Identifying central brain pathways driving cardiovascular reactivity during mental stress

Braun J[1], Patel M[1], Kameneva T[2,3], Keatch C[3], W Woods[4], Lambert E[1,2]

[1]School of Health Sciences, Swinburne University of Technology, Melbourne, Australia
[2]Iverson Health Innovation Research Institute, Swinburne University of Technology, Melbourne, Australia
[3]School of Science, Computing and Engineering Technologies, Swinburne University of Technology, Melbourne, Australia
[4]Centre for Mental Health and Brain Sciences, Swinburne University of Technology, Melbourne, Australia

Introduction: Substantial evidence indicates associations between mental stress, aberrant signalling through the sympathetic nervous system, and cardiovascular disease. We assessed how the brain and body respond to acute psychological stress by recording magnetoencephalography (MEG), and multiple peripheral physiological parameters simultaneously.

Methods: Healthy participants (n=13) underwent MEG, muscle sympathetic nerve activity (MSNA), heart rate, respiration, and blood pressure recordings, at rest, and during psychological stress. Whole head and region of interest (ROI) analyses were used and brain response to stress and rest conditions were compared across delta (1-4Hz), theta (4-8Hz), alpha (8-13Hz), beta (13-30Hz) and low gamma (30-80Hz) bands. Participants were divided into high responders and low responders.

Results: Whole head analysis in alpha band showed that stress elicits a significant increase in activity in the left and right anterior cingulate gyrus, both posteriorly and anteriorly, the insular cortex, hippocampus, central opercular cortex, and the right caudate and brainstem. ROI analysis showed significant differences between stress and rest conditions in various brain regions in alpha and delta bands, including the regions mentioned above, and in the left frontal medial cortex and the left and right amygdala. For the high responders' group, whole head analysis showed stronger activation in the above regions, and increased activity in the right orbital frontal cortex and the right accumbens, during stress.

Conclusion: Our results demonstrate that MEG is an effective neuroimaging tool for analysing brain-body cardiovascular reactivity during acute mental stress and presents exciting novel avenues towards identifying the underlying central stress pathways, that are critically associated with neurogenic cardiovascular pathophysiology.

Analysis of neuromuscular function and cell distribution in the Hirschsprung disease colon

Carbone SE[1], Mahdavian N[1], Rajasekhar P[2], Lam T[1], King S[3,4,5], Poole DP[1]

[1]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia
[2]Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia
[3]Department of Paediatric Surgery, The Royal Children's Hospital, Melbourne, VIC, Australia
[4]Surgical Research, Murdoch Children's Research Institute, Melbourne, VIC, Australia
[5]Department of Paediatrics, The University of Melbourne, VIC, Australia

Introduction: Hirschsprung disease (HD) occurs when enteric neurons fail to develop in the distal colon, requiring removal of this aganglionic region. Postsurgical symptoms including chronic constipation affect 30-50% of patients. This suggests factors other than neuron loss affect the ganglionated bowel.

Aim: To correlate neuromuscular function and the distribution of cells involved in motility in HD compared to the healthy colon.

Methods: Smooth muscle contractions were measured in segments spanning the resected bowel (17 HD and 10 control patients HREC: 38262). Neuromuscular function was assessed in response to electrical stimulation and pharmacological stimulation by contractants (e.g., ACh), relaxants (e.g., SNP), and donepezil (cholinesterase inhibitor). The distributions of nerves, interstitial cells of Cajal (ICC) and PDGFRα+ cells (PC) were assessed by immunofluorescence.

Results: Contractions in response to stimulation were larger and more variable in the ganglionated HD bowel compared to control ($P<0.05$, n=8-13). In 10 of 13 HD patients, stimulated contractions were larger in the most proximal compared to the most distal bowel. In 11 of 13 patients, the greatest responses to stimulation occurred in segments of the resected colon where ganglia were present but not fully formed. Innervation and PC densities were reduced in the ganglionated HD compared to healthy controls ($P<0.05$, n=7-8).

Conclusions: This study demonstrates differences in cell distribution and neuromuscular function in the ganglionated HD versus the healthy bowel and may explain why some HD patients experience postsurgical problems.

Cardiovascular and locomotor responses elicited by chemogenetic activation of the medial and lateral hypothalamus under two different promoters.

