GALWAY NEUROSCIENCE

Galway Neuroscience Centre

Research Day

10th December 2021

B001 Small Lecture Theatre, Human Biology Building

Time	
10:15	**Arrival, Registration and Covid Cert Check**
10:30	**Dr Derek Morris**: Welcome to GNC Research Day
10:35	**Meeting opening by Prof Jim Livesey, Vice-President for Research and Innovation**
Session 1 Chair: Dr Michelle Roche & Dr Ciara Egan	
10:45	**Dr Una Fitzgerald, NUI Galway** Lab greening & how neuroscientists can play their part!
11:20	*Rosanna Rossi* Investigating Acute Ischemic Stroke clots from COVID-19 patients: insights on thrombo-inflammatory process involved in COVID-19 pathology
11:25	*Rebecca Mahoney* Using Cellular Deconvolution to Investigate Cell Subtype Proportions in Cortical Gene Expression Data in Schizophrenia
11:30	*Rachel Kelly* The Small Molecule Alpha-Synuclein Aggregator, FN075, Enhances Alpha-Synuclein Pathology in Subclinical AAV Rat Models
11:35	*Michael Mackey* Systematic Review and Meta-analysis of Damage Associated Molecular Patterns HMGB1 and S100B in Schizophrenia.
11:40	*Q&A with short oral presenters*
11:50	**Prof Gianpiero Cavalleri, Royal College of Surgeons, Dublin** The role of common and rare genetic variation in the epilepsies
12:30	**Posters & Lunch**

Session 2 Chair: Dr Derek Morris & Dr Sinead King	
14:00	**Dr Gerard Clarke, University College Cork** Of bowels, brain and behavior: The Microbiota-gut-brain Axis in Psychiatry and Beyond
14:40	*Shane O'Connell* Magnetic Resonance Imaging-based Deep Learning Predictive Models of Brain Disorders: A Systematic Review of Modelling Practices, Transparency, and Interpretability
14:45	*Sinead King* Childhood trauma, inflammation and brain default mode network dysregulation in Schizophrenia. Recent findings from the Immune Response and Social Cognition (iRELATE) project
14:50	*Q&A with short oral presenters*
14:55	**Ass Prof Iracema Leroi, Trinity College Dublin** Dementia Trials Ireland (DTI)
15:15	**Karen Meenan, DTI PPI Lead, Trinity College Dublin** 'From Drama to Dementia' Patient & Public Involvement (PPI) in Dementia
15:30	**A conversation with Naomi about living with Lewy body dementia**
16:00	**Prize-giving and meeting closing**

Poster Presentations: Seminar Room G001, Human Biology Building

Odd-numbered posters present at 12:30-1:00pm

Even-numbered posters present at 1:00-1:30pm

Poster Number	Name	Abstract title
1	Laura Boullon	INVESTIGATION OF SEXUAL DIMORPHISM IN PAIN-RELATED BEHAVIOURS AFTER FATTY ACID AMIDE HYDROLASE AND MONOACYL GLYCEROL LIPASE INHIBITION IN AN ANIMAL MODEL OF PERIPHERAL NEUROPATHIC PAIN
2	Emma Corley	MICROGLIAL-EXPRESSED GENES, COGNITIVE FUNCTION AND BRAIN VOLUME IN PATIENTS WITH SCHIZOPHRENIA AND HEALTHY CONTROLS
3	Stephanie Bourke	ALTERATIONS IN PLASMA ENDOCANNABINOID LEVELS IN PATIENTS WITH NEUROPATHIC PAIN
4	Mary Hopkins	EVIDENCE FOR A SEXUALLY-DIMORPHIC RELATIONSHIP BETWEEN PAIN, NEGATIVE AFFECT AND CIRCULATING ENDOCANNABINOIDS/N-ACYLETHANOLAMINES IN PATIENTS WITH CHRONIC LOW BACK PAIN.
5	Mehnaz Ferdousi	CHARACTERISATION OF PAIN- AND ANXIETY-RELATED BEHAVIOURS IN THE SODIUM MONOIODOACETATEMODEL OF OSTEOARTHRITIS IN MALE SPRAGUE-DAWLEY RATS
6	Giulia Comini	INVESTIGATING THE POTENTIAL OF BIOMATERIALS FOR STEM CELLS-DERIVED BRAIN REPAIR FOR PARKINSON'S DISEASE: POLYHEDRIN-BASED PODS® MICROCARRIERS.
7	Kaushik Narasimhan	ASSESSMENT OF THE BIOCOMPATIBILITY AND EFFICACY OF POLYHEDRIN-BASED MICROCARRIERS (PODS®) FOR DELIVERY OF BDNF TO THE RAT BRAIN IN THE CONTEXT OF IPSC-DERIVED BRAIN REPAIR IN PARKINSON'S DISEASE.
8	Xi Luo	DEVELOPMENT OF METABOLIC BIOMARKERS FOR PARKINSON'S DISEASE DIAGNOSIS AND PROGRESSION BASED ON CLINICAL DATA AND COMPUTATIONAL MODELLING.
9	Aodan Laighneach	SOCIAL ISOLATION INDUCES TRANSCRIPTOMIC CHANGES IN FEMALE MOUSE HIPPOCAMPUS
10	Brendan Harhen	DETERMINATION OF CANNABINOID-LIKE PHARMACOLOGICAL ACTIVITY OF LEELAMINE IN RATS AND DISCOVERY OF NOVEL LEELAMINE METABOLITES
11	Duaa Jabrah	INVESTIGATING THE EXPRESSION OF DIFFERENT WHITE BLOOD CELL SUBTYPES IN ISCHEMIC STROKE THROMBI AND CORRELATING IT TO THE ETIOLOGY
12	Shane Crinion	IS CHRONOTYPE A RISK FACTOR FOR NEUROPSYCHIATRIC DISORDERS? A TWO-SAMPLE, MULTIVARIABLE MENDELIAN RANDOMISATION STUDY
13	Ciara Shortiss	PROTEOGLYCAN SYNTHESIS FOR SPINAL CORD INJURY REGENERATION
14	Daniela Costa	CHARACTERISATION OF NEURONAL SUBPOPULATIONS AND ASSESSMENT OF AN EXCITOTOXIC VULNERABILITY IN RAT CORTICAL CELL CULTURES

Speaker Bios

Dr Una Fitzgerald, NUI Galway
Lab greening & how neuroscientists can play their part!

After completing a BE (Ind Eng) and MSc in Biotechnology at NUIG, Dr FitzGerald worked in the pharmaceutical sector for five years in France and the UK, before embarking on a PhD in Molecular Biology at the University of Strathclyde, Glasgow. Following a brief spell in cancer research, on joining Prof. Sue Barnett's lab in Glasgow Uni. she discovered her true passion – neuroscience and in particular, research on brain disorders including multiple sclerosis and Parkinson's disease. Since returning to NUIG, Una built a track record in MS research; she is a funded investigator in CÚRAM, the SFI Centre for Research on Medical Devices and is ex-director of the Galway Neuroscience Centre. Supported by funding from the EU and SFI, her current research focus is on developing better models of progressive phase of MS and on precision medicine for people with MS. In 2019 she led the CÚRAM lab to be the first in Europe to gain Green Lab Certification from 'My Green Lab' and she now chairs a national working group on sustainable public sector labs. Dr FitzGerald's talk will explain how all members of the GNC can contribute to national and international efforts at mitigating against our climate and ecological emergency.

