ABSTRACTS FOR 2015 – Inter-University Neuroscience and Mental Health Conference, Poster Presentations

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The role of executive functioning and alcohol cue reactivity in the regulation of alcohol consumption and drinking consequences in social drinkers

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Introduction: Previous research has implicated a potential relationship between executive functioning (EF) and cue reactivity (CR) to salient alcohol stimuli in the regulation of alcohol consumption and related negative consequences - albeit in clinical samples and usually only employing a single, complex EF task. This study implements discrete domain-specific EF tasks in a social drinking sample.

Objective: We aim to demonstrate that heavier social drinkers who have experienced negative drinking consequences will show reduced EF and physiological regulation during a CR challenge task.

Method: 60 social drinkers were administered domain-specific EF (response inhibition; set-shifting; updating) tasks and a CR challenge whereby a control (H₂O) and preferred alcohol beverage were presented. Subjective alcohol craving and physiological measures (e.g. heart rate variability: HRV) were recorded during the CR challenge, and measures of alcohol consumption and negative consequences.

Results: Preliminary analyses show heavier drinkers experienced more negative consequences and had lower EF scores compared to lighter social drinkers, and demonstrated reduced high-frequency HRV during the CR task.

Conclusion: This is one of the first studies to investigate physiological responses (i.e. High-frequency HRV) during the CR task and EF as a potential moderator of the feedback systems relating to alcohol consumption and consequences of drinking.
A rodent model of single-inescapable foot shock induces long-term reduction of microglial expression in the amygdala

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Background: Post-traumatic stress disorder (PTSD) is characteristically associated with disruption of grey matter in regions critical to stress and fear processing. This disruption has been attributed to alterations in dendritic spines in various rodent models of stress. Recent evidence suggests that microglia play a role in synapse turnover through interaction with dendritic spines. Therefore we hypothesised that microglia expression would be altered in the stress and fear-critical regions; the hippocampus, the amygdala and prefrontal cortex. Additionally, given recent evidence of p-glycoprotein SNPs in depression we also included a p-glycoprotein strain of knockout mice to examine a possible role in PTSD susceptibility.

Methods: Utilizing a single foot-shock model of PTSD, Mdr1a/b (-/-) mice and FVB mice were sorted into shock and non-shock conditions. After shock exposure, both short-term and long-term behavioural changes was assessed. Mice were culled at 30 days, then their brains were immunohistochemically stained for Iba-1, to mark for microglia, and its expression was quantified in the stress and fear-critical regions.

Results: The mice administered the foot-shock displayed a greater fear response and reduced social interaction both in the short-term and long-term. P-glycoprotein deficient mice displayed a variety of significantly blunted behaviours when compared to FVB mice, suggesting potential resilience to PTSD. Expression of microglia was reduced in nuclei of the amygdala, where increased spine density had been found.

Conclusions: Microglial activation is demonstrably part of the PTSD pathology and is likely involved in, if not responsible for changes in dendritic spine density in key fear conditioning regions.
Type-D Personality is associated with increased levels of depression, anxiety, and stress; increased posttraumatic stress; and less security in relationships in severe burn injury patients

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**Background:** Type-D, or distressed personality, is characterised by a tendency to experience negative emotions and thought processes while simultaneously inhibiting the expression of these emotions and thoughts in social interaction. Type-D has been linked to a number of negative health outcomes in a variety of medical and mental health areas as well as in response to, and recovery from, trauma. It has also been associated with increased symptomology in depression, anxiety and stress, and reduced attachment security.

**Methods:** Data was obtained in the Adjustment to Burns Pilot Study (2009-2014) conducted at Royal North Shore Hospital looking at various psychosocial measures in severe burns patients. Comparisons were made between the Depression Anxiety and Stress Scale (DASS) scores, and Davidson Trauma Scale (DTS) scores between participants characterised as having Type-D personality and those not considered to have Type-D personality. Relationship security was also measured by the Relationship Questionnaire (RQ).

**Results:** Total DASS scores for Type-D participants (M=65.8, SD=35.84), were significantly higher than non-Type-D participants (M=17.10, SD=15.55), t(52)=7.01, p=<.001. This trend carries over all sub scales. On the DTS, Type-D participants scored significantly higher (M=64.50, SD=34.94), compared to non-Type-D participants (M=15.0256, SD=16.03694), t(51)=7.08, p=<.001. Type-D participants also scored significantly lower on the security subscale of the RQ, χ²(1, N= 50)=5.00, p=0.019.

**Conclusions:** This analysis further supports the notion that Type-D personality is linked to depression, anxiety, and stress, strengthening the association between Type-D and negative health outcomes. Findings also suggest a link between lower attachment security and Type-D personality.
Vasopressin V1a receptor antagonism blocks oxytocin’s attenuation of reinstatement of methamphetamine-seeking behaviour in rats

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Methamphetamine (METH) is highly addictive psychostimulant which is increasingly abused in Australia. Systemic administration of the neuropeptide oxytocin attenuates METH-related reward and METH-seeking behaviour. However, the oxytocin receptor within neural substrates of reward is only modestly involved in oxytocin’s inhibitory effects on relapse, suggesting mediation by other receptors. Oxytocin has a high affinity for the vasopressin V1a receptor, which has been shown to be involved in some of oxytocin’s prosocial effects. Using a self-administration paradigm of drug reinstatement, we investigated whether oxytocin’s attenuation of METH-primed reinstatement of METH-seeking behaviour could be reduced by systemically co-administering SR49059, a selective vasopressin V1a receptor antagonist. 13 male Sprague-Dawley rats were surgically implanted with an intravenous jugular vein catheter and trained to self-administer METH by lever pressing during daily 2-hr fixed ratio 1 scheduled sessions for 20 days. Following extinction of a preference for the active lever, rats were then tested for the effects of systemically administered oxytocin, SR49059, Compound 25 (oxytocin antagonist), or co-administration of oxytocin with either SR49059 or Compound 25 on METH-primed reinstatement of METH-seeking behaviour. As expected, systemic oxytocin substantially reduced METH-primed reinstatement of lever pressing, as well as METH-induced hyperactivity. Importantly, systemic blockade of the V1a receptor but not the oxytocin receptor prevented the inhibitory effects of oxytocin on METH primed reinstatement and METH-induced hyperactivity. These findings demonstrate that in its important modulatory effect on relapse to methamphetamine-seeking behaviours, oxytocin is mediated by receptors other than its own. Understanding oxytocin’s interaction with these receptors has significant implications for the development of small oxytocin-like molecules with improved brain penetration.
Adjunctive selective estrogen receptor modulator treatment increases bilateral hippocampus and inferior frontal gyrus activity during facial emotion processing in schizophrenia

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Background: Estrogen has been implicated in the development and course of schizophrenia with most evidence suggesting a neuroprotective effect. The selective estrogen receptor modulator raloxifene has been shown to reduce symptom severity, improve cognition and normalize brain activity during learning in schizophrenia. The present study was designed to determine the extent to which adjunctive raloxifene treatment would alter abnormal neural activity during facial emotion processing in schizophrenia.

Methods: Twenty people with schizophrenia (14 men, 6 women) participated in a thirteen-week, randomized, double-blind, placebo-controlled, crossover trial of adjunctive raloxifene treatment (120 mg/day orally) during a facial emotion identification task assessed with fMRI. Region of interest analyses were performed to identify brain areas typically associated with the task that show differential activity between the raloxifene and placebo conditions during the processing of angry versus neutral faces.