Carrive P[1], Dracup J[2], Assareh N[2], Prasad A[2], McNally G[1]

[1]School of Medical Sciences and [2] School of Psychology, University of New South Wales, Kensington, Australia

Introduction: Disinhibition or activation of the tuberal hypothalamus with pharmacological agents can evoke marked cardiovascular and locomotor effects. Can the same effects be evoked by chemogenetic activation with the hM3Dq DREADD?

Methods: Adeno-Associated Virus (AAV) vectors encoding the hM3Dq DREADD driven by CaMKIIα or human Synapsin (hSyn) promotors were injected bilaterally in the medial or lateral hypothalamus (MH or LH) of male Sprague Dawley rats implanted with telemetric probes (n=24). One week later the rats were injected with clozapine-N-oxide (CNO, 3 mg/kg, i.p.).

Results: CNO evoked long lasting increases in locomotor activity, heart rate and mean blood pressure with stronger effects evoked from the MH than the LH (+8.1 vs +5.6 activity units n.s., +128 vs +73 bpm, $p<0.05$, +18 vs +13 mmHg, $p<0.05$, respectively). These were significantly reduced by half with the dual orexin receptor antagonist Almorexant (100 mg/kg, i.p.). The CamKIIα and hSyn promoters had similar effects in the MH, but the effect on locomotion was greater with CaMKIIα than with hSyn. RNAscope analysis revealed a greater density of neurons in MH than LH, a greater proportion of glutamate neurons in MH than LH, a greater tropism for glutamatergic neurons with CamKIIα and a greater tropism for GABAergic neurons with hSyn.

Conclusion: In summary, chemogenetic activation of the hypothalamus has the same effect as activation or disinhibition with pharmacological agents. Both glutamatergic and GABAergic neurons would have been activated, but glutamatergic neurons may be the main driver. Specific promoters are needed to tease out the role of these two populations of neurons.

Prenatal maternal amoxicillin exposure influences perinatal enteric nervous system development

Czapla BJ, Poon SSB, Parathan P, Bornstein JC, Foong JPP

Department of Anatomy & Physiology, The University of Melbourne, Parkville, VIC, Australia

Introduction: Antibiotic usage during pregnancy can increase to over 40% immediately prior to parturition (1). Antibiotics disrupt gut microbiota and central nervous system development (2), but their effect on the perinatal enteric nervous system (ENS), the primary controller of gastrointestinal functions, is not well understood.

Methods: Mouse offspring, aged either at embryonic day (E)18.5 or postnatal day (P)0, were taken from dams exposed to the commonly used antibiotic amoxicillin (0.02g/100mL or water as control) between E12.5-E18.5 of their pregnancy *ad libitum* via their water bottle. The pups' colons were immunohistochemically labelled for the pan-neuronal marker Hu, and enteric neuronal subtype markers, calbindin (subdivided into bright and dim immunoreactivities) and neuronal nitric oxide synthase (nNOS).

Results: Female amoxicillin-treated E18.5 embryos had a reduced proportion of calbindin+ myenteric neurons ($p<0.01$), particularly the bright subtype ($p<0.001$), compared to control. However, P0 amoxicillin-treated males had an increased proportion of calbindin+ and nNOS+ myenteric neurons (calbindin: $p<0.05$; nNOS: $p<0.05$), although the change in calbindin could not be attributed to a particular subtype. No statistically significant differences were observed between female P0 amoxicillin-treated and female P0 control pups. P0 control neonates had fewer Hu+ enteric neurons ($p<0.0001$) and Sox10+/S100β+ enteric glial ($p<0.05$) counts per image area than E18.5 control embryos, consistent with intestinal growth. However, enteric subtypes demonstrated no statistically significant differences.

Conclusion: We demonstrated that amoxicillin treatment during pregnancy impacts ENS development differentially across parturition with perturbations more evident in the newborn ENS highlighting the susceptibility of perinatal ENS development to maternal amoxicillin.

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Premotor organisation of laryngeal control in mice.

