International Society for
Autonomic Neuroscience
established 1995
www.autonomicneuroscience.info

13th Congress of
The International Society for Autonomic Neuroscience (ISAN)

Abstract Book (Birmingham)

Thursday 25th - Saturday 27th July 2024

UNIVERSITY OF BIRMINGHAM

Contents

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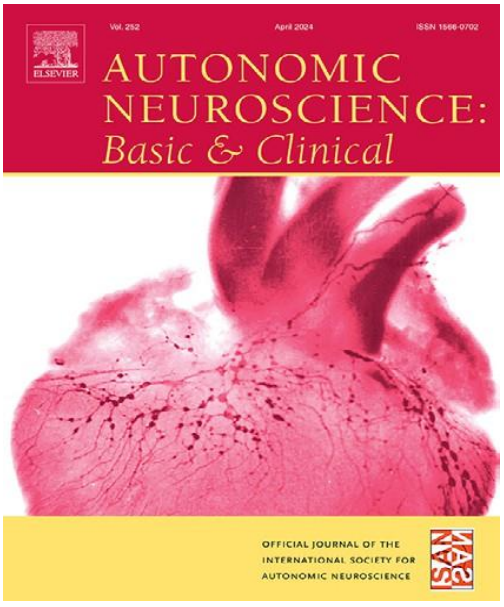

Welcome to ISAN 2024

Welcome to an English summer at the University of Birmingham. Together with our colleagues in Oxford, we're delighted to welcome you, and look forward to sharing the most recent advances in Autonomic Neuroscience. While the Oxford part of the meeting has had a cardiovascular focus, the Birmingham part is truly pan-autonomic, from fundamental science to clinical practice.

Birmingham has long had an interest in autonomic control. John Coote, former Bowman Professor of Physiology here (1983–2003), was well-known for the central control the cardiovascular system, and had a particular interest in exercise and high-altitude physiology. That field of work is also active in our modern 'School of Sport, Exercise and Rehabilitation Sciences', and respiratory pathophysiology as it relates to the cardiovascular system in our 'Department of Biomedical Sciences'. Professor Janice Marshall (our last Professor of Physiology and Bowman Professor of Physiology), who has recently been awarded for 50 years of service to the university, is our transgenerational autonomic representative, with her work covering both animal and human research. I particularly recommend her published work on the autonomic regulation of the vascular supply of blood vessels and her work on cardiovascular disease markers.

It's a particular pleasure for me to welcome back to Birmingham several former colleagues and former students who are now based overseas, having spread their wings and now returning, if fleetingly, to share their work. We have a large contingent of researchers from both New Zealand and Australia, and it will be great to hear how the field has grown there (both organically and through acquisitions!) over the last few years.

For those of you embarking on a career in the field, or approaching it tangentially from other fields, can I recommend the International Society for Autonomic Neuroscience: it's a run with a small, international team who have plans for additional research-focussed and education-focussed activities between more. Come along to the AGM to find out more, and to find out where we might be in 2026! The local organizing committee would also like to thank ISAN for the 10 early career research scholarships, proving financial assistance to attend this meeting.

The Biotechnology and Biological Sciences Research Council (BBSRC) has also generously sponsored one of Keynotes, and also provided support for ECRs; it will be great to hear more about their new initiative to support gut-brain-inflammation interactions. Our official journal, 'Autonomic Neuroscience: Basic and Clinical', also continues to support our Burnstock prizes, and this year also provides support to some of two of the keynote speaker. The Physiological Society has provided key support, particularly for the Oxford part of the meeting; long may it remain a bastion of autonomic research, not just in the UK but internationally! Let's hope that their journal does the same …!

It's also great to have the support of ADInstruments and CED, laboratory (and teaching!) favourites of ours here in Birmingham. Here in Birmingham we're particularly wedded to the ADInstruments data acquisition and teaching resources, while several of us have off-the-shelf and custom-designed equipment from CED.

So, whatever your subspecialty interest, we hope you'll find much of interest in our sessions here in Birmingham, and that you enjoy your time in the historic heart of the University!

Dr Keith Brain
Chair of the Birmingham ISAN 2024 LOC, on behalf of its members

Organising Committee

LOC (Birmingham)

Prof Janice Marshall
Dr Keith Brain
Dr Andy Holmes
Dr Andy Coney
Dr Davor Pavlovic

LOC (Oxford)

Prof David Paterson
Prof Neil Herring

IPC Chair

Prof Julian Paton

Key Information

Conference Secretariat Opening Hours

Day	Opening Times
Thursday 25th July	1pm – 8pm
Friday 26th May	8am – 5:30pm
Saturday 27th May	8am – 4:30pm

Conference Social Events

Event	Date/Time	Venue
Welcome Reception	**Thursday 25th July** 6pm – 8pm	Great Hall, Aston Webb, University of Birmingham R6, Red Zone – shown on Campus Map, page 33
Conference Dinner	**Friday 26th July** 7pm - Midnight	Council House, Victoria Square, Birmingham, B1 1BB

Conference Programme

Thursday 25th July 2024

Time	Session/ Activity	Venue
13:00 - 14:00	**Welcome Refreshments and Registration**	Great Hall
14:00 - 15:10	**Keynote: Andrew Allen, University of Melbourne, Australia** Vital interactions: Exploring the relationships between respiratory and autonomic neural networks. Sponsor: Elsevier (with an introduction to Autonomic Neuroscience: Basic and Clinical)	Bramall
15:10 - 16:00	**Poster Session 1** **Theme: Basic - Bioelectronic Medicine** **P1 - Alberto Esteban-Linares** – A microfabricated Parylene cuff electrode for branched nerve stimulation **P2 - Dzifa Kwaku** – Exploring Hemodynamic Responses to Electrical Stimulation of Renal Nerves: A Potential Therapeutic Approach for Drug-Resistant Hypertension **Theme: Basic - Cardiovascular** **P5 - Aparaajita Bhatnaagar** – Microgravity induced impairment of baroreflex sensitivity in rats is associated	Great Hall

	with sympathovagal imbalance but not with changes in structure of carotid artery **P6 - Larissa Correa** – Temporal profile of changes in cholinesterase activity induced by Ketamine-Xylazine anaesthesia **P7 - Yu-Wen Dai** – A study of Schwann cells in human and murine heart **P8 - Mohanad Mahdi** – Correlation of staging and risk factors with cardiovascular autonomic neuropathy in patients with type II diabetes mellitus **P9 - Thais Silva** – Galectin-3 Inhibitor Modulates Autonomic Nervous System **P10 - Finbar Argus** - A Computation Model of the Postganglionic Sympathetic Neuron for predicting drug response **Theme: Basic - Gut and metabolism** **P25 - Tomoya Sawamura** – Evidence that inhibitory regulation of oxytocinergic neurons to the spinal defecation center is manifested by hindpaw inflammatory pain in rats **Theme: Basic - Integrative Control** **P29 - Joost Wagenar** – Towards sustainable scientific data management solutions in the age of scale and multi-modal data-integration **P30 - Mabelle Lin** – Scaffold Mapping Tools for Mapping Data to Anatomical Scaffolds **P31 - Christian Reynolds** – Exposure to a diet rich in linoleic acid promotes nociceptive hypersensitivity and elevated systemic blood pressure in both spinal-intact and spinalized rats **P32 - Deborah Romeu** – Exploring the connections between C1 and liver-related DMV neurons involved in the autonomic control of glucose homeostasis **P33 - Karla Sampaio** – Volatile and injectable anaesthetics effects on cardiorespiratory and biochemical parameters in rats: enlightening anaesthetic choice according to the outcome studied	

	Theme: Basic - Neuroscience **P41 - Elise Collard** – Methodologies for Vagus ElectroNeuroGram (VENG) analysis **P42 - Anna Katharina Kau** – Food perception promotes autonomic response to anticipate consumption and guides motivated behaviour **P43 - Ko Yamanaka** – Gene expression and cardiovascular effects of nucleus tractus solitarii dopamine D1 receptors in stress-induced hypertension and its counteraction by exercise **P51 - Pippa Wittenberg** – On the regulation of arterial blood pressure by an intracranial baroreceptor **Theme: Clinical - Cardiovascular** **P52 - Angelica Carandina** – Indoor air pollution impacts cardiovascular autonomic control during sleep and inflammatory profile **P53 - Peter Latchman** – Neurologically based cardiovascular risk in young men after COVID-19 **Theme: Clinical - Integrative Control** **P59 - Thalia Babbage** – Effect of YMCA GoldFit exercise participation on the chemoreflex control of breathing in older adults **Theme: Clinical - Neuroscience** **P60 - Costanza Scatà** – Characterization of dysautonomia in patients with Ehlers-Danlos Syndrome and its relationship with anxiety and sleep quality. The importance of comprehensive care	
15:30 - 16:00	**Afternoon Refreshments**	Great Hall
16:00 - 18:00	**Symposium Presentations Session 1** **Theme: Bioelectronic Medicine**	Bramall

	Symposium Title: Bioelectronic Medicine *Chairs: Ellis Meng, University of Southern California and Victor Pikov, Medipace Inc* **O1 - Olujimi Ajijola, University of California** – Axonal modulation therapy for bioelectronic treatment of cardiovascular diseases (20 minutes) **O2 - Chris Wilson, Loma Linda University** – Saving premature infants from sudden death using vagus nerve stimulation (20 minutes) **O3 – Oliver Armitage, BIOS Health - Personalised bioelectronic stimulation using real-time biomarkers** (20 minutes) **O4 - Daniel Chew, Galvani Bioelectronics** – Recent studies on splenic nerve stimulation in pigs and humans (20 minutes) **O5 - Victor Pikov, Medipace** – Inc preliminary data on sacral nerve stimulation in humans to treat colitis (20 minutes)	
16:00 - 18:00	**Symposium Presentations Session 1** **Theme: Cardiovascular** **Symposium Title: Central nervous control of blood pressure, brain blood flow, and cognitive health** *Chairs: Emma Hart, University of Bristol and Sam Lucas, University of Birmingham* **O6 - Alex Gourine, University College London** – Regulation of arterial blood pressure by an intracranial baroreceptor (20 minutes) **O7 - Fiona McBryde, University of Auckland** – Defending blood flow to the brain in hypertension, diabetes and ischemic stroke (20 minutes) **O8 - Emma Hart, University of Bristol** – Cerebrovascular variants and the role of the selfish brain in hypertension (20 minutes) **O9 - Sam Lucas, University of Birmingham** – Cerebral blood flow, aging and physiological stress (20	G33

	minutes) **<u>Selected Abstracts</u>** **O10 - Emi Narai** – Divergent roles of orexinergic and non-orexinergic neurons in the hypothalamic perifornical area in eliciting behavioral and autonomic cardiovascular responses (20 minutes) **O11 - Tina Vrabec** – Closed loop control of Norepinephrine to attenuate sympathetic response to cardiac stressors (20 minutes)	
16:00 - 18:00	**Symposium Presentations Session 1** **Theme: Integrative control** **Symposium Title: Brainstem integrator for viscerosensation and autonomic regulation** *Chairs: Andrew M Allen, University of Melbourne and Julian FR Paton, University of Auckland* **O12 - Ambre Linossier, Aix-Marseille University** – GABAergic neurons of the pre-Bötzinger complex regulate respiratory sinus arrhythmia and blood pressure via the autonomic nervous system (20 minutes) **O13 - Zoe Adams, University of Bristol** – New insights into deep stimulation for correcting autonomic imbalance (20 minutes) **O14 - James P Fisher, University of Auckland** – Sympathetic neurocirculatory responses to central chemoreflex activation in human hypertension (20 minutes) **O15 - Stefan Trapp, University College London, UK** – The two faces of GLP-1: gut hormone and NTS neurotransmitter – does it matter for physiology? (20 minutes) **O16 - Stuart McDougall, University of Melbourne, Australia** – CaMPing in the brainstem (20 minutes) **<u>Selected Abstracts</u>** **O17 - Beata Graff** – Higher periodicity and irregularity of respiratory pattern during wakefulness in subjects with newly diagnosed sleep apnea (20 minutes)	WG5

18:00 - 20:00	**Welcome Reception**	Great Hall

Friday 26th July 2024

Time	Session/ Activity	Venue
08:00 - 09:00	**Registration and Refreshments**	Great Hall
09:00 - 11:00	**Symposium Presentations Session 2** **Theme: Bioelectronics** **Symposium Title: Utilising NIH SPARC resources for ANS research** *Chairs: Peter Hunter and David Paterson, Auckland Bioengineering Institute* **O18 - Jack Cheng, Ariege Bizanti & Mabelle Lin** – Spatial mapping of neural data with 3D scaffolds (20 minutes) **O19 - Nicole Pelot & Joost Wagenaar** – Dashboard of the human vagus: from gross to micro anatomy (20 minutes) **O20 - Igor Efimov, David Brooks & Alan Garny** – Data visualisation and modelling to support cardiovascular control studies (20 minutes) **O21 - John Osborn & Maryann Martone** – SPARC Infrastructure supporting Functional studies of vagal stimulation (20 minutes) **Selected Abstracts** **O22 - Zoe Adams** – Sympathetic action potential recruitment during transient cessation of deep brain stimulation for severe refractory hypertension (20 minutes)	Bramall

	O23 - David Nickerson – Interactive maps of nerve-organ anatomy and function on the SPARC Portal (20 minutes)	
09:00 - 11:00	**Symposium Presentations Session 2** **Theme: Cardiovascular** **Symposium Title: You're so vein" – new insights into the function and autonomic regulation of the 'forgotten' venous circulation** *Chairs: Fiona McBryde and James Fisher, University of Auckland* **O24 - Tonja Emans, University of Auckland** – Sympathetic regulation of the 'forgotten' venous circulation – a new therapeutic target for blood pressure control? (20 minutes) **O25 - Mickey Fan, University of Auckland** – Venous capacity and compliance in hypertensive adults: influence of hypoxia and hyperoxia (20 minutes) **O26 - Melanie Dani, Imperial College London** – New horizons in the ageing autonomic nervous system: orthostatic hypotension and supine hypertension (20 minutes) **Selected Abstracts** **O27 - Wolfgang Kummer** – Tuft cells trigger neurogenic inflammation in the urethra (20 minutes) **O28 - Kayleigh Scotcher** – Immunohistochemical characterisation of the superior hypogastric plexus and hypogastric nerves in the adult human (20 minutes) **O29 - Adriana Barbosa Ribeiro** – The treatment of oral inflammation influenced the regulation of hemodynamic responses such as blood pressure and heart rate variability (20 minutes)	G33

09:00 - 11:00	**Symposium Presentations Session 2** **Theme: Integrative control** **Symposium Title: Anatomical, functional, and molecular mapping of autonomic innervation of organs** *Chairs: Jack Cheng and John Tompkins* **O30 - Jack Cheng, University of Central Florida** - Spinal afferent innervation in flat-mounts of the rat heart and stomach: anterograde tracing (20 minutes) **O31 - Jerry Yu, USA** – Integration of Molecular, Morphological, and Physiological Aspects of Mechanosensors in the Lung (20 minutes) **O32 - John Tompkins, USA** – Morphology, synaptics, and membrane excitability of intracardiac neurons from mice, pigs and humans: targets of clinical neuromodulation for cardiac disease (20 minutes) **O33 - Hanjun Wang, USA** – Cardiac Spinal Afferents: A New Therapeutic Target in Treating Chronic Heart Failure (20 minutes)	WG5
11:00 - 11:30	**Mid-morning Refreshments**	Great Hall
11:30 - 13:00	**Poster Session 2** **Theme: Basic - Bioelectronic Medicine** **P3 - Sue Tappan** – The collaborative SPARC Portal for peripheral neuromodulation data, modelling and device design	Great Hall

Theme: Basic - Cardiovascular

P11 - Amatul Ahmad – Plasticity in human intracardiac neurons from patients with Atrial Fibrillation

P12 - Om Lata Bhagat – Heart Rate Variability during Short-Term Head-Down Tilt

P13 - Vera K. Jandackova – Heart rate variability and air pollution

P14 - Yuma Sato – The midbrain dopaminergic areas mediate the cardiovascular response induced by the activation of the lateral habenula

P15 - Thais Silva – Mice with overexpression of vesicular acetylcholine transporter have increased cardiac parasympathetic activity

P16 - Hidefumi Waki – Exercise Mitigates Stress-Induced Hypertension and Brain Inflammation by Modulating Molecular Pathways in the Amygdala and Hypothalamus

P17 - Jinan Saboune – Muscle sympathetic nerve activity responses to the cold pressor test in women across the third and fourth decades of life

P18 - Thatiany Jardim Batista – Liraglutide improvement of chemoreflex function in ovariectomized female rats is associated with a reduction in oxidative stress

Theme: Basic - Gut and metabolism

P26 - Natsufu Yuki – Involvement of neurons projecting from the hypothalamus to the medullary raphe in stress-induced defecation in rats

Theme: Basic - Integrative Control

P34 - Maryann Martone – The SPARC SCKAN multi-species knowledge base of ANS connectivity

	P35 - Vitor Minassa – Comparing cardiorespiratory responses after organophosphate poisoning in Wistar and pre-hypertensive SHR in situ **P36 - Zeljka Minic** – Supratentorial inhibition of regional sympathetic nerve activity **P37 - Simon McMullan** – Inhibitory control of motor and respiratory components of orienting by the substantia nigra pars reticulata is state-dependent **Theme: Basic - Neuroscience** **P44 - Jack Cheng** – Quantitative Analysis of CGRP-IR Afferent Axons in the Mouse Stomach Using Zeiss Arivis Vision4D for Automated Tracing **P45 - Maci Heal** – Automated 3D stereology for cell counting using artificial intelligence technology yields rapid, unbiased results analogous to manual stereological methods **P46 - Alla Korsak** – On the mechanisms of exercise-induced autonomic neuroplasticity **P47 - Davi Oliveira** – Neuroanatomy of the Thoracic Sympathetic Neural Networks revealed by whole-torso imaging **Theme: Clinical - Cardiovascular** **P54 - Daniel D. Hodgkiss** – Ergogenic effects of invasive and non-invasive spinal cord stimulation strategies following spinal cord injury: a case-series **P55 - Gabriel Rodrigues** – Cardiac vagal modulation and inflammation are upregulated in exceptional human longevity **P64 - Sakshi Gupta** – What is the most effective treatment for congenital Long QT syndrome?	

	P65 - Amna Nazzar – Examining sex differences in autonomic function and its influence on cardiovascular disease prevalence in women; particularly stress induced cardiomyopathy **P66 - Georgia Pratley** – The use of selective noradrenaline reuptake inhibitors and serotonin-noradrenaline reuptake inhibitors to treat psychiatric disorders as a cause of Takotsubo Syndrome **Theme: Clinical - Neuroscience** **P61 - Jin Yong Jeong** – Thoracic sympathetic nerve block before sympathectomy for irreversible treatment of primary hyperhidrosis	
13:00 - 14:00	**Lunch**	Great Hall
14:00 - 15:00	**Keynote 2: Melanie Gareau, University of California, USA** “It takes guts: The developing microbiota-gut-brain axis” Sponsor: BBSRC **BBSRC Presentation**	Bramall
15:00 - 15:30	**Afternoon Refreshments**	Great Hall
15:30 - 17:30	**Symposium Presentations Session 3** **Theme: Integrative control** **Symposium Title: Neuroimaging of cardiovascular and respiratory control in humans**	Great Hall

	Chair: Vaughan Macefield, Monash University **O34 - Luke Henderson, University of Sydney** – Identification of the sympathetic connectome in humans (20 minutes) **O35 - Rebecca Glarin, University of Melbourne** – Functional brainstem imaging of sympathetic drive using MSNA coupled fMRI at ultra-high field (20 minutes) **O36 - Kevin Shoemaker, University of Western Ontario** – The roles of the forebrain in cardiovascular control in exercising humans (20 minutes) **O37 - Olivia Harrison, University of Otago** – Ultra-high-field imaging of networks related to breathing and breathlessness (20 minutes) **Selected Abstracts** **O38 - Marie-Claire Seeley** – The manifestation of Autonomic Disorders in Post-Acute Sequelae of Covid-19 (PASC) (20 minutes) **O39 - Mariya Patel** – Resting-state brain activity, sympathetic outflow and vascular function in adiposity – investigating the link using combined magnetoencephalography and microneurography (20 minutes)	
15:30 - 17:30	**Symposium Presentations Session 3** **Theme: Bioelectronics** **Symposium Title: Working towards selective vagus nerve stimulation to modulate autonomic function**	Bramall

	Chairs: Lindsea Booth, Florey Institute and Alexander Gourine, University College London **O40 - James Fallon, University of Melbourne** – Stimulation parameters for directional vagus nerve stimulation (20 minutes) **O41 - Nicole Thompson, University College London** – Deciphering the anatomical and functional organisation of the cervical vagus for spatially selective neuromodulation (20 minutes) **O42 - Stuart McDougall, The Florey** - Selectively targeting the afferent vagus (20 minutes) **Selected Abstracts** **O43 - Svetlana Mastitskaya** – Neural mechanisms of cardioprotection (20 minutes) **O44 - Julia Shanks** – Cardiac vagal nerve activity increases during exercise to enhance coronary artery blood flow (20 minutes)	
15:30 - 17:30	**Symposium Presentations Session 3** **Theme: Cardiovascular** **Symposium Title: Breaking news in cardiac autonomic regulation** *Chair: Keith Brain, University of Birmingham* **Selected Abstracts** **O45 - Elizabeth Akin** – Use of high-resolution, live-cell imaging to investigate the molecular mechanisms of sympathoexcitation in cardiac sympathetic neurons (15 minutes)	G33

	O46 - Mark Badrov – Sympathetic response to exercise predicts exercise capacity of patients with heart failure across the left ventricular ejection fraction spectrum (15 minutes) **O47 - Nadja Zeltner** – A modular platform to generate functional sympathetic neuron-innervated heart assembloids (15 minutes) **O48 - Carol Bussey** – Anti-arrhythmic potential of P2X3 inhibition (15 minutes) **O49 - Claire Feetham** – PrRP/GPR10 signalling has an important role in energy balance and cardiovascular regulation (15 minutes) **O50 - Shaoping Hou** – Transplanting embryonic neural progenitor cells to restore cardio-electric disorders following spinal cord injury (15 minutes) **O51 - Mridula Pachen** – Addressing the enigma of treating heart failure with preserved ejection fraction with P2X3 receptor antagonism (15 minutes) **O52 - Arianna Scalco** – Cardiac sympathetic innervation is disrupted in a mouse model of hypertension-induced heart failure (15 minutes)	
15:30 - 17:30	**Symposium Presentations Session 3** **Theme: Gut and Metabolism** **Symposium Title: Recent insights into the role of the vagus nerve in brain-gut communication and therapeutic implications of vagus nerve stimulation in the treatment of gastrointestinal disorders** *Chairs: Valentin Pavlov, Feinstein Institutes for Medical Research and Bruno Bonaz, CHU Grenoble*	WG5

	O53 - Nicole Pelot, Duke University – Quantified anatomy of human vagus nerves from brainstem to abdomen (20 minutes) **O54 - Sophie Payne, Bionics Institute** – Fixing faulty plumbing with better wiring: current and future vagus nerve stimulation approaches for Crohn's disease (20 minutes) **O55 - Qasim Aziz, Queen Mary University of London** – Role of the vagus nerve in modulating visceral pain hypersensitivity, intestinal permeability and inflammation in health and GI disease (20 minutes) **O56 - Bruno Bonaz, CHU Grenoble** – Invasive vagus nerve stimulation in Crohn's disease: A 10-year prospective study follow-up (20 minutes) **Selected Abstracts** **O57 - Warren Grill** – Sacral nerve stimulation to control colonic motility (20 minutes) **O58 - Yvonne Hsu** – Differential developmental blueprints of organ-intrinsic nervous systems (20 minutes)	
17:30 - 19:00	**Free Time**	
19:00 - 00:00	**Dinner**	Council House

Saturday 27th July 2024

Time	Session/ Activity	Venue
08:00 - 09:00	**Registration and Refreshments**	Great Hall
09:00 - 11:00	**Symposium Presentations Session 4** **Theme: ECR focus: Breaking abstracts** *Chair: Dimitris Nathanael, James Saleeb-Mousa and Andy Holmes* **Selected Abstracts** **O59 - Ariege Bizanti** – Remodeling of Ventricular Catecholaminergic Axons Following Chronic Intermittent Hypoxia in Mice (15 minutes) **O60 - Demitris Nathanael** – CD73 inhibition reverses chronic hypoxia induced carotid body hyperactivity (15 minutes) **O61 - Joe Braun** – A neural footprint of central cardiovascular control during acute mental stress in humans (15 minutes) **O62 - Giada Cattelan** – In vitro modelling of the neurocardiac junction with a novel human iPSC-based co-culture system: new perspectives for the investigation of cardiac autonomic regulation (15 minutes) **O63 - Olivia Gold** – The accelerator and brake modulatory system of carotid body sensitivity (15 minutes)	Bramall

	O64 - Marie-Claire Seeley – Functional Brain Imaging Reveals Cerebral Hypoperfusion Patterns in Postural Orthostatic Tachycardia Syndrome (POTS): A Retrospective Study (15 minutes) **O65 - Nirupama Unnikrishnan** – Stromal cell derived factor-1 (SDF-1) acts on CXCR4 and CXCR7 in the rostral ventrolateral medulla (RVLM) to regulate blood pressure (15 minutes)	
09:00 - 11:00	**Symposium Presentations Session 4** **Theme: Gut and Metabolism** **Symposium Title: Targeting GI vasodilatory hormones for the treatment of postprandial syndromes in autonomic disorders** *Chair: Cyndya A. Shibao, Vanderbilt Autonomic Dysfunction Center* **O66 - Christopher Mathias, Queen Square Institute of Neurology** – Postprandial syndromes in autonomic disorders: pathophysiology and treatment (20 minutes) **O67 - Cyndya A. Shibao, Vanderbilt Autonomic Dysfunction Center** – Increased Glucose-dependent insulinotropic polypeptide (GIP) in postprandial syndromes (20 minutes) **O68 - Simon Veedfald, University of Copenhagen** – Neural modulation of entero-pancreatic hormone secretion (20 minutes) **O69 - Sophie Woge Nielsen** – Glucose-dependent insulinotropic polypeptide receptor antagonism in humans (20 minutes)	G33

09:00 - 11:00	**Symposium Presentations Session 4** **Theme: Integrative control** **Symposium Title: Neural control & autonomic regulation during exercise: recent innovations** *Chairs: Satoshi Koba, Tottori University and Marc Kaufman, Penn State College of Medicine* **O70 - Masaki Mizuno, University of Texas Southwestern Medical Center** – An integrative approach to better understand the mechanisms of the exercise pressor reflex in health and disease (20 minutes) **O71 - Markus Amann, University of Utah** – The exercise pressor reflex: a flow-raising or a pressure-raising mechanism? (20 minutes) **O72 - Satoshi Koba, Tottori University** – Subcortical circuit mechanisms for central command regulation of sympatho-motor coordination (20 minutes) **O73 - Vaughan Macefield, Monash University** – The relative contributions of central command and the metaboreflex to the increases in sympathetic vasoconstrictor drive to contracting muscle (20 minutes) **Selected Abstracts** **O74 - Jonathan Moore** – Effect of pulmonary artery mechanoreceptor input on sympathetic vasomotor outflow during exercise in healthy humans (20 minutes) **O75 - Julia Shanks** – Reinstating respiratory sinus arrhythmia in heart failure improves cardiac responses to exercise (20 minutes)	WG5
11:00 - 11:15	**Mid-morning Refreshments**	Great Hall

11:15 - 13:15	**Symposium Presentations Session 5** **Theme: Gut and Metabolism** **Symposium Title: Glucose sensing affecting autonomic activity** *Chairs: Fiona McBryde and Pratik Thakkar, University of Auckland* **O76 - Stefan Trapp, University College London** – Are GLP-1 producing pre-proglucagon neurons of the lower brainstem a useful target for obesity and diabetes treatment? (20 minutes) **O77 - Silvia V Conde, NOVA Medical School** – Carotid body, autonomic function and dysmetabolism: is there something new under the sun? (20 minutes) **O78 - Pratik Thakkar, University of Auckland –** GLP1 receptor agonist ameliorates high blood pressure and high blood sugar in a rat model of “glucotension” (20 minutes) **Selected Abstracts** **O80 - Alessandra Occhinegro** – The blockade of sympathetic mediated inhibition of immunity improves bacterial clearance in pigs (20 minutes)	Bramall
11:15 - 13:15	**Symposium Presentations Session 5** **Theme: Integrative control** **Symposium Title: Bidirectional association between depression and autonomic nervous system alteration: new insights into therapeutic strategies** *Chairs: Nicola Montano, University of Milan and Caroline Sévoz-Couche, Sorbonne Université*	G33

	O81 - Andrea Sgoifo, University of Parma – Antidepressant activity and cardioprotective effects of endocannabinoid neuromodulation enhancement in socially stressed rats (20 minutes) **O82 - Caroline Sévoz-Couche, Sorbonne Université** – Evaluation of Ketamine effects on autonomic nervous system in patients with depressive disorders (20 minutes) **O83 - Xiaoran Zhang, Sun Yat-sen University** – Mesenchymal Stromal Cells Alleviate Murine Depressive and Anxiety-like Behaviors via a Lung Vagal-to-Brain Axis (20 minutes) **O84 - Angelica Carandina, University of Milan** – The effects of repetitive transcranial magnetic stimulation (rTMS) and transcutaneous auricular vagal nerve stimulation (tVNS) on depressive symptoms: evidence from DEPONEST and DIGEST studies (20 minutes) **Selected Abstracts** **O85 - Ayse Dereli** – Cardiorespiratory Responses to Hypercapnia Are Altered Differentially in Different Types of Epilepsy Models (20 minutes) **O86 - Amanda Marshall** – Exploring the interaction of hypermobility with brain fog induction in people with Postural Orthostatic Tachycardia using lower body negative pressure (20 minutes)	
11:15 - 13:15	**Symposium Presentations Session 5** **Theme: Bioelectronics** **Symposium Title: Interrogating the physiology of the human vagus nerve** *Chair: Vaughan Macefield Monash University* **O87 - Nicole Pelot, Duke University** – Anatomical parameterization and physiological validation of	WG5

	computational modelling of vagus nerve stimulation (20 minutes) **O88 - Matteo Maria Ottaviani, University of Ancona** – Ultrasound-guided microneurography of the human vagus nerve (20 minutes) **O89 - David Farmer, Monash University** – 'Single-unit recordings of vagal neurones with cardiac rhythmicity in the human (20 minutes) **O90 - Mikaela Patros, Monash University** – Activation of vagal axons by vagal nerve stimulation (20 minutes) **Selected Abstracts** **O91 - Joana Sacramento** – Blockade of P2X3 ATP receptors decreases carotid body-mediated hypoxic ventilatory responses in high-fat rats (20 minutes)	
13:15 - 14:00	**Lunch**	Great Hall
13:15 - 14:00	**ISAN AGM** *Chair: Valentine Pavlov* International Secretary - Vaughan Macefield	Great Hall
14:00 - 15:00	**Keynote 3: Jessica Filosa, Augusta University, USA** Blood pressure variability impaired neurovascular outputs in middle-aged mice	Bramall

15:00 - 16:00	**Poster Session 3** **Theme: Basic - Bioelectronic Medicine** **P4 - Dzifa Kwaku** – Three-Dimensional Reconstruction of Renal Tissue: Mapping Renal Nerve Trajectories **Theme: Basic - Cardiovascular** **P19 - Jack Cheng** – Identification of Spinal Afferent Innervation in the Rat Heart: Atria and Ventricles: Anterograde Tracing **P20 - Carol T. Bussey** – Autonomic mechanisms of disturbed circadian rhythm in the diabetic heart **P21 - Rubens Fazan Jr.** – Cardiovascular variability and baroreflex function are altered in rats with femoral artery catheterization **P22 - Gabriel Gavazza Noé** – Distinct autonomic effects of single and intermittent chlorpyrifos exposure in the contextual fear conditioning test in rats **P23 - Daryl Briggs, James Hunt, Spardha Raut** – High-resolution ex-vivo structural and functional analysis of sympathetic innervation using a novel confocal fluorescence technique **P24 - Katharina Scherschel** – Neuro-glial interaction in the heart **Theme: Basic - Integrative Control** **P38 - Samantha Kraft** – Integrated Dashboard for large-scale visualization of the anatomical connectivity of the human Vagus Nerve **P39 - Mabelle Lin** – Mapping the Vagus Nerve with Anatomical Scaffolds	Great Hall

	P40 - Karla Rodrigues – Respiratory pattern and responses to hypercapnia of adenosine A2A knockout mice submitted to sustained hypoxia **<u>Theme: Basic - Neuroscience</u>** **P48 - Rui Chang** – Differential developmental blueprints of organ-intrinsic nervous systems **P49 - Olivia Gold** – Mechanisms underlying long-term facilitation in the carotid body **P50 - Song Yao** – Blockade of CCR2 receptors in the brain prevents hypertension in renovascular hypertensive rats **<u>Theme: Clinical - Cardiovascular</u>** **P56 - Riccardo Asnaghi** – Autonomic Impairment in Parkinson's Disease and Multiple System Atrophy Patients during Valsalva Maneuver **P57 - Helio Salgado** – Modulation of Oral Microbiota and Inflammatory Cytokines in Hypertensive and Healthy Complete Denture Wearers **P58 - Harvey Walsh** – Effects of interval versus continuous exercise on cerebral vascular flow-mediated dilatation **<u>Theme: Clinical - Neuroscience</u>** **P62 - Sae Uchida** – The basal forebrain cholinergic system linking olfaction and cognitive function: from basic studies to clinical application **<u>Theme: Clinical - Gut and Metabolism</u>** **P63 - Rasmus Syberg Rasmussen** – The gut hormone GIP contributes to the postprandial gastrointestinal hyperaemia in humans	

15:30 - 16:00	**Afternoon Refreshments**	Great Hall
16:00 - 16:30	**Closing Ceremony**	Bramall

1

- Senate Chamber/Senate Meeting Room
- Great Hall Rotunda
- Stairs
- Toilets
- Disabled Toilets
- Lift

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- Great Hall
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- Beale Room
- The Bramall
- Lecture Theatre – G33, WG5
- Stairs
- Toilets
- Disabled Toilets
- Lift

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- Disabled Toilets
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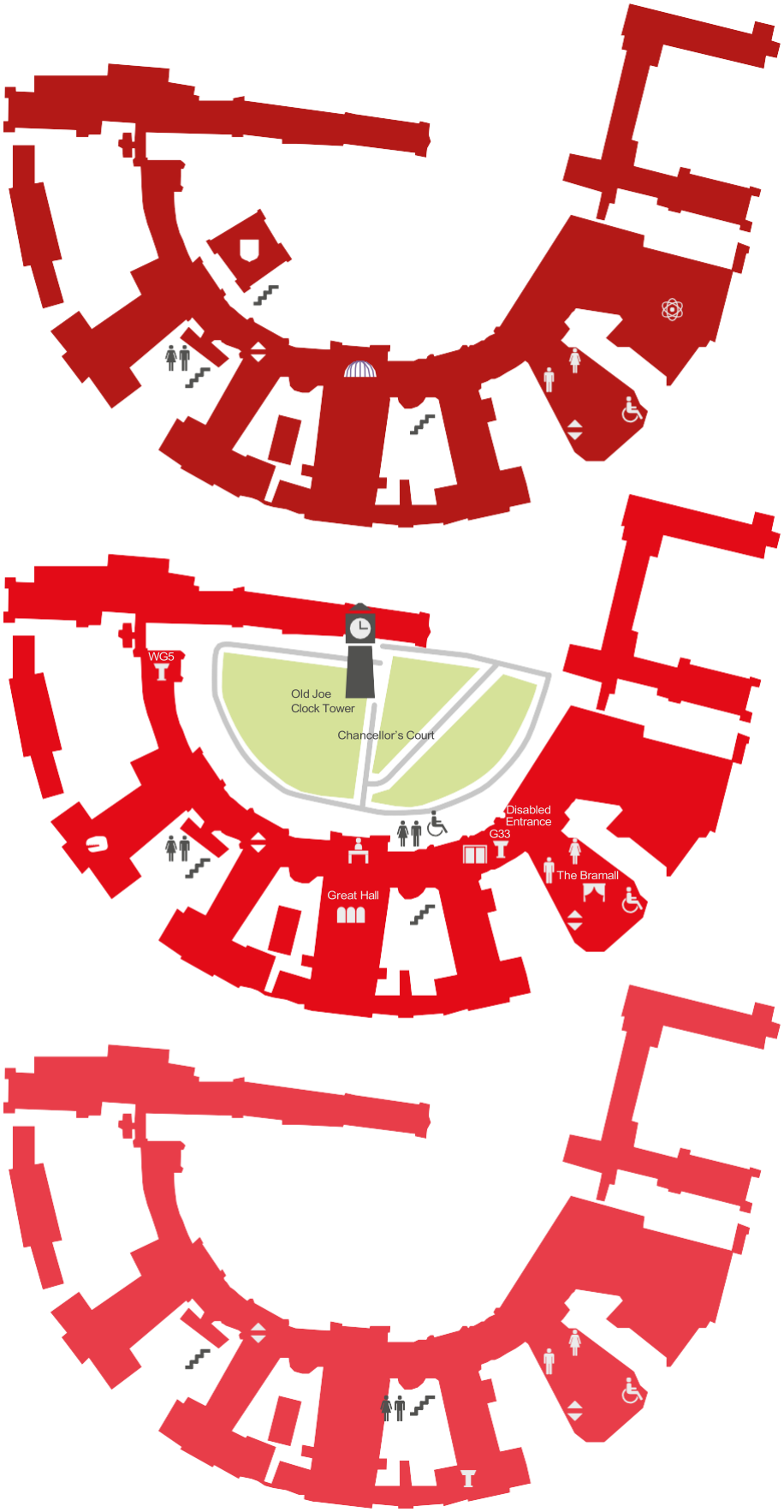


Campus Map

Edgbaston Campus Map

Red Zone
- R0 The Harding Building
- R1 Law Building
- R2 Frankland Building
- R3 Hills Building
- R4 Aston Webb – Lapworth Museum
- R5 Aston Webb – B Block
- R6 Aston Webb – Great Hall
- R7 Aston Webb – Student Hub
- R8 Physics West
- R9 Nuffield
- R10 Physics East
- R11 Medical Physics
- R12 Bramall Music Building
- R13 Poynting Building
- R14 Barber Institute of Fine Arts
- R15 Watson Building
- R16 Arts Building
- R17 Ashley Building
- R18 Strathcona Building
- R19 Education Building
- R20 J G Smith Building
- R21 Muirhead Tower
- R23 University Centre
- R24 Staff House
- R26 Geography
- R27 Biosciences Building
- R28 Murray Learning Centre
- R29 The Alan Walters Building
- R30 Main Library
- R31 Collaborative Teaching Laboratory
- R32 Teaching and Learning Building
- R33 Fry Building
- R34 Cuore

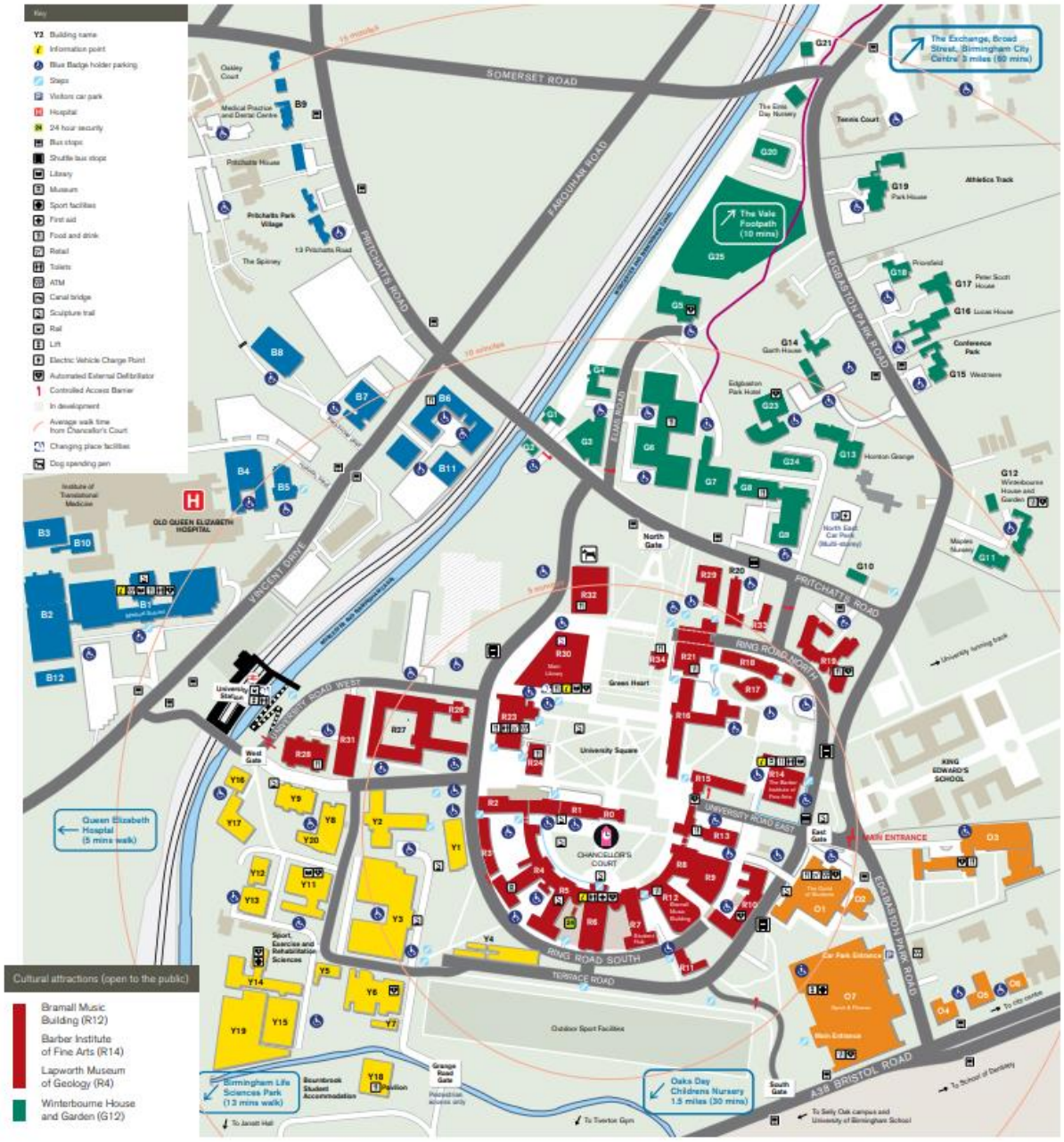
Blue Zone
- B1 Medical School
- B2 Institute of Biomedical Research including IBR West
- B3 Wellcome Clinical Research Facility
- B4 Robert Aitken Institute for Clinical Research
- B5 CRUK Institute for Cancer Studies and Denis Howell Building
- B6 Research Park
- B7 90 Vincent Drive
- B8 Henry Wellcome Building for Biomolecular NMR Spectroscopy
- B9 Medical Practice and Dental Centre
- B10 Advanced Therapies Facility
- B11 BioHub Birmingham
- B12 Health Sciences Research Centre (HSRC)

UNIVERSITY OF BIRMINGHAM

Orange Zone
- O1 The Guild of Students
- O2 St Francis Hall
- O3 University House
- O4 Ash House
- O5 Beech House
- O6 Cedar House
- O7 Sport & Fitness

Green Zone
- G1 32 Pritchatts Road
- G2 31 Pritchatts Road
- G3 European Research Institute
- G4 3 Elms Road
- G5 Computer Centre
- G6 Metallurgy and Materials
- G7 IRC Net Shape Laboratory
- G8 Gisbert Kapp Building
- G9 52 Pritchatts Road
- G10 54 Pritchatts Road – Institute for Global Innovation
- G11 Maples Nursery
- G12 Winterbourne House and Garden
- G13 Hornton Grange
- G14 Garth House
- G15 Westmere
- G16 Lucas House
- G18 Priorsfield
- G19 Park House
- G20 Wolfson Advanced Glasshouses
- G22 Elms Day Nursery
- G23 Edgbaston Park Hotel and Conference Centre
- G24 Centre for Human Brain Health
- G25 EcoLab

Yellow Zone
- Y1 The Old Gym
- Y2 Haworth Building
- Y3 Engineering Building
- Y4 Terrace Huts
- Y5 Estates West
- Y6 Maintenance Building
- Y7 Grounds and Gardens
- Y8 The School of Engineering
- Y9 Computer Science
- Y11 Chemical Engineering
- Y12 Biochemical Engineering
- Y13 Chemical Engineering Workshop
- Y14 Sport, Exercise and Rehabilitation Sciences
- Y15 Civil Engineering Laboratories
- Y16 Institute of Occupational and Environmental Medicine
- Y17 Public Health
- Y18 Bournbrook Student Accommodation
- Y19 NBIF
- Y20 UKRRIN



Keynote Speakers

Keynote 1: Andrew Allen

University of Melbourne, Australia

Title: Vital interactions: Exploring the relationships between respiratory and autonomic neural networks.

