SA01

The Hedgehog Signalling pathway: insights from flies and fish

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Analyses using genetically tractable model organisms over the past 30 years have made key contributions to the elucidation of the intercellular signaling systems that underpin animal development. A prime example is the Hedgehog signalling pathway: originally characterized in *Drosophila* through analyses of the members of the segment polarity class of mutations, the discovery of vertebrate homologues of the *Drosophila hedgehog* gene led to the unveiling of its involvement in a plethora of processes in vertebrate development and to the recognition of its role as a key driver of certain types of cancer, in particular basal cell carcinoma (BCC).
A striking feature of the Hedgehog pathway is its intimate relationship with sterols: the Hedgehog proteins themselves are covalently coupled to cholesterol; their receptor Patched is a multipass transmembrane protein that contains a Sterol Sensing Domain (SSD), first defined in proteins involved in cholesterol synthesis or transport; and the obligate transducer of the Hedgehog activity, the G-protein coupled receptor (GPCR)-like protein Smoothened is activated by cholesterol. Another defining feature of the pathway – at least in vertebrates - is its dependence upon the integrity of the primary cilium.
In my talk, I will review our current understanding of this enigmatic pathway and illustrate some of its functions, highlighting insights based on studies in *Drosophila* and the zebrafish *Danio rerio.*

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA02

How much less would we understand about human physiology if we didn't use the mouse as a research model?

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The mouse has not always been the model of choice to study physiology. Over many years a whole array of animal models have been used including rat, dog, rabbit, guinea pig, chick, zebrafish and fruit fly, providing valuable information about cardiovascular physiology, the immune system, embryonic development, circadian rhythm and the nervous system, to name a few. It was the advent of

transgenesis and the ability to genetically manipulate the mouse genome that led to a revolution in the use of the mouse as the go-to model in which to study mammalian gene function *in vivo*. However, does the fact that we can readily manipulate the genome of the mouse make it an appropriate model in which to study human physiology and pathophysiology? It is clear that humans and mice are very different but they do have important essential similarities. They share considerable genetic homology, with 99% of their genes being the same, and with key physiological and cellular similarities for example in their cardiovascular, nervous, musculoskeletal, endocrine and immune systems. That said, it is important at this point to insert the caveat that there are also a number of physiological differences in these systems. Whilst recognising the inherent limitations of any model system I will argue that the mouse is our most valuable model to further our understanding of physiology and pathophysiology.

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SA03

Of mice, rats and men: using rodent models and human stem cell models to understand cellular and behavioural deficits in monogenic neurodevelopmental disorders

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Mice have been used extensively to model human neurodevelopment disorders (NDDs) and have contributed richly to our understanding of the pathophysiology seen in NDDs. Nevertheless, a key step to realizing the potential of translating findings from pre-clinical models to treatment of human disease is to assess whether cellular and behavioural deficits seen in, for example, mouse models are also present in other rodent models and in addition whether such deficits can also be modelled using human-derived tissue.

The ability to generate genetically-modified rats permits extended modelling of NDDs allowing for greater assessment of complex cognitive and social functions and non-invasive imaging. Moreover, as mice and rats last shared a common evolutionary ancestor over 12 million years ago this provides us with an opportunity to test whether the same disease-associated null mutations in these species lead to similar cellular and behavioural deficits. Indeed, although models may have structural validity to human disease, face validity (i.e. models having an equivalent manifestation of a cellular or behavioural outcome) is not necessarily expected given the evolutionary pressures experienced by these two species. Simply put, if mice

and rats do not show similar deficits there is little expectation that such models should display face validity with the human condition. Thus, it can be argued that for assessment of NDD models the study of behaviours that rely on brain regions and circuits that are considered to be affected in the human condition is desirable. Furthermore, where robust deficits are present this allows for the assessment of whether pharmacological intervention can ameliorate or reverse dysfunction.
In this presentation I will review findings from a large collaborative research programme conducted by colleagues based in the UK and in India that has focussed on cellular and behavioural phenotyping of eight rat models of NDDs. One of these, a model of fragile X syndrome (FXS) which is a major heritable cause of intellectual disability, will be used as an exemplar to illustrate the extent to which some cellular pathophysiologies are conserved in both mouse and rat models but where behavioural deficits are not conserved. In addition, using this rat model of FXS I will present data that indicates that early and brief pharmacological intervention prevents the emergence of a complex associative memory deficit. Furthermore, such early intervention leads to a long-lasting correction of the deficit and is also associated with a rescue of some key cellular pathophysiologies seen in rodent models of FXS. These studies are complemented by experiments with FXS patient induced pluripotent stem cell-derived and isogenic stem cell-derived cortical neurones that reveal altered network excitability and which also highlight the importance of considering both cell autonomous and cell non-autonomous origins of cellular pathophysiology.

The work to be described in this presentation has been generously funded by the Medical Research Council (MRC), the Biotechnology and Biological Sciences Research Council (BBSRC), the Simons Foundation Autism Research Initiative (SFARI) and the Department of Biotechnology (DBT), Government of India.

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SA04

The fish within – remodelling the pharynx in development and evolution

A. Graham

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The pharynx is an important region of the vertebrate body that is involved in many key processes including feeding, respiratory and vascular functions, immune and endocrine activities. However, with the colonisation of land by vertebrates, the pharynx underwent substantial modification and it is this that we wish to understand. In this talk, I will discuss the development of the pharynx and how this has been altered to facilitate terrestrial life. To understand how the development of the pharynx has been modified through evolution, we take a comparative approach.

This involves analysing pharyngeal development using a range of cellular and molecular approaches: immunostaining, in situ hybridisation, morpholino knock-downs, lineage tracing and pharmacological inhibition of signalling pathways, in embryos that are chosen for the phylogenetic position. These include shark, zebrafish, chick and mouse embryos. We have considered how features have been lost; the gills and their covering, the operculum, and how new structures; the parathyroid glands, have emerged. We find that although the gills were lost from the adult forms, the gill primordia persist in land vertebrates and that they have been transformed into the parathyroid glands. We further find that the operculum was also not completely lost but that it exists transiently at embryonic stages. This work is significant as it lays out the progressive nature of evolutionary change and shows that the modification underpinning the emergence of the tetrapods were not as abrupt as previously believed. Finally, this work also highlights the utility of comparative approaches involving embryos of multiple species to address key problems in vertebrate biology.

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We would like to thank the Leverhulme Trust and the Anatomical Society for funding

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SA05

Birds as model systems to understand the evolution of physiological control of behaviour.

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Physiological mechanisms are studied in a wide variety of organisms, either because we are intrinsically interested in these organisms, or because they provide a good “model system” in which to study the mechanisms under investigation. Such a phylogenetically broad approach allows us to derive general principles of physiological functions and adaptations to similar ecological challenges across different clades. Birds can have advantages over mammals for studying some aspects of physiology. For example, the developing embryo is much more physiologically independent from its mother, allowing for easier access and manipulation. Some

bird species are also better models of humans than rodents are, because of their diurnal lifestyle, social monogamy and relatively long life-span. Here, however, I will focus on using birds as a system in which we can investigate the physiological mechanisms underlying well-studied behavioural adaptations to ecological challenges.

Food-hoarding behaviour in titmice has been studied for over 4 decades as a model system of behavioural adaptation to harsh winter conditions[1] and a model of the evolution of cognitive systems (spatial memory and the hippocampus)[2]. To date, however, very little is known about the physiological mechanisms that drive the motivation to hoard food in these birds. We have started this investigation from observations about the environmental conditions that drive food hoarding behaviour. We note that the same short day and low temperature conditions that drive the motivation to eat also drive the motivation to hoard. This makes us hypothesize that the mechanisms underlying the motivation to eat and the motivation to hoard are probably at least partially shared. In support of this, chronic moderate experimental elevation of corticosterone increased both food intake and hoarding motivation[3], while systemic injection of ghrelin or leptin suppressed food intake and food hoarding in these animals[4]. In birds, these peripheral hormones have different functions from the way they work in mammals. Understanding how birds and mammals have solved similar evolutionary problems, using similar ingredients, but in different physiological ways gives us deeper insight into how the evolution of physiological mechanisms works.

We are currently investigating the gene expression of small peptides in the brain and peripheral tissues that we hypothesize to signal energetic state and gut fill in titmice. Once we have identified genes signalling nutritional state, we will investigate the effect of their encoded peptides on food consumption and hoarding behaviour.

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Thank you to Tim Boswell for our long-term collaboration on this topic and to Lindsay Henderson, Rowan Cockcroft and Bedour Al Sayegh for working on this project. Tim and Lindsay also provided important feedback on the abstract.

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SA06

Modelling the insect flight motor using *in vivo*, time-resolved microtomography

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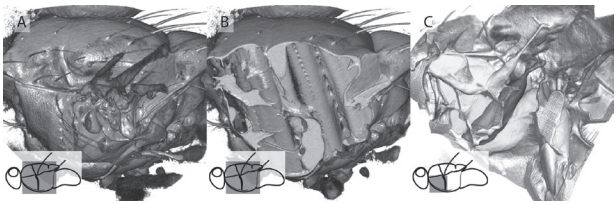
The insect flight motor serves as a useful model for understanding complex biomechanical systems. The insect body contains numerous evolutionary adaptations that provide them with incredible aerial manoeuvrability and precision. However, we still have a limited understanding of how the muscles, thorax and sensory structures operate together to provide power and fine control during flight. This is perhaps unsurprising as the insect flight motor forms ones of the most intricate mechanical designs found in nature. Small, essentially linear, strains produced by the indirect power muscles are amplified and transformed into the large, nonlinear flapping motion of the wings through subtle deformations of the insect thorax and wing hinge. Our lack of knowledge stems at least in part from the extraordinary difficulty in measuring, or even visualizing, micron-scale muscle movements *in vivo* at frequencies in excess of 100 Hz. Matters are made more problematic as the many flight muscles are hidden from view behind the thick thoracic shell, which itself forms an integral part of the flight motor.

High-speed, time-resolved microtomography provides a method for visualising and measuring the internal and external movements of otherwise inaccessible structures in small organisms[1,2]. Here I will present the latest research using this technique to investigate multiple aspects of the dipteran flight motor[1]. Diptera (true flies) form a useful model order as they are united in having a single pair of wings, making many measurements and modelling work simpler, and yet it includes some of the most agile fliers and economically important insect species. This work is providing unprecedented insights into the structure and function of the myriad components that form the insect flight motor, which can then be modelled to answer fundamental questions in biomechanics related muscle function, sensory control and mechanical design. The outcomes from this research will be important for understanding how natural selection has shaped the insect flight motor, while also providing inspiration for engineers interest in building bio-inspired, micro-actuators and air vehicles. Furthermore, high-speed, time-resolved microtomography is a powerful method that can be used to investigate other small-scale, complex biomechanical systems, which undergo repetitive or controllable motions.



Three-dimensional visualisation of blowfly thorax created using *in vivo*, time-resolved microtomography. A, external view of thorax, B, cut-away view showing parts pf thorax (shaded green to blue) and power muscles (shaded yellow to red). C, internal view showing steering muscles.

Walker SM, Schwyn DA, Mokso R, Wicklein M, Müller T, Doube M, Stampanoni M, Krapp HG & Taylor GK. (2014), *In vivo* time-resolved microtomography reveals the mechanics of the blowfly flight motor. *PLoS Biol* 12(3): e1001823. doi:10.1371/journal.pbio.1001823

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Prof. Graham Taylor, University of Oxford

Dr. Anna Chabokdast, University of Oxford

Mr. Jonathan Page, University of Oxford

Prof Holger Krapp, Imperial College

Dr Patrik Christen, ETH Zurich

Dr Rajmund Mokso, Lund University

Prof Marco Stampanoni, Paul Scherrer Institute

Dr Christian Schepütz, Paul Scherrer Institute

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SA07

Sodium nitroprusside prevents glucose-induced impairment of cerebrovascular development and function in zebrafish

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Diabetes is well-known to cause both macrovascular and microvascular disease, but is increasingly associated with neurological dysfunction including dementia. Neurovascular coupling (increased regional cerebral blood flow in response to neural activation) is impaired in many neurological diseases including dementia.