Eymael AN[1], Burke P[1], McMullan S[1], Dempsey B[1]

[1]Faculty of Medicine, Health & Human Sciences (FMHHS), Macquarie University, Macquarie Park, NSW, Australia

Introduction: Laryngeal muscles support upper airway integrity (tone/patency), speech production, and play a key protective role in closing the airway during feeding. When contracted, the thyroarytenoid (TA) muscles adduct the vocal folds and valve the airway, serving as a pivotal point of control for intentional behaviours, such as vocalisation, and critical autonomic processes like swallowing and breathing. Thus, the action of the TA must be both finely tuned and tightly regulated to permit seamless transition between various aerodigestive behaviours.

Methods: In this study, we mapped the premotor neural inputs to the TA to determine the cell types and connections that underpin its versatile action. Rabies based monosynaptic retrograde tracing was used to identify the sources of presynaptic drive to the TA, revealing an extensive and genetically diverse premotor network, that spanned from the brainstem to cervical spinal cord.

Results: Key premotor pools included: neurons expressing paired-like homeobox 2b (Phox2b) within the nucleus tractus solitarius (NTS), forkhead box protein P2 (FOXP2) within the Kölliker-Fuse (KF), and tryptophan-hydroxylase (TPH) within caudal raphe and cholinergic spinal interneurons. Furthermore, we identified axon collaterals originating from within this network, that terminated on the phrenic, infrahyoid, suprahyoid and lingual motor pools, indicating the existence of highly collateralised premotor neurons that 'hardwire' the larynx with key respiratory and oral effectors.

Conclusion: We propose this premotor hardwiring facilitates the unified and stereotyped motor pattern of swallowing.

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The wanderer's heart: identification of cardiac-rhythmic neurons in microneurographic recordings of the human vagus nerve

Farmer DGS[1], Patros M[1], Ottaviani MM[2], Wright L[3], Dawood T[3], Macefield VG[1]

[1]Department of Neuroscience, Monash University, Melbourne, Australia
[2]Department of Neurosurgery, Università Politecnica delle Marche, Ancona, Italy
[3]Baker Heart and Diabetes Institute, Melbourne, Australia

Introduction: Recently, our laboratory performed the first microneurographic recordings from the cervical vagus nerve in humans. Many multi-unit intrafascicular sites exhibited cardiac rhythmicity. Here, we sought to characterise the firing properties of neurones with cardiac rhythmicity by making single-unit recordings from the human vagus nerve.

Methods: Using ultrasound guidance, a tungsten microelectrode was inserted into the left (n=3) or right (n=7) vagus nerve in 9 participants. Single-units were discriminated (Spike2, CED) from the resulting recordings.

Results: Single-unit recordings were obtained from 48 axons that displayed cardiac rhythmicity: 8 from the left vagus nerve and 40 from the right. Based on action potential polarity, 26 were defined as myelinated and 22 as unmyelinated axons. Cross-correlation of single unit firing with the R-wave of the ECG revealed that 34 neurones showed detectable cardiac rhythmicity at baseline, while 10 showed minimal activity at baseline but increased their rate of firing and displayed cardiac rhythmicity on commencement of slow, deep breathing.

Conclusion: We have shown that it is possible to examine the firing properties of individual myelinated and unmyelinated axons in the human cervical vagus nerve. By careful examination of their firing properties, we hope to be able to identify different subtypes of afferent and efferent cardiovascular vagal neurones.

Disentangling myelinated visceral afferents of the pelvic plexus using adeno-associated viral transfection

Fuller-Jackson JP, Bowden LD, Chan J, Osborne PB, Keast JR

Department of Anatomy and Physiology, School of Biomedical Sciences, University of Melbourne, Parkville, Victoria, Australia, 3010

Introduction: In rodents, the pelvic nerve (pelvic splanchnic nerve homolog) contains most of the visceral sensory axons innervating pelvic organs, in addition to sacral preganglionic and paravertebral sympathetic axons. In the urinary bladder, myelinated Aδ-fibres are mechanosensory and critical in the detection of filling but lack an immunohistochemical marker. We have recently found that, in rats, peripheral neuron-tropic adeno-associated virus, AAV-PHP.S, preferentially transfects ~20% of these afferents. Using this selective, sparse labelling approach, we aimed to characterize myelinated bladder afferents by combining AAV labelling with cholera toxin subunit B (CTB) retrograde tracing.