Summary: Since the earliest recordings of sympathetic innervation of the cardiovascular system, investigators have been aware of the influence of breathing on this activity. Prior to that, humans have known that breathing can be manipulated to affect cardiovasculardiseases, emotional well-being, exercise performance and many other states.

In experiments conducted in humans and experimental animal models we have examined this interaction between breathing and sympathetic nerve activity in the development of high blood pressure and begun to understand the identity of the neural circuits involved. This work has been aided by the use of replication-deficient viral vectors, in combination with opto- and chemo-genetic methods, that enable high temporal-resolution manipulation of the activity of specific neuronal cell groups. The contribution of this information to forming an understanding of the factors involved in the development of hypertension will be discussed.

Keynote 2: Melanie Gareau

University of California, USA

Title: It takes guts: The developing microbiota-gut-brain axis

Summary: During early neonatal development, the microbiota, gut, and brain (MGB) mature concurrently to promote bi-directional communication along this newly characterized MGB axis. While the exact pathways of communication between the gut and brain remain incompletely delineated, we know that the microbes that reside within the gut play an important role. Increasing evidence suggests that a combination of humoral, neural, and immune pathways work in tandem to ensure proper signaling across the MGB axis. Our lab is focused on identifying the mechanisms involved in regulating MGB axis communication, particularly as they develop in early life.

Keynote 3: Jessica Filosa

Augusta University, USA

Title: Blood pressure variability impaired neurovascular outputs in middle-aged mice.

Summary: Perenkita J. Mendiola, Philip O'Herron, Kun, Xie, Valeria Di Stefano, Michael W. Brands, Jessica A. Filosa. Department of Physiology, Medical College of Georgia, Augusta University, Augusta, Georgia, USA

A growing body of evidence underscores the significance of elevated blood pressure variability (BPV) as an emerging risk factor for cardiovascular events, cognitive decline, and end-organ damage. Yet, the specific cellular targets and underlying mechanisms governing intravascular pressure fluctuations-induced neuronal dysfunction remain elusive. To address this critical knowledge gap, we developed an innovative murine model of BPV.

Our study aims to determine the impact of chronic BPV on neurovascular-dependent processes, encompassing both steady-state and stimulus-evoked alterations in cerebral blood flow. Middel aged mice (12-15 months old) underwent surgical implantation of a chronic cranial window, and were injected with an AAV5-GCaMP6f virus to facilitate the monitoring of cortical astrocyte Ca^{2+} events. Mice were then equipped with a programmable infusion pump and telemetry transmitter. Intermittent Angiotensin II (Ang II) infusions induced blood pressure (BP) fluctuations, effectively increasing the average real variability ($P<0.02$) and coefficient of variation ($P<0.0001$). Notably, the chronic BPV protocol (20-25 days) did not induce hypertension but did attenuate the bradycardic reflex response to Ang II-evoked increases in blood pressure ($P<0.03$).

Following 20 days of BPV, we assessed parenchymal arteriole diameter responses to Ang II-induced pressure increases using two-photon imaging. In the same mouse, we also measured the sensory-evoked response (whisker stimulation) when the pump was off (low-BP period) and during the Ang II infusion (high-BP period). Mice subjected to chronic BPV exhibited blunted myogenic-induced constrictions ($P<0.04$). In control mice, sensory-evoked responses displayed a significant pressure dependency ($P<0.0001$), with a greater response during higher mean arterial pressure (~92 mmHg vs 78 mmHg); this pressure dependency was significantly compromised ($P<0.002$) in the BPV group. Contrary to our expectations, acute pressure increases in BPV mice exhibited no changes in the magnitude of the astrocyte Ca^{2+} response but a significant increase in the frequency of the Ca^{2+} events ($P<0.01$).

In summary, chronic BPV led to a substantial decrease in the ability of parenchymal arterioles to respond to acute pressure increases, a blunted neurovascular coupling response, and altered astrocyte Ca^{2+} dynamics. These findings collectively support the hypothesis that increased BPV detrimentally affects elements of the neurovascular unit, representing a likely early event contributing to cognitive decline.

Oral Presentations

Symposium Presentations Session 1

Symposium Title: Bioelectronic Medicine

O1 - Axonal modulation therapy for bioelectronic treatment of cardiovascular diseases

Theme: Bioelectronic Medicine

Dr. Olujimi Ajijola, Dr. Jeff Ardell[1]
[1]University of California Los Angeles, Los Angeles, United States

Cardiac control is mediated via nested-feedback reflex control networks involving the intrinsic cardiac ganglia, intra-thoracic extra-cardiac ganglia, spinal cord, brainstem, and higher centers. It is now recognized that cardiac disease progression reflects the dynamic interplay between adverse remodeling of the cardiac substrate coupled with autonomic dysregulation. Focusing on myocardial infarction, excessive reflex activation of the sympathetic nervous system contributes to adverse remodeling following myocardial infarction (MI). Axonal modulation therapy (AMT), directed at the paravertebral chain, blocks sympathetic efferent outflow to the heart and is a potential strategy to transiently and controllably mitigate chronic MI-associated sympatho-excitation to reduce ventricular arrhythmias.

In porcine models with chronic MI, we evaluated scalable AMT, directed at the right paravertebral chain (T1-T2 level), in blocking reflex-mediated pacing-induced sympatho-excitation post-MI. Level of sympatho-excitation was assessed by dynamic interstitial measurement of norepinephrine (NE) and neuropeptide Y (NPY). Programmed pacing evoked differential NE and NPY release in both remote and MI border zones of the left ventricle. Right-sided AMT mitigated NE and NPY pacing-induced release in remote left ventricle tissues with a positive correlation to increasing AMT levels. Pacing-induced NE and NPY release in the MI border zone was not mitigated by AMT. From these data we conclude that AMT mitigation of regional NE and NPY release may underlie the anti-arrhythmic effects of partial stellate ganglion block in the setting of chronic MI.

O2 - Saving premature infants from death and disability using vagus nerve stimulation: pre-clinical models

Theme: Bioelectronic Medicine

Professor Christopher Wilson[1], Associate Professor Arlin Blood[1]
[1]Loma Linda University, Loma Linda, United States

Preterm infants are subject to a wide array of debilitating diseases, many of which are due to infection and cardiorespiratory dysregulation. Our laboratories have, collectively spent the last two decades studying these perinatal diseases and sought treatments to improve outcome and prevent death in these most vulnerable infants. In this presentation, we provide an overview of our past and ongoing work on neonatal lung disease, apnea of prematurity, neonatal inflammation, and necrotizing enterocolitis (NEC). Over the past ten years, we have moved from traditional harmacologic therapies to the use of bioelectric stimulation, mainly vagus nerve stimulation (VNS), as an intervention in animal models of prematurity, chiefly ovine and murine. Our ultimate goal is to deploy VNS in neonatal care centers to reduce early life mortality and long-term morbidity.

O3 - Bioelectronics for Treating Diabetes

Theme: Bioelectronic Medicine

Director Of Research Jon Waataja[1], VP Clinical, Regulatory and Opperations Dov Gal[1]
[1]Reshape Lifesciences Inc., Irvine, United States

Approximately ten percent of the world's population suffers from diabetes. Current therapeutic options, including GLP-1 RAs, are marred by compliance, cost, and side effects. The Vagus nerve influences organ systems that are responsible for blood glucose regulation and its modulation holds promise as a new therapy for treatment of both T1- and T2DM. Ample research has demonstrated that non-invasive and non-electrical stimulation of the Vagus nerve at various locations modifies glycemia. However, non-invasive approaches carry a risk of non-compliance, and many non-electrical modulation methodologies are currently clinically unrealistic. Direct vagal electrical modulation has proven to be safe and is used clinically. Many studies spanning decades using different stimulation parameters and Vagus stimulation sites have demonstrated effectiveness, yet the best stimulation approach to pursue clinically remains uncertain. Development of neuromodulation technology that utilizes, or combines, successful stimulation modalities (optimizing a personalized medicine approach) while negating off-target effects (particularly cardiac) would be desirable. To address this, we are developing a sub-diaphragmatic Vagus nerve neuromodulation platform with the flexibility of multi-site multi-frequency glycemic neuromodulation while limiting cardiac off-target effects. We have demonstrated that using simultaneous 5 kHz modulation of the vagal hepatic branch with 1 Hz stimulation of the vagal celiac branch improves glycemic control in animal models of T2DM. This is demonstrated through increased performance on glucose tolerance tests, decreased glucose coefficient of variation and decreased fasting plasma glucose. Interestingly, stimulation from 5-15 Hz of the celiac branch produces the opposite effect and has the potential as a treatment for sever hypoglycemia. Sub-diaphragmatic stimulation does not show signs of off-target effects such as the absence of changes in heart rate, blood pressure or oxygen saturation. Neuromodulation shows a bright future for the treatment of glycemic dysregulation with a possible more attractive approach to its management than dependence of perpetual use of drugs.

O4 - Recent studies on splenic nerve stimulation in pigs and humans

Theme: Bioelectronic Medicine

Dr Daniel Chew[1]
[1]Galvani Bioelectronics, Stevenage, United Kingdom

Neuromodulation of the parasympathetic nervous system is now a well-known therapeutic approach for numerous clinical conditions. Modulation of the immune system via the sympathetic autonomic nervous system for the treatment of immune and inflammatory conditions, is however a more recent therapeutic strategy in Galvani Bioelectronics. Targeting post-ganglionic sympathetic nerves directly innervating the spleen achieves direct nerve activation at the organ, which may provide an improved therapeutic window in the treatment of immune and inflammatory conditions. Described herein is the Research and Development progress of a concept-to-clinic implantable medical device treatment for Rheumatoid Arthritis. The presentation will provide; i) in vivo mechanistic evidence of action of noradrenaline on monocytes, suppressing cytokine release, and reversing a collagen-induced arthritis disease state; ii) in silico and ex vivo translation characterisation of clinical target anatomy and setting requirements and stimulation parameter estimations for the device design; iii) the development of translational large animal in vivo models to provide suitable evidence of chronic safety of the medical device and proof of target engagement through physiology and disease-relevant biomarkers; iv) this finalised alongside First-In-Human feasibility assessments of surgery and sympathetic nervous system stimulation via a splenic neurovascular cuff interface provides an example of industrial R&D process for regulatory approval of an Early Clinical Feasibility Study of a novel neuroimmune-modulating Class III medical device.

O5 - Preliminary data on sacral nerve stimulation to treat ulcerative colitis

Theme: Bioelectronic Medicine

Dr Victor Pikov[1]
[1]Medipace Inc, Pasadena, United States

The field of bioelectronic medicine involves stimulation of the vagus nerve (VNS) and sacral nerve (SNS). Medipace is developing implantable neuromodulation devices for VNS and SNS, including an open-source closed-loop OpenNerve device that is developed in collaboration with University of Southern California and Med-Ally. The OpenNerve device includes the implantable stimulation leads: helical cuff for VNS and 4-contact tined lead for SNS. The OpenNerve device also includes implantable sensing leads for electrocardiography, electromyography, and motion sensing. Additional mplantable sensing leads under development include electrochemical sensing for acetylcholine and atecholamines and physiological sensing for strain and internal organ temperature. The OpenNerve device uses Bluetooth communication, rechargeable battery, and can run advanced algorithms for real-time signal processing of data from the implantable sensing leads.

In parallel, Medipace participates in a multi-center clinical trial using an existing SNS device to evaluate its therapeutic effect on ulcerative colitis. We will present preliminary data from the first cohort of patients after 3 months of implantation.

Symposium Presentations Session 1

Symposium Title: Central nervous control of blood pressure, brain blood flow, and cognitive health

O6 - Regulation of arterial blood pressure by an intracranial baroreceptor

Theme: Cardiovascular

Dr Alexander Gourine[1]
[1]University College London, United Kingdom

There is significant evidence for the existence of an intracranial baroreceptor mechanism(s) capable of sensing physiological changes in cerebral blood flow. The presentation will focus on the sensitivity of this mechanism to changes in brain perfusion and its interaction with inputs from the peripheral (arterial) baroreceptors. The data will be presented on the underlying cellular and molecular mechanisms. The hypothesis of cerebral blood flow being an important determinant of systemic arterial blood pressure will be discussed.

O7 - Defending blood flow to the brain in hypertension, diabetes and ischemic stroke

Theme: Cardiovascular

Dr Fiona McBryde[1], Dr Tonja Emans[1], Dr Suma Thampi[1], Ms Sryana Sukdev[1]
[1]University of Auckland, Auckland, New Zealand

The brain is our most metabolically demanding organ, and requires a constant, uninterrupted supply of blood, glucose and oxygen in order to maintain normal function. I will present some of our most recent research, which utilizes preclinical models of cardiovascular disease to help us understand the dynamic relationships between blood pressure, the sympathetic nervous system and brain blood flow under conditions of hypertension, stroke and cardiometabolic disease.

O8 - Cerebrovascular variants and the role of the selfish brain in hypertension

Theme: Cardiovascular

Emma Hart, University of Bristol

Abstract not received

O9 - Cerebral blood flow, aging and physiological stress

Theme: Cardiovascular

Sam Lucas, University of Birmingham

Abstract not received

O10 - Divergent roles of orexinergic and non-orexinergic neurons in the hypothalamic perifornical area in eliciting behavioral and autonomic cardiovascular responses

Theme: Cardiovascular

Ph. D Emi Narai[1], Dr. Satoshi Koba[1]
[1]Tottori University, Tottori, Japan

A descending motor signal from the forebrain to the subcortical neural circuits, termed central command, contributes to the precise regulation of cardiovascular system during exercise, yet hypothalamic involvement in the central command-mediated autonomic adjustments remained undetermined. Neurons that secret orexin, a neuropeptide pivotal in the long-term regulation of various organismic functions such as wakefulness, are densely distributed in the hypothalamic perifornical area (PeFA). In a series of studies, we elucidated the role of orexinergic and non-orexinergic neurons in central command signaling to coordinate somatomotor and autonomic cardiovascular controls for exercise. Initially, we observed activation of both orexinergic and non-orexinergic PeFA neurons in rats engaging in voluntary wheel running, as shown by an increased expression of Fos, a marker of neural activation. Subsequently, we investigated the role of orexinergic PeFA neurons using transgenic Orexin-Cre rats, and found that optogenetic excitation of orexinergic neurons rapidly caused renal sympathoexcitation under anesthesia and elicited locomotor-like exploratory behaviors along with pressor and tachycardiac responses under freely-moving, conscious states. Moreover, orexinergic neuron inhibition during voluntary wheel running immediately suppressed locomotor activities and blood pressure elevation. These observations suggest that orexinergic neurons contribute to autonomic cardiovascular regulation for locomotor exercise (Narai et al., J Physiol, 2024). We also examined the role of non-orexinergic neurons by utilizing optogenetic manipulation in neurons other than orexinergic neurons in Sprague-Dawley rats. Optogenetic excitation of non-orexinergic PeFA neurons elicited rapid sympathoexcitation immediately followed by sympathoinhibition under anesthesia, and randomly induced locomotion or biting behavior, accompanied by pressor and tachycardiac responses, irrespective of the observed behaviors, under conscious states (Narai & Koba, in revision). Altogether, our observations demonstrate that orexinergic and non-orexinergic PeFA neurons play divergent roles in driving behavioral and autonomic cardiovascular responses. We suggest that orexinergic neurons boost locomotor activities, while non-orexinergic neurons stochastically promote locomotor or biting behavior.

O11 - Closed loop control of Norepinephrine to attenuate sympathetic response to cardiac stressors

Theme: Cardiovascular

Shane Bender[3], Dr Corey Smith[4], Dr Shyue-An Chan[4], Dr. Jeff Ardell[2], **Assistant Professor Tina Vrabec**[1]
[1]Physical Medicine and Rehabilitation, MetroHealth Medical Center, Cleveland, United States, [2]UCLA Cardiac Arrhythmia Center, David Geffen School of Medicine, Los Angeles, United States, [3]Biomedical Engineering, Case Western Reserve University, Cleveland, United States, [4]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, United States

Chronic elevated sympathetic stress has been implicated in the pathogenesis of several cardiovascular disorders including arrhythmias and heart failure[1]. Sympathetic control of cardiac function occurs through postganglionic innervation from stellate ganglia. Downregulation of sympathetic tone can be achieved via catheter ablation or surgical resection of the paravertebral chain[2]; however, these techniques are irreversible. Alternatively, electrical sympathetic nerve block is a treatment that can be applied instantly, is rapidly reversible, and can be titrated to customizable dosages[3] [4]. Norepinephrine (NE) is the primary neurotransmitter released from sympathetic efferent nerves, and causes increased cardiac mechanical and electrical function. NE levels were measured in real-time using fast scanning cyclic voltammetry (FSCV)[5] via platinum sensor electrodes placed in the myocardium of healthy porcine models. A closed-loop controller was used to detect changes in NE levels and respond with graded electrical nerve block of the paravertebral chain. Direct current (DC) electrical nerve block was provided using a carbon separated interface nerve electrode (CSINE)[6]. A fuzzy logic controller (FLC)[7] was designed to titrate the level of nerve block to maintain the NE release at a pre-defined threshold (the "setpoint"). Programmed ventricular pacing was used as stressors to elicit NE release and the controller was used to attenuate sympathetic drive to clamp the NE output at the setpoint. NE levels in response to programmed pacing were first measured with no nerve block. Setpoints were determined to be 66% and 33% of this maximum level. Programmed pacing was then repeated with the controller activated at one of these thresholds and the resultant NE release and controller output were recorded. NE was successfully maintained at the selected thresholds for the duration of the pacing and the NE levels resumed the initial values after the intervention was completed.

Symposium Presentations Session 1

Symposium Title: Brainstem integrator for viscerosensation and autonomic regulation

O12 - GABAergic neurons of the pre-Bötzinger complex regulate respiratory sinus arrhythmia and blood pressure via the autonomic nervous system

Theme: Integrative control

Ms Ambre Linossier[1], Dr Julie Buron[1,2], Pr Christian Gestreau[1], Dr Clément Menuet[1]
[1]INMED, INSERM, Aix-Marseille Université, Marseille, France, [2]UNIL, Université de Lausanne, Lausanne, Suisse

The respiratory and cardiovascular systems act together to maintain the body's homeostasis. Their physiological efficiency is improved by respiratory-cardiovascular coupling (RCC), where heart rate and blood pressure oscillate in phase with respiratory activity, which optimizes pulmonary gas exchanges and cardiac energetic cost. RCC is mainly due to an interaction between neurons generating the respiratory command and neurons regulating cardiovascular activity, which are located in the brainstem. Specifically, it was shown that preBötzinger complex (preBötC) neurons, the group that generates the inspiratory rhythm, also directly modulate the activity of autonomic neurons that regulate heart rate and blood pressure. The preBötC is a highly heterogenous neuronal group, with excitatory and inhibitory neurons. Previous studies suggested that the inhibitory GABAergic neurons of the preBötC participate in the generation of the RCC. To test this hypothesis, we injected adeno-associated viruses with floxed expression cassettes in the preBötC of GAD-Cre rats, to selectively express proteins for neuronal tracing (tdTomato and synaptophysin-GFP) or bidirectional optogenetics (somBiPOLES) in preBötC GABAergic neurons. We found that preBötC GABAergic neurons make presynaptic contacts with neurons in autonomic regions involved in regulating cardiovascular activity, the nucleus Ambiguus (nA) and the Rostral Ventral Lateral Medulla (RVLM). Using the in situ Working Heart-Brainstem Preparation and in vivo anesthetized rats, photoinhibition of preBötC GABAergic neurons increased the amplitude of respiratory oscillations in heart rate (respiratory sinus arrhythmia) and blood pressure (Traube-Hering waves), decreased mean heart rate and increased mean blood pressure, while photostimulation of these neurons induced the opposite effects. Photomodulations of preBötC GABAergic neurons had little effect on respiratory activity. Systemic injection of the muscarinic antagonist atropine blocked the heart rate effects but not the blood pressure effects. This work shows that preBötC GABAergic neurons are involved in the generation of RCC.

O13 - New insights into deep stimulation for correcting autonomic imbalance

Theme: Integrative control

Zoe Adams, University of Bristol

Abstract not received

O14 - Sympathetic neurocirculatory responses to central chemoreflex activation in human hypertension

Theme: Integrative control

Associate Professor James Fisher[1], Dr Ana L. C. Sayegh[1], Dr Jui-Lin (Mickey) Fan[1], Dr Matthew Dawes[1], Prof. Julian F. R. Paton[1]
[1]University of Auckland, Auckland, New Zealand

The central chemoreceptors respond to elevations in local tissue PCO2/[H+] by causing powerful reflex increases in ventilation and sympathetic nerve activity. An augmented central chemoreflex sensitivity has been observed in the spontaneously hypertensive rat where is linked to the development of high blood pressure (BP) (Li et al., 2016). This presentation will describe our recent work examining whether central chemoreflex sensitivity is augmented in hypertensive humans. In fifteen HTN participants (68±5 years; mean±SD) and 13 normotensives (NT; 65±6 years) ventilation ($\dot{V}E$; pneumotachometer) and muscle sympathetic nerve activity (MSNA; microneurography) were recorded during two modified rebreathing trials where the partial pressure of end-tidal carbon dioxide (PETCO2) was progressively and similarly increased. In the first trial, the partial pressure of end-tidal oxygen (PETO2) was clamped at 150 mmHg (central chemoreflex activation) and in the second PETCO2 was clamped at 50 mmHg (combined central and peripheral chemoreflex activation). Ventilatory ($\dot{V}E$ vs. PETCO2 slope) and sympathetic (MSNA vs. PETCO2 slope) chemoreflex sensitivities and recruitment thresholds (breakpoints) were calculated. Central ventilatory and sympathetic chemoreflex sensitivities were greater in HTN than NT (2.48±1.33 vs. 1.58±0.42 L·min-1·mmHg-1, P=0.030: 3.32±1.90 vs. 1.77±0.62 a.u.·mmHg-1, P=0.034, respectively), while recruitment thresholds were not different between groups. Combined central and peripheral ventilatory and sympathetic chemoreflex sensitivities and recruitment thresholds were similar in HTN and NT. The results of these investigations suggest that central ventilatory and sympathetic chemoreflex sensitivities are increased in humans with HTN, perhaps indicating that therapeutic targeting the central chemoreflex may aid some forms of HTN.

Funding provided by the Auckland Medical Research Foundation (Ref. no. 1119008. JPF, JFRP, ALCS), the Health Research Council of New Zealand (Ref. no. 19/687. JPF, JFRP), and the Sydney Taylor Trust (JFRP).

O15 - Are GLP-1 producing pre-proglucagon neurons of the lower brainstem a useful target for obesity and diabetes treatment?

Theme: Integrative control

Stefan Trapp, University College London

GLP-1 receptors are found in many parts of the brain, and particularly in those areas implemented in control of food intake and in the endogenous reward system. Systemically administered GLP-1 receptor agonists are currently the most efficacious weight loss medication. However, various lines of evidence suggest that their brain access is limited to circumventricular organs and possibly a few more select sites. This leaves many brain GLP-1 receptors inaccessible and endogenous brain GLP-1, produced by preproglucagon (PPG) neurons of the lower brainstem, seems the likely relevant ligand. From this the question arises of whether PPG neurons are capable of eliciting a reduction in food intake and bodyweight independent of and in addition to systemic GLP-1 receptor agonists, and if so, whether this can be achieved by pharmacological means. This presentation will consider the experimental evidence and additionally ask the question whether there are qualitative differences in the reduction of food intake achieved by PPG neuron activation compared to GLP-1 receptor agonists, e.g. is nausea, which is a mayor side effect of GLP-1 receptor agonist therapy, also an issue for PPG neuron mediated hypophagia? Finally, to address the translational relevance of PPG neuron activation the possibility of combination therapy with GLP-1 receptor agonists will be addressed.

O16 - CaMPing in the brainstem

Theme: Integrative control

Andrew G. Butler[1,2], Kimberly R. Thek[1], Jespreet Bassi[2] Angela Connely-Huf[2], Andrew Allen[2] & **Stuart McDougall[1]**

[1] The Florey, University of Melbourne, VIC 3010

[2] Department of Physiology and Anatomy, University of Melbourne, VIC 3010

Central processing of vagal afferent sensory information is critical for maintaining homeostasis and co-ordinating organ function with neuroendocrine and behavioural outputs. How vagal mediated reflex signals are integrated in the brainstem remains poorly understood. Here, we recorded neurons of the nucleus of solitary tract (NTS), the site that first receives vagal afferent synaptic input, by calcium imaging in the working heart-brainstem preparation (WHBP). Female and male 20-22 day old Sprague Dawley rats (n=10 animals) were anesthetised and the commissural or medial NTS injected with (AAV)1-syn-GCaMP6f-WPRE followed by six to nine days recovery. NTS neuronal activity was recorded in response to initiation of the chemo, baro and diving reflexes, synchronous with phrenic, vagus efferent and cardiac measurements. Reflexes evoked GCaMP6f neuronal activity and was highly specific such that distinct sensory drives activated heterogenous populations of NTS neurons in line with phrenic nerve activity. Chemoreflex evoked NTS GCaMP6f excitatory responses (n=32 neurons) and inhibitory responses (n=5 neurons) compared to baroreflex excitatory (n =10 neurons) and inhibitory responses (n=8 neurons). Chemosensitive NTS neurons were classified into clusters dependent upon the pattern of GCaMP6f responses; early responsive (n=8), late responsive (n=15), long-lasting (n=9), as well as inhibitory neurons (n=5). Barosensitive NTS neurons were classified as excitatory (n=10) or inhibitory responsive neurons (n=8). In addition, few NTS neurons (n=4) were found to be responsive to both the chemo and baroreflex. These data indicate reflexes are mostly mediated by dedicated populations of neurons in the initial phase of information integration.

O17 - Higher periodicity and irregularity of respiratory pattern during wakefulness in subjects with newly diagnosed sleep apnea

Theme: Integrative control

M.D., Ph.D. Beata Graff[1], Professor Grzegorz Graff[2], Phd, DSc Paweł Pilarczyk[2], MD Marta Szymańska[1], Professor Krzysztof Narkiewicz[1]
[1]Medical University of Gdansk, Gdansk, Polska, [2]Gdansk University of Technology, Gdansk, Poland

In sleep apnea patients increased blood pressure variability and decreased heart rate variability reflecting autonomic nervous system dysfunction have been documented and linked to adverse cardiovascular effects including hypertension.

The aim of the present study was to assess whether sleep disordered breathing is related to changes in respiratory pattern assessed in awake subjects. Besides standard parameters characterizing variations in respiratory cycle length and amplitude we have proposed novel analytical methods.
The Hilbert Transform (HT) was applied to create an analytic signal from a real-valued respiratory signal. Then, the analytic signal was used to analyze its envelope and phase characteristics, each providing unique insights into the respiratory behavior. As the additional step, the HT analysis has been visualized using special diagrams based on which a quantitative assessment of respiratory pattern variability was carried out.

Protocol: 165 consecutive subjects (49±12 years; 84 male) were included in the study. Among them 80 had the history of hypertension, but NONE were previously diagnosed with sleep apnea. In each subject 20-minutes recordings of respiration with respiratory belt were performed in the sitting position during wakefulness. Then, type III home sleep testing was performed.

Results:
1. Apnea-hypopnea index (AHI) ≥5/hour was identified in a substantial number of individuals (79 persons, 48%). In 58 subjects (35%) average saturation (AvSat) during the night was lower than 94%.
2. There were no significant differences according to mean values of standard parameters characterizing respiratory cycle.
3. Both subjects with AHI≥5/hour and subjects with AvSat<94% presented with higher periodicity and irregularity of respiratory pattern defined using novel parameters based on the HT application.

Conclusion: Subjects with desaturations and sleep apnea episodes during the night characterize with altered variability of parameters describing breathing pattern during wakefulness including higher periodicity and irregularity which might suggest altered respiratory regulation even in mild sleep apnea.

O18 - Spatial mapping of data onto 3D heart and stomach scaffolds

Theme: Bioelectronics

Dr Ariege Bizanti[1], **Dr Mabelle Lin**[2], Dr Yuanyuan Zhang[1], Ms Duyen Nguyen[1], Dr Richard Christie[2], Dr David Nickerson[2], Ms Maci Heal[3], **Dr Zixi Jack Cheng**[1], Prof. Peter Hunter[2]
[1]University of Central Florida, United States , [2]University of Auckland, New Zealand, [3]MBF Bioscience, United States

Comparison and integration of data from multiple samples, modalities, and/or species have always been challenging. Under the SPARC program, we developed a methodology to map spatial data to a common coordinate framework using examples of flat-mounts of heart and stomach data onto their respective scaffolds. Through the application of diverse neural markers and tracer injections into peripheral ganglia, we achieved integration of peripheral neural innervation within 3D heart and stomach scaffolds.

In the heart, we used tyrosine hydroxylase as a marker for sympathetic postganglionic axons and applied flat-mount tissue processing, confocal microscopy (Zeiss), automatic axonal tracing (Neurolucida 360), and integration of tracing data onto a 3D heart scaffold (Scaffold Mapping Tool). We obtained a comprehensive topographical map of catecholaminergic axon distribution of the whole heart at single cell/axon/varicosity scale that will be used to create a cardiac sympathetic-brain atlas.

In the stomach, calcitonin gene-related peptide-immunoreactive axon innervation retrieved from imaging a flat-mount half stomach was also spatially mapped to a 3D stomach scaffold with the same techniques. Multiple tissue samples can be embedded in a common coordinate framework for comparison. Beside the distribution of neural markers, we can also map vasculature data obtained from micro-CT scans of whole rat stomach to 3D generic stomach scaffold. Continuous fields representing the distribution of gastric enteroendocrine cells obtained from immunohistochemistry studies can also be mapped to the stomach scaffold.

With data from different samples/modalities mapped onto a generic organ scaffold, we can now integrate multiple data on a common coordinate framework for comparison on the SPARC Portal. This will not only demonstrate how data from different experiments interact, but it also identifies knowledge gaps and advances our understanding of the heart- and stomach-brain connectome. This also enables us to evaluate the cardiac and gastric neural control in pathological conditions.

O19 - Dashboard of the human vagus: from gross to micro anatomy

Theme: Bioelectronics

Dr Nicole Pelot[1], **Dr Joost Wagenaar**[2]
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Impactful scientific data sharing requires meaningful interfaces to query and visualize data in repositories without the need to download the full original dataset. As part of the NIH SPARC program, we are quantifying the gross, macro, and micro anatomy of 100 cadaveric human vagus nerves, from brainstem to abdomen, using multiple imaging modalities: MRI, photography, 3D tracing of the gross anatomy, computed tomography (CT), microCT, histology, immunohistochemistry, and 3D-MUSE (microscopy with ultraviolet surface excitation) block-face imaging. We segment the imaging data and add annotations with standardized anatomical terms. The resulting data files are large and numerous: for the first 25 of 50 subjects, we have >120 TB of raw imaging data across >1M files. Therefore, we are developing computational tools to handle image analyses, annotations, co-registration, exploration, and visualization. We standardized the data formats and organization for each imaging modality consistent with the SPARC Data Standards to enable data upload to the Pennsieve platform, followed by publication on the SPARC Portal.

To facilitate data interrogation, we are developing dashboard functionality to support two specific use cases: 1) to browse data across subjects and anatomical regions, and 2) to visualize derived quantitative data. The dashboard is developed as an open-source project and has a modular approach to enable independent widgets to display a variety of information and to support interactive data exploration.

The ability to interrogate the Vagus Atlas will have important implications for informing surgical procedures in the vicinity of the vagus nerve to avoid damage and for improving neuromodulation therapies, including computational modeling of vagus nerve stimulation and identification of new points of intervention. We expect that the Vagus Atlas will provide a impactful resource for the scientific community and demonstrate the necessity of tight integration of data and infrastructure to accelerate our scientific efforts.

O20 - Data visualisation and modelling to support cardiovascular control studies

Theme: Bioelectronics

Dr Alan Garny[1], **Dr David Brooks**[1], Dr David Nickerson[1], **Professor Igor Efimov**[2], Prof. Peter Hunter[1]
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The SPARC portal (https://sparc.science/) enables interactive exploration of data and knowledge collected and curated by SPARC and other scientists in many different ways. One new approach is the Functional Connectivity (FC) map (https://sparc.science/apps/maps?type=fc), which attempts to provide a visual representation of functional connectivity across the whole body – specifically designed to provide a physiological systems level overview of all organ systems in the body. The FC map, and its associated user interface, enables users to zoom into organ, tissue, and in some cases cellular and protein scale, while rendering the known neuronal connectivity retrieved from the SPARC Connectivity Knowledge Base (SCKAN; https://sparc.science/tools-and-resources/6eg3VpJbwQR4B84CjrvmyD).

By leveraging the comprehensive semantic links underpinning the SPARC portal, we are working to use the FC map to guide users to mathematical models relevant to their current journey through the map. Relevant models can be retrieved from the SPARC portal or other connected repositories, such as the Physiome Model Repository (https://models.physiomeproject.org/welcome). Following a standards-based approach encouraged by SPARC, individual models may be integrated to link the function of proteins to models of whole systems physiology and to link models to relevant data published on the SPARC portal.

In this presentation, we will demonstrate this capability via the driving use-case in which a model of cardiac cell contractile function is linked with cardiac output in a model of blood flow to a vascular bed that is subject to sympathetic control. The driving use-case arose in support of the REVEAL project (https://reporter.nih.gov/project-details/10610556) under SPARC Phase 2. We will also show how these methods and tools can be used by other projects such as the Bioelectronics for Neurocardiology - Diagnosis & Therapeutics project

(https://fondationleducq.org/network/bioelectronics-for-neurocardiology-diagnosis-therapeutics/), funded by the Leducq Foundation (https://fondationleducq.org/).

O21 - SPARC Infrastructure supporting functional studies of vagal nerve stimulation

Dr John Osborn[1], **Prof Maryann Martone**[2]
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Despite over 100,000 patients implanted with vagal nerve stimulation (VNS) devices for treatment of depression and epilepsy, the physiological effects VNS on peripheral organs in humans remains poorly understood. The overarching goal of REVEAL Project (Research Evaluating Vagal Excitation and Anatomical Linkages) is to measure the responses of multiple peripheral organ systems to VNS and their dependence on specific stimulation parameters. We will measure autonomic, cardiovascular, immune, and metabolic responses to VNS in 144 participants implanted with a standard LivaNova device on the left vagus. In addition, there are three ancillary projects: 1) in-vivo human recording of vagal nerve activity during VNS; 2) measurements of the effects of VNS on gastric emptying and accommodation; and 3) responses in participants implanted with Microburst, a novel LivaNova device which enables a wider range of stimulation parameters. We will examine the effects of acute and chronic VNS on the physiology of the organ systems described above by measuring: arterial pressure, cardiacsympatho-vagal tone, muscle sympathetic nerve activity, autonomic reflex function, cardiac mechanics, glucose and lipid metabolism, and immune function. We have assembled a multi-disciplinary team of highly qualified investigators across 7 sites globally. We will produce a first-of-its-kind data set to share rapidly and broadly to the neuroscience and autonomic clinical communities. These data will provide a multi-system view of the vagus nerve functional connectivity in humans, filling a critical knowledge gap and leading to new therapies. These data will be made available through the SPARC portal (sparc.science), which provides search, visualization and computational tools to explore a rich collection of data, maps and models of the ANS. To help support the REVEAL data set, SPARC is developing a quantitative database to house physiological and structural parameters from this dataset that will provide these parameters for search and for import into SPARC modeling tools.

