaviour in a novel BDNF val66met rat model. After weaning at three weeks, the animals were held in dedicated running wheel cages (LaFayette, USA) or remained sedentary in standard individually-ventilated IVC cages. From 8 weeks of age, the animals were tested in a battery of behavioural paradigms for anxiety-like behaviour and cognition. The number of running-wheel revolutions increased with age, was higher in female than in male rats, and not dependent on genotype. Time spent on the open arms of the elevated plus maze was higher in female rats than in male rats. Unexpectedly, chronic exercise rats showed a marked reduction of open-arm time, suggesting increased anxiety. This effect was similar in males and females and independent of the val66met genotype. Similar results were found with time spent in the centre of an open-field, another measure of anxiety-like behaviour. Other behavioural tests showed little effect of exercise. In conclusion, despite the common belief that chronic exercise reduces anxiety, our results suggest a specific but paradoxical increase in anxiety. The data so far show little influence of the val66met genotype on these behaviours or the effect of exercise.

id #11689

AUTONOMIC AND RESPIRATORY MODULATION VIA OPTOGENETIC STIMULATION OF LEFT VS RIGHT VAGAL NEURONS OF DIFFERENT EMBRYOLOGICAL LINEAGES

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The vagus nerve contains axons derived from neurons of two distinct embryonic origins, namely the neural crest (Wnt1-expressing) and epibranchial placodes (Phox2b-expressing). We hypothesise that embryonic origin dictates the neuroanatomical and neurophysiological properties of vagal axons. Using transgenic mice expressing channelrhodopsin-2 (ChR2) under Wnt1 (Wnt1xChR2) or Phox2b (Phox2bxChR2) promoter we have investigated the autonomic cardiorespiratory effects of selectively stimulating neural crest- or placodal-derived sensory and motor vagal axons, and determined whether optical stimulation of left and right vagus nerves produces comparable responses. Under isoflurane anaesthesia, carotid blood pressure (BP), heart rate (HR), diaphragm electromyography and oesophageal pressure were recorded during optogenetic cervical vagal nerve stimulation (opto-VNS). In Phox2b x ChR2 mice, both left and right opto-VNS induced a significant decrease in BP and HR and increase in oesophageal pressure. Left opto-VNS (>20Hz) decreased respiratory rate (RR) while right opto-VNS induced frequency-dependent biphasic change in RR (increase with 5-20 Hz and decrease with >30 Hz). In Wnt1 x ChR2 mice, a

small but significant increase in RR was observed only with right opto-VNS (20-50Hz) while HR, BP and oesophageal pressure were not altered by both right and left opto-VNS. Ex vivo electrophysiology recordings with opto-VNS also revealed differential recruitment of nerve fibre types (A, B and C in PhoxbxChR2 and only C in WntxChR2 mice) which underlies the observed physiological changes. These findings indicate that vagal fibres of neural crest or placodal origins from left and right side provide differential sensorimotor effects on autonomic outflow and respiratory patterning.

id #11695

Recurrent SCN2A autism variant S1758R shows a loss-of-function phenotype

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SCN2A is the most commonly mutated gene associated with neurodevelopmental disorders, including intellectual disability, epilepsy, and autism. *SCN2A* encodes the Nav1.2 voltage-gated sodium channel subunit that underlies action potential electrogenesis in excitatory neurons. The S1758R Nav1.2 variant has been described in two cases of *de novo* autism, with patients exhibiting similar clinical symptoms including developmental delay, restricted-repetitive behaviours, atypical sensory processing and no history of seizures. We heterologously expressed and electrophysiologically characterised the S1758R variant. Whole-cell patch clamp experiments revealed the S1758R mutation causes almost complete channel loss-of-function, reducing cell current density to less than 10% of wild-type currents. Behavioural assessment of an S1759R knock-in mouse model, the equivalent position in mouse *Scn2a*, is underway. S1759R mice have no spontaneous seizures, however they demonstrate a higher susceptibility in the pentylenetetrazol-induced seizure assay compared to control animals. S1759R mice also exhibit male-specific behavioural changes, demonstrating cognitive deficits across several tests. These results corroborate the patient phenotype and the literature, with all previously reported *SCN2A* autism mutations demonstrating a loss-of-function. To account for genetic modifiers of S1758R autism, we have obtained a skin biopsy from one patient and generated induced pluripotent stem cells (iPSCs), simultaneously correcting the mutation using a CRISPR/Cas9 approach to obtain an isogenic control iPSC line. The identified disease markers in iPSC-derived neurons will be used to screen and develop novel therapies that reverse the mechanism of disease, having implications for not only the two identified S1758R patients but all who carry a loss-of-function mutation in *SCN2A*.

id #11722

Altered neuronal regulation of mucosal barrier function in the Neuroigin-3^{R451C} mouse model of autism

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Up to 90% of autism individuals experience gastrointestinal (GI) dysfunction. Autism patients expressing a point mutation (R451C) in the Nlgn3 gene encoding the Neuroligin-3 (NL3) synaptic adhesion protein also show GI dysfunction. The intestinal mucosal barrier is regulated by the enteric nervous system (ENS) and when compromised, antigenic materials translocate into the body; a potential cause for GI distress associated with autism. Nlgn3 expression is well characterised in the brain but not in the gut. We therefore first localised Nlgn3 mRNA in the ileal mucosa using RNAScope *in situ* hybridization. Next, we evaluated ileal mucosal barrier function by assessing transepithelial resistance and paracellular permeability using an Ussing chamber. To investigate whether this mutation affects ileal submucosal neuronal populations, neurons expressing Choline Acetyltransferase (ChAT) and Vasoactive intestinal peptide (VIP) were quantified using immunofluorescence in WT and NL3^{R451C} mice. In the ileum, RNAScope showed that Nlgn3 is predominantly expressed in submucosal neurons, glia and PYY, GLP-1 and 5-HT enteroendocrine cells. Ussing chamber experiments showed increased intestinal permeability ($p=0.001$) and decreased transepithelial resistance in NL3^{R451C} ileal tissue compared to controls ($p<0.0001$). In the ileal submucosal plexus, NL3^{R451C} mice had more VIP-positive neurons (WT ($n=3$), $54.3\pm 1.5\%$, NL3 ($n=6$) $61.9\pm 0.9\%$; $p=0.0029$) and fewer ChAT-positive neurons (WT ($n=3$), $45.2\pm 1.5\%$, NL3 ($n=6$) $38.3\pm 1.2\%$ $p=0.012$) compared to wild types. These findings reveal that Nlgn3 is expressed in the enteric mucosal neuronal circuitry and alters neuronal numbers and the regulation of mucosal barrier function in mice.

id #11724

Selective inhibition of ROCK2 does not cause neurotrophic changes in cultured mouse astrocytes

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Astrocytes are highly plastic cells, responding rapidly to promote brain homeostasis and neuronal viability. Impaired astrocytic function contributes to neuronal death underlying neurodegenerative diseases, and thus modulation of astrocytic behavior represents a therapeutic target. Astrocytic morphology alters rapidly following physiological or pathological stimuli, some of which also alter glutamate transporter (EAAT) activity. These morphological changes are produced by remodelling of the actin cytoskeleton, which is effected by Rho-associated coiled-coil containing kinases (ROCK). Drugs that non-selectively inhibit ROCK cause stellation of astrocytes and have shown therapeutic potential, however the functions of the two isoforms, ROCK1 and ROCK2, in astrocytes are not understood. This study aimed to determine the effect of selectively inhibiting ROCK2 in astrocytes compared to inhibiting both isoforms non-selectively. Primary cultures of mouse

astrocytes were treated with non-selective ROCK inhibitors Fasudil or Y27632, or the ROCK2 specific inhibitor KD025 for 24 hours. Morphology was assessed with immunostaining for GFAP, and actin dynamics were determined with fluorescent probes for G-actin and F-actin. Lactate dehydrogenase (LDH) and MTT assays were performed on culture medium to investigate cytotoxicity. Both Fasudil and Y27632 induced stellation of astrocytes, accompanied by rearrangement of F-actin fibres from distinct, brightly stained rings to diffuse and disorganized labelling, indicative of their disassembly. Inhibition of ROCK2 with KD025 did not reproduce these changes, and produced cytotoxic effects. These findings suggest that ROCK2 inhibition is not responsible for the beneficial changes associated with astrocyte stellation caused by pan ROCK inhibition.

id #11733

Habitual behaviour resulting from high-calorie food is prevented by an orexin-receptor antagonist

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We have previously shown that giving rats access to sugary-fatty milk shifts food-seeking from flexible and goal-directed, to automated and habitual (1). Orexin is a neuropeptide implicated in addiction-related behaviour, and its receptors are located within the neurocircuitry that mediate habitual actions. The aim of this study was to determine if the orexin system can be utilised to restore goal-directed food seeking following high-calorie diet, and to demonstrate that this effect is mediated by the substantia nigra. Rats were given access to either sweetened condensed milk or regular chow. Animals were then trained to press a lever for a pellet outcome and tested using a sensory specific satiety procedure. At test, rats were peripherally administered the orexin-A-receptor antagonist, SB334867 (10mg/kg), or vehicle solution. In a second study, these solutions were administered directly into the substantia nigra. Control animals demonstrated goal-directed behaviour reducing lever response rates under devalued conditions compared to non-devalued conditions. In contrast, HCD-exposed animals pressed the lever at equal rates under devalued and non-devalued conditions, thus demonstrating habitual responding for food. Importantly, both peripheral and intra-nigral administration of SB334867 restored goal-directed behaviour following HCD. This study demonstrates that antagonism of orexin-A receptors reduces poor decision-making capacity, and thus may be a potential treatment for regaining control of food intake. It also implicates the orexin system in the regulation of habitual actions. The substantia nigra is known to project to the dorsolateral striatum to mediate habitual actions, and thus orexin is upstream of this neurocircuitry.

1. Furlong, T. M., Jayaweera, H. K., Balleine, B. W. & Corbit, L. H. (2014). Binge-Like Consumption of a Palatable Food Accelerates Habitual Control of Behavior and Is Dependent on Activation of the Dorsolateral Striatum. *Journal of Neuroscience*, 34(14), 5012-5022. <http://doi.org/10.1523/Jneurosci.3707-13.2014>

id #11740

Increased inflammation and macrophage infiltration is associated with altered subependymal zone neurogenesis in schizophrenia but not bipolar disorder

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Background: Inflammation is implicated in the pathogenesis of schizophrenia and bipolar disorder. Inflammation regulates neurogenesis, and markers for stem cells and neuronal progenitors are reduced in schizophrenia and bipolar disorder in the subependymal zone (SEZ) – the brain’s largest region of neurogenesis.

Methods: We performed total RNA sequencing in the SEZ of 20 post-mortem schizophrenia and 21 control brains, validated by quantitative PCR in 32 schizophrenia and 32 controls that overlapped and 29 bipolar disorder cases. Immunohistochemistry was used for quantification and localisation of CD163+ macrophages. Cluster-analysis of *IL6*, *IL6R*, *IL1R1* and *SERPINA3* expression defined low and high inflammation groups, which were used to compare neurogenesis marker expression.

Results: Out of >60,000 genes, the most significantly differentially expressed gene in schizophrenia was *CD163*, a macrophage marker, which was increased 3.3 times compared to controls and confirmed by quantitative PCR. CD163+ macrophages were located where SEZ neurogenic cells typically reside, and their density was increased in schizophrenia compared to controls and bipolar disorder (> 29%; $p < 0.03$). *CD163* expression positively correlated with the neural stem cell marker *GFAP δ* , but negatively correlated with neuronal progenitor marker *ASCL1* in schizophrenia but not bipolar disorder. Forty percent of schizophrenia cases were designated as high inflammation; they had even greater *CD163* expression and increased *GFAP δ* but decreased *ASCL1* expression.