We therefore established a novel non-invasive zebrafish model allowing simultaneous quantification of cerebrovascular anatomy, neural activation, and cerebral vessel haemodynamics in response to visual stimulus and examined the effect of glucose exposure on cerebrovascular patterning and neurovascular coupling. Combining lightsheet microscopy and compound transgenics to simultaneously visualize neuronal calcium activity and blood flow, we find that zebrafish larvae exhibit neurovascular coupling by 8 days post fertilisation ($n=40$, $p<0.0001$ for neuronal calcium activations and $p=0.0006$ for RBC speed in response to visual stimulation). Using this model, we demonstrate continuous exposure to glucose at levels seen in the blood of poorly controlled diabetics (20mM or 360 mg/dL) impairs neurovascular coupling with reduced RBC speed ($n=25$, $p=0.0008$, two-way ANOVA) in the blood vessels of optic tectum (visual processing area in zebrafish). We also show associated cerebrovascular patterning defects such as reduced branch point number ($n=20$, $p<0.0001$), vessel length ($n=20$, $p=0.0013$) and vessel radius ($n=20$, $p=0.0003$), upon glucose treatment. Multiple reports show that nitric oxide (NO) generation is impaired by diabetes. Since both vascular development and neurovascular coupling are NO-dependent, we examined the effect of exposure to the NO donor, Sodium Nitroprusside (SNP), widely used to treat hypertension in humans. Administration of SNP resulted in a marked improvement in the impairment of neurovascular coupling induced by glucose exposure ($n=20$, $p<0.0001$) and prevented the glucose induced vascular defects ($n=30$, $p<0.001$). Our results establish the first non-mammalian model of neurovascular coupling and reveal a potential strategy to ameliorate the effects of hyperglycemia on cerebrovascular function.

We are very grateful to the aquarium staff of the Bateson Centre for expert husbandry and advice. This work was funded by a Project Grant from the National Centre for Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) NC/P001173/1. C.H. is the recipient of a Sir Henry Dale Fellowship jointly funded by the Welcome Trust and the Royal Society (Grant Number 105586/Z/14/Z). The Zeiss Z1 lightsheet microscope was funded via British Heart Foundation Infrastructure Award IG/15/1/31328 awarded to T.C.

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SA08

Fish as models for extreme cardiac (patho)physiology

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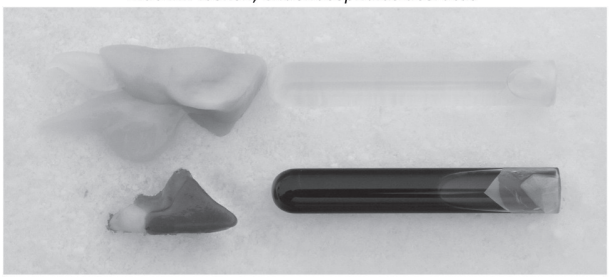
Cardiovascular adaptations to changing environments such as climate change may include short term phenotypic responses, longer term effects such as developmental programming, or evolutionary adaptations to associated physiological

challenges. Within this context, and given the degree of similarity among biological processes across the vertebrates, those species with unusual anatomical or physiological traits help us explore limits to function in ways not feasible with experimental interventions using model species.

Fish have been at the forefront of many novel biomedical research opportunities, including discovery of cardiac stem cells in zebrafish that permit ventricular regeneration. In general, fish have a low metabolic rate and corresponding low heart rate, which in the absence of compensatory factors regulating sinus rhythm would be expected to increase the risk of arrhythmogenesis. Orthologs of mammalian K^+ channels regulate action potential duration at low temperatures. The two-chambered heart powers a single-pass circulation, meaning that ventricular myocytes derive their oxygen uptake from venous return in those species lacking a coronary circulation. By comparing species that have an additional limitation, i.e. genetic mutations that have resulted in a lack of facilitated oxygen transport due to absence of myoglobin and/or haemoglobin (*Chaenocephalus aceratus*), with sympatric red-blooded species (*Notothenia coriiceps*) allows unique insight into extreme cardiovascular physiology. These icefishes live in the frigid waters around Antarctica (<0ºC all year round), and the associated increase in oxygen solubility together with wholesale remodelling of the cardiovascular system (including cardiomegaly and large diameter blood vessels, which in other contexts are associated with various pathologies) and autonomic control (having a low catecholamine synthetic capacity but high vagal tone), support healthy populations across the Southern Ocean. Their large hearts display a normal ECG waveform, and generate an impressive stroke volume/cardiac output, but the lack of respiratory pigments is associated with a poor tolerance of afterload when measured using an *in situ* perfused heart preparation.

Extant organisms are the product of selection pressure, and we need to understand mechanisms underlying both the drivers of change and resultant physiology, in order to assess how well the genetic plasticity and phenotype capacity may limit response to future environmental change. We reasoned that these animals would be more sensitive than others to current rises in seawater temperature, so our latest expedition explored their thermal sensitivity. Instrumented animals showed an impressive resilience to an acute temperature ramp, with loss of sinus rhythm only seen >13ºC due to failure of atrioventricular conductance. *N. coriiceps* (Hb^+Mb^+) had a higher routine and maximal heart rate than *C. aceratus* (Hb^-Mb^-), but similar critical thermal maximum (14-16ºC). Comparison of *in situ* function with a species having an intermediate phenotype, *Chionodraco rastrospinosus* (Hb^-Mb^+), suggest that loss of Hb conveys poor pressure generating ability, but additional loss of cardiac Mb reduces intrinsic heart rate and maximum cardiac output, which may limit resilience to near-future ocean warming.

Blackfin icefish, *Chaenocephalus aceratus*



Yellowbelly rockcod, *Notothenia coriiceps*

All procedures accorded with current UK legislation. Thanks to Tony Farrell (UBC), Kristin O'Brien (Alaska) and Lisa Crockett (Ohio) for help and discussions. Supported by NERC and NSF.

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SA09

A systems approach to skeletal muscle adaptation

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Skeletal muscle comprises ~ 45% of the healthy human body mass. It is critical for development, growth, metabolism, posture, locomotion, thermoregulation and the provision of energy. Ageing, muscular dystrophies and cachexia are associated with muscle wasting and weakness, however, the mechanisms underpinning these losses may differ. Muscles hypertrophy (increase in size through an increase in the cross sectional area of individual fibres) when protein synthesis exceeds protein degradation, in response to e.g. loading, which ultimately leads to an increase in maximal force generating capacity. Conversely, muscles atrophy (decline in fibre size and cross sectional area) following disuse, unloading or disease, which culminates in a decline in peak force generating capacity - when protein degradation dominates. The adaptability of skeletal muscle, given its terminally differentiated state, is thought to be achieved via activation of resident muscle stem cells. The regulators of synthesis, degradation and ultimately muscle mass are therefore likely to involve complex cellular, biochemical and genetic controllers. Our research focuses on using and developing stem cell cultures to model the interactions of skeletal muscle cells with anabolic (insulin-like growth factors) and catabolic (tumour necrosis factor-alpha, interleukin-6) agents. Models span age, disease and injury and provide us with a means to understand the regulators (e.g. IGFBPs, PI3 kinase, MAP kinase, Adra1d, caspases and sirtuins),

which influence survival, differentiation, migration or death of these cells. Key human studies complement our work (age, nutrition and exercise) and are critical, since severe loss of functional muscle mass contributes to increased morbidity and early mortality. This presentation will provide information on the use of our model systems to provide some insight into the regulators of muscle adaptation.

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SA10

Replicating human disease in rodents: the good and the bad of CRISPR/Cas9 genome editing

G. Pavlovic[1,2,3]

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Even if rodents allowed major breakthroughs for biomedical research, a striking issue in modern biology is certainly some failure of studies in mice and rats to be replicated or translated to humans.
With the advent of new genome editing techniques such as CRISPR/Cas9, it has become quicker and cheaper to generate a panel of mutations, lot easier to generate more complex models and possible to target different backgrounds in rodents.
This speech will discuss possible strategies that are now available thank to CRISPR/Cas9 mediated genome editing to develop better animal models of human disease.
We will present our results for generating complex mouse or rat models. We developed the CRISpr Mediated REarrangement (CRISMERE) strategy, which takes advantage of the CRISPR/Cas9 system, to generate easily most of the desired structural variant & CNV rearrangements. We were indeed able to achieve deletions, duplications, and inversions of genomic regions as large as 24.4 Mb in rat and mouse (the good).
We will also present how we can combine CRISPR and ES cells to achieve whole gene humanization. Finally, we will show that very careful validation of the lines generated by CRISPR/Cas9 is requested (the bad) and how some unexpected event can be used to generate targeted overexpressing models.

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Birling, M.-C., Herault, Y., and Pavlovic, G. (2017).

Modeling human disease in rodents by CRISPR/Cas9 genome editing. Mamm. Genome.

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SA11

A Quantitative Systems Pharmacology Approach to Understanding Drug Mechanisms of Action

A.E. Roashan[1,2], A. Bithell[1], M. Tindall[2], M. Bazelot[3], B. Whalley[3], W. Hind[3], R. Gray[3] and J. Brodie[3]

[1]School of Pharmacy, University of Reading, Reading, UK, [2]Department of Mathematics & Statistics, University of Reading, Reading, UK and [3]GW Research Ltd, Cambridge, UK

Within the past decade, Quantitative Systems Pharmacology (QSP) has emerged as a new field seeking to improve pharmaceutical research and development (R&D). It combines mathematical modelling of subcellular and whole body scale events with experimental data to provide insight into the mechanisms of action underlying drug efficacy, and to test predicted therapeutic strategies likely to achieve clinical validation. These models can also be used to predict causes of drug failure, identify new drug targets, and help improve clinical trial design and optimisation [1].
The growing investigation into the therapeutic indications for phytocannabinoids found in, and derived from, *Cannabis sativa* (cannabis) makes it an interesting case for a QSP approach. Despite the conclusive data on the therapeutic effects of cannabinoids upon diseases such as childhood epilepsy, there is a need to further explore their mechanisms of action and their therapeutic potential for other indications. To begin to address this, we are taking a QSP approach by modelling mechanisms of action possibly underlying the biological effects of these phytochemicals.
Through proprietary data, we have identified the molecular targets that cannabidiol (CBD), a major compound of cannabis [2], significantly binds to and activates/inhibits. We have connected these targets to the biomarkers of two diseases of interest, Tuberous Sclerosis Complex (TSC) and Rett Syndrome (RTT), via relevant cell signalling pathways. Using Michaelis-Menten kinetics and the Law of Mass Action, we have created a mathematical model integrating the molecular interactions in the pathways with body scale information and clinical data. This model will be used to test the efficacy of CBD for both TSC and RTT, and predict the mechanisms through which it exerts its effect.
To summarise, we discuss the advantages of integrating mathematical models into pharmaceutical R&D, and will demonstrate the utility of these models to gain insight into the therapeutic potential of a drug and the mechanisms by which it acts.

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SA12

Seasonal and circadian contributions to mental health and wellbeing in the UK Biobank cohort

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Background: Disruption of circadian rhythmicity is associated with increased vulnerability to mood disorders. Previous studies have used subjective reports of activity patterns but the availability of accelerometer-based data in large numbers of participants of the UK Biobank cohort permits the derivation and analysis of new, objectively-ascertained rhythmicity parameters. Seasonal factors have been linked to circadian disruption and increased depressive symptoms, but previous studies have rarely accounted for effects of lifestyle and environmental factors. Using extensive health and lifestyle data from the UK Biobank, we tested for associations between objectively–assessed circadian rhythmicity and mental health and wellbeing phenotypes, including history of mood disorder. We then examined evidence of seasonal patterns in recent depressive symptoms.
Methods: Using wrist-worn accelerometry data from 91,105 UK Biobank participants, a circadian relative amplitude (RA) variable was derived, reflecting the distinction between the most and least active periods of the day. Cross-sectional associations between low RA and mood disorder, wellbeing and cognitive variables were examined. To test for seasonality of depressive symptoms, we fitted cosinor models to a total depressive symptoms score and scores of low mood, anhedonia, tenseness and tiredness scores in >150,000 participants. Associations of depressive symptoms with day length were then examined.
Findings: A quintile reduction in RA was associated with increased risk of lifetime major depressive disorder (odds ratio (OR) = 1.06, 95% CI 1.04, 1.08) and bipolar

disorder (OR = 1.11, 95% CI 1.03, 1.20), as well as with greater mood instability, higher neuroticism, more subjective loneliness, lower happiness and health satisfaction, and slower reaction time. The associations were independent of demographic, lifestyle, education and overall activity confounders. Seasonality of total depressive symptom scores, anhedonia and tiredness scores was observed in women but not men, with peaks in winter. In women, increased day length was associated with reduced low mood (incidence rate ratio (IRR) = 0.997, SE = 0.001) and anhedonia scores (IRR = 0.996, SE = 0.001), but longer day length was associated with increased tiredness (IRR = 1.004, SE = 0.001).
Interpretation: This large, population-based study provides evidence that circadian disruption is associated with a range of adverse mental health and wellbeing outcomes. We also provide evidence of seasonal variation in depressive symptoms in women, with association between shorter days and increased feelings of low mood and anhedonia. The depth and breadth of physical and mental health, sociodemographic, lifestyle, environmental and activity data in UK Biobank affords the opportunity to examine chronobiological contributions to mood disorder on an unprecedented scale.

