Workshop Booklet

13–14 February 2020

ARMI, Monash University
Melbourne

OptogeneticsAustralia

MONASH University

ARMI AUSTRALIAN REGENERATIVE MEDICINE INSTITUTE

10 YEARS 2009–19

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PROGRAM

Thursday, February 13, 2020

13:30 Welcome and Acknowledgement of Country
Harald Janovjak (Monash University)

13:40 Keynote Lecture: “Using optogenetics to unravel complex brain functions”
Andrew Allen (University of Melbourne)

14:30 Break with coffee & tea

SESSION 1 - Chair: Sarah Lockie (Monash University)

14:50 Invited Talk: “Optogenetic tool development for neuroscience: Success, limitations and prospective”
John Lin (University of Tasmania)

15:30 Short Talk: “Activation of the medial-prefrontal cortex to lateral hypothalamic circuit reduces food intake and motivation to obtain sucrose reward”
Rachel Clarke (Monash University)

15:45 Short Talk: “A closed loop optogenetic brain stimulation device for major depressive disorder”
Lekshmy Sudha Kumari (Deakin University)

16:00 Short Talk: “Development of a novel optogenetic transgenic quail line for use in light-activated gene function”
Hila Barzilai-Tutsch (Monash University)

16:15 Break with coffee & tea

SESSION 2 - Chair: Elliot Gerrard (Monash University)

16:35 Invited Talk: “Novel engineering approaches for the development of optogenetic tools”
Alexandra Tichy (Monash University)

17:15 Short Talk: “Development of optogenetic approaches to inhibit neuronal GPCR signalling”
Jayde Lockyer (University of Tasmania)

17:30 Short Talk: “Characterisation of lattice light sheet microscopy based photo-manipulation of proteins”
Sanjeev Uthishtran (Monash University)

17:45 Group Photo

17:50 Dinner in the Foyer with Best Talk Award (*sponsored by TrendBio*)

19:00 End

Friday, February 14, 2020

9:10 Keynote Lecture: "Wireless optogenetic control of the vertebrate nervous system in conscious & freely-moving animals"
Nick Spencer (Flinders University)

10:00 Split-Up in Groups and Hands-on Workshop
Group 1: Module 1: Design of non-neuronal optogenetic tools
Group 2: Module 2: Optogenetic manipulation of cell signaling *ex vivo*
Group 3: Module 3: *In vivo* wireless optogenetics

12:00 Lunch in the Foyer

13:00 Hands-on Workshop
Group 1: Module 2: Optogenetic manipulation of cell signaling *ex vivo*
Group 2: Module 3: *In vivo* wireless optogenetics
Group 3: Module 1: Design of non-neuronal optogenetic tools

15:00 Break with coffee & tea

15:30 Hands-on Workshop
Group 1: Module 3: *In vivo* wireless optogenetics
Group 2: Module 1: Design of non-neuronal optogenetic tools
Group 3: Module 2: Optogenetic manipulation of cell signaling *ex vivo*

17:30 End

ABSTRACTS

Keynote Lecture: "Using optogenetics to unravel complex brain functions"

Andrew Allen
Department of Physiology, University of Melbourne

The use of optogenetics to provide remote control of neuronal activity enables dissection of the involvement of specific neurons in behaviours. Optogenetic activation provides an understanding of what a cell type is able to do, whilst optogenetic inhibition provides an understanding of what the cells are doing. Together these powerful approaches provide great insight. The presentation will discuss some of the pitfalls and challenges involved in using optogenetics *in vivo* and how these have been addressed within the context of 3 research snapshots that have used optogenetics to:

1. Demonstrate the first known function for the C3 group of adrenergic neurons
2. Examine the widespread effects of the brainstem neuronal oscillator characterised as the kernel responsible for generating breathing, using opsins targeted to different neuronal compartments
3. Develop strategies to selectively activate axons in peripheral nerves.