Conclusions: Macrophage infiltration is a key difference in schizophrenia pathology and potentially drives inflammation. Increased macrophages and inflammation seem to sustain neural stem cells but reduce neuronal differentiation in schizophrenia, but not bipolar disorder.

id #11741

Effects of maternal immune activation and adolescent cannabinoid exposure on GABAergic and BDNF gene expression in the prefrontal cortex

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Schizophrenia is a neurodevelopmental disorder associated with genetic and environmental factors that can perturb brain development resulting in symptom emergence in adolescence and early adulthood. The multiple-hit hypothesis of schizophrenia postulates that schizophrenia is caused by a combination of multiple risk factors across different developmental periods that contribute to disease onset. As both maternal immune activation (MIA) and adolescent cannabinoid exposure (ACE) are risk factors for schizophrenia, we investigated the separate (MIA or ACE) and synergistic (combined MIA and ACE; two-hit) effects on GABAergic interneurons and brain-derived neurotrophic factor (BDNF). Pregnant Wistar rats were given either Poly(I:C) (5mg/kg) or saline intravenously at gestation day 19. Offspring received either synthetic cannabinoid HU210 (75 or 100µg/kg) or vehicle injections intraperitoneally for 14 days, from P35. We quantified GABAergic- and BDNF-related genes from the medial prefrontal cortex (mPFC) collected at either P50 or P90 ($n=20$ /group). At P50, GABA-synthesizing enzyme (*Gad1*) mRNA was significantly decreased in MIA and ACE groups, while mRNA encoding GABA transporter (*Slc6a1*) was decreased in MIA only. Similarly, K-Cl cotransporter 2 (*Slc12a5*) mRNA was decreased in MIA, ACE and two-hit groups at P50. Interestingly, these genes were unchanged at P90. BDNF-related genes like *Trkb T1* (truncated BDNF receptor) mRNA was increased at P50, whilst *Bdnf I* and *Bdnf IV* mRNA were increased at P90 in two-hit groups. Our results suggest that MIA or ACE may lead to transient depression of cortical interneuron function and that synthesis of more truncated BDNF receptor may lead to a block in neurotropic support.

id #11742

Pre-synaptic effects of general anaesthetics: from single molecule dynamics to whole-brain connectivity

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Since its discovery over 180 years ago, general anaesthesia has revolutionised the medical world by allowing safe and painless operations to be performed. Despite their critical importance, the complete mechanism by which these lipophilic drugs work is still unknown. Anaesthetic potentiation of post-synaptic sleep circuits in the brain inducing unconsciousness is well described, however widespread loss of responsiveness to painful stimuli is unaccounted for in this model. Recent insights into the mechanisms of general anaesthesia have largely paralleled our growing understanding of the brain, especially regarding synaptic neurobiology. Here we present evidence of a pre-synaptic mechanism for general anaesthetic drugs. Several studies have now shown that neurotransmission is disrupted by targeting the synaptic fusion machinery (SNARE proteins) by clinical intravenous and volatile anaesthetics, both *in vitro* and *in vivo*. Using super-resolution microscopy and single molecule tracking techniques (sptPALM), we investigated the effect of general anaesthetics on SNARE proteins in the brains of *Drosophila melanogaster* fruit flies. The mobility of a genetically encoded SNARE protein, syntaxin1A, fused with the photoconvertible mEos2 tag in intact adult fly brains was tracked using sptPALM. Syntaxin1A-mEos2 was consistently and significantly immobilised in the presence of clinically relevant concentrations of general anaesthetics, such as propofol and etomidate. We contrast these single molecule results with whole-brain imaging in live animals, which show that general anaesthetic drugs cause a reduction in neurotransmission and decreases functional connectivity across the brain. Together, these results help explain how local effects across millions of synapses translate to global effects on brain function.

id #11743

ENTERIC NEURAL MECHANISMS OF GUINEA PIG FAECAL PELLET FORMATION

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Objective: Propulsion of the faecal pellets in the guinea pig involves enteric neural circuits. However, the extent to which enteric circuits shape the colonic contents and transform the homogeneous semisolid contents into faecal pellets is largely unknown. We investigated the underlying enteric neural mechanisms in isolated preparations of guinea-pig colon by comparing the motor patterns associated with propulsion of natural contents compared with those evoked by infusion of contents of varying viscosity.

Methods: The full proximal colon, colonic flexure and part of the distal colon taken from 12 adult guinea pigs euthanized according to the Institutional AWC were placed in an organ bath containing carbogenated Krebs solution at 36.5°. Combined recordings were made to construct spatiotemporal maps of movements by video and electromyography of smooth muscle activity with suction electrodes.

Findings: Upon slow infusion of 4% methyl cellulose into the proximal colon, individual pellets with similar size to natural ones were generated at the flexure region at intervals. The formation of these pellets from methylcellulose coincided with each of the proximal colon migrating motor complexes as they slowly reached the colonic flexure. Lesions to the enteric circuits in the proximal colon by crushing did not prevent the formation of pellets at the flexure, whereas lesions to the colonic flexure changed the single pellets formation into a series of continuous fluid pellets in the distal colon.

Conclusion: The guinea pig colon contains neural circuits sufficient, not only to propel, but also to actually shape the faecal content at the colonic flexure.

id #11771

SV2A surface pool nanoclustering and recycling in synaptic vesicles is controlled by synaptotagmin-1 in hippocampal nerve terminals

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Following the fusion of synaptic vesicles (SV) with the plasma membrane, recycling of vesicular proteins by endocytosis requires interaction between multiple vesicular cargoes to facilitate their efficient retrieval from the plasma membrane and help maintain the protein composition of SVs. It is unclear whether vesicular proteins are sequestered transiently just prior to internalization, or whether they are organised in functional nanoclusters on the plasma membrane that help package and prime proteins for retrieval. In this study, we examined the mechanisms that regulate the surface clustering of synaptic vesicle protein 2A (SV2A), a common vesicular cargo. Using the single molecular imaging technique, universal Point Accumulation Imaging in Nanoscale Topography (uPAINT), to image the atto647N-nanobody-labelled SV2A-pHluorin mobility on the plasma membrane in cultured hippocampal neurons, we reveal a fraction of SV2A molecules are immobile and define nanoclusters. A mutation designed to prevent synaptotagmin-1 (SYT1) binding (T84A) significantly reduced SV2A clustering. In contrast, a mutation which ablated the interaction of SV2A with AP2 (Y46A) did not significantly impact SV2A mobility, suggesting that SV2A clustering occurs independently of its incorporation into endocytic pits. In addition, we examined the impact of an epilepsy-conferring mutant (R383Q) on SV2A mobility and observed no effect on SV2A surface nanoclustering. We also performed sub-diffractive Tracking of Internalised Vesicles (sdTIM) and showed that interactions with both SYT1 and AP2 were required for retrieval of SV2A in recycling SVs. Our results demonstrate that SYT1 controls SV2A surface pool nanoclustering and, along with AP2, is critical for its internalisation into recycling SVs.

id #11774

CHARACTERIZING A MOUSE MODEL OF *HCN1* GENETIC EPILEPSY

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A variety of mutations in HCN ion channels cause epilepsy, including the HCN1 p.M305L variant which has been found in two unrelated people with severe early-onset developmental and epileptic encephalopathy¹. The Hcn1 p.M294L knock-in mouse model was engineered to study the pathophysiological mechanisms behind how this mutation causes epilepsy. Hcn1 p.M294L heterozygous knock-in mice are significantly smaller than their wild-type littermates and show a propensity to sudden death around weaning age. Thermogenic and pentylenetetrazol seizure assays showed that Hcn1 p.M294L heterozygous knock-in mice are more susceptible to seizures than their wild-type littermates. EEG traces recorded from Hcn1 p.M294L heterozygous knock-in mice show frequent high-amplitude excitable spikes which are absent from recordings of wild-type mice. Preliminary voltage clamp recordings from layer V somatosensory cortex pyramidal neurons suggest that Hcn1 p.M294L channels have lost voltage dependence and remain open at depolarized membrane potentials. This is consistent with observations of HCN1 p.M305L channel function in the *Xenopus* oocyte expression system. Current clamp recordings showed neurons from Hcn1 p.M294L heterozygous knock-in mice sit at significantly more depolarized resting membrane potentials than those from wild-type mice, potentially leading to increased excitability. Cells from knock-in mice also have a significantly more depolarized action potential threshold, presumably an adaptation to the more depolarized membrane potential. These results provide insight into how HCN1 channel dysfunction can cause epilepsy and position the Hcn1 p.M294L heterozygous knock-in mouse as a good preclinical model of *HCN1* disease.

1. Marini C, Porro A, Rastetter A, et al. HCN1 mutation spectrum: from neonatal epileptic encephalopathy to benign generalized epilepsy and beyond. *Brain* 2018; 141:3160-3178.

id #11777

The voltage gated calcium channel CaV1.2 promotes adult oligodendrocyte progenitor cell survival in the mouse corpus callosum but not motor cortex

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Throughout life, oligodendrocyte progenitor cells (OPCs), also known as NG2-glia, proliferate and differentiate into myelinating oligodendrocytes. OPCs express cell surface receptors and channels that allow them to detect and respond to neuronal activity, including voltage-gated calcium channel (VGCC)s. The major L-type VGCC expressed by OPCs is

CaV1.2, which has been shown to regulate developmental OPC differentiation. However, it is unclear whether CaV1.2 similarly regulates OPC behaviour in the healthy adult central nervous system (CNS). To examine the role of CaV1.2 in adulthood, we conditionally deleted this channel from OPCs by administering tamoxifen to P60 *Cacna1cfl/fl* (wildtype) and *Pdgfra-CreERTM* :: *Cacna1cfl/fl* (CaV1.2-deleted) mice. Whole cell patch clamp analysis revealed that CaV1.2 deletion reduced L-type voltage-gated calcium entry into adult OPCs by ~59%. However, CaV1.2 deletion did not affect the number of new oligodendrocytes produced or influence the length or number of internodes they elaborated. Unexpectedly, CaV1.2 deletion resulted in the dramatic loss of OPCs from the corpus callosum, such that 7 days after tamoxifen administration CaV1.2-deleted mice had an OPC density ~42% that of wildtype mice. OPC density recovered within two weeks of CaV1.2 deletion, due to replacement from surviving CaV1.2-deleted OPCs. As the density of OPCs in the motor cortex and spinal cord was not affected at any time-point examined, we conclude that calcium entry through CaV1.2 is a critical survival signal for a subpopulation of callosal OPCs but not for all OPCs in the mature CNS.

id #11783

The kappa opioid receptor agonist ethoxymethyl ether Salvinorin B promotes functional recovery and remyelination in preclinical models of multiple sclerosis

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While there are a wide range of disease-modifying therapies that are effective at targeting the immune system during relapsing-remitting multiple sclerosis (MS), there are no therapies that can enhance the repair of damage in the central nervous system. Treatments that promote remyelination are urgently needed to prevent progressive disability and enable recovery in all forms of MS. The kappa opioid receptor agonist Ethoxymethyl ether Salvinorin B (EOM), a C-2 analogue of Salvinorin A, is highly effective at promoting functional recovery and remyelination in two models of MS. When administered therapeutically after disease onset in the experimental autoimmune encephalomyelitis (EAE) model, paralysis was completely resolved in over 85% of EOM-treated mice compared to 28% of those treated with vehicle alone ($p < 0.01$ by Chi-square test). This protection could be partially blocked by inhibition of the kappa opioid receptor suggesting that this pathway contributes to the disease-modifying activity of EOM. In the cuprizone model of non-immune-mediated demyelination, therapeutic administration of EOM promoted remyelination. Transmission electron microscopy revealed that EOM increased the number of myelinated axons in the corpus callosum ($p < 0.002$, Student's t-test) and significantly reduced g-ratios (0.77 ± 0.003 ; mean \pm SEM) compared to vehicle treated controls (0.84 ± 0.01 ; $p < 0.0001$, Student's t-test). Moreover, g-ratios were restored to levels seen in healthy controls (0.78 ± 0.003). Given the proven analgesic and anti-inflammatory effects of EOM and its reduced side effect profile in

comparison to other known kappa opioid receptor agonists, this unique compound shows promise as a new therapeutic strategy to enable repair and remyelination during MS.

