# Abstracts for the 253rd Otago Medical School Research Society Summer Student Sepeaker Awards, Wednesday 22 April 2020

## The developmental effects of disrupting DONSON – a gene involved in genetic microcephaly.

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The clinical features of many genetic disorders are often caused by complex mechanisms that are poorly understood. Recently, mutations in DONSON, a DNA replication protein, have been found to cause microcephaly (reduced brain size) in children. The developmental processes that cause this reduction in size remain unknown. This project aimed to study the effects of disrupting Donson using the CRISPR Cas9 editing tool in the developing brain of Xenopus laevis (African clawed frog).

To study how disruption of Donson affects the developing brain, Xenopus embryos were microinjected with Donson sgRNA at 400 or 800 pg/embryo plus Cas9 protein, with control embryos uninjected or injected with only Cas9 protein. PCR of the cleavage site and T7 endonuclease was used to confirm editing status in DNA from injected tadpoles. Four tadpole heads from each condition were fixed and sectioned into 4 μm sections, which underwent haematoxylin and eosin (H&E) staining to analyse general histology. Expression profiles of Sox2 and Tubb2b, markers of neuronal proliferation, were examined by immunohistochemistry and qPCR.

There was a 55% decrease in Sox2 and a 69% decrease in Tubb2b mRNA (markers of neural proliferation) in anterior tissues in the Donson 800 pg edited tadpoles relative to the controls. H&E staining alongside markers of neuronal proliferation, suggested a reduction in the area of the fourth ventricle and decreased thickness and organisation of the developing brain, specifically in the Donson 800 pg tadpoles, relative to the controls.

The morphological changes suggested alterations to proliferation, which would fit with the role of DONSON in DNA replication. This is further supported by a reduction in transcripts for neural proliferation. Further studies will provide further insight into the exact mechanism underlying such a reduction in proliferation, to understand the link between DONSON and microcephaly.

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## High definition transcranial infraslow pink noise stimulation for improving executive functioning in healthy older adults – A pilot safety trial

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Dementia, characterised by progressive deterioration of cognitive functions, is a significant and growing health challenge, with a huge social and economic impact. Current available treatments have limited efficacy, warranting new targeted interventions. Several studies demonstrate dysfunctional electrical activity in brain-wide functional networks, particularly involving the dorsal anterior cingulate cortex (dACC), in individuals with cognitive impairments. Non-invasive transcranial electrical stimulation, can normalize dysfunctional brain activity, thereby improve cognition. This pilot, double-blinded (participants and assessor) randomised sham-controlled parallel trial evaluated the safety and explored the immediate trend of effect of a novel, dACC targeted, high-definition transcranial infraslow pink noise stimulation technique (HD-tIPNS) on cognition in healthy older adults.

Ten cognitively healthy participants were randomised to receive either HD-tIPNS or Sham stimulation, for a single session of 30 minutes. Adverse effects were recorded throughout the intervention period. Cognitive tests [Stroop tests (ST), trail making test (TMT), and digit span recall test (DSRT)] and resting-state electroencephalography (EEG) were conducted at baseline and immediately post-intervention. Descriptive statistics were used to explore

the trend of effect of HD-tIPNS on cognitive outcomes. EEG data were analysed using sLORETA to explore changes in brain activity (BA) and functional connectivity (FC).

No adverse outcomes were reported. All ST variables improved post-treatment, irrespective of the group. TMT demonstrated a positive trend of effect in the HD-tIPNS group. No effect was observed in DSRT. A trend towards significant effects of HD-tIPNS was evident on the infraslow, alpha, and theta bands in dACC network ($P$ = 0.06), compared to sham ($P$ = 0.93). Increased and decreased FC of dACC with the dorsolateral prefrontal cortex and the posterior cingulate cortex respectively, was observed in HD-tIPNS group ($P$ < 0.001).

These results implicate the safety and ability of HD-tIPNS technique to modulate the activity and FC of dACC, suggesting that it has potential for use as a treatment tool.

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## Pilot study of ketamine in phobic participants using virtual reality stimuli.

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Treatment for specific phobia, an anxiety disorder characterized by unreasonable fears related to specific objects or situations, is limited to psychological interventions with no approved drug treatments. Recent research identified an incidental effect of low-dose injected ketamine on anxiety ratings for a specific phobia (blood/needle phobia). This study aimed to extend this finding by using oral ketamine (minimal side effects), a more common phobia (spider phobia), and a more controllable experimental paradigm (virtual reality (VR) exposure), in a cross-over active-controlled design.

Participants received one of the following medications (0.5 mg/kg or 1.5 mg/kg oral ketamine or 0.02mg/kg oral midazolam (psychoactive control)). One hour later, they experienced a 5-level VR spider simulation. At higher simulation levels, spiders numbers increased, as did their size and movement. We obtained vital signs measurements and a visual analogue scale (VAS) for anxiety (range 0–100) before and at each level of the VR spider simulation. Participants returned for all three sessions. The study is ongoing, with data presented for 11 participants.