Prof Gianpiero Cavalleri, Royal College of Surgeons, Dublin
The role of common and rare genetic variation in the epilepsies

Gianpiero Cavalleri is Professor of Human Genetics at RCSI and Deputy Director of the SFI FutureNeuro Research Centre. His research group works at the interface of computational biology, clinical research and human evolution. His key scientific discoveries include the identification of one of the strongest signatures of natural selection detected in the human genome to date, characterizing genetic predictors of cutaneous adverse reactions to anti-epileptic drugs and describing fine scale population structure across Ireland, and within the indigenous Irish Traveller community

Dr Gerard Clarke, University College Cork
Of bowels, brain and behavior: The Micobiota-gut-brain Axis in Psychiatry and Beyond

Dr Gerard Clarke is a lecturer in the Department of Psychiatry and Neurobehavioural Science. Gerard graduated with a B.Sc and M.Sc fromNUI Galway and Ph.D from University College Cork (UCC). Gerard's research focus takes a translational approach to the assessment of neurpharmacological indices of stress-related disorders such as depression and irritable bowel syndrome (IBS). His has a particular interest on the impact of the gut microbiome on brain and behaviour across the life span and microbial regulation of tryptophan metabolism. He has received numerous awards and accolades for his research has been included in the Clarivate Most Highly Cited Researchers list for the past 3 years.

Dr Iracema Leroi, Trinity College Dublin
Dementia Trials Ireland (DTI)

Iracema is an academic geriatric psychiatrist with a special interest in pragmatic interventions for the cognitive and neuropsychiatric aspects of neurodegenerative disorders, particularly Parkinson's disease and Alzheimer disease. Iracema is Chief Investigator for the SENSE-Cog programme which aims to understand the links among hearing, vision and cognitive impairment in older people in five European countries. Iracema leads the 'Mind and Memory' clinic, which aims to support people with cognitive and behavioral complications in Parkinson's-related conditions, including Lewy body dementia. Iracema and her team at School of Medicine and the Global Brain Health Institute (GBHI), Trinity College Dublin, were recently awarded a Health Research Board (HRB)'s Clinical Research Network Award. The funding will allow Iracema and her team to launch Dementia Trials Ireland (DTI).

Karen Meenan, Trinity College Dublin
From Drama to Dementia' Patient & Public Involvement (PPI) in Dementia

Karen Meenan is a Senior Atlantic Fellow for Equity in Brain Health at the Global Brain Health Institute (GBHI) based in Trinity College Dublin and is director and co-creator of Lewy Body Ireland. She is the founder of Making Hay Reminiscence Theatre and founder volunteer with the Forget-Me-Nots dementia-inclusive choir. She is also a volunteer researcher, broadcaster and producer of award-winning dementia-inclusive radio programmes at NearFM in Ireland and LLARC in the UK. She has recently been appointed to the role of Patient and Public Involvement and Communications/Dissemination Coordinator with HRB Dementia Clinical Trials Network Ireland 'Dementia Trials Ireland' (DTI).

A conversation with Naomi about living with Lewy body dementia

Naomi Gleeson (top left) will join us to speak about her experience of living with Lewy Body Dementia (LBD). Naomi was only diagnosed with LBD this year, in summer 2021 and she has become an 'expert patient' learning as much as she can about the disease online and by forming connections with people who are based in the USA.

First, we will kick off with a short <10 min recorded conversation between Naomi, Karen Meenan (top right) and Heckle (bottom) from Heckle and Jeckle. Heckle and Jeckle are two Mental Health professionals diagnosed with Early Onset Dementia. They are funny, serious and smart as they explore all facets of daily life living with this baffling and challenging disease. Heckle & Jeckle podcasts have become hits on Spotify: see more here: https://backtracks.fm/discover/s/talking-dementia-with-heckle-jeckle/2128dc7c2cb8c942. Here, the group will discuss the importance of finding people who understand the disease outside of your own family group or caregiving support group.

We then very much welcome Naomi who will be joining us to speak with us in person on the day, to discuss her journey and experience with her diagnosis of Lewy Body Dementia.

Oral Abstracts

Investigating Acute Ischemic Stroke clots from COVID-19 patients: insights on thrombo-inflammatory process involved in COVID-19 pathology

<u>Rosanna Rossi</u>[1,2], Oana Madalina Mereuta[1,2,10], Sara Molina Gil[1,2], Andrew Douglas[1,2], Duaa Jabrah[1], Abhay Pandit[2], Ray McCarthy[3], Michael Gilvarry[3], István Szikora[4], Georgios Tsivgoulis[5], Klearchos Psychogios[6], John Thornton[7], Alexandros Rentzos[8], Turgut Tatlisumak[9], Waleed Brijinkji[10], Karen M. Doyle[1, 2]

Background: COVID-19 is a major health concern and can be devastating, especially for the elderly. Although much is known about the mortality of the clinical disease, much less is known about its pathobiology. Blood clotting is a significant cause of death in COVID-19 patients, as recognized early in the pandemic, with patients experiencing blood clots in both deep veins and arteries and leading sometimes to acute ischemic stroke (AIS).

Aim: We aimed to characterize acute ischemic stroke (AIS) blood clots from COVID-19 patients and compare them with matched control AIS clots from the RESTORE Registry. We studied similarities and differences in clot composition between the two groups to provide insights into the thrombo-inflammation process in COVID-19.

Methods: Six mechanically extracted clots from four COVID-19 patients were analysed and compared to matched mechanically extracted AIS clots from 8 non COVID-19 patients from the RESTORE registry. Martius Scarlet Blue (MSB) staining was used to visualise the main histological clot components (red blood cell (RBC), white blood cells (WBC), fibrin (FIB), platelets/other (PTL) and collagen (Coll)). Immunohistochemistry (IHC) was used to stain platelets (CD42b), von Willebrand factor (vWf), fibrinogen and specific WBC markers, i.e. T-lymphocytes (CD3) and neutrophils (CD66b). Additionally, citrullinated histone 3 (CitH3), a marker for Neutrophil Extracellular Traps (NETs) and other inflammatory markers such as C5b-9 complement complex and C-reactive protein (CRP) were analysed by IHC. Quantification of scanned images (Olympus VS120) was carried out using Orbit Image Analysis software. Data was expressed as median [IQ1-IQ3]. The non-parametric Kruskal-Wallis test was used for statistical analysis.

Results: MSB staining revealed significantly more WBC in COVID-19 clots compared to matched controls (P=0.009*), while no difference was found in terms of RBC(P=0.191), FIB (P=0.228), PTL (P=0.546) and Coll (P=0.070). COVID-19 clots were also significantly richer in CD42b (P=0.012*), vWf (P=0.002*), C5b-9 (P=0.021*) and CRP (P=0.0005*). We did not find any significant difference in the expression of CD3 (P=0.108), CD66b (P=0.269), fibrinogen (P=0.315) and CitH3 (P=0.920). However, we observed a trend suggesting CD3 and fibrinogen expression may be higher in COVID-19 cases compared to control.

Conclusions: AIS clots from COVID-19 patients show characteristics that may provide insight into thrombo-inflammatory processes involved in COVID-19 pathology. We observed significantly raised levels of several biomarkers involved in inflammation and the coagulation process in COVID-19 cases compared to matched controls. However, analysis of more cases is needed moving forward.

Using Cellular Deconvolution to Investigate Cell Subtype Proportions in Cortical Gene Expression Data in Schizophrenia

<u>Rebecca Mahoney</u>[1,2] Cathal Seoighe[2] Derek Morris[1]

Background: Schizophrenia is a psychiatric disorder that affects 1% of adults and is a major global health problem in that only 13.5% of affected individuals achieve full recovery criteria. Altered gene expression in the brain has previously been associated with neuropsychiatric disorders but this research has primarily focused on bulk tissue. Further analysis of data from bulk tissue to isolate and study predicted cell types based on gene expression profiles could uncover further insights into the altered expression of genes and pathways that contribute to schizophrenia etiology.

Aim: This research aimed to integrate new single-cell data to improve the cell type deconvolution of the PsychENCODE bulk expression data. It also aimed to identify cell type proportions in the PsychENCODE data and compare to previous analyses.

Methods: Cell subtype deconvolution is used to estimate the proportion of cell subtypes present in bulk expression data. Here, we reanalyzed gene expression data from the PsychENCODE consortium (n= 558 schizophrenia cases and 1039 controls; cortical samples) by using new single cell sequencing data that allowed for an improved cell subtype deconvolution analysis.