id #11802

INVESTIGATING THE ROLE OF THETA FREQUENCY OSCILLATIONS IN VISUAL PROCESSING DURING READING

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The auditory *temporal sampling hypothesis* (TSH) indicates that attending to speech-sounds entrains oscillations in the auditory domain to a theta frequency, thus enabling coding of the linguistic knowledge required for reading (Goswami, 2011). We suggest that theta oscillations might also be involved in visual processing during reading in a *visual correlate* to the auditory TSH. It is possible that sequential fixations and saccades during reading occur at a theta frequency, providing a rhythmic stimulus for oscillatory entrainment in the visual domain. We conducted a within-subjects ($N = 17$) experiment to gather eye-tracking data while participants completed natural reading tasks. We demonstrated that, as hypothesised, fixations during reading occurred at a theta frequency rate. In the experimental condition a 5Hz electrical stimulation (tACS) was conducted over the frontal eye fields, with the aim of improving the efficiency of eye-movements during reading. As hypothesised, theta stimulation decreased fixation frequency rate ($p < .001$), the number of progressive fixations per second ($p < .05$), and the length of time that the eye remained still during fixations ($p < .001$). The increased speed of these eye-movements did not incur a cost to reading comprehension or increase the need for readers to re-read sections of text. Remarkably, the temporal frequency of fixations increased from 3Hz to 5Hz, suggesting that theta oscillations may drive the oculomotor mechanics of reading. These results indicate that theta frequency oscillations in the visual domain are involved in reading and lends support to a visual correlate to the auditory TSH.

id #11803

DISRUPTION OF NEURITE NETWORKS IN DORSAL ROOT GANGLION NEURONS FOLLOWING TREATMENT WITH THE CHEMOTHERAPEUTIC DRUG PACLITAXEL

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Paclitaxel is a microtubule stabilising agent used as a successful anti-neoplastic therapy for the treatment of solid tumours. A common side effect of paclitaxel treatment is the development of chemotherapy-induced peripheral neuropathy characterised by debilitating sensory symptoms such as numbness, tingling, and ongoing pain. In this study, we tested the effects of paclitaxel on neurite networks of the dorsal root ganglion (DRG), which house the peripheral sensory afferents, using 3D explants and dissociated DRG neuronal cultures. DRGs were dissected from 5-week-old C57BL/6J male mice and were either cultured *ex vivo* for several days or were further dissociated for *in vitro* culture of primary sensory neurons before treatment with paclitaxel, or vehicle control.

The 3D DRG explants were analysed by neurite outgrowth assay using the ImageJ plugin, Neurite-J, and the dissociated neurons by Sholl analysis to identify morphological differences between groups.

In DRG explants, treatment with paclitaxel (25-200nM) induced an almost 85% reduction in axonal outgrowth ($p < 0.0001$). Sholl analysis of DRG neurons showed around a 50% decrease in the size of the neurite networks and a clear shift toward a multipolar network within the small, medium and large cell populations. Paclitaxel treated neurons had an extensive number of retraction bulbs and axonal bulges with disorganised microtubules within their networks.

These results demonstrate that paclitaxel acting in the whole DRG explant alters the morphology of sensory neurons by impairing their ability to support and maintain large and extensive neurite networks, pushing the individual neuron to adopt a dysfunctional and multipolar network instead.

id #11805

Modelling cell-cell interactions in the olfactory nerve using a novel 3D culture method

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We have developed a three-dimensional (3D) culture method suitable for modelling cell-cell interactions; the naked liquid marble (NLM)¹. In this system, cells are cultured in a droplet (the NLM) on a superhydrophobic plate. Inside NLMs, cells interact freely with each other and naturally form 3D spheroids in less than 24 h. Here, we determined how cells found in the olfactory nervous system arranged themselves when co-cultured in NLMs. We combined primary mouse olfactory ensheathing cells (OECs; olfactory glia) with other cell types, and analysed the location of the different cell populations using the spot identification tool in

IMARIS software. When co-cultured with astrocytes, OECs formed the spheroid core with astrocytes forming a distinct outer layer, resembling the *glia limitans* separating the central and peripheral part of the olfactory nervous system. When OECs, fibroblasts and macrophages were co-cultured, the 3D arrangement resembled that in the olfactory nerve (without axons), with an OEC core surrounded by fibroblasts and macrophages largely on the outside of spheroids. Using this 3D model, in combination with two-dimensional (2D) confrontation assays, we showed that macrophage migration inhibitory factor (MIF) is crucial for the arrangement of OECs, fibroblasts and macrophages. In 2D cultures, OECs and macrophages co-mingled only when MIF was inhibited (using the tautomerase inhibitor ISO-1). Inhibition of MIF also led to displacement of macrophages towards the interior of 3D spheroids. Thus, the NLM system is suitable for modelling specific regions of the nervous system and for assessing the roles of specific molecules/factors in regulating cell-cell interactions.

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id #11843

Phosphorylated Tau Interactome in the Human Alzheimer's Disease Brain

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Accumulation of phosphorylated tau (pTau) is a key pathological feature of Alzheimer's disease. The pathological actions of pTau are mediated by surrounding neuronal proteins, however our knowledge of the specific proteins that pTau interacts with in Alzheimer's disease is surprisingly limited. Therefore, the aim of this study was to map the pTau interactome in the human brain for the first time. To do this we used two complementary proteomics approaches. First, quantitative proteomics was performed on neurofibrillary tangles microdissected from patients with advanced Alzheimer's disease. Second, affinity purification-mass spectrometry was used to identify which of these proteins specifically bound to pTau. 542 proteins were identified in neurofibrillary tangles including abundant detection of proteins known to be present in neurofibrillary tangles including tau, ubiquitin, neurofilament proteins and apolipoprotein E. Affinity purification-mass spectrometry confirmed that 75 of these proteins directly interacted with pTau. 34 of these proteins are known to interact with tau, therefore validating our approach. More excitingly, 34 proteins had previously been associated with Alzheimer's disease, but not linked directly to tau (e.g. synaptic protein VAMP2, RNA binding protein HNRNPA1), therefore we provide evidence that their involvement in Alzheimer's disease likely involves their interaction with pTau. We also identified 7 novel proteins, not previously known to be associated with Alzheimer's disease. Our results reveal novel potential drug targets for the treatment of tauopathies and provide insight into how pTau mediates its pathological effects in Alzheimer's disease.

id #11869

A novel cell-ablation strategy for studying the role of oligodendrocyte-forming stem cells in myelin homeostasis and regeneration

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Adult oligodendrocyte progenitor cells (OPCs) and subventricular zone (SVZ)-derived neural precursor cells (NPCs) are two endogenous stem cell populations in the mouse brain with the capacity of differentiating into oligodendrocytes (OLs) following cuprizone-induced demyelination. To further determine the precise role of OPCs and NPCs in homeostasis and to explore whether spatial competition exists between them for effective remyelination in the corpus callosum, we have developed novel transgenic mouse models of conditional OPC or NPC ablation, which can be achieved by crossing a Cre-dependent *Sox10-DTA* mouse line with *Pdgfra-CreER^{T2}* or *Nestin-CreER^{T2}* mice, respectively. Our pharmacogenetic approach resulted in a complete loss of OPCs throughout the entire brain of *PdgfraCreER^{T2}; Sox10-DTA* transgenic mice for up to 10 days. This OPC ablation initiated a complex sequence of cellular responses characterised by inflammatory and neurovascular changes accompanied by the presentation of an anxiety-like phenotype. Restoration of normal behaviour after 10 days of OPC loss coincided with the return of normal microglial densities and the onset of Pdgfr α + cell regeneration, which arose first in regions adjacent to the SVZ. This suggests that OPC ablation in the normally myelinated brain could mobilise SVZ-derived NPCs to efficiently migrate to the brain parenchyma where they produce new OPC-like cells. The sole contribution of NPCs to remyelination in the OPC-deficient mice, and vice versa, are currently under investigation. Overall, our study will provide fundamental understanding of the repertoire of functions that OPCs and NPCs serve in the adult central nervous system in both health and disease.

id #12121

Development and validation of a novel circuit-based chemogenetic inhibitory approach.

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Background: Optogenetic and chemogenetic approaches are vital tools for studying neural function. The insect allatostatin (Ast)/allatostatin receptor (AstR) system is a highly specific and effective inhibitory chemogenetic approach, but its adoption has been limited as the peptide, Ast, needs to be delivered into the brain. However, unlike ligands for DREADDs or light for optogenetics, Ast is genetically encoded. We have taken advantage of this to develop a viral approach to express Ast in neurons, and thus extend the chemogenetic approach to provide circuit information. We tested this in the vagal viscerosensory system due to the clear anatomical separation between the sensory cell bodies, in the nodose ganglia (NDG) in the neck, and their projections to the nucleus of the solitary tract (NTS) in the medulla.

Objective: To develop and validate a novel circuit-based chemogenetic approach. **Methods:** Sterile surgeries were performed under ketamine/medetomidine anesthesia with appropriate analgesia. An adeno-associated virus expressing Ast was injected into both NDG of Spontaneously Hypertensive Rats. Two weeks later blood pressure (BP) telemeters were implanted and two weeks after that a lentivirus, expressing either AstR or GFP, was injected into the NTS. **Results:** We observed inhibition of baroreceptor reflex sensitivity for the entire 31-day observation period post AstR expression. We also observed a more transient increase in BP, increased BP variability and decreased heart rate variability. Body weight decreased for the entire period. **Discussion:** We observed physiological changes consistent with inhibition of vagal afferent input to the NTS induced by our novel chemogenetic construct.

id #12128

The novel gene PHF21B associated with major depression exhibits sexually dimorphic responses to chronic stress in a transgenic preclinical model

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We aim to determine the role PHF21B plays in responding to stress and progression to depression-like behaviour. A gene wide association study of rare variants found PHF21B to be associated with Major Depressive Disorder.

Targeted mutations in PHF21B by CRISPR-Cas9 founded two transgenic mouse lines on a C57BL/6J background. Male and female animals were subjected to chronic mild stress and contrasted to non-stressed control groups. Anhedonia, anxiety and despair like behaviours were assessed by sucrose preference, open field and the Porsolt swim tests respectively. The latter two tests were assessed using Noldus Observer XT video tracking. Tissues were collected and quantitative gene expression was measured by qRT-PCR. Associations between sex, treatment and genotype were statistically tested for behavioural and differential gene expressions.

Behavioural responses to chronic stress differed between male and female wild-type and PHF21B transgenic animals. Immobility in the Porsolt swim test found females exhibited more despair-like behaviour than males both before and after stress. Numerous genes were also found to be differentially expressed between sexes and genotypes, in various brain regions.

Our transgenic preclinical model demonstrates PHF21B does play a role in depression-like behaviour. Males and females exhibit different depression-like behaviours in response to chronic stress. Gene expressions also vary between sexes and genotypes at baseline and following chronic stress. Further study is required to determine the interaction of PHF21B with anti-depressant treatments and identify sex specific pathways to explore therapeutic targets.

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id #12143

Does dopamine mis-metabolism underlie preclinical hyposmia in Parkinson's disease?