Significantly more simulation levels were completed after 1.5 mg/kg ketamine compared with midazolam dosing (mean (SD) 1.0 (1.2), t = 2.6, $P$ = 0.03). A two-way repeated measures analysis of variance identified significant treatment x time interactions for VAS anxiety and respiration rate (RR) during the simulation (VAS anxiety F = 2.02, df = 8, $P$ = 0.05; RR F = 2.94, df=8, $P$ = 0.007). Heart rate changes were not statistically significant between dose groups.

Most of the assessments reported support our hypothesis that ketamine has an anti-phobic effect in patients with spider phobia, compared with midazolam. This is consistent with our earlier research and supports a theory that ketamine may work across a broad range of internalizing disorders via effects on a central neuroticism process. This study adds support to the use of ketamine as an acute treatment for specific phobia.

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## Factors related to the presence of toxicological investigation following fatal injury: a retrospective quantitative review of Coronial records in New Zealand.

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The National Coronial Information System (NCIS) is a repository containing investigative data of all deaths reported to a Coroner in Australasia. A Coronial investigation can include toxicological analysis at the discretion of the Coroner. Little is known both within New Zealand, and internationally, about potential biases related to toxicology report requests.

This study undertook a retrospective review of New Zealand injury deaths in 2014. Data was collected from the NCIS and the Mortality Collection. Variables of interest, such as circumstances of death and characteristics of decedents, were extracted from the Coronial files. Eligible injury-related deaths referred to the Coroner that had toxicological investigations were compared to those that did not.

Of the 744 unintentional injury Coronial cases in those under 85 years of age, 560 (75%) received toxicological analysis. Females were less likely to have a toxicology report (67%; males 78%; 95%CI for difference -5%, -18%), as were the young and the elderly (58% and 55% respectively compared to 88% for those aged 25–34). Māori were more likely to recieve toxicological analysis compared to NZ European (83% and 73% respectively; 95%CI for difference 3%, 17%). Only 44% of decedents that died as a result of a fall received a toxicology report. There was a clear relationship between receiving a toxicology report and the time between injury and death; 86% for those that died on the same day as their injury compared to 13% in those that this period was over a week. Opioids were detected in 23% of cases that received toxicological screening tests.

It is currently unknown whether the observed patterns are justifiable or whether they relate to subconscious biases. Follow-up qualitative research is required to understand this

further. Better understanding of Coronial processes helps inform policymakers, researchers, and practitioners, and contributes to injury prevention and improved health policies.

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## ABM300, a new negative allosteric modulator of the $CB_1$ cannabinoid receptor, exhibits a similar mechanism of action as ORG27569.

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The extensive role of the endocannabinoid system in homeostatic regulation and the fact that G protein-coupled receptors (GPCRs) are the most common drug targets makes the cannabinoid receptor 1 (CB1) a suitable subject for development of novel therapeutics. Recently, research on CB1-targeting drugs has shifted to allosteric modulators in the hope that they offer fewer adverse effects than orthosteric ligands. Allosteric binding sites are distinct from orthosteric sites and are less conserved in comparison, allowing allosteric ligands to exhibit a greater receptor selectivity. In contrast to promising in vitro studies, the existing allosteric modulators such as ORG27569 have not demonstrated substantial activity in vivo. ABM300, a novel allosteric modulator has been shown to alleviate abnormal behaviours in hyperdopaminergic mice models. We aimed to characterise ABM300 in vitro (in HEK293 cells), in comparison with ORG27569.

Pilot data (n = 3) suggested that ORG27569 and ABM300 exert similar effects on CB1 cAMP signalling, as the co-administration of either allosteric modulator with potent CB1 agonist CP55,940 leads to blockade of agonism, and subsequent inverse agonism. Radioligand binding competition assays performed demonstrated that ABM300 has an enhanced ability (pEC50 7.133 ± 0.201) compared to ORG27569 (pEC50 6.316 ± 0.101) in increasing the binding of CP55,940 to the CB1. We also found that ABM300 (10 μM) abolishes the effect of CP55,940 on phosphorylation of ERK1/2 (PerkinElmer AlphaLISA Surefire kit), resulting in pERK1/2 levels at 23% ± 6.8 of maximum CP55,940-induced ERK1/2 phosphorylation, similar to ORG27569. Furthermore, pilot data indicated that both ABM300 and ORG2769 lead to a concentration-dependent decrease in CP55,940-mediated β2-arrestin recruitment.

This study has made a start in understanding the ABM300 mechanism of action and found strong similarities to that of ORG27569; that is, that the allosteric modulator prevents the activity of the orthosteric agonists.

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## Thyroid cancer recurrence prediction using regression approaches.

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Prediction of thyroid cancer recurrence is important information for planning post-surgical follow-ups. Cancer stage is a well-known predictor of thyroid cancer and the American Thyroid Association offers a thyroid cancer staging calculator that helps predict the stage of thyroid cancer using preoperative clinical and demographical information. The current calculator, however, is missing information on serum thyroglobulin which is a critical predictor to evaluate the cancer stage of patients. The objective of this study was to develop a regression model that updates the calculator with serum thyroglobulin information.