Results: Adjunctive raloxifene significantly increased activation in the hippocampus and inferior frontal gyrus compared to the placebo condition (FWE p<.05). There was no significant difference in performance accuracy or reaction time between active and placebo conditions.

Conclusions: This study provides the first evidence that adjunctive raloxifene treatment can alter neural activity in brain regions associated with emotional face recognition deficits in schizophrenia. These findings support the hypothesis suggesting a role for estrogen in schizophrenia and provide evidence for the potential role of adjunctive raloxifene in reversing abnormal neural activity during emotion face recognition which is relevant to impaired social functioning in men and women with schizophrenia.
Cocaine unmasks Group III Metabotropic Glutamate Receptor (GP III mGluR) function & evokes long lasting increases in excitatory drive to lateral hypothalamic orexin neurons

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Background: The lateral hypothalamic (LH) orexin system is strongly implicated in pathologically enhanced reward-seeking behaviour. For example, orexin-1 receptor (Orx-1) antagonism profoundly suppresses drug-seeking. Further, vulnerability for renewed drug-seeking remains sensitive to orexin receptor antagonism even after protracted abstinence, suggesting that potent rewards may promote lasting changes in LH-orexin circuits. A mechanistic framework for how LH circuitry is sufficiently modified to sustain lasting increases in drug-seeking behaviour is lacking.

Methods: GFP-orexin mice were injected with cocaine (15 mg/kg/7 days) and the activity of GFP neurons was recorded using whole cell patch clamp techniques on day 1 (D1) and day 14 (D14) after final cocaine exposure. We also tested whether pharmacological activation of GPIII mGluRs reverses cocaine-induced maladaptations in the LH.

Results: We found that cocaine caused functional remodeling of excitatory, glutamate synapses onto orexin neurons in GFP-orexin mice that is evident 1 day following drug withdrawal and persists for up to 14 days. This remodelling involved an increase in both release probability and the AMPA:NMDA ratio. These changes were accompanied by an up-regulation of presynaptic GPIII mGluR function. Pharmacological activation of these mGluRs decreased glutamate release onto orexin neurons.

Conclusion: These findings demonstrate that cocaine causes a protracted increase in excitatory synaptic drive to orexin neurons and a potentially compensatory increase in GP III mGluRs. The latter may offer a prospective entry-point for therapeutic strategies aimed at reversing the effects of enhanced orexin neuron activity in animal models of drug addiction.
Mitochondrial DNA perturbations in the vestibular neuroepithelium of the aged rat

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Background: Our ability to maintain balance declines as we age, often leading to falls requiring hospitalisation, with an ageing population this is becoming a common occurrence. A contributing factor is thought to be impaired vestibular function, however, it is currently unknown whether age-related vestibular dysfunction is due to peripheral and/or central components of the vestibular system. Our study sought to determine whether peripheral vestibular organ function is compromised with aging. Inner ear vestibular hair cells detect head motion through an energetically demanding process, in part due to the necessity to continuously remove calcium from the cell. Mitochondrial activity is critical for normal hair cell and vestibular system function. Ageing has previously been associated with increased mtDNA alterations, thought to compromise respiratory chain activity in a number of tissues. However, whether aging alters mtDNA in vestibular hair cells, potentially underlying age-related balance loss, is currently unknown.

Methods: Vestibular hair cells were laser microdissected from 7 young adult (4-6 months) and 6 old (23-26 months) Fisher-344 rats. DNA was extracted and qPCR analyses carried out to compare relative abundance of four mtDNA genes between the two age groups. In mtDNA genomes that do not contain duplications or deletions, the relative abundance of the various genes contained in the genome will be equal.

Results: Our investigations have found a decline in mitochondrial genome copy number, and an interesting increase in the replication initiation site suggesting complications with mitophagy.

Conclusions: These data indicate mtDNA alterations may contribute to age-related changes in vestibular system function.
The effect of pharmacological reduction of delta-opioid receptor expression in the nucleus accumbens shell on specific Pavlovian-instrumental transfer

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Background: Animals can use information from their environment to guide their choice between actions. This ability is studied in the laboratory through outcome-specific Pavlovian-instrumental transfer (PIT), in which a stimulus associated with a particular reward biases the choice between actions towards the response that earned that same reward. Evidence from our laboratory indicates that this bias in action selection relies on delta-opioid receptor (DOR) accumulation at the membrane of cholinergic interneurons (mCINs) within the nucleus accumbens (NAc) shell, which occurs during Pavlovian learning.

Methods: The effect of reduced and recovered (to an accumulated state) DOR expression in the NAc shell was assessed using the selective DOR agonist SNC80. SNC80 produces reduced DOR expression 24 hours after administration, but DOR accumulation on shell mCINs recovers after 48 hours. SNC80 was injected systemically into mice (dose: 10 mg/kg), or infused directly into the NAc shell of rats (concentration: 1 mg/mL). Mice were subjected to one PIT test 24h (DOR reduced) after SNC80 injection. Rats received two PIT tests, at 24h (DOR reduced) and 48h (DOR accumulation recovered) after SNC80 infusion.

Results: Systemic SNC80 in mice, and intra-NAc shell SNC80 in rats, impaired specific PIT expression 24h after administration. In rats, PIT expression recovered at 48h. That is, when NAc shell DOR expression was reduced, specific PIT was impaired, but when DOR had recovered to its accumulated state, PIT expression also recovered.

Conclusions: These results suggest that DOR accumulation, not simply DOR function, in the shell is necessary for the expression of specific PIT.
Severe apathy is related to poorer prognosis in amyotrophic lateral sclerosis

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Background: Apathy of varying severity is highly prevalent in amyotrophic lateral sclerosis (ALS). However, the degree to which it affects prognosis and overlaps with depression is unknown. The current study examined the relationship between level of apathy and survival time and whether apathy was linked to specific symptom clusters of depression.

Method: A cohort of 76 consecutive ALS patients attending specialised multidisciplinary clinics were classified according to level of apathy. The effect of clinical factors and apathy on survival time were analysed using univariate and multivariate methods.

Results: The majority of patients with moderate-severe apathy died during the study ($P = .003$) and had a median survival time of 21.7 months, considerably shorter than patients with mild apathy (46.9 months) and no apathy (51.9 months) ($P = .0001$). Apathy remained a significant predictor of survival even after controlling for clinical factors and symptom duration at the time of study entry (hazard ratio 3.5, 95% confidence interval 1.9-6.5, $P = .0001$). Depression with demoralisation was not related to level of apathy ($P = .172$) whereas depression with anhedonia was more common in patients with apathy than in those without apathy ($P = .006$).

Conclusions: The presence of severe apathy is an independent, negative prognostic factor in ALS.
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Characterisation of glial and neuronal pathology in non-Alzheimer’s Disease tauopathies

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Background: Accumulation of a hyperphosphorylated form of the microtubule-associated protein tau is a pathological feature of frontotemporal lobar degeneration with tau-immunopositive inclusions (FTLD-tau). Abnormal tau aggregates with three (3-repeat) or four (4-repeat) microtubule repeat domains occur in neurons, astrocytes and oligodendroglia. The distribution, molecular profile and cellular compartment where tau aggregates differs substantially to produce four main pathological subtypes: Pick’s Disease (PiD) with 3-repeat tau, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP) and globular glial tauopathy (GGT) each with 4-repeat tau. Differences in the phosphorylation of tau may underlie the distinct neuropathological features observed in each FTLD-tau subtype and may prove informative for characterisation and diagnosis.