Results: New single cell data for ~30,000 cells was added to the original dataset to give an increased sample of ~62,000 cells. In comparison to the original analysis, we observed alterations in astrocyte proportions between cases and controls, particularly for two distinct astrocyte populations, only one of which had significantly different proportions between cases and controls ($p=<2.2e-16$) with a lower proportion of cells inferred in cases. In addition, neurons, microglial cells and endothelial cells had significantly different proportions between cases and controls ($p<0.05$). Oligodendrocytes did not have significantly different proportions between cases and controls ($p=0.588$) as an overall subtype but four distinct subtypes of oligodendrocytes did appear to exhibit differences in proportions ($p<0.05$).

Conclusions: Overall, this analysis leveraged new single cell data to perform a more detailed cellular deconvolution analysis of gene expression data from PsychENCODE and identified a number of distinct cell subtypes that differ in proportion between schizophrenia cases and controls. These can be followed up to uncover new insights into the biological functions that influence schizophrenia.

The Small Molecule Alpha-Synuclein Aggregator, FN075, Enhances Alpha-Synuclein Pathology in Subclinical AAV Rat Models

Rachel Kelly [1], Andrew G. Cairns [2], Jörgen Ådén [2], Fredrik Almqvist [2], Alexis-Pierre Bemelmans [3], Emmanuel Brouillet [3], Tommy Patton [1], Declan P. McKernan [1] and Eilís Dowd [1]

Background: Parkinson's disease (PD) models which overexpress human alpha-synuclein in the rat brain are considered some of the most valid currently available. However, they are limited by their slowly developing pathology and high degree of variability. We have recently developed a novel small molecule (FN075) that is capable of promoting α-synuclein oligomerisation and progressive fibril formation[1] and inducing pathology after intra-nigral injection in mice[2] and rats[3].

Aim: Therefore, we aimed to determine if combining viral-mediated overexpression of α-synuclein with FN075-mediated aggregation of α-synuclein resulted in a more rapidly developing and consistent model of PD.

Methods: 80 Sprague-Dawley rats were given unilateral intra-nigral injections of AAV-GFP or AAV-α-synuclein, of either the wild-type or A53T mutant variety. Four weeks later, the rats were given unilateral intra-nigral injections of FN075 or vehicle. 16 weeks later, animals were sacrificed and immunohistochemical analyses were used to assess α-synuclein expression and aggregation as well as nigrostriatal degeneration.

Results: At post mortem, widespread expression of α-synuclein was observed throughout the ventral midbrain and the striatum on the ipsilesional side in groups which received either wild-type AAV-α-synuclein or A53T AAV-α-synuclein infusions. The α-synuclein aggregating molecule FN075 combined with the AAV-α-synuclein vectors caused a significant increase in the expression of the pathogenic phosphorylated form of α-synuclein, with visible anomalous accumulations. However, there was no significant degeneration of the nigrostriatal dopamine neurons in any group.

Conclusions: A viral vector that independently induces neuronal death is required to investigate the true potential of this combined model. Nevertheless, the presence of the large intracellular accumulations of phosphorylated α-synuclein which were formed by the combination of AAV-α-synuclein and FN075 is a promising result, and this potential model decidedly warrants further investigation.

Systematic Review and Meta-analysis of Damage Associated Molecular Patterns HMGB1 and S100B in Schizophrenia.

Michael Mackey[1], Gary Donohoe[2], Laurena Holleran[2] Declan McKernan[1]

Background: Immune system dysregulation is hypothesised to be central to the aetiopathogenisis of schizophrenia, however the role of sterile inflammation remains unclear. Damage associated molecular patterns (DAMPs) are key initiators of sterile inflammation that are detectable in peripheral blood and possess the potential to serve as clinically relevant biomarkers in schizophrenia.

Aim: The aim of this study was to integrate literature reporting quantitative data of DAMPs (HMGB1 and S100B), in adults diagnosed with schizophrenia and healthy controls to quantify inter-group differences.

Methods: A defined systematic search of the Web of Science, PubMed and Scopus was performed to identify adult case-control studies published between 01Jan1990 and 01Nov2021. Three studies consisting of 242 cases and 83 controls met inclusion for the systematic review and meta-analysis of HMGB1. The systematic review and meta-analysis of S100B included twenty-eight studies consisting of 1544 cases and 1248 controls. The standardised mean difference of peripheral blood S100B and HMGB1 concentrations between adults diagnosed with schizophrenia and healthy controls served as the main outcome parameter, with Hedges g effect size and 95% confidence intervals reported. The potential presence of publication bias was measured using the Fail-Safe N calculation.

Results: A significant standardised mean difference in peripheral S100B (g=0.87[0.57, 1.16] P<.001) and HMGB1 (g=0.92[0.52, 1.32] P<.001) concentrations was detected between cases and controls. S100B subgroup analysis determined the largest significant effect size for unmedicated individuals diagnosed with schizophrenia (g=1.08[.68, 1.48] P<.001), but a smaller significant mean difference for medicated individuals diagnosed with schizophrenia (g=0.73[.01, 1.46] P=0.05) when compared with healthy controls.

Conclusions: This study has provided evidence that peripheral S100B and HMGB1 concentrations are elevated in individuals diagnosed with schizophrenia when compared with healthy controls. Novel findings indicate that antipsychotic medication may influence peripheral S100B concentrations. These results should be interpreted with caution as significant heterogeneity was present during whole and subgroup meta-analysis of S100B, while a smaller pooled sample size with moderate heterogeneity was detected during HMGB1 meta-analysis. The persistence of significant heterogeneity throughout subgroup analysis indicates that the current diagnostic groupings may be a barrier to understanding human thoughts, behaviours and emotions.

Magnetic Resonance Imaging-based Deep Learning Predictive Models of Brain Disorders: A Systematic Review of Modelling Practices, Transparency, and Interpretability

Shane O'Connell[1], Dara M Cannon[2], Pilib Ó Broin[1]

Background: The recent rise in the use of deep learning approaches on neuroimaging data has led to the reporting of impressive predictive performances as well as indications of potential clinical utility. Deep learning models however, have a number of characteristics that make their effective implementation difficult. These can include 1) a variety of network architecture choices and stochastic initialisation of network weights leading to poorly defined convergence and sub-optimal solutions, 2) a large number of hyperparameters that require significant computational resources for tuning and repeat experiments for robust performance evaluation, 3) interpretability issues, which make it difficult to understand what information is being used by the model, and thus limiting its potential clinical utility. Additionally, studies implementing these models can suffer from poor data separation practices during model training leading to information leakage and issues around reproducibility due to a lack of sufficiently detailed methodologies and code availability. In our work, we systematically review the: 1) modelling practices, 2) degree of transparency, and 3) interpretability of 55 studies that apply deep learning approaches to neuroimaging data, specifically brain MRI studies

Aim: We sought to evaluate the state of modelling practices, transparency, and interpretability across studies applying deep learning models to structural MRI data for prediction tasks. We aimed to relate the findings to existing best practices, discuss the implications of the results in the context of clinical integration and reproducibility, and make practical recommendations to researchers carrying out experiments in this field.

Methods: We conducted a systematic review of 55 papers arising from a search of the Web of Science and Pubmed databases. We evaluated their modelling practices, transparency, and reproducibility according to a standardised questionnaire applied to each study.

Results: Our results show a lack of repeat experiments (23/55), code sharing (49/55), interpretability (36/55), and potential information leakage (28/55) across the selected articles. These findings suggest study reproducibility may be hampered by non-adherence to the outlined principles; furthermore, the clinical utility of models from studies not observing these principles may be limited.

Conclusions: We conducted a systematic literature review of 55 studies carrying out CNN-based predictive modelling of brain disorders using structural brain imaging data and found issues with modelling practices, transparency, and interpretability. Careful consideration of these principles will inform a patient care framework that can effectively incorporate deep learning into diagnostic and prognostic systems, which could further our physiological understanding of these disorders and lead to improved patient care.