O22 - Sympathetic action potential recruitment during transient cessation of deep brain stimulation for severe refractory hypertension

Theme: Bioelectronics

Dr Zoe Adams[1,2], Dr Angus K. Nightingale[2,3], Dr Richard P. Baker[2,4], Dr Stephen A. Klassen[5], Professor Kevin Shoemaker[6], Prof. Julian F. R. Paton[7], Dr Emma Hart[2,9], Mr. Nikunj K. Patel[8,9]
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Deep brain stimulation (DBS) of the ventrolateral periaqueductal gray (vlPAG) and other medial thalamic, epithalamic, and midbrain regions, is under evaluation as a treatment for severe, drug-resistant/refractory hypertension. The depressor effect may be mediated by a tempering of sympathetic outflow, but this is yet to be fully optimised in patients. We aimed to measure the sympathetic nerve activity (SNA) response to transient DBS cessation in a 57-year-old male patient with severe refractory hypertension (>200 mmHg despite maximal pharmacological treatment) in whom DBS of the vlPAG and mediodorsal thalamus had successfully reduced blood pressure (248±17/123±16 versus 116±15/73±11 mmHg 11 months later). Muscle SNA (MSNA; microneurography and action potential (AP) detection (continuous wavelet transform)) and heart rate (ECG) were recorded at 14-month follow-up during a DBS-on-off-on protocol (140-s off period). Peak response to DBS-off (with data averaged every 30-s from the start of DBS-off) was compared to a five-minute DBS-on baseline. MSNA burst incidence and normalised burst amplitude increased with DBS-off (93 versus 82 bursts/100 heartbeats, 14% increase; 50±25% versus 37±17% (median ± IQR), 35% increase). Sympathetic AP firing increased with DBS-off (359 versus 131 AP/100 heartbeats, 174%; 208 versus 77 AP/min, 171%; 3.9 versus 1.6 AP/burst, 140%). APs across the DBS-on-off-on protocol were sorted into amplitude bins (small, medium, large). Smallest amplitude APs were active at baseline but fired less frequently with DBS-off, whereas those of medium amplitude were active at baseline and fired more frequently with DBS-off. In contrast, the largest amplitude APs were silent at baseline and activated with DBS-off. In conclusion, transient DBS cessation was associated with sympathoexcitation and the recruitment of large, previously silent APs. In this patient, vlPAG/mediodorsal thalamic DBS evoked a depressor effect by dampening sympathetic outflow (inhibiting medium-AP firing and de-recruiting large APs). This supports a role for these regions in controlling human hypertension.

O23 - Interactive maps of nerve-organ anatomy and function on the SPARC Portal

Theme: Bioelectronics

Man Chung Wu[1], Dr Alan Garny[1], Aung Kyaw Hein[1], Dr David Brooks[1], David Yu[1], Mr Hugh Sorby[1], Keeran Balachandran[1], Jesse Khorasanee[1], Prof. Peter Hunter[1], **Dr David Nickerson**[1]
[1]Auckland Bioengineering Institute, University Of Auckland, Auckland, New Zealand

The integrated maps viewer of the SPARC Portal (https://sparc.science) provides interactive, modular, and continually-updated visualizations of nerve-organ anatomy and function.

SPARC data and knowledge is registered onto two-dimensional flatmaps, and three-dimensional anatomical organ and whole-body scaffolds. Flatmaps are zoomable maps built from a range of sources and portray anatomy and nerve knowledge of a given species, and scaffold maps are geometric models able to represent the spatial distribution of connectivity knowledge, observed data, and computational models. These maps, and their annotations, are published as SPARC datasets and they are available for public viewing using the map viewers on the SPARC Portal (https://sparc.science/maps).

The map viewers provide visual interfaces for exploring data and tools in the context of knowledge about neural connectivity. Visualization on the viewer can be customized using different settings. Search can be conducted on both the flatmap and scaffold maps to find and highlight specific features. Data and literature sources and other relevant information of a network and connectivity can be explored comprehensively based on standardized annotations. Multiple maps can be visualized simultaneously using a split-pane display system. Users can dynamically create permalinks to specific views, allowing knowledge and corresponding visualization to be shared and cited.

Development of the map viewers are on-going and new features continue to be deployed to the SPARC Portal.

This work is supported by the NIH SPARC program under award number OT3OD025347.

Symposium Presentations Session 2

Symposium Title: You're so vein" – new insights into the function and autonomic regulation of the 'forgotten' venous circulation

O24 - Sympathetic regulation of the 'forgotten' venous circulation – a new therapeutic target for blood pressure control?

Theme: Cardiovascular

Dr Tonja Emans[1], Mr Davi JA Moraes[2], Dr Alona Ben-Tal[1,3], Dr Carolyn Barrett[1], Prof. Julian F. R. Paton[1], Dr Fiona McBryde[1]

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The mesenteric venous reservoir plays a vital role in mediating blood volume and/or pressure changes and is richly innervated by sympathetic nerves; however, the precise nature of venous sympathetic regulation and its role during hypertension remains unclear. We hypothesized that sympathetic drive to mesenteric veins in spontaneously hypertensive (SH) rats is raised, increasing mean circulatory filling pressure (MCFP), and impairing mesenteric capacitance.

Arterial pressure, central venous pressure, mesenteric arterial and venous blood flow were measured simultaneously in conscious male Wistar and SH rats. MCFP was assessed using an intra-atrial balloon. Hemodynamic responses to volume changes (±20%) were measured before and after ganglionic blockade and carotid body denervation (CBD). Sympathetic venoconstrictor activity was measured in situ.

MCFP in vivo (10.8±1.6 vs 8.0±2.1 mmHg; P=0.0005) and sympathetic venoconstrictor drive in situ (18±1 vs 10±2 µV; P<0.0001) were higher in SH rats; MCFP decreased in SH rats after hexamethonium and CBD (7.6±1.4; P<0.0001 and 8.5±1.0 mmHg; P=0.0045). During volume changes, arterial pressure remained stable. With blood loss, net efflux of blood from the mesenteric bed was measured in both strains. However, during volume infusion, we observed net influx in Wistar (+2.3±2.6 ml/min) but efflux in SH rats (-1.0±1.0 ml/min; P=0.0032); this counterintuitive efflux was abolished by hexamethonium and CBD (+0.3±1.7 and 0.5±1.6 ml/min, respectively).

In SH rats, excessive sympathetic venoconstriction elevates MCFP and reduces capacitance, impairing volume buffering by mesenteric veins. We propose selective targeting of mesenteric veins through sympathetic drive reduction as a novel therapeutic opportunity for hypertension.

O25 - Venous responses to hypoxia in older normotensive and hypertensive adults

Theme: Cardiovascular

Dr Jui-Lin (Mickey) Fan[1], Dr Ana L. C. Sayegh[1], Miss Thalia Babbage[1], Dr Matthew Dawes[1], Prof. Julian F. R. Paton[1], Associate Professor James Fisher[1]
[1]University Of Auckland, New Zealand

The venous circulation holds approximately two-thirds of the blood volume and plays a crucial role in the control of cardiac output. Both age and hypertension are well-known to impair arterial function, their effects on the venous circulation remains unclear. Since oxygen is an important modulator of vascular function, in a series of recent studies we compared the effects of hypoxia on lower limb venous function in healthy young (YH), healthy older individuals (OH), and patients with hypertension (HTN). It was hypothesised that the venomotor responses to hypoxia would be attenuated with ageing and hypertension.

In fifteen YH (age: 29±8 years, mean±SD), eleven OH (68±8 years) and ten HTN (72±4years), we assessed the great saphenous vein cross-sectional area (GSV CSA; Doppler ultrasound) during a standard thigh cuff inflation-deflation protocol (11 min) while breathing either room air or hypoxia (fraction in inspired oxygen [FIO2]: 0.10) in a single-blinded, randomized manner. Pressure-CSA relationships were modelled using an established quadratic regression equation and GSV compliance derived[1].

Compared to room air values, hypoxia decreased GSV CSA (index of capacity) in YH (4.3±1.8 mm2 vs. 5.4±2.4 mm2, $p<0.001$) and HTN groups (5.6±3.7 mm2 vs. 7.3±7.9 mm2, $p=0.035$), while it was unchanged in OH (9.2±8.3 mm2 vs. 9.5±8.2 mm2, $p=0.299$). Hypoxia enhanced GSV compliance in HTN ($p=0.004$ vs. room air), but did not affect GSV compliance in YH or OH (both $p>0.05$).
Our observation that hypoxia increased venous tone (i.e., decreased venous capacity) in YH and HTN groups, but not OH group, indicates that lower limb venous function is impaired with 'healthy' ageing. We further postulate that the increased venous compliance observed in HTN with hypoxia may serve as a compensatory response to restore venous volume at higher transmural pressure. Whether local and/or chemoreflex-mediated sympathetic mechanisms underpin such alterations in venous function warrants further investigation.

O26 - New horizons in the ageing autonomic nervous system: orthostatic hypotension and supine hypertension

Theme: Cardiovascular

Melanie Dani, Imperial College London

Abstract not received

O27 - Tuft cells trigger neurogenic inflammation in the urethra

Theme: Cardiovascular

Dr. Patricia Schmidt[1,2], Dr. Uwe Pfeil[1], MD Mahmoud Lafee[1,3], Swantje Petersen[1], Dr. Alexander Perniss[1,4], Dr. Maryam Keshavarz[1,5], Prof. Dr. Burkhard Schütz[6], **Prof. Dr. Wolfgang Kummer**[1], PD Dr. Klaus Deckmann[1]
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Background: In the urethra, we previously identified a rare epithelial cell type – urethral tuft cells (UTC) - that produces acetylcholine and expresses the canonical taste transduction cascade, including the cation channel TRPM5. We consider them as sentinels monitoring the presence of danger-indicating microbial products and metabolites and triggering a protective micturition reflex (“flushing”) upon activation (1). We here hypothesized that acetylcholine released by stimulated UTC leads to neurogenic inflammation by triggering neuropeptide (SP, CGRP) release from nearby sensory nerve terminals.

Methods: Cleared urethrae from appropriate reporter mouse strains were immunolabelled for SP and CGRP; explanted urethrae were stimulated optogenetically (Chat-ChR2-YFP mice) or chemically (denatonium) and neuropeptide release was measured by ELISA; the UTC stimulus denatonium was applied into the urethra in vivo and inflammation was assessed using Evans Blue plasma extravasation as readout.

Results: Confocal analysis of cleared whole urethrae revealed a rich network of sub- and intraepithelial peptidergic nerve fibres (SP/CGRP: 42%; SP only: 48%, CGRP only: 9%) approaching UTC. LED illumination (460 nm, 10 min) evoked SP release from urethrae of mice expressing the blue light sensitive cation channel ChR2 selectively in tuft cells. Chemical stimulation with denatonium evoked SP and CGRP release in wildtype mice, which was largely reduced in Trpm5 gene-deficient mice, in contrast to that induced by capsaicin, a direct activator of peptidergic fibres. Denatonium-induced SP-release was reduced, although not abolished, by the nicotinic receptor blocker mecamylamine, but not by atropine. Accordingly, about 30% of SP-positive fibres expressed the nicotinic receptor alpha3-subunit (Chrna3-eGFP). Intraurethral denatonium led to Evans Blue plasma extravasation in vivo, which was sensitive to mecamylamine and to the SP-receptor blocker CP 96345 and was not seen in Trpm5-deficient mice.

Conclusion: Stimulated UTC not only trigger long-distance reflexes involving the bladder, but also evoke neurogenic inflammation, representing a local defence reaction.

O28 - Immunohistochemical characterisation of the superior hypogastric plexus and hypogastric nerves in the adult human

Theme: Cardiovascular

Ms Kayleigh Scotcher[1], Janet Keast[1], Peregrine Osborne[1], John-Paul Fuller-Jackson[1], Martin Bertrand[2]
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The superior hypogastric plexus (SHP) is part of the sympathetic nervous system and comprises an unpaired ganglionated plexus superficial to the distal abdominal aorta. Adjoining the SHP are bilateral hypogastric nerves (HGn) which project to the inferior hypogastric plexus (IHP) in the pelvic cavity; the ganglia in the IHP are responsible for most of the autonomic regulation of pelvic viscera. Functional clinical studies and neuroanatomical studies in rodents indicate that the HGn also contains visceral afferents and axons from sympathetic preganglionic neurons relevant to pelvic organ regulation, however the cellular components and microscopic organization of the SHP and HGn have been poorly defined in humans. This is essential to understand their functional elements and the pathophysiology of genitourinary disorders, consequences of postoperative injuries, and associated treatments, such as neuromodulation. The aim of our study was to define functional classes of neurons and axon trajectories within the adult human SHP and HGn by applying immunohistochemistry with multi-scale imaging, including large-volume imaging of cleared tissues (modified iDISCO with light sheet microscopy) and high-resolution confocal microscopy of tissue sections. Our studies have identified that most SHP neurons are immunoreactive for tyrosine hydroxylase (TH) and therefore considered as noradrenergic. Several large aggregates of TH neurons were embedded within the HGn. Paraganglia were also observed close to both the SHP and proximal HGn. Additional neural markers visualised in cryosections were identified in subclasses of ganglion neurons, synaptic boutons (potential preganglionic terminals) and axon tracts. These include neuropeptide Y, neuronal nitric oxide synthase, somatostatin, and vasoactive intestinal peptide. Putative afferent fibres (calcitonin gene-related peptide and substance P) traversed the SHP and HGn and commonly encircled individual ganglion neurons, raising the possibility of direct sensory-motor communication at these sites. This study has provided new insights into the structure and neural elements within the human SHP and HGn.

O29 - The treatment of oral inflammation influenced the regulation of hemodynamic responses such as blood pressure and heart rate variability

Theme: Cardiovascular

Dr. Adriana Barbosa Ribeiro[1,3], PHd Student Lorena Clemente[1], Professor Rubens Fazan Jr[2], Professor Helio Salgado[2], Dr. Adriana Barbosa Ribeiro[2], Prof Eleonora Tobaldini[3], Prof Nicola Montano[3], Professor Cláudia Silva-Lovato[1]
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Tooth loss and oral inflammation, such as denture stomatitis (DS), have been related to complications within the cardiovascular system, particularly hypertension and endothelial dysfunction. The aim was to evaluate the effect of a hygiene protocol on DS remission, arterial pressure, and heart rate variability (HRV). Thirty-three individuals were enrolled (mean age 66 ± 5.6 years). The outcomes were measured before (T0) and after 10 days (T1) of palate and denture brushing, and immersion of the dentures in 0.25% sodium hypochlorite solution. Data were analyzed by Wilcoxon and Friedman tests for DS remission and Paired t-tests for arterial pressure and HRV ($p < 0.05$). A significant reduction in the DS [T0: 3.00 (2.00-3.00); T1: 2.00 (1.00-3.00); $p < 0.001$]. There was a decrease in systolic (T0: 142 ± 3; T1: 136 ± 3; $p = 0.012$) and mean arterial pressure (T0: 105 ± 2; T1: 100 ± 1; $p = 0.02$), and heart rate (T0: 68 ± 2; T1: 64 ± 2 bpm; $p = 0.02$). No alterations were observed in the time domain. A decrease in the power of the normalized LF band (T0:53 ± 3; T1:48 ± 3 nu; $p = 0.02$). The power of the HF band in absolute units (T0:120 ± 24; T1:156 ± 30; $p = 0.3$), and the LF/HF ratio (T0:2 ± 0.3; T1:1.7 ± 0.3; $p = 0.1$) were similar at different at the time points. There was a decreased occurrence of 0V (T0:15 ± 1; T1:12 ± 1%; $p = 0.04$) and an increase of 2UV (T0:28 ± 2; T1:32 ± 2 %; $p = 0.01$). The hygiene protocol was effective for the treatment of DS and had a positive impact on systolic and mean arterial pressure, heart rate, and the sympathovagal balance by sympathetic attenuation and parasympathetic activation of the autonomic system.

Symposium Presentations Session 2

Symposium Title: Anatomical, functional, and molecular mapping of autonomic innervation of organs

O30 - Spinal afferent innervation in flat-mounts of the rat heart and stomach: anterograde tracing

Theme: Integrative control

Dr Ariege Bizanti[1], Dr Jichao Ma[1], Ms Duyen Nguyen[1], Dr John Furness[2], Dr Madeleine Di Natale[2], Dr Nick Spencer[3], **Dr Zixi Jack Cheng**[1]
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The dorsal root ganglia (DRG) project spinal afferent axons to the stomach and heart. However, the distribution and morphology of spinal afferent axons in these organs have not been well characterized. Therefore, we used a combination of state-of-the-art techniques, including anterograde tracer injection into the left DRG C8-T3 (for the heart) and T7-T11 (for the stomach), avidin–biotin and Cuprolinic Blue labeling, Zeiss M2 Imager, and Neurolucida to characterize spinal afferent terminal structures in flat-mounts of the whole rat heart and stomach muscular wall. Finally, we integrated our tracing data onto 3D organ scaffold using SPARC mapping tool.
Our findings showed that spinal afferent axons innervated all regions with various distinct terminal structures innervating different cardiac and gastric targets.

In the heart, parent axons entered the left atrium potentially via the left precaval vein, branching extensively and projecting in diverse orientations within the atrial tissue and extended to the base of the ventricles travelling downward toward the apex. There was a noticeable predominance in the innervation of spinal afferent axons within both the left ventricles and atria.
In the stomach, most axons ran in parallel with the longitudinal and circular muscles and expressed spherical varicosities. Complex terminal structures were observed within the circular muscle layer. Some individual varicose axons innervated both myenteric neurons, the circular muscle and intramural arteries. Very near all the axons were CGRP+ve. Extrinsic denervation showed that there were no or very rare intrinsic CGRP fibers.

In both the heart and stomach, there were simple, branching and complex types of spinal afferent axons. Additionally, DB-labeled axons innervated various cardiac and gastric targets, including muscle, ganglia, fat, and vasculature.

This work provides a foundation for future topographical anatomical and functional mapping of spinal afferent axon innervation of the heart and stomach under normal and pathophysiological conditions.

O31 - Integration of Molecular, Morphological, and Physiological Aspects of Mechanosensors in the Lung

Theme: Integrative control

Dr Jerry Yu[1]
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For more than a century, the lung vagal sensory system has undergone extensive investigation, yet our grasp of it remains incomplete. Even the most detailed mechanosensory information is shifting due to multiple-sensory theories (MST). This presentation interprets and integrates mechanosensory morphology, electrophysiology, molecular genetics, and reflex responses. Conventionally, investigators have adhered to two doctrines: the one-sensor theory (OST) and the line-labeled theory. According to these doctrines, one type of sensor sends signals to the brain to cause specific reflex responses, and different types of sensors cause different reflex responses. In contrast, recent physiological and morphological studies combined with anatomical approaches demonstrate that numerous different types of mechanosensors may be housed within a sensory unit, i.e., an afferent fiber may connect to multiple homogeneous or heterogeneous sensors (MST). Since each type of sensor detects a specific force and generates a unique response, MST violates conventional theories and requires a conceptual shift. Mechanosensors and their reflex functions need redefinition. Therefore, data generated over the last eight decades under OST require reinterpretation. The emergence of genetic tools further enhances our understanding of this sensory system. However, their data interpretation needs to be within the MST framework. Understanding MST may solve longstanding issues like mechanically induced cough. It should facilitate research on central reflex integration and the design of molecular and genetic approaches to the sensory system.

O32 - Morphology, synaptics, and excitability of intracardiac neurons from mice, pigs and humans: targets for neuromodulation in cardiac disease

Theme: Integrative control

Dr. John Tompkins[1]
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Intrinsic cardiac neurons (ICNs) play a crucial role in the functioning of the heart; yet there are limited data on human ICNs. To bridge this gap, we have used a multidisciplinary approach to conduct a comprehensive cellular comparison of ICNs across three species: mice, pigs, and humans. Our methodology included immunohistochemistry of whole and sectioned ganglia, transmission electron microscopy, intracellular microelectrode recording, and dye filling for quantitative morphometry, enabling us to delineate the neurophysiology, histochemistry, and ultrastructure of these cells across species. I will present key findings of this recent study and showcase highlights of the multimodal atlas for mouse, pig, and human ICNs. In summary, the densely packed, smaller ICNs of mice lacked dendrites, formed axosomatic connections, and had high synaptic efficacy resembling an obligatory synapse. At Pig ICNs, a convergence of subthreshold cholinergic inputs onto extensive dendritic arbors supported greater summation and integration of synaptic input. Human ICNs were tonically firing, with synaptic stimulation evoking suprathreshold excitatory postsynaptic potentials like mouse, and subthreshold potentials like pig. Ultrastructural examination of synaptic terminals revealed conserved architecture, yet small clear vesicles were larger in pigs and humans. The presence and localization of ganglionic neuropeptides was distinct, with VIP observed in human but not pig or mouse ganglia, and little SP or CGRP in pig ganglia. Action potential waveforms were similar, but human ICNs had larger after-hyperpolarizations. Intrinsic excitability differed; 93% of human cells were tonic, all pig neurons were phasic, and both phasic and tonic phenotypes were observed in mouse. In combination, this publicly accessible atlas of ICNs from mice, pigs, and humans documents similarities and differences in the evolution of ICNs. We hope the work inspires future experimental investigations and theoretical modeling aimed at advancing therapeutic strategies targeting these cells for treatment of cardiac disease.

O33 - Cardiac Spinal Afferents: A New Therapeutic Target in Treating Chronic Heart Failure

Theme: Integrative control

Dr Hanjun Wang[1]
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The enhanced cardiac sympathetic afferent reflex (CSAR), a sympatho-excitatory reflex originating in the heart, contributes to elevated sympathetic tone as well as cardiac remodeling in chronic heart failure (CHF). Neuromodulation of the cardiac spinal afferent pathway is a potentially new therapeutic approach to treating CHF. Selective ablation of cardiac TRPV1 (transient receptor potential cation channel, subfamily V member 1)-containing spinal afferents, while retaining all other sensory modalities and motor function, represents a novel therapeutic strategy to reduce cardiac sympathetic tone while avoiding off-target side effects. In the last decade, our laboratory has explored the therapeutic potential of selective ablation of cardiac TRPV1-expressing afferents by administration of the ultra-potent TRPV1 agonist resiniferatoxin (RTX) in CHF. Earlier work from our team demonstrated that cardiac afferent ablation by epicardial application of RTX at the time of myocardial infarction (MI) reduced cardiac fibrosis, inflammation, and apoptosis, and improved cardiac diastolic dysfunction in post-MI rats. A subsequent study reported that cardiac afferent ablation prevented the development of renal dysfunction post-MI. More recent work from our laboratory focuses on pharmaceutical approaches to suppressing cardiac afferent sensitization by targeting the neural inflammation cascade at the level of T1-T4 DRGs post-MI. Our data suggests that pro-inflammatory macrophage infiltration occurs in T1-T4 DRGs post-MI, which was associated with downregulated protein expressions of A-type Kv channels as well as decreased Kv currents in T1-T4 DRG neurons. Both macrophage infiltration and Kv channels dysfunction in T1-T4 DRGs were largely prevented by chronic oral administration of minocycline (Mino). Moreover, Mino attenuated the exaggerated CSAR in MI rats. Therefore, our work suggests the translational potential of cardiac spinal afferent ablation in treating cardiac remodeling and renal dysfunction in CHF. A new anti-inflammatory approach could be more promising for functionally suppressing cardiac spinal afferent sensitization without requiring cardiac afferent ablation in CHF.

Symposium Presentations Session 3

Symposium Title: Neuroimaging of cardiovascular and respiratory control in humans

O34 - Identification of the human sympathetic connectome involved in blood pressure regulation

Theme: Integrative control

Prof Luke Henderson

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We review our recent data obtained on the cortical and subcortical components of the human sympathetic connectome - the network of regions involved in the sympathetic control of blood pressure (Macefield & Henderson, 2019). Specifically, we functionally identified the human homologue of the rostral ventrolateral medulla (RVLM), the primary premotor sympathetic nucleus in the medulla responsible for generating sympathetic vasoconstrictor drive. By performing functional magnetic resonance imaging (fMRI) of the brain at the same time as recording muscle sympathetic nerve activity (MSNA), via a microelectrode inserted into the common peroneal nerve, we are able to identify areas of the brain involved in the generation of sympathetic outflow to the muscle vascular bed, a major contributor to blood pressure regulation. Together with functional connectivity analysis of areas identified through MSNA-coupled fMRI, we have established key components of the human sympathetic connectome and their roles in the control of blood pressure. Whilst our studies confirm the role of lower brainstem regions such as the NTS, CVLM and RVLM in baroreflex control of MSNA, our findings indicate that the insula – hypothalamus – PAG – RVLM circuitry is tightly coupled to MSNA at rest. This fits with data obtained from experimental animals, but also emphasizes the role of areas above the brainstem in the regulation of blood pressure.

O35 - Functional brainstem imaging of sympathetic drive using MSNA coupled fMRI at ultra-high field

Theme: Integrative control

Ms Rebecca Glarin[1], Mr Donggyu Rim[2], Prof Vaughan Macefield[3], Prof Vaughan Macefield[2]
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Introduction: Muscle Sympathetic Nerve Activity (MSNA) contributes to the beat-to-beat regulation of blood pressure and originates within a specific nucleus of the brainstem - the rostral ventrolateral medulla (RVLM). Using MSNA-coupled functional Magnetic Resonance Imaging (fMRI) - in which we record MSNA while simultaneously performing fMRI - our lab previously identified the human RVLM and associated medullary nuclei at 3T. We are now extending this work using Ultra-High Field (7 Tesla), with the promise of higher spatial resolution and signal-to-noise, we aim to functionally identify the brainstem nuclei responsible for generating sympathetic drive using high-resolution 7T fMRI coupled with direct recordings of MSNA.

Methods: Recording: A tungsten micro electrode was inserted into a muscle fascicle of the left common peroneal nerve of 10 healthy participants. Neural activity was amplified and spontaneous bursts of MSNA identified and measured.
Imaging: Blood Oxygen Level Dependent (BOLD) contrast, echo-planar images were continuously collected in a 4 s ON, 4 s OFF protocol, with 1 mm isotropic voxels (210 volumes) (Magnetom Plus, Siemens). The data were divided into 4 x 1 s epochs and the analysis model couples the presence of a nerve burst per epoch with the equivalent epoch in the BOLD signal.

Results: Fluctuations in BOLD signal intensity covaried with intensity of the concurrently recorded bursts of MSNA. A group level analysis has identified MSNA-coupled BOLD activation increases in RVLM, Raphe Obscurus (ROb) and Periaqueductal Gray (PAG), along with a decrease in BOLD signal in the Dorsal Motor Nucleus of the vagus nerve (DMV).

Conclusion: Ultra-High Field MSNA-coupled fMRI has shown spontaneous bursts of MSNA covary with spontaneous changes in BOLD signal intensity within the RVLM, ROb, PAG and DMV. Improved signal-to-noise and higher spatial resolution available at Ultra-High Field provides highly specific imaging of small medullary nuclei responsible for generating sympathetic drive.

O36 - The role of the forebrain in cardiovascular control in exercising humans

Theme: Integrative control

Professor Kevin Shoemaker[1]
[1]Western University, London, Canada

The concept of a central, or cortical, autonomic network (CAN) has emerged over the past century. More recently, studies exploring the temporal and spatial features of such a network in conscious humans became feasible with neuroimaging technologies. Using blood oxygenation level dependent (BOLD) imaging (or functional magnetic resonance imaging) our studies regarding the CAN have focused on the whole-brain activation patterns that correspond to cardiac function in conscious humans (1). For example, rapid increases in heart rate at the exercise onset represent the impact of vagal withdrawal with minimal change in sympathetic outflow (2). Therefore, this model of short-duration, moderate intensity handgrip exercise provides an opportunity to target brain regions associated with cardiovagal control. Using BOLD imaging we have explored the CAN associated with dynamic heart rate changes at the exercise onset. Our studies highlight strong associations between the heart rate response and activity changes in the medial prefrontal cortex, insula cortex, dorsal anterior cingulate, posterior cingulate, amygdala and hippocampus (1). Connectivity analyses indicate that subnetworks, such as the hippocampus – medial prefrontal cortex axis may exist (3). Submotor electrical stimulation of wrist flexor muscle proprioceptive afferents a) induces CAN activity patterns that, generally, are opposite to those elicited by volitional exercise, and b) corresponds to evidence of elevated cardiovagal influence (4). Direct electrical recordings of CAN sites using surgically implanted stereo-encephalographic electrodes confirm the BOLD patterns. The combined studies provide evidence supporting a CAN in humans that influences cardiac functional responses to exercise.

O37 - Ultra-high-field imaging of networks related to breathing and breathlessness

Theme: Integrative control

Dr Olivia Harrison[1], Professor Kyle T S Pattinson[2], Professor Klaas E Stephan[3]
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Capturing the brain dynamics involved in the control and perception of human respiration remains immensely challenging. Almost all currently available, non-invasive technologies struggle to separate the physiological and movement artefacts from the neural underpinnings of breathing control, and many (such as electroencephalography; or EEG) cannot accurately localise the integral deep subcortical and brainstem structures. With the advent of ultra-high field magnetic resonance imaging (i.e., 7 Tesla and above), reaching these previously inaccessible structures is now possible. We have developed neuroimaging protocols and analysis methods to quantify breathing-related signal within key autonomic circuitry structures such as the midbrain periaqueductal gray, which we have shown to respond to breath holds as well as both anticipation and perception of mechanical breathing loads. Moreover, we have considered the wider circuitry of breathing perception and control across a range of populations, from healthy volunteers, endurance athletes, those with lung conditions such as asthma and chronic lung disease, and more recently within the field of mental health and anxiety. This work now also incorporates computational models to better understand how individuals learn about breathing stimuli, and how predictions and prediction errors towards breathing may be altered under different physiological and psychological conditions.

O38 - The manifestation of Autonomic Disorders in Post-Acute Sequelae of Covid-19 (PASC)

Theme: Integrative control

Ms Marie-claire Seeley[1,2], Dr Celine Gallagher[1,2], Ms Gemma Wilson[1,2], Professor Amanda Page[1,2], Prof Dennis Lau[1,2]
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Background: Post-acute sequelae of COVID-19 [PASC] affects 10% of all COVID infections and has been associated with postural orthostatic tachycardia syndrome [POTS].

Purpose: To assess prevalence of autonomic disorders in those with PASC.

Methods: Consecutive patients reporting unexplained symptoms persisting for ≥3 months after SARS-CoV-2 infection were recruited through social media platforms. Beat to beat plethysmography was used to determine autonomic haemodynamic response to 10-minute active stand test. International criteria were used to diagnose POTS, initial orthostatic hypotension (IOH), and classical orthostatic hypotension (COH). Validated patient reported outcome measures were used to determine symptom severity and health related quality of life (HrQoL) including, composite autonomic symptom score [COMPASS-31], Euroquol 5-Dimension [EQ-5D] and fatigue severity score (FSS).

Results: A total of 150 PASC participants (79% female, 93% Caucasian) with a mean age of 40.7 ± 11.5 years and BMI 25.8 ± 6.7 were enrolled. In total 75% met the criteria for an autonomic disorder with the most common diagnoses being POTS (55%), initial orthostatic hypotension (38%), and classic orthostatic hypotension (5%). Autonomic symptoms were more severe in women than men (Total COMPASS-31; p = .018) and those with a COMPASS-31 score >40 (49%) had worse fatigue severity (p<.001) and poorer health utility scores (p =.008). Fatigue was universally problematic with 92% experiencing FSS score >36. A significant 91.3% reported missing work due to PASC symptoms and 50.3% had been unable to return to work.

Conclusion: Autonomic disorders are prevalent in PASC and greatly impact on working capacity and health related quality of life.

O39 - Resting-state brain activity, sympathetic outflow and vascular function in adiposity – investigating the link using combined magnetoencephalography and microneurography

Theme: Integrative control

Miss Mariya Patel[1], Mr Joe Braun[1], Dr. Will Woods[2], Dr. Charlotte Keatch[3], A/Prof. Tatiana Kameneva[1,3,4], A/Prof. Elisabeth Lambert[1,4]
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Keywords: magnetoencephalography, sympathetic nerve activity, obesity

Introduction: Obesity-associated cardiometabolic complications are associated with sympathetic overactivity. Whether alterations in neural activity are implicated in these outcomes remain unknown; therefore, we investigated resting-state brain dynamics and its link with muscle sympathetic nerve activity (MSNA) and vascular function in individuals with various levels of adiposity.

Methods: Concurrent recordings of brain activity using magnetoencephalography (MEG) and MSNA using microneurography were obtained in 29 healthy participants (BMI 16.7 to 33.8kg/m2 and total body fat percentage 7 to 45.3%). MEG data was co-registered with structural T1-MRI and source activity determined using Linearly Constrained Minimum Variance (LCMV) beamformer. Source activity was expressed as neural activity index (NAI) for regions of interest, selected based on their role in MSNA generation or obesity-related cardiometabolic dysfunction, in physiological frequency bands, delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz) and gamma (30-120Hz). Vascular function was assessed using SphygmoCor XCEL to derive central blood pressure, augmentation index and pulse wave velocity.

Results: NAI in the right medial prefrontal cortex (mPFC), anterior cingulate, caudate (all gamma band) and precuneus (alpha band) negatively correlated with fat% but not BMI. Moreover, NAI in the right precuneus and caudate (delta, theta and gamma bands) showed correlations with MSNA burst incidence (bursts per 100 heartbeats). NAI in right precuneus, left amygdala (both alpha band) and left orbitofrontal cortex (theta band) also correlated with central BP, in the left and right mPFC (delta-gamma bands) correlated with augmentation index and, in the brainstem (delta-gamma bands) and left caudate (theta, alpha bands) correlated with pulse wave velocity.

Conclusion: We have shown that key regions including the right precuneus, caudate and mPFC may play an important role in sympathetic outflow control and vascular dysfunction in adiposity. Thus, our results may help in evaluating the central network in obesity-associated excess sympathetic activity.

Symposium Presentations Session 3

Symposium Title: Working towards selective vagus nerve stimulation to modulate autonomic function

O40 - Stimulation parameters for directional vagus nerve stimulation

Theme: Bioelectronics

James Fallon, University of Melbourne

Abstract not received

O41 - Deciphering the anatomical and functional organisation of the cervical vagus for spatially selective neuromodulation

Theme: Bioelectronics

Dr Nicole Thompson[1], Dr Enrico Ravagli, Dr Svetlana Mastitskaya, Dr Ronald Challita, Dr Joseph Hadaya, Dr Francesco Iacoviello, Mr Justin Perkins, Mr Ahmad Shah Idil, Prof Paul R Shearing, Dr. Olujimi Ajijola, Dr. Jeff Ardell, Prof Kalyanam Shivkumar, Professor David Holder, Dr Kirill Aristovich
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Spatially selective vagus nerve stimulation (sVNS) offers a promising solution to mitigate the off-target effects associated with traditional VNS. Our research focuses on elucidating the anatomical and functional organisation of the vagus nerve to enable sVNS, allowing for not only enhancing therapeutic outcomes in epilepsy and depression, but extending its application beyond, to treat other conditions, such as heart failure and lung injury.

We have mapped the fascicular anatomy of the recurrent laryngeal, cardiac, and pulmonary branches of the vagus nerve at the mid-cervical level in pigs using in vivo Fast Neural Electrical Impedance Tomography (FN-EIT) and selective neuromodulation with a 28-electrode array, cross-validated with ex vivo micro-computed tomography (microCT). Our findings revealed that these fascicles remain discrete with minimal overlap and sVNS was shown to successfully modulate physiological activities in the larynx, lungs, and heart separately, confirming organotopic organisation of the cervical vagus nerve (1).

Furthermore, we investigated the spatial organisation of afferent and efferent cardiac-related fibres specifically by combining the above techniques with vagotomies. Significant separation between cardiac afferent and efferent regions was identified, localised to opposite sides of the nerve. Stimulation of these regions elicited distinct heart rate responses, confirming the ability to selectively target therapeutic-related cardiac efferent effects without affecting afferent-related reflexes, promising for the treatment of chronic heart failure (2).

Now, we are performing sVNS in humans to determine if the same functional organisation exists. So far, selective cardiac efferent neuromodulation (~10% bradycardia) has been observed.

Our research demonstrates that the cervical vagus nerve exhibits a distinct anatomical and functional organisation. This knowledge can be leveraged to develop more precise VNS therapies, offering enhanced efficacy by minimising off-target effects and eliminating the need for extensive titration. This work represents a significant advancement towards the realisation of spatially selective neuromodulation for treating a range of disorders.

O42 - Selectively targeting the afferent vagus

Theme: Bioelectronics

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What impact does vagal nerve stimulation have on brainstem circuits. Are these associated with changes in behaviour (interoception) and neuronal function and is there a dose response relationship? We determined if VNS delivered at different intensities, indexed to the Hering-Breuer Reflex (HBR), affects anxiety-like behaviour in rats. Electrodes for VNS delivery and recording diaphragm muscle and electrocardiogram activity were implanted in rats. Anxiety-like behaviour was assessed after VNS using the elevated plus maze, open field test, and novelty suppressed feeding test. A place preference assay was performed to determine if VNS is rewarding or aversive. Rats were perfused to assess c-Fos expression in the nucleus of the solitary tract and locus coeruleus. VNS at 1.5x HBR reduced anxiety-like behaviour in the elevated plus maze and open field test, increasing open area times. Conversely, 3x HBR VNS heightened anxiety, prolonging centre avoidance. Higher intensity VNS was aversive in the place preference assay. Both VNS intensities increased c-Fos expression in the NTS, with 1.5xHBR elevating it in catecholaminergic neurons and reducing it in the locus coeruleus. Depending upon the strength of stimulation current, physiological indexed VNS has different and reproducible effects on anxiety-like behaviour in the rat.

O43 - Neural mechanisms of cardioprotection

Theme: Bioelectronics

Dr Svetlana Mastitskaya[1], Dr Felipe Freitas[2], Prof David Attwell[2]
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Vagus nerve plays a crucial role in protecting the heart during myocardial infarction, preserving cardiac function in heart failure, and enhancing exercise capacity (Mastitskaya et al., 2012; Machhada et al., 2020). The signaling pathway from the vagus to the heart is complex and constitutes a brain-gut-heart communication axis. The majority of vagal efferent fibers project to the gut, where they control digestion and the secretion of various gut hormones, including the incretin hormone glucagon-like peptide-1 (GLP-1). GLP-1 exhibits significant positive effects on the cardiovascular system, such as vasodilation, reduced inflammation, improved myocardial blood flow, and enhanced cardiomyocyte survival. Our research has demonstrated that the cardioprotective action of vagus nerve stimulation on the heart is mediated, at least in part, by an increased release of GLP-1 from the gut (Basalay et al., 2016; Mastitskaya et al., 2016). The stimulation of GLP-1 receptors in the heart activates pro-survival signaling pathways (Basalay et al., 2016) and reduces myocardial susceptibility to ventricular arrhythmias (Ang et al., 2018). Furthermore, vagus nerve stimulation prevents no-reflow after myocardial ischaemia, a phenomenon where, after reopening the culprit artery in myocardial infarction, blood flow at the microvascular level is not fully restored. This contributes heavily to poor healing of the infarct, the development of heart failure, malignant arrhythmias, and even cardiac rupture. Microvascular no-reflow is mediated by pericytes – contractile cells wrapped around capillaries. In ischaemia, pericytes contract and constrict the underlying capillaries (O'Farrell et al., 2017). Our most recent data suggest that this constriction can be prevented by the stimulation of GLP-1Rs on microvasculature. The downstream molecular mechanism of GLP-1R activation involves the opening of KATP channels. Therefore, GLP-1R agonists and KATP channel openers are novel therapeutic targets to prevent no-reflow, reduce infarct size, and decrease the incidence of ventricular arrhythmias in patients with ischaemic heart disease.

O44 - Cardiac vagal nerve activity increases during exercise to enhance coronary artery blood flow

Theme: Bioelectronics

Dr Julia Shanks[1], Dr Mridula Pachen[1], Dr Rohit Ramchandra[1]
[1]Manaaki Manawa - The Centre for Heart Research, , Department of Physiology, New Zealand

There is a strong association between parasympathetic nervous system activity (vagal tone) and physical fitness. Evidence for vagal withdrawal during exercise comes from historic studies examining heart rate control and the use of cholinergic blockers. However, more recent studies have challenged this assumption. We hypothesized that cardiac vagal activity increases during exercise and maintains cardiac function via neurotransmitters other than acetylcholine.

During whole-body treadmill exercise, chronic direct recordings of cardiac vagal nerve activity (CVNA), cardiac output, coronary artery blood flow, and heart rate were recorded in conscious adult sheep (n = 6 - 9). Sheep were exercised with pharmacological blockers of acetylcholine (atropine, 250 mg), vasoactive intestinal peptide (VIP) ([4Cl-D-Phe6,Leu17]-VIP 25 µg), or saline control, randomized on different days. In a subset of sheep, the left cardiac vagal branch was denervated.

Directly recorded CVNA increases during the lowest exercise intensity and plateaus as exercise intensity increases. Left cardiac vagal branch, denervation attenuated the maximum changes in coronary artery blood flow (control: 63.5±5.9 ml/min, n = 8, denervated: 32.7±5.6 ml/min, n = 6. $P < 0.01$) cardiac output (control: 5.6±0.9 L/min, n = 8, denervated: 4.5±0.7 L/min, n = 6. $P < 0.05$), and heart rate, during exercise, and rate of heart rate recovery post-exercise. Atropine did not affect any cardiac parameters during exercise, but VIP antagonism significantly reduced coronary artery blood flow during exercise to a similar level to vagal denervation (maximum exercise, paired: control: 68.2±6.0 ml/min, VIP antagonism: 44.9±3.9 ml/min, n = 6. $P < 0.01$).