Methods: This study compared the glial (astrocytic and oligodendroglial) and neuronal phosphorylation profile of tau in 41 pathologically confirmed FTLD-tau cases with PiD (n=10), CBD (n=12), PSP (n=10) and GGT (n=9) subtypes from the Sydney and Cambridge Brain Banks. The type and distribution of subtype-specific features was compared in sections immunostained with phosphorylated tau (AT8) and seven phosphorylation-dependent tau antibodies (pS202, pT205, pS214, pT231, pS396, pS404 and pS422).

Results: The neuropathological phenotypes immunolabelled with each phosphorylated tau antibody varied within, and between each FTLD-tau subtype. All antibodies immunolabelled Pick bodies in PiD. The phosphorylation profile of astrocytic and oligodendroglial inclusions was heterogeneous across FTLD-tau subtypes.

Conclusions: This study reveals important differences in the phosphorylation profile between neurons and glia in FTLD-tau, which is informative for neuropathological diagnosis and provides insights into how changes in one protein can potentially lead to distinct neuropathological subtypes.
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Statistical modelling of post-stroke aphasia recovery

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Background: Aphasia is a communication disorder caused by damage to the language centres of the brain due to Traumatic Brain Injury or Stroke. The Western Aphasia Battery Aphasia Quotient (AQ) is a standardised test battery with high test-retest reliability but is negatively affected by a ceiling effect. Maximal Potential Recovery (MPR) is calculated from the AQ by dividing the improvement in AQ (from baseline) by the maximum achievable improvement. The MPR was introduced to overcome the ceiling effect of the AQ. This work assesses the use of MPR to model post-stroke aphasia recovery to determine the most statistically appropriate model.

Methods: Secondary analysis of data from two Australian randomised controlled trials was used to compare competing statistical models. Data from study 1 (N=59) was used to develop the models with different outcome measures: a) AQ and b) MPR. Data from study 2 (N=20) was used to validate and compare the models. Quasi Information Criterion (QIC), Residual plots and Residual Sum of Squares (RSS) were compared across the two models to determine the optimal aphasia measure.

Results: The MPR model demonstrates substantially lower values for QIC and RSS. It also demonstrates considerable improvement in residual scores compared to the AQ model. The lower values of QIC together with the residual plot indicate a better statistical model fit; the lower value of RSS indicates greater accuracy in predicting recovery.

Conclusions: Modelling aphasia recovery on MPR provides a better fit than AQ. This provides a more accurate prediction of recovery.
Early changes in the caudate of young people with affective and psychotic disorders

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Background: Affective and psychotic disorders are among the most common disorders affecting young people. Early intervention is effective in helping young people before more severe symptoms arise, however objective measures tracking illness progression are lacking. The present study used neuroimaging to investigate subcortical volume differences between controls, young people at an early disorder stage and those with more advanced illness, to identify relationships between neuroimaging markers and functional outcomes.

Methods: Young females presenting to youth mental health services with admixtures of depressive, manic and psychotic symptoms (n=108), and control females (n=37) aged 18-25 were recruited. Participants underwent magnetic resonance imaging and standardised clinical assessments rating their current illness stage. Sixty-four patients were identified at the early illness stage and 44 were classified in more advanced illness stages. Automated segmentation was performed using NeuroQuant® to determine six subcortical structure volumes which were compared between groups using MANOVA.

Results: Caudate volume was significantly lower in the early staged group compared to controls (p=0.015), but not the later staged group (p=0.279). Reduced caudate volume correlated with worse functional outcomes including higher depression ratings, self-reported psychological distress and disability, and poorer social and occupational functioning across all groups (p<0.05).

Conclusion: Abnormal caudate volumes correlate with affective and psychotic disorder development in young people, even at an early stage. Poorer functional outcomes are already evident in this early stage, possibly relating to caudate structural changes. Further investigation into the relationship between illness progression and the caudate may reveal its role in these disorders’ later stages.
The role of STAT1 serine 727 phosphorylation as a key regulator in Experimental Autoimmune Encephalomyelitis

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Interferon-beta (IFN-β) is a commonly used treatment for multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS). However, many patients with MS do not respond to IFN-β and show exacerbations and disease progression. The signal transducer and activator of transcription 1 (STAT1) is a key transcription factor that mediates IFN-β signalling. STAT1 activity is regulated by phosphorylation of tyrosine 701 (pY-STAT1) which is essential for STAT1 activation, and serine 727 (pS-STAT1). The function of pS-STAT1 remains unclear but is thought to be required for regulating STAT1 transcriptional activity. Importantly, reduced pS-STAT1 levels have recently been linked to MS pathogenesis. The present study investigates the role of pS-STAT1 in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Transgenic mice that express a serine727-alanine mutant STAT1 (STAT1 KI mice), on average, develop more severe symptoms of EAE during acute and chronic phases compared to WT mice. Inflammatory lesions which consisted of perivascular cuffs and CNS infiltrates were observed in the spinal cord, brain stem, cerebellum and meninges of both STAT1 KI and WT mice. Although the lesions and degree of demyelination were more widespread with more severe EAE, the histological changes in STAT1 KI mice could not be distinguished from that of WT mice with similar clinical symptoms. However, differences were observed in the expression of various proinflammatory cytokines and chemokines, and in transcription factor activation. This argues for a role of pS-STAT1 in EAE and MS and may present a target for developing novel therapeutics.
Glutamine modulates glycine neurotransmission in the superficial dorsal horn in the spinal cord of 14-21 day old Sprague-Dawley rats

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Background: The two fast inhibitory neurotransmitters, GABA and glycine control inhibition in the spinal chord. These neurotransmitters are co-released, as they are loaded into synaptic vesicles via a shared vesicular transporter, VIAAT. However, the mechanisms that control and modulate this process are not well understood. Glutamine is in high concentrations in the cerebrospinal fluid and supplies presynaptic terminals with GABA and glutamate. Modulation of glutamine supply to inhibitory spinal chord neurons could alter presynaptic concentrations of GABA, altering neurotransmitter release.

Methods: Whole-cell patch clamping was used to take recordings from inhibitory neurons taken from coronal spinal cord slices and glycine transmission was isolated using CNQX (5µM) and SR93551 (10µM). Extracellular application of glutamine (2mM) was applied to the slice in order to characterize is effect on glycine transmission.

Results: Compared to controls of glycine transmission, application of 2mM glutamine causes a greater variation in glycine inhibitory postsynaptic currents (IPSC). This variation shows a small reduction of glycine transmission 3-4 minutes after glutamine application (80.85±5.06% of Glycine IPSC, p=0.0018, n=16). However, this group includes a subset of cells with no or very little response to glutamine.

Conclusions: Whilst glutamine appears to reduce some examples glycine transmission at high concentrations, the result remains inconsistent. Further research is needed to determine the conditions in which this inhibition occurs. This research influences our understanding of inhibitory control of nociceptive neurotransmission in the spinal cord and how it might affect chronic pain states.
Determining the fate of motor neurons that have been deprived of their supraspinal input by a spinal cord injury

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Background: One significant outcome of a spinal cord injury is the abolishment of the supraspinal input to the motor neurons below the lesion, therefore resulting in paralysis. The majority of research has focused on understanding the pathophysiological events surrounding a lesion, however, there is little knowledge regarding what changes occur to motor neurons below a lesion. Therefore, the aim of this project is to determine the morphological changes to motor neurons below a spinal cord injury.