Methods: Adult Sprague-Dawley rats (n = 3 per sex) were injected intravenously with AAV, and the bladder microinjected with CTB 19 days later. At 21 days the major pelvic ganglia (MPG) with associated nerves and bladder were fixed for further analysis. Bladder afferents in the pelvic nerve were confined to three of the five major fascicles.

Results: Using neurofascin as an immunohistochemical marker for paranodes (myelination) we determined internode length (115.0-1890.8 μm) and diameter (0.5-1.5 μm) of myelinated AAV+ bladder afferents; no relationship between these two parameters was identified. Individual AAV+ axons were followed within nerves in the bladder wall, revealing that nodes were rare, suggesting that that myelination does not extend to the most peripheral components of bladder afferents.

Conclusion: In summary, we have defined new structural features of myelinated bladder afferent projections. This approach can be applied to myelinated afferents of other organs and will inform computational modelling for neuromodulation and bioelectronic therapies.

Interrogating gastric enteric neurons to align genotype and phenotype

Furness JB[1,2], Laddach AC[3], Gajanaike C[1], Hunne B[1], Di Natale MR[1,2], Oliveira A[3], Progatzky F[3], Heanue TA[3], Adams CD[2] and Pachnis V[3]

[1]Department of Anatomy & Physiology, University of Melbourne, Parkville, VIC 3010, Australia
[2]Florey Institute of Neuroscience and Mental Health, Parkville, VIC 3010, Australia
[3]The Francis Crick Institute, London, UK

Introduction: Several recent single cell RNAseq studies to characterise enteric neuron populations in the mouse small and large intestines have revealed nerve cell clusters that are not yet unambiguously aligned with neurons that have been classified by functions. We reasoned that investigation of gastric enteric neurons would provide further insights and would be likely to successfully identify genotype/ phenotype alignments.

Methods: We utilised single cell RNAseq analysis of 744 enteric neurons from the mouse stomach; regional localisation of markers in enteric neuron somas with and without colchicine treatment to enhance peptide levels; localisation of markers in nerve terminals related to targets; and distinction between sources of terminals using nerve lesion surgery, to provide insights into genotype/ phenotype alignment.

Results: Through studies still in progress, we have identified the relations between genotype and phenotype for motor neurons to the muscle and identified for the first time two possible classes of interneuron (immunoreactive for and expressing VIP (*Vip*) and Tachykinins (*Tac1*). Intrinsic primary afferent neurons, identified by NMU (*Nmu*) in the intestines were rare in the stomach. Classes of neurons corresponding to secretomotor neurons, including acid secretion stimulatory neurons, have been tentatively identified as VIP/ GRP expressing. Unlike in the intestine, the stomach does not appear to harbour Somatostatin (*Sst*), CGRP (*Calca* or *Calcb*) or serotonin (*Tph1* or *2*) intrinsic neurons.

Conclusion: In conclusion, our hypothesis that investigation of genotype / phenotype relationships in the stomach will clarify the identification of functional classes of enteric neuron is supported by our investigations in the mouse stomach.

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

Glarin R[1], Rim D[2], Henderson LA[3], Macefield VG[2]

[1]Melbourne Brain Centre Imaging Unit, University of Melbourne; [2]Department of Neuroscience, Monash University; [3]Brain and Mind Centre, University of Sydney

Introduction: Muscle Sympathetic Nerve Activity (MSNA) contributes to the beat-to-beat regulation of blood pressure. Using MSNA-coupled functional Magnetic Resonance Imaging (fMRI) – in which we record MSNA at the same time as performing fMRI – we previously identified the human homologue of the rostral ventrolateral medulla (RVLM) and associated medullary nuclei (Macefield & Henderson, 2010). This work was conducted at 3T, and we are now using Ultra-High Field fMRI (7 T) with the promise of higher spatial resolution and signal-to-noise within the brainstem.