Childhood trauma, inflammation and brain default mode network dysregulation in Schizophrenia. Recent findings from the Immune Response and Social Cognition (iRELATE) project

Sinead King[1*], David Mothersill[3,1], Laurena Holleran[1], Saahithh Patlola[1], Ross McManus[5], Marcus Kenyon[5], Colm McDonald[6], Brian Hallahan[1,6], Aiden Corvin[6], Derek W. Morris[1,4], John Kelly[2], Declan McKernan[2], Gary Donohoe[1**].

Background: Childhood trauma (CT) is associated with cognitive and social cognitive dysfunction in schizophrenia. The neurobiological mechanism by which this occurs is unclear, but recent studies conducted in our lab and others suggest that the relationship between CT and cognition is mediated by both excessive inflammatory pathways and dysconnectivity of the default mode network (DMN) *during resting state*.

Aims This study sought to test whether higher CT was associated with any observed DMN connectivity changes *during task based activity*. In turn, we sought to investigate whether IL-6 mediated the association between a past history of CT and DMN dysconnectivity *during task based activity*. Our hypothesis is that CT would predict increased DMN alterations during facial emotion recognition, and that this relationship would be mediated by an increased inflammatory response.

Methods: 53 individuals with schizophrenia (SZ) or schizoaffective disorder (SZA) and 176 healthy participants were recruited from the Immune Response and Social Cognition (iRELATE) project. Pro-inflammatory cytokine interleukin 6 (IL-6) was measured in plasma using ELISA. CT was collected using the Childhood trauma questionnaire (CTQ). DMN connectivity was measured during an fMRI social cognitive task. fMRI Functional connectivity was measured based on DMN connectivity using the toolbox software CONN, during a social cognitive face processing task.

Results: Patients showed significantly increased connectivity between the left lateral parietal cortex (LLP) and the cerebellum and between the left LP and left angular gyrus compared to healthy participants ($p < .0125$). Across the entire sample, IL-6 predicted increased functional connectivity between the LLP and cerebellum ($r = .205$, $p = .002$), LLP and precuneus, and between the mPFC and bilateral precentral gyri and left postcentral gyrus ($P_{FWE} < .05$ and $P_{FWE} < .05$ height threshold). In turn, IL-6 mediated the relationship between physical neglect and connectivity between the LLP and cerebellum ($\beta_{INDIRECT} = .0179$, CI: .0007 to .0422). Again, after accounting for age, BMI and IQ, both physical neglect ($F_{(1, 208)} = 5.912$, $P < .05$) and emotional neglect ($F_{1,208} = 3.920$, $p < .05$) scores also significantly predicted the positive association between IL-6 and LLP-precuneus connectivity.

Discussion: This is the first study to our knowledge that provides evidence that higher plasma IL-6 mediates the association between higher childhood neglect and increased DMN connectivity during task based activity. Consistent with our hypothesis, exposure to trauma is associated with weaker suppression of the DMN during a face processing task, and this association was mediated via increased inflammatory response. The findings may represent part of the biological mechanism by which CT and cognitive performance are related. More specifically, the findings in this study suggest that low-grade systematic inflammation (i.e., elevated IL-6) may bridge the temporal gap between childhood neglect and failure to deactivate the DMN in adulthood. It is therefore likely that IL-6 has been elevated for years after such trauma in childhood, a theory supported by a series of longitudinal studies on prolonged proinflammatory cytokine elevation post trauma.

POSTER ABSTRACTS:

Poster #1:

Investigation of sexual dimorphism in pain-related behaviours after Fatty Acid Amide Hydrolase and Monoacyl Glycerol Lipase inhibition in an animal model of peripheral neuropathic pain

Laura Boullon[1,2,3], Sarah Crudden[1], David P. Finn[1,2,3], Alvaro Llorente-Berzal[1,2,3]

Introduction and Aims: The endogenous cannabinoid system (ECS) has been proposed to play a key role in the pathophysiology of neuropathic pain, and associated comorbidities [1]. Activation of cannabinoid receptors on presynaptic nerve terminals inhibits neurotransmission, resulting in antinociception [2], and cannabinoid-induced antinociception exhibits sexual dimorphism [3]. The aim of the present experiment was to investigate the effects of pharmacological inhibition of the anandamide-catabolizing enzyme Fatty acid amide hydrolase (FAAH) and the 2-arachidonylglycerol (2-AG)-catabolizing enzyme Monoacylglycerol lipase (MGL) on pain-, anxiety- and depression-related behaviours in male and female rats following peripheral nerve injury.

Methods: Sham or Spared Nerve Injury (SNI) surgery was performed in male and female Sprague-Dawley rats. At 7 days post-injury, the FAAH inhibitor URB597 (0.3mg/kg; i.p.) and the MGL inhibitor MJN110 (1mg/kg; i.p.) were administrated systemically, once daily, for 15 consecutive days. 2h post-drug injection, von Frey and acetone drop tests were used to investigate mechanical and cold allodynia, respectively, on post-surgery days (PSD) 7, 12, 17 and 22. The Open Field and the Elevated Plus Maze tests examined the effect of chronic URB597/MJN110 administration on locomotor activity and anxiety-related behaviours on PSD 14. In addition, depression-related behaviours were investigated with the Sucrose Preference test at PSD 19 and 20. Pain-related behaviours were analysed using Kruskal-Wallis followed by Mann-Whitney U tests with Bonferroni-Holm corrections. The remaining behavioural tests were analysed using 3-way ANOVA.

Results: As previously reported by our group [4], SNI induced mechanical and cold allodynia in male and female animals, with females showing earlier onset and greater sensitivity to mechanical and cold stimulation. URB597 induced a significant increase in the mechanical paw withdrawal threshold (PWT) in female-SNI animals over the full duration of the experiment (PSD7: $H(11)=43.504$, $p<0.001$). URB597 also induced robust reduction of acetone-induced nociceptive responses in Female-SNI rats compared to their vehicle counterparts (Latency to the first response on PSD12: $H(11)=49.493$, $p=0.006$. Frequency of responses PSD17: $H(11)=41.194$, $p=0.005$). This antinociceptive effect was only observed in females. MJN110 had no significant effect on SNI-induced mechanical or cold allodynia in animals of either sex at any of the time points studied. However, trends were observed for MJN110-induced pro-nociceptive effects from PSD12, with a significant reduction in the PWT in male-Sham rats on PSD22 ($H(11)=48.88$, $p=0.004$), compared to vehicle-treated counterparts. No significant differences in behaviours in the Open Field, Elevated Plus Maze and Sucrose Preference tests were observed between any of the groups investigated.

Conclusions: Our results provide evidence for sexual dimorphism in the antinociceptive response to chronic administration of a FAAH inhibitor following peripheral nerve injury, with females, but not males, reduced nociceptive behaviour. Trends for pro-nociceptive trends following chronic administration of the MGL inhibitor in males but not females further support sex-dependent endocannabinoid modulation of nociceptive behaviours following peripheral nerve injury. There were no effects of the FAAH and MGL inhibitors on anxiety- and depression-related behaviours in rats of either sex.

Poster #2:

Microglial-Expressed Genes, Cognitive Function and Brain Volume in Patients with Schizophrenia and Healthy Controls

Emma Corley[1,2], Laurena Holleran[1,2], Laura Fahey[2,3,], Aiden Corvin[4], Derek W. Morris[2,3], Gary Donohoe[1,2]

Background: Schizophrenia (SZ) is a complex heritable neuropsychiatric disorder, in which level of disability is strongly predicted by impairments in cognitive function. This variation in cognition has previously been associated with immune-relevant genetic loci, including genes of the complement system. In addition, microglia have also been associated with SZ pathophysiology and cognition; findings which have supported the growing evidence of immune involvement in variation in cognitive functioning and brain structure in patients with SZ.

Aim: Given that microglia are the primary innate immune cells in the brain, with known roles in synaptic functioning, we sought to investigate the effects of a SZ microglial-based polygenic score (PGS) on SZ risk, cognitive performance, and brain structure in healthy participants and in patients with SZ.