Method: Rats were subjected to a unilateral partial transection at C3-4 spinal cord segments. Animals were sacrificed one, three, seven and fourteen days post-lesion and the C5-6 and L2-3 spinal cords segments were dissected and histologically processed. Using the Optical Fractionator workflow component of the Stereo Investigator software, the morphology and number of motor neurons were quantified.

Results: Analysis revealed that the motor neurons of both C5-6 and L2-3 were bilaterally affected after a lesion. Across all time points, the number of large motor neurons fluctuate at C5-6 and L2-3. Initially, the number of large motor neurons decrease on both sides, however, as time progresses the number of motor neurons then increase bilaterally.

Conclusion: A unilateral partial transection of the spinal cord results in anatomical changes to the motor neuron morphology below a lesion. Such changes to viable motor neurons will have functional implications to their nerve excitability and innervation of muscle fibre subsets. This knowledge is critical for future therapeutic scenarios that aim to restore the function of motor neurons below an injury.
Translocation of delta-opioid receptors on striatal cholinergic interneurons is mediated by a local dopamine - substance P interface and afferent projections from the basolateral amygdala

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Introduction: The δ-opioid receptor (DOR) is heavily expressed in the striatum particularly on cholinergic interneurons (CINs). These neurons contain large amounts of receptor sequestered in intracellular pools. During Pavlovian conditioning, DOR in the nucleus accumbens shell of DOR-eGFP mice translocates from the cytoplasm to the somatic membrane of CINs. This study investigates the role of both striatal microcircuits and afferent projections from the BLA in this learning-related insertion.

Methods: Substance P (SP), an NK1R antagonist, and D1- and D2-receptor agonists were administered to discrete striatal subregions of DOR-eGFP mice. NK1R antagonists were also administered during learning. Projections from the BLA to the ventral striatum were selectively manipulated using Designer Receptor Exclusively Activated by Designer Drug (DREADDs). DOR-eGFP distribution was analysed by confocal microscopy.

Results: Intrastriatal administration of SP and co-administration of D1- and D2-receptor agonists induced an increase in DOR at the membrane of striatal CINs in an NK1R-dependent fashion. However, NK1R antagonists could not block learning-induced DOR translocation. Activation of BLA projections to the ventral striatum also drives an increase in levels of DOR.

Conclusions: While SP signalling and concurrent D1- and D2-receptor activation triggers the translocation of DOR on CINs in the striatum via NK1R, NK1R inhibition is insufficient to block DOR translocation induced by Pavlovian conditioning. A separate mechanism may promote long-term learning-induced redistribution of DOR. Activation of BLA projections to the ventral striatum also triggers DOR translocation, either by activating the local dopamine-SP microcircuit or in an NK1R-independent manner that may be relevant during learning.
Calcium Imaging of Retinal Ganglion Cell Responses to Extracellular Microelectrode Stimulation

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Background: To improve existing stimulation strategies for prosthetic vision, it is essential to assess the effectiveness of proposed stimulation protocols by measuring the resulting activation profiles of populations of retinal neurons. Calcium imaging enables signals to be recorded simultaneously from a large number of neurons and thereby allows retinal spatial activation profiles to be determined for different stimulation strategies.

Methods: All procedures were approved by the UNSW Animal Ethics Committee. The eyes of wild-type mice (C57BL/6J) were enucleated and retinas extracted. Electroporation was used to load calcium dye Cal520 into retinal ganglion cells (RGCs) of the excised retina. After electroporation, the retinal section was mounted onto an imaging chamber, placed under a microscope and perfused with standard Ames’ solution or a solution containing a cocktail of presynaptic blocker agents. Sub-retinal stimulation was delivered by multi-electrode arrays (Multi-Channel Systems).

Results: With normal Ames’ solution, increasing stimulus strength resulted in increased spatial activation profiles and higher signal peaks in RGCs. With presynaptic blocker agents in the solution, signal peaks still increased as the stimulation strength increased, however the number of activated RGCs and signal peaks were greatly reduced. In addition, rise times to signal peaks were significantly longer in Ames’ perfusion.

Conclusions: Calcium imaging is an effective method to simultaneously assess spatial activation profiles and response properties in populations of RGCs. These data will improve our understanding of the effectiveness of proposed retinal stimulation protocols and enhance our design of stimulation strategies for prosthetic vision.
The Direct Action of Cannabidiol at GABA-A Receptors

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Background: Cannabidiol (CBD) is considered the major non-psychoactive component of cannabis. It has been found to possess anti-epileptic, anxiolytic and anti-psychotic properties in humans, which may be suggestive of GABAergic involvement and makes such elucidation compelling. Endogenous and synthetic cannabinoids have been shown to act at various GABA-A receptors, however, the actions of the phyto-cannabinoids have not been wellexplicated. Here we aim to assess for the activity of CBD and 2-arachidonyl glycerol (2AG) upon GABA-A receptors. Specifically, to contrast the selectivity of these compounds between the alpha and beta subunits of synaptic GABA-A receptors.

Methods: Recombinant DNA techniques and two-electrode voltage clamp electrophysiology of receptors expressed in Xenopus laevis oocytes.

Results: CBD and 2AG modulate GABA significantly, with CBD more efficacious. In terms of alpha subunit selectivity, selectivity for the alpha 2 subunit was observed, with greater than fourfold modulation of GABA EC₅₀ with 100µM CBD. At 10µM CBD, the modulation of the GABA EC₅₀ upon receptors was approximately twice that of other alpha subunit receptor combinations (i.e. 241% vs 160-180%, p<0.05, n=6). In terms of beta subunit selectivity, modulatory activity was abolished when beta 1 was introduced. At 10µM CBD, the modulation of a GABA EC₅₀ upon some receptors was approximately the same (i.e. 241-246%) however upon others was 0%. CBD and 2AG were weak partial agonists at some GABA-A receptors.

Discussion: CBD directly activates and modulates GABA when applied upon specific synaptic GABA-A receptor combinations. CBD modulates these receptors more efficaciously than 2AG, the major central endocannabinoid. Selectivity for the alpha 2 and beta 2/3 subunits was discovered for synaptic GABA-A receptor combinations which may account for some of the intriguing central effects seen with CBD.
Maternal immune activation alters molecular indices of the NMDA receptor in the striatum

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Background: Schizophrenia has many risk factors, one of which includes prenatal infection that leads to Maternal Immune Activation (MIA). Offspring of pregnant rats subjected to MIA show deficits paralleling schizophrenic individuals, particularly cognitive dysfunction and pathology in the striatum. The striatum may be involved in regulating some of the cognitive-related behaviours impaired in both MIA-offspring and people with schizophrenia. Male MIA-offspring exhibit exacerbated cognitive deficits and pathology, and may exhibit altered neurobiological changes versus female littermates. We determined if MIA alters striatal NMDA glutamate receptor (NMDAR) indices within progeny based on (1) gestational-timing of MIA, and (2) offspring sex.

Methods: Brains of adult offspring from dams treated with the viral infection mimic polyriboinosinic:polyribocytidilic acid or saline at gestational day 10 (GD10) and 19 (GD19) were processed for binding to NMDAR channel ([\textsuperscript{3}H]MK801), NR2A ([\textsuperscript{3}H]CGP39653) and NR2B ([\textsuperscript{3}H]Ifenprodil) NMDAR subunits, and in-situ hybridisation for NR1 and NR2A mRNA levels, within the dorsal striatum and nucleus accumbens (shell and core separately).