Methods: A tungsten microelectrode was inserted into a muscle fascicle of the left common peroneal nerve of 10 healthy participants. Neural activity was recorded using an MR-compatible low-noise headstage (NeuroAmpEX, ADInstruments) and spontaneous bursts of MSNA identified. Blood Oxygen Level Dependent (BOLD) contrast gradient echo, echo-planar images were continuously collected in a 4 s ON, 4 s OFF (210 volumes) sparse-sampling protocol, acquired at 7T with 1 mm isotropic voxels (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 the intensity of the concurrently recorded bursts of MSNA. A group level analysis identified MSNA-coupled BOLD signal increases at the level of the RVLM, midbrain periaqueductal gray (PAG) and raphe obscurus (RO). A decrease in signal was detected in dorsal motor nucleus of the vagus (DMX).

Conclusion: The improved signal-to-noise and higher spatial resolution available at Ultra-High Field allows us to identify additional brainstem nuclei involved in the generation of muscle sympathetic nerve activity and control of blood pressure.

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Critical role of lateral habenula circuits in the control of stress-induced palatable food consumption

Herzog H

Neuroscience Division, Garvan Institute of Medical Research, St Vincent's Hospital

Introduction: Chronic stress fuels the consumption of palatable food and can enhance obesity development. While stress and feeding controlling pathways have been identified, how stress-induced feeding is orchestrated remains not known. **Results:** Here we identify lateral habenula (LHb) Npy1r-expressing neurons as the critical node for promoting hedonic feeding under stress, since lack of Npy1r in these neurons alleviates the obesifying effects caused by combined stress and high fat feeding (HFDS) in mice. Mechanistically, this is due to a circuit originating from central amygdala NPY neurons, with the upregulation of NPY-induced by HFDS initiating a dual inhibitory effect via Npy1r signalling onto LHb and lateral hypothalamus neurons, thereby reducing the homeostatic satiety effect through action on the downstream ventral tegmental area.

Conclusion: Together, these results identify LHb-Npy1r neurons as a critical node to adapt the response to chronic stress by driving palatable food intake in an attempt to overcome the negative valence of stress.

Gastrointestinal dysfunction in a mouse model of autism spectrum disorder and potential therapeutics

Hill-Yardin EL[1, 2], Lin V[1], Li Y[1], Mohsenipour M[1], Abo-Shaban T[1], Mou K[1], Filappone RT[3], Alamoudi MU[1], Perez AJ[1], Munira MS[1], Williams JK[1], Hosie S[1], Balasuriya GK[1], Bornstein JC[2], Nurgali K [3], Franks AE[4]

[1]School of Health and Biomedical Sciences, Bundoora West Campus, RMIT University
[2]Department of Anatomy and Physiology, The University of Melbourne
[3]Institute for Health and Sport, Victoria University, Western Centre for Health, Research and Education, Sunshine Hospital.
[4]Department of Microbiology, Anatomy, Physiology and Pharmacology, School of Life Sciences, La Trobe University.

Introduction: Individuals diagnosed with autism spectrum disorder (ASD; autism) commonly experience gastrointestinal (GI) comorbidities including altered GI motility and permeability. We assessed GI function in the preclinical model of autism, Neuroligin-3^{R451C} (*Nlgn3^{R451C}*) mice.

Methods: Male wildtype and *Nlgn3^{R451C}* C57BL/6 mice (8-14-weeks-old, fasted for 18h) were used for *ex vivo* permeability and motility experiments. Permeability was assessed in duodenum, jejunum, distal ileum and colon segments (~4-5cm long), closed using suture thread. Tissue preparations were injected with 1mg/ml 4-kDa FITC (Fluorescein isothiocyanate; 40µL/cm), placed in DMEM and incubated at 37°C for 2h. 10mM caffeine, or 30 or 90mM of L-glutamine was delivered to some preparations. Every 30 min, external solution absorbance was measured via a FlexStation3 microplate reader (490-519nm). We characterised motility patterns in *Nlgn3^{R451C}* mice using *ex vivo* organ bath video imaging and our novel edge detection software interface ('GutMap'). During motility experiments, the nitric oxide synthase inhibitor, N-nitro-L-arginine (NOLA) or the $GABA_A$ antagonist, gabazine, were applied to small intestinal and colon preparations, respectively.

Results: Fasted *Nlgn3^{R451C}* mice showed increased GI permeability across gut regions. Caffeine and L-glutamine administration restored permeability in *Nlgn3^{R451C}* mice. *Nlgn3^{R451C}* mice had shorter ileal contraction durations and more frequent short colonic contractions. NOLA increased jejunal contractions in wildtypes and mutants with no effect in ileal preparations. Gabazine application reduced short contraction frequency in *Nlgn3^{R451C}* colons.