Methods: The microglial-based PGS (*n*= 294 genes) was generated using recent GWAS summary data for SZ. We examined whether this PGS explained variation in cognition in an Irish sample of SZ patients and controls (*n* = 1,234) and tested whether grey matter (GM) volume mediated this association. The performance of the microglial PGS was then compared to that of neuronal *(n*=375) and astroglial (*n* = 286) PGSs, and MAGMA was used to test for enrichment of these gene-sets with SZ risk. Subsequently, results were examined in a large independent sample of UK Biobank participants (*n* =134,827). Throughout, analyses were corrected for age, sex and intracranial volume.

Results: The microglial SZ-PGS was significantly associated with lower performance across several measures of cognitive functioning (R^2 range= 0.8-1.8) in both samples; associations which were then found to be mediated via total GM volume in the UK Biobank (β = -0.004). In a post-hoc analysis, the association between the microglial SZ-PGS and episodic memory remained significant in cases (β=-0.096, *p*= 0.028). No significant enrichment of association was observed between the microglial genes and SZ risk (β=-0.0087, *p*= 0.860), unlike neuronal genes which did show evidence of enrichment. Further, the difference in magnitude between the microglial, neuronal and astroglial SZ-PGSs across the cognitive tests was not statistically significant.

Conclusions: Results from our study provide evidence that increased numbers of SZ risk variants in microglial genes, as captured by our SZ-PGS, are associated with a decrease in cognitive performance in both patients and healthy controls. We further highlight that variation in GM volume mediates the association between the microglial SZ-PGS and cognition, supporting evidence of immune processes being associated with variation in brain structure. We also highlight both the non-illness specificity of the findings and the absence of enrichment for SZ risk genes in microglia, and interpret this to reflect the relevance of microglial-expressed genetic variation is for neurodevelopmental processes related more generally to cognition.

Poster #3:

Alterations in plasma endocannabinoid levels in patients with neuropathic pain

<u>Stephanie Bourke</u>[1,2], Barira Islam[3,4], Maurizio Manca[3,4], *David P. Finn[1,2] and *Patrick C. McHugh[3,4]

Background: Chronic pain is a debilitating condition that remains a major unmet clinical need and is associated with high social and economic costs. Chronic pain with neuropathic characteristics is estimated to affect 7-10% of the population and has a higher prevalence in women than in men. The endogenous cannabinoid (endocannabinoid) system plays a key role in pain modulation.

Aim: The aim of the present study was to investigate whether chronic neuropathic pain is associated with alterations in circulating levels of endocannabinoids.

Methods: Patients with NP (n= 96) and healthy control (n=91) participants were recruited via pain clinics in two centres – Seacroft Hospital, Leeds and Galway University Hospital. Pain scores were measured using Leeds Assessment of Neuropathic Symptoms and Signs (S-LANSS) and Graded Chronic Pain Scale (GCPS). Blood samples were taken and plasma endocannabinoids, 2-arachidonoylglycerol (2-AG) and anandamide (AEA), and *N*-acylethanolamines, oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), were quantified by HPLC-tandem mass spectrometry. Data were analysed by Mann-Whitney U tests, or Spearman's correlation analysis ($p<0.05$ considered significant).

Results: Plasma 2-AG levels were higher in patients with NP compared to controls ($p<0.0001$). AEA, PEA and OEA did not differ between controls and patients with NP and were not correlated with S-LANSS. PHQ-9 ($p<0.0001$), state anxiety ($p<0.0001$) and trait anxiety ($p<0.005$) scores were all significantly higher in patients with NP compared to controls. GCPS and S-LANSS scores were positively correlated to PHQ-9 ($p<0.0001$), state anxiety ($p<0.0001$ and $p<0.05$, respectively) and trait anxiety ($p<0.05$) scores.

Conclusions: The findings of this study were consistent with the literature in so far as neuropathic pain was accompanied with higher anxiety and depression scores compared to healthy controls. The increased 2-AG levels found in patients with NP could indicate the development of a compensatory mechanism to modulate pain.

Poster #4:

EVIDENCE FOR A SEXUALLY-DIMORPHIC RELATIONSHIP BETWEEN PAIN, NEGATIVE AFFECT AND CIRCULATING ENDOCANNABINOIDS/N-ACYLETHANOLAMINES IN PATIENTS WITH CHRONIC LOW BACK PAIN.

<u>Mary Hopkins</u>[1,2,3], Stephanie Bourke [1,2,3], *Patrick C McHugh[4], *David Finn[1,2,3]

Background: Chronic low back pain (CLBP) is a major unmet clinical need with significant socioeconomic impact, and a large contributor to disability[1,2]. Research has revealed significant genetic alterations in the endocannabinoid system in patients with acute and chronic LBP vs healthy individuals[3,4].

Aim: Our aim was to investigate the relationship between circulating endocannabinoids, pain, somatosensory measures, comorbidities, and lifestyle factors in CLBP patients vs healthy controls (HCs).

Methods: 135 CLBP patients and 88 HCs were recruited and consented to approved procedures. Blood samples and QST scores were taken, while BMI, smoking and alcohol use were recorded. Pain Detect (PD), Pain Catastrophising Scale (PCS) and S-LANSS were administered to the patient cohort, while Beck Depression Inventory-II (BDI-II), Patient Health Questionnaire-9 (PHQ-9), Fatigue Severity Scale (FSS), Visual Analogue Fatigue Scale (VAFS) scores were taken for all participants. Plasma endocannabinoids (2-AG, anandamide [AEA]) and N-acylethanolamines (OEA, PEA) were quantified using LC-MS/MS.

Results: CLBP patients had higher BMI scores than HCs. Levels of 2-AG were higher in CLBP patients. BMI scores were positively correlated with circulating 2-AG levels in both cohorts and both sexes. However, AEA levels were positively correlated with BMI scores only in CLBP patients. BDI-II, PHQ-9, FSS and VAFS scores were significantly higher in CLBP patients than HCs. AEA and OEA levels in female CLBP patients, but not males, were positively correlated with higher PCS scores. Additionally, circulating PEA levels were negatively correlated with PD scores in male CLBP patients only.

Conclusions: Correlations reveal a sexually-dimorphic relationship between pain, negative affect and circulating endocannabinoids/N-acylethanolamines in CLBP.

Poster #5:

Characterisation of pain- and anxiety-related behaviours in the sodium monoiodoacetate model of osteoarthritis in male Sprague-Dawley rats

Mehnaz Ferdousi[1,3], Reena Irvine[1], Barry McDermott[2], Alison Liddy, David Finn[1]

Background: Osteoarthritis (OA), a degenerative joint disorder, is one of the most prevalent causes of chronic pain. In addition to pain, anxiety disorders are often comorbid in chronic pain associated with OA, further worsening the quality of life of the patient. One of the most widely used animal models of OA involves intra-articular (i.a.) injection of sodium monoiodoacetate (MIA) into the joint capsule that results in cartilage degeneration, thus modelling the pathological changes observed in the joints of patients with OA. However, the dose of MIA to induce OA in rats varies in the literature (0.5-4.8 mg).

Aim: To characterise the pain- and anxiety-related behaviours induced by MIA (1 and 2 mg) at the behavioural level in rats.

Methods: Male Sprague-Dawley rats (7-8 weeks old, n=5-6/group) were used in this study. Following an i.a. injection of MIA or saline into the left knee joint under anaesthesia, rats were assessed for static weight bearing asymmetry, mechanical and cold hypersensitivities, and anxiety-like behaviours over a 35-day period.

Results: MIA injection at both doses (1 and 2 mg) resulted in weight bearing asymmetry and mechanical and cold hypersensitivities. MIA 2 mg induced greater and more stable weight bearing asymmetry and mechanical hypersensitivity than MIA 1 mg throughout the study period. No MIA-induced anxiety-like behaviours were observed on Day 31 post-injection.

Conclusions: Overall, MIA 2 mg is an optimal dose to produce consistent chronic pain-related behavioural changes in a rat model. However, we did not find any evidence of MIA-induced anxiety-related behaviours in male rats at the time point tested.