Invited Talk: "Optogenetic tool development for neuroscience: Success, limitations and prospective"

John Lin
School of Medicine, University of Tasmania

Optogenetic tools have been utilised to study the function of neurons and brain regions with tremendous success. In neuroscientific research, 2 main types of optogenetic tools have been the developed and utilised: microbial opsin variants to manipulate membrane excitability, and tools that are capable of directly manipulate biochemical signalling. Thus far, microbial opsin tools have been widely adapted and have been used successfully in many situations even with their small photocurrent amplitude, inconsistent level of desensitization and complicated spectral response. The limited understandings of photocycles and dynamic structures of the opsins limit the rational engineering of new variants but the exploration of natural variants in nature has been successful at generating improved variants. For the tools that manipulate biochemical pathways, both rational designs and exploration of natural proteins have been successful at generating useful tools, however, many of these still need improvement and better tweaking. In the next stage of optogenetic tools development and utilization for neuroscientific research, improved viral delivery, more precise genetic targeting, specific subcellular localization and better hardware instrumentation would likely be to have critical roles in answering novel biological questions, in addition to more vigorous characterisation and testing by the developers and the users to benchmark the different tools.

Short Talk: "Activation of the medial-prefrontal cortex to lateral hypothalamic circuit reduces food intake and motivation to obtain sucrose reward"

Rachel Clarke, Romana Stark and Zane B. Andrews
Department of Physiology, Monash Biomedicine Discovery Institute, Monash University

The medial pre-frontal cortex (mPFC) is understood to be a key brain region encoding for the reward value of a food. Human imaging studies demonstrate that mPFC activity is positively associated with BMI when choosing between images of healthy and unhealthy foods. The mPFC directly projects to the lateral hypothalamus – a brain region known to coordinate both feeding and reward seeking. Our aim in this study is to manipulate mPFC-LH projecting neurons in mice to determine the effect of this circuit on feeding and motivation to obtain food reward.

Using a dual viral approach, we ablated the mPFC-LH circuit in C57BL/6 mice by targeting a genetically engineered caspase to the mPFC projection neurons. Circuit ablation significantly increased body weight on a chow diet and also increased food intake on an acute palatable diet. We then activated the mPFC-LH circuit using hMd3q designer receptors exclusively activated by designer drugs (DREADDs) and observed decreased chow intake in response to clozapine-N-oxide and reduced motivation to obtain sucrose reward in an operant conditioning task. Next, we used wireless optogenetic stimulation and a retrogradely transported ChR2 to acutely activate excitatory mPFC-LH projection neurons. Activation of this circuit inhibited fasting induced feeding, reduced motivation for sucrose reward and was aversive, as assessed by real-time place preference. Our results highlight a previously unknown role for the mPFC-LH circuit in body weight, feeding and motivated behaviour. Our findings support recent human imaging data and suggest this circuitry has relevance to human feeding behaviour and eating disorders.

Short Talk: "A closed loop optogenetic brain stimulation device for major depressive disorder"

Lekshmy Sudha Kumari and Abbas Kouzani
School of Engineering, Deakin University

Over the years, major depressive disorder (MDD) has become increasingly common in people of all age groups. Rather than just feeling mood swings or general sadness, MDD adversely affects day-to-day life and could lead to serious life threatening conditions in those affected. The scientific world is still in search of a complete explanation for the reasons of its occurrence with multiple researchers suggesting alterations in alpha, theta, and gamma bands in the electrophysiological recordings to be possible biomarkers of MDD. Optogenetics, providing the advantage of single neuron manipulation capability even in large networks, is a competent brain stimulation technique in understanding the physiological variations in the brain leading to MDD. In this work, a tether-less head-mountable closed-loop optogenetics brain stimulation device capable of detecting key MDD biomarkers, and modulating the light stimulations based on the state of the detected biomarkers is reported. The device consists of: (i) an optrode (with a µLED, a temperature sensors, and recording pads) and a brain sensor to measure the MDD biomarkers, and an optogenetic stimulator to deliver stimulation pulses, and (ii) a feature extractor, a binary classifier, and a control algorithm. Benchtop and in-vitro test results are presented.

Short Talk: "Development of a novel optogenetic transgenic quail line for use in light-activated gene function"

Hila Barzilai-Tutsch[1], Valerie Morin[2], Claire Hirst[1], Olivier Serralbo[1], Harald Janovjak[1] and Christophe Marcelle[1,2]
[1]EMBL Australia, Australian Regenerative Medicine Institute (ARMI), Monash University
[2]Institut NeuroMyoGène (INMG), University Claude Bernard Lyon1, CNRS UMR 5310, INSERM U1217