Conclusion: We highlight increased GI permeability and dysmotility in *Nlgn3^{R451C}* mice. These changes are modified by treatment with caffeine or L-glutamine (permeability), and NOLA or gabazine (motility). These findings enhance our understanding of GI dysfunction associated with autism spectrum disorder.

Central pathways of the inflammatory reflex

McAllen RM[1], McKinley MJ[1], Martelli D[2]

[1]Florey Institute of Neuroscience and Mental Health
[2]Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

Introduction: The inflammatory reflex exerts an inhibitory influence on inflammatory cytokine responses to infection or systemic inflammation. It is mediated by the splanchnic sympathetic nerves. When those nerves are cut or blocked to disable the reflex, inflammatory challenge (by systemic lipopolysaccharide -LPS) results in substantially higher blood levels of pro-inflammatory cytokines (e.g. TNFα and IL- 6) with reduced levels of the anti-inflammatory cytokine IL-10. We have been investigating the central pathways that drive this reflex in urethane-anaesthetised rats.

Results: A series of neuraxial transections have revealed that the isolated spinal cord does not support the reflex, but the brain stem does. The caudal-most transections that preserved the reflex were at the level of the facial nuclei. Microinjections of the inhibitory agent muscimol (2%, 50nL) bilaterally into the RVLM/RVMM region prevented the reflex suppression of TNFα in response to LPS challenge. Microinjections into the rostral medullary raphé were ineffective.

Conclusion: These findings support the hypothesis that basic inflammatory reflex circuitry resides within the rostral medulla, and the sympathetic premotor neurons that drive its output via the splanchnic nerves are located in the RVLM/RVMM region. We do not exclude influences on the reflex from more rostral regions of the brain.

Electrical stimulation of the dorsolateral prefrontal cortex inhibits vestibular signalling and vestibulosympathetic reflexes in humans

McCarthy B[1,2], Rim D[3], Sesa-Ashton G[1], Datta S[1,2], Wong R[1,2], Crawford LS[4], Dawood T[1,2], Henderson LA[4], Macefield VG[1,2,3]

[1]Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

[2]Baker Department of Cardiometabolic Health, The University of Melbourne, VIC, Australia

[3]Department of Neuroscience, Monash University, VIC, Australia

[4]School of Medical Sciences (Neuroscience), Brain and Mind Centre, The University of Sydney, NSW, Australia

Introduction: We aimed to determine the effects of combining sinusoidal galvanic vestibular stimulation (sGVS) with transcranial alternating current stimulation (tACS) of the dorsolateral prefrontal cortex (dlPFC) on generating vestibular sensations and vestibulosympathetic reflexes, alongside determining the neural pathways between the systems.

Methods: Sinusoidal stimuli (±2 mA, 0.08 Hz, 100 cycles) were applied in a randomised order to 36 human participants: (i) tACS of the dlPFC at electroencephalogram site F3 or F4, (ii) bilateral sGVS via the mastoid processes, and (iii) tACS and sGVS together. In 11 of these participants, muscle sympathetic nerve activity (MSNA) was recorded via a microelectrode inserted percutaneously into the right common peroneal nerve near the fibular head. In a separate study, the same areas were stimulated (±2 mA, 0.2 Hz, 60 cycles) in 20 participants while performing fMRI of the brain.

Results: tACS of the dlPFC abolished almost all vestibular perceptions of motion and nausea when delivered concurrently with sGVS. MSNA cross-correlation analysis revealed that the amplitude and pattern of cyclic modulation produced by combined stimulation was similar to that produced by tACS of the dlPFC alone. Increases in blood-oxygen-level-dependent signal intensity occurred within the parietal operculum and thalamus during sGVS and tACS, but not when the stimuli were applied concurrently.

Conclusion: Perceptions of sway and nausea during sGVS were diminished with concurrent tACS of the dlPFC, along with activation of core vestibular areas in the thalamus and parietal operculum. This, coupled with inhibition of the vestibulosympathetic reflexes, suggests that the dlPFC exerts top-down inhibitory control of vestibular processing.