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SA13

From experiment to computational model and back: flipsides of the physiology coin

J.A. Jeneson

University Medical Center Groningen, Groningen, Netherlands

Since early day biologists, biochemists and physiologists have been conducting experiments in animals and their tissues to gain understanding of the principles of life. Putting humpty dumpty back together again has proven to be yet another thing. Here, computational modeling presents a valuable investigative tool by offering a precise framework to capture and integrate what has been learned about a particular subject matter and generate testable predictions that may be then be verified against experimental observation. Importantly, such numerical models can help experimentalists identify what particular measurement should next be made in order to rigorously test a particular hypothesis. This talk will highlight these beneficial uses of computational modeling in physiology.

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SA14

My local AWERB - what's in it for me?

P. Hawkins

Research Animals, RSPCA, Southwater, UK

What are your views and experiences of your local Animal Welfare and Ethical Review Body (AWERB)? It should be a valuable resource to help optimise the benefits of scientific research, identify associated ethical issues, minimise harms to animals, and prepare project licence applications so that they are 'Home Office ready'. It should also provide you with the opportunities to improve animal welfare alongside your science, help to develop and shape your establishment's Culture of Care, and get to know your animal technologists, Named Veterinary Surgeon and other named persons better, facilitating good communications. However, if the AWERB is not structured well, is not very inclusive of scientific and other staff in its approach to its tasks, or is poor at communicating, it may be seen negatively as a source of stress rather than as the source of useful information and advice that is intended.

It is in everyone's interests for AWERBs to function well, yet there have been few opportunities to gather personal and project licence holders' views on how the AWERB affects them - either positively or negatively - and to feed this back to AWERBs more widely. Neither have there been many forums where the benefits of a well run, thoughtfully organised AWERB can be set out for scientists who are not closely engaged with the process.

This interactive lunchtime workshop will cover both aspects. The aims are:

- to provide the opportunity for you to set out what you have always wanted to tell your AWERB (anonymously if you wish!) which can then be fed into AWERB guidance notes;
- to review the benefits the AWERB should deliver and why it is important to be more engaged; and
- to discuss ideas and action points to help participants become more involved with their AWERBs.

Why not join us and have your say? You can bring your lunch with you, and drop in for as long or as short a time as you like over the lunch break.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA15

Cardiovascular & renal disease insights from the rat

R. Menzies

University of Edinburgh, Edinburgh, UK

The rat has classically been the species of choice for pharmacological and toxicological investigations during the early *in vivo* phase of drug discovery, providing high-quality physiological datasets on cardiovascular and renal pathophysiology over many decades. Recent genome engineering advances, particularly with zinc finger nucleases and CRISPR/Cas9, are now enabling us to measure these typically challenging physiological studies in gene-targeted strains, previously restricted to murine models. This presentation will focus our acquired understanding of rat models of hypertension, diabetic nephropathy, and acute and chronic kidney disease. These models have made important contributions to our understanding of cardio-renal diseases, revealing key genetic drivers such as *Ace* and *P2rx7* involved in pathogenic processes. By targeting these genes of interest, we are gaining a better understanding of the aetiology of cardiovascular and renal pathologies, with the promised potential of slowing, or even reversing, disease progression. Some, but not all of the identified targets, have proven to be translationally important. These advances are under intensive investigation in my lab to identify new druggable targets and develop targeting strategies.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA16

Using zebrafish to model joint physiology in development, ageing and disease

C. Hammond

Phsyiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK

Zebrafish are a well-established model to study developmental biology and early physiology. Their optical translucency, genetic tractability and the availability of fluorescent reporter lines has been extensively used to give insights into cell behaviour, organogenesis and many disease states. However, they can also be used to model diseases associated with ageing. The degenerative joint disease osteoarthritis affects around 1 in 8 of the population worldwide. Wear and tear through joint use and genetic factors are known to play a role, yet we still know relatively little about the interplay between genes and mechanics in the early stages of disease pathology. Using genome editing we have generated 10 zebrafish lines carrying mutations in genes identified from human genome wide association studies (GWAS) as osteoarthritis susceptibility genes (*runx2a, barx1,*

gdf5, *col11a2, col9a1, dot1l, chsy1, ncoa3*, *wnt16* and *mcfl2a*). Using a range of techniques (e.g. histology, confocal of transgenic lines, second harmonic generation, micro computed tomography, functional analysis) we show that not only do these mutants develop osteoarthritis as they age but that most mutants show changes to cell behaviour, joint shape and joint function that are detectable early in development, by 5 days post fertilisation. Using Finite Element Analysis on wild type and mutant larval joints we have tested the relative impact of joint shape and of cartilage properties on skeletal strain and show that shape has a greater impact on joint performance. To facilitate high throughput screening of CRISPR mutants we have developed an automated 3D analysis tool to segment skeletal elements through which we can define alpha shape, volume and shape variation allowing us to group genes by their mutant phenotypes. I will also, briefly, discuss our plans to test the effects of altered gravitational fields on zebrafish joint physiology.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA17

Towards human kidneys in culture: the power and limitations of self-organization

J.A. Davies

Deanery of Biomedical Sciences, University of Edinburgh, Edinburgh, UK

Inter-species differences in the development, physiology, and pathology limit the utility of animal models in the study of human disease and development of new treatments. Human-focused researchers therefore have a strong need for realistic, human-derived tissues and mini-organs that can be studied in culture. The shortage of transplantable organs, and the desirability of having perfect immune compatibility between transplant and host, creates an additional demand for production of human tissues and organs from patient-specific stem cells especially for commonly transplanted organs such as the kidney.

Typical approaches to making human tissues work by exposing pluripotent stem cells (e.g. hiPS cells) to a sequence of signalling environments that mimic what cells would experience in an embryo, if they happened to end up developing into the desired tissue. The result, when the procedure works, is the differentiation and self-organization of cells into tissue elements that are anatomically and physiologically realistic at a micro-level, but the system is symmetrical (the same everywhere), and lacks macroscopic (organ-scale) organization. Kidney tissue produced this way, for example, produces multiple nephron and collecting duct tubules with none of the natural organization of the kidney around a single collecting duct tree, and no exit ureter.

We have been using murine and human renal systems to explore the reasons for this limitation. Careful analysis of embryonic development has identified

localized cues that break the symmetry of the naturally developing system. We have designed renal differentiation systems to include artificial versions of these symmetry-breaking cues, and ave shown that their inclusion greatly improves the realism of the result. Instead of producing jumbled renal tissues, these systems can produce mini-organs with cortex and medulla, proper organization around a single collecting duct tree, and a urothelial exit tube. We have also used fluorescent tracers to verify that these mini-organs show realistic tubular transport physiology, and have used reporter genes to enable cultures to report automatically their exposure to nephrotoxic compounds in a blind-coded screen.
This lecture will conclude by indicating how its concepts can be extended to other systems and also how the science of synthetic biology can be used to extend further the capabilities of tissue engineering.

Mills CG, Lawrence ML, Munro DAD, Elhendawi M, Mullins JJ, Davies JA. (2017) Asymmetric BMP4 signalling improves the realism of kidney organoids. Sci Rep. 2017 Nov 1;7(1):14824. doi: 10.1038/s41598-017-14809-8.

Davies JA. (2015) Biological techniques: Kidney tissue grown from induced stem cells. Nature. 2015 Oct 22;526(7574):512-3. doi: 10.1038/nature15639.

Lawrence ML, Chang CH, Davies JA. (2015) Transport of organic anions and cations in murine embryonic kidney development and in serially-reaggregated engineered kidneys. Sci Rep. 2015 Mar 13;5:9092. doi: 10.1038/srep09092.

Work described in this lecture was funded by BBSRC, Kidney Research UK, Leverhulme Trust, MRC and Wellcome Trust.

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SA18

Cutaneous photoageing: Systems for modelling repair of human skin

R.E. Watson

Centre for Dermatology Research, University of Manchester, Manchester, UK

Skin ageing is a complex process involving the convergence of two distinct mechanisms: the subtle effects of time-dependent intrinsic ageing, and the changes brought to bear on our skin by its constant interaction with the external environment, predominantly chronic sun exposure. This photoaged skin has a distinctive clinical appearance, exhibiting coarse and fine wrinkles, a sallowness of complexion and reduced ability to recoil. One major dermal alteration is loss of fibrillin-rich microfibrils – key components of the dermal elastic fibre matrix - at the dermal-epidermal junction. Topical all-*trans* retinoic acid (RA) improves the clinical appearance of photoaged skin; however, the time period for improvements to occur may be up to 6-months. Targeting improvements in photoaged skin is a commercial focus for personal care companies; with a shift in public perception around the scientific background to product claims, we designed a short-term *in vivo* screening

assay fibrillin-rich microfibrils as a marker for outcome of repair to provide additional confidence during product design prior to commercial launch. Clinically assessed, severely photoaged individuals were recruited to the study (n = 8) and were subject to an occluded patch test on their photoaged extensor forearm (0.025% RA (positive control); 5% sodium lauryl sulphate (SLS, as an irritant control) or vehicle alone); a fourth untreated control area was also occluded to assess baseline expression of the biomarker. After 4-days, 4 mm biopsies were taken and probed for the occurrence of fibrillin-rich microfibrils (immunohistochemistry and *in situ* hybridization). In this 4-day patch test assay, RA and SLS significantly increased fibrillin-rich microfibril protein content compared to control and vehicle groups ($p<0.05$), with RA treatment significantly increasing fibrillin content over that of SLS ($p<0.001$). This was also observed at the mRNA level; RA-treatment producing highly significant increases in fibrillin-1 mRNA in epidermal keratinocytes ($p<0.001$) over SLS-treatment ($p<0.05$). This study indicates that RA can significantly affect fibrillin-rich microfibril content in photoaged skin and can be used as a biomarker of repair in short-term assays for testing the utility of topical products targeting photoageing.

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SA19

Exploring the regulation of materno-fetal resource allocation and its implication for development, physiology and disease

A. Sferruzzi-Perri

University of Cambridge, Cambridge, UK

In eutherian mammals, the successful outcome of pregnancy depends on balancing the genetically-determined fetal drive for growth with the maternal nutrient requirements to support the pregnancy and the subsequent lactation. Failure to achieve the right balance of nutrient allocation can lead to pregnancy complications for the mother and abnormal development of the fetus with long-term consequences for both maternal and infant health. The placenta, which forms the functional interface between the mother and fetus is thought to be central to the allocation of nutrients during pregnancy; it controls materno-fetal nutrient exchange and secretes hormones with metabolic impacts[1]. My talk will focus on our findings using environmental and genetic manipulations of the insulin-like growth factor-2-phosphoinositide-3 kinase (IGF2-PI3K) system in mice which explore the regulation of materno-fetal nutrient allocation by the placenta during pregnancy. I will present data demonstrating that the placenta is able to respond to both maternal signals of resource availability, as well as, to fetal signals of nutrient demand, by adapting its transport phenotype. I will also demonstrate that maternal nutrient allocation to the fetus can be additionally modified by the

endocrine actions of the placenta on maternal physiology. By studying the placenta and materno-fetal nutrient allocation, our work aims to understand the aetiology of pregnancy complications, developmental mechanisms and origins of health and disease.