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Parkinson's disease (PD) is a neurodegenerative disorder with an extensive preclinical phase that is aligned with non-motor symptoms, including olfactory impairment. Hyposmia is a highly prevalent, canonical symptom that precedes the characteristic motor dysfunctions of PD. Despite the overt nature of hyposmia, the biological basis has yet to be elucidated, hampering its utility as an aid to early diagnosis. Currently, our characterization of postmortem PD olfactory bulb (OB) has demonstrated alterations in dopamine metabolism. There is an increase in the expression of tyrosine hydroxylase (TH), despite this not translating into an increase in dopamine. Simultaneously, there are significantly reduced levels of homovanillic acid, suggesting a failure of dopamine breakdown by COMT. Metal analysis has implicated a potential role of environmental factors, indicated by an increase in the level of lead. We have recently shown that the tau KO mice have pre-motor hyposmia¹. Expanding on these findings here we show that the tau KO mice show features of dopamine mis-metabolism synonymous with the human tissue, such as increased TH not translated to increased dopamine. Furthermore, the hyposmia present in these animals can be rescued using the D₂R antagonist Haloperidol, and hyposmia can be induced in healthy mice using cocaine, further supporting our hypothesis that dopamine modulation can directly affect olfaction. Taken together, these studies highlight the utility of tau KO mice in pre-motor PD studies and suggest that hyposmia in PD may be driven by changes in dopamine metabolism in the olfactory bulb.

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id #12146

Characterisation of CLN5 Batten disease in induced pluripotent stem cell derived human neurons.

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Background: Batten disease is a group of fatal childhood neurodegenerative disease caused by mutations in one of at least 13 genes. One of the late-infantile onset Batten disease forms is caused by mutations in CLN5 and currently, there is no cure for this disease. CLN5 is a soluble lysosomal protein with yet unknown function.

Objective: To understand the molecular mechanisms underlying lysosomal dysfunction in CLN5 Batten disease using human induced pluripotent stem cell (iPSC) derived human neurons.

Brief method: A neuronal transcriptional regulator neurogenin-2 (NGN2) was stably integrated in iPSCs to induce differentiation into pure functional human glutamatergic neurons (i3Ns). Using CRISPR interference (CRISPRi), CLN5 was knocked down (CLN5i) in the iPSCs followed by differentiation into i3Ns and subsequent characterisation.

Key findings: CRISPRi knockdown of CLN5, using guide RNAs against 3 different regions around the CLN5 transcriptional start site, showed significant knockdown in iPSCs and differentiated i3Ns, both at transcript and protein levels. All three CLN5i i3Ns lines showed loss in LysoTracker fluorescence intensity suggesting change in lysosomal acidity. CLN5i i3Ns showed impaired lysosomal trafficking as observed by aberrant lysosomal movement, decreased velocity and impaired directionality. CLN5i i3Ns showed accumulation of p62, indicating inhibition of autophagy.

Conclusion: The retarded lysosomal trafficking in CLN5i i3Ns suggests inhibition of normal lysosomal function, thereby hampering recycling of neuronal wastes. Our iPSC-derived i3Ns CRISPRi model provides an efficient tool to test lysosomal dysfunction, not only in CLN5 Batten disease, but also in other neurodegenerative diseases and hence, offers high-throughput drug library screening for therapies.

id #12150

Does a nasal injury increase bacterial infection of your brain?

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There has been a recent growing body of research investigating the correlation between bacteria and chronic diseases of the central nervous system (CNS), such as neurodegenerative disorders, in particular Alzheimer's disease. Our previous work has shown that the bacterium *Burkholderia pseudomallei*, following intranasal inoculation, can enter the olfactory nerve and the intranasal branch of the trigeminal nerve, reaching the spinal cord within 24 hours of inoculation. To date, what has remained largely uncharacterised is the consequence of injury to the nasal olfactory epithelium for the invasion of the CNS by bacteria. In the current study, we investigated this by using an established olfactory epithelial injury model, where mice were administered methimazole via intraperitoneal injection, inducing patchy epithelial degradation. Then mice, both with and without epithelial degradation, were intranasally inoculated with *B. pseudomallei* for seven days. We have now discovered that epithelial injury greatly increases the invasion of *B. pseudomallei* within the olfactory epithelium. This led to bacterial invasion of the olfactory nerve fascicles within the lamina propria underlying the olfactory epithelium, continuing to the nerve fiber layer and then the glomerular layer of the olfactory bulb (CNS). Our work here shows, for the first time, that prior injury to the nasal epithelium increases the risk of olfactory nerve and olfactory bulb invasion by bacteria. Thus, these findings open the possibility that other bacterial species may also use this route to invade the CNS, contributing to the growing correlation between bacteria and chronic diseases of the CNS, such as Alzheimer's disease.

id #12152

Novel transgenic click chemistry tool enables the identification of distinct *de novo* proteomic changes during memory formation specifically in the hippocampus

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Spatial long-term memory (LTM) formation requires the *de novo* synthesis of distinct sets of proteins; however, a non-biased examination of the *de novo* proteome in this process has yet to be undertaken. Here, we generated a novel mouse strain, RC3, which enables cell-type-specific labelling of newly synthesised proteins with non-canonical amino acids (NCAAs) by genetically restricting the expression of the mutant tRNA synthetase, NLL-MetRS. Combining hippocampal targeting with a click chemistry technique termed fluorescent non-canonical amino acid tagging (FUNCAT), we revealed increased *de novo* protein synthesis in the hippocampus during spatial LTM formation. Furthermore, by performing bio-orthogonal non-canonical amino acid tagging (BONCAT) followed by SWATH quantitative mass

spectrometry, we identified a number of proteins which were altered in synthesis during spatial memory consolidation. These regulated proteins formed clusters associated with endocytosis, the modulation of synaptic transmission, synaptic vesicle recycling, and glycolysis, which provides further evidence that these processes are involved in spatial LTM formation. We anticipate that the RC3 strain will enable future cell-type or tissue specific examination of new protein synthesis in response to a wide range of stimuli.

id #12153

Activity-dependent recruitment and nanoscale organization of dynamin I isoforms in neurosecretory cells

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Dynamin family of GTPase proteins, required for clathrin-mediated endocytosis, mediate vesicular fission at the neck of clathrin-coated pits. Brain specific dynamin I comprise of eight structurally different isoforms. Whether and how these different dynamin isoforms are recruited to the endocytic sites remains unclear. Here, we used total internal reflection fluorescence (TIRF) microscopy to investigate the recruitment of EGFP-tagged dynamin I-bb and -aa (Dyn1bb/aa-EGFP) isoforms to the plasma membrane (PM) of neurosecretory PC12 cells in response to secretagogue stimulation. We detected a significant recruitment of Dyn1bb-EGFP, while Dyn1aa-EGFP was recruited to a lesser extent. Interestingly, Dyn1bb-EGFP recruitment was spatiotemporally non-homogeneous, forming discrete areas on the PM where the peak fluorescence intensity occurred at a later time point than elsewhere on the PM. Importantly, the formation of these high intensity areas was prevented by Dyngo-4a-treatment suggesting a role for dynamin GTPase activity. Such differential increase in dynamin I local concentration suggested an involvement of dual mechanism, first a non-specific recruitment to the PM and second, an accumulation of Dyn1bb-EGFP via lateral trapping. We therefore used single particle tracking photoactivated localization microscopy (sptPALM) to study the role of lateral trapping in the recruitment of Dyn1bb. We demonstrate that Dyn1bb-mEos2 molecules become more confined in response to stimulation and are organised in nanoclusters. Dyn1aa-mEos2 mobility was lower and not affected by stimulation suggesting that they form long-term nanoclusters on the PM. Together, our results suggest that Dyn1bb undergoes a two-phase recruitment; Dyn1aa, however, does not appear to follow similar recruitment mechanism.

id #12154

***Neisseria meningitidis* induces pathology-associated cellular and molecular changes in trigeminal Schwann cells**

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Neisseria meningitidis, an important cause of bacterial meningitis globally, can infect the central nervous system (CNS) via the nerves extending between the nasal cavity and the brain. While *N. meningitidis* is a commensal bacterium found frequently in the nasopharynx, the cellular mechanisms of CNS invasion remain unknown. Here, we show that *N. meningitidis* serogroup B can infect trigeminal Schwann cells (the main glial cells of the trigeminal nerve) *in vitro* in both two-dimensional and three-dimensional cultures. Infection of the cells led to nuclear alterations, with the presence of the atypical nuclei increasing over time. Using SWATH-MS proteomics followed by bioinformatics pathway analysis, we demonstrated that *N. meningitidis* serogroup B induced protein expression changes in the glia associated with altered intercellular signalling and cellular interactions. The analysis also showed that the alterations in protein levels were consistent with the carcinogenesis changes. Overall, these results demonstrate that *N. meningitidis* serogroup B can initiate the molecular and cellular changes in trigeminal Schwann cells including nuclear morphology such as nuclear atypia and also altered levels of proteins responsible for cellular haemostasis and proliferation. Thus infection of the trigeminal nerve by *N. meningitidis* serogroup B may have ongoing adverse effects on the biology of Schwann cells, which may lead to pathology.

id #12158

Brain-wide visual habituation networks in wild type and *fmr1* zebrafish

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Habituation is a form of learning during which animals stop responding to repetitive stimuli, and deficits in habituation are characteristics of several psychiatric disorders. Due to the technical challenges of measuring brain activity comprehensively and at cellular resolution, the brain-wide networks mediating habituation are poorly understood. Therefore, we decided to exploit the optical advantages and genetic toolkits of zebrafish larvae to investigate whole-brain visual habituation dynamics. By presenting larvae repeated threatening looming stimuli in behavioural and brain-wide calcium imaging experiments, we are able to analyse how stimuli features, like saliency and timing, modulate habituation. We show that different functional categories of loom-sensitive neurons habituate at different rates and are located in characteristic locations throughout the brain. Using graph theory, we are able to observe that the stimulus features also modulate brain network dynamics. These modulating properties uncover a principally visual circuit that habituates minimally, a moderately habituating midbrain population proposed to mediate the sensorimotor transformation, and downstream circuit elements responsible for higher order representations and the delivery of behaviour. We then extend our study to zebrafish larvae carrying a mutation in the *fmr1* gene, which present slower behavioural habituation and maintained network activity. This represents the first description of a visual learning network across the brain at cellular resolution, and provides insights into the circuit-level changes that may occur during normal and altered habituation.

id #12161

Activation of CRF2 receptor increases gastric vagal afferent mechanosensitivity

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Gastric vagal afferent (GVA) sensing of food related mechanical stimuli is a crucial mechanism in the control of feeding behavior and gastric function. Stress is an important factor contributing to eating disorder and gastric diseases. Chronic stress has been shown to increase the mechanosensitivity of GVAs in mice, which may contribute to the reduced food intake and body weight. Whether the mechanosensitivity of GVAs is modulated by stress hormones is not known. This study aimed to determine the effect of stress hormones on GVA mechanosensitivity. The expression of stress hormone receptors in GVA cell bodies was determined in 8 week old male C57BL/6 mice using qRT-PCR combined with laser capture microdissection. The mechanosensitivity of GVAs was determined in the absence and presence of stress hormones using an *in vitro* single fiber recording preparation. NR3C1 and CRHR2 (mRNA isoforms of glucocorticoid receptor and CRF2 receptor respectively) were expressed in GVA neurons. The glucocorticoid receptor agonist, corticosterone, had no effect

on the mechanosensitivity of either tension or mucosal GVAs. The CRF2 specific analogue, UCN3, significantly increased the mechanosensitivity of both tension and mucosal GVAs, an effect prevented by the CRF2 receptor antagonist astressin 2B. In conclusion, activation of CRF2 receptor increases the mechanosensitivity of GVAs. This may contribute to the stress and CRF2 receptor associated changes in feeding behavior and gastric function, possibly contributing to the hypersensitivity of GVAs in chronic stress conditions.