Our study demonstrates that cardiac vagal nerve activity increases and is crucial for maintaining cardiac function during exercise, challenging the conventional view. Further, our results show that acetylcholine has no role in modulating cardiac function during exercise but that the dynamic modulation of coronary artery blood flow during exercise is mediated by parasympathetic co-transmitter VIP.

O45 - Use of high-resolution, live-cell imaging to investigate the molecular mechanisms of sympathoexcitation in cardiac sympathetic neurons

Theme: Cardiovascular

Jie Zhao[1], Ruqaiyah Lokhandwala[1], **Dr. Elizabeth Akin**[1]
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Sympathoexcitation, or overactivation of the sympathetic nervous system, contributes to numerous cardiovascular diseases. One characteristic of sympathoexcitation is increased release of neurotransmitters and neuropeptides from cardiac post-ganglionic neurons. Numerous studies have demonstrated that increased plasma levels of neuropeptide Y (NPY) correlates with worse clinical outcomes in patients. NPY is thought to arrhythmogenic and contribute to sudden cardiac death. Despite the importance of NPY as a novel therapeutic target, little is known about the cellular and molecular mechanisms that contribute to the enhanced release of NPY under conditions of sympathoexcitation. Here, we use high-resolution, live-cell imaging to investigate the real-time dynamics of cultured neonatal cardiac sympathetic neurons to test the hypothesis that axonal transport and synaptic release of NPY-containing vesicles is enhanced under conditions of sympathoexcitation. Using microfluidic chambers, we have established a compartmentalized co-culture system with the sympathetic neuron cell bodies localized within the somatic chamber, while their axons extend through microbarriers into a chamber containing cultured cardiomyocytes. Preliminary experiments using calcium imaging suggest the presence of functional neuro-cardiac junctions. In order to investigate the long-distance axonal trafficking of NPY-containing vesicles, or dense core vesicles, we use imaging of neurons transfected with fluorescently-tagged NPY. Future experiments will use spinning-disk confocal microscopy to characterize the transport, synaptic capture, and exocytic release of NPY-containing vesicles under control conditions and conditions of sympathoexcitation. The long-term goal of this project is to identify molecular pathways that contribute to the pathophysiological increase of NPY during cardiovascular disease to provide targets for therapeutic interventions.

O46 - Sympathetic response to exercise predicts exercise capacity of patients with heart failure across the left ventricular ejection fraction spectrum

Theme: Cardiovascular

Dr. Mark B. Badrov[1], Dr. Catherine F. Notarius[1], Dr. Daniel A. Keir[1,2], Dr. Tomoyuki Tobushi[1], Dr. Philip J. Millar[1,3], Dr. David Z. Cherney[1], Dr. Susanna Mak[1], Dr. John S. Floras[1]
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In heart failure (HF), both sympathetic excess and exercise intolerance impair quality of life. We recently discovered a 'paradoxical' reflex increase in muscle sympathetic nerve activity (MSNA) during one-leg cycling exercise in patients with HF with reduced ejection fraction (HFrEF), which related inversely to exercise capacity. It remains unknown if this disturbance is specific to HFrEF, or whether it is evident in all with HF, regardless of left ventricular ejection fraction (LVEF). We investigated data from 55 HF patients (16 females; 64±9 yrs), across the LVEF spectrum (mean 38±16%; range 15-73%), and 49 healthy controls (23 females; 60±9 yrs). We measured, in the seated position, heart rate (HR), blood pressure (BP), and MSNA (microneurography) during 2 min of baseline and 4 min of one-leg cycling (2 min each at mild and moderate intensity). Exercise capacity (peak oxygen uptake; $\dot{V}O_2$peak) was obtained by open circuit spirometry. Resting MSNA was elevated in HF (50±11 bursts/min) vs. HC (39±10 bursts/min; P<0.0001), whereas both relative (19±7 ml/kg/min) and percent-predicted (79±24%) $\dot{V}O_2$peak were lower vs. HC (30±9 ml/kg/min and 116±26%, respectively; both P<0.0001). During cycling, HR and BP increased similarly in both groups (P>0.05). Compared to baseline, MSNA decreased during mild (−5±6 bursts/min; P<0.001) and moderate (−6±7 bursts/min; P<0.001) cycling in HC, as anticipated, yet increased in HF during both mild (+5±8 bursts/min; P<0.001) and moderate (+5±10 bursts/min; P<0.001) cycling. In HF, the MSNA response to moderate cycling was unrelated to LVEF (R2=0.01; P=0.46), but related inversely to both relative (R2=0.21; P<0.001) and percent-predicted (R2=0.19; P=0.001) $\dot{V}O_2$peak. No such relationships existed in HC (P>0.05). Thus, unlike healthy individuals, exercise stimulates a 'paradoxical' sympatho-excitatory response in most with HF, independent of LVEF, that predicts patients' $\dot{V}O_2$peak. Our findings offer unifying evidence for a neurogenic, vasoconstrictor limit on exercise in HF, regardless of reduced or preserved phenotype.

O47 - A modular platform to generate functional sympathetic neuron-innervated heart assembloids

Theme: Cardiovascular

Dr. Hsueh FuW Wu[1,2], Dr. Kenyi Saito-Diaz[1,2], Xin Sun[3], Ming Song[4], Tripti Saini[1,2], Courtney Grant[5], Christina James[1,2], Kimata Thomas[1], Yohannes Abate[6], Elizabeth Howerth[7], Peter Kner[4], Bingqian Xu[3], **Dr. Nadja Zeltner**[1,2,8]
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The technology of human pluripotent stem cell (hPSC)-based 3D organoid/assembloid cultures has become a powerful tool for the study of human embryonic development, disease modeling and drug discovery in recent years. The autonomic sympathetic nervous system innervates and regulates almost all organs in the body, including the heart. Yet, most reported organoids to date are not innervated, thus lacking proper neural regulation, and hindering reciprocal tissue maturation. Here, we developed a simple and versatile sympathetic neuron (symN)-innervated cardiac assembloid without the need for bioengineering. Our human sympathetic cardiac assembloids (hSCAs) showed mature muscle structures, atrial to ventricular patterning, and spontaneous beating. hSCA-innervating symNs displayed neurotransmitter synthesis and functional regulation of the cardiac beating rate, which could be manipulated pharmacologically or optogenetically. We modeled symN-mediated cardiac development and myocardial infarction, where we detected fibrosis, stiffening of the tissue that could be pharmacologically rescued. This hSCAs provides a tool for future neurocardiotoxicity screening approaches and is highly versatile and modular, where the types of neuron (symN or parasympathetic or sensory neuron) and organoid (heart, lung, kidney) to be innervated may be interchanged.

O48 - Anti-arrhythmic potential of P2X3 inhibition

Theme: Cardiovascular

Dr Carol T Bussey[1], Dr Rexson Tse[2], Assoc/Prof Martin K Stiles[3], Professor David Paterson[4], Prof. Julian F. R. Paton[1]
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Cardiovascular diseases are characterised by elevated sympathetic nerve activity, which contributes to end-organ damage, morbidity and mortality. Surgical removal of the stellate ganglion to short-circuit sympathetic nerve overactivity can eradicate arrhythmias, however this is a highly invasive approach with significant side-effects, necessitating discovery of novel non-invasive druggable targets. Recent transcriptomic data shows upregulation of P2X3 purinergic receptors in the stellate ganglia of Spontaneously Hypertensive (SHR) compared to Wistar rats (Bardsley et al. Sci Rep. 2018). We hypothesise that these purinergic receptors within cardiac stellate ganglia contribute to sympathetic overactivity and the development of cardiovascular diseases such as hypertension and arrhythmias.

We have confirmed in both human and rat stellate ganglia that P2X3 receptors co-localise with tyrosine hydroxylase-expressing sympathetic cells via immunofluorescent staining. Upregulation of P2X3 expression in stellate ganglia of SHR is demonstrated via qPCR (Wistar 1.03±0.10, SHR 3.77±0.78 fold, n=7-8, $p<0.01$). Cardiac responses to stellate ganglion P2X3 receptors were investigated in the decerebrated working heart-brainstem rat preparation (4-5 week old). Microinjection of stable ATP analogue αβmethylene-ATP (100µg) directly into the stellate ganglion causes tachycardia (Wistar 45.6±8.26; SHR 62.5±14.14 Δbpm), which is attenuated by P2X3 inhibition with AF353 (Wistar 25.0±7.46; SHR 19.5±7.48 Δbpm; n=4-5, $p<0.05$). Further, SHR, which exhibit increased arrhythmogenicity, were triggered with atropine (30µM) and caffeine (100µM) followed by electrical stimulation of the stellate ganglion. Arrhythmias were observed in the ECG of 61% of experiments (n=13), particularly AV block, and fragmented QRS complexes, with bundle block and bradyarrhythmia also observed. Of these arrhythmias, 70% were attenuated or abolished following blockade of P2X3 receptors (n=9).

Stellate ganglion P2X3 purinergic receptors regulate cardiac function, and P2X3 overexpression likely contributes to sympathetic overactivity in cardiovascular disease. P2X3 inhibition rapidly and profoundly recovers arrhythmic heart rhythms, and reverses electrical instability.

Health Research Council of New Zealand and Sidney Taylor Trust funded research.

O49 - PrRP/GPR10 signalling has an important role in energy balance and cardiovascular regulation

Theme: Cardiovascular

Dr Claire H. Feetham[1], Dr Fleur Talbot[2], Professor Sadaf Farooqi[2], Professor Simon M. Luckman[1]
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Prolactin-releasing peptide (PrRP) is expressed in three neuronal populations, including one in the dorsomedial hypothalamus (DMH). The receptor for PrRP, GPR10, is expressed at sites involved in appetite and autonomic regulation. We have found polymorphisms in human GPR10 linked to both obesity and blood pressure (BP) (1,2). We have shown also that knock-out of PrRP reduces energy expenditure and causes obesity, but that this can be rescued by re-expression in DMH neurones (3). Furthermore, in rats, central administration of PrRP increases mean arterial blood pressure (BP) (4).

Here, we report that null Gpr10-/- mice exhibit an obese phenotype. Importantly, pre-obese Gpr10-/- mice have reduced energy expenditure, measured as a difference in oxygen consumption (VO_2) measured by indirect calorimetry, with no difference in food intake. This reduction persists with age compared with wild-type (WT) littermates. Baseline BP recordings were made in conscious, pre-obese Gpr10-/- mice by tail-cuff plethysmography, and were found to be hypotensive compared with WT littermates. To test the physiological consequence of human variants on body weight, we generated a knock-in mouse model harbouring the most common functional GPR10 variant found in individuals with severe obesity (Gpr10P193S). Gpr10P193S mice exhibit greater weight gain than WT littermates. As with Gpr10-/- mice, pre-obese Gpr10P193S mice show no difference in food intake, but reduced energy expenditure.

Additionally, we have used PrRP-cre mice injected into the DMH with a cre-dependent stimulatory designer receptor, AAV-hM3Dq-mCherry (DMH-PrRPhm3Dq). Injection with clozapine N-oxide (CNO) is sufficient to cause an acute increase in VO_2 and BP.

Together, the data suggests that PrRP/GPR10 signalling plays an important role in energy balance and cardiovascular regulation.

1. Bhattacharyya et al., 2003, Diabetes 52(5):1296–1299.
2. Talbot*, Feetham* et al., 2023, Nat Commun 14: 1450.
3. Dodd et al., 2014, Cell Metab 20: 639.
4. Samson et al., 2000, Brain Research, 858(1):19-25.

O50 - Transplanting embryonic neural progenitor cells to restore cardio-electric disorders following spinal cord injury

Theme: Cardiovascular

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Traumatic spinal cord injury (SCI) at high levels often interrupts the supraspinal vasomotor pathway to cause reduced sympathetic regulation of the heart. The loss of balance between autonomic components triggers the occurrence of cardio-electric conduction disorders. To determine if transplanting serotonergic, early neurons restores the compromised neuronal regulation to improve cardiac dysfunction, embryonic day 14 (E14) raphe nuclei derived neural progenitor cells (RN-NPCs) were transplanted into the lesion of a crushed rat upper thoracic spinal cord. Spinal cord (SC)--derived NPCs, injury alone, and intact rats served as controls. After 9 weeks, a radio-telemetric system was used to record electrocardiogram (ECG) and hemodynamic parameters, including 24-h recording for heart rate variability (HRV), cardiac arrhythmias during colorectal distention (CRD)-induced autonomic dysreflexia, and dobutamine stress tests. As a result, transplanting RN or SC-NPCs significantly increased HRV which was illustrated in both time and frequency domains, and decreased the number of arrhythmias during dobutamine delivery compared to the injury control. Subsequently, pharmacological interventions showed that grafting either RN or SC-NPCs reconstituted the sympathetic tone to the heart and reestablished sympathetic support of blood pressure. However, cell grafting did neither reduce arrhythmias during autonomic dysreflexia nor improve resting hemodynamics. NPC grafting did not reverse the injury-induced vagal gain and enhanced dependence on the renin-angiotensin system of blood pressure. Histological analysis revealed that grafted cells were well-integrated with the host tissue and extended axons onto the host caudal autonomic regions. Ultimately, the results indicate that NPC transplantation, regardless of their phenotype, improves neuronal control of the heart to partially restore cardiac electrical activity after SCI.

O51 - Addressing the enigma of treating heart failure with preserved ejection fraction with P2X3 receptor antagonism

Theme: Cardiovascular

Dr Mridula Pachen[1], Dr Siyi Chen[1], Dr Joshua Chang[1], Professor Julian F.R Paton[1], Associate Professor Rohit Ramchandra[1]
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Heart failure with preserved ejection fraction (HFpEF) is a major health burden and a primary cause of death globally, yet there remains no curative treatment. Previous studies have indicated an important role for P2X3 receptors (P2X3R) in mediating high levels of sympathetic drive in hypertension (Pijacka et al., 2016). Given the high sympathetic drive in HFpEF (Seravalle et al., 2019), we investigated whether P2X3R antagonism would improve this condition. We tested the hypotheses that in HFpEF, P2X3R activation causes high levels of blood pressure and excessive vasoconstriction in the hindlimb vasculature during exercise.

Following unilateral renal artery clipping, an ovine model of HFpEF was established in female sheep displaying hypertension and diastolic dysfunction. A sham-operated group acted as a control. Mean arterial pressure (MAP), heart rate (HR), and hindlimb blood flow (HLBF) were recorded before and during treadmill exercise, and the effect of either a P2X3R antagonist (L227, 10mg/kg) or vehicle was assessed.

The clipping of one renal artery increased MAP (91±5 vs. 131±6, $p \leq 0.05$). The administration of the P2X3R antagonist reduced both MAP (-6±7 mmHg, $p \leq 0.05$) and HR (-6±6 bpm, $p < 0.05$) in HFpEF sheep but had no effect in sham-operated animals. HLBF during exercise was attenuated in the HFpEF model compared to sham-operated sheep (an increase of 2±1 vs. 4±2 L/min, respectively, $p < 0.05$). Importantly, P2X3R blockade improved the change in HLBF during exercise from 1±0.1 to 2±0.4 L/min in HFpEF sheep, but there was no change in the sham-operated animals. There was no change in the MAP or HR response to the exercise with the P2X3R blockade.
P2X3R plays a role in the aetiology of hypertension and in the attenuation of HLBF responses during exercise in HFpEF. P2X3R antagonism may provide a novel target to alleviate symptoms of HFpEF and improve exercise tolerance through increased HLBF.

O52 - Cardiac sympathetic innervation is disrupted in a mouse model of hypertension-induced heart failure

Theme: Cardiovascular

Ph.D. Arianna Scalco[1], Ethan N. Lee[1,2], Morgan Johnson[1], Michelle L. Sorensen[1], Thomas Hilton[1], Riley K. Omonaka[1,3], Shae Zeimantz[1], Sue Aicher[1], Ph.D. William R. Woodward[1], Professor Beth Habecker[1]
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Heart failure (HF) is a leading cause of cardiovascular morbidity and mortality, estimated to affect 26 million people worldwide[1]. Hypertension (HTN) contributes significantly to the incidence of HF, being the primary cause in 25% of these cases[2]. Autonomic dysfunction and sympathetic hyperactivity play an important role in the development and evolution of HF[3] [4]. However, changes in cardiac sympathetic innervation in HF are not well understood. We hypothesized that cardiac sympathetic innervation is disrupted in a mouse model of HTN-induced HF.

To test this hypothesis, C57BL6/J mice were implanted with minipumps releasing either Angiotensin II (AngII) or saline for 4 weeks. AngII-treated mice developed HTN, as shown by significantly increased mean arterial pressure. Four weeks of AngII treatment led to HF, as indicated by reduced ejection fraction and fractional shortening. AngII HF mice also displayed cardiac hypertrophy and fibrosis, as reflected by increased heart weight: body weight ratio and trichrome staining, respectively. AngII HF mice had significantly reduced sympathetic nerve density in the left ventricle (LV), intraventricular septum, and right ventricle. In the LV, the subepicardium remained normally innervated while the subendocardium was almost devoid of sympathetic neurons. Loss of sympathetic fibers led to loss of norepinephrine content in the LV. AngII HF mice had a trend toward increased arrhythmia susceptibility after isoproterenol injection. Although HF can induce a cholinergic phenotype and neuronal hypertrophy[5] [6] in stellate ganglia, AngII HF did not induce a cholinergic phenotype or increase soma size in cardiac sympathetic neurons. Cardiac neurons in the left stellate ganglion were significantly smaller in AngII HF mice, while neurons in the right stellate were unchanged.

Our findings show that AngII HF disrupts endocardial sympathetic innervation. Further investigations are imperative to unveil the mechanisms of cardiac sympathetic denervation in HF and to develop neuromodulatory therapies for patients with autonomic imbalance.

Symposium Presentations Session 3

Symposium Title: Recent insights into the role of the vagus nerve in brain-gut communication and therapeutic implications of vagus nerve stimulation in the treatment of gastrointestinal disorders

O53 - Quantified anatomy of human vagus nerves from brainstem to abdomen

Theme: Gut and Metabolism

Dr Nicole Pelot[1]
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The vagus nerve (VN) connects the brainstem to cervical, thoracic, and abdominal organs. Mapping the gross anatomy, morphology, and fiber types of the VN has tremendous potential to improve the efficacy and specificity of autonomic neuromodulation therapies. We are conducting the most comprehensive mapping of the VN to date, from the perspectives of sample size, nerve length, imaging modalities, and image resolution.

Embalmed cadavers (N=25) were imaged with a 3T MR scanner. We developed a novel dissection approach to access the vagus from brainstem to abdomen without moving the body. Using an optical stylus, we digitized the 3D coordinates of the vagal trunk, the proximal ~2 cm of each branch, and standardized anatomical landmarks. We stained the excised nerve with 3% phosphotungstic acid (PTA) as a contrast agent for micro-computed tomography (microCT) imaging (11.4 µm isotropic voxels). Portions of the nerve were prepared for microscopy.

The entire bilateral vagal complex, including the cervical, thoracic, and abdominal branches, was successfully removed as one interconnected piece of tissue; we labeled each branch with its target structure. Branch counts and patterns varied substantially across individuals. PTA staining enhanced microCT contrast of fascicles against the surrounding epineurium, enabling automated segmentation of the complex 3D morphology. Histology resolved small fascicles and perineurium that were not captured by microCT, while immunohistochemistry enabled quantification of the spatial organization of fiber types. These MRI, 3D tracing, microCT, and microscopy imaging data provide essential inputs to computational models of autonomic nerve stimulation to analyze and improve neuromodulation therapies.

We quantified the branching patterns, morphology, and microstructure of the human VN to advance the VN anatomical knowledge base, and to identify novel neuromodulation targets, for neural stimulation therapies with high specificity through computational modeling. Our ongoing project will ultimately yield high-resolution imaging from N=50 cadavers.

O54 - Fixing faulty plumbing with better wiring: current and future vagus nerve stimulation approaches for Crohn's disease

Theme: Gut and Metabolism

Dr Sophie Payne[1]
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Despite medical advancements over 70% of patients with Crohn's Disease (CD) relapse and experience debilitating symptoms. At the Bionics Institute we have translated a medical device to deliver electrical stimulation to the abdominal vagus nerve for the benefits of 1) avoiding off-target affects seen during cervical vagus nerve stimulation (VNS); 2) allowing a larger therapeutic window; 3) being closer to the end organ (Payne 2019a).

Our abdominal vagus nerve stimulation (aVNS) array allows recording of electrically evoked neural responses and verification that stimulation is above threshold and activating appropriate fiber populations. In an rat efficacy study (Payne 2019b), we showed improvements in stool quality and reduced histopathological damage in the ileum during aVNS following in a experimental ileitis model. In a sheep safety study (Payne 2018), the device was scaled up to human size and stimulated over 12 weeks (30 Hz, 2 mA, continuous). There were no changes to the animal's behavior, histopathology of the nerve was benign and no changes to evoked potential thresholds. The safety/efficacy of the aVNS System is now in a first-in-human clinical trial NCT05469607, A/Prof Peter De Cruz, Austin Health, Melbourne, Australia) that aims to assess device safety and prevention of inflammatory recurrence at the anastomosis site in post-operative Crohn's Disease patients. Our first patient has been successfully implanted and I will discuss the exciting data after 6 months of stimulation (3 hrs/day; 10 Hz; 2 mA).

Although promising, delivering 'fixed' stimulation fails to adapt to the rapidly changing conditions of CD. The next broad aim of our research program is to develop 'adaptive' aVNS technology that rapidly adjusts to the level of inflammation. Here I describe pilot rat data that supports the use of an innovative neural recording/analysis system (Payne 2023) to extract vagal activity induced during intestinal inflammation.

O55 - Role of the vagus nerve in modulating visceral pain hypersensitivity, intestinal permeability and inflammation in health and GI disease

Theme: Gut and Metabolism

Qasim Aziz, Queen Mary University of London

Abstract not received

O56 - Invasive vagus nerve stimulation in Crohn's disease: A 10-year prospective study follow-up

Theme: Gut and Metabolism

Professor Bruno Bonaz[1]
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Crohn's disease (CD) is a chronic inflammatory disorder affecting all the gastro-intestinal tract. CD treatments focus on biologics and small molecules, not devoid of side-effects and with unmet needs. The vagus nerve (VN) has anti-inflammatory properties through its afferents (HPA axis) and efferents (cholinergic anti-inflammatory pathway) (Bonaz, 2017). The VN inhibits the release of pro-inflammatory cytokines such as TNFα and IL-6, produced by peripheral immune cells (Pavlov, 2018). VN activity is decreased in chronic inflammatory diseases, thus it is possible to use electrical VN stimulation (VNS) to modulate peripheral inflammation in CD patients (Bonaz, 2021).

We have reported a pilot study of a one year-VNS performed in 9 patients with active CD. The first patient was implanted in 2012 and the last one in 2016. Seven patients completed the study, two were retrieved at 3 month-VNS (surgery or biologic therapy), but all kept active stimulation. Five patients were in clinical remission, 6 in endoscopic remission, 6 decreased their CRP, 5 decreased their fecal calprotectin, 7 rebalanced their sympatho-vagal balance. Pro-inflammatory cytokinergic profile evolved towards a normal profile (decrease in IL6, IL23, IL12, and TNFα). There was a correlation between CRP/TNFα and metabolites of the gut mucosa (taurine, lactate, alanine, and □hydroxybutyrate) (Sinniger, 2020).

All the patients were then regularly followed in consultation twice a year with clinical, biological (CRP, fecal calprotectin), and morphological (abdominal ultrasound, ileo-colonoscopy) evaluation. At the end of follow-up, 3 patients under VNS were in remission without treatment. Five patients had to be treated with an immunosuppressant and/or anti-TNF or anti-Il12/IL23 agent (after a minimum of 3.5 years of active VNS alone), but all wanted to keep an active VNS. VNS was interrupted in one patient for pregnancy desire (after 3 years of VNS), without treatment at 8 years of follow-up. VNS was safe for all patients.

O57 - Sacral nerve stimulation to control colonic motility

Theme: Gut and Metabolism

Bradley Barth, **Professor Warren Grill**
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Disorders of gut-brain interaction disrupt control of colonic motility. Electrical stimulation of peripheral nerves can restore control and provides some relief of gastroparesis, fecal incontinence, and inflammatory bowel disease. However, sacral nerve stimulation failed to treat slow-transit constipation more effectively than sham stimulation in a randomized, double-blind, placebo-controlled, crossover study. The objective of our study was to restore colonic motility by electrical stimulation of the sacral nerves. We hypothesized that intermittent bursts of sacral nerve stimulation, based on the refractory period of the colonic motor complex, would more effectively increase prokinetic motility compared to conventional continuous sacral nerve stimulation. Burst-patterned stimulation of the pelvic nerve was more effective at evoking colonic motor complexes in the mouse colon ex vivo than was continuous pelvic nerve stimulation. We used anorectal manometry to reveal that burst stimulation of the L6/S1 nerve evoked larger and more consistent pressure responses in the urethane-anesthetized rat than continuous stimulation, and used this preparation to quantify the sensitivity of evoked pressures to burst parameters. Subsequently, we quantified changes in fecal output in awake, behaving rats constipated by loperamide gavage under three conditions: no stimulation, continuous stimulation, and burst stimulation of the L6/S1 nerve. Burst stimulation increased fecal output more effectively than continuous stimulation and reversed the constipation phenotype despite continued loperamide gavage. The results of this preclinical study demonstrate the sensitivity of colonic responses to the temporal pattern of sacral nerve stimulation and reveal that burst-patterned stimulation is effective and is superior to conventional, continuous sacral nerve stimulation in treating constipation. This study suggests sacral nerve stimulation may be a viable clinical treatment for slow-transit constipation.

O58 - Differential developmental blueprints of organ-intrinsic nervous systems

Theme: Gut and Metabolism

Dr. I-Uen (Yvonne) Hsu[1,2], Dr. Yingxin Lin[6], Yunshan Guo[1,2,6], Qian J. Xu[1,2,3], Yuancheng Shao[1,2], Ruiqi L. Wang[1,2,3], Dominic Yin[4], Jie Zhao[6], Lawrence H. Young[2,5], Hongyu Zhao[6], Dr. Le Zhang[1,3,4,7], Assistant Professor Rui Chang[1,2,3,7]

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The organ-intrinsic nervous system is a major interface between visceral organs and the brain, mediating important sensory and regulatory functions in the body-brain axis and serving as critical local processors for organ homeostasis. Molecularly, anatomically, and functionally, organ-intrinsic neurons are highly specialized for their host organs. However, the underlying mechanism that drives this specialization is largely unknown. Here, we describe the differential strategies utilized to achieve organ-specific organization between the enteric nervous system (ENS) and the intrinsic cardiac nervous system (ICNS), a neuronal network essential for heart performance but poorly characterized. Integrating high-resolution whole-embryo imaging, single-cell genomics, spatial transcriptomics, proteomics, and bioinformatics, we uncover that unlike the ENS which is highly mobile and colonizes the entire gastrointestinal (GI) tract, the ICNS uses a rich set of extracellular matrix (ECM) genes that match with surrounding heart cells and an intermediate dedicated neuronal progenitor state to stabilize itself for a 'beads-on-the-necklace' organization on heart atria. While ICNS- and ENS-precursors are genetically similar, their differentiation paths are influenced by their host-organs, leading to distinct mature neuron types. Co-culturing ENS-precursors with heart cells shifts their identity towards the ICNS and induces the expression of heart-matching ECM genes. Our cross-organ study thus reveals fundamental principles for the maturation and specialization of organ-intrinsic neurons.

O59 - Remodeling of Ventricular Catecholaminergic Axons Following Chronic Intermittent Hypoxia in Mice

Theme: ECR focus: Breaking abstracts

Dr Ariege Bizanti[1], Dr Yuanyuan Zhang[1], Mr Kohlton T Bendowski[1], Mr Jazune Madas[1], Ms Zulema Toledo[1], Ms Duyen Nguyen[1], Mr Lucas Qu[1], Dr Jin Chen[1], Ms Maci Heal[2], Dr Richard Christie[3], Prof. Peter Hunter[3], Dr Yulong Li[4], Dr Zixi Jack Cheng[1]
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Chronic intermittent hypoxia (CIH) is a widely employed model for sleep apnea. The imbalance of autonomic control may contribute to CIH-induced cardiovascular diseases. Previously, we demonstrated that CIH increases sympathetic innervation of the atria. In this study, we determined whether CIH remodels peripheral cardiac tyrosine hydroxylase (TH, a marker for sympathetic efferent axons) innervation in the whole ventricles. The two challenges here were: 1)labeling the entire TH-IR axon innervation in the full thickness (~500 µm) of whole flat-mount ventricles. 2)Quantifying the topographical distribution of TH-IR axons through the thick ventricular walls at the single axon/varicosity scale. In this study, C57BL/6J mice (male, n=7/group) were exposed to room air (RA) or CIH for 8-10 weeks. We prepared flat-mounts of the whole ventricles and septum and processed them with immunohistochemical labeling for TH. Using Zeiss M2 Imager, confocal microscope, Zeiss Arivis Vision 4D, and Neurolucida system, we traced and digitized the TH-IR axons. We found that: 1)In RA and CIH mice, several large TH-immunoreactive (TH-IR) bundles from the base area entered the ventricles and septum. These large bundles branched into numerous smaller bundles as they traveled downward toward the apex and finally developed into fine varicose axons and terminals in the epicardial and myocardial layers. 2) Using Vision 4D, we were able to accurately detect, trace, and digitize all TH-IR axons in the ventricle walls with high accuracy. 3) CIH increased overall TH-IR axon density and network complexity of the whole ventricles and septum. 3)Abnormal CIH TH-IR axon terminal structures were frequently seen. 4)We geometrically and precisely registered the tracing data into a scaffold map for presentation and comparison in 3D. Altogether, CIH increased sympathetic innervation of the heart which may contribute to the augmented sympathetic drive. Our success in mapping data in the 3D heart scaffold will establish a heart-brain connectome/atlas.

O60 - CD73 inhibition reverses chronic hypoxia induced carotid body hyperactivity

Theme: ECR focus: Breaking abstracts

Mr Demitris Nathanael[1], Dr Andrew Coney[1,2], Mr Prem Kumar[1], Dr Andrew Holmes[1,2]
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Carotid body (CB) hyperactivity in patients with chronic obstructive pulmonary disease (COPD) is associated with autonomic imbalance and cardiovascular disease [1]. A key feature of COPD is chronic hypoxia (CH). Mechanisms of CB hyperactivity in response to CH are unknown but could be dependent on increased CD73 activity (an enzyme that generates adenosine) [2-4]. Here, we investigated if pharmacological blockade of CD73 attenuated CH mediated CB hyperactivity.

Chemoafferent activity was measured ex vivo from CBs dissected from adult male Wistar rats (150-250g) exposed to either 10 days of normoxia (N, n=13 animals) or CH (FiO_2=12%, n=14 animals). Concentration-response experiments with AOPCP (CD73 inhibitor) ranged between 10nM-330µM in normoxia. Activity was measured continuously as the superfusate PO_2 was decreased in the absence or the presence of either 15µM or 100µM AOPCP. Results are expressed as mean ±SEM and significance ($P<0.05$) was established by unpaired t-test or one-way ANOVA.

CH increased basal CB discharge (0.55±0.06 Hz vs 1.50±0.17 Hz, $P<0.001$) and increased hypoxic sensitivity. The IC50 for AOPCP in N and CH were 1.4±0.3µM and 7.8±4µM respectively. At a concentration of 15µM, AOPCP reduced basal discharge by 89±2% in N and 53±3% in CH. However, 100µM AOPCP had no additional effect on basal discharge in N but reduced it in CH to 91±1%. 100µM AOPCP caused a leftward shift in the hypoxic response curves in N and CH. Sensitivity of the hypoxic response to AOPCP was enhanced in CH ($P<0.01$) reversing the exaggerated hypoxic responses back to those seen in N animals.

This data suggests that CH leads to elevated CB sensory discharge at baseline and during hypoxia, which can be reversed by ex vivo pharmacological inhibition of CD73. Whether in vivo CD73 inhibition can dampen augmented autonomic reflexes caused by CH to prevent cardiovascular disease warrants future investigation.

O61 - A neural footprint of central cardiovascular control during acute mental stress in humans

Theme: ECR focus: Breaking abstracts

Mr Joe Braun[1], Miss Mariya Patel[1], Dr. Will Woods[2], A/Prof. Tatiana Kameneva[3,4,5], Dr. Charlotte Keatch[5], A/Prof. Elisabeth Lambert[1,4,5]
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Keywords: Central cardiovascular control, mental stress, functional neuroimaging

Substantial evidence indicates associations among mental stress, aberrant signaling through the sympathetic nervous system and cardiovascular disease in certain individuals. We assessed the relationship between brain activity and cardiovascular responses to acute mental stress.

Simultaneous magnetoencephalography (MEG), sympathetic nerve activity (SNA), heart rate, blood pressure (BP) and respiration were recorded at rest, and during stress, in 29 healthy participants. T1 weighted magnetic resonance imaging and linearly constrained minimum variance (LCMV) beamforming were applied. Activity was filtered through delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz) and low gamma (30-80Hz) bands. Whole head, region of interest (ROI) and correlation analyses were performed. Participants were divided into high and low cardiovascular responders.

Stress induced a significant increase in SNA, 4.0±8.0 bursts per minute, heart rate, 4.1±4.8bpm, and mean BP, 4.1±5.1mmHg, $P<05$. Whole head analysis showed that stress significantly increased activity in the right medial frontal cortex and brainstem in delta band and decreased activity temporally in alpha band, $P<0.05$. However, ROI analyses revealed that stress increased activity in alpha band in the left and right thalamus, insular, precuneus and the right hippocampus and cingulate gyrus both anteriorly and posteriorly, but only in the low responders. Correlation analysis revealed that during stress, increased brain activity in the right insular, in alpha band, was significantly correlated with lower systolic ($r=0.446$) and mean ($r=0.456$) BP, while increased activity in the left amygdala, in alpha band, was significantly correlated with lower State ($r=0.647$), Trait ($r=0.442$), BDI-II ($r=0.446$) and systolic BP ($r=0.392$), $P<05$. Alternatively, increased brain activity during stress in the right amygdala, in delta band, was significantly correlated with increased SNA ($r=0.474$), $P<05$.

Regionalized and frequency specific brain activity plays a crucial and possibly protective role in the control of cardiovascular responses to acute mental stress in humans.

O62 - In vitro modelling of the neurocardiac junction with a novel human iPSC-based co-culture system: new perspectives for the investigation of cardiac autonomic regulation

Theme: ECR focus: Breaking abstracts

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The cardiac autonomic nervous system is known to play a key role in many cardiac disorders. However, many details of the neuronal regulation of the human heart remain elusive due to the lack of reliable experimental models. Here, we describe the generation of an in vitro neurocardiac model based on human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) and sympathetic neurons (iPSC-SNs). iPSC-SNs in monoculture expressed MAP-2 (neuronal marker), TH, DBH (adrenergic lineage markers), and peripherin (peripheral nervous system marker) as evaluated by flow cytometry, immunofluorescence and western blot analyses. Furthermore, iPSC-SNs exhibited spontaneous firing and burst activity, measured using the Maestro Edge Multi-Electrode Array (MEA). iPSC-CMs and iPSC-SNs were co-cultured in two chambers separated by a silicon insert, and after insert removal, iPSC-SNs formed axons projecting towards iPSC-CMs. While the beat rate of iPSC-CMs was stable after 7 days of co-culture, iPSC-SNs significantly increased their firing activity after 7 days of co-culture. Nicotine treatment was used to trigger iPSC-SN activity. A significant increase in the beat rate of iPSC-CMs in co-culture was observed after nicotine treatment, which had no effect on iPSC-CMs in mono-culture. The increased beat rate of iPSC-CMs in response to nicotine stimulation was reduced upon administration of the β-blocker Propranolol, while it remained unaffected by α-bungarotoxin, which blocks nicotinic receptors. After 7 days of co-culture, a significant decrease in FFN270 (a fluorescent tracer of norepinephrine)-stained vesicles was observed in nicotine-treated cells, indicating effective neurotransmitter release and functional exocytosis. Collectively, these data confirm the ability of iPSC-SNs to establish functional connections with iPSC-CMs. The proposed co-culture system represents a valuable model to study diseases with compromised neuro-cardiac interactions, facilitating both disease modeling and pharmacological testing.

Funding:
Interreg: ITAT1047

O63 - The accelerator and brake modulatory system of carotid body sensitivity

Theme: ECR focus: Breaking abstracts

Miss Olivia Gold[1], Dr Igor Felippe[1], Dr Audrys G Pauza[1], Prof. Julian F. R. Paton[1]
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The carotid body (CB) is the primary sensor of arterial gases and maintains cardiovascular and respiratory homeostasis. CB chemosensitivity increases upon repeated stimulation and in disease states; however, the molecular basis for determining CB sensitivity remains elusive. We hypothesise that an interplay between glutamate and γ-aminobutyric acid (GABA) determines CB sensitivity.

Electrophysiological recordings of carotid sinus nerve (CSN) afferent discharge were made from an in vitro arterially perfused carotid artery bifurcation preparation. An in situ, double-perfused working-heart brainstem preparation was used to determine CB motor responses during glutamatergic receptor modulation of CB. Drug modulatory effects on CB sensitivity were assessed by changes in cyanide (CN-)-evoked response magnitude (1.23 µmol bolus).

The standard CSN response consisted of a rapid high-amplitude discharge that was quenched abruptly, leading to a low-amplitude tail. Glutamate (15 mM) augmented the CSN response by 2-fold compared to baseline ($P<0.001$) whereas GABA (15 mM) suppressed the response by 2-fold ($P<0.001$). An N-methyl-D-aspartic acid (NMDA) receptor (R) antagonist (MK801; 100 µM) increased the response amplitude, resulting in a single high amplitude long duration discharge (160% increase from baseline), abolishing the low amplitude tail. A similar effect was observed after GABAA-R blockade with bicuculline (500 µM) (i.e., 80% increase from baseline). However, the CN- response was blocked by PPADS (P2 antagonist; 100 µM), thus suggesting it to be solely mediated by purinergic transmission. Interestingly, in situ, glutamate stimulation augmented CB ventilatory response ($p=0.009$) and NMDA-R antagonism led to further amplification of the hypoxia-evoked CB ventilatory response ($p=0.006$) only, i.e., without effect on blood pressure and sympathetic responses.

We suggest an intrinsic ‘accelerator and brake’ inter-cellular paracrine mechanism within the CB involving glutamate activation of NMDA receptors causing GABA release, respectively; we propose that this is fundamental in determining CB sensitivity set-point.

HRC_NZ and Sidney Taylor Trust funded research.

O64 - Functional Brain Imaging Reveals Cerebral Hypoperfusion Patterns in Postural Orthostatic Tachycardia Syndrome (POTS): A Retrospective Study

Theme: ECR focus: Breaking abstracts

Ms Marie-claire Seeley[1,2], Dr Celine Gallagher[1,2], Mr Howard O'Brien[3], Mr Kevin Kickson[4], Ms Claire Coat[4], Ms Tess Smith[4], Dr Reymond Casse[4], Professor Amanda Page[1,2], Prof Dennis Lau[1,2]
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Background: Cerebral hypoperfusion in POTS has previously been reported using transcranial doppler. However, this tool is not clinically useful for routine assessment.

Purpose: To explore whether single photon emission computed tomography (SPECT) is clinically useful for detection of reduced cerebral blood flow (rdCBF) in POTS.

Methods: A retrospective record review of the Australian POTS registry was undertaken for evidence of functional imaging using brain SPECT. Significant regional rdCBF was analysed using NEUROSTAT3D-SSP statistical parametric mapping using regional z-scores. Validated patient reported outcome measures were used to determine symptom severity and health related quality of life including, composite autonomic symptom score (COMPASS-31), Euroquol 5-Dimension (EQ-5D) and the gastroparesis cardinal symptom index score (GCSI).

Results: A total of 56 participants (female 87%, Caucasian 95%) were included. Mean age was 34.8±10.7 years. Most were tertiary educated (71%), however, only 21% were working full time. In total, 61% (34) of POTS patients demonstrated rdCBF on brain SPECT. Of these 41% (n = 23) of participants had at least three brain regions with z-scores outside two standard deviations of normal. rdCBF of the left and right prefrontal and left and right sensorimotor cortices was most prevalent throughout the cohort (41.1%, 33.9%, 32.1%, 26.8% respectively). The mean z-score of the cohort was lowest in the prefrontal lateral right (-1.69±0.92) and the prefrontal lateral left (-1.67±0.99) regions. The mean number of brain regions affected by rdCBF as well as the total COMPASS-31 and GCSI scores explained 51% of the total variances in EQ-5D quality of life scores ($F [5, 50] = 10.24$, $p<0.001$).

Conclusion: Cerebral hypoperfusion in POTS is clinically detectable utilising brain SPECT. Further investigation into the role of autonomic regulation in driving functional brain changes within this cohort is warranted.