1. Sferruzzi-Perri, A. N. & Camm, E. J. The programming power of the placenta. *Front Physiol* 7:33, DOI: 10.3389/fphys.2016.00033 (2016).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

SA20

In vitro modelling of respiratory infection in cystic fibrosis related diabetes

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The barrier function of the airway epithelium is central to the prevention of infection; in patients with chronic respiratory disease the integrity of this barrier is compromised leading to an increase in nutrient leakage into the airway surface liquid (ASL). Not only does this provide a nutrient source for bacterial growth but it is also likely to cause a change in the metabolism of the epithelial cells and resident immune cells thereby affecting their ability to control the infection.

Healthy human ASL only contains around 0.4 mM glucose - this increases in patients with respiratory diseases such as COPD and cystic fibrosis, with the greatest increase in patients with both respiratory disease and diabetes (2-6 mM). The increase in ASL glucose seen in hyperglycaemia is associated with increased risk of respiratory *S.aureus* and *P.aeruginosa* infections and poorer outcomes for the patients.

Cystic fibrosis affects more than 10,400 people in the UK with approximately half of adult patients developing hyperglycaemia (CF-related diabetes) and the associated increased risk of pulmonary infection. We propose that regulating the glucose concertation of ASL may provide an antibiotic free therapy for respiratory infections in CF and CFRD. However it is vital to fully investigate the consequences of modulating glucose in terms of infection as glucose is required for the function of phagocytic cells located within the lungs. Consequently the key aim of this project is to develop a disease relevant *in vitro* co-culture model of airway infections including epithelial cells, immune cells and bacteria to investigate the effect of controlling glucose levels on infection severity.

In order to develop this complex in vitro model we need to first determine the metabolic requirements of phagocytic cells. Initial results suggest that neutrophils show no loss of their ability to kill live bacteria across a wide range of glucose concentrations (0-15 mM). Monocyte derived dendritic cell (moDC) maturation

and antigen uptake are unaffected by acute changes to glucose concentration. Further work is ongoing to look at moDC cytokine secretion and capacity to stimulate T cells.
To generate epithelium for co-culture models human tissue samples were obtained from routine nasal surgeries. Basal epithelial cells isolated from this tissue were grown on Transwell supports at air-liquid-interface (ALI) into fully differentiated airway epithelia. These cell cultures mimic much of the function and complexity of human airways. Preliminary data shows that elevated glucose promotes *S.aureus* and *P.aeruginosa* growth in ALI epithelial-bacterial co-cultures and increases their binding to airway epithelial monolayers. The addition of neutrophils to the co-culture reduced apical *P.aeruginosa* growth under all glucose concentrations, consistent with a limited influence of glucose on neutrophil phagocytic capacity. Next we need to determine optimum conditions for the addition of multiple phagocytic cells (neutrophils, moDCs and monocyte-derived macrophages) in addition to bacteria to the ALI cultures to generate a physiological human in vitro airway infection model to further investigate the influence of glucose, hyperglycaemia and CF on bacterial killing/growth. Development of this co-culture model will be of huge value for modelling human respiratory disease without the need for animal models in accordance with the NC3Rs.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C01

P130Cas regulates both venous endothelial angiogenesis in the caudal vein plexus and lymphatic development in the trunk of zebrafish

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Bcar1/p130Cas is an important adaptor protein downstream of receptor tyrosine kinases, G-protein coupled receptors, and integrin signalling. It is required for the migration of endothelial cells and vascular smooth muscle cells in response to VEGF or PDGF stimulation *in vitro*. While global knockout causes embryonic lethality in the mouse due to severe cardiovascular defects, no further reports discuss its role in the cardiovascular system *in vivo*. To address this, we generated a novel knockout using the zebrafish model and used a combination of vascular reporters to interrogate p130Cas function during embryonic, regenerative and pathophysiological angiogenesis. We used confocal and light sheet microscopy to image live zebrafish embryos throughout development. A variety of transgenic vascular reporters, including the *tg(fli1a:nEGFP)*^{y7} line, allowed tracking of single endothelial cells.

Unlike the mouse model, *bcar1-/-* zebrafish were viable and fertile, showing no gross morphological defects, neither in embryonic development nor adulthood. Furthermore, they were able to regenerate the caudal fin upon amputation. However, we observed a failure of venous endothelial cell sprouting and migration in p130Cas-deficient embryos in the caudal vein plexus starting from 26 hours post fertilisation (hpf) (see figure 01).

This plexus resolved by 48hpf but maintained the reduced ventral extension already seen at 30hpf. We further observed a severe reduction in the development of the parachordal lymphangioblasts, the first lymphatic structures in the zebrafish trunk. P130Cas-deficient zebrafish embryos formed far fewer parachordal lymphangioblasts and these were also delayed in their migration (see figure 02).

We present here a novel model for interrogating p130Cas function *in vivo*. While the zebrafish *bcar1-/-* mutant is not lethal, this allows investigation of more subtle effects in physiological and pathophysiological angiogenesis. We find a specific requirement for venous sprouting in the caudal vein plexus; this also impacts the initial development of the lymphatic system in the trunk. We are currently investigating other vascular beds dominated by venous sprouting and the signalling mechanism underlying this defect. Preliminary data shows increased phosphorylation of p130Cas in response to BMP2 treatment in vitro and we hypothesise that p130Cas could be a key downstream mediator of BMP signalling which is known to be an important regulator of venous angiogenesis Wiley *et al.* (2011).

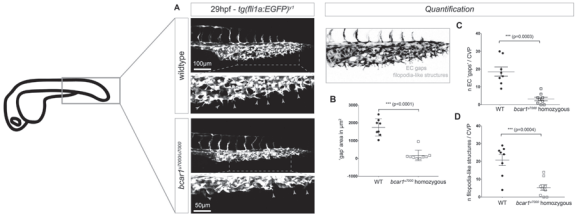

Figure 1: p130Cas is required for venous angiogenic sprouting in the caudal vein plexus.
(A) Representative confocal microscopy images of live *bcar1*^{tv1000} and wildtype embryos at the indicated time points. Images are maximum intensity projections generated using FIJI software, lateral views. At 29hpf, venous endothelial cells of *bcar1*^{tv1000} embryos failed to form filopodia (indicated by arrowheads in zoom-in), resulting in a reduced ventral extension and decreased complexity of the caudal vein plexus. **(B)** Quantification of the free area between actively migrating venous ECs and filopodia-like structures at the vascular front confirmed that these parameters are significantly decreased in *bcar1*^{tv1000} homozygous mutant embryos. Analysis was performed on n=8 WT and n=9 *bcar1*^{tv1000} embryos, from n=2 clutches; scale bars as indicated. Statistical analysis of data showing normal distribution (D'Agostino-Pearson test) was performed with unpaired t-test, not normally distributed data was analysed using non-parametric Mann-Whitney test, bars show mean ± SEM.

Figure 01

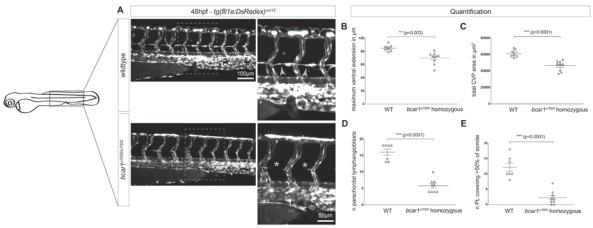

Figure 2: p130Cas is required for caudal vein plexus remodelling and initial lymphatic development in the trunk at 48hpf.
(A-E) Representative lightsheet microscopy images of live *bcar1*^{tv1000} and wildtype transgenic embryos at 48hpf and quantification of vascular parameters. Images are maximum intensity projections generated using FIJI software, lateral views of the trunk around the anal pore. *bcar1*^{tv1000} embryos fail to resolve the caudal vein plexus properly; the total vascularised area and ventral extension [indicated by blue arrow] from the dorsal aorta are significantly decreased (C, D). Furthermore, *bcar1*^{tv1000} embryos form only a reduced number of parachordal lymphangioblasts at the horizontal myoseptum (indicated by arrowheads/asterisks in zoom-in of A, area indicated by dashed box) and the majority of PL formed does not extend across the entire somite (E, F). Analysis was performed on 8 somites, 4 distal and 4 proximal from the ISV at the anal pore. 48hpf, n=8 wildtype, n=9 homozygous mutant embryos from n=2 clutches; scale bars as indicated. Statistical analysis of data showing normal distribution (D'Agostino-Pearson test) was performed with unpaired t-test, not normally distributed data was analysed using non-parametric Mann-Whitney test, bars show mean ± SEM.

Figure 02

Wiley DM, Kim JD, Hao J, Hong CC, Bautch VL, Jin SW. (2011) *Distinct signalling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein.* Nat. Cell Biol., 13, pp.686-692

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C02

The assembly of a mass spectrometry-based library of the guinea pig proteome as a tool for physiology research

P. Palmowski[1], R. Watson[1], N. Europe-finner[1], A. Treumann[2] and M. Taggart[1]

[1]Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK and [2]Newcastle University Protein and Proteome Analysis, Newcastle University, Newcastle upon Tyne, UK

Recent advances in liquid chromatography-mass spectrometry (LC-MS) approaches have facilitated the incorporation of proteomic studies to many experimental workflows. This has increased our capabilities to assess the changes of hundreds of proteins (abundances or post-translational modifications) occurring with particular insults; in turn, enabling one to interrogate the dynamic proteome-phenotype relationships underlying adaptations to cellular, tissue and organ physiology and pathophysiology challenges. In pursuing experiments to assess broad proteomic profiles from complex cells/tissues, particularly those seeking to take advantage of the high-throughput capabilities and reproducibility of Data-Independent Acquisition LC-MS platforms such as SWATH (Schubert OT *et al*., 2015), it is desirable to have accessible for reference an extensive background data resource. This is not always readily available if working with species/cells outside of the few very commonly used model systems. For example, the guinea pig is an excellent experimental model for many aspects of human physiology and pathophysiology - including maternal and fetal adapatations to pregnancy (Taggart & Mitchell 2009), cardiac excitation-contraction coupling (Taggart *et al*., 2014), airway drug responsiveness (Lexmond *et al*., 2018) and auditory somatosensory processes (Marks *et al*., 2018) - yet there is limited experimental information regarding the proteome available for this species. In an effort to overcome this obstacle, we sought to generate, via LC-MS/MS measurements, a spectral library of the guinea pig proteome.
Homogenates were prepared from 15 tissues (heart, skeletal muscle, brain, uterus, colon, placenta, ovaries, liver, pancreas, lung, kidney, intestines, duodenum, adipose) isolated from sacrificed guinea pigs (fetal- and adult) and each digested to peptides with exposure to trypsin. These tryptic digests were subjected to >200 LC-MS/MS runs to (i) extract peptide-specific information including retention time, m/z value, fragmentation pattern and amino acid sequence; and (ii) thereby, with reference to the guinea pig genome (version: January 2016), identify protein constituents of the proteome.

Analysis of >250,000 peptide-spectrum matches resulted in the construction of a library of 74,828 peptides (unique to individual proteins) that corresponded to 7692 proteins. This experimentally validated spectral library increases coverage of the guinea pig proteome >50-fold beyond that publicly available (Uniprot, Dec 2017). It thereby will furnish the research community with a comprehensive proteomic resource to enable (i) exploration of future molecular-phenotypic studies using (re-engaging) the guinea pig as an experimental model and (ii) assessment of cross-species responses to consistent physiological and pathophysiological challenges.

Schubert OT *et al*., (2015) Nat Protocol 10: 426-441.

Taggart MJ & Mithcell BF (2009) *Am J Physiol* 297, R525-545.

Taggart *et al*., (2014) Cardiovasc Res 104: 226-227.

Lexmond AJ *et al*., (2018) Drug Deliv Transl Res doi: 10.1007/s13346-018-0490-z.