In this study, we used both the logistic regression and the LASSO methods for model building. Records from 3962 thyroid patients were analysed for training models for recurrence prediction. A-priori twelve variables were investigated (age at operation, sex, number of carcinomas presented in the operation, size of the greatest tumour, histologic type of carcinoma, extrathyroidal extension status of tumours, pathologic staging of the primary tumour, presence of venous invasion of the primary tumour, immunohistochemistry for the primary tumour, presence of extranodal spread, number of lymph nodes and serum thyroglobulin level presented in the scans) as predictors of recurrence.

Both approaches demonstrated excellent performance. Logistic regression (with an AUC of 0.874) had a slightly better performance, whereas lasso regression (with an AUC of 0.856) utilized fewer variables to achieve a similar result. The LASSO method had identified five crucial variables for predicting structural recurrence of thyroid cancer. These include extrathyroidal extension status of tumors, the pathologic staging of the primary tumor, the presence of extranodal spread, the number of lymph nodes that are involved by carcinoma and the serum thyroglobulin level presented in the scans.

Recurrence of thyroid cancer can be predicted with reasonable accuracy with as little as five clinical variables. This information can be used to help plan post-surgical follow-up of patients. Future work will investigate whether these variables also predict survival.

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## Upregulation of GAD65 in somatosensory cortex of stargazer absence epileptic mice.

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Childhood absence epilepsy is one of the most common paediatric epilepsies. It is characterised by frequent non-convulsive seizures causing brief loss of consciousness. Absence seizures arise within the cortico-thalamo-cortical (CTC) network; however, the precise molecular mechanisms are not fully understood and are likely multifactorial. This may account for the variability in patients' responses to antiepileptic drugs (AEDs). Although AEDs greatly improve quality of life for most patients, one third cannot be effectively treated and there is no cure for epilepsy. Understanding underlying molecular causes is imperative. The stargazer mouse model of absence epilepsy has a mutation reducing excitatory input to feed-forward inhibitory (FFI) interneurons; FFI prevents runaway excitation in networks. The stargazer mutation specifically affects CTC parvalbumin containing GABA (γ-Aminobutyric acid) interneurons, causing FFI deficits and altered GABA levels. This study aimed to investigate whether changes in GABA levels are due to altered expression of its production enzymes, glutamate decarboxylases (GADs 65&67), and/or transport proteins, GABA transporters (GATs 1&3).

Confocal immunohistochemistry of sections double labelled for parvalbumin and GAD/GAT showed no differences in cortical expression pattern between epileptic and non-epileptic animals. However, semi-quantitative western blotting detected significantly increased GAD65 in somatosensory cortex of epileptic mice compared to non-epileptic littermates ($P$ = 0.0324, Mann-Whitney U test, N = 11 Epileptic/13 Non-Epileptic). No significant change in GAT3 or GAD67 levels was detected ($P$ > 0.05, Mann-Whitney U test, N = 9 Epileptic/14 Non-Epileptic or 10 Epileptic/14 Non-Epileptic respectively); although GAD67 trended towards elevation in epileptic animals above controls.

GAD65 upregulation may be a mechanism of compensating for reduced FFI, and account for the heightened somatosensory cortex GABA levels previously observed. This discovery provides the foundation for investigating the cell-type specificity of GAD65 elevation and interrogating its role in absence seizure genesis and maintenance over the developmental timespan. GAD65 may itself constitute a therapeutic target or may allow discovery of an underlying mechanism to therapeutically target.

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## Investigation of exhaled nitric oxide as a measure of left atrial pressure.

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During left-sided heart failure, left atrial pressure (LAP) increases, producing pulmonary congestion. Previous studies examining patients with symptomatic heart failure or rheumatic heart disease suggest a relationship between increased LAP and fractional exhaled nitric oxide (FeNO), which can be measured by a reliable portable hand-held analyzer. If such a relationship existed, a portable diagnostic test could be used to supplement current assessments.

This observational study examined a subset of the OxVALVE cohort, a UK population-based cohort aged 65 years and older. Participants were eligible for inclusion if they did not have a previous diagnosis of valvular heart disease, and could provide informed consent. Each participant had echocardiography performed at their local general practice. The E/e' ratio, measured via transthoracic echocardiography, was used as a surrogate of LAP. A consecutive subset of 277 participants had FeNO measurement attempted using a NIOX VERO electrochemical analyzer.

Mean age of participants was 73.5 ± 6.3 years, with 45% female. Only 10 participants had an NYHA class of III or more (New York Heart Association classification of heart failure patients). FeNO was successfully measured in 227 participants, with a mean FeNO of 24.2 ± 15.6 ppb. Mean E/e' was 11.5 ± 3.5. There were 58 participants with high E/e' (>14), and these were older than those with normal E/e' (<8) (76.8 ± 7.4 years vs 72.0 ± 5.5 years, $P$ < 0.001). No relationship between FeNO and E/e' was seen on regression analysis (linear regression $R^2$= 0.007).

These results illustrate that FeNO is not an accurate predictor of elevated E/e' in predominantly asymptomatic patients in a general practice setting.

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