Results: Repeated measures ANOVA in male offspring revealed an effect of MIA increasing NMDAR channel and NR2A binding, and NR2A:NR2B ratio across all striatal subregions (F(1, 24)=21.503, p<0.001; F(1, 24)=7.27, p<0.05; F(1, 24)=6.608, p<0.05). Analysis in female offspring revealed an overall effect of MIA decreasing NR1 mRNA levels (F(1, 24)=7.975, p<0.01). There were no significant GD effects, or GD-interactions, across either sex.

Conclusions: These findings suggest MIA affects striatal NMDAR-related indices in offspring at either exposure times. This may substantiate that subcortical changes may contribute to the behavioural deficits found in the MIA-exposed rats.
If I can’t have that snack then... : Cues associated with the specific or general absence of food rewards produce distinct influences on food-seeking actions.

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Background: Cues associated with the delivery of specific food rewards have been shown to influence food-seeking behaviour - increasing motivation and biasing action-selection in both humans and rats. Recent research in rats has demonstrated that cues associated with the absence of specific food rewards can also bias action-selection, however this effect has not yet been examined in humans.

Methods: We developed a novel behavioural task to examine the ways in which cues associated with the delivery or absence of different food rewards influenced both participants’ motivation to engage in food-seeking behaviours and action-selection.

Results: Cues associated with the delivery of a specific food reward selectively elevated responding on an action that had previously produced that outcome. In contrast, cues trained to predict the absence of specific outcomes produced an opposing bias in action-selection. Further analysis found in ‘General Learners’, where participants learned the inhibitory cues signalled the non-specific absence of food, these cues produced a suppression of responding on both available outcomes. However ‘Specific Learners’, who learned that the inhibitory cues predicted the absence of a specific outcome, showed elevated responding on the action associated with the delivery of an alternate outcome.

Conclusions: These findings provide the first evidence that cues associated with the absence of a specific outcome can promote responding associated with an alternate outcome in humans. The differences in cue-elicited responding between learner subgroups provides greater insight into the mechanisms driving this effect, highlighting the complex ways that cues in our environment can influence our food-seeking behaviours.
Suppression of spontaneous activity in a noise-induced hearing loss model


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Hearing loss results in changes in the auditory system including increased spontaneous activity and increased synchronization of neural activity. These changes have been proposed as a neural mechanism responsible for tinnitus. Increases in spontaneous activity have been reported in many different auditory nuclei, which leads us to question whether this is a general property of the auditory system following hearing loss, or whether spontaneous activity is generated in one particular nucleus and then propagated throughout the rest of the auditory pathway. Hearing loss was induced in adult Long Evans rats by unilaterally exposing them to a 115 dB-SPL, 16kHz 1/10th octave bandpass noise for 1-hour. At least three months later we recorded extracellular activity simultaneously from three auditory nuclei (dorsal cochlear nucleus, inferior colliculus and auditory cortex) using 32 and 64 channel electrode arrays. Data were sorted into discrete, multi-unit clusters. Baseline spontaneous activity was recorded for 10 minutes, and then the animals were exposed for 5 minutes to an 80dB-SPL noise stimulus, which suppressed spontaneous activity. We then tracked the re-emergence of spontaneous activity in the three nuclei. The effect of the noise exposure on ensuing spontaneous activity varied between the two groups. In controls, spontaneous activity increased relative to baseline levels, whereas in noise-exposed animals there was a continued suppressive effect. These effects were greatest in the dorsal cochlear nucleus and the inferior colliculus.
Tactile aftereffect strength depends on the speed of adapting motion


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Perception of movement across the skin relies on processing of tactile sensory inputs to the nervous system. We used adaptation to investigate how speed of motion is represented; the amount of adaptation depends on the amount of neural activation elicited by the adapting stimulus, and this is evident in the strength of resulting perceptual aftereffects. We varied the speed of adapting motion and measured the strength of two such aftereffects.

We induced the tactile speed aftereffect (tSAE), in which the speed of motion appears slower following exposure to a moving surface. We also induced the tactile motion aftereffect (tMAE), in which a direction-neutral test stimulus appears to move in the opposite direction to previously felt adapting motion. Participants judged either the speed (n = 13) or direction (n = 8) of a test stimulus applied to the finger following adaptation to a variety of speeds (19 – 136 mm/s).

For the tSAE, perceived speed showed a greater reduction when the adapting stimulus was faster than the test, compared to when they were matched; this result was independent of the rate at which texture features crossed the skin. For the tMAE, participants were more likely to report that the test stimulus moved in the opposite direction to the adapting motion when the adapting speed was faster.

In both cases, faster adapting speeds resulted in stronger aftereffects. This suggests that in the neural populations encoding speed and direction, an ‘intensive’ code for speed is present, in which faster speeds cause greater neural activation.
Structural changes in the cognitive control network are associated with improvements in cognitive performance during development

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Background: Cognitive control is the process of performing self-regulatory behavior towards a predefined goal. Recent findings suggest a superordinate cognitive control network (CCN) made up of the dorsolateral prefrontal, anterior cingulate and posterior parietal cortices is responsible for a range of cognitive control related behaviors including sustained attention, working memory and other executive functions. This study examines the developmental trajectory of these behaviors from late childhood to early adulthood and associated structural changes in the brain.

Methods: 141 participants aged 8 to 18 completed a computerized battery of 12 tests measuring cognitive control and underwent structural MRI on a 1.5T scanner. Cognitive measures were analysed using a General Linear Model (GLM) to evaluate age trends controlling for gender. Grey matter volume information of brain regions in the CCN was extracted from structural magnetic resonance imaging data (MRI-T1) and a GLM was applied to elucidate age and cognitive performance relationships.

Results: All, except two, cognitive control measures had a significant improvement in performance with age (p<0.0080). MRI-T1 data revealed a significant decrease in gray matter as age increased (p<0.0001). Grey matter volume and performance in cognitive tasks were associated across all domains. Attention and working memory performance were associated with parietal and anterior cingulate cortex volumes after controlling for age.

Conclusion: Improvement in cognitive performance during childhood and adolescence appears to be linked to age-related decreases in gray matter volume throughout the CCN. This may be the result of synaptic pruning and network refinement occurring during this time.
The blood-brain barrier may be more permeable in individuals who have schizophrenia with elevated inflammatory markers

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Background: The blood-brain barrier (BBB) regulates the transport of circulating molecules into the brain. BBB dysfunction is implicated in a variety of neurological diseases and also associated with a disturbed neuroimmune response. Inflammation may be involved in schizophrenia and we previously identified a subset of people with schizophrenia (40\%) with elevated inflammatory markers. Whether peripheral inflammation in schizophrenia can directly impact brain endothelial cells, which form the first point of contact with the brain is unknown.

Method: Next generation sequencing was used to identify changes in endothelial cell related gene expression in the dorsolateral prefrontal cortex from 20 individuals with schizophrenia and 20 healthy controls. Individuals were previously classified as having a high or low inflammatory profile (Filman et al, Mol Psych). To validate changes observed by next generation sequencing, qPCR was performed on our full cohort of 37 individuals with schizophrenia and 37 healthy controls.