Marks KL *et al*., (2018) Sci Transl Med 10: eaal3175

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C03

Spatial coding deficits in mouse models of dementia

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[1]Medical School, University of Exeter, Exeter, UK and [2]Eli Lilly UK, Surrey, UK

Dementias are associated with severe spatial memory deficits which likely arises due to dysfunction in hippocampal and parahippocampal circuits. These circuits rely on precise encoding of directional and velocity information for spatially-sensitive neurons, such as grid cells in the medial entorhinal cortex (mEC), to faithfully represent the environment. In recent years, rodent models in particular have allowed for the detailed understanding of this 'internal GPS'. In this study, high density silicon probe electrode arrays were stereotaxically implanted to examine the firing rate coding properties of mEC neurons in male rTg4510 mice (7-8 month old), a mouse model of tauopathy which displays severe spatial memory deficits. Animals were anaesthetised using isoflurane (4%) and maintained at 1-2% during surgery. After recovery, single-unit activity was recorded while animals explored either a linear track or open field. Speed, head direction and spatial firing scores were calculated for each cell and compared to a distribution of scores produced from shuffled data. Cells were classified as significant if their score was greater than the 95th percentile of the shuffled distribution.

Grid cell firing patterns were largely absent in rTg4510 mice (fig. 1), suggesting some, or all, of the information required for neural path integration is deficient

(WT: 36/150, rTg4510: 3/129, χ^2 (1) =18.63 p <0.0001, Chi-Square test). Interestingly, we found that head direction cells, a key component of any path integration system, are largely functionally intact (WT: 19/150, rTg4510: 12/129, χ^2 (1) =0.79 p=0.37, Chi-Square test). In contrast, neural representation of running speed information was significantly disturbed in a number of ways. Firstly, a significantly smaller proportion of mEC cells recorded from rTg4510 mice had firing rates correlated with running speed, when compared to the 95th centile of a shuffled distribution of data produced from 250 shuffles for each cell (WT: 96/150, rTg4510: 17/129 χ^2 (1) =74.3 p <0.05, Chi-Square test). Secondly, of those cells which are modulated by running speed a much greater proportion are negatively modulated (WT: 11/96, rTg4510: 6/17, χ^2 (1) =6.42, p =0.011, Chi-Square test). Finally, the power of local field potential oscillations in the theta and gamma frequency bands, which in wildtype mice are tightly linked to running speed, was shown to be invariant in rTg4510 mice, with increased locomotor activity having little effect on oscillatory properties (linear regression; WT: R^2=0.75, p<0.001, n=3, rTg4510: R^2=0.15, p=0.03, n=5). Our results using rodent models reveal deficits in locomotor speed encoding that are likely to severely impact the ability of animals to continuously update positional information and thus disrupt path integration systems in these mice and in dementia patients.

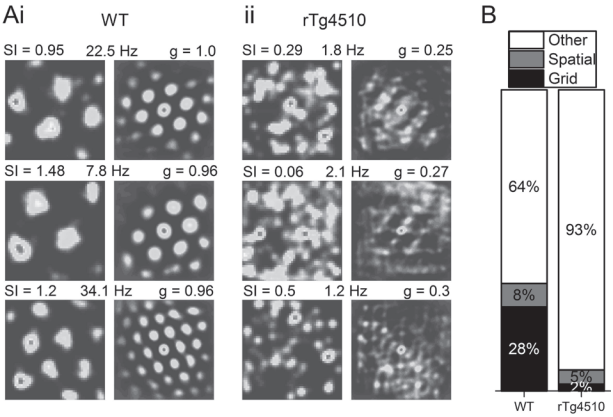

Figure1: Breakdown of grid cell periodicity in rTg4510 mice. A) Example spatial firing patterns of cells from WT (i) and rTg4510 (ii) mice in a 1.25 m square arena, displayed with grid score (g), spatial information content (SI) and peak firing rate across recording environment.. **B)** Proportions of grid and spatial non-grid cells greater than threshold (95th centile of score produced from shuffled data) in WT and rTg4510 mice.

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C04

Adult myocardial slices: a viable and highly functional platform to bridge the gap between *in vitro* and *in vivo* cardiac studies.

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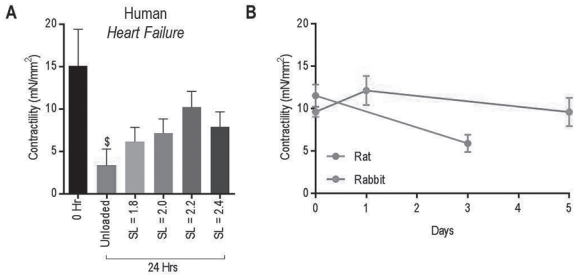
NHLI, Imperial College London, London, UK

Cardiovascular diseases remain the leading cause of mortality worldwide and their global burden is increasing. Over the last few decades, progress in cardiovascular research has been significantly hindered by a lack of appropriate research models. The vast majority of research is currently conducted using isolated cell preparations or *in vivo* animal studies. However, the data collected using current *in vitro* approaches is often oversimplified and this makes the translation of results from the laboratory to clinical trials challenging. Additionally, cardiac tissue undergoes a rapid remodelling when removed from the body, which has limited *in vitro* studies to acute time points. To date, there are no cardiovascular models that can be cultured without significant changes in cardiac structure and function. These factors have significantly hampered translational cardiac research and the development of novel therapeutics for cardiovascular diseases; it is imperative that this issue is addressed. What is required is a model that bridges the gap between *in vitro* and *in vivo* studies and that can be maintained in a physiological state for prolonged periods in culture. Myocardial slices are 100- to 400-μm-thick slices of living adult ventricular myocardium, prepared using a high-precision vibratome. They retain the multicellularity, complex architecture and physiology of adult cardiac tissue, and their thinness allows the diffusion of oxygen and other metabolic substrates into their innermost cells, maintaining viability in the absence of coronary perfusion *in vitro* (Nature Protocols,2017). Additionally, as multiple slices can be produced from a single specimen, throughput can be increased and the number of animals used can be reduced. We hypothesised that recreating the physiological *in vivo* cardiac environment was fundamental to maintain the structure and function of myocardial slices and prevent the remodelling that occurs in culture. By applying electromechanical stimulation and physiological preload to myocardial slices for prolonged periods we showed that numerous cardiac structural, functional and transcriptional properties are optimally maintained at a specific preload (sarcomere length (SL)=2.2μm) and this allowed us to culture myocardial slices generated from a preclinical animal model (rabbit) for 5 days without a loss of contractile function. When cultured at other SLs, to simulate cardiac unload and overload (SL=1.8, 2 or 2.4μm), slices displayed adaptive responses without changes in viability. Finally we have demonstrated that this platform can be applied to maintain human heart failure myocardial slices *in vitro*, making it particularly useful for translational and regenerative medicine research. This is the first approach that can successfully maintain adult cardiac tissue in a physiological state *in vitro*.



A) Contractility of human heart failure myocardial slices cultured for 24 hours (N=6) B) Contractility of chronically cultured myocardial slices. Both rat (grey) and rabbit (blue) myocardial slices were cultured with a preload of SL=2.2μm and were stimulated continuously at 1Hz. Rat myocardial slices were cultured for 3 days (72 hours, Day 0 – N=10, Day 3 – N=4) and rabbit myocardial slices were cultured for 5 days (120 hours, Day 0 – N=8, Day 1 - N=11, Day 5 – N=5)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C05

The regenerating zebrafish heart following cryoinjury: role for neuropilin 1 in epicardial activation and revascularisation.

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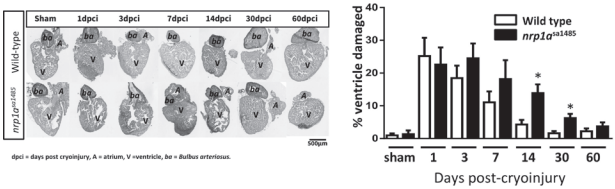
Unlike adult mammals, zebrafish (*Danio rerio*) are able to naturally regenerate their heart. We have used the cryoinjury model of myocardial infarction (MI) in the zebrafish to study heart regeneration. In this model, a copper filament is pre-cooled in liquid nitrogen and applied to the apex of the ventricle. Cryoinjury triggers in tissue necrosis and results in a severe apoptotic response and the generation of a scar. A key mechanism in zebrafish heart regeneration is the activation of the epicardium, leading to the establishment of a supporting scaffold for newly formed cardiomyocytes, angiogenesis and cytokine secretion. Neuropilins (NRPs) are cell surface co-receptors mediating functional signalling of kinase receptors for cytokines known to play critical roles in zebrafish heart regeneration, including Platelet-derived growth factor (PDGF), Vascular endothelial growth factor (VEGF), and Fibroblast growth factor (FGF). Herein, we investigated the role of neuropilins in the response of the zebrafish heart to cryoinjury and its subsequent regeneration. Zebrafish have four neuropilin isoforms, *nrp* 1a, 1b, 2a and 2b. We found that all isoforms were upregulated following cardiac cryoinjury and were strongly expressed by the activated epicardium. A *nrp1a* mutant, coding for a truncated, non-functional protein, shows a significant delay in heart regeneration in compar-

ison to wild type fish and displays a lasting collagen deposition. Importantly, epicardial cells from *nrp1a* mutant zebrafish heart explants display an impaired response to activation by cryoinjury and have a lower re-expression of the developmental gene Wilms' tumour 1. Moreover, the revascularisation of the heart is compromised: less new vessels are seen invading the injured region of the mutant hearts in comparison to wild-type. These results identify a key role for Nrp1 in zebrafish heart regeneration, mediated through epicardial activation and migration and revascularisation of the damaged area of the heart.



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We would like to thank the Bristish Heart Foundation for funding (PG/16/84/32464).

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C06

Consequences of glycocalyx destruction on renal hemodynamics and oxygen consumption in healthy rats

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Worldwide chronic kidney disease (CKD) is a fast-growing public health and socio-economic problem[1,2]. It is a multifaceted disease often coinciding with cardiovascular pathology and metabolic disorders and recognized as a cardiovascular risk factor[1]. Degradation of the endothelial glycocalyx (glx) is associated with CKD and disease progression. A final pathological progression of CKD is renal fibrosis which arises from glx damage and dysfunction[3]. Although many studies have demonstrated glx destruction in several pathological conditions[4, 5] a link between glx integrity and kidney function needs to be established in relation to CKD. The aim of the study was to assess the renal hemodynamics and oxygen homeostasis with and without the glx barrier. The hypothesis is the renal function and oxygen consumption will be altered.
Male Wistar Rats (250-350g, n=3) were anaesthetised with Isoflurane (inhaled, 2.5%), cannulas were placed in the left femoral artery for blood pressure (BP)

and in the vein for saline/FITC-inulin infusion for glomerular filtration rate (GFR) measurements; the left ureter was cannulated for left kidney urine collection. The glx removal was accomplished by venous infusion of an enzyme bolus mix (chondroitinase 0.087mU/g and hyaluronidase 15mU/g, a final volume of 200 μl in saline (0.9%)) 30 minutes before the experimental period. Blood and urine samples were collected before and after the enzyme mix bolus infusion. Spike2 Software was used to record BP, renal blood flow (RBF, Doppler flow probe placed around the left renal artery) and heart rate (HR). Renal oxygen consumption (QO2) was calculated from the arterio-venous difference in oxygen content and the oxygen delivery rate was obtained using the product of arterial oxygen content and RBF. Comparisons before and after enzyme mix bolus was performed using paired ttest. Values are means and ± S.E.M.

Glycocalyx destruction did not seem to affect BP (95.0±1.7 vs 87.1±4.1 mmHg, p>0.05), HR (313.4±22.2 vs 301.7±21.2 bpm, p>0.05), RBF (4.5±0.3 vs 5.5±0.9 ml/min, p>0.05), oxygen delivery rate (89.7±5.4 vs. 102.9±16.5 mmol/ml/min, p>0.05) and left kidney GFR (0.5±0.1 vs 0.7±0.1 ml/min, p>0.05). However, QO2 (6.3±0.7 vs 16.1±4.1 mmol/min, p>0.05) was numerical higher for each animal before and after enzyme mix bolus, although not significantly (P=0.x).

These preliminary data demonstrate that we have an experimental model in which we can quantify basic kidney physiology, i.e. renal hemodynamics and oxygen consumption. Although further experiments are needed (and currently ongoing) to elucidate the link

between glx degradation and renal function the data is suggesting that glx destruction could increase renal oxygen consumption and hence alters oxygen homeostasis in healthy kidneys.