O65 - Stromal cell derived factor-1 (SDF-1) acts on CXCR4 and CXCR7 in the rostral ventrolateral medulla (RVLM) to regulate blood pressure

Theme: ECR focus: Breaking abstracts

Miss Nirupama Unnikrishnan[1], Dr Willian Korim[1], Dr Song Yao[1]
[1]University Of Melbourne, Parkville, Australia

Although essential hypertension was first recognised several decades ago, there still is no identifiable cause[1]. Significant progress has been made in linking neuroinflammation[2-3], sympathetic nerve activity (SNA)[4], and hypertension, yet the exact mechanisms remain unclear. Evidence suggests that a chemokine, stromal cell-derived factor-1 (SDF-1), can cause increases in blood pressure (BP) and SNA[5]. Increased activation of neurons in the rostral ventrolateral medulla (RVLM), a brainstem region critical for the regulation of sympathetic tone and blood pressure, is linked to increases in both SNA and BP[6].

This study was designed to determine whether: 1) SDF-1 microinjections into the RVLM of normotensive rats increases BP; and blockade of the two receptors, CXCR4 and CXCR7, can inhibit this response. 2) Blocking CXCR4 and CXCR7 in the RVLM of hypertensive rats decreases BP via decreases in SNA. 3) CXCR4 and CXCR7 receptor expression is changed in the RVLM neurons of hypertensive rats.

Microinjecting SDF-1 into the RVLM of anaesthetised, normotensive, Sprague-Dawley rats significantly increased BP, via activation of CXCR4 and/or CXCR7 receptors; blocking either receptor was sufficient to inhibit the SDF-1-mediated increase in BP. Furthermore, blocking both CXCR4 and CXCR7 significantly decreased BP in spontaneously hypertensive rats (SHRs); BP of normotensive Wistar Kyoto rats was unaffected. Preliminary evidence demonstrates that blockade of these receptors in SHRs is likely decreasing BP by reducing renal SNA.

In summary, these data suggest that neuroinflammation, partly mediated by the SDF-1/CXCR4/CXCR7 axis, may contribute to the hypertensive state by increasing SNA via increased activation of RVLM neurons.

Symposium Presentations Session 4

Symposium Title: Targeting GI vasodilatory hormones for the treatment of postprandial syndromes in autonomic disorders

O66 - Postprandial syndromes in autonomic disorders: pathophysiology and treatment

Theme: Gut and Metabolism

Christopher Mathias, University College London

The physiological responses to food are numerous, affecting cardiovascular and autonomic systems and release of gut peptides. In 1953 it was reported that hypertensive patients given the ganglionic blocker Pentolinium had a marked fall in blood pressure (BP) after meals but this was not considered a clinical problem until the early 1980's, with observations in chronic autonomic failure (AF). Postprandial hypotension (PPH), a 20 mmHg fall in systolic BP after food ingestion, can contribute to morbidity in many with primary AF, and is also present in common neurological disorders (Parkinson's disease and Diabetes Mellitus), when autonomic function is impaired. PPH can contribute to syncope and falls, of particular importance in the elderly, with reports of PPH in 25-40% of residents in aged care facilities. Food can exacerbate tachycardia in the postural tachycardia syndrome (PoTS), and probably also in post-viral syndromes such as Long Covid where 30% may have autonomic dysfunction. In all these groups there are implications following food ingestion when seated, and especially when standing upright, compounded by impaired autonomic function.

The cardiovascular autonomic mechanisms causing postprandial syndromes in AF have been extensively described, with the splanchnic circulation playing a prominent role. The volume and composition of food is of relevance, with carbohydrate causing greater hypotension (probably linked to glucose and insulin, amongst other gut peptides), than lipid and protein. The role of gut peptides and allied hormones has been increasingly studied along with therapeutic implications of peptide inhibition, as with the beneficial use of Octreotide. More specific targeting is likely to benefit particular groups with postprandial syndromes.

An overview on pathophysiology and current treatment will provide a background to presentations to follow, on recent advances in GI peptides and specific peptide antagonists.

O67 - Increased Glucose-dependent insulinotropic polypeptide (GIP) in postprandial syndromes

Theme: Gut and Metabolism

Cyndya A. Shibao, Nashville, TN, USA

The splanchnic circulation is the largest blood volume reservoir of the human body, storing ≈25% of the total blood volume and contributing to sudden, and large, fluctuations in the stroke volume (SV) and blood pressure (BP). These orthostatic changes in systemic hemodynamics are particularly magnified after meals, due to increased blood volume sequestration triggered by the release of gastrointestinal peptides with vasodilatory properties. Previous studies using doppler flow assessment of the Superior Mesenteric Artery (SMA) showed significant SMA increases between 58% and 250% after meals. Important to note, is that a high-carbohydrate meal rapidly increases SMA blood flow (BF) compared to a high- protein or high-fat meal, whereas a high-fat meal is accompanied by a slow and prolonged fall in this vascular bed. To counteract meal-induced blood pooling in the splanchnic circulation and maintain blood pressure within normal levels, a series of compensatory hemodynamic changes must occur, including an increase in heart rate (HR), stroke volume (SV), and cardiac output (CO), mainly driven by sympathetic activation. A previous study found a different response on the muscle nerve sympathetic activity (MSNA), HR and BP after glucose, fat, protein, and a mixed meal with similar caloric content was ingested. We previously reported that in Postural Orthostatic Tachycardia Syndrome (POTS) symptoms increased after large and carbohydrate right meals, these patients developed worsening post-prandial symptoms and orthostatic tachycardia. We also showed elevated glucose-dependent insulinotropic (GIP) polypeptide secretion, which is a strong splanchnic vasodilator. Furthermore, we have preliminary data indicating excessive GIP secretion in patients with neurogenic orthostatic hypotension and Parkinson disease.

O68 - Neural modulation of entero-pancreatic hormone secretion

Theme: Gut and Metabolism

Simon Veedfald, University of Copenhagen

Abstract not received

O69 - Glucose-dependent insulinotropic polypeptide receptor antagonism in humans

Theme: Gut and Metabolism

Lærke Smidt, **Sophie Woge Nielsen**
University of Copenhagen,

Abstract not received

Symposium Presentations Session 4

Symposium Title: Neural control & autonomic regulation during exercise: recent innovations

O70 - An integrative approach to better understand the mechanisms of the exercise pressor reflex in health and disease

Theme: Integrative control

Dr Masaki Mizuno[1]
[1]University of Texas Southwestern Medical Center, Dallas, United States

Exercise induces abnormal increases in blood pressure in patients with cardiovascular disease or diabetes. Since the exaggerated cardiovascular responses to exercise are associated with an increased risk of adverse cardiovascular events during exercise, elucidation of the mechanisms responsible is clinically and physiologically important. In addition, the abnormal circulatory response limits the safety of exercise prescription, which is problematic as habitual physical activity is known to be a therapy with demonstrated potential for improving overall cardiovascular health. Afferent signals from working skeletal muscle are one of the major sources of neural input to cardiovascular centers that control cardiovascular function during physical activity (exercise pressor reflex, EPR). Increasing evidence suggests that abnormal EPR function contributes significantly to the generation of exaggerated circulatory responses in these diseases. Furthermore, EPR dysfunction has been shown to be mediated by both mechanosensitive ion channels (e.g. Piezo1 or TRPV4) and chemically sensitive ion channels (e.g. TRPV1). To this end, we have recently developed an integrative research approach using in vivo (measurement of cardiovascular and sympathetic responses to physical stress such as mechanical and chemical stimuli), ex vivo (measurement of action potential activity to mechanical and chemical stimuli from single fibers in muscle-nerve preparations), and in vitro approaches. The symposium presentation will focus on recent findings aimed at determining the mechanisms underlying the increased BP response to exercise in cardiovascular disease or diabetes.

O71 - The exercise pressor reflex: a flow-raising or a pressure-raising mechanism?

Theme: Integrative control

Markus Amann, University of Utah

Abstract not received

O72 - Subcortical circuit mechanisms for central command regulation of sympatho-motor coordination

Theme: Integrative control

Dr. Satoshi Koba[1]
[1]Tottori University, Japan

Early studies suggested that a feedforward descending motor signal from the forebrain, known as central command, contributes to cardiovascular control during exercise. However, the brain substrates underlying central command-mediated autonomic cardiovascular adjustments have yet to be fully elucidated. Our recent research has focused on elucidating the central circuit mechanisms that relay volitional central command signals to coordinate autonomic cardiovascular control and somatomotor limb control. We employed functional neuroanatomy and in vivo physiological experiments to record peripheral nerve discharges, cardiovascular changes, and behaviors, in combination with optogenetic techniques to manipulate specific neuronal populations in rats. We identified the essential role of RVLM-projecting neurons in the mesencephalic locomotor region and the orexinergic nervous system in transmitting central command signals for locomotor exercise. This was demonstrated by the excitation of these neuronal populations associated with voluntary running, the effects of their optogenetic excitation to drive both locomotor activities and sympathetic cardiovascular responses, and the effects of their inhibition to suppress locomotor activity and cardiovascular responses during voluntary running exercise. In the presentation, current findings will be compiled to organize our understanding of central circuit mechanisms for central command regulation of sympatho-motor coordination. Moreover, we recently generated FosTRAP rats via CRISPR/Cas9 system, enabling us to investigate the roles played by running exercise-excited hypothalamic neurons in eliciting autonomic cardiovascular responses. Our recent efforts to dissect neural circuit functions using the rat FosTRAP method to provide deeper insights into the central circuity underlying central command will also be discussed.

O73 - The relative contributions of central command and the metaboreflex to the increases in sympathetic vasoconstrictor drive to contracting muscle

Theme: Integrative control

Dr Daniel Boulton[2], Assoc Prof Chloe Taylor[3], Assoc Prof Simon Green[3], Prof Luke Henderson[4], **Prof Vaughan Macefield**[1]
[1]Monash University, Melbourne, Australia, [2]Neuroscience Research Australia, Sydney, Australia, [3]Western Sydney University, Sydney, Australia, [4]University of Sydney, Sydney, Australia

Both central command and metaboreceptor inputs from contracting muscles increase muscle sympathetic nerve activity (MSNA) to non-contracting muscle during sustained isometric exercise. By inserting a tungsten microelectrode into the common peroneal nerve supplying the contracting dorsiflexor muscles we can extract negative-going spikes of MSNA from the nerve signal and quantify sympathetic outflow despite the presence of ongoing activity in motor axons, muscle spindle and Golgi tendon organ afferents and EMG from the contracting muscles¬. Using this approach we have shown that MSNA to contracting muscle also increases in an intensity-dependent manner. Moreover, this increase in MSNA was stronger than in the non-contracting muscle and occurred within the first minute of contraction, whereas it occurred in the second minute in non-contracting muscle. Interestingly, six minutes of post-exercise ischaemia (PEI) maintained the elevated MSNA to the non-contracting muscle but not to the contracting muscle, which returned to baseline levels at the conclusion of the static isometric exercise. These findings indicate that during sustained contractions only central command is responsible for the increase in MSNA to contracting muscles. By performing these manouevres during functional magnetic resonance imaging (fMRI) of the brain, we showed that parallel increases in BOLD (Blood Oxygen Level Dependent) signal intensity occurred in the contralateral primary motor cortex and cerebellum; these matched the central command profile and ceased at the conclusion of the contraction [1]. Using MSNA-coupled fMRI, significant increases in BOLD signal intensity time-locked to bursts of MSNA were identified in the right inferior frontal cortex, bilateral insula, right angular gyrus, and left somatosensory cortex. Together, given that MSNA increases earlier and more strongly to contracting muscle, these data allow us to conclude that these changes in MSNA-coupled BOLD signal intensity primarily reflect the parallel outflow of central command to the regions involved in the control of MSNA.

O74 - Effect of pulmonary artery mechanoreceptor input on sympathetic vasomotor outflow during exercise in healthy humans

Theme: Integrative control

Mr Michiel Ewalts[1], Dr Samuel Oliver[1], Dr Mike Stembridge[2], **Dr Jonathan Moore**[1]
[1]Bangor University, Bangor, United Kingdom, [2]Cardiff Metropolitan University, Cardiff, United Kingdom

Previous studies indicate that pulmonary artery mechanoreceptor stimulation effects sympathetic vasomotor outflow. Dynamic exercise increases arterial pressure in the pulmonary circulation, which suggests that pulmonary artery mechanoreceptors may contribute to exercise-induced activation of sympathetic vasomotor outflow (i.e., muscle sympathetic nerve activity, MSNA). This study examined whether lowering pulmonary artery pressure during sub-maximal exercise, achieved by inhaled nitric oxide (iNO), could translate into an attenuated MSNA response and a reduction in the operating point of the vascular sympathetic baroreflex. Twelve healthy individuals (28 ± 7yrs, 2 females) completed a randomized cross-over trial. All breathed hypoxic air (FiO_2=12.5%) to elevate pulmonary artery pressure above normal. Pulmonary artery systolic pressure (echocardiography, PASP), MSNA (microneurography), blood pressure (finger photoplethysmography, BP), heart rate (HR), oxygen saturation (SaO_2) and minute ventilation (VE) were measured at rest, and during sub maximal cycling exercise in the absence and presence of iNO (40 parts per million). By design, PASP was significantly reduced during exercise by iNO (42 ± 9 vs. 36 ± 8 mmHg; P = 0.018). This reduction was accompanied by significant attenuation in MSNA (34 ± 9 vs. 30 ± 9 bursts/min; P = 0.027) and a downward shift in the MSNA operating point of the spontaneous vascular sympathetic baroreflex (28 ± 9 vs. 25 ± 8 bursts/100 heartbeats; P = 0.03). There was no difference in corresponding diastolic pressure (79 ± 16 vs. 78 ± 12 mmHg) or baroreflex sensitivity (slope). MAP, HR, SaO_2 and VE did not differ between exercise conditions. These data indicate that reducing PASP during exercise does translate into attenuated sympathetic outflow and downward resetting of baroreflex control of MSNA in healthy humans. This supports our view that afferent input from pulmonary artery mechanoreceptors contributes to changes in sympathetic vasomotor outflow during exercise, at least under hypoxic conditions of this study.

O75 - Reinstating respiratory sinus arrhythmia in heart failure improves cardiac responses to exercise

Theme: Integrative control

Dr Julia Shanks[1], Dr Mridula Pachen[1], Dr Nigel Lever[1,2], Prof. Julian F. R. Paton[1], Assistant Professor Rohit Ramchandra[1]
[1]Manaaki Manawa - The Centre for Heart Research, New Zealand, [2]Te Whatu Ora, Auckland DHB and Green Lane Cardiovascular Service, Auckland, New Zealand

Heart failure is characterised by a loss of autonomic balance and reduced heart rate variability (HRV). One aspect of HRV is the modulation of heart rate with breathing: respiratory sinus arrhythmia (RSA). We have shown that reinstating RSA in reduced ejection heart failure can improve ejection fraction and myocyte structure compared to monotonic pacing. For individuals living with heart failure, one of the most life-limiting symptoms is reduced exercise capacity. We hypothesised that reinstating RSA in heart failure would not only improve cardiac function at rest but would improve cardiac responses to exercise.

Heart failure was induced in adult sheep by a microembolisation technique. Eight weeks after embolisation, sheep were split into two groups: RSA-paced (n = 5-6) and monotonically-paced (n = 5-7). After instrumentation with chronic arterial pressure and cardiac flow probes sheep underwent a one-week baseline recording and were paced daily for two weeks. Respiratory modulated pacing was generated by a proprietary device (Ceryx Medical). At baseline and after two weeks of pacing, direct recordings of cardiac output, coronary artery blood flow, and heart rate were recorded in conscious adult sheep during exercise.

RSA pacing for two weeks increased cardiac output ($P<0.01$) with no change in coronary artery blood flow at rest. At baseline, there were no differences in the hemodynamic and cardiac responses to graded exercise between groups. After two weeks of RSA, sheep showed an increase in coronary artery blood flow (pre-pace: 61.4±10.1 ml/min, post-pace: 90.6±11.3 ml/min, n = 5. $P<0.01$) exercise and an increase in the rate of heart rate recovery post-exercise. After two weeks of monotonic pacing, there was no change in cardiac function during exercise compared to baseline.

Reinstalling RSA may be a novel therapeutic target for improving outcomes in heart failure, including reduced exercise capacity.

Symposium Presentations Session 5

Symposium Title: Glucose sensing affecting autonomic activity

Theme: Gut and Metabolism

O76 - Are GLP-1 producing pre-proglucagon neurons of the lower brainstem a useful target for obesity and diabetes treatment?

Stefan Trapp, University College London

GLP-1 receptors are found in many parts of the brain, and particularly in those areas implemented in control of food intake and in the endogenous reward system. Systemically administered GLP-1 receptor agonists are currently the most efficacious weight loss medication. However, various lines of evidence suggest that their brain access is limited to circumventricular organs and possibly a few more select sites. This leaves many brain GLP-1 receptors inaccessible and endogenous brain GLP-1, produced by preproglucagon (PPG) neurons of the lower brainstem, seems the likely relevant ligand. From this the question arises of whether PPG neurons are capable of eliciting a reduction in food intake and bodyweight independent of and in addition to systemic GLP-1 receptor agonists, and if so, whether this can be achieved by pharmacological means. This presentation will consider the experimental evidence and additionally ask the question whether there are qualitative differences in the reduction of food intake achieved by PPG neuron activation compared to GLP-1 receptor agonists, e.g. is nausea, which is a mayor side effect of GLP-1 receptor agonist therapy, also an issue for PPG neuron mediated hypophagia? Finally, to address the translational relevance of PPG neuron activation the possibility.

O77 - Carotid body, autonomic function and dysmetabolism: is there something new under the sun?

Theme: Gut and Metabolism

Silvia V Conde, NOVA Medical School

Abstract not received

O78 - GLP1 receptor agonist ameliorates high blood pressure and high blood sugar in a rat model of "glucotension"

Theme: Gut and Metabolism

Dr Pratik Thakkar[1], Mr Aeson Chappell[1], Dr Fiona McBryde[1], Prof. Julian F. R. Paton[1]
[1]Department of Physiology, Manaaki Manawa – the Centre for Heart Research, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

Diabetes is the fastest-growing disease in New Zealand. Most (75%) patients with T2D have hypertension, and half of hypertensives exhibit dysfunctional glucose metabolism, such a condition we call "glucotension". Glucagon-like peptide type -1 (GLP-1) has an essential role in regulating glucose homeostasis but its efficacy has not fully established in glucotension. Given the recent finding of GLP1R expression in the carotid body and hyperactivity of this organ in hypertension and diabetes, we have sought to test the hypothesis that GLP1R stimulation will modulate glucotension.

A high-fat diet (HFD) induced diabetic rat model (Wistar control, Wistar-HFD, SHR control, SHR-HFD) was used in this study. GLP1 agonist (Exendin-4) was given (acutely & chronically) and chemoreflex testing, blood glucose, glucose tolerance (GTT), cognitive function, blood pressure (BP), sympathetic nerve activity (SNA) and ultrasound assessments of cardiac and renal function were assessed.

Acute study showed Exendin-4 attenuates the chemoreflex evoked SNA response along with BP in HFD fed conscious SH and Wistar rats ($p<0.05$). In chronic treatment with Exendin-4, SHR-HFD rats showed improvement in cognitive functions compared to pre-drug values suggesting that improved contextual memory, indicating improvement in cerebral blood flow. In addition, baseline renal artery resistance index (RI) was higher ($P=0.081$) in SHR-HFD compared to SHR. SHR-HFD group showed higher systolic dysfunction compared to all other groups and Exendin-4 paused this acceleration with no further decline in dysfunction ($P=0.0018$). Post-drug treatment, the SHR-HFD group showed an improvement in glucose tolerance to a level seen in SHR controls. Wistar-HFD showed a significant reduction in rate of weight gain post-drug compared to pre-drug while SHR-HFD group did not show any change.

Chronic HFD exposure worsens cardiac, renal and cognitive function in SH rats that is ameliorated by chronic treatment with a GLP-1 agonist. We conclude that GLP-1 agonist provides a new way to control glucotension.

O80 - The blockade of sympathetic mediated inhibition of immunity improves bacterial clearance in pigs

Theme: Gut and Metabolism

Dr. Alessandra Occhinegro[1], Dr. Domenico Ventrella[2], Prof. Alessandra Cappellini[1], Dr. Silvia Felici[3], Dr. Marco Luppi[1], Dr . Emiliana Piscitiello[1], Ludovico Taddei[1], Prof. Maria Laura Bacci[2], Prof. Tiziana Lazzarotto[3], Prof. Davide Martellli[1]
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The intricate interplay between the nervous system and the immune system underscores a dynamic relationship crucial for maintaining health. During infections, a pivotal neural reflex known as the inflammatory reflex comes into play, aiming to temper the inflammatory response. This reflex operates through the efferent arm, coursing along the splanchnic sympathetic nerves, which can modulate the immune system via β2-adrenergic receptors on leukocytes.

Recent studies have shed light on the potential of the inflammatory reflex. Notably, experimental findings demonstrate the swift resolution of systemic Escherichia coli (E. coli) infection in sheep with surgically ablated nerves, compared to those with intact splanchnic nerves, emphasizing the profound impact of this reflex.

Considering this evidence, the aim of the study was to investigate whether prophylactic pharmacological interventions targeting the communication between sympathetic nerves and leukocytes could enhance the innate immune response and promote early recovery from an eventual infection.

Female pigs were divided into vehicle (control) and propranolol-treated (non-selective β-blocker, 3mg/kg, 3 x day, orally) groups (n=4 each). Propranolol (or vehicle) treatment started two days before bacterial challenge and continued for the entire duration of the study. All animals were injected i.v. with a bolus of E. coli (1.4 x 104 CFUs/Kg) and followed for 14 days.
Results revealed that propranolol-treated pigs exhibited decreased plasma levels of the anti-inflammatory cytokine interleukin-10, alongside heightened neutrophil and platelet activation. Notably, these animals cleared bacteremia more swiftly than controls, suggesting a disinhibited, more responsive innate immune system.

In conclusion, propranolol emerges as a promising avenue for enhancing immune responses against infections. Our findings propose a novel strategy for managing bacterial infections, potentially mitigating the risk of hospital-acquired infections.

Keywords: inflammatory reflex; systemic infection; propranolol; bacterial clearance; pharmacological prophylaxis

Symposium Presentations Session 5

Symposium Title: Bidirectional association between depression and autonomic nervous system alteration: new insights into therapeutic strategies

O81 - Antidepressant activity and cardioprotective effects of endocannabinoid neuromodulation enhancement in socially stressed rats

Theme: Integrative control

Dr Andrea Sgoifo[1]
[1]Stress Physiology Lab - University of Parma, Parma, Italy

Antidepressant activity and cardioprotective effects of endocannabinoid neuromodulation enhancement in socially stressed rats

In humans, depression is often triggered by prolonged exposure to psychosocial stressors and is often associated with cardiovascular comorbidity. Mounting evidence suggests a role for endocannabinoid signaling in the regulation of both emotional behavior and cardiovascular function. We examined cardiac activity in a rodent model of social stress-induced depression and investigated whether pharmacological inhibition of the enzyme fatty acid amide hydrolase (FAAH), which terminates the signaling of the endocannabinoid anandamide, exerts antidepressant-like and cardioprotective effects. Male rats were exposed to five weeks of repeated social stress or control procedure. Starting from the third week, they received daily administration of the selective FAAH inhibitor URB694 (0.1 mg/kg, i.p.) or vehicle. Cardiac electrical activity was recorded via radiotelemetry. Repeated social stress triggered biological and behavioral changes that mirror symptoms of human depression, namely (i) reductions in body weight gain and sucrose solution preference, (ii) hyperactivity of the hypothalamic-pituitary-adrenocortical axis, and (iii) increased immobility in the forced swim test. Moreover, stressed rats showed (i) alterations in heart rate daily rhythm and cardiac autonomic neural regulation, (ii) a larger incidence of spontaneous arrhythmias, and (iii) signs of cardiac hypertrophy. Daily treatment with URB694 (i) increased central and peripheral anandamide levels, (ii) corrected stress-induced alterations of physiological and behavioral parameters, and (iii) protected the heart against the adverse effects of social stress. Repeated social stress in rats appears to mimick reliably aspects of human depression/cardiovascular comorbidity and pharmacological enhancement of anandamide signaling might be a promising strategy for the treatment of these comorbid conditions.

O82 - Evaluation of Ketamine effects on autonomic nervous system in patients with depressive disorders

Theme: Integrative control

Caroline Sevoz-Couche[1], MD Hugo Bottemanne[1]
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Introduction: Ketamine is a fast-acting antidepressant treatment whose therapeutic mechanisms are only partially known. Disruptions in interoception, referring to the brain's processing of bodily signals, are frequently found in depression, and could be linked to cardinal symptoms such as anhedonia and motor slowing. These interoceptive signals are linked to vagal transmission; and these interoceptive disturbances could involve modifications in the sympathovagal balance. However, no study has evaluated autonomic and interoceptive changes during antidepressant treatment with ketamine.

Patients and Methods or Materials and Methods: We conducted a longitudinal, open study exploring autonomic and interoceptive changes during ketamine treatment in a group of patients suffering from severe depressive episodes treated with intravenous ketamine, in comparison with a control group. without psychiatric history. The assessments were carried out 24 hours before, 4 hours and 1 week after taking ketamine. Each assessment included measures of interoception (Schandry test, heart rate variability), interoceptive awareness (MAIA-2, THISQ), autonomic system (HRV), and dissociation (CADSS).

Results: We found interoceptive and autonomic alterations in subjects suffering from depression compared to control subjects. A few hours after the administration of intravenous ketamine, we observed a rapid improvement in interoception (particularly interoceptive attention, emotional regulation and body confidence) with an absence of statistically significant difference on interoception scores and on autonomic variables between patients and healthy subjects. These changes persisted one week after the first ketamine administration.

Conclusion: This exploratory study highlights the importance of exploring interoceptive and autonomic variables to study the effect of rapid-acting antidepressant treatments such as ketamine.

O83 - Mesenchymal Stromal Cells Alleviate Murine Depressive and Anxiety-like Behaviors via a Lung Vagal-to-Brain Axis

Theme: Integrative control

Xiaoran Zhang, Sun Yat-sen University

Abstract not received

O84 - The effects of repetitive transcranial magnetic stimulation (rTMS) and transcutaneous auricular vagal nerve stimulation (tVNS) on depressive symptoms: evidence from DEPONEST and DIGEST studies

Theme: Integrative control

Dr Angelica Carandina[1], Dr Costanza Scatà[1], Dr Francesca Rapella[1,2], Dr Greta Salafia[1], Dr Giandomenico Schiena[3], Prof Paolo Brambilla[3,4], Prof Eleonora Tobaldini[1,2], Prof Nicola Montano[1,2]
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The global burden of depression still represents a hot topic for public health, largely due to the prevalence of treatment-resistant depression (TRD) to conventional pharmacotherapies1. This situation underscores the need for alternative treatment options. The frontal-vagal network has recently emerged as a promising target for such interventions and neuromodulation techniques have gained attention, particularly repetitive transcranial magnetic stimulation (rTMS) and transcutaneous auricular vagal nerve stimulation (tVNS)2.

We aim to compare the antidepressant effects of in-hospital rTMS treatment with at-home tVNS treatment in patients with depressive symptoms. Participants were recruited from the Psychiatry Unit at Policlinico Hospital (Milan) and randomized into two treatment groups. One group received rTMS over the left dorsolateral prefrontal cortex for 20 sessions of 40 minutes over four weeks. The other group received tVNS applied to the left cymba concha for 4 hours/day over four weeks. Both the neuromodulation techniques were performed as add-on treatments to standard pharmacotherapy. Depressive symptoms were assessed using the 21-item Hamilton Depression Rating Scale (HAM-D) and the self-report Beck Depression Inventory (BDI-II). Additionally, cardiac autonomic control at rest was evaluated via ECG recordings.

Sixteen patients completed the treatment, with seven having eating disorders and nine having mood disorders. Sixty-nine percent of patients had severe depressive symptoms (HAM-D>23). The baseline characteristics of the two groups were comparable. The tVNS group showed a significant reduction in both clinician- and self-reported depressive symptoms (mean ΔHAM-D=-13.3±9.8, $p<0.001$; mean ΔBDI-II=-10.0±8.7, $p=0.003$). In contrast, the rTMS group did not achieve statistically significant results. Although there was a general reduction in resting heart rate, post-hoc analysis did not reveal treatment-specific differences (mean Δ=-6.0±4.8, $p=0.049$).

These preliminary results show that tVNS could represent an effective and valid additional treatment for depressive symptoms, offering the advantages of reduced personnel costs and greater convenience for patients compared to rTMS.

O85 - Cardiorespiratory Responses to Hypercapnia Are Altered Differentially in Different Types of Epilepsy Models

Theme: Integrative control

Dr Ayse Dereli[1], Ms Auriane Apaire[1,3], Ms Nibiyizi Abigail[1], Dr Germany Enrique[1,3], Ms Elise Collard[1], M Antoine Nonclercq[4], Prof Riem El Tahry[1,2,3]
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Both tonic-clonic and absence seizures are classified as generalised epilepsy and are characterised by autonomic anomalies. Tonic-clonic seizures, in particular, pose a significant risk for Sudden Unexpected Death in Epilepsy (SUDEP) and may contribute to respiratory arrest. Emerging evidence suggests that SUDEP may involve impaired central CO_2 chemoreception (CCR) drive for breathing, coupled with cardiovascular dysregulation. CCR is typically assessed through ventilatory response to hypercapnic challenge. This study aims to explore cardiorespiratory responses to acute hypercapnia in tonic-clonic versus absence epilepsy models.

We used Wistar kainic acid (KA) rats with severe spontaneous tonic-clonic seizures (approximately 5 months after KA injection, intraperitoneal), Genetic Absence Epilepsy Rat from Strasbourg (GAERS) rats, and healthy Wistar control rats. The photoplethysmography method was employed to measure interictal ventilatory frequency (fB) and heart rate (HR) before, during, and after a 1-hour, 10% CO_2 challenge, followed by statistical analysis (2-way ANOVA, Fisher's LSD test).

Both control and GAERS groups demonstrated normal ventilatory responses to hypercapnia, showing increased fB ($p < 0.01$) compared to baseline. While the control group exhibited a decrease in HR ($p < 0.0001$) during 10% CO_2 exposure, the GAERS group displayed a delayed response with gradual HR reduction during hypercapnia, eventually reaching levels comparable to the control group by the end of the 1-hour challenge ($p < 0.001$). Conversely, in KA rats, both fB and HR responses to CO_2 were significantly attenuated compared to healthy (HR: $p < 0.0001$, fB: $p < 0.01$) and GAERS (HR: $p < 0.1$, fB: $p < 0.01$) groups, without significant deviation from baseline.

Overall, these results suggest that rats experiencing tonic-clonic seizures exhibit impaired cardiorespiratory responses to hypercapnia compared to those with absence seizures. These findings underscore the influence of tonic-clonic seizures on cardiorespiratory dysfunction and elucidate their association with increased SUDEP risk.

Keywords: Brain fog, Cognitive dysfunction, Hypermobility, Orthostatic intolerance, Postural orthostatic tachycardia syndrome (POTS)

O86 - Exploring the interaction of hypermobility with brain fog induction in people with Postural Orthostatic Tachycardia using lower body negative pressure

Theme: Integrative control

Miss Amanda Marshall[1], Miss Amy Kartar[1], Mr Joel Patchitt[1], Mr Giovanni Calcagnini[2], Mr Hugo D Critchley[1,3,4], Ms Jessica A Eccles[1,3,4]
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Most people with Postural Orthostatic Tachycardia Syndrome (POTS) report difficulty thinking or 'brain fog', but the mechanisms that cause these symptoms are poorly understood. People with POTS also frequently experience symptomatic joint hypermobility, but the effects of orthostatic stress on brain fog in POTS and concurrent hypermobility have not been tested. The main aims of this study were to determine if: 1) orthostatic stress induced a greater change in brain fog for people with POTS, 2) there was an interaction of hypermobility with brain fog induction after an orthostatic challenge, and 3) connective tissue features mediated the relationship between POTS and orthostatic intolerance. A lower body negative pressure chamber (LBNPC) was used to mimic orthostatic stress. Participants lay supine in an MRI as this study was part of a larger neuroimaging study. There were 27 POTS participants and 27 healthy controls. Participants were assessed for hypermobility using the hEDS (hypermobile Ehlers-Danlos Syndrome) 2017 criteria. Brain fog VAS (Visual Analogue Scale) scores were self-reported, and interactions were tested with a repeated measures general linear model. The POTS group had a greater change in brain fog (rbs=0.41, p=0.003) after orthostatic challenge compared to controls. There was a significant interaction between brain fog induction after LBNP and POTS when controlling for age and sex [$F(1,1)=4.35$, $p=0.042$, $\eta^2=0.080$]. There was also a significant interaction between brain fog induction after LBNP and hEDS [$F(1,1)=7.72$, $p=0.008$, $\eta^2=0.134$] when controlling for age and sex. The relationship between POTS and orthostatic intolerance was mediated by the number of connective tissue features (b=4.47, 95% CI 0.01–8.54). These findings suggest that people with POTS and symptomatic hypermobility experience brain fog and these symptoms are worsened by orthostatic stress. Further research is required to elucidate the neurobiological mechanisms of brain fog so that more individualised treatments can be developed.

Symposium Presentations Session 5

Symposium Title: Interrogating the physiology of the human vagus nerve

O87 - Anatomical parameterization and physiological validation of computational modeling of vagus nerve stimulation

Theme: Bioelectronics

Dr Nicole Pelot[1]
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Vagus nerve stimulation (VNS) is used clinically to treat many conditions. Computational modeling of neural stimulation therapies enables analysis, design, translation, and patient programming, but many modeling tools are computationally demanding and lack important anatomical realism.

We are using multiple imaging modalities to digitize and quantify the gross, macro, and micro anatomy of 100 cadaveric human vagus nerves, from brainstem to abdomen. The segmented imaging data provide the inputs required to generate a large population of anatomically-realistic models of vagus nerve stimulation, at new and existing points of intervention.

To improve model accuracy, we modeled VNS with 3D fascicular nerve morphology based on microCT imaging, and we developed novel models of autonomic and cutaneous C-fibers. To reduce computational demands, we designed a surrogate nerve fiber model (S-MF) that enables simulation of activation thresholds orders of magnitude faster while maintaining accuracy and biophysical detail. To inform design of closed-loop therapies and to complement our models of neural stimulation, we developed a pipeline for efficient modeling of neural recording.

We applied these modeling tools for analysis and design. For example, we examined the mechanisms by which fibers within smaller fascicles have lower activation thresholds than fibers in larger fascicles, and we used S-MF to optimize stimulation parameters for selective activation within minutes rather than days. To validate these models, we conducted intraoperative recordings of laryngeal muscle activation and changes in heart rate in response to systematic changes in VNS parameters.

Our advances in computational modeling of nerve stimulation will accelerate the design process in preclinical stages and will be invaluable in the clinical programming of nerve stimulation devices. Efficient approaches are essential to tractable modeling of multimodal imaging data being collected across numerous individuals and nerve locations.

O88 - Ultrasound-guided microneurography of the human vagus nerve

Theme: Bioelectronics

MD, PhD Matteo Maria Ottaviani[1,2], **PhD Lea Wright**[3], **Dr Tye Dawood**[3], **Prof Vaughan Macefield**[2]
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The name "vagus" comes from the Latin word for "wandering." This name aptly describes the vagus nerve (VN) because, as the tenth cranial nerve, it wanders extensively from the brainstem through various parts of the body providing bidirectional innervation to numerous visceral organs and influencing a broad range of physiological functions. Its portrayal as a superhighway of information is depicted by the myriad of afferent and efferent fibers that contribute to homeostasis. Like peripheral nerves, these fibers are organized into 5-10 fascicles in humans, in contrast to a single fascicle in the rat and up to 30 in the pig. Surprisingly, the cervical VN has been extensively targeted for neuromodulation interventions due to its favorable anatomical features but with little previous investigations upon vagal electrophysiology in humans. The lack of human data in respect of an abundant literature from animal studies boosted our interest of narrowing this gap. Microneurography has the uniqueness of allowing directly recording of nerve activity in awake participants, offering a window into the real-time communication occurring within peripheral nerves. Intraneural microelectrodes have been used extensively to record from single somatosensory axons supplying muscle, tendons, joints and skin in peripheral nerves, but never in the VN. Here, we describe how we developed our approach using an ultrasound-guided insertion of tungsten microelectrodes into cervical vagal fascicles to get the first ever unitary recordings from the human cervical VN. We demonstrated the feasibility of the approach as we obtained neuronal activity identifying phasically- and tonically-active axons with cardiac and/or respiratory-related activity. With such a weapon in our hands, we seek to expand the horizons of vagal electrophysiology and to unveil potential mechanisms of VN stimulation.

O89 - Single-unit recordings of vagal neurones with cardiac rhythmicity in the human

Theme: Bioelectronics

Dr. David Farmer[1], Ms Mikaela Patros[1], Dr. Matteo Ottaviani[2], Dr Tye Dawood[3], Dr Marko Kumric[4], Professor Josko Bozic[4], Mr Mattew Badour[5], Dr Anthony Bain[5], Professor Otto Barak[6], Professor Zeljko Dujic[4], Prof Vaughan Macefield[1,3]
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Using microneurography, multi-unit neural activity with both cardiac and respiratory rhythmicity has been documented in the cervical vagus of awake humans. The presence of cardiac rhythmicity is of particular interest, as it suggests that the physiology of vagal neurones that have cardiovascular regulatory function can be investigated using this technique. Here, the activity of cardiac rhythmic single units was discriminated from recordings of the human cervical vagus nerve. The functional identification of these neurones was then attempted based on their pattern of firing with respect to the cardiac or the respiratory cycle.

Twenty-two neurones with positive-going spikes (indicative of myelinated axons) and 22 with negative-going spikes (indicative of unmyelinated axons) were isolated across 9 awake participants. Of particular note is the observation of 7 cardiac rhythmic neurones with myelinated axons that showed increased activity during slow, deep breathing; fired predominantly during expiration, and during the minima in heart rate associated with respiratory sinus arrhythmia. This is consistent with the known firing properties of cardioinhibitory efferent neurones. The remaining 15 cardiac rhythmic neurones with myelinated axons were classified as either cardiopulmonary receptors or baroreceptors based on the average position of their peak in firing with respect to the R wave of the cardiac cycle.

Three neurones with unmyelinated axons showed expiratory rhythmicity, implicating them as cardiac-projecting efferent neurones. Classification of the remaining 19 unmyelinated neurones is challenging, as their slow and variable conduction velocities renders an analysis based on the temporal relationship between their peak in firing and the cardiac cycle futile. However, this population is likely dominated by arterial baroreceptors.

In conclusion, the activity of single neurones with putative cardiovascular function has been discriminated from recordings of the human cervical vagus for the first time.

O90 - Activation of vagal axons by vagal nerve stimulation

Theme: Bioelectronics

Mikaela Patros, Monash University

Vagus nerve stimulation (VNS) is an effective treatment for people with drug-resistant epilepsy. However, its mechanisms of action are poorly understood, including which nerve fibres are activated in humans during VNS at typical clinical settings and which are required for clinical efficacy. In particular, there have been no studies of vagus nerve fibre activation in awake ambulant humans undergoing chronic VNS. In this study, for the first time, we report recordings from the vagus nerve in this setting. The recordings were performed using a microelectrode inserted percutaneously into the cervical vagus nerve under ultrasound guidance. The clinical VNS systems were used to deliver stimulation while activity in the vagus nerve was recorded. In addition to activating myelinated axons at low currents, we provide evidence that VNS can also activate unmyelinated C-fibres in the vagus nerve at currents < 1 mA. Given that much of the vagus nerve is composed of C-fibres, these results add to our understanding of how VNS exerts its beneficial effects in drug-resistant epilepsy.

O91 - Blockade of P2X3 ATP receptors decreases carotid body-mediated hypoxic ventilatory responses in high-fat rats

Theme: Bioelectronics

Joana F. Sacramento[1], Prof. Dr Silvia Vilares Conde[1]
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The carotid body (CB) is a metabolic sensor whose dysfunction is implicated in the development of metabolic diseases. Previously we showed that high-fat (HF) animals exhibit an increased spontaneous and hypoxia-evoked carotid sinus nerve activity that is mediated by P2X3 ATP and A2A adenosine receptors. Aiming to explore the potential of CB ATP and adenosine modulation for metabolic diseases, we investigated the role of ATP and adenosine in ventilatory responses in dysmetabolic animals and the contribution of the CB.

Wistar rats fed a standard diet or a HF diet (60% energy from fat, 21 days). Animals were anesthetized (sodium pentobarbital, 60mg/kg, i.p.) and basal ventilation and ventilatory responses to hypoxia (10%O2) were tested in the presence or absence of SCH58261 (A2A adenosine receptor antagonist, 20 nmol/kg) and AF353 (P2X3 ATP receptor antagonist, 1.5 nmol/kg), before and after bilateral CSN resection. Experiments followed the 2010/63/EU European Union Directive and were approved by NMS Ethics Committee.