Poster #6:

Investigating the potential of biomaterials for stem cells-derived brain repair for Parkinson's disease: polyhedrin-based PODS® microcarriers.

Giulia Comini, Kaushik Narasimhan, Rachel Kelly, Sarah Jarrin, Eilis Dowd.

Background: Although iPSC-derived brain repair for Parkinson's disease has already reached clinical trial, preclinical studies have shown that most of the implanted progenitors are not reaching dopaminergic maturation because of the lack of appropriate neurotrophin support.

Aim: Therefore, the aim of this study was to investigate the potential of a commercially-available polyhedrin-based microcarrier system (PODS®) for delivery and retention of the dopaminergic neurotrophin, GDNF, in the rat brain.

Methods: 36 male Sprague Dawley rats received bilateral implantation of PODS® loaded with human GDNF either alone or in a collagen hydrogel. They were sacrificed either by terminal anaesthesia followed by transcardial perfusion-fixation at Days 1, 4 & 7 post-implantation for immunohistochemical analyses (6 rats per timepoint) or by transient isofluorane anaesthesia and rapid decapitation at Days 4, 7 & 14 post-implantation for ELISA analyses (6 rats per timepoint). The biocompatibility of the PODS® was assessed by quantitative immunohistochemical staining for microgliosis and astrocytosis at the implant site, while efficacy was assessed by quantitative immunohistochemistry and ELISA for GDNF release and retention.

Results: The polyhedrin microcarriers were detectable in the rat brain at all timepoints and were clearly identifiable by their crystalline structure. The immunohistochemical analyses revealed that the PODS® induced a neuroinflammatory response in the brain that was not affected by implantation within the collagen hydrogel. Human GDNF was not detected by immunohistochemistry at any of the timepoints but ELISA analyses revealed the successful delivery and retention of the neurotrophin in the rat brain for up to 14 days.

Conclusions: This study demonstrates the potential of the commercially-available polyhedrin-based microcarrier system (PODS®) for delivery and retention of the dopaminergic neurotrophin, GDNF, in the rat brain. Ongoing studies are focussing on the potential of the PODS® to improve the *in situ* maturation of iPSC-derived dopaminergic progenitors in the rat brain.

Poster #7:

Assessment of the biocompatibility and efficacy of polyhedrin-based microcarriers (PODS®) for delivery of BDNF to the rat brain in the context of iPSC-derived brain repair in Parkinson's disease.

Kaushik Narasimhan, Giulia Comini, Sarah Jarrin, Rachel Kelly, Eilis Dowd

Background: In iPSC-derived dopaminergic progenitor cell transplantation studies, lack of, or rapid degradation of, neurotrophins contribute to poor survival of the cells post-grafting. Longer retention of neurotrophins like BDNF are essential for better survival and maturation of the grafted cells.

Aim: Therefore the aim of this study was to assess the biocompatibility and efficacy of commercially available polyhedron-based microcarriers (PODS®), alone or encapsulated within a collagen hydrogel, for delivery of BDNF to the rat brain.

Methods: 18 male Sprague Dawley rats received bilateral implantation of PODS® loaded with human BDNF either alone or in a collagen hydrogel. They were sacrificed by terminal anaesthesia followed by transcardial perfusion-fixation at Days 1, 4, 7 post-implantation (6 rats per timepoint). The biocompatibility of the PODS® was assessed by quantitative immunohistochemical staining for microgliosis and astrocytosis at the implant site, while efficacy was assessed by quantitative immunohistochemistry for BDNF release and retention.

Results: PODS® crystals were clearly visible in the brain at all timepoints. Staining for microglia and astrocytes revealed that the PODS® induced a transient neuroinflammatory response in the brain that was not affected by implantation within the collagen hydrogel. Human BDNF was not detected by immunohistochemistry at any of the time points – possibly because the PODS® did not release it or the levels were below the limits of detection for this methodology.

Conclusions: Although the polyhedron-based microcarriers (PODS®) are biocompatible with the rat brain, their ability to deliver and retain BDNF in the rat brain remains to be determined. Ongoing studies are assessing PODS®-mediated BDNF delivery to the rat brain using ELISA.

Poster #8:

Development of metabolic biomarkers for Parkinson's Disease diagnosis and progression based on clinical data and computational modelling.

<u>Xi Luo</u>[1], Ronan M.T. Fleming[1]

Background: Parkinson's disease (PD) is a progressive, multi-focal neurodegenerative disorder, the number of patients with PD increased as a result of the increase of old people. Since PD is a complex disease with multiple molecular mechanisms, the possible pathogenesis of PD is still not elucidated. Although a range of biomarkers derived from clinical, neuroimaging, genetic studies have been proposed, cumulating evidence indicates metabolism contributes to disease development and progression. Therefore, the metabolite biomarkers of may play an important role in the diagnosis and progression of PD patients. It's necessary to effectively integrate metabolomic biomarkers with constraint-based computational modeling to enable context-specific biochemical interpretation of metabolomic data and generate mechanistic hypotheses as to the metabolic pathways that are dysfunctional.

Aim: To reveal consistent and inconsistent metabolomic changes of diagnosis and progression across PD patients.

Methods: The latest Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines, were used to search for clinical studies of PD until March 2021 from two public databases PubMed and Web of Science. The QUADOMICS tool was used to assess the risk of bias and the research quality of each diagnostic-related study. The name of metabolites was converted into the corresponding Virtual Metabolic Human (VMH) IDs to provide a link between separated metabolites with the Human metabolism model (Recon3D) and Dopaminergic Neuronal(DN) model.

Results: A total of 62 studies were eligible for our systematic review, 61 of which contained diagnostic-related metabolites and 15 involved PD progression. QUADOMICS assessment of most studies revealed their high quality. Among 61 studies involving diagnosis, a total of 840 metabolites had changed abundance in PD versus controls, 323 of which with VMH ids. Total 479 increased and 460 decreased metabolites, 99 overlapped metabolites have inconsistently changed trends in different studies. For cross-match with Recon3D model, 313 metabolites were matched. However, only a small subset of metabolites with high frequency, of which 93 increased and 56 decreased in more than one study, 20 of which with inconsistent changes in different studies. For cross-match with DN model, 123 metabolites can be matched. A total of 50 increased and 37 decreased metabolites appeared in more than one study, 17 overlapped metabolites with inconsistent changes. The rest of 200 metabolites with VMH id can't be found in the DN model. Among 15 studies involving progression, a total of 183 changed metabolites were identified, only 74 of them with VMH ids. There are no more than ten metabolites with high frequency that cross-match with both two models.

Conclusions: Metabolite levels are affected by diet, environment, genetic background, activity level, and different human biofluids. Considering that, the duplicate metabolites that appeared in more than one study are the most important biomarkers for diagnosis, which will be further used for exploring the metabolic pathways that are dysfunctional of PD. More primary research studies are needed for the metabolomics of PD progression.

Poster #9:

Social Isolation Induces Transcriptomic Changes in Female Mouse Hippocampus

Aodán Laighneach[1], Lieve Desbonnet[2], Laurena Holleran[1], Declan McKernan[2], John Kelly[2], Gary Donohoe[1], Derek W. Morris[1]

Background: Early life stress is among the known environmental risk factors for neurodevelopmental disorders such as schizophrenia. Social isolation (SI) is used to model early life stress in animal models by depriving animals of crucial social interactions at vulnerable periods of neurodevelopment. Using next generation sequencing (NGS) techniques such as RNA-seq, the effect of SI on brain gene expression can be investigated.

Aim: Measure gene expression changes caused by social isolation in hippocampus and investigate the biological relevance in relation to human disorder and cognitive phenotypes.

Methods: Using RNA-seq, we investigated brain gene expression changes in the SI model. Paired-end sequencing was performed on RNA extracted from the hippocampus of group-housed (n=4) and post-weaning socially-isolated (n=5) adult female C57BL/6J mice. Differential expression analysis was performed on reads and gene ontology (GO) analysis was performed on sets of differentially-expressed genes (DEGs) with absolute fold change > 1.2 meeting thresholds of FDR < 0.05 and FDR < 0.01.