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Kidney Research UK (D_005_20170330), British Heart Foundation (FS/14/2/30630 and PG/15/68/31717)

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C07

Male AKR1D1 (5β-reductase) knockout mice have altered pancreatic islet morphology and hormone secretion

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The enzyme 5β-reductase (AKR1D1) catalyses an essential step in bile acid synthesis, but in addition, controls intra-cellular steroid hormone availability by inactivation and the generation of 5β-reduced dihydrosteroid metabolites. Steroid hormones and bile acids are regulators of global lipid and carbohydrate metabolism. As disturbances in steroid hormone and bile acid metabolism have potent effects on metabolic health, we hypothesize that AKR1D1 may play a role in metabolic homeostasis. Secretion of the pancreatic hormones, insulin and glucagon, are dysregulated in metabolic disorders and the role of AKR1D1 in regulating glucose homeostasis and pancreatic function remains unexplored.

We generated a global AKR1D1 knockout (KO) mouse. After euthanasia, the pancreas was removed and weighed. Immunohistochemical and stereological techniques were used to define whole pancreas and islet morphology in KO (n=5) mice at 12 weeks of age (12w) compared against wild-type (WT; n=5) controls. Additionally, pancreatic islets were isolated from male WT (n=3) and KO (n=3) mice at 30w and insulin and glucagon secretion were assessed in static incubations. Data (mean±SEM) were assessed by Student's t-test.

At 12w, relative pancreas mass was decreased in AKR1D1 KO male mice compared to WT controls (g/kg: WT: 12.7±1.3, KO: 7.5±1.0, $P<0.05$), while there was no change in pancreatic mass in female mice. Pancreatic islet volume and relative beta-cell mass were decreased in male KO mice only, however, there was no alteration in alpha-cell mass. At 30w, insulin secretion was increased in isolated KO islets upon treatment with 1mM (basal) glucose (mean as % islet content: WT: 0.07±0.01, KO: 0.12±0.01, $P<0.05$), without any change in total islet insulin content. However, in response to 20mM glucose, the increase in insulin secretion was lower in KO islets when expressed relative to basal levels (WT: 3.5-fold change, KO: 2.6-fold change, $P=0.08$). Compared to WT controls, the KO islets failed to suppress glucagon release in the presence of 20mM glucose (mean as % change in glucagon secretion: WT: -29±20, KO: 61±14). Indeed, we observed a paradoxical increase in glucagon secretion with increasing glucose concentration (1mM glucose; WT: 5.8±1.1, KO: 7.4±3.9 pg/islet/hr. 20mM glucose; WT: 4.0±0.7, KO: 8.7±3.0 pg/islet/hr).

Whilst endogenous expression of AKR1D1 in the murine pancreatic islet is very low, alterations in steroid hormone and bile acid exposure have been shown to modify pancreatic islet cell function; AKR1D1 KO male mice have a dysregulation of insulin

and glucagon secretion, which may have profound effects on normal glucose homeostasis. The mechanisms underpinning the changes observed remain to be determined. Further characterization is warranted to define the role of AKR1D1 and to determine whether it has potential as a therapeutic target in metabolic disease.

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C08

Hypothyroidism induces hyperplasia of unilocular adipocytes in perirenal adipose tissue of the ovine fetus

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Thyroid hormones are important regulators of fetal growth, although their mechanism of action remains unclear. In the sheep fetus, thyroid hormone deficiency increases plasma insulin and leptin concentrations (1). This study investigated the effects of hypothyroidism on perirenal adipose tissue (PAT) development and adipose insulin signalling pathways in fetal sheep.

All procedures were performed under the UK Animals (Scientific Procedures) Act 1986. In 10 twin-bearing pregnant ewes at 105-110 days of gestation (d; term~145d) and under general anaesthesia (1.5% isoflurane in O_2/N_2O), one fetus was thyroidectomised (TX), while the other was sham-operated. At 143d, fetuses were delivered by Caesarean section under general anaesthesia (20 mg kg^{-1} maternal body weight sodium pentobarbitone i.v.). After maternal and fetal euthanasia (200 mg kg^{-1} sodium pentobarbitone i.v.), PAT was collected, weighed, and frozen or processed for histology and stereological assessment. Protein and mRNA content were determined by Western blotting and qRT-PCR. Data (mean±SEM) were assessed by Student's t-test.

Absolute and relative PAT mass was increased in TX fetuses compared to sham fetuses (absolute: sham 10.9±1.1g, TX 14.7±1.1g, $P<0.05$; relative: sham 3.1±0.3g/kg, TX 4.8±0.4g/kg $P<0.05$). This was due to a 2-fold increase in absolute and relative mass of unilocular (white) adipocytes (absolute: sham 3.3±0.6g, TX 7.2±0.7g, $P<0.001$; relative: sham 1.1±0.2g/kg, TX 2.3±0.3g/kg, $P<0.05$), with no change in the mass of multilocular (brown) adipocytes. Relative unilocular adipocyte mass correlated positively with plasma insulin ($r=0.76$, $P<0.001$) and leptin ($r=0.64$, $P<0.002$). Unilocular adipocyte perimeter was unaffected by TX which indicated that thyroid hormone deficiency in utero induced hyperplasia rather than hypertrophy of unilocular adipocytes. In PAT from TX fetuses, increases were observed in protein levels of proliferating cell nuclear antigen, the insulin-sensitive glucose

transporter-4 and phosphorylated S6-kinase, and in mRNA and protein levels of the differentiation marker, peroxisome proliferator-activated receptor-γ ($P<0.05$). In the ovine fetus, development of unilocular adipocyte mass in PAT is sensitive to changes in thyroid hormones, which may be related, in part, to altered insulin concentrations in utero. These findings have implications for the control of adipose function and leptin secretion before and after birth

Harris, S. E., Blasio, M. J., Davis, M. A., Kelly, A. C., Davenport, H. M., Wooding, F. B., Blache, D., Meredith, D., Anderson, M., Fowden, A. L., Limesand, S. W. and Forhead, A. J. (2017), Hypothyroidism *in utero* stimulates pancreatic beta cell proliferation and hyperinsulinaemia in the ovine fetus during late gestation. J Physiol, 595: 3331-3343.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

C09

Defining an efficient model for inducing obesity and metabolic syndrome in Wistar rats

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Obesity and metabolic syndrome are major health problems which often present a confounding therapeutic challenge. The composition of diet consumed has been implicated in these metabolic phenotypes, and rats have been used extensively to study them. However, different dietary models in both male and female rats have been stated in literature. The aim of this study was to explore different models and determine the most efficient model(s) of achieving diet-induced metabolic syndrome in Wistar rats. Wistar rats (n=48) of both sexes, weighing between 100-120 g, were used in this study. Rats were divided into gender pairs consisting of male and female pairs in four dietary groups: standard rats' chow, standard rats' chow + 60% sucrose-in-water solution, high-fat (60% margarine) and high-fat (60% pure butter) (n=6 for each gender), and fed for nine (9) weeks. Blood samples were collected via the retro-orbital sinus and then animals were euthanized with sodium pentobarbital. Body weight was measured using an electronic weighing scale, and body-mass Index (BMI) calculated from this. Fasting blood glucose was assessed, using blood obtained from a tail puncture, by the use of a glucometer, based on the glucose oxidase method of determination, while insulin concentration was determined using ELISA kits following manufacturer's protocols and read spectrophotometrically at a wavelength of 450 nm using a microplate reader. HDL- and total cholesterol were both determined using Randox® (UK) Lipid Profile Kit, as described by Roeschlau *et al*., 1974 and Young, 1995, respectively. Values

are expressed as means ± S.E.M., compared by one-way ANOVA. Body weight (185.5±4.3 vs 168.3±8.7g in control, $p<0.05$) and BMI (0.46±0.02 vs 0.46±0.02, $p<0.05$) were significantly raised in male rats fed with HFD (butter), while HDL cholesterol (28±1.2 vs 33±0.8mg/dl, $p<0.05$). Male rats fed on HFD (butter) also showed increases in other components of metabolic syndrome (Glucose=161.2±0.8 vs 99.8±0.4 mg/dl, Insulin=800±69 vs 512±78 ρmol/l, total cholesterol=96±3.2 vs 84±4.9mg/dl, $p<0.05$). The results of this study suggest that male rats fed on a high fat diet, with the fatty component gotten from pure butter, are an efficient means of creating a model for the study of diet-induced obesity and metabolic syndrome in Wistar rats.

Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. Z Klin Chem Klin Biochem. 1974;12(5):226.

Young DS. Quantitative determination of triglycerides. In effects of drugs on clinical lab. Tests, 4th ed. AACC Press; 1995. 20. Jacobs DR, Jr, Mebane IL, Bangdiwala

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PC01

C. elegans as a model for biology in space

M. Cooke[2,1], C. Deane[1], A. pollard[2], B. Philips[2], T. Etheridge[1] and N. Szewczyk[2]

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Survival of humanity is likely dependent on our ability to leave Earth and colonise other planetary bodies. However, space environment presents several environmental stressors that prove deleterious to human health, for example high cosmic radiation and prolonged microgravity. As such, a major obstacle preventing long duration space exploration is exponential decline in multiple physiological systems that would ultimately pose a serious risk to astronaut health. There is a need to understand how life responds to the challenges associated with life in space and develop effective countermeasures. However, elucidating mechanistic insight and initial demonstrations of pharmacologic countermeasures against flight-induced health decline are not possible in humans. Utilizing alternate systems remain at the forefront of understanding flight-induced health effects and determining the efficacy of new therapeutic strategies.

The microscopic worm *C. elegans* present several advantages as a model organism. As the first multicellular organism to have its genome sequenced, there exists a wealth of genetic tools available for studying pathways of interest. Sequencing of higher organisms' genome has shown 35% of *C. elegans* genes have homologues in humans and at least 42% of human disease-related genes have *C. elegans* homologues, with essential and highly connected genes being most frequently conserved. Importantly, the architecture of major organs (e.g. muscle) is almost

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PC01

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identical between *C. elegans* and mammals and many of the major molecular signalling and metabolic pathways are present in both systems.
C. elegans recapitulate the most consistently observed effects of spaceflight in rodents and astronauts. This worm has set developmental timings, allowing selection of developmental stages for controlled experimentation, which can also be designed for minimal astronaut input to reduce risk of experimental failure. Short generation time (3-4 d) also provide large sample size for studying population level effects of spaceflight in short time frames. Small hardware volume and low up-mass can minimise cost and launch requirements and be considered for inclusion in exploratory missions beyond the Van Allen belts to understand the adaptation/ survivability of life in deep space. *C. elegans* is, therefore, highly suited to the rapid exploration of novel biological pathways, particularly in the context of the experimental constraints present in spaceflight. We have successfully studied *C. elegans* adaptation to spaceflight on multiple previous missions on-board the ISS and, in our current flight experiment (Molecular Muscle Experiment, MME; launch Nov. 2018), use *C. elegans* to establish time, mechanisms of and countermeasures against neuromuscular decline in flight. Helping understand and counter health decline in low Earth orbit and beyond, studies in *C. elegans* can ultimately help prepare humanity for long-term habitation of space.