HF diet increased basal respiratory frequency (Rf) by 14% ($p=0.057$), tidal volume (TV) by 24% ($p<0.05$) and minute volume (MV) by 24% ($p<0.05$) (basal Rf= 52.55±3.44 bpm; basal TV=4.07±0.27 ml/kg; basal MV=223.80±9.35 ml/min/kg). SCH58261 and AF353 did not modify basal ventilation in control and HF animals. In control animals, SCH58261 decreased by 41% ($p<0.01$) the response to hypoxia, with no effects of AF353. In HF animals, the hypoxic ventilatory response increased by 25% ($p<0.05$) (MV=129.04±8.09%), an effect that only modified significantly in the presence of AF353 (38% decrease, $p<0.05$). CSN resection abolished the hypoxic ventilatory response in all groups.

We conclude that the contribution of adenosine and ATP to CB-mediated hypoxic ventilatory responses shifts between control and HF animals, with the P2X3 receptor playing a more significant role in HF animals. The use of P2X3 receptor antagonists as a therapy for metabolic diseases might be limited.

Poster Session 1

P1 - A microfabricated Parylene cuff electrode for branched nerve stimulation

Theme: Basic - Bioelectronic Medicine

Dr Alberto Esteban-linares[1], Brianna Thielen[1], Dr Quentin Rezard[1], Dr Artin Petrossians[1,2], Jared Wells[3], Jayme Coates[3], Dr Sahar Elyahoodayan[1], Dr Dong Song[1], Raja Hitti[3], Dr Victor Pikov[4], Professor Ellis Meng[1]
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Peripheral nerve interfaces provide a therapeutic avenue for bioelectronic medicine applications and pain management, typically employing small electrodes on a target nerve. Minimally invasive interfaces, notably cuff electrodes, wrap around the nerve circumference to avoid nerve trauma and extend implant longevity compared to more invasive nerve interfaces that breach the epineurium and/or perineurium. However, existing designs produced using thick polymer substrates are not suitable for abdominal vagal nerve branches, which are desirable therapeutic targets in bioelectronic medicine due to their proximity to and greater selectivity in controlling internal organs.

We developed a thin-film cuff electrode suitable for stimulating sub-millimeter diameter nerves. This thin film cuff comprises a metal layer sandwiched between two insulating layers of Parylene C polymer and is enabled by advanced microfabrication methods. The structured and cut-out thin films are fixtured and thermoformed into a soft-closing and self-sizing cuff configuration. We systematically assessed the electrochemical performance of the electrode sites with and without PtIr coatings to meet charge injection capacity requirements.

Concurrently, we developed a novel interconnect component that permits the cuff electrodes be mechanically and electrically connected to standard clinical-grade leads for interfacing with implantable pulse generators. The interconnect is a rigid substrate consisting of metal pads patterned on an adhered patterned platinum foil that allows reliable attachment of the lead wires (using welding) and thin film electrodes (using low temperature bonding).

We designed several cuff placement tools for cuff deployment using different surgical techniques (e.g. open surgery and laparoscopic surgery). Placement and stimulation will be evaluated in acute experiments using the rat sciatic nerve. Additionally, benchtop accelerated lifetime testing will be conducted to evaluate device longevity prior to chronic animal experiments. We report current results on cuff development towards the realization of an open-source implantable cuff electrode capable of targeting sub-millimeter abdominal vagal nerve branches.

P2 - Exploring Hemodynamic Responses to Electrical Stimulation of Renal Nerves: A Potential Therapeutic Approach for Drug-Resistant Hypertension

Theme: Basic - Bioelectronic Medicine

Ms. Dzifa Kwaku[1], Joan Dao[1], Dr John Osborn[1], Dr Matthew Johnson[1]
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Despite the availability of safe and effective high blood pressure medications, many people still struggle to control their hypertension. Drug-resistant hypertension has been linked to persistent overactivity of the renal nerves, resulting in decreased renal blood flow, which makes hypertension regulation difficult to control. Recent therapeutic strategies show that catheter-based renal denervation (RDN) successfully lowers arterial pressure in hypertensive patients by interfering with the brain-kidney link, which reduces central sympathetic outflow. However, a critical drawback associated with RDN is its irreversibility and lack of adaptability over time. Animal studies have suggested the potential reconnection of renal nerves, prompting the need for alternative strategies. Addressing this concern, our study explores the application of kilohertz-frequency trains of electrical stimulation as a promising approach to inhibit nerve activity to the kidneys. In this investigation, we explored the effects of electrical stimulation on renal nerves utilizing an acute swine model. Using hook electrodes around the renal artery complex, we performed a preliminary study to investigate how stimulation frequency (5 Hz - 15 kHz) and stimulation current (1 - 6 mA) affect hemodynamics through the kidney. Our findings revealed that stimulation at low frequencies (<100 Hz) activated renal nerves, leading to a reduction in renal blood flow. Notably, kilohertz-frequency stimulation induced a carryover effect on renal nerves, influencing the effects of subsequent low-frequency stimulation (pre-stimulation reduction of 25% and post-stimulation reduction of 13%). This observation suggests a complex interplay between different stimulation frequencies, highlighting the need for further exploration into the dynamic responses of renal nerves to electrical stimulation. Our results hold promise for the development of targeted and efficient strategies to address drug-resistant hypertension using kidney neuromodulation.

P5 - Microgravity induced impairment of baroreflex sensitivity in rats is associated with sympathovagal imbalance but not with changes in structure of carotid artery

Theme: Basic - Cardiovascular

Dr. Aparaajita Bhatnaagar[1], Dr. Ritesh Netam[2], Dr. Atanu Roy[4], Dr. Kamal Kishore Deepak[3]
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Introduction: In space, cephalad shift of body fluid equalizes the blood pressure throughout the body leading to cardiovascular deconditioning. Effects of microgravity on humans can be studied on earth by simulating microgravity by using hindlimb unloading (HU) rat models. This study was planned to examine the effect of 14 days HU on baroreflex sensitivity, heart rate variability (HRV) and carotid artery structure in rats.

Material and methods: 12 male Wistar rats weighing 250-300 g were randomly divided into control and HU groups and housed in separate cages for 14 days. Body weight, food and water intake was measured every day. The pelvic suspension method was used to simulate microgravity. To record HRV, ECG was recorded in lead II configuration on the 14th day. Then blood pressure was recorded directly from the carotid artery. Linear regression of mean arterial pressure and RR interval was used to derive the BRS. Vessel wall thickness, intimal thickness, smooth muscle cell layer thickness & luminal diameter were compared after H&E staining. Statistical analysis was done using GraphPad Prism (version 9.0). Unpaired t-test, Mann Whitney and 2 way ANOVA were used according to groups and distribution of parameters.

Results: We found significant differences in Food intake ($p < 0.001$), Body weight ($p < 0.001$), Heart rate ($p=0.031$), BRS ($p= 0.026$), R-R interval ($p=0.0246$) and HRV parameters {SDSD($p=0.0001$), RMSD ($p= 0.0001$), VLF($p=0.001$),HF ($p=0.0054$), LF/HF Ratio ($p= 0.0001$)}. We did not find significant changes in water intake, MAP, SBP, DBP as well as vessel wall thickness(µm), luminal diameter (µm), intimal thickness(µm) and smooth muscle cell layer thickness(µm) of carotid artery.

Conclusion: HU for 14 days showed a decrease in BRS however it was not sufficient to produce any structural changes in the carotid artery. BRS changes were accompanied by alteration in the sympathetic and parasympathetic outflow.

P6 - Temporal profile of changes in cholinesterase activity induced by Ketamine-Xylazine anaesthesia

Theme: Basic - Cardiovascular

Miss Larissa Correa[1], Mr Gabriel Gavazza Noé[1], Doctor Thatiany Jardim Batista[1], MSc Vitor Minassa[1], Dr Vanessa Beijamini[1], MSc Rodolpho Jose Silva Barros[1], Dr Juliana Barbosa Coitinho[1], Dr Karla Sampaio[1]
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Introduction: The synthesis and breakdown of acetylcholine (ACh) determines the cholinergic status. Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) are responsible for the degradation of ACh, serving as indirect markers of cholinergic status as well as biomarkers of cardiac malfunctioning. Evidence suggests that lower AChE and BChE activity may be associated with post-surgical complications and anaesthesia seems to be the key factor in this issue. Thus, we assessed the effects of anaesthesia with ketamine/xylazine (K/X) on plasmatic BChE and erythrocyte AChE activity during the perioperative and postoperative period in rats.

Methodology: Blood samples were collected from male Wistar rats (10 weeks old; 0.2 mL maximum blood volume/sample) over four days (Ethical Committee Approval number 17/2023). On day 1, a blood sample was collected via the tail vein (control sample). On day 2, the animals were anaesthetized with K/X (80/10 mg/kg, i.p.) and submitted to a surgical procedure for artery catheterization, and a new blood sample was collected through the animal's cannula 30 minutes after induction of anaesthesia. On days 3 and 4, 24 and 48 hours after surgery recovery, blood samples were also collected. Erythrocyte AChE activity and plasmatic BChE activity were measured following the method described by Ellman et al, 1961. Data was analyzed by one-way ANOVA with repeated measures, followed by the Dunnet post hoc test. Results: K/X reduced plasmatic BChE activity after 24h and 48h and erythrocyte AChE activity after 48h of anaesthesia induction when compared to the control condition ($p<0.01$).

Conclusion: Our data shows that K/X anaesthesia induces a persistent reduction in cholinesterase activity, which may affect in the long-term the cholinergic status and autonomic balance during perioperative and postoperative periods.

Keywords: Acetylcholinesterase, Butyrylcholinesterase, Cholinergic Status, Anaesthesia.

Funding: Fapes/PROAPEM, Grant code 2022- 78KWB; Capes Foundation Scholarship (Grant Code 01). Ellmann et al, 1961. DOI: 10.1016/0006-2952(61)90145-9

P7 - A study of Schwann cells in human and murine heart

Theme: Basic - Cardiovascular

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Background: The autonomic nervous system regulates cardiac rhythms and functions on a beat-to-beat basis. Evidence suggests the presence of glial cells associated with cardiac autonomic innervation. However, to date the diversity of the cardiac glia remains unexplored. Therefore, we investigated Schwann cells, the most common type of glia in human and murine heart.

Methods: Human left ventricular heart samples were obtained from patients receiving heart transplantation. In addition, adult C57BL/6 mice of both sexes were used. We investigated ventricular cross sections from humans, as well as murine epicardium and whole hearts by staining them with FluoroMyelin and immunohistochemistry for the glial marker S100B and the sympathetic nerve marker tyrosine hydroxylase (TH).

Results and conclusion: In the human heart, Schwann cells were detected in few sympathetic nerve fibers of the left ventricular epicardium, whilst most of the nerve fibers contained only non-myelinating Schwann cells. In the working myocardium, only non-myelinating Schwann cells were detected. In contrast, no FluoroMyelin-positive nerve fibers were found in the murine heart, despite the presence of non-myelinating Schwann cells and nerve fibers within the epicardium.

Our data indicate that most of the Schwann cells in the heart are non-myelinating and that species-specific differences regarding glial diversity exist between humans and mice. As glia have an emerging role in the autonomic control of the heart, further studies regarding their heterogeneity and functionality are needed.

P8 - Correlation of staging and risk factors with cardiovascular autonomic neuropathy in patients with type II diabetes mellitus

Theme: Basic - Cardiovascular

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The impairment of cardiovascular autonomic control among the underdiagnosed complication of diabetes mellitus (DM) with a high prevalence rate of up to 60% in type 2 DM (T2DM). Cardiac autonomic neuropathy (CAN) is an independent risk factor for cardiovascular mortality, arrhythmia, silent ischemia, any major cardiovascular event, and heart failure. We aimed to evaluate cardiovascular autonomic activity by different physiological maneuvers, study risk factors for diabetic CAN including age, gender, duration of diabetes, body mass index (BMI), and glycemic control, and correlate CAN stage with risk factors. One hundred and forty-two T2DM patients consisted of 62 males and 80 females and 100 volunteers as a control sample. Cardiac autonomic functions were assessed by Ewing's tests. Glycated hemoglobin (HbA1c), body weight, height, body mass index (BMI), and waist-hip ratio (WHR) were also measured. Cardiovascular autonomic functions and Ewing scores were significantly different in people with diabetes when compared with control healthy subjects. Ewings test values and Ewing scores were significantly different between diabetics with and without CAN and within patients with different CAN staging. People with diabetes with CAN have a significantly longer duration of disease when compared to those without CAN. A strong association has been found between CAN severity and patient age, duration of disease, HbA1c severity, and the WHR ($P < 0.001$) but not with BMI. The duration of disease and HbA1c level appear to be associated with the development of CAN ($P = 0.001$ and $P = 0.008$, respectively). The poorer glycemic control and the longer the duration of the disease, the higher the prevalence of CAN in T2DM. Age, duration of disease, WHR, and HbA1c are well correlated with the severity of CAN. Parasympathetic impairment is more sensitive to the detection of autonomic dysfunctions than do sympathetic impairment.

P9 - Galectin-3 Inhibitor Modulates Autonomic Nervous System

Theme: Basic - Cardiovascular

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Sympathetic hyperactivity and chronic inflammation are critical factors for the pathogenesis of hypertension. Galectin-3 (Gal-3) inhibitors, known for their anti-inflammatory properties, have shown promise in treating hypertension. Despite this, no studies have explored whether Gal-3 inhibitors effectively mitigate sympathetic hyperactivation in hypertension. Therefore, the present study aimed to evaluate the arterial pressure (AP), heart rate (HR), and heart rate variability (HRV), over time, to different doses of Gal-3 inhibitor, and its response elicited by angiotensin II (Ang II) administration. This research seeks to provide insight into the temporal dynamics of cardiovascular parameters in response to Gal-3 inhibition, particularly in the context of Ang II-induced effects. Rats were anesthetized, and the femoral artery and veins were cannulated. The next day, AP was recorded in the unanesthetized animals during basal time. Subsequently, Gal-3 inhibitor (0.5 or 1 mg/kg, oral gavage) or saline was administrated. Following 30 min of the AP recording, Angio II (200 ng/kg, iv) was administrated. The Gal-3 inhibitor 1 mg/kg increased HRV global compared to the Saline baseline (48 ± 21 vs. 14 ± 5 ms) in the time domain. Following administration of Angio II, all groups increased AP and decreased HRV in the time domain, however, Gal-3 inhibitor administration of 0.5 and 1 mg/kg reduced HR compared to Saline baseline (329 ± 15 and 322 ± 6 vs. 426 ± 26 bpm). Moreover, Angio II increased HRV parameters that suggest sympathetic overactivity in the frequency domain (power of the LF band), or through symbolic analysis through (pattern 0V) in Saline rats. Nevertheless, these HRV parameters were not altered in rats that received previous Gal-3 inhibitors in both doses compared to the Saline baseline. Thus, this study showed that Gal-3 inhibitor administration can modulate the autonomic nervous system, representing an important tool for investigating new therapies against autonomic alterations.

P10 - A Computation Model of the Postganglionic Sympathetic Neuron for predicting drug response

Theme: Basic - Cardiovascular

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Hypertension affects over 1 billion people, increases the risk of cardiovascular disease, and strains healthcare systems globally [1,2]. Currently, treatment follows a one-size-fits-all approach that does not optimise for individual patients. The sympathetic nervous system has a critical regulatory role in hypertension, with many treatments attempting to directly modify the sensitivity to and the activity of sympathetic firing (beta-blockers [3], renal denervation [4]).

We have developed a computational model of the postganglionic sympathetic neuron to optimise treatment choice for hypertensive patients. This computational model was calibrated to data from stellate ganglia neurons excised from spontaneous hypertensive rats, a good experimental model for hypertensive patients. Model parameters were further calibrated with human iPSC-derived sympathetic neuron action potentials to better represent a human sympathetic neuron. Bayesian calibration techniques were used to provide uncertainties in the prediction response that account for experimental error, model error, and calibration uncertainty.

We validated the simulated response to sodium channel blockers, calcium channel blockers, and m-type potassium channel up-regulators to demonstrate the accurate response to drug effects. We effectively showed the neuron changing between a tonic firing phenotype and a phasic firing with the increase of drug concentration. The effects of combining drugs on firing rate and rheobase were also accurately predicted, demonstrating that this model could have utility for optimising drug combinations and predicting treatment outcomes.

The model predicts norepinephrine release at the pre-synaptic synapse and, in future work, will be coupled to i) cardiomyocyte models for predicting cardiac electrophysiological response and to ii) vascular smooth muscle models for predicting constriction response in arterioles, venules, veins, and the renal system. This baseline sympathetic neuron model forms the foundation for creating digital twin models of circulatory system sympathetic control, aiming to predict individual patient responses to hypertension treatment, ultimately advancing personalised medicine.

P25 - Evidence that inhibitory regulation of oxytocinergic neurons to the spinal defecation center is manifested by hindpaw inflammatory pain in rats

Theme: Basic - Gut and metabolism

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We have previously shown that nociceptive stimulation to the colonic lumen enhances colorectal motility via descending dopaminergic and descending serotonergic neurons that project from the brain to the spinal cord. These monoaminergic neurons constitute a descending pain regulatory system, suggesting that pain and colorectal motility share regulatory pathways. In this study, we aimed to elucidate whether possible alteration of the descending pain regulatory pathway due to the presence of chronic pain can affect central regulatory pathway of colorectal motility. Complete Freund's adjuvant (CFA) was administered to the unilateral hindpaw of male SD rats to induce inflammation. Sham rats were treated with saline in the hindpaw. Colorectal motility was measured in vivo in anesthetized rats 3 days after hindpaw administration. In sham rats, noxious stimuli in the colonic lumen enhanced colorectal motility. In contrast, the noxious stimuli failed to enhance colorectal motility in the CFA-treated rats. When L-368,899, an oxytocin (OXT) receptor inhibitor, was administered to the lumbosacral spinal cord, where the spinal defecation center is located, colorectal motility was induced by noxious stimuli in CFA-treated rats, in a similar manner to the responses in sham rats. The results suggest that oxytocinergic neurons are associated with inhibitory effects of hind paw inflammation on colorectal motility. Then, we investigated the involvement of oxytocinergic pathways using OXT-hM3Dq rats, which express the facilitative Designer Receptors Exclusively Activated by Designer Drugs (DREADD) hM3Dq in the oxytocinergic neurons. Intrathecal administration of clozapine N-oxide to activate oxytocinergic neurons abolished colorectal motility induced by noxious stimuli in OXT-hM3Dq rats. This indicates that oxytocinergic neurons projecting to the spinal cord inhibit colorectal motility. In conclusion, these results indicate that inflammatory pain might cause alterations in the central regulation of colorectal motility via activation of oxytocinergic neurons projecting to the spinal defecation center.

P29 - Towards sustainable scientific data management solutions in the age of scale and multi-modal data-integration

Theme: Basic - Integrative Control

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Open data does not equal free data. This distinction will prove to be increasingly important when we discuss the requirements and needs for FAIR and sustainable data management. With the rise of ultra-large multi-modal data acquisition, we are reaching an inflection point where simple file-based data repositories will no longer support the needs of the scientific community as they do not scale nor capture the intricacies of integrated multimodal datasets. Software technologies can and will play a significant role to ensure sustainability of newly created data resources, but only when implemented with rigor and sufficient levels of commitment and effort. Ensuring sustainability requires de-risking cost to the repository at scale, and providing a user experience that invites investigators to integrate the platform within their scientific workflow.

Over the last 8 years, we developed the Pennsieve platform with these ideas in mind. At its core, it is a scientific data management platform with repository functionalities and workflows. It supports files, and complex metadata side-by-side, has curation workflows, and supports 'peer-reviewed' publishing and versioning of FAIR datasets. The platform is currently used by several NIH programs including SPARC, HEAL RE-JOIN, HEAL-PRECISION and Epilepsy.Science. It leverages the latest industry standards for software engineering to facilitate the scale and sustainability of the resource while providing a meaningful platform for data users with different areas of expertise.

In context of the SPARC program, the data management platform is one component of the SPARC Data Resource Center (DRC) which includes a Data-core, Simulation-Core, Curation-Core and Mapping-core. The DRC provides a unique distributed data ecosystem supporting high quality data publishing for projects that bridge the body and the brain and to enable scalable data analytics and integrations.

Funding Information: This project is supported by NIH SPARC project, under award number OT3OD025357.

P30 - Scaffold Mapping Tools for Mapping Data to Anatomical Scaffolds

Theme: Basic - Integrative Control

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Reproducible comparison and integration of data from multiple samples, modalities, and/or species has always been challenging. To address this, we developed a methodology to map data to a common coordinate framework using the Scaffold Mapping Tools.

The Scaffold Mapping Tools is an application with capabilities for building anatomical models or scaffolds, fitting to make individualized models, registering data in standard material coordinate systems, visualization, and enriching datasets with metadata and renderings for publication to repositories.

The tools allow complex workflows to be defined by a series of connected steps. The workflow and parameters used are captured for reproducibility and re-use across multiple specimens or populations.

Within the tools, the Scaffold Creator package constructs high quality anatomical scaffolds which are annotated geometric models of organs or whole body, using modular scripts and component re-use. The Geometry Fitter fits scaffold geometry to similarly annotated specimen data to make individualized model. Using a series of user-defined align and fit steps, material coordinate smoothness can be maintained. This simplifies production and ensures validity of population studies. The Data Embedder defines scaffold material coordinates on data and sub-structures from their locations in the fitted scaffold. It can be used for embedding maps, thereby allowing multiple datasets to be interpreted in a common coordinate system and aids in multi-scale modelling. The Field Fitter fits continuous fields over a scaffold from discrete data to describe the spatial variation of non-coordinate data. High quality graphics of mapped data can be generated using the Argon Viewer for web export, while the SPARC Curation Tool generates export of the workflow and related metadata for submission to standard repositories (for example, SPARC Portal or the Physiome Model Repository), hence providing accessibility and reproducibility of the mapping process. The finite element meshes can also be exported from the scaffolds for computational studies.

P31 - Exposure to a diet rich in linoleic acid promotes nociceptive hypersensitivity and elevated systemic blood pressure in both spinal-intact and spiralized rats

Theme: Basic - Integrative Control

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Pain is associated with the development of cardiovascular disease, and nociceptive hypersensitivity may contribute to hypertension. Various fatty acyl lipid mediators (e.g. oxylipins) are derived from dietary polyunsaturated fatty acids (PUFAs) and modulate nociception. The modern diet is rich in the omega-6 PUFA, linoleic acid (LA), which may present a risk factor for developing pain conditions and cardiovascular disease. In this study rats were randomized, at the time of weaning, to one of two modified AIN-76A diets each containing 5.1% fat. The standard corn oil was replaced with a custom triglyceride blend rich in either LA or oleic acid (OA; 18:1n-9), a monounsaturated fatty acid that is not metabolized to form oxylipin lipid mediators. In general, rats maintained on the LA-rich diet displayed greater plasma accumulation of pro-nociceptive oxylipins when compared to rats maintained on the OA-rich diet. The accumulation of pro-nociceptive oxylipins was associated with a significant increase in thermal nociceptive hypersensitivity. Using an unanesthetized, decerebrate preparation splanchnic sympathetic nerve activity (sSNA), arterial blood pressure and heart rate were measured at baseline and in response to ganglionic blockade. Rats maintained on the LA-rich diet displayed higher baseline mean arterial pressure (MAP) compared to littermates maintained on the OA-rich diet (94±16 vs 78±14mmHg; $P<0.02$), while baseline heart rate was not influenced by diet. Ganglionic blockade with hexamethonium, (20mg/kg, i.v.) produced a larger fall in baseline MAP in rats maintained on the LA-rich diet compared to littermates maintained on the OA-rich diet (-54±15 vs -41±12 mmHg, respectively; $P<0.05$). Moreover, the effects of diet on nociceptive hypersensitivity and baseline MAP were preserved in chronic spinalized (T2 transection) rats. These findings illustrate the potential of intraspinal circuits to modulate systemic blood pressure and support the notion that hypertension associated with chronic pain may involve intraspinal circuitry.

P32 - Exploring the connections between C1 and liver-related DMV neurons involved in the autonomic control of glucose homeostasis

Theme: Basic - Integrative Control

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Glucose is the main energy substrate used by the most of body's cells, particularly to the brain, and its plasma level is finely regulated through complex interaction of hormonal and neuronal autonomic signals, from and to organs. Parasympathetic fibers innervating the liver originate from preganglionic neurons of the dorsal motor nucleus of the vagus (DMV), that receives monosynaptic glutamatergic neurotransmission from the C1 neurons in the ventrolateral medulla, being reasonable to suppose that this connection from C1 to DMV may comprise a neuronal circuitry as part of the glycemic control. We sought to access whether there is apposition of the terminals of C1 neurons with the efferent hepatic nerves, and which glycemic stimulus may activate this pathway. The experimental procedures were approved by the Ethics Committee of Animals Welfare of the ICB-USP (#9141070319). Male wistar rats, 8 weeks old, received an anterograde lentiviral vector PRSx8-ChR2-eYFP, delivered stereotaxically into the C1 region, and 30 days after they received injections of FluoroGold (FG) into the liver. To test which glycemic stimuli could activate the C1-DMV pathway, the rats received an injection of either insulin (1UI/kg, i.p), glucose (3.6g/kg; i.p.) or a vehicle (0,9% NaCl). After 90 min, rats were deeply anesthetized and transcardially perfused for immunoreaction to detect the c-Fos protein. We observed varicosities and fibers originating from the C1 neurons with apposition with the DMV-FG+ neurons. Glucose overload led to an expressive c-Fos staining in the dorsal-vagal complex, including the DMV, and a slight detection in c-Fos+ neurons in the rostral portion of C1 region; more expressive in the C1/A1 region overlap, when compared to insulin-induced hypoglycemia and control groups, suggesting that hyperglycemia might be a potential stimulus that recruits the C1-DMV connection, activating the parasympathetic efferent pathway to the liver. Financial support: FAPESP #22/02895-4; #23/08762-9.

P33 - Volatile and injectable anaesthetics effects on cardiorespiratory and biochemical parameters in rats: enlightening anaesthetic choice according to the outcome studied

Theme: Basic - Integrative Control

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In a pilot study, we showed an interference of Ketamine and Xylazine (K/X) on the activity of a cardiac injury biomarker enzyme (unpublished observations). In the present study, we evaluated the effects of isoflurane (ISO, 4% induction; 2% maintenance; inhalation), thiopental (TP, 80 mg/kg; i.p.), K/X (80/10mg/kg; i.p.), tribromoethanol (TBE, 250 mg/kg; i.p.) and chloral hydrate (CH, 400 mg/kg; i.p.), in adult Wistar rats (300-400 g) on blood pressure (BP) and heart rate (HR), on respiratory dynamics and cardiac injury biomarker enzymes (Ethics Committee Approval number 29/2015; 17/2023).

Under anaesthesia, the femoral artery was catheterized to allow BP recordings and blood sampling. On the next day, recordings of BP, HR, and respiration (plethysmography) were performed in awake animals. After 30 minutes, the anaesthetic was administered, and cardiorespiratory changes were monitored until full recovery. Blood samples were collected before and after induction of anaesthesia and the activity of butyrylcholinesterase (BuChE), creatine kinase fraction MB (CK-MB), troponin I (cTnI), and lactate dehydrogenase (LDH) were monitored. Baseline BP values remained unchanged by TBE and TP ($p>0.05$), but all anaesthetics changed HR levels during anaesthesia induction and/or recovery phase ($p<0.05$). All anaesthetics reduced minute volume and respiratory rate, while tidal volume was reduced only by K/X and TBE ($p<0.05$). LDH and TnI activity remained unchanged after induction with all anaesthetics tested ($p>0.05$). In contrast, the activity of the BuChE enzyme was reduced by anaesthesia with K/X, TBE, and ISO ($p<0.05$). CK-MB was elevated after induction with HC and KX ($p<0.05$). Our findings unraveled some specific effects of general anaesthetics of clinical and preclinical use on basal cardiorespiratory function and cardiac biochemical biomarkers. These results can help enlighten more appropriate anaesthetic choices according to the experimental protocol studied. Keywords: anaesthesia; cardiovascular; enzyme biomarkers. Funding: Fapes (Grant code 2022- 78KWB); Capes Foundation Scholarship (Grant Code 01).

P41 - Methodologies for Vagus ElectroNeuroGram (VENG) analysis

Theme: Basic – Neuroscience

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The vagus nerve plays a crucial role in the parasympathetic system with an inhibitory role in homeostasis processes to restore internal stability in response to sympathetic system [1]. At the cervical level, around 80% of the fibers consist of somatic or visceral afferents, while efferent fibers are the primary conduit for the parasympathetic system's output. Several diseases manifest autonomic symptoms, thus using vagus nerve activity information could unveil new avenues for diagnosis and monitoring purposes. Indeed, while reduced heart rate variability is a significant predictor for conditions like diabetes[2], cardiovascular disorders, and premature mortality, it represents only a fraction of the information conveyed by the vagus nerve. Correlations between poor vagal tone and inflammatory conditions such as rheumatoid arthritis[3], atherosclerosis[4], and Crohn's disease[5] have been established, highlighting the broad impact of vagus nerve activity. Recent studies have explored compound action potentials transmitted by the vagus nerve, particularly in healthy rats and in humans, revealing respiratory and ECG-related burst activity modulated by both afferent and efferent components[6–8].

Extraneural vagus nerve recording remains challenging as movement artefacts can interfere with signal to noise ratio and potential gliosis between the electrode may decrease electrode-nerve contact[9], which is essential for the quality of the recording. We compare several time-domain, frequential and morphological analysis methods, including Root Mean Square[10], Spike frequency[9], spike sorting/clustering[11], and entropy-based analysis[12], to establish the advantages and disadvantages of each signal processing method.

Investigating parasympathetic activity through VENG recordings is crucial for developing robust tools to monitor the autonomic system, understand diseases better, and optimize treatments like vagus nerve stimulation.

[1] Teckentrup, 2023
[2] Wulsin, 2015
[3] Janse van Rensburg, 2012
[4] Simula, 2014
[5] Pellissier, 2014
[6] Ottaviani, 2020
[7] Sevcencu, 2018
[8] Macefield 2023
[9] Stumpp, 2021
[10] Andreasen, 2002
[11] Smets, 2021

P42 - Food perception promotes autonomic response to anticipate consumption and guides motivated behaviour

Theme: Basic – Neuroscience

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While many homeostatic mechanisms operate based on internal feedback regulation, organisms have also evolved systems to anticipate the impact of food consumption on homeostasis. The sight, smell, and taste of food trigger numerous autonomic physiological changes, including increased heart rate, saliva production, the release of enteroendocrine peptides, and changes in lipid metabolism. These predictive bodily responses ensure that nutrients are metabolized rapidly and are more efficiently removed from circulation. Food perception also enables organisms to adapt their behaviour in anticipation of a change in physiological need.

Our study takes a unique approach to understanding anticipatory homeostatic regulation. Building on recent findings in rodents, we conducted a randomized cross-over study in overnight fasted healthy human participants (N = 30). We explored the interplay between sensory perception (food anticipation), gut mediators (food ingestion), and adaptation of motivated behaviour.

Contrasting food presentation relative to a neutral condition, our results show significantly higher hunger ratings upon the expectation and detection of the (visual and olfactory) food cue ($F(1,48) = 10.45$, $p = .002$). Moreover, heart rate activity and blood pressure analyses suggest an autonomic nervous system activation. Specifically, the pre-ejection period measured by impedance cardiography shows a decrease after food presentation relative to the neutral condition, reflecting an increase in sympathetic activity. Moreover, in the food cue condition, learning asymmetry between positive and negative feedback is reduced ($F(2,117) = 3.99$, $p = .02$), indicating a shift in exploitation-exploration trade-off in motivated behaviour. Collectively, our study unravels that sensory food perception coordinately primes autonomic bodily responses and affects behavioural adaptation, demonstrating that both internal physiological state and external environmental cues are anticipating metabolic changes.

P43 - Gene expression and cardiovascular effects of nucleus tractus solitarii dopamine D1 receptors in stress-induced hypertension and its counteraction by exercise

Theme: Basic – Neuroscience

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Chronic stress induces hypertension; however, it can be effectively prevented by daily exercise. Although the medulla oblongata nucleus tractus solitarii (NTS) is involved in the development of hypertension, the molecular and physiological mechanisms underlying the effects of stress and exercise remain unclear. This study examined whether gene expression in the NTS is altered by stress and daily exercise and its involvement in cardiovascular regulation. We performed RT2 profiler polymerase chain reaction (PCR) arrays targeting neurotransmitter receptor genes in the NTS of Wistar rats subjected to chronic restraint-stress (1 h daily for 3 weeks) with or without voluntary wheel exercise (Exp. 1). Immunohistochemistry was performed to determine whether the identified molecules were expressed at the protein level (Exp. 2). Additionally, microinjection was performed under urethane anaesthesia in naïve, 3-week sedentary, restraint-stressed, or stressed + exercised rats to determine whether the validated molecules exhibited physiological roles in the cardiovascular regulation of the NTS (Exp. 3). In Exp. 1, we observed that blood pressure was significantly increased by stress but was suppressed by exercise. PCR analysis revealed that the expression levels of four NTS genes, including the dopamine receptor D1 gene (Drd1), were significantly affected by stress and suppressed by exercise. In Exp. 2, we examined dopamine D1 receptor (D1R) expression in NTS neurons and found a significantly higher expression in stressed animals than in non-stressed animals. In Exp. 3, D1R agonist microinjection into the NTS of anaesthetized rats induced hypotensive effects, which was not significantly different among non-stressed, stressed, and stressed + exercised rats. These results suggest that NTS D1R may play a role in counteracting stress-induced hypertension, whereas D1R sensitivity may be reduced in stressed rats without exercise.

P51 - On the regulation of arterial blood pressure by an intracranial baroreceptor

Theme: Basic - Neuroscience

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There is evidence for the existence of an intracranial baroreceptor mechanism(s) capable of sensing changes in cerebral blood flow; however, little is known about the sensitivity of this mechanism and its interaction with peripheral baroreceptors. The aim was to characterise the cardiovascular responses to changes in cerebral perfusion induced by manipulations of intracranial pressure (ICP).

The experiments were performed in adult Sprague-Dawley rats, anaesthetised with urethane (induction: 1.3 g kg−1, i.p.; maintenance: 10–25 mg kg−1 h−1, i.v.). The femoral artery and vein were cannulated for measurement of arterial blood pressure (ABP) and administration of anaesthetic. The trachea was cannulated, and mechanically ventilated with room air. The lateral cerebral ventricles were cannulated to record and manipulate ICP. The experiments included the denervation of the arterial baroreceptors, stimulations of the aortic depressor nerve and recordings of renal sympathetic nerve activity.

The resting ICP in rats anaesthetised with urethane was 6.2 ± 0.7 mmHg (n=8). Following a craniotomy that reduced ICP to 0 mmHg, ABP decreased. Restoring the integrity of the intracranial space increased ABP to the baseline level. Progressive increases in ABP were observed in response to increases in ICP, revealing a linear relationship. In the absence of inputs from the arterial baroreceptors the ABP responses to ICP increases were preserved. Analysis of the cardiovascular responses to the electrical stimulation of the aortic depressor nerve suggested baroreflex re-setting at raised ICP. Renal nerve recordings demonstrated increased sympathetic activity at raised ICP (172 ± 34.91 % at 20 mmHg ICP ($p=0.003$; n=7) with markedly enhanced peaks of activity during the inspiratory phase of the respiratory cycle.

These data demonstrate that the intracranial baroreceptor mechanism is highly sensitive to changes in cerebral perfusion and at raised ICP, the baroreflex control of sympathetic vasomotor activity is reset, compensating for reduced brain perfusion.

P52 - Indoor air pollution impacts cardiovascular autonomic control during sleep and inflammatory profile

Theme: Clinical – Cardiovascular

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The present study explores the modifications of cardiovascular autonomic control (CAC) during wake and sleep time and the systemic inflammatory profile associated with exposure to indoor air pollution (IAP) in a cohort of healthy subjects.

Twenty healthy volunteers were enrolled. Indoor levels of fine particulate matter (PM2.5), nitrogen dioxide (NO2) and volatile organic compounds (VOCs) were monitored using a portable detector for 7 days. At the same time, a 7-day monitoring was performed through a wireless patch that continuously recorded ECG, respiratory activity and actigraphy. Indexes of CAC during wake and sleep time were derived from the acquired biosignals: heart rate and LF/HF, which represents the sympathovagal balance with higher values corresponding to a predominance of the sympathetic branch. Cyclic variation of heart rate index (CVHRI n°events/hour) during sleep, a proxy for the evaluation of sleep apnea events, was assessed for each night. After the monitoring, blood samples were collected to assess the inflammatory profile. Regression and correlation analyses were performed.

A positive association between daily exposure values to VOCs and the CVHRI (Δ%=+0.2% for 1µg/m3 VOCs, CI95% [0-0.3%]; p=0.008) was found. The CVHRI was also positively associated with LF/HF during sleep, thus higher CVHRI values corresponded to a shift of the sympathovagal balance towards a sympathetic predominance (r=0.52; p=0.018). Daily exposure values to NO2 were positively associated with both the pro-inflammatory biomarker TREM-1 and the anti-inflammatory biomarker IL-10 (Δ%=+1.2%, CI95% [0.4-1.9%] and Δ%=+2.4%, CI95% [0.4-4.4%], for 1µg/m3 NO2; p=0.005 and p=0.022, respectively).

The study highlights a possible causal relationship between IAP exposure and a higher risk of sleep apnea events, associated with altered CAC during sleep. Moreover, IAP exposure resulted in a pro-inflammatory state counterbalanced by an increased anti-inflammatory response in healthy subjects. This process may be disrupted in vulnerable populations, leading to a harmful chronic pro-inflammatory profile.

P53 - Neurologically based cardiovascular risk in young men after COVID-19

Theme: Clinical – Cardiovascular

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It has been suggested that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) could invade the central nervous system (CNS) via baroreflex trans-synaptic transmission. This invasion could result in increased sympathetic vasomotor activity (SVMA) and central systolic blood pressure (CSBP), in addition to reduced baroreflex sensitivity (BRS) and parasympathetic modulation (PM) of heart rate in young men who had COVID-19. These conditions could increase cardiovascular risk for young men who have had COVID-19 and who are currently experiencing Long-COVID. There is a need to know if these conditions exist in young men after COVID-19. This study aimed to compare SVMA, CSBP, BRS and PM between individuals who had COVID-19 versus controls.

Method: Forty-seven young men aged 18-27 were recruited and divided into two groups (COVID-19-22; Matched Controls-25) approximately six months after testing positive for COVID-19. Participants were examined for SVMA-as systolic blood pressure peaks within the low-frequency (LFSBP) band via power spectral density analysis, CSBP, BRS-as the alpha index (α-index), and PM-as high-frequency band of the R-R intervals (lnHFR-R).

Results: Mann-Whitney U tests indicated no significant difference between groups in SVMA (P = 0.15), CSBP (P =0.02), α-index (P =0.17), and PM (P = 0.26). Significance level was 0.01.
Conclusion: Findings suggest that there are no differences in SVMA, CSBP, α-index, and PM between young, healthy men who had COVID-19 and controls. This suggests that young, healthy men who had COVID-19 and are experiencing Long-COVID might not be at increased cardiovascular risk from baroreflex-mediated impairment.

P59 - Effect of YMCA GoldFit exercise participation on the chemoreflex control of breathing in older adults

Theme: Clinical - Integrative Control

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Ageing is a significant risk factor for the development of cardiovascular disease, while exercise training has many physiological benefits including reduced risk of cardiovascular and all-cause mortality. In animal models of disease (e.g., heart failure), exercise training reduces peripheral and central chemoreflex sensitivities. This study sought to determine whether peripheral and central chemoreflex sensitivities, along with peripheral chemoreflex tonicity, would be lowered in older adults participating in a community-based exercise intervention (GoldFit) compared to their sedentary peers.

In ten sedentary Controls (4 men, age 68±9yr, body mass index [BMI] 25±4 kg.m^{-2}) and thirteen regularly exercising adults (YMCA GoldFit participants; 3 men, age 72±6 yr, BMI 26±4 kg.m^{-2}), minute ventilation ($\dot{V}E$), end-tidal oxygen and carbon dioxide ($PETO_2$ and $PETCO_2$) and oxygen saturation (SaO_2) were continuously measured during three protocols. 1) Hyperoxic hypercapnic rebreathing (95% O_2-5% CO_2) where central chemoreflex sensitivity was determined from the slope of the relationship between $\dot{V}E$ and $PETCO_2$; 2) transient hyperoxia (100% O_2) where peripheral chemoreflex tonicity was calculated as the change in $\dot{V}E$ from baseline to nadir, and; 3) isocapnic hypoxic rebreathing (21% O_2-balance N_2), where peripheral chemoreflex sensitivity was taken as the increase in $\dot{V}E$ from baseline to peak rebreathing expressed relative to the fall in SaO_2.