Results: Endothelial cell related gene expression changes present by both next generation sequencing and qPCR include downregulation of transporters (ATP2B2, $F(2,67)=5.139$, $p<0.01$ and ABCG2, $F(2,64)=11.09$, $p<0.01$), and upregulation of adhesion molecule (ICAM-1, $F(2,65)=14.91$, $p<0.01$) in people with schizophrenia and high inflammation compared to both schizophrenia with low inflammation and healthy controls.

Conclusions: Endothelial cell transporters regulate the levels of molecules into and out of the brain while adhesion molecules are involved with leukocyte attachment to blood vessel endothelium. The observed changes in gene expression suggest the BBB may be more permeable in individuals with schizophrenia and high inflammation.
The PI3K/Akt/GSK3β Pathway is not involved in early Alzheimer’s disease

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Alzheimer’s disease (AD) and diabetes are two common diseases that have reached epidemic proportions in Australia. Epidemiological studies suggest that type 2 diabetes (T2D) is also a risk factor for AD. The AD brain is pathologically characterised by plaques and tangles, with the latter being made up of hyperphosphorylated forms of the protein, tau. T2D results in a paradoxical decrease in insulin signalling in the brain, that is predicted to decrease signalling through the PI3K/Akt pathway and lead to increased activation of the major tau kinase, glycogen synthase 3beta (GSK3β). Studies using post-mortem brain tissue are inherently retrospective with the most susceptible areas of the AD brain suffering major neuronal loss and compensatory changes. In contrast, areas such as the superior temporal gyrus display plaques but few tangles and retain nearly all their neurons. These regions may be equivalent to severely affected regions earlier in the disease. Here we explored the levels of AKT and GSK3β in the superior temporal gyrus, precuneus and primary visual cortex of 20 AD cases and 20 age-, gender- and APOE ε4 genotype-matched controls. There were no differences in the levels of active AKT or GSK3β between cases and controls in the STG. GSK3β was decreased in the AD-precuneus and primary visual cortex but it was coupled to levels of the major tau phosphatase, PP2A. Our results suggest that there is no linear relationship between decreased insulin signalling, GSK3β and increased tau hyperphosphorylation in early phases of clinical AD.
Effects of IL-35 gene therapy on neuroinflammation and neuropathic pain following peripheral nerve injury

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Background: Peripheral nerve injury often results in neuropathic pain that involves neuroimmune responses. While pro-inflammatory cytokines contribute to pain hypersensitivity, anti-inflammatory cytokines have been shown to resolve neuroinflammation and inhibit pain hypersensitivity. Interleukin (IL)-35, an Ebi3/p35 heterodimer, is a novel anti-inflammatory cytokine produced by regulatory T and B cells, and is known to suppress autoimmunity and inflammation in animal models.

Methods: Following intrathecal delivery (days 1 and 4) of the pVAX–IL-35, expression of p35/EBi3 mRNA and IL-35 protein in the lumbar spinal cord was detected by polymerase chain reaction (PCR) and ELISA in naïve C57BL6/J mice at day 5 (n=3-4). Following chronic constriction injury (CCI) of the sciatic nerve (Day 0), mice were treated with either pVAX-IL-35 plasmid or pVAX control at days 1 and 4 post-CCI, and mechanical pain hypersensitivity was tested in the hind paws. Additionally, spinal glial cell reactivity was assessed by immunohistochemistry, and the prevalence of regulatory T cells and intracellular cytokines in the lymph nodes were assessed by flow cytometry (n=3-5).

Results: Increased expression of Ebi3/p35 mRNA and IL-35 protein in the spinal cord confirmed the successful in vivo transfection. Intrathecal IL-35 gene delivery following CCI significantly abolished spinal cord microgliosis, but failed to modify pain hypersensitivity. This treatment had no effect on serum IL-35 expression, prevalence of systemic regulatory T cells and intracellular cytokine levels.

Conclusions: These results challenge the widely accepted link between microglia and pain hypersensitivity and suggest that microglial activation is not necessary for mice to develop neuropathic pain symptoms.
Homeostatic STAT1 signalling restricts endogenous retroviral gene expression in the CNS

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Endogenous retroviruses (ERV) occupy approximately eight percent of the human and murine genomes and their reactivation has been linked with cancer. Although the role of the transcription factor signal transducers and activators of transcription 1 (STAT1) in the host response against exogenous viruses is well established, whether STAT1 has a role in the regulation of ERV is unknown and was the focus of this study. Microarray analysis of total RNA from murine mixed glial cells revealed a strong induction of the expression of the ERV, melanoma antigen (Mela), in STAT1-deficient cells. Follow-up studies revealed Mela mRNA was significantly increased in STAT1-deficient primary murine microglia compared with WT counterparts and was not detectable in astrocytes. To localise Mela mRNA in vivo, dual-label in situ hybridisation and immunohistochemistry was performed. This showed colocalisation of Mela mRNA in microglia and astrocytes in a punctate pattern, with an incidence of astrogliosis, throughout the CNS of STAT1 KO mice but not in the WT CNS. To further elucidate the mechanism of regulation of Mela, its expression was analysed in mice deficient in other interferon signalling pathway components. STAT1 and to a lesser extent interferon-α/β receptor deficiency but not interferon-γ receptor, STAT2 or interferon regulatory factor 9 (IRF9) deficiency caused induction of Mela expression in the brain. These findings show that homeostatic STAT1-dependent non-canonical type I interferon signalling is involved in suppressing the re-emergence of Mela mRNA. Hence, in the healthy CNS, STAT1 may have an important role in restricting the expression of ERV.
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Extending the viability of acute brain slices

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The lifespan of an acute brain slices is approximately 6–12 hours, this limits the potential experimentation time. We have recently designed a new recovery incubation system which tightly controls the external environment and is capable of extending the lifespan of the slices to more than 36 hours. Brain slice viability was validated through electrophysiological recordings as well as live/dead cell assays and imaging of calcium dynamics. We found that the calcium dynamics of individual cells and the network activity remained intact long time after loading (>24 hrs). This system benefits researchers by monitoring incubation conditions and standardizing the artificial environment conditions. It further provides viable tissue for two experimental days, reducing the time spent preparing brain slices and the number of animals required for research.
Exploring the neurobiobehavioural basis of baclofen in the treatment of alcohol dependence.

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Background: The medication, baclofen, a GABAβ-R agonist, is showing promise as an effective pharmacotherapy for alcohol dependence. However, the widespread use of the medication is beyond the evidence base and the mechanism of action of any effect remains to be determined. Our previous work has suggested it to be effective for reducing relapse in alcohol dependent patients with comorbid anxiety problems.

Objective: We aim to explore the neurobehavioural basis of baclofen in the treatment of alcohol dependence.

Methods: Alcohol dependent patients will be randomised to receive baclofen (10mg tid, 20mg tid) or matched placebo. Following 14 days of treatment, patients will undergo a series of neuroimaging experiments including resting state (rsfMRI), functional magnetic resonance imaging (fMRI) and magnetic resonance spectroscopy (MRS) following baclofen administration. Specifically, we will examine i) regional brain neurotransmitter levels including GABA and glutamate (using MRS), ii) functional connectivity (using rsfMRI) and iii) activity in the medial prefrontal cortex (using fMRI) following exposure to an alcohol cue (craving) and stress (anxiety) task.

Results: Recruitment began in late 2014 and will continue until early 2016.