Developmental biology studies seek to understand the complex events leading to the formation of an organism. Many fundamental discoveries of the mechanisms controlling tissue and organ generation have come from analysing their cellular origins. However, in the ever-changing environment of the developing embryo, cell-tracing has always posed a challenge. Several technologies have been developed to address the dynamics of morphogenesis in vertebrates. However, they all present important limitations, leaving several basic questions on the embryonic origin of some of the major tissues and organs still unknown, along with unexplored contributions of minor cell populations to various organs. Our research project aims to overcome these limitations by developing the optogenetic transgenic Japanese quail ("Opto-Quail"). The avian embryo model has been at the origin of numerous seminal discoveries in a diverse range of topics, due to its high accessibility to manipulations and high-end imaging. Combining these advantages with a novel, polyvalent, light-activated (optogenetics) tool, will allow non-invasive, tight spatiotemporal control of gene activation in any specific cell or tissue of the

developing embryo. This unique tool will be used here to investigate unknown aspects of the differentiation of the mesoderm, which is one of the three embryonic layers composing the early embryo.

Invited Talk: "Novel engineering approaches for the development of optogenetic tools"

Alexandra-Madelaine Tichy[1,2], Elliot J. Gerrard[1,2,3] and Harald Janovjak[1,2]
[1]Australian Regenerative Medicine Institute (ARMI), Monash University
[2]European Molecular Biology Laboratory Australia (EMBL Australia), Monash University
[3]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Synthetic Biology FSP

Optogenetics enables spatio-temporally precise control of cell and animal behavior. To achieve light-control of a target process, many options are available, and it is often necessary to engineer a protein of interest to respond to light. This talk will focus on two commonly used approaches and to repurpose naturally light-sensitive photoreceptors for the development of optogenetic tools.
In the first part, optogenetic tools driven by light-controlled protein-protein interactions will be discussed. Applying light-controlled PPIs to new target proteins is challenging because it is difficult to predict which of the many available photoreceptors, if any, will yield robust light regulation. Consequently, multiple fusion protein variants need to be prepared and tested, but methods and platforms to facilitate this process are currently not available. To address this issue, we developed a genetic engineering strategy and vector library for the rapid and simple generation of light-controlled PPIs. This work provides a new, publicly available and simple to use resource for optical regulation of a broad range of target proteins in cell and developmental biology.
The second part will focus on the engineering approaches of light-activated G-protein coupled receptors (OptoXRs). OptoXRs are chimeric multi-fusion proteins assembled from a target GPCR and light-sensitive photoreceptors. The availability of new high-resolution structures of GPCRs and a deeper understanding of GPCR function allows critically revisiting the current model of OptoXR design. This revisitation highlights the limitation of the current design, while providing multiple opportunities for improving the next generation of OptoXRs.

Short Talk: "Development of optogenetic approaches to inhibit neuronal GPCR signalling"

Jayde Lockyer, Owen Marshall, Lisa Foa and John Y. Lin
College of Health and Medicine, University of Tasmania

The G protein-coupled receptor (GPCR) sub-family comprises the largest class of cell-surface receptors in the human genome, including over 370 non-sensory GPCRs. More than 90% of these are expressed within the brain, contributing to a vast array of neuronal functions through the transduction of signals from neuromodulators. Until recently, neuronal GPCRs have been manipulated by either pharmacological or chemogenetic means. However, these methods have limited spatiotemporal precision and are often irreversible. This becomes problematic for experiments requiring precise, controlled modulation of rapid intracellular events. Consequently, there is a need for a mechanism through which to modulate GPCRs that allows for the precise and reversible control of intracellular signalling events, both *in vitro* and *in vivo*. To address this issue, we have developed a new suite of optogenetic tools for the targeted inhibition of GPCR signalling cascades in the brain. This technique offers unprecedented spatiotemporal resolution; genetic targeting for cell-type-specific expression; and non-invasive manipulation of cultured cells and tissues, as well as awake, behaving animals. To achieve this, modified regulatory proteins were fused to photosensory domains, allowing for the controlled localization and inhibition of target GPCRs following blue light exposure. We have shown that the use of these tools allows for the precise manipulation of specific GPCR signalling cascades. This was demonstrated in cultured cells using live-cell imaging techniques to visualise the inhibition of defined downstream signalling pathways. The tools were further

confirmed through the modification of GPCR-dependent behaviors in the nematode, C. elegans. Finally, we validated the tools in the brains of awake, behaving fruit flies, achieving targeted manipulation of GPCR-associated learning processes.