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PC02

Purinergic modulation of cell proliferation within the spinal cord stem cell niche

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There is some controversy over the neurogenic potential of the central canal region of the mammalian spinal cord. After injury, this region displays increased proliferation with migration of the newly-proliferated cells to the site of injury and differentiation into astrocytes and oligodendrocytes (1,2). It is thought that ependymal cells are involved in these functions but the role of the cerebrospinal fluid contacting cells (CSFcCs) is not known. Following injury, adenosine triphosphate (ATP) is released and it is known that there are purinergic $P2X_2$ receptors in this region (3), which could be involved as part of a damage recognition system, initiating cell proliferation. This study will look at the role of purinergic signalling in the ependymal cells and CSF-contacting cells (CSFcCs).
Spinal cord slices were obtained from Wistar rats anaesthetised with either sodium pentobarbitone 120 mg kg^{-1} or urethane 2 g kg^{-1} i.p. and transcardially perfused with sucrose artificial CSF. In acute slices, whole cell patch clamp recordings were made from both ependymal cells and CSFcCs. Local application of ATP (300 μM)

elicited fast depolarisations in a subgroup of CSFcCs (21.50 ± 5.16 mV), which was reduced by application of the broad-spectrum purinergic antagonist suramin (50 μM; 9.73 ± 3.03 mV). The $P2X_{2/3,3}$-specific antagonist A317491 (1 μM) had variable effects on the magnitude of the ATP-triggered depolarisations, with no effect in some, partial reduction and complete reduction of depolarisation in others. Immunohistochemical analysis of the P2X subtypes in this area revealed a sub-population of CSFcCs, predominantly in the ventral area of the region around the central canal that expressed $P2X_3$ in addition to $P2X_2$-containing receptors indicated by previous research. The remainder of the CSFcC population and the ependymal stem cells responded to ATP or UTP with a hyperpolarisation (-5.64 ± 1.98 mV) which was not affected by application of suramin (-5.64 ± 1.98 mV).
Modulation of purinergic signalling had no effect on proliferation rate in spinal cord slices over a 4 hour time period, nor did it affect the survival rate of the newly-proliferated cells over 5 days in organotypic slice cultures. In this model, inhibition of purinergic signalling with suramin reduced the migration of newly-proliferated ependymal cells away from the central canal, while inhibition of the breakdown of ATP by ARL 67156 facilitated this migration.
The presence of fast acute responses to ATP and a spatial variation in receptor subtypes suggests a role for purinergic signalling in the functioning of the cell types in this area including the response of the cell types to damage.

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This work was fiunded by the BBSRC

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PC03

Live Imaging of a Zebrafish Model to Investigate Mechanisms Underlying Foreign Body Reaction

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Introduction: When a suture is placed in a patient the foreign body reaction (FBR) is initiated. The tissue response to a foreign body following implantation of a biomaterial leads to matrix deposition and fibrous encapsulation. This process is

overlaid by the same inflammatory response triggered by any acute wound and if the foreign body is left in situ develops into a "chronic wound". This pathway is of obvious interest to the surgeon (and patient) in terms of pathological process that may result in fibrosis, adhesions, contractures, excessive scarring and even failure of implanted devices. So far the study of complex interactions between cells and molecules during FBR has been limited due to the lack of live *in vivo* imaging opportunities. The genetic tractability of the zebrafish combined with its translucency and amenability for imaging make it an excellent candidate model organism for studying the mechanisms underlying FBR.

Methods: An 8-0 nylon or vicryl suture was placed anterior to the tail fin of a wild type or transgenic adult zebrafish expressing various fluorescent reporter genes. A small "pull through" wound simulated the acute condition as a control. Macrophage, neutrophil, blood vessel and inflammatory cytokine response to the site was visualised upto 28days. The colony stimulating factor 1 receptor (csf1ra) mutant zebrafish was used, which has a limited immune response. Confocal and multiphoton microscopy was used to capture images.

Results: Real time *in vivo* imaging analysis showed a prolonged and exaggerated inflammatory response to a suture compared to an acute wound. The inflammatory response is exaggerated and extended with increased numbers of macrophages, many of which have assumed a foreign body giant cell-like phenotype near the suture. We also observe a higher number, and increased activity, of neutrophils around the suture compared to an acute wound- this is maintained for the full 28days of our study. The observed inflammatory response to the more bio-reactive vicryl suture was exacerbated in comparison to the less immune-stimulating nylon suture. We also observe an avascular region adjacent to the foreign body, not maintained in acutely wounded fish. The avascular zone correlates with a fibrous encapsulated region surrounding the suture. In immune deficient fish we see a more limited FBR and thus smaller avascular zone.

Implications: This excessive inflammatory response may be contributing towards adverse outcomes associated with FBR such as fibrosis and scarring. We can gain mechanistic insight into inflammation, the FBR avascular zone and excessive matrix deposition around a foreign body in real time using the zebrafish model. We have shown that the bio-reactivity of a material dictates the scale of FBR and that the host response also modifies this. This has implications for the use and design of surgically implantable devices.

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PC04

Impairments in neural correlates of contextual memory in a mouse model of amyloidopathy

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Impairments in spatial navigation and memory are present at an early stage in Alzheimer's disease (AD). One of the brain areas affected earliest in AD is the medial entorhinal cortex (MEC), which plays a key role in the neural encoding of spatial information via a variety of functional subclasses of neurons. The activity of many of these cell types is strongly modulated by theta frequency (6-12 Hz) oscillations, the power and frequency of which increase with the speed at which the animal is moving. Gamma frequency (30-120 Hz) oscillations are also dependent on running speed. Previous work suggests that the running speed-theta frequency relationship is steeper in familiar environments than in novel ones, suggesting a neural correlate of contextual spatial memory. Despite the important role that the MEC plays in spatial navigation and memory, the effects of novel and familiar environmental stimuli on the MEC neuronal dynamics in mouse models of amyloidopathy remain unexplored. We have investigated this electrophysiological correlate of contextual memory in the J20 mice.

5-6 months old male J20 (n=8) and wild-type (WT) (n=7) mice were anaesthetised via isoflurane (2%) inhalation and surgically implanted with recording probes in the dorsal MEC. Following a recovery period, mice were placed in an open arena and encouraged to explore it for 15 minutes twice a day, for 4 days. On the fifth day all mice were placed in a novel arena. During each of these exploration periods, mice were tethered to a multi-channel recording system and the local field potential signals in the MEC acquired. All data are presented as mean ± SEM.

A repeated measures ANOVA revealed a significant interaction between genotype and recording day in the slope of theta frequency vs running speed relationships ($p<0.005$). Pairwise comparisons showed that the slope of theta frequency vs running speed increased with familiarity in WT mice (0.001 ± 0.007 Hz/cms^{-1} day 1 vs. 0.032 ± 0.006 Hz/cms^{-1} day 4, $p<0.005$; 0.032 ± 0.006 Hz/cms^{-1} day 4 vs. 0.008 ± 0.004 Hz/cms^{-1} day 5, $p<0.05$). In contrast, in J20 mice there was no significant pairwise difference between recording days, suggesting significant impairments in the neural mechanisms encoding environmental familiarity. Equivalent effects were observed in the high gamma power (60-120 Hz) vs running speed relationship, whereby there was a significant interaction between genotype and

day ($p<0.05$, repeated measures ANOVA). Similarly, pairwise comparisons showed an increase of the slope with familiarity in WT mice (8.3 ± 6.5 mV^2Hz^{-1}/cms^{-1} day 1 vs. 36.7 ± 12.3 $mV^2Hz\text{-}1/cms^{-1}$ day 4, $p<0.005$; 36.7 ± 12.3 $mV^2Hz\text{-}1/cms^{-1}$ day 4 vs. 20.4 ± 9.6 mV^2Hz^{-1}/cms^{-1} day 5, $p<0.05$) whilst in J20 mice there were no significant changes. These results provide evidence of a significant disruption to the neuronal network mechanism underlying spatial contextual memory in J20 mice.

Where applicable, the authors confirm that the experiments described here conform with the Physiological Society ethical requirements.

PC05

The effects of TNF-α and IL-1β on cardiac cellular calcium handling and contractility

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Cytokines including tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) are known to mediate systolic and diastolic myocardial dysfunction in sepsis. To provide a cellular basis we previously measured the effects of TNF-α on rat cardiac myocytes. Only changes to systolic function were observed [1]. In the present, more detailed study we separately measured the effects of 50 ng/ml TNF-α and IL-1β on intracellular Ca handling and contractility in sheep cardiac myocytes. Our aim was to provide further insight into the cellular basis of cytokine mediated myocardial dysfunction while using a translationally more relevant large mammal model.

All procedures used accord with the Animals (Scientific Procedures) Act, UK, 1986 and Directive 2010/63/EU of the European Parliament. Ventricular myocytes were isolated from 18 month sheep, loaded with the Ca indicator Fura-2 then field stimulated at 0.5 Hz. Ca and contractility dynamics were measured by epi-fluorescent photometry and video sarcomere detection respectively.

50 ng/ml TNF-α and IL-1β reduced SR Ca content by 27 % and 41 % respectively, accounting for a 17 % and 24 % reduction each in the amplitude of systolic Ca. With TNF-α, the reduction of systolic Ca was associated with a 20 % reduction in sarcomere shortening, however IL-1β increased sarcomere shortening. The rate constant of systolic Ca decay was unaffected by both TNF-α and IL-1β. In response to both TNF-α and IL-1β the onset of systolic Ca decrease was rapid (<10 s), however, in 71 % of TNF-α treated cells and 52 % IL-1β treated cells, this was preceded by an immediate increase (58 and 52 % respectively) in systolic Ca, lasting for only 1-3 beats. TNF-α decreased diastolic Ca by 4 %, which was unchanged by IL-1β whilst diastolic sarcomere length was decreased by both cytokines.

Though both cytokines reduced SR thence systolic Ca, only with TNF-α did this translate to reduced cell contractility. Given contractility was enhanced by IL-1β, and the fact that resting sarcomere length was decreased despite no increase

in diastolic Ca suggest both cytokines may increase myofilament sensitivity. The mechanism by which SR Ca content is reduced remains unclear. Whilst SERCA impairment does not appear to play a role, the initial and short-lived increase of systolic Ca may suggest a role for ryanodine receptor potentiation [2]; a phenomenon we are currently investigating. While the changes to cell function produced by both cytokines can account for certain aspects of myocardial depression in sepsis, their individual contribution to the clinical phenotype may be more complex than previously thought. It is interesting that changes to diastolic function were only observed in sheep, highlighting the need to consider translationally important species dependent effects.

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Thank you to Professor Andrew Trafford for provision of sheep ventricular myocytes.

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PC06

Stress and altered pubertal timing: is the limbic brain the key?

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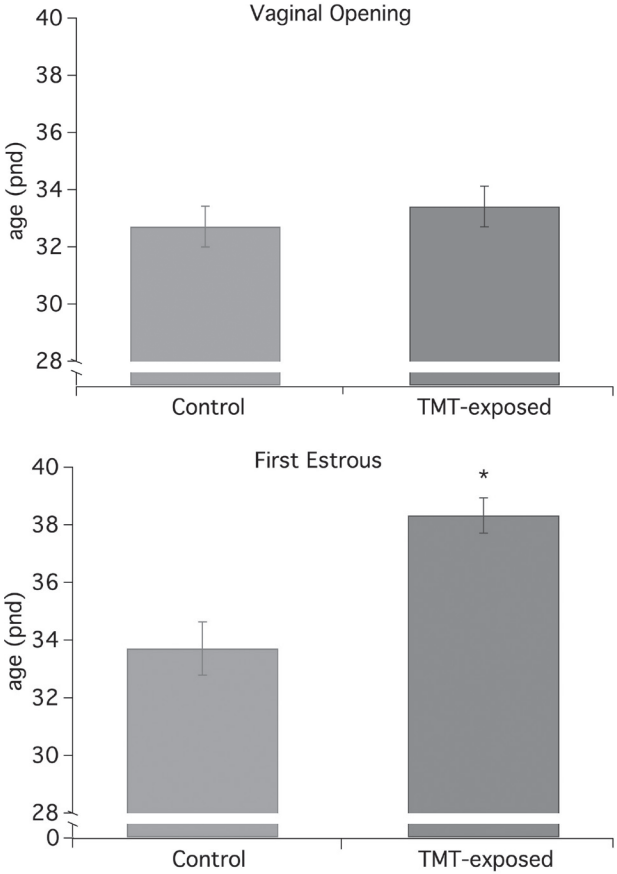
In humans and animals, exposure to post-traumatic stress (PTSD) is associated with altered pubertal timing, however the underlying neural mechanisms have yet to be discovered. Hypothalamic kisspeptin, a core component of the gonadotropin-releasing hormone (GnRH) pulse generator regulating the hypothalamus-pituitary-gonadal axis (HPG), is crucial for pubertal development (Li *et al.*, 2012). Extra-hypothalamic kisspeptin neuronal populations exist in the medial amygdala (MeA), a key limbic brain structure implicated in anxiety and emotional processing. The amygdala modulates the HPG axis, by exerting an inhibitory brake over pubertal timing (Li *et al.*, 2015). Exposure to psychosocial stressors delays puberty in rodents (Kinsey-Jones *et al.*, 2010). We will test the hypothesis that psychosocial stress, processed by the MeA, is relayed to the hypothalamus GnRH pulse generator to delay puberty. Female C57BL/6Jmice were exposed to chronic psychosocial stress (predator odour; an extract from fox faeces; 2,4,5-Trimethylthiazole (TMT)) for 14 days from postnatal day (pnd) 21. The onset of puberty was monitored from pnd 28 using classic markers, such as vaginal opening (VO) and first vaginal estrous (FE). Anxiety-related behaviour was assessed using behavioural tests including, the Elevated Plus Maze (EPM), Light/Dark box and Social Interaction (sniffing, following, grooming and mounting). These behaviour tests were performed before (pnd 19-20), during (pnd 28-29) and after exposure to TMT (pnd 40-41). The TMT-exposed mice showed a significant delay of 5 days

to the onset of FE. There was no effect of TMT exposure on the onset of VO. The TMT-exposed mice spent significantly less time in the open arm of the EPM during and after TMT exposure. Furthermore, the TMT-exposed mice spent significantly more time in the dark compartment of the Light/Dark box on pnd 28 only. Social Interaction was not affected by TMT exposure. These data suggest that psychosocial stress impacts negatively on the timing of puberty onset. Early exposure to predator odour, a classic model for PTSD, delays puberty in female mice and haslong term consequences of enhanced anxiety behaviour.