Conclusion: The proposal will also aid our understanding of the role of the GABAb receptor in the stress-response biological system that can be altered in alcohol dependent individuals. The results will also further elucidate the role of the GABAb receptor in craving.
Effects of chemogenetic and optogenetic manipulation of the striatopallidal pathway on the renewal and reacquisition of extinguished alcohol seeking

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The ventral pallidum (VP) is a key component of the neural circuitry mediating relapse to drug seeking. Using designer receptor exclusively activated by a designer drug (DREADD), we bi-directionally manipulated the VP. We transduced VP with an adeno-associated virus expressing hM3Dq (excitatory) and hM4Di (inhibitory) DREADD. Rats were trained to self-administer alcoholic beer in one context (A), extinguished in a second context (B), tested in the extinction (ABB), in context (A) for renewal and reacquisition. VP contributed to relapse and reacquisition of alcohol seeking. We further studied the role of the nucleus accumbens core (AcbC) to the VP pathway in ABA renewal and reacquisition of alcohol seeking. Rats received application of adenoviral vectors encoding eYFP, ChR2(H134R), or eNpHR3.0 to AcbC and implantation of fibre optic cannulae into VP to permit photostimulation of AcbC terminals there. There was evidence for ABA renewal of alcohol seeking but neither optogenetic excitation nor inhibition of the AcbC-VP pathway affected this renewal. In contrast, optogenetic inhibition of the AcbC-VP striatopallidal pathway did reduce reacquisition of alcohol seeking - as measured either by the number of active nosepokes emitted or by the number of alcohol rewards earned and consumed. Moreover, optogenetic excitation of the AcbC-VP striatopallidal pathway increased the number of magazine entries during reacquisition test. This finding shows the importance of the AcbC-VP pathway in controlling relapse when the drug reinforcer is present on test and is consistent with a role for the AcbC-VP pathway in regulating the hedonic or incentive motivational properties of drug reinforcers.
The role of different phosphorylation sites in μ-opioid receptor desensitization

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Phosphorylation of residues in the C-terminal tail of the μ-opioid receptor (MOPr) is thought to be a key step in desensitization and internalization. Phosphorylation of C-terminal S/T residues is required for internalization (Just et al., 2013) but its role in desensitization is unknown. This study examined the influence of C-terminal phosphorylation sites on rapid desensitization of MOPr. Wild type MOPr, a 3S/T-A mutant (S363A, T370A, S375A) that maintains internalization, 6 S/T-A (S363A, T370A, S375A, T376A, T379A, T383A) and 11S/T-A (all Cterminal S/T residues mutated) mutants not internalized by MOPr agonists were stably expressed in AtT20 cells. Perforated patch-clamp recordings of MOPr-mediated activation of GIRK (Kir3.X) conductance by submaximal concentrations of Met5-Enkephalin (ME) and somatostatin (SST coupling to native SSTR) were used to examine desensitization induced by exposure to ME and morphine for 5 min at 37oC. The rates of ME- and morphine-induced desensitization did not correlate with phosphorylation using phosphorylation site specific antibodies. ME-induced MOPr desensitization and resensitization did not differ from wild-type for 3S/T-A and 6S/T-A but was abolished in 11S/T-A. Morphine-induced desensitization was unaffected in all three mutants, as was heterologous desensitization of SSTR. Morphine-induced desensitization (but not ME) was reduced by protein kinase C inhibition in wild type MOPr and abolished in the 11S/T-A mutant, as was heterologous desensitization. These findings establish that MOPr desensitization can occur independently of S/T phosphorylation and internalization. However, Cterminal phosphorylation is necessary for some forms of desensitization because mutation of all C-terminal sites (11S/T-A) abolishes desensitization induced by ME.
Differential attenuation of auditory and visual evoked potentials for sensations generated by hand and eye movements

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Background: Feeling ownership over one’s thoughts and actions is a central feature of everyday life, but for people who suffer from schizophrenia, hearing intrusive voices and feeling as if strangers control their body are common, debilitating experiences. Evidence suggests that these bizarre psychotic symptoms could be related to a cognitive mechanism that attenuates sensations produced by our own actions, distinguishing them from sensations produced by the external world. Consequently, patients with deficits in sensory attenuation could misinterpret self-produced sensations as external in origin. However, though psychotic symptoms occur across all sensory domains, attenuation studies are mostly limited to stimuli from one sensory domain (typically auditory) produced by one motor area (typically the hand, during button-pressing).

Methods: We measured the electroencephalogram (EEG) of 34 participants exposed to both auditory (pure tone) and visual (unstructured flash) stimuli. In addition, using eye-tracking technology, participants were able to produce these stimuli by either pressing a button or saccadic movements.

Results: We found that attenuation of self-produced sensations, indexed by auditory and visual N1-components, significantly differed by sensory domain and motor area, and was strongest when there was a natural link between action and sensation (i.e. hand–auditory and eye–visual).

Conclusions: The pattern of results is consistent with the fact that hands can produce auditory sensations (for instance, by clapping), but eyes normally only evoke visual sensations. Our research reveals important mediators of sensory attenuation, which, along with future clinical studies, could improve our understanding of characteristic psychotic symptoms.
The role of the basolateral amygdala in the consolidation of second-order conditioned fear

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In first-order Pavlovian fear conditioning, laboratory rats are able to quickly acquire fear to an innocuous stimulus that signals an innate source of danger. Rats are also able to learn to fear stimuli which signal learned sources of danger (second-order conditioning). The basolateral amygdala (BLA) is important for the acquisition and consolidation of first-order conditioned fear. However, very little is known about its role in the consolidation of second-order fear. Rats were first exposed to pairings of a stimulus (S1: auditory or visual) and foot-shock and then to pairings of a second stimulus (S2: visual or auditory, respectively) and S1. Finally, rats were tested for fear (freezing) to S2. In Experiment 1, functionally inactivating the BLA immediately after S2-S1 pairings with the sodium channel blocker bupivacaine attenuated levels of freezing to S2 relative to vehicle-treated rats. In Experiment 2, inhibiting ERK/MAPK activity with the MEK inhibitor U0126 immediately after second-order conditioning had no effect on the consolidation of second-order fear. However, when re-trained as a first-order cue (that is, S2-shock pairings), post-training infusion of U0126 impaired consolidation of the fear memory. The same pattern of results were found in Experiment 3 when kinases upstream of the ERK/MAPK pathway (that is, PKA/PKC) were inhibited using a broad spectrum protein kinase inhibitor, H7. There are at least two explanations for these findings. The first is that the presence of shock recruits these kinases in the consolidation process; the second is that the second-order association is consolidated elsewhere in the brain.
Modulation of hippocampal and olfactory neurogenesis in the adult mouse brain

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Background: The subgranular and subventricular zones (SGZ and SVZ) are the two primary neurogenic regions in the adult brain. In mice, new neurons generated in the SGZ integrate into the hippocampus, whereas those originating from the SVZ migrate into the olfactory bulb. Adult neurogenesis modifies existing neural circuits and thus has adaptive functions. Altered neurogenesis has also been linked to the pathogenesis of neurodegenerative diseases and schizophrenia. Modulation of adult neurogenesis, therefore, may have therapeutic potential.