Results: Differential expression analysis revealed 21 DEGs between SI and group-housed animals. Seven DEGs had an adjusted p-value < 0.01: Inppl1, Tbc1d24, Gm20517, Dusp11, Cmtr1, Vmn1r90 and Ccdc120. The full DEG set showed enrichments in cell projection, plasma membrane projection and nucleotidyltransferase activity. DEGs meeting FDR < 0.01 showed enrichments in axon/distal axon structure, nuclear structure, plasma membrane structure and phosphatase activity. MAGMA geneset analysis reveals enrichments in genes contributing to cognitive phenotypes in full DEG set and anxiety phenotype in FDR < 0.01 set.

Conclusions: Using RNA-seq, we have gained insight into some of the underlying gene expression changes in the brain as a result of SI. Results suggest that animal SI leads to molecular changes involving connectivity through axon structure. Enrichments in DEGs for genes contributing to human phenotype of cognition anxiety agrees with behavioural data in the same animals, which supports molecular validity of SI model of anxiety.

Poster #10:

DETERMINATION OF CANNABINOID-LIKE PHARMACOLOGICAL ACTIVITY OF LEELAMINE IN RATS AND DISCOVERY OF NOVEL LEELAMINE METABOLITES

Brendan Harhen [1,3,4], Alvaro Llorente-Berzal[1,3,4], Michelle Roche, [2,3,4] Howard Fearnhead[1,] and David P Finn.[1,3,4]

Background: Leelamine is a diterpenoid which exhibits cannabinoid-like pharmacological activity and yields one major oxidized metabolite in mice.

Aim: Determination of cannabinoid-like activity and leelamine metabolism in the rat was the focus of the present study.

Methods: Adult male Sprague-Dawley rats received a single acute i.p. injection of leelamine (25mg/kg) or vehicle (n=7 per group) and behaviour in the tetrad test for cannabinoid activity was assessed. The animals were euthanised at 30, 60 or 120 mins post-injection, and tissues including liver and various brain regions were harvested. Tissue homogenates were analysed with liquid chromatography mass spectrometry

Results: Leelamine treatment induced hypothermia, hypomobility and antinociception, but not catalepsy, compared to vehicle-treated groups. Unique signals occurred in the livers of leelamine-treated rats at m/z 286 (unmetabolized leelamine), 302 (monooxygenated leelamine+16) and 318 (dioxygenated leelamine+32). Tandem mass spectra of the oxygenated metabolites were similar to that for leelamine, although the fragment daughter ion m/z series increased by 16 and 32 respectively. A desaturated signal at m/z 284 was observed and, as above, daughter ion m/z series decreased by 2 m/z. Liver metabolites were detected at high intensity relative to the leelamine signal for the 30, 60- and 120-minute cohorts indicating a rapid metabolic rate for leelamine. The desaturation and oxygenation pathways bifurcate. Finally, the above metabolites were also present in brain tissue, though at lower levels.

Conclusions: Leelamine exhibits partial cannabinoid-like activity in rats and two separate metabolic pathways exist for leelamine transformation *in vivo*.

Poster #11:

INVESTIGATING THE EXPRESSION OF DIFFERENT WHITE BLOOD CELL SUBTYPES IN ISCHEMIC STROKE THROMBI AND CORRELATING IT TO THE ETIOLOGY

Duaa Jabrah[1,2], S. Fitzgerald[1,2], A. Douglas[1,2], R. Rossi[1,2], O.M. Mereuta[1,2] and K.M. Doyle[1,2].

Background: Endovascular mechanical thrombectomy has made studying inflammatory cells in ischemic stroke clots possible. Neutrophils are known to influence heart diseases, and macrophages have been found to play an important role in plaque instability that might lead to carotid clot formation. We hypothesized that the expression of sub populations of white blood cells may vary with stroke etiology.

Aim: To quantify neutrophil, lymphocyte and macrophage expression in acute ischemic stroke clots and correlate their expression with stroke etiology.

Methods: Clots from both Cardioembolic (CE) (n=73) and Large Artery Atherosclerosis (LAA) (n=92) origin were collected from three different hospitals in Europe. 3µm thick sections were immunohistochemically stained to investigate the three major white blood cell populations using a Leica Bond III autostainer. The primary antibodies used were CD66b antibody to detect neutrophils (rabbit polyclonal, 1:100, ab197678, Abcam), CD3 to detect lymphocytes (rabbit polyclonal, 1:100, ab5690, Abcam) and CD68 to detect macrophages (mouse monoclonal [KP1], 1:50, ab955, Abcam). The staining was visualized using the Bond Polymer Refine Detection Kit. Slides were scanned using the Olympus VS120 Digital Slide Scanner and quantification of the stained cells was performed on Orbit Image Analysis Software. Data was not normal, and it was analysed by Mann-Whitney U test. Results were expressed as median ± interquartile range (IQR).

Results: Results are reported in the following table

Stroke Etiology	% Expression of Neutrophils (Median ± IQR)	% Expression of Lymphocytes (Median ± IQR)	% Expression of Macrophages (Median ± IQR)
CE Clots (n=72)	5.5 ± 6.6	0.1 ± 0.17	0.15 ± 0.3
LAA Clots (n=91)	4.8 ± 4.5	0.1 ± 0.18	0.2 ± 0.3
Statistical Analysis	P=0.4535	P=0.1878	P=0.6065
Mann-Whitney U	3129	2956	3201

Conclusions: No significant differences in neutrophils, lymphocytes or macrophages were found between CE and LAA clots, but we can see trends of higher neutrophils in CE clots and higher macrophages in LAA clots. Our ongoing study will analyse larger numbers to reach sufficient power for firm conclusions.

Poster #12:

Is chronotype a risk factor for neuropsychiatric disorders? A two-sample, multivariable Mendelian randomisation study

Shane Crinion[1], Lorna M. Lopez[2], Derek Morris[1]

Background: Disruption of circadian rhythm is a common feature in many neuropsychiatric disorders including autism and schizophrenia. Chronotype, an individual's synchronisation to the 24 hour day, is commonly used as a proxy for circadian rhythm disruption. Being a morning person, someone who prefers waking and going to bed earlier, is genetically correlated with increased well-being and decreased risk of neuropsychiatric disorders.

Aim: The aim of this study is to test for causal effects between chronotype and neuropsychiatric disorders using MR analysis.

Methods: We performed a two-sample Mendelian randomisation (MR) study to determine the effect of chronotype on risk of six neuropsychiatric disorders. We used 351 independent genome-wide significance loci ($p < 5e-18$) from a genome-wide association study (GWAS) of chronotype in 697,828 European individuals as genetic instruments.

Results: Summary-level GWAS data was obtained for the six neuropsychiatric disorders from the largest available studies to date. Results from inverse-variance weighted MR indicated a causal relationship between the morning chronotype and lower risk of autism spectrum disorder (OR = 0.88, 95% CI 0.81, 0.94, IVW p = 0.0004), major depressive disorder (OR = 0.95, 95% CI 0.9, 0.99, IVW p = 0.03) and schizophrenia (OR = 0.9, 95% CI 0.83, 0.96, IVW p = 0.002). Sensitivity tests found no evidence for the presence of horizontal pleiotropy. Further research will be performed to correct for bias due to weak instruments and sample overlap.

Conclusions: This study gives further evidence for the role of circadian rhythm disruption in neuropsychiatric disorders. Establishing its causal role can demonstrate the potential for circadian misalignment as a new modifiable risk factor that could be targeted for treatment of neuropsychiatric disorders.