id #12162

Superior colliculus modulates cortical coding of somatosensory information

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The cortex sends a direct projection to the superior colliculus. What is largely unknown is whether (and if so how) the superior colliculus modulates activity in the cortex. Here, we directly investigate this issue, showing that optogenetic activation of superior colliculus changes the input-output relationship of neurons in somatosensory cortex during whisker movement, enhancing responses to low amplitude whisker deflections. While there is no direct pathway from superior colliculus to somatosensory cortex, we found that activation of superior colliculus drives spiking in the posterior medial (POm) nucleus of the thalamus via a powerful monosynaptic pathway. Furthermore, POm neurons receiving input from superior colliculus provide excitatory input to somatosensory cortex. Silencing POm abolished the capacity of superior colliculus to modulate cortical whisker responses. Our findings indicate that the superior colliculus, which plays a key role in attention, modulates sensory processing in somatosensory cortex via a powerful disinaptic pathway through the thalamus.

id #12163

Group I metabotropic glutamate receptors are involved in neural control of colonic motility

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Group I metabotropic glutamate receptors (mGluRs) comprise mGluR1 and mGluR5 and have well established roles in excitatory neurotransmission and synaptic plasticity in the central nervous system (CNS). The enteric nervous system (ENS) is a network of neurons and glia in the gut wall that controls gastrointestinal function. The major excitatory neurotransmitter in the ENS is acetylcholine, but there is evidence that glutamate also has a role in enteric neurotransmission. However, the individual roles of mGluR1 and mGluR5 in

gastrointestinal function remain unknown. Here, we examined roles of mGluR1 and mGluR5 on neurally-mediated propagating contractions in the isolated colon of the mouse, known as colonic migrating motor complexes (CMMCs). CMMCs were examined in colons of male and female C57BL/6 mice in the presence of the Group I mGluR antagonist PHCCC (30 μ M), as well as specific antagonists against mGluR1 (BAY 36-7620, 10 μ M) and mGluR5 (MPEP, 10 μ M). Exposure to PHCCC produced a significant decrease in frequency of CMMCs ($p=0.01$, $n=10$), CMMC length ($p=0.01$, $n=10$), and CMMC speed ($p=0.01$, $n=10$). Similarly, when mGluR1 was blocked with BAY 36-7620, CMMC frequency ($p=0.03$, $n=8$), CMMC length ($p=0.03$, $n=8$), and CMMC speed ($p=0.03$, $n=8$) were decreased significantly. Decreased CMMC frequency was also observed when mGluR5 was blocked with MPEP ($p=0.03$, $n=7$), but there was no change in CMMC length ($p=0.11$, $n=7$) or speed ($p=0.18$, $n=7$). These data suggest that both mGluR1 and mGluR5 are involved in the initiation of colonic contractions in the mouse, but only mGluR1 is involved in propagation of contractions.

id #12165

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

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Myelination of central nervous system axons increases action potential conduction speed. However, it is unclear whether myelination optimizes action potential conduction to facilitate information processing within the brain. To investigate this, we altered neuronal activity in adult mice (P60-90) through low-intensity repetitive transcranial magnetic stimulation (rTMS; 120mT rodent coil, 3 minutes daily), or spatial learning (radial arm maze) over 14 days. We found that rTMS reduced the average length of nodes of Ranvier by ~10% in the cortex, and by ~8% in the corpus callosum (CC), but that spatial learning increased node of Ranvier length by ~31% in the hippocampal fimbria. This modulation of node length 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, that was primarily associated with a ~31% increase in the space between the myelin the axon known as the periaxonal space. Conversely, spatial learning induced a ~5.8% increase in g-ratio and a ~19% decrease in the size of the periaxonal space within the fimbria. Functionally, the myelin and nodal changes induced by rTMS were associated with a ~20% reduction in myelinated axon conduction velocity and a ~30% increase in the compound action potential amplitude of the myelinated axon peak. These data are the first to demonstrate that this kind of change in axon-glia configuration, which is independent of oligodendrogenesis, tunes conduction velocity to increase the synchronicity of action potential conduction.

id #12180

A Retrospective Analysis of 5 Years of Laboratory Data to Identify Interrelationships Between Outcome Measures in Experimental Stroke

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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 parameters are difficult to identify. We performed a retrospective large-scale analysis on 716 control C57BL/6 mice subjected to either transient (1h) middle cerebral artery occlusion (tMCAO) or permanent MCAO (pMCAO). Structural equation modelling (SEM) was used to construct models to identify cause-effect relationships among numerous variables recorded at 24h post-stroke. Our collective data demonstrate that following tMCAO, infarct volume is fully developed within 24h, whereas brain edema continues to evolve. Eighty-five percent of lung infections resolved within 48h of tMCAO, accompanied by improved functional outcome. There was greater leukocyte infiltration in brains of mice receiving pMCAO, and more severe leukopenia. Multivariable analyses revealed that edema was positively correlated with infarct volume (Beta=0.778) and clinical score (Beta=0.365) in tMCAO, but not pMCAO. Age (during young adulthood; i.e. 6-40 weeks old) was correlated positively with lung infection (Beta=0.540), and negatively with mobility (Beta=-0.322) following tMCAO. However, in pMCAO mice, age did not appear to affect infarct volume, functional outcomes or lung infection. Bivariable analysis (Spearman's rank correlation) showed clinical score to be negatively correlated with circulating leukocytes ($\rho=-0.286$), but positively correlated with brain leukocytes ($\rho=0.332$) following tMCAO, but not pMCAO. This large-scale analysis of animal data has thus provided insight into relationships between variables not available when studies are analysed in isolation.

id #12181

Dynamic Within-Subject Functional Connectivity in the Resting State Using High Temporal-Resolution Simultaneous BOLD-fMRI FDG-PET

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Objective: To compare resting-state functional connectomes acquired using BOLD-fMRI and FDG-PET simultaneously. Recent developments in acquisition protocols have made it possible to introduce a temporal dimension to FDG-PET acquisitions. We employ these protocols to probe metabolic and haemodynamic connectivity.

Brief method: 27 healthy right-handed volunteers underwent a simultaneous MR-PET scan using a 3 Tesla Siemens Biograph. fMRI was acquired with 2.45 second volumes in 6 blocks of 10 minutes. A continuous infusion of PET tracer (FDG) was administered to allow dynamic modelling of the PET signal in 16 second frames. A gradient filter was applied to model the instantaneous change in the PET signal. Timeseries were extracted in brain regions and correlated to construct metabolic (FDG-PET) and haemodynamic (BOLD-fMRI) functional connectomes for each subject. Group average connectomes were compared.

Key findings: Subject-level functional connectomes were obtainable with FDG-PET with a temporal resolution of 16 seconds. The metabolic and haemodynamic connectomes were correlated ($R=0.44$) with strong similarities in some brain regions (frontal and parietal lobe) and divergence in others (occipital lobe). Strong metabolic connectomes were matched in the BOLD-fMRI connectome, however many highly correlated BOLD-fMRI regions did not appear to be metabolically correlated.

Conclusion: Metabolic and haemodynamic connectomes can be acquired simultaneously at a high temporal resolution. These complimentary measures provide a new avenue for probing cerebral function in health and disease.

id #12182

Ventral pallidum subpopulations in reward seeking behavior

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Ventral pallidum (VP) is a well established locus for the reinforcing effects of drugs of abuse and reinstatement of drug seeking. However, VP neurons are at the origin of multiple output pathways, with strong projections to ventral tegmental area (VTA), subthalamic nucleus (STN) and the lateral hypothalamus (LH), among others, and the roles of these VP output pathways in reinstatement of drug seeking remain poorly understood. Here we addressed these issues using a combination of neuroanatomical tracing, chemogenetic and optogenetic approaches. VP subpopulations of the GABAergic and parvalbumin (PV) showed activity during relapse but not glutamate or cholinergic neurons. Retrograde tracing showed VP neurons projecting to the LH are recruited during context-induced reinstatement of extinguished alcohol seeking in rats. To determine the causal roles of VP-LH pathway in context-induced reinstatement we used complementary approaches of chemogenetic contralateral disconnection or bilateral optogenetic inhibition of VP terminals in LH. Silencing the VP-LH pathway using either approach prevented context-induced reinstatement. Selective chemogenetic inhibition VP Gad1 neurons contribute to contextual control over relapse (renewal), but not to relapse during reacquisition, via projections to LH where they converge with ventral striatal inputs onto LH Gad 1 neurons. In contrast, VP PV neurons contribute to both renewal and reacquisition via projections to VTA but not LH. These findings show complementary roles for different VP cell types and their projections in relapse. Targeting these different ventral striatopallidal pathways may provide effective, tailored interventions for different forms of relapse.

id #12187

The betacellulin knock-out mouse: Behavioural characterisation of a novel model with relevance to schizophrenia

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Clozapine relieves psychotic symptoms in 30-50% of treatment resistant (TR) schizophrenia patients, however, the underlying mechanism remains unclear. Our body of work indicates that clozapine may augment signalling through the epidermal growth factor (EGF) system. Betacellulin (BTC) is a protein of this system and is markedly decreased in SZ patients, particularly in the TR subpopulation; and in SZ patients with severe cognitive impairment, suggesting its important role in these aspects of schizophrenia.

Here, we characterized adult BTC KO mice using a behavioural battery of tests to measure short-term memory, social recognition, anxiety, and locomotor activity. To investigate whether BTC interferes with the dopaminergic or the glutamatergic system, mice were challenged with a single i.p. injection of either the dopamine releaser, amphetamine (5mg/kg), or the glutamatergic antagonist, MK-801 (0.25mg/kg) during the locomotor test.

While male BTC KO mice showed spatial memory impairment, female BTC KO mice showed significantly decreased social preference as compared to WT controls. No difference

in LC activity was detected at baseline and after MK-801 administration. Amphetamine attenuated LC activity in female BTC KO mice as compared to controls suggesting that BTC may play a significant role in dopaminergic transmission, particularly in females.

Our results show for the first time that BTC regulates specific behavioural domains with relevance to SZ in a sex specific manner and BTC KO mouse may represent a new model relevant to treatment resistant SZ, enabling the investigation of more targeted treatment options for this debilitating mental illness.

id #12193

Novel patterns of axonal plasticity in congenital and acquired developmental callosal absence

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Humans born without the main neocortical commissure, the corpus callosum (CC), often retain many functions requiring bilateral coordination, especially when compared with adults who have undergone surgical severance of the CC. These findings raise the question of whether and how brains with developmental absence of the CC can undergo plastic change during development to retain callosal-related functions. By precisely labelling subsets of neocortical connections with in-utero electroporation, we quantified and characterised the patterns and degrees of long-range plasticity in the highly comparable models of developmentally- and adult-callosotomised mice, as well as a congenital model of agenesis of the CC (BTBR), measuring axonal fluorescence in candidate regions for >10 animals per group. Despite the acquired versus congenital cause of callosal absence, we found similar novel axon tracts in both the developmentally-callosotomised and BTBR mice, including from the neocortex to the septum, that were not present in the adult-callosotomised group. In contrast, we did not find evidence of plasticity through alternative commissures, as has previously been suggested in humans. We next applied developmental brain electroporation to a marsupial model, the fat-tailed dunnart, which naturally lacks a CC, its axons instead coursing through a larger anterior commissure. While many patterns of axonal plasticity found in developmentally acallosal mice are absent from the marsupial, such as Probst bundles, others are conserved, including from the neocortex to the septum. These results suggest that varied causes of developmental loss or absence of a corpus callosum can result in evolutionarily conserved and stereotypical patterns of axonal routing/plasticity.