Central chemoreflex sensitivity was lower in Controls compared to GoldFit participants (1.33±0.68 vs. 2.30±0.92 L.min^{-1}.mmHg^{-1}, P=0.015). Peripheral chemoreflex tonicity was greater in GoldFit compared to Controls (-2.2±1.5 vs. -1.0±1.0 L.min^{-1}, P=0.041), while peripheral chemoreflex sensitivity was not different between Controls and GoldFit participants (-0.45±0.19 vs. -0.49±0.21 L.min^{-1}.%$^{-1}$, P=0.654).

These results show that contrary to expectation, central chemoreflex sensitivity and peripheral chemoreflex tonicity are greater in older adults who regularly participate in physical activity compared to their sedentary counterparts. It is possible that exercise training helps defend against age-related declines in central chemoreflex sensitivity and peripheral chemoreflex tonicity.

P60 - Characterization of dysautonomia in patients with Ehlers-Danlos Syndrome and its relationship with anxiety and sleep quality. The importance of comprehensive care

Theme: Clinical – Neuroscience

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Ehlers-Danlos Syndrome (EDS) is a group of rare inherited connective tissue diseases characterized by impaired collagen production, resulting in the laxity of skin, ligaments, and blood vessels, with significant impact on morbidities and quality of life. EDS has been associated with low quality of sleep, high levels of anxiety and depression, and overall dysautonomia (autonomic nervous system, ANS, dysfunction). ANS, mostly through its role in the brain-heart axis, is gaining more and more attention in understanding the relationship between the mind and the body, and, broadening perspectives, other privileged communication pathways, such as the brain-gut axis, have been explored. However, the link between psychological features and dysautonomia, as well as a deep inquiry among different forms of EDS have not yet been thoroughly studied. 21 patients (9 with hypermobile EDS, 6 with classical form, and 6 with hypermobility spectrum disorder; HDS) were enrolled at the EDS clinic, Policlinico Hospital (Milan). Dysautonomia was evaluated through the Composite Autonomic Symptom Score-31 (COMPASS-31). Sleep quality was assessed through the Pittsburgh Sleep Quality Index (PSQI), and anxiety through the Patient-Health Questionnaire 4 (PHQ-4). One-way ANOVA was used to explore differences among different forms of the disease. Correlation analysis was performed to investigate the relationship between variables. 67% of patients reported sleep disturbances and 33,3% mild or moderate anxiety. The hypermobile group showed greater overall dysautonomia compared to HDS ($p < .05$). Classical ($p < .05$) and hypermobile ($p < .001$) patients reported more gastrointestinal dysfunction than HDS. A positive significant correlation was found between COMPASS-31, PHQ-4 anxiety score ($r = 0.53$; $p < .05$), and PSQI score ($r = 0.58$; $p = 0.009$), as well as between gastrointestinal sub score and anxiety ($r = 0,49$; $p < 0.05$). Dysautonomia resulted to be an important feature to consider for both physical and psychological health. Delving into its role could help in disease management by providing patients with personalized and holistic treatment.

Poster Session 2

P3 - The collaborative SPARC Portal for peripheral neuromodulation data, modeling and device design

Theme: Basic - Bioelectronic Medicine

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Stimulating the autonomic nervous system to modulate organ physiological function holds enormous therapeutic promise, but the underlying mechanisms are poorly understood. The NIH Stimulating Peripheral Activity to Relieve Conditions (SPARC) program aims to improve targeted therapies by providing public access to foundational research into nerve-target organ interactions.

The SPARC Data and Resource Center provides a cloud-based data management platform to manage, curate, and publish scientific datasets, while the SPARC Portal (https://sparc.science) is the community platform to discover and use these high-value models and datasets to support the development of neuromodulation devices. As a result of professional scientific curation, datasets and models are scientifically interpretable, and compliant with the 2023 NIH Data Management and Sharing Policy. Datasets are augmented with viewers, maps, and integrated computational platforms, making the Portal much more than a data repository.

We report on the development of the SPARC Portal and its growing number of technical apps. To highlight open-source device contributions, we are preparing novel dataset types that support GitHub releases and CAD model publication. Connectivity maps are automatically populated using the knowledge obtained from SCKAN, the SPARC connectivity knowledge base, allowing us to expand as our knowledge evolves. The annotation mode for maps allows Portal users to comment on knowledge surfaced, which then can be curated and contributed to SCKAN. Enhanced pipelines are being created to more efficiently port data directly to o²S²PARC, a cloud-based, open computational platform so the effects of neuromodulation on organ function can be predicted. We introduce a centralized client-based infrastructure to allow access to essential functions in python modules from DRC services from one entry point and centralized authentication. This continued integration of SPARC platform resources will accelerate progress in the field of bioelectronic medicine and beyond as data contribution from the wider community expands.

Acknowledgements: 1OT3OD025348-01, 1OT3OD025347-01, 1OT3OD025349-01, 1OT2OD030541-01

P11 - Plasticity in human intracardiac neurons from patients with Atrial Fibrillation

Theme: Basic - Cardiovascular

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Changes in the autonomic nervous system cause abnormalities in heart rhythm including Atrial Fibrillation (AF). The structural and functional properties of the autonomic neurons present in the ganglionated plexi (GP) of the human heart remain unknown. Here we aim to bridge this gap in knowledge by studying GP neurons of both AF and non-AF patients.

Epicardial adipose tissue samples from the right atrium were obtained from consented patients during open-heart surgery. Confocal imaging analysis on fixed tissue revealed the structural complexities of neurons with branched neurite outgrowth. The GP were predominantly cholinergic with a very few noradrenergic and dual phenotypes. Furthermore, the GP from AF patients showed increased synaptic density along with an increase in the number of noradrenergic neurons, whereas the number of dual and cholinergic neurons was decreased. Electrophysiological analysis showed a significant increase in excitability in neurons from AF patients as compared to non-AF neurons. GP neurons also require a lower current to induce action potential firing and exhibit higher action potential rates and decreased action potential accommodation.

The presence of both structural and functional differences between the GP neurons of the non-AF and AF patients could cause altered autonomic influence on atrial rhythm. Furthermore, these findings identify decreasing excitability in GP neurons as a potential target for AF therapy.

P12 - Heart Rate Variability during Short-Term Head-Down Tilt

Theme: Basic - Cardiovascular

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Introduction: The head-down tilt (HDT) technique can imitate the immediate effects of microgravity. A change in HRV (Heart Rate Variability) results from the redistribution of fluid from the lower to the upper body caused by HDT. However, the effect of short term HDT with various degrees on HRV is not well documented and needs to be explored.

Objective: To evaluate the effect of short-term HDT at 6°, 15°, and 30° on HRV in healthy adults.
Methods: After approval from Institutional Ethics Committee (AIIMS/IEC/2021/3551), we enrolled 50 healthy subjects (Age = 30.38±6.63 (mean ± SD)). Short-term HDT for 5min was administered using a motorized tilt table with continuous recoding of Lead II ECG and chest movements. HRV was analysed during baseline and each degree of tilt. Values are expressed as the median (interquartile range) and Friedman test was applied.

Result: We observed that the mean RR interval was increasing in all three degrees of tilt compared to the baseline. It was observed that, as compared to baseline, there is an increase in SDNN (BL-39.54 (23.14-93.06); HDT = 6°-48.12(22.44-85.74); 15°-45.73(21.03-107.2); 30°-46.02(18.92-106.4); p-value =0.0185*) and RMSSD (BL-31.74(14.50-106.9); HDT = 6°-40.81(11.56-128.5); 15°-39.73(12.94-119.4); 30°-38.78(11.33-104.5); p-value =0.0241*), which is suggestive of parasympathetic stimulation during tilt. Total power also showed a significant increase with each degree of tilt, which is representative of an increase in overall autonomic reactivity during tilt (BL-1426(507.4-8096); HDT = 6°-2283(514.9-9610); 15°-1909(426.7-9976); 30°-1714(254.6-8258); p-value =0.0268*).

Conclusion: Short-term HDT leads to increased HRV due to improved autonomic reactivity during tilt with significant involvement of parasympathetic activation.

P13 - Heart rate variability and air pollution

Theme: Basic - Cardiovascular

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Exposure to air pollutants can decrease heart rate variability (HRV), an index of cardiac vagal modulation of parasympathetic activity and a predictor of cardiac morbidity and mortality. Conversely, higher levels of physical fitness are cardioprotective. Whether the benefits of exercise and fitness on the autonomic nervous system extend to situations where habitual exercise is performed in polluted air is unknown. We examined whether cardiorespiratory fitness modify the association between life-time exposure to air pollution and HRV. Data from the Czech Study Healthy Aging in Industrial Environment (HAIE) were utilized comprising 890 adults aged 18-65 years (mean age 37.4yrs,SE±0.41). For each participant an individualized level of the lifetime exposure to PM2.5 and NO2 were calculated. Main HRV variables of our interest were RMSSD and HF-HRV, as they reflects vagal tone. Cardiorespiratory fitness was determined by peak aerobic power. Participants were categorized into fitness groups according to American College of Sports Medicine guidelines. Kendal´s partial Tau showed that after controling for age, sex, education and economy status, higher life-time exposures to PM2.5 and NO2 were associated with lower RMSSD and HF-HRV ($\tau \leq -0.04; p \leq 0.047$). Stratified analysis by fitness level revealed an association between exposure to PM2.5 and NO2 and lower RMSSD and HF-HRV in participants with very poor/poor fitness($\tau \leq -0.11; p \leq 0.038$), whereas the negative effects of air pollution on RMSSD were abrogated in those with fair/good or excellent/superior fitness ($\tau \geq -0.05; p \geq 0.111$). Additional adjustments for presence of cardiometabolic condition and medication use had little effect on the observed associations. We showed that physical fitness may modify the association of air pollution with HRV indices. We conclude that physical fitness may be protective against the negative effects of air pollution on the autonomic nervous system.

P14 - The midbrain dopaminergic areas mediate the cardiovascular response induced by the activation of the lateral habenula

Theme: Basic - Cardiovascular

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The cardiovascular response is essential for stress coping. The lateral habenula (LHb) in the epithalamus has been implicated in stress-related autonomic regulation. In the previous study, we reported that activation of the LHb caused phasic bradycardia and a pressor response. The LHb regulates midbrain dopaminergic areas such as the ventral tegmental area (VTA) and ventrolateral periaqueductal grey (vlPAG). However, it remains unclear whether the midbrain dopaminergic regions are involved in the cardiovascular response induced by the activation of the neurons in the LHb. In this study, we investigated the participation of the dopaminergic system, the VTA and the vlPAG, in generating the cardiovascular response originating from the LHb.

We used Wistar male rats (250~350 g) anaesthetized by urethane. Arterial pressure was measured from a catheter inserted into the femoral artery. The femoral vein was also cannulated to administer a dopamine receptor antagonist (clozapine, 1 mg/kg, i.v.). Heart rate was calculated from the electrocardiography. First, we observed the cardiovascular response induced by the electrical stimulation to the LHb with the administration of clozapine. Administration of clozapine attenuated the LHb-induced bradycardia and pressor responses. Next, we injected muscimol, a GABAA receptor agonist, into the VTA or the vlPAG to inactivate these areas. We found that inactivation of the VTA attenuated both the LHb-induced bradycardia and pressor responses. On the other hand, inactivation of the vlPAG enhanced the LHb-induced bradycardia and reduced the pressor response. These results suggested that the neurons in the VTA would mediate the excitation of the sympathetic vasoconstrictor fibers and the cardiac vagus nerves in the LHb-originated cardiovascular response. Neurons in the vlPAG mediate the excitation of cardiovascular sympathetic nerves. The dopamine neurons in the VTA and vlPAG may mediate autonomic cardiovascular regulation in stress situations.

P15 - Mice with overexpression of vesicular acetylcholine transporter have increased cardiac parasympathetic activity

Theme: Basic - Cardiovascular

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Inhibitory parasympathetic influence on the heart contributes to the maintenance of a steady and healthy resting heart rate and effectively decreases the cardiac workload, lowering the risk of cardiovascular complications. Furthermore, parasympathetic activity has been demonstrated to reduce inflammation throughout the body. Therefore, strategies to enhance parasympathetic drive reduce cardiac risk. Mice genetically engineered to overexpress the vesicular acetylcholine transporter (VAchT+) may exhibit heightened cardiac parasympathetic activity, potentially leading to increased cardiovascular health and reduced cardiac risk. We aimed to evaluate cardiac autonomic tone and heart rate variability (HRV) in VAchT+ mice. Conscious, freely moving VAchT+ or their non-transgenic littermates, referred to as WT mice, equipped with ECG electrodes and jugular vein catheters, underwent basal ECG recording followed by administration of methylatropine (1 mg/kg, iv) to block muscarinic receptors. This procedure enabled the assessment of HRV indices and cardiac vagal tone. In VAchT+ mice compared to their WT littermates, basal heart rate was significantly lower (506±17 vs. 569±17 bpm), while vagal tone was markedly higher (146±18 vs. 72±16 bpm). VAchT+ mice exhibited higher SDNN (9.0±1.2 vs. 5.5±1.1 ms) and RMSSD (5.7 ± 1.3 vs. 2.5 ± 0.6 ms) than WT littermates mice. Sample entropy was also elevated across scales 1 to 5 (1.4±0.1 vs. 1.1±0.1) and 6 to 10 (1.6±0.1 vs. 1.3±0.1) in VAchT+. Heart rate fragmentation, as indicated by the percentage of inflection points (PIP) and the percentage of words with 3 inflection points (W3), was increased in VAchT+ mice (PIP: 70.4±3.8 vs. 54.8±5.1 %, W3: 39.0±3.0 vs. 22.8±4.2 %). HRV indices and pharmacological blockade of muscarinic receptors indicate heightened cardiac vagal activity in genetically modified mice. Our study suggests that VAChT overexpression enhances vagal drive, rendering VAChT+ mice more responsive to the cardioprotective effects associated with increased cholinergic activity.

P16 - Exercise Mitigates Stress-Induced Hypertension and Brain Inflammation by Modulating Molecular Pathways in the Amygdala and Hypothalamus

Theme: Basic - Cardiovascular

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Background: Chronic stress elevates blood pressure (BP) and promotes brain inflammation, while regular exercise exerts antistress, antihypertensive, and anti-inflammatory effects. The specific mechanisms underlying these effects, particularly in brain regions involved in autonomic BP regulation, remain unclear. This study investigates the molecular basis of these processes, focusing on the amygdala and the paraventricular nucleus (PVN) of the hypothalamus.

Methods: Male Wistar rats were assigned to control, restraint stress, and stress plus daily exercise (SE) groups. Cardiovascular parameters were monitored, and gene expression profiles in the amygdala, hypothalamus, and bone marrow (BM) were examined using microarray and RT-PCR. The inflammatory blood cell population was analyzed via flow cytometry. Immunohistochemical staining examined the presence of BM-derived C-C chemokine receptor type 2 (CCR2)-expressing microglial cells in the PVN and amygdala. The role of identified genes in cardiovascular regulation was assessed using siRNA transfection.

Results: Stress increased gene expression of inflammatory factors such as interleukin 1 beta and CCR2 in the BM, and recruited BM-derived CCR2-expressing microglial cells into the PVN, which exercise prevented. Stress also upregulated hypothalamic matrix metalloproteinase 3 (MMP3), increasing blood-brain barrier (BBB) permeability, while exercise normalized MMP3 expression and downregulated genes facilitating cell migration. Additionally, chronic restraint stress decreased signal transducer and activator of transcription 3 (Stat3) expression in the amygdala, which exercise normalized. STAT3 was expressed in neurons in the amygdala, and its inhibition increased BP without affecting baroreflex gain.

Conclusions: STAT3 in the amygdala plays a crucial role in BP regulation and mediates the hypertensive effects of chronic stress, which are counteracted by exercise. In the hypothalamus, daily exercise prevents stress-induced recruitment of inflammatory cells and changes in gene expression, reducing BBB permeability and inflammation. These findings highlight STAT3 and MMP3 as potential targets for managing stress-related hypertension and inflammation, emphasizing the protective role of regular exercise.

P17 - Muscle sympathetic nerve activity responses to the cold pressor test in women across the third and fourth decades of life

Theme: Basic - Cardiovascular

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A recent retrospective analysis of muscle sympathetic nerve activity (MSNA) across the premenopausal lifespan suggested that MSNA declines in women from the ages of 18 to 30, before increasing from the age of 30 and onwards, challenging dogmatic views of menopause-induced increases in MSNA. However, there has yet to be prospective study of the impact of premenopausal aging on MSNA in healthy women. Therefore, the purpose of this study was to examine MSNA and sympathetic responses to an acute stressor (the cold pressor test; CPT) across the third (i.e., twenties) and fourth (i.e., thirties) decades of life. In this pilot analysis, we assessed 6 healthy premenopausal women (3rd decade: n=3; age: 23±1yrs, range 20-28; 4th decade: n=3; age: 31±5yrs, range 30-39). Blood pressure (finger photoplethysmography calibrated to manual sphygmometry-derived values) and MSNA (peroneal nerve microneurography) were assessed at rest and during a 3-min CPT. CPT data were divided into 1-min bins and peak mean arterial pressure (MAP) and MSNA responses were extracted for further analyses. Resting MAP was not different between groups (3rd decade: 90±6; 4th decade: 88±10mmHg; P=0.8). Likewise, neither resting MSNA burst frequency (17±5 vs 12±4bursts/min; P=0.3) nor burst incidence (14±3 vs 11±4bursts/100hb; P=0.4) were different between 3rd and 4th decades. Relative increases in MAP tended to be higher in the 3rd decade than the 4th (17±8 vs. 9±4mmHg; P=0.16). However, neither relative increases in MSNA burst frequency (8±18 vs 13±8bursts/min; P=0.7) nor burst incidence (17±20 vs 17±9bursts/100hb; P=0.6) were different between groups. In this preliminary cohort of participants, we observed a trend towards higher blood pressure responses to the CPT in women who were in their twenties relative to women in their thirties. However, this was not coupled to group differences in MSNA, suggesting a possible age-dependency in the neurovascular control of blood pressure.

P18 - Liraglutide improvement of chemoreflex function in ovariectomized female rats is associated with a reduction in oxidative stress

Theme: Basic - Cardiovascular

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Liraglutide improves cardiovascular outcomes in diabetics, but its effectiveness on chemoreflex and cardiac oxidative stress in spontaneously hypertensive (SH) ovariectomized female rats is unknown. SH female rats at 8 weeks of age were ovariectomized (O) or SHAM (S) operated and treated for 30 days, subcutaneously, with Liraglutide 0.6 mg/kg twice daily (OL, n=11; SL, n=10) or saline (O, n=11; S, n=10). Body weight (g) and glycemic curve (mmol/L) were recorded during treatment. After treatment, under anaesthesia (ketamine/xylazine 70/10 mg/kg, i.p.), the femoral artery and vein were catheterized to allow blood pressure (BP) recordings and chemoreflex activation through potassium cyanide injection (20 □g, i.v.). Heart rate (HR) recordings were derived from pulse pressure and respiratory parameters obtained through plethysmography. HR variability in the time domain was obtained from resting recordings and the root mean square of successive interpulse interval differences (RMSSD) was used as a cardiac vagal tonus index. After recordings, animals were euthanized, and samples of the heart and brainstem were collected to determine advanced oxidation protein products (AOPP) (µmol/mg of protein) and lipid peroxidation (µmol/mg of protein). Data was analyzed by Two-way ANOVA or Generalized Estimating Equations and level of significance was set as $p<0.05$. (Project Ethical Committee Approval nº11/2019). The OL group showed greater chemoreflex bradycardia and RMSSD index; lower AOPP levels in the cardiac ventricle and brainstem; in addition to less weight gain. Furthermore, O group increased the weight gain and lipid peroxidation in the cardiac ventricle, while Liraglutide prevented these changes. Our results indicate that Liraglutide can protect cardiac function by enhancing vagal tone through peripheral and central mechanisms in a condition of hypertension associated with ovarian hormone deficiency.

Keywords: Liraglutide/GLP-1, Hypertension, Menopause, Chemoreflex, Oxidative Stress
Funding: FAPES/PRONEM 23/2018, TO: 591/2018; FAPES/PROAPEM Grant code 2022-78KWB), UFES, Capes Foundation Scholarship.

P26 - Involvement of neurons projecting from the hypothalamus to the medullary raphe in stress-induced defecation in rats

Theme: Basic - Gut and metabolism

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Background & Aim: The mechanism by which acute stress elicits defecation reflex is not well understood. In rats, it has been reported that activation of descending serotonergic neurons projecting from the medullary raphe to the lumbosacral spinal cord cause stress-induced defecation. In this study, we focused on the neurons located upstream of the medullary raphe to identify the neural pathways involving stress-induced defecation.

Methods: Rats were injected with a retrograde tracer, FluoroGold (FG), into the medullary raphe and neurons projecting to the nucleus were labelled. To verify whether the FG-labelled brain regions regulate colorectal motility, we microinjected of (S)-AMPA, a glutamate agonist, to the regions in urethane-anesthetized rats. Then colorectal motility was assessed in vivo by measuring intraluminal pressure and expelled fluid volume from the anus.

Results: In the hypothalamus, FG-positive cells were observed in the paraventricular hypothalamic nucleus (PVH) and the dorsomedial hypothalamus (DMH). When (S)-AMPA was unilaterally administered to the PVH or the DMH, a marked increase in colorectal intraluminal pressure that was associated with increased expelled fluid was observed. The increased motility was suppressed by prior administration of serotonergic inhibitors into the lumbosacral spinal cord. Specific inhibition of the PVH→medullary raphe pathway or the DMH→medullary raphe pathway by the DREADD (designer receptors exclusively activated by designer drugs) suppressed the increased colorectal motility due to AMPA injection. Similarly, defecation induced by water avoidance stress was suppressed by DREADD-based inhibition of these pathways in conscious rats.

Conclusions: Stress-responsive neurons in the PVH and the DMH activate the medullary raphe, causing enhancement of colorectal motility through a mediation of descending serotonergic neurons that project to the lumbosacral spinal cord. These pathways are suggested to be novel neural pathways inducing defecation under stressful conditions.

P34 - The SPARC SCKAN multi-species knowledge base of ANS connectivity

Theme: Basic - Integrative Control

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The Stimulating Peripheral Activity to Relieve Conditions (SPARC) program is an NIH-funded consortium whose overarching goal is to improve the understanding of how the autonomic nervous system (ANS) interacts with end organs and the central nervous system. A major objective of SPARC is to use this knowledge to develop the next generation of neuromodulator devices as effective therapies for diseases of the nervous system. To assist in that effort, the SPARC Data and Resource Center (DRC) is generating infrastructure for sharing data and tools for mapping neural connectivity of the peripheral nervous system (PNS). A centerpiece of this infrastructure is the SCKAN and associated visual maps of connectivity. SCKAN contains knowledge about circuitry derived from SPARC data and scientific literature, in a form that supports reasoning. The SCKAN currently contains two levels of semantic connectivity knowledge: circuit and individual connections. A circuit represents a detailed model of connectivity associated with a particular organ, e.g., bladder or functional circuits, e.g., defensive breathing. These circuits are created through interviews with SPARC investigators, anatomical experts, and the scientific literature. They contain detailed representations of neuron populations giving rise to ANS connections, including mappings of the locations of cell bodies, dendrites, axon segments, and synaptic endings involved in a particular circuit. Existing neuronal populations within SCKAN have been enriched with details and supporting citations and new knowledge about the circuitry of female and male reproductive systems have recently been added.

Acknowledgements: SPARC 1OT2OD030541.

P35 - Comparing cardiorespiratory responses after organophosphate poisoning in Wistar and pre-hypertensive SHR in situ

Theme: Basic - Integrative Control

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Cardiorespiratory complications are the main cause of hospitalization and death caused by organophosphate (OP) poisoning among farmers. Previously, we showed that acute exposure to chlorpyrifos (CPF), a commonly used OP, attenuated cardiorespiratory reflexes, such as the baro- and chemo- reflexes. Interestingly, mortality rate by OP is greater in spontaneously hypertensive rats (SHR) than normotensive animals (Aitken et al 2024). However, whether this susceptibility is due to high blood pressure or rat strain differences is unknown. Using the working heart-brainstem preparation, we recorded heart rate, phrenic (PN) and thoracic sympathetic (tSNA) nerve activities in both Wistar and pre-hypertensive SHR (4-5 week-old; ethical committee approval number 17/2022).

After 15 min baseline recording, chlorpyrifos-oxon (CPO; 15 mg/Kg), the active form of CPF, was added to the preparation. The peripheral chemoreflex was evoked via arterial bolus injection of potassium cyanide (75 µL of 0.03% i.a.). CPO evoked hypopnea (i.e., reduction in PN amplitude ≥ 10%) and apnoeas (i.e., 5-fold increase in expiratory time) accompanied by an increase in tSNA. These responses were not different between Wistar and SHR ($P>0.05$). In addition, CPO reduced the carotid body chemoreflex sympathetic (35%, $P<0.05$) and tachypneic reflexes (20%, $P<0.05$), whereas it exaggerated the bradycardic response (24% increase, $P<0.05$). Only the latter response was different between strains with a less intense bradycardia seen in Wistar rats (-160.9 bpm vs – 205.6 bpm, Wistar vs SHR, respectively). Our study shows that differences in rat strain are not a predictor of CPO-induced respiratory failure. We suggest that the greater susceptibility of adult SHR to CPF is due to the elevated blood pressure. We predict that farmers with a medical history of hypertension may be at greater risk if exposed to OP. Keywords: Organophosphate poisoning, Chlorpyrifos, Cardiorespiratory and Hypertension. Funding: FAPES foundation (Grant CODE:2022-78KWB), CAPES (Finance Code 01). Aitken et al: 10.1016/j.cbi.2023.110821

P36 - Supratentorial inhibition of regional sympathetic nerve activity

Theme: Basic - Integrative Control

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Central control of sympathetic nerve activity has been studied extensively in the past. There is an abundance of information available about the central control of regional sympathetic nerve activity by the brainstem and lower brain centers, however, the influence of higher brain centers, those located above the superior colliculus, on sympathetic nerve activity is less well understood. Some higher brain structures directly influence the activity of sympathetic preganglionic neurons and others modulate sympathetic nerve activity indirectly, via medullary relay centers. The purpose of this study was to test the effect of removal of all brain regions rostral to the superior colliculus on splanchnic sympathetic nerve activity (SNA) in male Spraque-Dawley rats. SNA was recorded in urethane anesthetized, pancuronium paralyzed, and ventilated rats before and after performing precollicular decerebration. Twenty minutes of sympathetic and hemodynamic recordings were quantified before and after decerebration in (i) intact, (ii) baroreceptor denervated, and (iii) chronically spinalized rats. Power spectral density and coherence analyses were used to quantify frequency and correlation as a function of frequency, in sympathetic and hemodynamic recording. In both intact and baroreceptor denervated animals, precollicular decerebration resulted in an immediate and dramatic increase in SNA of 126% ± 34% and 93% ± 38%, respectively. Decerebration induced increases in SNA were not observed in chronically spinalized rats. The effect of precollicular decerebration on SNA firing frequency and coherence between SNA and hemodynamic parameters is presented. These data suggest that supratentorial structures serve as a source of tonic descending inhibition of regional SNA and that this inhibition exists independently of central baroreflex processing. Pilot studies suggest the extent of tonic supratentorial inhibition varies across regional sympathetic beds (adrenal vs splanchnic vs lumbar).

P37 - Inhibitory control of motor and respiratory components of orienting by the substantia nigra pars reticulata is state-dependent

Theme: Basic - Integrative Control

Prof Simon McMullan[1], Stephanie Kennett[1], Thays Maria Vieira Costa[1], Anita Turner[1], Professor Andrew M Allen[2], Roger AL Dampney[3], Bowen Dempsey[1], Peter GR Burke[1]
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The ability to respond appropriately to salient environmental stimuli is vital to animal survival. The superior colliculus (SC) is a highly conserved midbrain structure that plays a key role in coordinating responses to external cues by integrating multisensory inputs and recruiting behavioural (e.g. orienting) and physiological outputs (e.g. changes to respiration, blood pressure). Tonic GABAergic drive is suggested to play an important role in modulating the excitability of the SC.

Here we investigate a potential role for GABAergic neurons in the substantia nigra pars reticulata (SNr) in controlling orienting behaviours in the rat. We selectively targeted SNr GABAergic neurons using a viral vector under the control of the GAD1 gene. Anterograde tracing of SNr^{GAD1} neurons revealed dense terminal fields within the deep SC, confirming innervation of regions known to mediate orienting responses, and the ventromedial and paracentral/centrolateral thalamic nuclei. No labelling was observed in the pons, medulla, cerebral cortex or striatum.

Inhibitory optogenetics was then used to examine whether tonic drive from SNr^{GAD1} neurons gates orienting-like behaviours. In awake rats, unilateral SNr inhibition evoked contralateral orienting accompanied by rapid and variable breathing behaviour, consistent with previously reported responses to optogenetic SC stimulation during wakefulness.

However, responses to SNr^{GAD1} inhibition were notably state-dependent. In quiet wakefulness, SNr^{GAD1} inhibition evoked motor and respiratory responses in >70% trials, falling to 3% during NREM sleep. Under urethane or isofluorane anaesthesia, SNr^{GAD1} inhibition did not evoke respiratory, motor or autonomic (sympathetic nerve /blood pressure) responses under baseline conditions, or unmask motor autonomic responses to visual, auditory or somatosensory stimuli.

We conclude that responses evoked by SNr^{GAD1} inhibition likely result from disinhibition of SC outputs, as results were consistent with previously reported responses to SC activation. The state-dependency of responses may reflect variable baseline activity of SNr neurons in these conditions.

P44 - Quantitative Analysis of CGRP-IR Afferent Axons in the Mouse Stomach Using Zeiss Arivis Vision4D for Automated Tracing

Theme: Basic - Neuroscience

Ms Duyen Nguyen[1], Mr Jazune Madas[1], Dr Jichao Ma[1], Ms Kayla Barton[1], Mr Andrew M Kwiat[1], Mr Kohlton T Bendowski[1], Dr Jin Chen[1], **Dr Zixi Jack Cheng**[1]
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Gastric nociceptive afferents detect and respond to noxious stimuli by transmitting signals from the stomach to the brain. Calcitonin gene-related peptide (CGRP) is a nociceptive marker and is crucial to gastric functions. However, the topographical quantitative analysis of CGRP-immunoreactive (CGRP-IR) axons in the whole stomach is challenging due to the large amount of axon innervation in various targets = within the tissue. Manual tracing of axons is time-consuming and extremely laborious. Here, we used Zeiss Arivis Vision4D, a novel and sophisticated software with a user-guided machine learning capability, for automated tracing of CGRP-IR axons in flat-mounts of the whole mouse stomach muscular layers and thick serial sections of the antrum-pylorus-duodenum (APD) region (C57BL/6J, n=7). We utilized a combination of techniques, including the Zeiss M2 Imager for automated scanning, Zen3.3 for image processing, and Vision4D for stitching the entire stomach flat-mounts and sections, 3D fiber tracing, and density analysis. Our results demonstrated that: 1) Vision4D detected and traced almost all CGRP-IR axons of different gastric targets at different regions. 2) The x-y-z tracing allowed the visualization of axons in space, portraying its continuous trajectory across layers in the flat mounts and cross sections. 3) Vision4D also enabled quantitative morphological and laminar analysis. The axon density heatmap generated from the tracing data showed high CGRP-IR axon innervation in the blood vessels. After the removal of blood vessels, dense innervation was seen in the upper fundus and antrum-pylorus. 4) Vision4D is a powerful tool in handling large data sizes (hundreds of gigabytes). 5) The tracing data will be integrated into a 3D stomach scaffold. This study represents, for the first time, the automatically traced CGRP-IR axons using whole stomach preparations as well as serial sections and highlights the potential of Vision4D in creating axon tracing models in other organs and species.

P45 - Automated 3D stereology for cell counting using artificial intelligence technology yields rapid, unbiased results analogous to manual stereological methods

Theme: Basic - Neuroscience

Mrs Alissa Wilson[1], Dr Jorge Castro[1], **Ms Maci Heal**[1], Dr Dan Peruzzi[1], Nicolas Roussel[1], Art DeLuc[1], Dr Brian Eastwood, Paul Angstman[1], Jack Glaser[1]
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With the advent of artificial intelligence, automated stereology is rising to the forefront of unbiased cell counting as it dramatically reduces the labor and expertise involved in manual cell counting. The manual aspects of stereology have been a barrier to wider utilization of design-based stereology, the gold standard for unbiased cell counting. Stereo Investigator- AI (SI-AI) utilizes convolutional neural networks to replicate expert human observer judgments in cell identification using trained classifiers developed to consider cell shape, size, internal structure, and location, dramatically accelerating the process of stereological cell counting. The trained classifiers identify cells in 3D volumes throughout anatomical regions using the same observer criteria as a human expert, resulting in accurate estimates of a cell population within regions of interest. This innovative technology makes stereology easier and faster to perform, without sacrificing the accuracy of the data.

Within the application workflow, 3D image volumes are analyzed using the Optical Fractionator probe combined with novel 3D detection methods to ensure accurate cell detection and unbiased population estimates. SI-AI can differentiate between different cell types, sub-cellular objects, and non-cell objects.

In our study, to accommodate varying cell densities in different brain regions, we trained deep learning classifiers on both dense and sparse populations, across varying imaging modalities, for accurate identification of the following fluorescent markers: DAPI, NeuN, Iba1, and Tyrosine Hydroxylase (TH).

Cell counts were validated in the mouse brain by comparing automated stereology results with data collected by experts trained in manual stereology methods. Population estimates, coefficients of error, false positive, false negative, and true positive detection rates were quantified and compared between cell counting methods and imaging modalities.

In conclusion, stereological results generated by SI-AI are unbiased, accurate, repeatable, and comparable to that of an expert.

P46 - On the mechanisms of exercise-induced autonomic neuroplasticity

Theme: Basic - Neuroscience

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The brain controls the heart by dynamic recruitment and withdrawal of cardiac parasympathetic (vagal) and sympathetic activity. Autonomic control is essential for the development of cardiovascular responses during exercise, however, the patterns of changes in the activity of the two autonomic limbs, and their functional interactions in orchestrating physiological responses during exercise, are not fully understood. The aim of this study was to characterize changes in vagal parasympathetic drive in response to exercise and exercise training by directly recording the electrical activity of vagal preganglionic neurons in experimental animals (rats). Single unit recordings were made using carbon-fibre microelectrodes from the populations of vagal preganglionic neurons of the nucleus ambiguus (NA) and the dorsal vagal motor nucleus of the brainstem. It was found that (i) vagal preganglionic neurons of the NA and the dorsal vagal motor nucleus are strongly activated during bouts of acute exercise, and (ii) exercise training markedly increases the resting activity of both populations of vagal preganglionic neurons and augments the excitatory responses of NA neurons during exercise. These data show that central vagal drive increases during exercise and provide the first direct neurophysiological evidence that exercise training increases vagal tone. The data argue against the notion of exercise-induced central vagal withdrawal during exercise. We propose that robust increases in the activity of vagal preganglionic neurons during bouts of exercise underlie activity-dependent plasticity, leading to higher resting vagal tone that confers multiple health benefits associated with regular exercise.

P47 - Neuroanatomy of the Thoracic Sympathetic Neural Networks revealed by whole-torso imaging

Theme: Basic - Neuroscience

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The deep-seated location of the sympathetic ganglia (SG) has been a barrier to its comprehensive molecular analysis at a systemic level. Nonetheless, recent breakthroughs in tissue-clearing techniques and light-sheet microscopy have enabled the visualisation of large intact specimens. By integrating an innovative dissection method with cutting-edge tissue clearing and light-sheet microscopy, we have generated a realistic neuroanatomical blueprint of the sympathetic nervous system (SNS), a field largely reliant on broad and subjective descriptions from studies on larger animals. Our investigation unveils numerous fresh anatomical revelations, unveiling novel configurations of ganglionic arrangement and neural projections that challenge conventional understandings of autonomic regulation. Notably, our observations include an unexpected fusion of the stellate (St) and T2 ganglia on the left side, absent on the contralateral side, implying a lateralised control over cardiac functions. Additionally, we have identified local rami emanating from the ganglia to innervate the neighbouring paravertebral brown adipose tissue (pBAT), supplementing the traditional grey and white rami. Furthermore, we have uncovered supernumerary and accessory ganglia at T5-T8 levels, forming ganglionic plexi. Within these plexi, we have observed bypassing rami that circumvent ganglia, potentially indicating alternative neural pathways. Moreover, we have noted the presence of sympathetic fibres entering the dorsal root ganglia (DRG) via the grey ramus at each vertebral level, persisting towards target organs. These findings remain consistent across multiple mice (N=5). This neuroanatomical investigation enhances our comprehension of autonomic architecture through meticulous mapping, shedding light on its organisation as a neural network not previously elucidated in standard textbooks.

P54 - Ergogenic effects of invasive and non-invasive spinal cord stimulation strategies following spinal cord injury: a case-series

Theme: Clinical - Cardiovascular

Mr Daniel D Hodgkiss[1], Miss Alison MM Williams[2,3], Dr Claire S Shackleton[2,4], Dr Soshi Samejima[2,5], Dr Shane JT Balthazaar[1,2,6], Dr Tania Lam[2,3], Dr Andrei V Krassioukov[2,4,7], Dr Tom E Nightingale[1,2]
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Cervical or upper-thoracic spinal cord injury (SCI, ≥T6) often leads to low resting blood pressure (BP) and impaired cardiovascular responses to acute exercise due to disrupted supraspinal sympathetic drive. Epidural (implanted and invasive; ESCS) and transcutaneous spinal cord stimulation (skin surface and non-invasive; TSCS) have previously been used to target dormant sympathetic circuits below the lesion level and modulate cardiovascular responses [1-3]. This case-series compared the effects of cardiovascular-optimised ESCS and TSCS versus sham ESCS and TSCS on modulating cardiovascular responses and improving submaximal upper-body exercise performance in individuals with SCI. Seven males with a chronic, motor-complete SCI between C6-T4 underwent a mapping session to identify cardiovascular responses to spinal cord stimulation. Subsequently, four participants (two ESCS and two TSCS) completed submaximal exercise testing. Stimulation parameters (waveform, frequency, intensity, epidural electrode array configuration, transcutaneous electrode locations in the lumbosacral region) were optimized to elevate cardiovascular responses (CV-SCS). A sham condition (SHAM-SCS) served as a comparison. Participants performed arm-crank exercise to fatigue at a fixed workload corresponding to above ventilatory threshold, on separate days, with CV-SCS or SHAM-SCS. At rest, CV-SCS increased BP, left ventricular cardiac contractility and total peripheral resistance. During exercise, CV-SCS increased time to fatigue and peak oxygen pulse (a surrogate for stroke volume), relative to SHAM-SCS. Ratings of perceived exertion also tended to be lower with CV-SCS than SHAM-SCS. Comparable improvements in time to fatigue with ESCS and TSCS suggest that both approaches could be promising ergogenic aids to support exercise performance or rehabilitation, along with reducing fatigue during activities of daily living in individuals with SCI.

P55 - Cardiac vagal modulation and inflammation are upregulated in exceptional human longevity

Theme: Clinical - Cardiovascular

Dr. Gabriel Rodrigues[1,2], Dr Domenico Azzolino[3], Dr Valentina Manzini[1], Prof Marco Proietti[1,3], Dr Angelica Carandina[1], Dr Costanza Scatà[1], Dr Chiara Bellocchi[1,3], Prof Eleonora Tobaldini[1,3], Dr Evelyn Ferri[2], Prof Beatrice Arosio[1,3], Prof Matteo Cesari[1], Prof Nicola Montano[1,3]
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Exceptional human longevity highlights an extraordinary adaptive capacity to maintain homeostasis during lifespan. Persons with extreme longevity (PEL) have a molecular setup that may counteract the harmful effects of 'inflammaging'. The sympatho-vagal balance increases with aging, but, above 80 years old, a 'U' shape relationship was observed, showing a possible restoration of vagal modulation. Recently, vagal stimulation was directly related to a reduction in inflammation, described by the "inflammatory reflex". Thus, this preliminary study was designed to investigate the association of cardiovascular autonomic and inflammatory profiles in persons with extreme longevity (PEL), their direct offsprings (DO), and a group of controls matched for age and sex with the DO. We enrolled 30 participants recruited in the community (10 PEL, 99 ±2 yrs; 10 DO, 65 ±2 yrs; and 10 controls 63 ±10 yrs [i.e., community-dwelling volunteers matched with DO by age and sex]). Cardiac autonomic control was assessed through the analysis of heart rate variability (HRV) using spectral and symbolic analysis. The plasma concentration and gene expression of interleukin (IL)-10, IL-6, and TNF-α were quantified. In PEL, the sympatho-vagal is shifted to a vagal predominance, and both pro- and anti-inflammatory circulation cytokines are increased compared to DO and controls ($p<0.01$). No differences were found in HRV between DO and controls. Lastly, the cardiac vagal modulation (2UV%) was positively correlated with anti-inflammatory (IL-10; $r=0.72$; $r^2=0.52$; $p<0.01$) in PEL. Our preliminary results suggest that environmental factors, at least in our small sample, may overcome the impact of heritability on cardiac autonomic control. Finally, the positive correlation between cardiac vagal control and anti-inflammatory indexes highlights the interplay between the autonomic nervous system and inflammation in PEL. Key-words: Inflammatory reflex; Inflammaging; heart rate variability.