Poster #13:

Modulating Proteoglycan synthesis for Spinal Cord Injury regeneration

Ciara Shortiss[1,2], Siobhan S. McMahon [2] and Linda Howard [1]

Background: Spinal cord injury (SCI) is one of the most life changing injuries as it can leave a person completely paralysed below the injury level. Following injury in the spinal cord a glial scar forms. This scar poses a physical and chemical barrier to neuronal growth preventing regeneration after SCI The glial scar contains a variety of O-linked proteoglycans that are secreted by astrocytes and oligodendrocytes. These are established in the scar 7 days post injury yet undergo some turnover [1, 2]. One group of O-linked proteoglycans are Chondroitin sulphate proteoglycans (CSPGs). CSPGS are the subject of intense study in SCI as they are known to inhibit axon regeneration [3, 4]. Other O-linked proteoglycans are also produced after SCI, namely Heparan sulphate proteoglycans (HSPGs) and Dermatan Sulphate proteoglycans (DSPGs). These have been found to have varying effects on neuronal growth. Proteoglycans consist of a core protein with polysaccharide glycosaminoglycan side chains. These side chains are thought to be responsible for most of their effects on neuronal growth. Previously proteoglycans have been manipulated individually or their side chains have been altered to elicit positive effects on neuronal growth after injury with some success. However these strategies lack the ability to provide long term widespread reduction of inhibitory proteoglycan stimulus [5]. In my Ph.D. we propose to disrupt a common point in biosynthesis of several proteoglycans using novel lentiviral gene therapy vectors that will provide long term control over proteoglycan production in the hopes of aiding neuronal outgrowth after SCI. The enzymes xylosyltransferase I and II (XT-I, XT-II) catalyse the initial and rate limiting step in all O-linked proteoglycans synthesis. XT-I level reduction by a specific deoxyribozyme enhanced SCI repair providing proof of concept [5]. We are developing lentiviral vectors to deliver short hairpin RNA (shRNA) targeting XT-I and XT-II specific mRNA to reduce these enzymes and in turn reduce all O-linked proteoglycan production. HSPGs have been shown to be neuronal growth promotive [6, 7] and will also be reduced with this strategy. Therefore a second lentiviral shRNA vector in development in our lab targets the enzyme Chondroitin Sulphate N-acetylgalactoasminyl-transferase-1 (Csgal1), that is common to CSPG and DSPG synthesis only.

Aim: Validate lentiviral-shRNA knockdown of XT isoforms and Csgal1 at an RNA level and assess any changes in CSPG expression due to lentiviral-shRNA knockdown of XT1, XT2 and Csgal1. Observe changes in XT1, XT2 and Csgal1 after chemical and physical injury of spinal cord mixed glial culture cells as an *in vitro* model of spinal cord injury inflammation.

Methods: Briefly three targeting shRNAs plasmids against each target mRNA (XT1, XT2 or Csgal1) were bought commercially. One non-targeting control shRNA was also bought for each vector. Lentiviral vectors to deliver these shRNA to cells were made by co-transfecting HEK293T cells with shRNA plasmids and lentiviral packaging plasmids psPax, pMD2.G and pRSV-REV. Viral particles were secreted into the media following successful transfection with all four plasmids. Neu7 (astrocytic) or B104 (neuroblastoma) cell lines were transduced with lentiviral-shRNA vectors by plating with media containing unconcentrated viral particles. After three days cells underwent antibiotic selection for 7-10 days until all control untransduced cells had died. Total RNA was extracted from transduced cells and relative XT1, XT2 and Csgal1 mRNA levels were assessed by qPCR. Cells were stained with an antibody against CS56 to assess levels of CSPG expression. Semi-quantitative fluorescence intensity measurements of images were carried out using ImageJ and corrected total cell fluorescence (CTCF) formula; CTCF = Raw Integrated Density of image – (Area of image X Mean 'Mean grey value' of background readings). CTCF was divided by number of nuclei/image that contributed to fluorescence. Mixed Glial culture cells were obtained from P3/4 Sprague Dawley rat pup spinal cords. These were plated on PLL coated flasks and allowed to mature for 14 days. Three days after the first passage cells were inflamed with LPS (100ng) and/or a physical scratch in the cell monolayer. Cells were left after inflammation/scratch for 24hrs and 7days (media changed 72hrs after first inflamed). RNA was extracted and qPCRs were done to assess XT1, XT2 and Csgal1 mRNA levels. Cells were stained for GFAP, CD11B, CS56 and Oligo2. To confirm inflammation and glial scar formation relative levels GFAP and CD11b and CS56 respectively were assessed by obtaining the CTCF per nuclei of this staining using ImageJ (as above).

Results: Knockdown of XT1, XT2 and Csgal1 using Lentiviral-shRNA vectors has been achieved individually in either Neu7 or B104 cells on an RNA level. Cell lines with highest level of transcript knockdown were selected to be stained with CS56 to assess effect of this knockdown on CSPG levels. Initial results from MGC inflammation experiment indicate that XT1 and XT2 expression are increased when cells are inflamed via LPS and/or scratch. Highest levels of XT1 and XT2 were seen at 24hrs in MGC cells that were inflamed with LPS and Scratch. Analysis of relative immunostaining intensity to assess inflammation is ongoing.

Conclusions: mRNA knockdown of XT1, XT2 and Csgal1 has been achieved in cell lines. The effect of this knockdown on CSPGs and other proteoglycan groups remains to be determined. Expression of XT1 and XT2 at an RNA level is upregulated after inflammation of MGC cells taken from a spinal cord. This gives weight to the hypothesis that they are involved in the inflammatory response after spinal cord injury. Pending results from immunostaining of these MGC cells may confirm that the increase in XT1 and XT2 is seen in conjunction with an increase of CSPGs (CS56 staining).

Poster #14:

Characterisation of neuronal subpopulations and assessment of an excitotoxic vulnerability in rat cortical cell cultures

Daniela AD Costa[1], Jill McMahon[1], Tanja Kuhlmann[2], Una FitzGerald[1]

Background: Cortical pathology is defined as one major contributor to the severity of Multiple Sclerosis (MS), with noticeable decline of cognitive function of the patients. This entails pathological changes occurring within projection neurons (PN) and interneurons (IN) in an outer-to-inner crescent-shaped gradient of pathology. Evidence points towards the heightened vulnerability of cortical neuron subsets from cortical layers II/III. Half of demyelinated type 3 lesions occur in the supragranular layers of the cortex, accompanied by the loss of specific excitatory PNs and inhibitory INs (e.g., Cux^{2+} PNs, PV^+ and SST^+ INs). Intriguingly, specific loss of INs within layer II reaches 40% and 25% in layer III. We *hypothesize* that neuronal and synaptic damage may be due, in part, to excitotoxicity, as reported in acute MS lesions.

Aim: The aim is to establish an *in vitro* model of Progressive MS-like excitotoxicity, with particular focus on layer II/III and layer V cortical neurons as a screening platform for novel combinatory therapies targeting compromised cortical neuron populations.

Methods: Constituent cortical cell populations were characterised using 3- and 10-day post-natal rat cortical mixed cultures that were formalin-fixed and stained for 15 layer-specific markers using immunocytochemistry. Based on the literature and manufacturers' recommendations, three different antibody concentrations were tested. In parallel, high concentrations of glutamate (above 20 mM) were used to induce excitotoxicity on day *in vitro* (DIV) 13 cultures for 24 hours.

Results: The viability of rat mixed cortical cultures exposed to concentrations equal to or higher than 250 mM of glutamate was significantly reduced by more than 80%. From a panel of 15 commercially available antibodies tested, 7 had specifically bound the target protein; three antibodies labelled TLE4-, Cux1- and BRN2-expressing projection neurons, while four labelled SST-, PV-, CB- and CR-positive interneurons.

Conclusions: Preliminary conditions for mimicking excitotoxicity conditions were identified. Higher concentrations of glutamate produce toxicity to the cultures and that was measurable using bright-field imaging and alamarBlue assay. Immunocytochemistry validated the heterogenous phenotype of the mixed cortical cultures, including neuronal cells. Its quantification is underway. Further investigation is needed to define cell population-specific toxicity and synaptic pathologies. Further validation on rat and human tissue sections will bring some clarity regarding the temporal and spatial expression of supraganular cortical markers.