id #12194

PICK1 Regulates Presynaptic Vesicle Recycling in Primary Hippocampal Neurons

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Neuronal communication relies on the rapid release of neurotransmitters following the fusion of synaptic vesicles with the plasma membrane. Following exocytosis, these vesicles are retrieved through endocytosis, a process that 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 BAR (Bin/amphiphysin/Rvs) and PDZ (postsynaptic density-95/disc-large/zona-occluden-1) domain-containing molecule that regulates the vesicular trafficking of many postsynaptic neurotransmitter receptors and transporters. Interestingly, PICK1 is also expressed in the presynaptic terminals where its function remains unknown. To determine whether PICK1 regulates synaptic vesicle recycling, we employed a live-cell imaging technique to monitor the recycling of a resident synaptic vesicle protein, synaptophysin that is tagged with a pH-sensitive green fluorescent protein (SypHy), in primary hippocampal neurons. PICK1 loss of function specifically slows the kinetics of SypHy endocytosis without affecting its exocytosis when neurons are stimulated at 50 Hz, but not at 10 Hz. In addition, shRNA-mediated knockdown of PICK1 also causes surface stranding and mislocalisation of SypHy along the axon. Structure and function analyses reveal that a functional PDZ domain of PICK1 is required for the proper targeting of SypHy along synaptic boutons, whereas the interaction between PICK1 and clathrin is responsible for synaptic vesicle retrieval. Surprisingly, mutations in the lipid-binding BAR domain have little effect on SypHy localisation or recycling at synapses. Taken together, our data has uncovered a role for PICK1 as a novel regulator of presynaptic vesicle recycling through clathrin-mediated endocytosis in mammalian central neurons.

id #12195

Hippocampal network aberrations in P301S tau transgenic mice is linked to immediate early genes

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INTRODUCTION

Hyperphosphorylation and deposition of tau in the brain characterizes frontotemporal dementia and Alzheimer's disease. Disease-associated mutations in the tau-encoding *MAPT* gene have enabled the generation of transgenic mouse models that recapitulate aspects of these neurodegenerative diseases, including tau hyperphosphorylation and neurofibrillary tangle formation. The aim of this project was to characterize the effects of transgenic P301S mutant human tau expression on neuronal network function in the murine hippocampus.

METHODS

Mice expressing P301S mutant human tau under the control of a neuron-specific promoter as well as non-transgenic littermates were analysed at a range of ages using: behavioural testing, electrophysiological recordings, hippocampal EEG recordings, RNA sequencing and immunohistochemistry.

RESULTS

- The onset of progressive spatial learning deficits in P301S tau transgenic mice were paralleled by neuronal network aberrations.
- Gene expression profiling at onset of the neuronal deficits revealed a signature of immediate early genes.
- Increased immediate early gene activity was confined to neurons harbouring tau pathology.

CONCLUSIONS

This data suggests that tau pathology drives neuronal network dysfunction through hyperexcitation of individual neurons, thereby contributing to memory deficits.

id #12196

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

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

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

been recently identified in autism patients. However, the underlying molecular mechanism that control WRC recruitment to the PSD remain unknown.

Here we test the hypothesis that netrin/RGMA guidance receptor, Neogenin, spatially-restricts branched actin remodelling within dendritic spines, thereby enabling spine maturation. In support of this, we show that in the developmental context Neogenin controls branched actin polymerization in dendritic spines through its interaction with the WRC in cultured hippocampal neurons. Neogenin depletion via RNA interference and the inhibition of the direct interaction between Neogenin and the WRC both result in decreased spine maturation. Our most recent data suggest that the Neogenin ligand RGMA is also involved in this process. We will also discuss our recent studies investigating the role of Neogenin signalling in activity-dependent actin remodelling and spine expansion in response to LTP using in vitro assays.

id #12197

A Comparison of Peripheral and Central Biomarkers of Post-Stroke Secondary Neurodegeneration

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The progressive development of post-stroke neurodegeneration has implications for long-term functional outcome and increased risk of vascular dementia. Neuroinflammation and blood-brain barrier (BBB) disruption are well known to contribute to acute neuronal loss post-stroke, however their potential role in delayed secondary neurodegeneration is less established. As such, we sought to examine the relationship between the brain microenvironment and peripheral circulation through assessment of neuroinflammatory and BBB dysfunction biomarkers following stroke in a clinically-relevant ovine model.

Transient ischaemic stroke was induced by 2-hour middle cerebral artery occlusion with reperfusion (n=14 merino sheep, 7M;7F). Serum and CSF samples were obtained from naïve animals (n=4) and stroke animals pre-surgery, 1d, 3d, 6d and 28d post-stroke. Brain tissue was collected at 6d and 28d. Protein quantification for interleukin (IL)-6, IL-1 β TNF- α and matrix metalloproteinase-9 (MMP-9) was conducted using ELISA. Claudin-5, Caveolin-1 and Iba-1 was immunohistochemistry performed for quantification of BBB disruption and microglial expression within the infarct, peri-infarct area and regions prone to secondary neurodegeneration including both contralateral and ipsilateral thalamus.

Our preliminary findings indicate positive correlations between serum, both thalamic areas ($R^2=0.531$, $p<0.05$) peri-infarct zone ($R^2=0.827$, $p<0.005$) for IL-6 at 28d post-stroke. Furthermore, an increase in MMP-9 expression was observed within the serum ($p<0.005$) and peri-infarct zone ($p<0.005$) at 28d post-stroke.

Our results suggest a relationship between key inflammatory mediators and BBB breakdown in the neuroinflammatory processes in regions of secondary neurodegeneration out to 28d post-stroke.

id #12198

Fyn Kinase Inhibition as a Novel Therapeutic Target for Parkinson's Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease, with prevalence projected to more than double by 2030. Whilst the complex aetiology of PD is not fully understood, the neuroinflammatory hypothesis has gained increasing support in recent years. This suggests activation of microglia, the brain's immune cells, leads to the release of pro-inflammatory cytokines and subsequent death of neurons. Fyn kinase has recently been established as a major upstream regulator of neuroinflammation in PD, leading to microglial activation. This study aimed to determine if inhibition of Fyn kinase could lead to reduced neuroinflammation and subsequent improvements in motor and non-motor impairments in PD. An experimental model of preclinical PD was produced using intra-striatal injection of the neurotoxin 6-OH-DA (5µg/µl). Animals ($n=9/group$) were given either vehicle or 3mg/kg, 6mg/kg or 12mg/kg of a novel Fyn kinase inhibitor daily for 32 days via oral gavage and tested on a battery of tasks assessing motor function, learning, memory, depression and anxiety-like behaviour. A significant reduction in depressive-like behaviour was observed between all treatment groups and controls. Additionally, 6mg/kg and 12mg/kg treated animals showed significant improvement in recognition memory. Interestingly, pathologically, there were no significant differences in dopamine loss between Fyn treated groups, however, 3mg/kg animals were the only treatment group not to demonstrate increased microglial reactivity and exhibited significantly less microglial activation compared to shams, suggesting a reduced inflammatory response. Taken together, the results indicate a potential therapeutic benefit for the use of Fyn kinase inhibition to treat non-motor symptoms of PD.

id #12200

Synthesis and secretion of human lipoprotein beta-amyloid restricted to peripheral lipogenic organs in mice results in an early Alzheimer's-like neurodegenerative phenotype

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Epidemiological studies indicate a positive association between the chronic ingestion of Western styled diets relatively enriched in saturated fats (SFA) and the risk for developing

Alzheimer's disease in adulthood. However, the physiological mechanisms for this putative causal association are not established. Diets enriched in SFA stimulate secretion from peripheral lipogenic organs of amyloid-beta (A β), complexed with nascent triglyceride-rich lipoproteins. In this study, we report that genetic modification of C57BL6 mice engineered to secrete human amyloid-beta (A β) that is restricted exclusively to intestinal enterocytes and liver hepatocytes (HSHA1 strain), have marked neurodegeneration concomitant with onset of neurovascular inflammation. Moreover, the HSHA1 mice develop cognitive deficits preceding frank cerebral amyloidosis. Transmission electron microscopy reveals in HSHA1 mice, significant cerebral-capillary luminal lipofuscin aggregates; astrogliosis; abundance of dark activated glial cells and cellular loss compared to aged-matched controls. This study provides causal evidence of a lipoprotein-A β /capillary axis for onset and progression of a neurodegenerative disease process.

id #12203

Onset and early dynamics of calcium activity in the developing neocortex revealed by *in vivo* two-photon imaging of the marsupial fat-tailed dunnart.

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Spontaneous and evoked neural activity plays key roles in the formation and refinement of cortical circuits. This activity is characterised by transient patterns that engage spatially distinct regions in age-dependent fashions, and can be detected in postnatal rodents *in vivo*. While *in vitro* evidence indicates that these patterns begin prenatally in rodents, precise studies of embryonic cortical activity have been hindered by a lack of experimental paradigms *in vivo*. Here, we exploit marsupial development to characterise the onset and early dynamics of cortical activity, starting from stages equivalent to intrauterine rodents and humans. We overexpressed the calcium indicator GCaMP6s in pyramidal cortical neurons, via in pouch electroporation, in the Australian marsupial fat-tailed dunnart (*Sminthopsis crassicaudata*), and performed two-photon imaging of the developing cortex across the joeys' translucent skull. Calcium activity begins at stages equivalent to prenatal rodents (embryonic day 16.5 mice), as low amplitude, infrequent, but highly synchronised events within cortical areas. More complex features, such as travelling waves and non-propagating patchwork-type activity, appear towards stages equivalent to perinatal rodents. Importantly, somatosensory and visual cortices show different spatiotemporal patterns and developmental dynamics of activity, similar to findings in rodents, suggesting that they may represent an evolutionarily conserved phenomenon of mammalian neocortical development. These experiments highlight the versatility of dunnarts as experimental models of brain development and evolution. We anticipate this imaging paradigm will open the way for future investigations into the neurophysiology of early cortical activity and the role of such activity in the formation of functional circuits.

id #12204

Timing of SATB2 and CTIP2 expression differentially regulates development of cortical projection neurons in marsupial and eutherian mammals

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The formation of neocortical circuits is under tight control of transcription factors. In eutherian mammals, SATB2 and CTIP2 specify callosal and subcerebrally projecting neurons, respectively. However, as only mammals evolved a neocortex, and only eutherians evolved a corpus callosum, the origin and diversification of transcriptional networks involved in these structures remain largely unknown. Here we show that marsupials and eutherians share a transcriptional program that regulates commissural versus subcerebral projections, despite the route taken by neuronal axons. Moreover, the relative timing of expression of SATB2 and CTIP2 differs between marsupials and eutherians, and heterochronic manipulation of expression of these genes is sufficient to recapitulate marsupial-like axonal phenotypes in mice. Our results demonstrate the early establishment of neocortical transcriptional networks in ancestral mammals, before the emergence of the corpus callosum, and suggest that changes in the timing of expression of existing genes could contribute to the evolutionary origin of new brain tracts.

id #12206

Astroglia mediate the transfer of Parkinson's disease pathology through neuronal networks

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Neuropathology in Parkinson's disease initially develops in the caudal brainstem and gradually proceeds to rostral regions through anatomical connections. This process is thought to involve the spread of alpha-synuclein through "prion-like" mechanisms. Recent evidence is given for a role of astrocytes in this pathogenic spread of protein pathology. We hypothesise that disease processes, including oxidative stress and excitotoxicity, alters astroglia signaling and physiology to cause propagation of neurodegenerative pathology. This may be independent from the direct transfer of alpha-synuclein. To test our hypothesis, we developed a novel *in vitro* model of neuron-astrocyte networks, which is based on a microfluidic platform that allows physical and fluidic separation of two neuron/astrocyte co-cultures, bridged by a single population of pure astrocytes. The fluidic isolation of each cell compartment enables chemical manipulation of different cell populations within the network. Using this culture model, we have demonstrated the role of astrocytes in transferring pathogenic effects of excitotoxicity between segregated neuron populations. Addition of

1mM kainic acid to a single neuron/astrocyte population caused a 50% increase (n=3; p<0.05) in intracellular free calcium, as measured in astrocytes of the astrocyte-only compartment. The significance of this effect is underscored by the fact that astrocytes are resistant to excitotoxicity caused by kainic acid. In addition to effects on the astrocyte population, treatment of a single neuron/astrocyte population caused significant (p<0.05) axonal blebbing in neurons of the second neuronal/astrocyte population, consistent with excitotoxic effects. Collectively, these results indicate that astrocytes can mediate the transfer of neuropathology between segregated neuron populations.

id #12207

Epilepsy-causing Mutations to the $\beta 3$ Subunit of GABA type-A receptor Alter The Receptor Function and Trafficking

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Heritable human epilepsies are frequently caused by mutations in the GABA type-A receptor (GABAAR). We examined the functional effects of epilepsy-causing mutations to the $\beta 3$ subunits on inhibitory postsynaptic currents (IPSCs) mediated by synaptic GABAAR isoforms $\alpha 1\beta 3\gamma 2L$ and $\alpha 5\beta 3\gamma 2L$. We used 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 investigate changes in neuronal morphology, synapse formation and GABAAR mobility using super-resolution fluorescence microscopy. To simulate neuronal activity during epileptic seizures 4-Aminopyridine was applied.