Figure 1 and 2. The effect of psychosocial stress (TMT exposure) on day of VO and FE in mice. (A) Day of VO for control group (n=6) and TMT exposed group (n=10). For control group mean = 32.71; SEM = 0.71. For TMT exposed group mean = 33.42; SEM = 0.71. (B) Day of FE for control group (n=6) and TMT exposed group (n=10). For control group mean = 33.71; SEM = 0.92. For TMT exposed group mean = 38.33; SEM = 0.607196. Welch Test to compare control vs. TMT exposed for FE P=0.0004.



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PC07

Long term study of renovascular hypertension: renal oxygenation, blood flow and sympathetic nerve activity

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Hypertension has become a serious and expensive public health problem with more than 1.3 billion people affected worldwide. In ~90% of cases the cause of high blood pressure is unknown (primary or essential hypertension), and a significant proportion (~7-14%) of hypertensive patients remain drug resistance or intolerant to medication. Thus, better understanding of the control of blood pressure in physiological and pathological circumstances is necessary to understand basic mechanisms and unveil new therapeutic targets. Unequivocal evidence supports chronic activation of the sympathetic nervous system as a characteristic of hypertension. We and others have found evidence suggesting that hyperactivity in afferent nerves from the carotid bodies and renal dysfunction are mutually involved in the initiation, maintenance and progression of hypertension. We hypothesize that the kidney and carotid body cooperate synergistically to optimize organ blood flow and tissue oxygen homeostasis. Under pathological conditions of severe or prolonged (renal) tissue hypoxia, these sensors become over activated resulting in hypertension. We aim to map the time course of events during the genesis of hypertension and how it relates with long-term renal dysfunction and sympathetic hyperactivity with or without carotid body imput. In order to do this we use radio-telemetry for long-term recording of blood pressure, tissue partial pressure of oxygen, and sympathetic nerve activity in awake and freely moving rats with inducible renovascular hypertension (2-kidney-1-clip) and compare these with relevant sham-operated controls. A solid state pressure sensor is placed in the abdominal aorta to measure blood pressure in male Wistar Rats with or without 2-kidney-1-clip to generate renovascular hypertension, and with or without sectioning the carotid sinus nerve. In two sub-groups of animals, either partial

pressure of oxygen from the renal cortex or renal nerve activity is measured continuously. After 6 weeks of follow-up, renal hemodynamics and oxygen metabolism is evaluated under thiobutabarbital anaesthesia. This animal model of physiology and pathophysiology has been recently established in Exeter; with successful removal of the carotid body input resulting in 9±0.01% mean arterial pressure reduction. If we understand more about long-term regulation and the sequence of events leading to hypoxia, hypoperfusion and coincident elevation of sympathetic nervous system activity, we might be able to intervene earlier, more targeted, and change the progression of renal and cardiovascular disease.

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PC08

Novel electromechanical method for measuring toxic response in aquatic invertebrates

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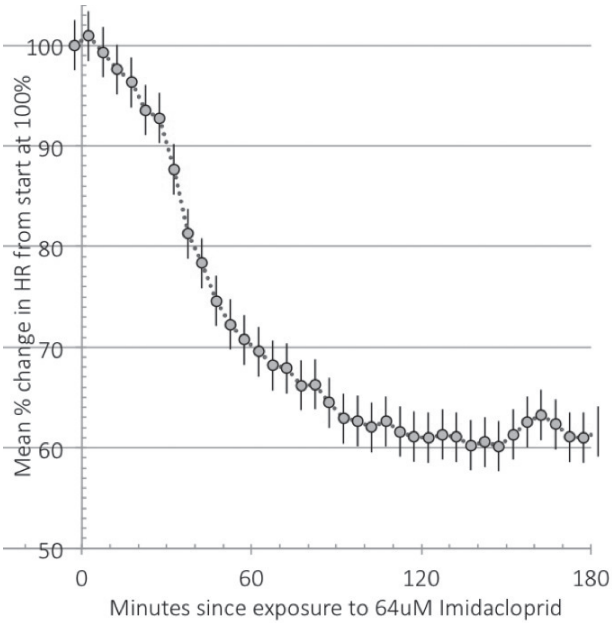
Using principles from ECG, a novel method of recording electromechanical movement (EMM) from whole *Daphnia* was created. Comparative investigation using simultaneous high speed film revealed EMM signals derived from feeding limbs rather than the heart (fig1). Dependent two-tailed t-tests were performed for paired samples. Null hypotheses assumed no difference in the randomness of the data, therefore paired data would be the same. Comparing heart vs EMM data $p=1.39x10^{-9}$, there is 99% confidence the EMM does not record the heart. Leg vs EMM data p=0.083, giving 95% confidence that EMM records leg movement. Leg vs heart data were also paired, $p=4.52Ex10^{-10}$, showing 99% confidence that the leg movement is not a proxy for heart activity.

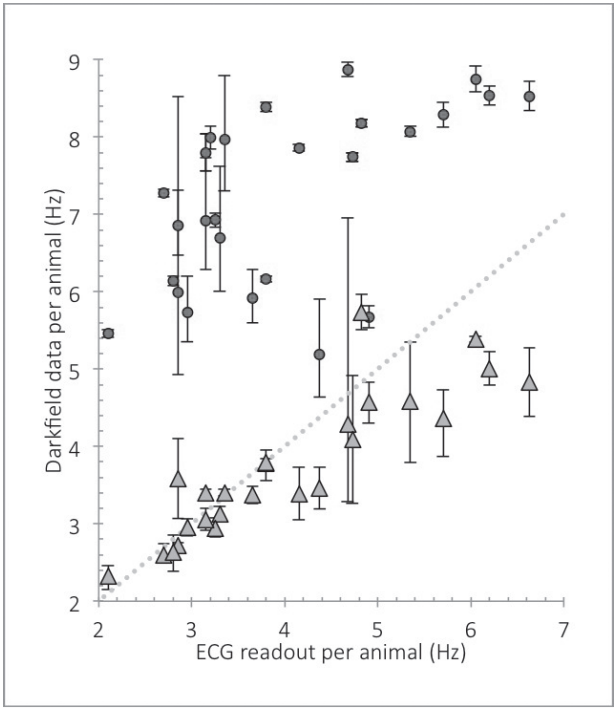
Observation of limb activity has been used as a toxicity measure in previous studies (Ren et al 2015, Lari et al 2017). Legs beat a continuous repeating rhythm to push waterborne food particles towards the digestive opening. EMM signals from entire swarms have been performed (Freund et al 2002) however this has never been done with individual *Daphnia*; dedicated equipment was built for the task. The EMM method presents an efficient real time method of recording limb beat patterns in *Daphnia.* It is here applied to the investigation of toxic chemicals in the freshwater environment.

The neonicotinoids' effect on pollinators are a hot topic both politically and scientifically. These nicotine receptor agonists show biphasic hormesis giving low dose stimulation and high dose inhibition. Imidacloprid is popular and well studied of these compounds, as a partial agonist, it specifically binds and activates post

synaptic nicotinergic acetylcholine receptors (nACHR) in the central nervous system. Binding is nearly irreversible, holding the channel open to cause continuous nervous system stimulation, which eventually leads to the death of the neuron. This affinity is equivalent across the neonicotinoids. Permanent effects accumulate with time. Neonicotinoids are primarily used in corn seed coating. There they form a prophylactic which is taken up by growing plant tissues to provide long-lasting toxicity against insect pests. However, these are also highly leachable compounds prone to water source contamination. The presence of neonicotinoids is frequent and long term due to their long 1000+ day half life in soil and freshwater streams. This work compares results obtained via the EMM method to current peer-reviewed data on imidacloprid exposure in *Daphnia* (Qi et al 2018, Zein et al 2014). Using equivalent doses we show that this method produces directly comparable results. The effects of various concentrations of imidacloprid over time are shown, an example is shown in figure 2. Whole *Daphnia* EMM measurement removes the manual/ observation step from current practice, which may improve accuracy as well as efficiency in toxicological studies.



Legend: Heart (circles) and leg (triangles) beats (Hz) of individual *Daphnia* filmed in darkfield are compared to EMM readouts of the same individuals (on the x-axis.) Error bars show standard deviation. The grey diagonal indicates a Line of Identity through which we would expect data to pass should the EMM readout, and either the heart, or leg, movement data be one and the same. Leg movement data is close to the line of identity while the heart data is not, suggesting that the legs, not the heart, are the source of Whole *Daphnia* EMM based readouts. P values are given in the main text.



Legend: Leg movement rate is normalised to 100% for individual starting values so that rate after exposure is given in comparison to the start point. Records were taken at 5 minute intervals. Circles show mean, error bars show standard error for population data. Here we see that leg rate declines and then plateaus at around 60.8% of the original mean rate when 64uM imidacloprid is applied.

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PC09

"Of Mice and Men" to "Animal Farm": Selecting animal models based on homologous protein primary sequences?

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The choice of rodents as primary models for the investigation and better understanding of human diseases has been challenged (1-2). Poor results in clinical trials of promising lead compounds have prompted calls to refocus at least some of translational research onto larger animals with closer molecular matches and disease-relevant physiology to human (3). 27-28 million cattle, sheep and pigs were slaughtered in the UK in 2014 (4) while large farm animals represented 1,3% of the 4 million total animals used in scientific procedures over the same time period – a fraction of the numbers slaughtered for food (5). Rodents represented 83% of all animals used in regulated experimental procedures. There is an opportunity to make better use of our resources and reduce our reliance on rodent models through the choice of large animal tissue where evidence exists to support its use. The molecular match of potential large animal target proteins to human homologs could form part of that evidence-based decision making process in choosing appropriate animal models. We have undertaken a preliminary study and present data obtained by comparing the primary sequence of ten distinct proteins in rodents (rat & mouse) and in large farm animals (pigs, cattle & sheep) with their homologous human sequences. The ten proteins were: kv2.1; kv1.5; BKCa; TREK-1; Kir6.2; vasopressin receptor V1a; TGFb1; CTLA-4; IL23; and CD55; and have roles ranging from regulating membrane potential to monitoring cellular metabolism or modulating inflammatory/immune responses. We used three separate approaches: protein primary sequence alignment; phylogenetic analysis; and similar protein searches (UniProt UniRef) to establish species ranking with respect to their match to human. All three approaches yielded similar patterns with farm animal homologs closer-sometimes dramatically so- to the human homolog than either of the rodent forms in 7 out of 9 proteins. CD55's poor homologous sequence alignment resulted in its exclusion from further study. The ranking of the two remaining proteins, kir6.2 and avpr1a, appeared to be approach-dependant- with both sequence alignment and phylogenetic analysis finding the rodent kir6.2 to be closer to the human than any of the farm animal homologs while phylogenetic analysis found only the rodent vasopressin receptor to be closer to human. As for the similar protein search method, a rodent homolog was never found to be closer to human than any of the farm animal homologs. We propose that bioinformatics approaches, as described here, can help inform decision making in the selection of appropriate model species by giving consideration to the molecular identity of the target proteins. Better use of abattoir tissues may be an ethical and effective route to improvements in translational and pharmaceutical research.

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