Methods: Eleven- to 12-week-old C57BL6J mice (24 males and 24 females) were used. The male mice were housed in either standard cages or in an enriched environment (i.e., cages equipped with toys and running wheels) for 4 weeks. The female group was exposed to either odorless filter paper or novel scents every morning for 4 weeks. At the end of the treatment, the numbers of neuronal and proliferating cells were determined in both the hippocampus and olfactory bulb, using the isotropic fractionator method.

Results: Enriched housing as well as odor stimulation significantly increased the number of neurons in both the hippocampus and olfactory bulb. The number of proliferating cells was also significantly increased in the hippocampi of mice housed in the enriched environment and in the olfactory bulbs of mice exposed to olfactory stimulation.

Conclusions: Our results indicate that specific interventions improve adult neurogenesis. We conclude that non-invasive stimuli may enhance neurogenesis in both primary neurogenic regions and may offer a new avenue for counteracting the negative effects of neurodegenerative diseases.
Addressing rare variant contributions to the genetic architecture of Bipolar Disorder, utilizing extended families with highly penetrant forms of illness

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Background: Bipolar disorder (BD) is a highly heritable illness, likely contributed to by a spectrum of common variants of small additive effect plus rare variants of higher penetrance. We hypothesise that pathogenic rare variants of moderate effect are present in the expressed protein-coding portion of the genome (exome), and shared amongst individuals with BD in unique families with a high density of illness.

Methods: We selected a cohort of 15 extended families, each containing 4 or more relatives with severe forms of BD. Whole exome sequencing (WES) was performed in 117 subjects, using the Proton platform, to detect rare single nucleotide variants (SNV). Copy number variants (CNVs) were detected via CytoScan HD Array in 2 affected subjects per family. Linkage analysis was performed using WES-derived genotypes. Genome-wide burdens of rare variation shared amongst related subjects were compared, and analysed for enrichment in gene ontology groups and KEGG pathways.

Results: We identify a number of novel candidate genes, bearing mutations shared amongst affected relatives and predicted to be damaging. Several genes have previously shown association with psychiatric disorders. We find heterogeneity across families with respect to biological pathways enriched. We identify novel candidate genes, including the brain-expressed X-linked IRS4. In a combined linkage analysis, the strongest peak was found on chromosome 10 (exLOD=2.87, 95%CI=51.5-70.4Mb), encompassing ~50 candidate genes including ANK3.

Discussion: Approaches that combine WES, CNV and linkage analyses in extended families are an effective method for detection of potential pathogenic variation, pinpointing genes and pathways that may contribute to the pathophysiology of BD.
Genetic variation in *GRM5* is associated with cognition, hippocampal volume and schizophrenia

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*GRM5* is implicated in hippocampal-dependent cognitive functions that are disrupted in schizophrenia. We therefore investigated the effects of two novel single nucleotide polymorphisms (SNPs) in *GRM5* on cognitive function and hippocampal volume in schizophrenia, as well as simple genetic association with the disorder. The two *GRM5* SNPs (rs60954128 [C>T] and rs3824927 [G>T]) were genotyped by Sequenom MassARRAY in 268 schizophrenia and 268 control Caucasian individuals, using DNA from the Australian Schizophrenia Research Bank. All participants completed standard neuropsychological assessments, and 78 controls/103 cases had high-resolution T1-weighted MPRAGE anatomical scans available. Scans were processed and hippocampal volumes extracted using Freesurfer v5.1. Chi-squared analyses revealed schizophrenia males were more likely to carry minor alleles for rs60954128 than male controls (\(p=0.011\)). Mixed design MANCOVA showed that genetic variants for this SNP also affected intelligence and delayed memory in males, and working memory in females with schizophrenia (\(p<0.042\)). A significant interaction of rs60954128 with diagnosis of schizophrenia in males affected hippocampal volumes, with carriers of the minor allele showing significantly reduced right hippocampal volume relative to major allele homozygotes and controls (\(p=0.013\)). For rs3824927, schizophrenia males carrying the minor allele showed reduced intelligence, working and delayed memory scores (\(p<0.025\)), but common variation of this SNP was not associated with schizophrenia *per se*. These findings converge with previous reports from animal and postmortem studies to implicate *GRM5* variants in the cognitive symptoms seen in individuals with schizophrenia, with these effects potentially mediated by anatomical integrity of the hippocampus, and possibly occurring in a sex-specific manner.
External electrical stimulation activates voltage-gated potassium currents

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Neuroprosthetic devices aim to activate neurons in the most efficient and specific way. To do this, these devices take advantage of the intrinsic expression of voltage-gated ion channels that open in response to a change in the cell membrane potential. The most widely studied of these are the voltage-gated sodium channels (Nav), as their activation usually gives rise to action potentials. However, several other classes of voltage-gated channels exist that likely contribute to the probability of successful neuronal stimulation. We specifically analysed the response of voltage-gated potassium channels (Kv) in retinal neurons to external electrical stimulation with patch-clamp electrophysiology (voltage-clamp) and computational modeling. We found that due to the brief nature of external electrical stimulation the currents elicited were essentially “tail currents”. The cell is depolarized long enough to open these potassium channels but the membrane potential is then quickly returned to resting levels due to the very brief nature of external electrical stimulation. The current recorded reflects the activation/deactivation kinetics of the specific channel(s). In non-spiking retinal neurons the magnitude of these externally activated potassium currents varies greatly between cells, irrespective of Kv current magnitudes elicited by a classical step protocol. Computational modeling and pharmacological dissection of these responses implicates calcium-activated potassium channels (KCa) as a major contributor, with the remaining currents completely blocked by both TEA and 4-AP. Consequently, the likelihood of activating a neuron with electrical stimulation relies on the expression levels of these Kv channels, and likely plays a large role in the probability of action potential generation in spiking neurons.
Chronic methamphetamine self-administration dysregulates oxytocin plasma levels and oxytocin receptor fibre density in the nucleus accumbens core and subthalamic nucleus of the rat

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The psychostimulant methamphetamine (METH) is an addictive illicit drug. The neuropeptide oxytocin modulates METH reward and abuse. Recent findings have implicated the nucleus accumbens core (NAcc) and subthalamic nucleus (STh) as key substrates involved in oxytocin modulation of acute METH reward and relapse to METH use. Surprisingly, we previously showed a modest involvement of the oxytocin receptor (OTR) in both regions in oxytocin modulation of METH-primed reinstatement. Considering this, the aim of the current study was to investigate whether there are cellular changes to the oxytocin system in the NAcc and STh, as well as changes to oxytocin plasma levels following chronic METH intravenous self-administration (IVSA) and after extinction. Male Sprague Dawley rats (n=32) underwent surgery for jugular vein catheter implantation. After recovery, rats were trained to lever press for intravenous METH (0.1mg/kg/infusion) under a fixed-ratio 1 schedule or received yoked saline infusions during 2-hour sessions for 20 days. Rats then underwent behavioural extinction for 15 days. Following the last day of IVSA or extinction, blood plasma was collected for enzyme immunoassay and immunofluorescence was conducted on NAcc and STh coronal sections. Rats that self-administered METH had higher oxytocin plasma levels and decreased OTR-immunoreactive fibres in the NAcc than controls. After extinction, oxytocin plasma levels remained elevated, OTR-immunoreactive fibres increased in the STh, and a trend towards normalisation of OTR-immunoreactive fibres was evident in the NAcc in rats previously experienced at METH IVSA compared to controls. These findings demonstrate that the oxytocin system, both centrally within the NAcc and STh, and peripherally through plasma measures, is dysregulated following METH abuse.