We found that IPSCs mediated by the mutant subunits induced different IPSCs than those mediated by wild type receptors in hetero-synapses. All studied mutations induced smaller peak amplitude and reduced decay time constant of IPSCs produced by GABAARs. Upon Diazepam treatment the currents became comparable to those produced by *wild-type* receptors. Super-resolution microscopy revealed changes the mobility of GABAARs found at synapse and in extra-synaptic regions as well as in the rate of exocytosis of GABAARs during 4AP-ivoked neuronal hyperexcitability. These results provide new insights into the mechanisms of epileptogenesis and suggest possible leads for improving treatments for patients harbouring mutations in GABAAR subunits.

id #12209

State dependent changes in perception and sensory coding in the somatosensory cortex

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An animal's behavioural state affects the dynamics of neuronal population activity in cortex, which in turn influences the population's capacity to process sensory information. Here, we quantify population activity in Layer 2/3 neurons across different behavioural states using two-photon calcium imaging in awake mice engaged in a sensory detection task. GCamp6f was expressed in the vibrissal primary somatosensory cortex of mice (n=7), which were habituated to head-fixation and trained to detect pulses of whisker-deflections, with each trial composed of 1 out of 5 possible deflection amplitudes. The same neurons (n=2480, average of 62 cells per session) were imaged within each session across different behavioural states, defined based on detection performance (quiet-wakeful - alert-behaving) and pupil diameter. As the mice transitioned towards active states, psychometric curves shifted towards lower stimulus intensities, revealing enhanced detection sensitivity. This change in sensitivity correlated with changes in pupil diameter - with stable, large dilation corresponding to alert states. The behavioural state also influenced neuronal synchronicity, with alert states exhibiting lower distant (<200µm) synchrony. Neuronal responses revealed parallel changes in detection sensitivity in the form of an increase in evoked response magnitude in alert states. Tracking whiskers showed no significant differences in movement. Changes in calcium fluorescence were predictive of the animal's choice outcome (hit versus miss), with larger evoked activity observed on hit trials (independently of state). Finally, a linear discriminate analysis allowed accurate classification of state and trial outcome. The observed state-dependent changes can underlie adaptive routing of relevant information through the sensorimotor system.

id #12210

Alzheimer's disease risk factor gene 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 #12212

Human Neural Tissues from Induced Pluripotent Stem Cells Using Conductive Biogel and Printed Polymer Microelectrode Arrays for 3D Electrical Stimulation

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Electricity is important in the physiology and development of human tissues such as embryonic and fetal development, and tissue regeneration for wound healing. Accordingly, electrical stimulation (ES) is increasingly being applied to influence cell behaviour and function for a biomimetic approach to in vitro cell culture and tissue engineering. We have previously described the application of conductive polymer (CP) poly(3,4-ethylenedioxythiophene)-polystyrenesulfonate (PEDOT:PSS) pillars, direct-write printed in an array format, for three-dimensional (3D) ES of maturing neural tissues that are derived from human neural stem cells (NSCs; Tomaskovic-Crook, et al., *Advanced Healthcare Materials*, 2019;8(15):e1900425). NSCs were initially encapsulated within a conductive polysaccharide-based biogel interfaced with the CP pillar (microelectrode) arrays (MEAs), followed by differentiation in situ to neurons and supporting neuroglia during stimulation. Electrochemical properties of the pillar electrodes and the biogel support their electrical performance. Remarkably, stimulated constructs are characterised by widespread tracts of high-density mature neurons and enhanced maturation of functional neural networks. Building on our previously published we have extended our use of the 3D MEA platform to the generation of 3D neural tissues from human induced pluripotent stem cells. Formation of tissues using the 3D MEAs further substantiates the platform for advanced clinically-relevant neural tissue induction, with the system likely amendable to diverse cell types to create other neural and non-neural tissues. The platform may be useful for both research and translation, including modelling tissue development, function and dysfunction, electroceuticals, drug screening and regenerative medicine.

id #12217

Intrauterine inflammation is associated with high PGE₂ expression and microgliosis in brainstem respiratory centres

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Background: Preterm infants exposed to inflammation during pregnancy are prone to apnoeas and require respiratory support at birth. These infants are also at increased risk of brain injury. The brainstem contains vital respiratory centres which can be inhibited by inflammation, particularly by prostaglandin E₂ (PGE₂). We aimed to investigate whether intrauterine inflammation alters fetal respiratory function, and whether this is associated with increases in PGE₂ and inflammation in brainstem respiratory centres.

Methods: Fetal lambs (125 days gestation) were exteriorised and instrumented to measure fetal respiratory function (fetal breathing movements; FBMs), heart rate and blood pressure before being returned in utero. At 128 days gestation, fetal lambs received saline or escalating doses of Lipopolysaccharide (LPS; 300ng, 600ng, 1.2ug intravenously) over 3 days. The duration of FBMs was assessed using LabChart. PGE₂ expression in the brainstem and cerebrospinal fluid (CSF) was assessed by immunohistochemistry (IHC) and ELISA. Inflammation was assessed by microglia and astrocyte IHC. T-test was used for statistical analysis.

Results: LPS caused a significant reduction in FBMs, decreased oxygen saturation, increased blood lactate levels and caused mild acidosis (all $p < 0.05$) on days 1 and 2 compared to the control group. LPS increased PGE₂ expression in brainstem respiratory centres and CSF ($p < 0.05$), increased hyper-ramified, reactive and amoeboid microglia ($p < 0.05$), and decreased the number of astrocytes ($p < 0.05$).

Conclusion: Acute exposure to LPS suppresses FBMs which coincides with increased PGE₂ in brainstem neurons and CSF and microgliosis, suggesting that brainstem inflammation may have a significant role in respiratory depression in preterm newborns.

id #12220

Low intensity repetitive magnetic stimulation alters gene expression in cultured mouse cortical astrocytes

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Repetitive transcranial magnetic stimulation (rTMS), a form of non-invasive brain stimulation, applies magnetic pulses over the cranium to induce electrical currents in the underlying brain. rTMS has been shown to modulate aspects of neural plasticity such as synaptic plasticity and structural reorganisation of neurons and is used to treat a variety of neurological conditions, such as depression and stroke. However, the impact of rTMS on glial cells such as astrocytes remains largely unknown. In this study, we investigated changes in RNA (qPCR array: 128 selected genes) and protein expression (immunohistochemistry) in cultured mouse astrocytes following a single session of low intensity repetitive magnetic stimulation (rMS). Purified neonatal cortical astrocyte cultures were stimulated with either 1Hz (600 pulses), 10Hz (600 or 6000 pulses) or sham (0 pulses) rMS, followed by RNA extraction at 5 hours post-stimulation, or fixation at either 5- or 24-hours post-stimulation. rMS resulted in a two-to-four-fold downregulation of mRNA transcripts related to calcium signalling (*Stim1* and *Orai3*), inflammatory molecules (*Icam1*) and neural plasticity (*Ncam*). 10Hz reduced expression of *Stim1* and *Orai3*, and *Ncam* mRNA, whereas 1Hz reduced expression of *Icam1* mRNA. Measuring immunofluorescence intensity, preliminary results indicate 10Hz rMS significantly increased *Stim1* by 74%, *Orai3* by 120%, whereas *Ncam* label decreased by 37%. These findings demonstrate the ability of rTMS to modulate astrocyte plasticity, highlighting the importance of glial cells in rTMS-induced plasticity, and their mechanistic involvement in the therapeutic benefits of rTMS treatment of neurological disease.

id #12221

Synaptic modulation of viscerosensory signals within the brainstem.

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Viscerosensory information is conveyed to the brainstem's nucleus of the solitary tract (NTS) where it initiates autonomic reflexes that ensure optimal internal organ function. Autonomic reflexes are dynamic. For example, the baroreceptor reflex, which rapidly modulates blood pressure, is re-set to different operating sensitivity and gain during stress. It is not known how this flexibility in reflex processing is achieved. We hypothesize that viscerosensory signals entering the brain at the NTS are modulated by efferent activity from other brain regions (hypothalamus) and the intrinsic inhibitory network within NTS. We have used a combination of optogenetic tools and slice electrophysiology to define the neural circuits and mechanisms that modulate viscerosensory signals within the NTS. We find hypothalamic input to the NTS to be exclusively excitatory, AMPA receptor mediated and these efferents facilitate viscerosensory signal throughput. In contrast, somatostatin-expressing (SST) NTS neurons utilized both GABAergic and glycinergic systems to effectively gate viscerosensory signal throughput within the NTS. SST-NTS neurons receive direct input from solitary tract afferents, indicating that they form part of a feed forward circuit where all recorded SST-negative NTS neurons received SST input. These results indicate synaptic modulation of viscerosensory signals occurs via excitation or inhibition of second order NTS neurons directly, with the potential to gate viscerosensory input to powerfully alter autonomic reflex function and other behaviours.

id #12222

A novel approach to identify functional genetic variants associated with persistent post-concussion symptoms

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Objective: This research aims to use a machine learning approach to investigate the genetic basis of concussion through the application of Whole Exome Sequencing (WES). To date, studies investigating the genetics of concussion have been limited to candidate gene association studies. Evidence suggest that variants in Ion channel genes (*CACNA1A*, *ATP1A2*, *KCNJ10*, *SLC26A4*) and neuronal structure genes (*BDNF*, *KIAA0319*, and *APOE*) are implicated in response to head trauma and recovery from post-concussion symptoms. **Methods:** This study utilised 56 participants with a history of multiple concussion, persistent post-concussion symptoms (PCS), severe responses to trivial head trauma, or mild Traumatic Brain Injury (mTBI) following major accidents/incidents, and 70 participants with neurological symptoms unrelated to head trauma or past concussion. WES variant files were filtered to only include variants predicted to be damaging by in-silico prediction tools (e.g. PredictSNP2). An unsupervised hierarchical clustering algorithm was then implemented to be followed by a supervised classification (gradient boosted trees). **Results:** Preliminary results indicate that variants predicted to be damaging/disease causing can cluster individuals with persistent post-concussion migraines, seizures, and memory problems from patients exhibiting similar symptoms due to a neurological condition. Family-based analysis of rare and novel variants in related genes is used to interpret the results of the classification. **Conclusion:** Using a machine learning approach to explore the protein-coding genetic signatures that classify response to head trauma and mTBI is a novel approach to close the current knowledge gaps in the genetics of concussion literature. This will inform better management and tailored treatment to concussion.