P64 - What is the most effective treatment for congenital Long QT syndrome? (An undergraduate review)

Theme: Clinical - Cardiovascular

Sakshi Gupta, Medical Student, University of Birmingham

Long QT syndrome is an inherited cardiac channelopathy associated with a poor prognosis. It is characterised by an abnormally extended QT interval, with some patients experiencing symptoms such as syncope. There are several subsets which can lead to dangerous arrhythmias and cardiac arrest. Fortunately, the risk of these outcomes can be significantly reduced with effective treatment. The first line treatment is usually pharmacological, including beta blockers, which slow down the activity of the heart. However, in cases of persistent symptoms, other treatments may be used. These include implantable devices, specifically pacemakers and implantable cardioverter defibrillators or surgery, namely Left Cardiac Sympathetic Denervation. Careful clinical evaluation of each patient is critical in deciding on the most appropriate course of action. Due to the rare nature of this condition, the pool of research examining the difference in successful outcomes using the mentioned treatments consists mainly of retrospective cohort and case studies. The aim of this research project is to use the available evidence to form conclusions on the most effective care for patients in avoiding sudden cardiac death due to Long QT syndrome. Newer developments and a shift towards personalised medicine has also opened other avenues for tailored treatment using genotypic testing, although the effects of this in comparison to the 'one size fits all approach' remains unclear. This suggests that although several advances in research have been made, further understanding and implementation of findings into practice is required to optimise treatment.

Keywords: Long QT Syndrome, Beta blockers, Implantable cardioverter defibrillator, Left Cardiac Sympathetic Denervation, Precision therapies

P65 - Examining sex differences in autonomic function and its influence on cardiovascular disease prevalence in women; particularly stress induced cardiomyopathy (An undergraduate review)

Theme: Clinical - Cardiovascular

Amna Nazzar, Medicine student, University of Birmingham

Cardiovascular disease is one of the leading causes of death in post-menopausal women. Takotsubo Syndrome (TTS), where a stressor causes a catecholamine rush resulting in ventricular dysfunction of the heart, affects a demographic of 80-90% post-menopausal women. This literature review examines sex differences in autonomic function and the influence of hormones, theorized to be implicated in the increased risk of cardiovascular disease; specifically, the loss of oestrogen and its cardioprotective effects in post-menopausal women. These factors are further considered by differences in menstrual cycle phases, and the effects of a lack of oestrogen on autonomic and cardiac functioning are also described. The loss of oestrogen observed in post-menopausal women has been shown to increase sympathetic drive and reduce cardioprotective effects. This may be implicated in the development of TTS or other cardiovascular disease. Oestrogen exhibits positive effects in reducing endothelial dysfunction, which is one of the hallmarks of TTS and is implicated in its pathophysiology. Sexual dimorphism in the expression of certain receptors in the brain has also been observed. Additionally, sex differences in expression of oestrogen receptors and the stress modulation response have been noted. HRT and other measures like mind-body interventions were evaluated as possible preventative treatments. An interesting finding was the incidence of increased complications for male TTS patients, providing another area for further research. There continues to be a lack of research into the underlying causes of increased cardiovascular disease in women. Future research into sex differences in autonomic functioning as well as extensive investigations into the role of oestrogen and the effects of its loss is required. Subsequently, research to design preventative measures that reduce cardiovascular risk in women is also necessary. TTS further requires investigation into the complications experienced by male patients and any preventative or treatment measures available should be assessed.

This work has been kindly supported by the Arthur Thomson Trust.

P66 - The use of selective noradrenaline reuptake inhibitors and serotonin-noradrenaline reuptake inhibitors to treat psychiatric disorders as a cause of Takotsubo Syndrome (An undergraduate review)

Theme: Clinical - Cardiovascular

Georgia Pratley, Medical student, University of Birmingham

Background: Takotsubo Syndrome (TS) has been long thought of as a spontaneous event in the event of abnormally heightened emotion. However, new research suggests that there is an underlying pathophysiology. Catecholamine theory describes the increased presence of catecholamines in these moments leads to the detrimental changes to the left ventricular wall which occur within TS. It seems that this heightened state can be artificially stimulated, thereby inducing TS.

Hypothesis/Aims/Purpose: This phenomenon can be seen in patients with psychiatric disorders, such as depression, who are taking pharmaceutical medications as treatment. This narrative review aimed to identify possible treatments that are associated with an increased risk of TS, propose a mechanism for this, and subsequently provide clinical implications.

Methods: I firstly researched the pathophysiology of TS, with a specific focus on the catecholamine theory. Selective noradrenaline reuptake inhibitors and serotonin-noradrenaline reuptake inhibitors were linked with an increased prevalence of TS in a variety of case studies and reports. Combining research surrounding the mechanisms of these drugs, along with the catecholamine theory of TS, provided a plausible explanation.

Results: 16 cases of TS associated with serotonin-noradrenaline reuptake inhibitors and 4 cases associated with selective noradrenaline reuptake inhibitors were identified. Both medications act to increase the plasma circulating volume of noradrenaline, subsequently providing the trigger for left ventricular dysfunction

Conclusions: Overall, whilst more research is required, the use of selective noradrenaline reuptake inhibitors and serotonin-noradrenaline reuptake inhibitors as the predisposing factor to the development of TS is a likely outcome. This provides direct support for the catecholamine theory of TS. It also provides important clinical implications. Healthcare professionals should be aware of this when prescribing a patient these medications (particularly in those with other cardiac risk factors) and should be aware of this when a patient presents with cardiac symptoms.

P61 - Thoracic sympathetic nerve block before sympathectomy for irreversible treatment of primary hyperhidrosis

Theme: Clinical – Neuroscience

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Background: Sympathectomy, one of the treatments for primary hyperhidrosis, can lead to a potentially irreversible complication, compensatory hyperhidrosis, which can significantly impact patient satisfaction. Therefore, to enhance overall treatment satisfaction, thoracoscopic sympathetic nerve block before sympathectomy can be performed.

Aim: The objective of this study is to review our recent experience with the nerve block procedure and assess patient satisfaction through our institution's treatment.

Methods: From March 2021 to August 2023, medical records of 52 patients who underwent thoracoscopic sympathetic nerve block before sympathectomy for primary palmar and craniofacial hyperhidrosis were retrospectively analyzed. Thoracoscopic sympathetic blocks are performed by injecting local analgesics through a 2-mm thoracoscope.

Results: The mean age of the 52 patients was 26.06 ± 9.0 years, with 31 males (59.62%). Ten patients (19.23%) had craniofacial hyperhidrosis, while 43 (82.69%) had palmar sweating, and 39 (75.0%) had symptoms in their feet. The mean procedure time of thoracoscopic sympathetic nerve block was 23.04 ± 8.05 minutes, with three patients (5.77%) undergoing the procedure only on the left sympathetic nerve. The average increase in temperature measured at the left index fingertip before and after the procedure was 3.61 ± 2.48 degrees Celsius. Relief of sweating symptoms was confirmed for an average of 1.02 days, with complications including pneumothorax in 2 patients (3.85%) and transient ptosis in 1 patient (1.92%). Compensatory hyperhidrosis occurred in 27 patients (51.92%) overall. Thirty-five patients (67.31%) ultimately underwent sympathectomy, among whom compensatory hyperhidrosis occurred in 18 individuals (51.43%). However, they exhibited a mean satisfaction score of 97.73 ± 19.41 out of 100.

Conclusions: Our recent experience indicates that thoracoscopic sympathetic nerve block performed before sympathectomy showed high satisfaction rates for irreversible treatment of primary hyperhidrosis. Further comparative studies are needed to establish conclusive evidence.

Keywords: hyperhidrosis, sympathectomy, complication, compensatory hyperhidrosis, sympathetic nerve block

Poster Session 3

P4 - Three-Dimensional Reconstruction of Renal Tissue: Mapping Renal Nerve Trajectories

Theme: Basic - Bioelectronic Medicine

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The autonomic system is hypothesized to have a role in the progression and pathogenesis of hypertension. Recent therapeutic strategies show that catheter-based renal denervation (RDN) successfully lowers arterial pressure in hypertensive patients by interfering with the brain-kidney link, reducing central sympathetic outflow. Consequently, there is a growing interest in understanding the anatomy and physiology of renal nerves. While previous studies have predominantly focused on quantifying nerve distribution around the renal artery, the three-dimensional (3D) spatial organization of renal nerves and surrounding tissue is often overlooked. To address this gap, we integrated histological analysis with microCT imaging from human, non-human primate, and pig samples to create comprehensive 3D reconstructions of the renal nerves in each species. These reconstructions provide visual insights and precise spatial mapping of nerves within the renal tissue, spanning from the descending aorta to the vessel bifurcation at the kidney. In the non-human primate reconstruction, for instance, the analysis revealed the presence of major nerve branches near the descending aorta and converging at the middle of the renal artery before branching into the kidney. The nerve counts were greatest proximally (46.34%) and declined gradually distally (middle 31.71%; distal 21.95%). The resulting 3D anatomical models provide foundational data for developing computational models aimed at optimizing renal nerve targeting. By combining species-specific models with diverse electrode designs and stimulation parameters, our approach facilitates the assessment of how design variables influence thresholds for electrical activation and blockade of renal nerves.

P19 - Identification of Spinal Afferent Innervation in the Rat Heart: Atria and Ventricles: Anterograde Tracing

Theme: Basic - Cardiovascular

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The spinal afferent innervation of the heart regulates cardiac functions by sending sensory information from the dorsal root ganglia (DRG) to the brain. However, the distribution and morphology of spinal afferents in the whole heart are not well characterized. The challenges here are: 1) difficulty in surgically accessing the upper thoracic DRG to label only cardiac spinal afferents. 2) Handling flat-mounts of whole atria and ventricles (thickness ~850 μm). To overcome these challenges, we injected anterograde tracer dextran biotin (DB) into the left DRG (C8-T3) of male SD rats and allowed them to recover for 14 days for tracer transportation. Flat-mounts of the atria and ventricles were prepared, and DAB stained, followed by imaging, tracing, and digitization using the Neurolucida system. Our findings are: In the atria, the DB-labeled axons entered the atria potentially through the left precaval vein, with predominant innervation of the left side of the atrial wall and extended their projection towards the auricles, middle region of the atrium, and pulmonary veins. Most of the spinal afferent axons were present in the myocardial layer. Varicose DB-labeled axons were also observed on cardiac targets at different regions, and distinct morphological structures were found. In the cardiac muscle, axons ran mainly in the direction of muscle fibers. Also, varicose axons ran along the small vasculature on the atria. Spinal afferents formed varicose contacts with individual principal neurons within some intrinsic cardiac ganglia. In the ventricles, DB-labeled axons ramified and expressed spherical-like varicosities on the cardiac muscle. In both atria and ventricles, spinal afferent axons formed simple, branching, and complex terminal structures. Thus, our work, for the first time, successfully labeled, traced, and digitized the spinal cardiac afferents in the whole atria and ventricles, which will provide a foundation for specific labeling of cardiac spinal afferent fibers.

P20 - Autonomic mechanisms of disturbed circadian rhythm in the diabetic heart

Theme: Basic - Cardiovascular

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The healthy heart displays a circadian rhythm in which heart rate and blood pressure decrease overnight. However, this rhythm is blunted, absent or even reversed in the diabetic heart, which is a crucial risk factor for the development of cardiovascular disease. The origin of the circadian rhythm in the heart and its dysregulation, has not yet been fully elucidated, but changes in autonomic neural control have been implicated.

We investigated autonomic regulation in 20-week old male Zucker type 2 Diabetic Fatty rats (DM) and their non-diabetic littermates (ND) at two timepoints, the start of the inactive and active periods (Zeitgeber times 3 and 15, respectively). We found a circadian rhythm in cardiac sensitivity to sympathetic stimulation (noradrenaline) in Langendorff-isolated hearts, in contrast to existing theories of parasympathetic regulation. However, in DM hearts, parasympathetic responsiveness (acetylcholine) was lower compared to ND, alongside potential attenuation of circadian rhythms in parasympathetic relaxation responses. While there was no significant difference in the protein expression of cardiac beta-adrenergic (β1, β2) or muscarinic (M2) autonomic receptors, circadian rhythms were observed in the expression of calcium handling proteins (SERCA2a, PLB), which were higher during the active period (ZT15). There was also a significantly lower expression CLOCK protein in the DM sinoatrial node. Further, we observed greater activation of sympathoregulatory brain regions (NTS, RVLM), which may contribute to sympathetic overactivation as well as disrupted circadian rhythms in DM. Overall, physiological circadian signalling was primarily associated with sympathetic regulation, while parasympathetic dysregulation in diabetes might signal an underestimated therapeutic target.

Funding: Heart Foundation of New Zealand.

P21 - Cardiovascular variability and baroreflex function are altered in rats with femoral artery catheterization

Theme: Basic - Cardiovascular

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Measurement of arterial pressure (AP) by catheterization of femoral artery is a routine procedure in cardiovascular studies performed in experimental animals. However, tissue ischemia due to artery obstruction and the inflammatory response in ischemic tissue may lead to changes in autonomic cardiovascular control, bringing consequences for interpreting data. Nevertheless, a non-occlusive catheterization of abdominal aorta is an alternative approach to direct record AP in experimental rats. We compared hemodynamics, variability of heart rate and AP and also baroreflex function in rats with catheters into femoral artery and in those with non-occlusive catheterization of abdominal aorta. Wistar rats were instrumented with polyethylene catheters into the femoral artery (N=10) or non-occlusive polyethylene catheters into the abdominal aorta (N=13). After the proper recovery time, conscious, freely moving rats had their AP recorded for 30 min. The AP recordings were processed to generate series of successive values of systolic AP and pulse intervals (PI). Rats with femoral catheters showed higher AP (116±4 mmHg) than rats with non-occlusive aortic catheterization (103±3 mmHg). Heart rate was similar between groups. Time and frequency domain indices of PI were not different in both groups. However, rats with femoral catheters showed higher standard deviation of AP and higher power of AP spectra (5.2±0.4 mmHg and 6.9±1.0 mmHg2) as compared to counterparts with non-occlusive aortic catheterization (4.2±0.4 mmHg and 3.2±0.5 mmHg2). Multiscale sample entropy was not different between groups. Nevertheless, the gain of spontaneous baroreflex, assessed by the sequence method, was lower in rats with femoral catheters (1.49±0.2 vs 1.10±0.2 ms/mmHg). These results show that catheterization of femoral artery does exert a significant influence on AP and its variability indices as well as on baroreflex function. Despite requiring major surgery, abdominal aortic catheterization is a good approach for AP recording in studies aiming to assess cardiovascular neural modulation.

P22 - Distinct autonomic effects of single and intermittent chlorpyrifos exposure in the contextual fear conditioning test in rats

Theme: Basic - Cardiovascular

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Repeated and acute exposure to organophosphorus (OP) compounds may lead to widespread health issues, including neurological, psychiatric, and cardiovascular abnormalities. Of note, chlorpyrifos (CPF), given every other day to adult rats, impairs spatial memory and prepulse inhibition associated with brain AChE inhibition. Our group showed that intermittent treatment with CPF, simulating occupational exposure, impairs the cardiorespiratory reflexes and causes cardiac hypertrophy. This study investigates the impact of acute or repeated intermittent exposure to CPF on autonomic and behavioral responses triggered by the contextual fear conditioning (CFC) test in adult male Wistar rats (Ethical approval nº 38/2018 and nº 04/2022). In protocol 1, rats received intraperitoneal injections of saline or CPF at doses of 4 (CPF4) and 7 mg/kg (CPF7), three times a week for 4 weeks, followed by the CFC test. In protocol 2, animals underwent a conditioning fear session (3 footshocks 0.75 mA) followed by treatment with a single dose of saline or CPF at 20 mg/kg (CPF20). Subsequently, all rats suffered surgery for femoral arterial cannulation to monitor mean arterial pressure (MAP) and heart rate (HR). Fear extinction sessions (21 min) were conducted 48 hours after conditioning to assess freezing behavior and hemodynamic responses under no footshock. Independent rat cohorts were euthanized 48 h post-CPF treatment to measure acetylcholinesterase (AChE) activity in the hippocampus and prefrontal cortex. Intermittent CPF7 treatment did not affect MAP but enhanced HR response whereas acute CPF20 exposure increased MAP and impaired HR response (2-way ANOVA). However, neither CPF treatment affected freezing levels over time. Both treatments decreased AChE activity in the prefrontal cortex and the hippocampus. Our findings suggest that intermittent and acute CPF exposures differently impair autonomic responses to CFC.
Funding: Fapes/PROAPEM, Grant code 2022-78KWB; Capes Foundation Scholarship (Grant Code 01).
Keywords: organophosphate; fear; autonomic; chlorpyrifos.

P23 - High-resolution ex-vivo structural and functional analysis of sympathetic innervation using a novel confocal fluorescence technique

Theme: Basic - Cardiovascular

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Neurotransmitter Transporter Uptake Assay (NTUA, Molecular Devices) is a fluorescent sympathomimetic that accumulates in sympathetic terminals by uptake via the noradrenaline transporter (NAT). We have utilised NTUA to visualise sympathetic innervation ex-vivo by confocal microscopy with single-terminal resolution. As proof-of-principle, we applied this technique to investigate inter-tissue differences in sympathetic innervation structure and NAT kinetics, as well as examine sympathetic remodelling in the heart in response to chronic intermittent hypoxia (CIH).
Tissues were obtained from male adult Wistar rats (200-300g) under non-recovery terminal inhalation isoflurane (3-5% in O2, 1.5L min-1). To investigate sympathetic nerve remodelling in response to CIH, left atrial appendages (LAAs) were obtained from normoxic rats (N; n=4) and CIH (FiO2=0.06-0.21, 15 cycles hour-1, 8 hours day-1, 21-24 days; n=3). In an additional group of N rats, LAAs (n=9) and vasa deferentia (VD; n=6) were excised to investigate inter-tissue differences in sympathetic innervation. Tissues were pinned flat, transferred to a confocal microscope and superfused with NTUA. Image stacks were recorded over 15 minutes of NTUA delivery. Innervation structure and NAT kinetics were quantified using FIJI (v1.53).

VD and LAA tissues showed similar sympathetic innervation structure in terms of overall terminal density, terminals per nerve fibre length, overall nerve fibre length and nerve branch density ($p>0.05$).

In the LAA, CIH exposure was associated with a significant decrease in overall terminal density ($p=0.014$), as well as a modest but statistically insignificant decrease in terminals per nerve fibre length ($p=0.072$), overall nerve fibre length ($p=0.078$) and nerve branch density ($p=0.10$).
NTUA uptake rates were not statistically different between the VD and LAA ($p=0.24$), nor following CIH exposure in the LAA ($p=0.73$).

Overall, this study provides a basis for application of the NTUA assay for rapid, ex-vivo investigation of sympathetic innervation. This may represent a useful tool for studying sympathetic remodelling.

P24 - Neuro-glial interaction in the heart

Theme: Basic - Cardiovascular

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The autonomic nervous system tightly regulates cardiac function in health and disease. Glia cells are indispensable for normal neuronal function in the central and peripheral nervous system, but the diversity and roles of glia in the heart are vastly understudied.

Using transgenic mouse lines under the control of different glia-specific promoters, as well as human and murine cardiac tissue, we characterized glial cells of the heart and its autonomic structures in mice and men using immunohistochemistry, gene expression analysis and ex vivo Langendorff studies.

Glial cells with different morphologies and protein expression accompany cardiac autonomic innervation on all levels. They are associated with endo- and epicardial nerve fibers, mirroring their distribution throughout the left and right ventricular myocardium. A corresponding low expression of typical glial markers can be detected. Glia react to cardiac nerve damage with release of the neurotrophic factor S100B.

Different types of glial cells are present throughout the heart and their role is just beginning to emerge. Whether and how they contribute to autonomic regulation or even regeneration upon cardiac nerve damage needs to be determined.

P38 - Integrated Dashboard for large-scale visualization of the anatomical connectivity of the human Vagus Nerve

Theme: Basic - Integrative Control

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The NIH SPARC-REVA program funds the development of high-quality datasets mapping the anatomical connectivity of the human Vagus Nerve and the physiological outcomes of modulating its activity. The SPARC Data Resource Center is developing scalable solutions to allow the larger research community to interact with these datasets through novel dashboard functionality within the SPARC Portal. It is designed to serve as an interface for navigating the SPARC public datasets, focusing on the anatomy of the Vagus nerve. The dashboard integrates various filters and mapped representations of the Vagus nerve, so that users can meaningfully explore the large collection of REVA's Vagus nerve images.

The effort allows users to create customized dashboards that empower researchers to tailor their data exploration and analysis processes to their specific needs. Key features include the ability to select anatomic locations within the Vagus nerve and seamlessly browse through corresponding images from the REVA dataset, filter these images by modality, and access annotated regions complete with labels, where available. The goal of the dashboard is not just to provide access to data, but to display the data in a intuitive, accessible, and responsive way. The dynamic loading, intuitive navigation, and fluid display of imaging data will cater to the user's specific investigational focus within the Vagus nerve's anatomy.

The Vagus Atlas Dashboard aims to significantly enhance user engagement and data accessibility. Through continuous user feedback and the integration of additional data as it becomes available, the platform is expected to evolve, further refining its capabilities and user interface to maximize its utility for scientific discovery and advancements toward optimally leveraging the Vagus nerve as a conduit for biomedical device strategies.

Funding Information: This project is supported by NIH SPARC project, under award number OT3OD025357.

P39 - Mapping the Vagus Nerve with Anatomical Scaffolds

Theme: Basic - Integrative Control

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Phase 2 of the Stimulating Peripheral Activity to Relieve Conditions (SPARC) program aims to build a comprehensive map of the anatomy and functional connectivity of the human vagus nerve, allowing comparisons of inter-subject variability, and helping guide experiments on vagus nerve stimulation and the development of neuromodulation devices.

Following approaches used in Phase 1 of the SPARC program, segmented nerve and fascicle data (using various imaging modalities) of vagus nerves from a large number of human subjects will be registered into an Anatomical Scaffold. This is a geometric model of the vagus nerve providing a common coordinate system across subjects. Registration is achieved by fitting a subject-specific vagus scaffold to digitized subject data, using standardized annotations for trunks, branches, fixed landmarks and orientation marks in the data and for the corresponding features of the Scaffold.

We report here on progress and issues in the early stages of the study. A particular complication of the vagus is inter-subject variability. While the left and right vagus trunk are quite consistent across subjects, the number and locations of branches innervating a particular organ or target is quite different from one subject to another. This dictates generating a scaffold for each subject with its own unique branching structure. Once data is available for a reasonable number of subjects these will be remapped to a common or representative vagus scaffold.

At the conclusion of this study all vagus imaging, plus derived data including the scaffold and embedded fascicles, their groupings and distributions will be freely available on the SPARC Portal. Mapping to a common scaffold allows these data to be compared and visualized at equivalent locations across all subjects in the study.

This work is supported by the NIH SPARC program under award number OT3OD025347.

P40 - Respiratory pattern and responses to hypercapnia of adenosine A2A knockout mice submitted to sustained hypoxia

Theme: Basic - Integrative Control

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There is evidence that adenosine A2A receptors modulate synaptic transmission in the neural pathways of cardiovascular and respiratory reflexes. We documented that adenosine A2A receptors knockout (KOA2A) mice submitted to normoxia or sustained hypoxia (SH) presented changes in the baseline ventilatory parameters, indicating a role for these receptors in the generating breathing. Herein, we evaluated the baseline respiratory pattern after SH and the respiratory responses to hypercapnia of the in situ working heart-brainstem preparation (WHBP) of Balb/C (WT) and KOA2A mice. WT and KOA2A mice (6-8 weeks old) were exposed to normoxia (control) or SH protocol (24h, FiO2 0.1). Using the WHBP we recorded the activities of phrenic (PN), abdominal (AbN), sympathetic (tSNA) and cervical vagus (cVN) nerves. Percentage of CO2 in the perfusate was increased from 5% (baseline) to 7% and then 10%. Experimental protocols were approved by the institutional ethical committee (#076/2021). In normocapnia (5% CO2), the frequency of PN was significantly higher in the KOA2A (n=8) than in WT (n=9) mice (2.05±0.45 vs 0.28±0.04 Hz, P<0.001). At 7% CO2, the frequency of PN was also higher in KOA2A than in WT mice (1.35±0.94 vs 0.31±0.06 Hz, P= 0.04). The incidence of AbN active expiration of KOA2A group (n=6) was significantly lower in both hypercapnic challenges [(7%: 6.1±3.1 vs 52.3±4.2 %, P=0.03) (10%: 15.7±6.7 vs 61.4±12.3 %, P=0.01)] than in WT group (n=8). However, the incidence of Late-E events in AbN activity under baseline conditions (5% CO2) in the KOA2A group submitted to SH was similar to that observed in the KOA2A exposed to normoxia. These findings provide additional support for the concept that adenosine acting on A2A receptors plays an important role in modulating the baseline respiratory frequency and in generating active expiration in response to hypercapnia.

P48 - Differential developmental blueprints of organ-intrinsic nervous systems

Theme: Basic - Neuroscience

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The organ-intrinsic nervous system is a major interface between visceral organs and the brain, mediating important sensory and regulatory functions in the body-brain axis and serving as critical local processors for organ homeostasis. Molecularly, anatomically, and functionally, organ-intrinsic neurons are highly specialized for their host organs. However, the underlying mechanism that drives this specialization is largely unknown. Here, we describe the differential strategies utilized to achieve organ-specific organization between the enteric nervous system (ENS) and the intrinsic cardiac nervous system (ICNS), a neuronal network essential for heart performance but poorly characterized. Integrating high-resolution whole-embryo imaging, single-cell genomics, spatial transcriptomics, proteomics, and bioinformatics, we uncover that unlike the ENS which is highly mobile and colonizes the entire gastrointestinal (GI) tract, the ICNS uses a rich set of extracellular matrix (ECM) genes that match with surrounding heart cells and an intermediate dedicated neuronal progenitor state to stabilize itself for a 'beads-on-the-necklace' organization on heart atria. While ICNS- and ENS-precursors are genetically similar, their differentiation paths are influenced by their host-organs, leading to distinct mature neuron types. Co-culturing ENS-precursors with heart cells shifts their identity towards the ICNS and induces the expression of heart-matching ECM genes. Our cross-organ study thus reveals fundamental principles for the maturation and specialization of organ-intrinsic neurons.

P49 - Mechanisms underlying long-term facilitation in the carotid body

Theme: Basic - Neuroscience

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Glutamate and γ-aminobutyric acid (GABA) are major modulators of excitatory and inhibitory transmission in the mammalian brain, respectively. Glutamate transmission is critical for neural plasticity, learning and memory, whereas GABA plays a fundamental role in regulating neuronal excitability and facilitating the generation of neural oscillations. Our data indicate both glutamate and GABA release in the carotid body (CB) modulates its sensitivity to hypoxia-evoked response. We hypothesised that glutamate mediates long-term facilitation (LTF) of CB afferent activity.

We mined high-throughput RNA sequence data and used immunohistochemistry to map components underlying neuroplasticity in the CB of Wistar rats. CSN discharge was measured as a functional readout of CB function from an ex vivo arterially perfused carotid artery-CB preparation.

We found N-methyl-D-aspartic acid receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR) and transporters to be localised to CB chemosensory cells. Targeted administration of glutamate (15 mM) activated the carotid sinus nerve ($p=0.003$). Repetitive [5 times, 2-minute duration, 5-minute intervals] application of glutamate evoked LTF of CB afferent discharge as demonstrated by both a 12-fold increase in basal firing frequency (0.78 ± 0.37 vs 1232 ± 596 spikes.s-1; $p=0.004$) over 60 minutes ($p=0.004$) and sensitisation of the evoked response to histotoxic hypoxia (CN-; 1.23 µmol bolus) by 2-fold ($p=0.009$). The stimulation paradigm was specific as neither application of glutamate, 5 times for 1-minute at 5-minute intervals, nor a single glutamate exposure evoked LTF. In contrast, repeated application of 15 mM GABA [3 times, 1-minute duration, 5-minute intervals] evoked long-term depression (LTD) of CB afferent activity (from 10.5 to 3.5 spikes.s-1 $p<0.001$) and a 2-fold suppression of the response to CN- ($p<0.001$).

It is conceivable that processes such as LTP and LTD operate in the CB and may modulate the set point of peripheral chemoreceptor sensitivity and its state-dependent tonicity.

HRC_NZ and Sidney Taylor Trust funded research.

P50 - Blockade of CCR2 receptors in the brain prevents hypertension in renovascular hypertensive rats

Theme: Basic - Neuroscience

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In cases of neurogenic hypertension, active macrophages migrate to the par
aventricular nucleus of the hypothalamus (PVN)[1]. The activation of the C-C motif chemokine receptor type 2 (CCR2) plays a role in both the activation and recruitment of macrophages and lymphocytes[2]. Blocking CCR2 receptors has been shown to decrease blood pressure (BP) in rodent models of hypertension[3]. However, whether blocking CCR2 receptors in the brain can reduce BP in hypertension is not known. Here, we tested whether blocking brain CCR2 receptors can lower BP in renovascular hypertensive rats.

The 2-kidney-1-clip model of renovascular hypertension was employed. Following renal artery clipping, rats received continuous intracerebroventricular infusion of a specific CCR2 antagonist (RS-102895; 70 µmol/L) via an osmotic minipump (2.5 µl/hour) for 8 weeks while BP was monitored using radio-telemetry.

Antagonizing brain CCR2 receptors prevented BP elevation and macrophage accumulation in the PVN. Furthermore, it notably decreased the number of activated microglia and neurons in the treated rats' brains. Conversely, administering CCL2 (71 nmol/L) into the lateral cerebral ventricle of naïve rats led to macrophage recruitment in the PVN and increased BP.

These findings suggest that in renovascular hypertension, blocking brain CCR2 receptors prevents macrophage recruitment and activation of microglia and neurons in the PVN, ultimately reducing BP. Therefore, macrophage recruitment in the PVN appears to be crucial in the development of renovascular hypertension.

P56 - Autonomic Impairment in Parkinson's Disease and Multiple System Atrophy Patients during ValsalvaManeuver

Theme: Clinical - Cardiovascular

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Introduction: Alpha-synucleinopathies, such as Parkinson's disease (PD) and Multiple System Atrophy (MSA), are known for their motor symptoms. However, recent studies have shown that dysautonomic symptoms can appear even decades before and often include autonomic failure. This
study aims to characterize the cardiovascular response to the Valsalva maneuver (VM) in PD and MSA patients.

Materials & Methods: A cohort of 28 patients with PD, PD with orthostatic hypotension (OH), MSA, and healthy subjects (n=9, 10, 4, 5 respectively) were monitored during VM, continuously
non-invasive blood pressure (BP) and ECG were recorded at 200Hz for the entire duration of the maneuver. We analyzed the systolic blood pressure (SBP) and RR intervals series by means of the multi-level uniform phase empirical mode decomposition (UPEMD), an extension of the empirical
mode decomposition, the spectral analysis of the obtained intrinsic mode functions (IMFs) is used to evaluate autonomic nervous system (ANS) response. We also calculated standard indices such as the Valsalva ratio, overshoot, and recovery in the II and IV phases.

Results: MSA and PD OH patients have attenuated values of overshoot and recovery, indicating an altered neurogenic vasomotor response, disease patients have lower amplitudes of the IMFs spectra from RR signal, with PD OH patients showing a more pronounced reduction. Finally, we observed
a shift of the spectral peak around 0.1Hz (Mayer's wave) toward lower frequencies for RR and SBP series in PD and PD OH patients, which may indicate a possible baroreflex impairment.

Conclusions: This study suggests that the cardiovascular system's response to the VM is altered in alpha-synucleinopathies patients. Specifically, MSA and PD OH, reported severe dysautonomic symptoms, suggesting that autonomic failure represents an additional risk factor. Future research, with larger cohorts and multiple maneuvers eliciting different ANS responses, will clarify the origins
of these dysfunctions (i.e. peripherical/central).

P57 - Modulation of Oral Microbiota and Inflammatory Cytokines in Hypertensive and Healthy Complete Denture Wearers

Theme: Clinical - Cardiovascular

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Tooth loss has been associated with increased mortality and hypertension. However, the influence of oral conditions on normotensive and hypertensive complete denture wearers remains unclear. This case-control study aimed to evaluate whether the oral clinical history, microbial load, and levels of inflammatory cytokines differ among complete denture wearers categorized into four groups: healthy (CG), controlled hypertensives (G1), underreported hypertensives (G2), and uncontrolled hypertensives (G3). The microbial load on the dentures and palate for Candida spp., Staphylococcus spp., enterobacteria, and mutans group streptococci was assessed by quantifying colony-forming units (CFU). Salivary cytokine levels (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17) were measured using flow cytometry. Participants were characterized by sociodemographic data, clinical information, and blood pressure measurements [systolic (SBP) and diastolic (DBP)]. Statistical analyses included ANOVA, Fisher's exact test, Kruskal-Wallis, Spearman, and Pearson's chi-square tests using SPSS 25.0, with significance set at $p<0.05$. No significant differences were found in sociodemographic characteristics among the 80 individuals (n=20 per group), with a mean age of 66±7.2 years. The duration of edentulism was longer in G3 ($p=0.031$) and significantly associated with SBP ($p=0.012$; $r=0.281$). The CFU count of mutans group streptococci on dentures was higher in G3 ($p=0.029$) and negatively associated with smoking habits ($p=0.017$; $r=-0.338$). Smoking was positively associated with elevated salivary levels of IL-4, IL-2, IL-17, and IFN-γ ($p<0.05$), as well as with diabetes ($p=0.033$; $r=0.304$) and cardiovascular diseases (CVD) ($p=0.048$; $r=0.284$). No differences were found in overall inflammatory cytokine levels. Patients in G3, using only upper dentures, had significantly higher SBP ($p=0.024$) and IL-2 levels ($p=0.024$). The duration of edentulism may impact hypertension, particularly in G3 subjects. Smoking behaviors were found to modulate microbiota and interleukin levels, especially in individuals with diabetes and CVD. Non-functional dentures were linked to uncontrolled hypertension, as evidenced by elevated SBP and IL-2.

P58 - Effects of interval versus continuous exercise on cerebral vascular flow-mediated dilatation

Theme: Clinical - Cardiovascular

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Aerobic exercise is well established to improve brain health outcomes. Among the proposed mechanisms is an exercise-induced increase in cerebral endothelial shear stress that leads to an improved vasodilator function. It has recently been reported that interval exercise, where low and high-intensity exercise bouts are alternated, induces greater cerebral shear stress than continuous exercise. In the current study, we hypothesized that interval exercise would enhance cerebral endothelial function to a greater extent than continuous exercise.

Ten healthy men (21±0.6 years) completed 32 min of interval exercise and work-equivalent continuous exercise on a semi-recumbent exercise bike on separate days. Cerebral vascular flow-mediated dilatation (cFMD) was assessed before exercise (Pre), 15 min (Post-15) and 40 min post-exercise (Post-40). cFMD was taken as the peak internal carotid artery vasodilatation (% change from baseline; duplex Doppler ultrasound) in response to a 30s hypercapnic exposure where end-tidal partial pressure of carbon dioxide was raised by ~9 mmHg.

cFMD was not different prior to the interval and continuous exercise trials (Pre, 5.58±2.82% vs. 4.92±3.14%). cFMD was unchanged from baseline following both interval and continuous exercise trials (Post-15, 7.47±4.92% vs. 5.66±4.21%; Post-40, 5.91±4.01% vs. 6.16±2.26%; p=0.442). In addition, exercise per se did not change cFMD (p=0.437). There were no differences in baseline and maximum internal carotid artery diameter between exercise types (p=0.243, p=0.16) nor across time (p=0.351, p=0.407).

In summary, these preliminary results indicate that interval and continuous aerobic exercise do not elicit differential effects on cerebral vascular function. Further investigations are required to fully elucidate the effects of aerobic exercise on the cerebral vasculature.

P62 - The basal forebrain cholinergic system linking olfaction and cognitive function: from basic studies to clinical application

Theme: Clinical – Neuroscience

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Olfactory dysfunction is a common aspect of normal aging. It is also an early symptom of Alzheimer's disease (AD). The olfactory bulb, the first processing station of olfactory information in the brain, receives cholinergic basal forebrain input, as does the neocortex and hippocampus contributing cognition and memory, respectively. This study aimed to clarify (1) the role of cholinergic input to the olfactory bulb in adult and aged rats, (2) the relationship between olfaction and cognitive function in older adults.

In both adult and aged rats, under anesthesia, unilateral olfactory nerve stimulation produced frequency-dependent increases in blood flow in the olfactory bulb ipsilateral to the stimulus. In adult rats, intravenous injection of nicotinic acetylcholine receptor agonist, nicotine, potentiated the olfactory bulb blood flow response to nerve stimulation. The potentiating effect of nicotine shown in adult rats was greatly reduced in old rats.

In the community-dwelling older adults, the relationship between olfactory identification ability and cognitive functions, including attention and discrimination, were assessed. All participants were able to identify the rose odor between steps 2 and 7. Participants with a higher olfactory threshold (≥5) declined more in the attention and discrimination abilities, compared to those with a lower threshold (≤4). Because both attention and discrimination abilities are related to the basal forebrain cholinergic system, our results suggest that olfactory impairment links to the decline in cognitive function relating the cholinergic system. Olfactory stimulation may be a useful intervention for older adults preventing cognitive impairment by activating cholinergic system.

P63 - The gut hormone GIP contributes to the postprandial gastrointestinal hyperaemia in humans

Theme: Clinical - Gut and Metabolism

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Background and aims: During eating, blood supply to the digestive organs increases. The gut hormone glucose-dependent insulinotropic polypeptide (GIP) is suggested to play a role in this functional hyperaemia due to vasodilation in the superior mesenteric artery (SMA) accompanied by increased heart rate and reduced blood pressure. Using flowsensitive magnetic resonance imaging (MRI), we studied the effect of exogenous and endogenous GIP on gastrointestinal blood flow with the perspective of targeting autonomic dysregulation of postprandial hyperaemia.

Materials and methods: Ten healthy men (age 21-46 years, BMI 20-26 kg/m2) participated in five randomised MRI scanning experiments (supine position) on separate days: Oral glucose tolerance test (OGTT)+saline infusion, OGTT+GIP receptor antagonist infusion (GIPR-An) (GIP(3-30)NH2 1,000 pmol/kg/min), oral water+GIPR-An, oral water+saline infusion, and oral water+GIP subcutaneous injection (40 nmol). Blood flow was measured repeatedly with phase contrast MRI. Plasma glucose (PG) and heart rate were measured repeatedly.

Results: Blood flow in the SMA and portal vein were stable during water+saline (666[495,838] and 1169[1070,1267] ml/min, mean[95%CI]) and water+GIPR-An (684[516,852] and 1088[971,1204] ml/min). Baseline divided maximum flow during water+GIP was 120[115,126]% and for OGTT+saline 184[168,199]%. OGTT+GIPR-An resulted in a 25[-57,6.3]% decrease in mean baseline divided blood flow of SMA compared to OGTT+saline (p=1.02e-10). Also, flow in the portal vein was stimulated by water+GIP and OGTT+saline (115[111,119] and 136[130,141], and OGTT+GIPR-An resulted in a 16[-23,-9]% lower blood flow than OGTT+saline (p=6.61e-07). Blood flow in the hepatic artery and coeliac trunc, and heart rate were unchanged. PG levels were stable during water+saline, water+GIPR-An, and water+GIP (5[4.8,5.1], 4.9[4.8,5.0], 4.7[4.6,4.8] mmol/l), whereas PG levels were higher during OGTT+GIPR-An than OGTT+saline (9.0[8.5,9.5] vs. 8.3[7.9,8.7] mmol/l (p=0.0132).

Conclusion: As confirmed by MRI, both endogenous and exogenous GIP increase gastrointestinal blood flow demonstrating that GIP contributes to postprandial hyperaemia. The consequences of GIP receptor targeting treatments remain to be proven.

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