Short Talk: "Characterisation of lattice light sheet microscopy based photo-manipulation of proteins"

Sanjeev Uthishtran, Finian Leyden, Ullhas Kaarthi Moorthi, Harrison York, Abhishek Patil and Senthil Arumugam
Monash Biomedicine Discovery Institute, Monash University

Optogenetic constructs can be utilized to study cellular processes at μm scale within the cell in a highly controlled manner. With the advent of Lattice Light Sheet, rapid volumetric imaging can be performed to study processes at whole-cell level. Here, I describe a combination of Lattice Light-Sheet Microscopy with Optogenetic excitation and describe different excitation strategies and their relevance for optogenetically excitable constructs. Out strategies involve both epi-like illumination as well as spatio-temporal precise control using the spatial light modulator. To effectively characterize the activity of optogenetically activated proteins and to find a balance between effective photoactivation and minimal photobleaching, variables such as laser power, spatial excitation, exposure time per frame and per volume were moderated. A variety of photo-manipulable proteins were looked at, such as the photo-converting protein Dendra2, isolated from octocoral Dendronoepthya, which converts from green-to-red upon activation or the co-clustering protein Cry2 which clusters upon photoactivation. Finally, I will describe PA Rac1 that was found to require continuous excitation, using which we studied the dorsal ruffling of the membrane.

Keynote Lecture: "Wireless optogenetic control of the vertebrate nervous system in conscious & freely-moving animals"

Nick Spencer
College of Medicine & Public Health, Flinders University

Significant technical advances have recently been made in the past few years in the field of optogenetics. The first part of this presentation will focus on technical advances that have been made in our laboratory regarding the use of optogenetics to control the neural function in the autonomic nervous system in vertebrates. This talk will involve studies on the intrinsic nervous system in the wall of GI-tract, known as the enteric nervous system (ENS). We investigated whether optogenetic techniques could be applied to the ENS to selectively express light-sensitive ion channels in specific neurochemical classes of enteric neurons. Since, there are limited effective therapies available for improving gastrointestinal (GI) transit in mammals, optogenetic approaches may prove useful for improving transit. This could be a superior approach compared to current therapeutics that require oral ingestion of drugs that usually activate receptors distributed over multiple organs, often leading to a range of unwanted side effects. There is a desperate need in the community to improve GI-transit selectively without effects on other organs. This study will demonstrate that optogenetic techniques can be used to potently control the activity of subsets of enteric neurons both *in vitro* and *in vivo*. Indeed, recently we developed wireless micro-light emitting diodes with appropriate dimensions to be suitably attached to the gut wall *in vivo*. This presentation will demonstrate that wireless optogenetics can now be used to selectively stimulate neurons in the wall of the colon of conscious and freely-moving, untethered mice - leading to a significant increase in transit of colonic content. The ability to selectively stimulate the ENS to modulate GI-transit in live mammals using light, avoids the need for oral consumption of non-specific agonists to stimulate the ENS. In the final part of this presentation, we will discuss some recent technical advances in our laboratory that facilitate the selective expression of opsins in single dorsal root ganglia of live mice, facilitating direct illumination of DRG by wireless custom-made micro-LEDs. This approach allows us, for the first time, to selectively control the noxious (pain) and innocuous (physiological) sensory pathways from internal organs, without using opiates or other non-specific therapeutic agents.

WORKSHOP MODULES

Materials for the workshop modules will be distributed to course participants one week before the course.

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INDUCTION SHEET

SEPARATE SHEET HERE

VISITOR INDUCTION & SAFETY ORIENTATION CHECKLIST

NAME: .....

DATE/S OF STAY: .....

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Once completed it should be returned to the Manager, Resources & Scientific Services and kept on file as a record of the visitor's induction.

General

Laboratory & PC2 safety rules - appropriate PPE	Yes	☐
Safety shower, eyewash station	Yes	☐
Spill kit	Yes	☐
Emergency shutdown of power & gas	Yes	☐
Emergency warden & evacuation procedures	Yes	☐
Location of first aid kits	Yes	☐

Signature of Visitor: ..... Date: .....