id #12223

Significance of POm thalamo-striatal terminals in the dorsolateral striatum

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Objective It is important to integrate the sensory stimuli within our environment to learn and interact harmoniously with our surroundings and exhibit an appropriate behaviour. Two brain structures, the thalamus and striatum, are key for sensory processing and learning respectively. Here, we investigated the influence of the posteromedial complex of the thalamus (POm) on the processing of sensory information in the dorsolateral striatum (DLS). **Brief method** We unilaterally injected an adeno-associated virus expressing the excitatory opsin (ChR2) in POm, in C57BL/6 mice. Whole cell recordings were then performed in striatal neurons *in vitro* and *in vivo* (urethane-anesthetized), while photostimulating (460 nm) POm axons projecting to the striatum. **Key findings** Photoactivation of POm projections in the striatum *in vivo* evoked a subthreshold response with an average amplitude of 1.9 ± 0.54 mV. These responses were monosynaptic as photoactivation of POm projections *in vitro* also evoked a subthreshold response in the presence of Na and K channel blockers (3.19 ± 2.42 mV). **Conclusion** We illustrate direct connectivity between the higher order POm thalamic

nucleus and the DLS that could influence sensory processing in the striatum. Due to the diverse targets of POM, this POM connectivity may convey information in a closed loop, binding several cortical brain areas.

id #12225

INVESTIGATING THE PATTERN OF AXONAL INJURY IN A MODEL OF MILD TRAUMATIC BRAIN INJURY IN A LARGE ANIMAL MODEL

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Even mild traumatic brain injury (mTBI) is associated with the development of scattered axonal injury (AI), the tearing of axons due to indirect shearing forces during acceleration, deceleration, and rotation of the brain. This drives loss of functional connectivity seen post-mTBI and explains symptoms such as fatigue, difficulty concentrating and irritability. AI is influenced by brain size, as inertial effect is dependent on brain mass. Thus, to facilitate a greater understanding of the pathophysiology of AI, modelling of mTBI within larger gyrencephalic brains is required. This study characterised the distribution of AI via amyloid precursor protein immunohistochemistry following TBI of varying intensities (11, 3 or 15-charge; n=3) to the right temporal area in anaesthetised Merino wethers. Invasive blood pressure (BP) was monitored for 4-hours post-injury, prior to formalin perfusion and brain removal. Injury led to an immediate decrease in BP compared to shams, as calculated relative to pre-injury baseline which was comparable across all injury groups (11=-21.47, 13=-11.32, 15=-13.66) before returning to baseline within 10-minutes. The 15-charge produced significant AI in the right internal capsule (sham=p<0.045, 11=p<0.002, 13=p<0.001) and cingulum (sham=p<0.03, 11=p<0.007, 13=p<0.004.). Preliminary analysis of neuroinflammation via IBA1 staining within the cingulum, found no significant differences in number of IBA1+ve cells or % area of staining compared to shams. This pilot study found evidence of minor scattered axonal injury at the highest charge level, which was not yet associated with microglial activation, suggesting this was resultant of the primary insult and not secondary injury mechanisms.

id #12226

Targeting glycans on glioblastoma cells with nanodiamonds

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Glycosylation is a post-translational modification that attaches glycan chains to cell surface proteins and lipids. Abnormal glycosylation is evident during multiple chronic brain pathophysiologies including brain cancer, making glycans excellent biomarkers for selective targeting of affected cells. We bio-conjugated three different types of lectins to 120 nm fluorescent nanodiamond particles with nitrogen vacancy centres in order to target glycans on the surface of glioblastoma phenotype astrocytes, neurons and microglia cells. Lectins are widely used to identify different major biological glycan residues and we investigated *in vitro* targeting of sialic acid (wheat germ agglutinin), fucose (*Aleuria aurantia*lectin), and N-acetylglucosamine (tomato lectin) glycan moieties on the surface of the mentioned brain cell lines. We evaluated binding of bioconjugated nanodiamonds to these glycans in standard 2D cell cultures and developed unique brain scaffold-based 3D culture system to assess cell targeting in each cell type via more natural *in vivo* cellular growth conditions as glycans are a major component of the extracellular matrix and highly important in cell-cell interactions. In 2D and 3D cell cultures we found that *Aleuria aurantia* lectin, targeting fucose sugar receptors, binds to glioblastoma phenotype human astrocyte cells with the highest affinity. This is in agreement with previous analysis of sugar receptor expression in the literature showing fucose is highly expressed on tumour cells including glioblastoma cells compared to other cell types. Our findings indicate that the bioconjugated nanodiamonds developed in this study could be used in future studies as drug delivery vehicles for targeting human brain cancer cells.

id #12232

Characterisation of descending interneuronal pathways in myenteric plexus of human colon

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Multiple labelling immunohistochemistry and *ex vivo* retrograde tracing have proven to be powerful tools for analysing enteric neural pathways in laboratory animals. Here we studied descending interneuronal pathways in human myenteric plexus, in isolated specimens of colon obtained with ethics approval at elective surgery (SAHLN #207.17). A small glass bead, coated with the carbocyanine dye, 1,1'-didodecyl-3,3,3',3'-tetramethyl indocarbocyanine perchlorate (DiI), was applied to the myenteric plexus or circular muscle

for 4 days in organ culture, before fixation and processing for immunohistochemical multiple labelling. Filling from circular muscle (n=6) revealed that 95% of descending motor neurons had projections shorter than 17mm. To analyse descending interneurons (DINs) selectively, we therefore restricted our attention to neurons filled from the myenteric plexus with projections longer than 17mm. Calbindin immunoreactive neurons accounted for 77% of descending interneurons in a set of 8 preparations (344 of 450 cells) stained for calbindin and enkephalin. In another set of preparations (n=5), we investigated NOS and 5-HT immunoreactivities. Approximately 65% of DINs were immunoreactive for NOS and 9% contained 5-HT. Lastly, we mapped 6 preparations treated with Calretinin and NOS antisera. Of 329 DINs, 66% contained NOS and 28% were immunoreactive for calretinin. The remaining 10% lacked both NOS and Calretinin. However, sequential staining of NOS-/Calretinin- cells revealed that 24/31 were calbindin-immunoreactive. This means that over 97% of descending interneurons in human colon are labelled with just 3 immunohistochemical markers - NOS, Calbindin, Calretinin. This will form the basis for a subsequent detailed subclassification.

id #12235

Characterizing the role of voltage-gated potassium channel Kv8.2 subunits in the retina using a novel mouse model

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Cone Dystrophy with Supernormal Rod Response is a rare autosomal recessive disorder, leading to severe visual impairment, but little is known about the pathophysiology of this disease. It is caused by mutations in the *KCNV2* gene which encodes the Kv8.2 subunit, a modulatory subunit of a voltage-gated potassium (Kv) channel. This study used gene knock-outs of the *Kcnv2* gene and the *Kcnbl1* gene which encodes the Kv2.1 subunit, the obligate partner of Kv8.2 in functional retinal Kv channels, to study the role of Kv subunits in the retina and the mechanism of disease progression.

Immunohistochemistry staining was used to examine the retinas of knockout (KO) and wild-type (WT) mice (2 months old, both sexes). Retinas were labelled with either a Kv8.2 or Kv2.1 antibody and co-stained with a number of cone and rod photoreceptor markers to localise gene expression. Both Kv8.2 and Kv2.1 were found to be exclusively expressed in the inner segments of mouse photoreceptors. By evaluating the severity of retinal dystrophy in these KO models, we have demonstrated that the retinas in the both KO mice have significantly higher apoptotic cells, a thinner outer nuclear cell layer and increased microglia cells in both the inner plexiform and subretinal layers when compared to WT retinas. Our results validate the specific location of Kv channels in mouse retina and indicate that the loss of either Kv subunit induces photoreceptor death, thinning of the outer nuclear layer and activation of retinal microglia response, which likely contributes significantly to the photoreceptor dystrophy.

id #12236

Beyond the central dogma: from junk DNA to Schizophrenia-associated cell functions.

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Genome-wide association studies have identified over 100 loci in which common genetic variants are associated with schizophrenia in the population. Other rare genetic risk variants have also been identified; however, the challenge to the field is to understand the functional consequences of combinations of common and rare variants in context of the individual's full array of genetic risks. We have developed a systems-based approach using multi-omics data and cellular function assays in schizophrenia patient-derived olfactory neural stem cells as our model system. We have identified protein-coding and non-coding genetic variants that contribute to schizophrenia-associated cell functions using individual patient cells. Our integrated analysis of gene networks and cell functions using the same individual patient and control cells is unique in the fields of schizophrenia, cell biology, and systems biology. Whole-genome sequencing data from this cohort of patient cells confirm that the patients have higher polygenic risk scores when compared to healthy controls. We also identified rare variants in individual patients within schizophrenia risk genes, including microRNA-137 locus, which have been validated to affect multiple regulatory layers and schizophrenia-associated cell functions (e.g. cell cycle, cell adhesion, cell motility, and protein synthesis). This systems-level analysis identified perturbation of regulation of gene networks and pathways leading to modulation of multiple cell functions. Our overarching goal is to develop an unbiased approach to integrate experimental data from patient stem cells for discovery of new therapeutic strategies tailored to individual's molecular and cellular signatures.

id #12237

Disruptions in GABAergic signaling caused by the neuronal growth regulator 1 (NEGR1) in high fat diet-induced obesity

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Inhibitory neurotransmission plays a key role in appetite regulation. High fat diet-induced obesity is associated with reduced synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). The molecular mechanisms of this reduction remain poorly understood. We demonstrate that the level of the neuronal growth regulator 1 (NEGR1), a protein coded by a gene associated with obesity in humans, is increased in brains of mice fed high fat diet and is associated with accumulation of the soluble proteolytic products of NEGR1. We show that soluble NEGR1 induces an impairment in GABA synthesis caused by mislocalization of GAD65, a GABA-synthesizing enzyme, which associates with synaptic vesicles. Soluble NEGR1 and NEGR1 overexpression cause disruptions in the recycling of synaptic vesicles and reduce synapse numbers in hypothalamic neurons. Our results indicate

that upregulation of NEGR1 levels and accumulation of the soluble NEGR1 induced by high fat diet cause changes in the appetite-regulating inhibitory signaling.

id #12238

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

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Spino-cerebellar ataxia type 1 (SCA1) is an incurable human neurodegenerative movement disorder caused by an unstable expanded CAG trinucleotide (Q) repeat (39-82Q) in the ataxin-1 gene. The disorder is characterized by progressive motor incoordination, disruption of cerebellar excitatory synapse morphology and degeneration of Purkinje neurons (PN), the main output neurons of the cerebellar cortex.

Metabotropic glutamate receptor type 1 (mGluR1) are highly expressed in PNs and critical for motor coordination. Overactive mGluR1 signalling occurs in an SCA1 82Q mouse model and normalisation of this activity with an mGluR1-specific negative allosteric modulator acutely restores motor coordination in the disorder.

To further explore the role of enhanced mGluR1 signalling during SCA1 progression we chronically enhanced mGluR1 signalling by administering an mGluR1-specific positive allosteric modulator to SCA1 mice. This treatment hastened SCA1 progression in the mice, manifested by decreased motor performance on an accelerating rotarod (two-way ANOVA, $P < 0.05$, $F(1, 14) = 6.0$), decreased precision of PN firing output (unpaired t-test, $P < 0.05$) and the synaptic morphology remained disrupted (unpaired t-test, $P > 0.05$) in comparison to vehicle treated SCA1 mice. In contrast, chronically decreasing mGluR1 signalling by administering an mGluR1-specific negative allosteric modulator significantly improved PN firing frequency and rescued the disrupted synaptic morphology ($P < 0.05$, unpaired t-test).

Our results demonstrate that enhanced mGluR1 signalling is a driving force for SCA1 disease progression and that successful targeting of mGluR1 is a promising approach to treat SCA1.