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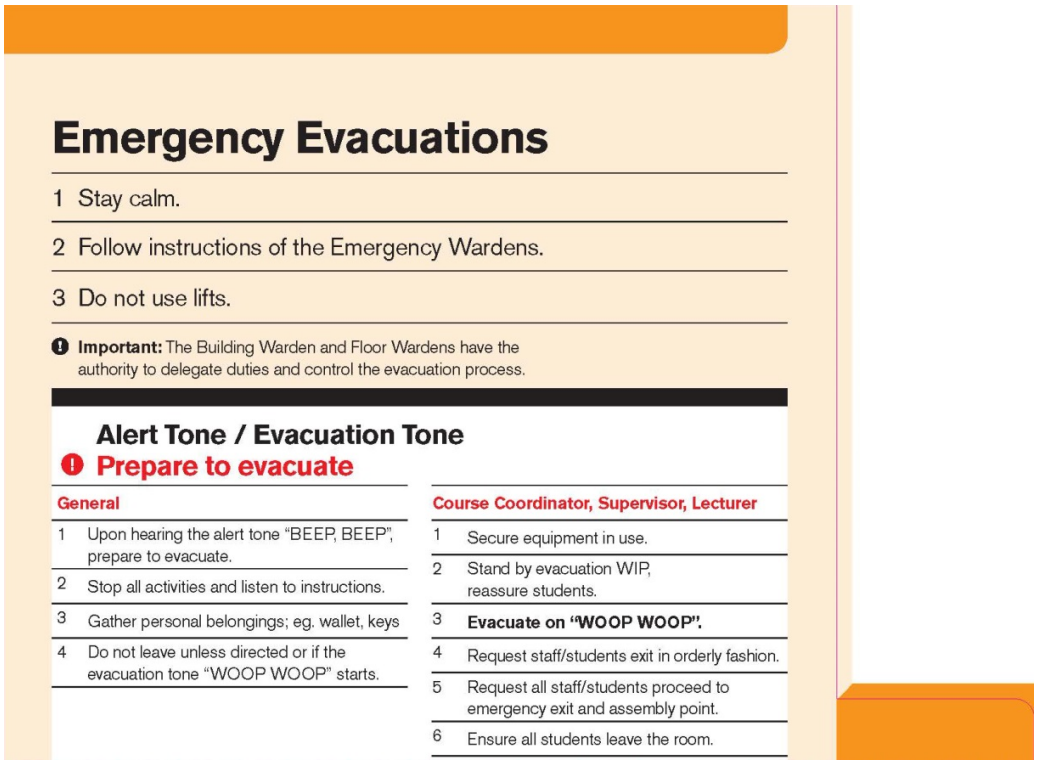

What's new at TrendBio?

Introducing Axion Biosystems Maestro Z Impedance and Lumos Optogenetics Systems

From the innovators in Multi-Electrode-Array (MEA) and Optogenetics (Lumos) technology comes Maestro Z, an impedance-based platform for cell analysis offering real-time, continuous, label-free monitoring of cells. Continuous data reveals the kinetics of cell-cell interactions and cell-drug responses for better mechanistic understanding without the time- and cost-intensive process of repeating multiple endpoint assays. Unlike conventional impedance systems, Maestro Z is equipped with a built-in precision environmental unit (software controllable temperature and CO2 gas) ensuring a high degree of reproducibility in intra and inter experimental conditions. Uniquely, by using three simultaneous sampling frequencies (1, 10 and 41.5kHz) the Maestro Z makes use of the mathematical relationship between impedance and sampling frequency enabling analysis of cell characteristics and cell quantitation. Matched with easy to use graphic based Axis software, the Maestro Z can provide a wealth of information about your cell model (e.g. growth, viability, senescence, cytotoxicity, drug response kinetics etc) and be used as a workhorse instrument for cell QC.

Lumos is a first-of-its-kind commercial instrument for optical stimulation and control of cultured cells in 24-, 48-, or 96-well microplates. Lumos provides the ability to simultaneously use up to four different wavelengths of light per well at user-specified, precisely defined output patterns with exquisite temporal control.

Introducing Yokogawa CQ1 High Content Imaging System


YOKOGAWA


From the inventors of the world leading spinning disk technology Yokogawa (Japan), the CQ1 is a laser based, small footprint benchtop automated confocal imager which produces images with the same quality of a high-end confocal microscope but is as easy to use as a microplate reader. The data generated can be analysed with Yokogawa's CellPathFinder (3D-4D analysis, machine learning, deep learning) and/or exported to downstream analysis software as needed. The CQ1 contains a built-in precision environmental unit (controllable O2, CO2 & humidity), is compatible with microplates (6-1536 well), petri dishes, tissue slides and chamber slides, and can be integrated with third party instrumentation.

For more information please contact Dr Marina Skrypnik (marina@trendbio.com.au)