Evidence for vagal sensory neural involvement in influenza pathogenesis and disease

McGovern AE[1], Verzele NAJ[1, 2], Chua BY[3], Short KR[2,4], Moe AAK[5], Edwards IN[2], Bielefeldt-Ohmann H[4], Reading PC[3, 6], Trewella MW[1], Mazzone SB[1]

[1]Department of Anatomy and Physiology, The University of Melbourne, Parkville, VIC, Australia. [2]School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia QLD, Australia.[3]The Peter Doherty Institute for Infection and Immunity, Department of Microbiology and Immunology, University of Melbourne, Parkville Victoria 3000, Australia.[4]Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, QLD, Australia. [5]Department of Medical Imaging and Radiation Sciences, Monash University, Clayton, VIC, Australia. [6]WHO Collaborating Centre for Reference and Research on Influenza, Victorian Infectious Disease Reference Laboratory, Peter Doherty Institute for Infection, and Immunity, Parkville, Australia.

Introduction: Influenza A virus (IAV) is a common respiratory pathogen causing significant and often severe morbidity. Although inflammatory immune responses to IAV infections are well described, little is known about how neuroimmune processes contribute to IAV pathogenesis.

Methods: Here, we employ surgical, genetic, and pharmacological approaches to manipulate pulmonary vagal sensory neuron innervation and activity in the lungs to explore potential crosstalk between neural and immune processes.

Results: Intranasal inoculation of mice with IAV resulted in stereotypical antiviral lung inflammation and tissue pathology, changes in respiration, loss of body weight and other clinical signs of severe IAV disease. Unilateral cervical vagotomy and genetic ablation of pulmonary vagal sensory neurons had moderate effects on pulmonary inflammation induced by IAV infection, but significantly worsened clinical disease presentation. Inhibition of pulmonary vagal sensory neuron activity via inhalation of the charged sodium channel blocker, QX-314, resulted in a moderate decrease in lung pathology, but was accompanied by a paradoxical worsening of clinical signs. Notably, vagal sensory ganglia neuroinflammation induced by IAV infection was significantly potentiated by QX-314 administration. This vagal ganglia hyperinflammation was characterized by alterations in IAV-induced host defense gene expression, increased neuropeptide gene and protein expression, and an increase in the number of inflammatory cells present within the ganglia.

Conclusion: These data suggest that pulmonary vagal sensory neurons play a role in the regulation of the inflammatory process during IAV infection and suggest that vagal neuroinflammation may be an important contributor to IAV pathogenesis and clinical presentation. Targeting these pathways could offer therapeutic opportunities to treat IAV-induced morbidity and mortality.

State-dependency of nocturnal swallowing in healthy adults: Incidence, motor function and coordination with breathing

Monroe M[1], Carter S[2], Eckert D[2,3] Bilston L[2], Hudson A[2], Butler J[2], Gandevia S[2], Kerr G[1], Burke P[2,4]

[1]Queensland University of Technology
[2]Neuroscience Research Australia, Sydney, Australia.
[3]Adelaide Institute for Sleep Health, Flinders Health and Medical Research Institute, Flinders University, Adelaide, Australia.
[4]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia.

Introduction: Swallowing involves a very complex motor pattern and coordination with breathing and other oro-motor behaviours. Sleep has major depressive effect on many of the motor neuronal pools that facilitate swallowing, particularly upper airway muscles. This study examines the state-dependence of the swallow motor program and coordination with breathing.

Methods: Adult subjects recruited from the community undertook an overnight sleep laboratory study. Subjects were instrumented to monitor sleep (EEG), breathing (nasal mask, pneumotach), swallowing / airway valving events and pharyngeal muscle activity (epiglottic pressure, submental EMG, peroral genioglosuss EMG). We report preliminary findings (N=16; 5 female; age: 20-63 yrs) from an ongoing study. Data reported mean ± SD.

Results: Nocturnal swallowing occurred intermittently throughout the night, the majority triggered during an arousal from sleep (43-100% of all nocturnal swallows across participants). Pharyngeal swallowing pressure was 85 ± 47 cmH_2O during wake, 78 ± 45 cmH_2O during arousal from sleep, and 58 ± 48 cmH_2O in stable sleep (X^2 = 68.05, *df* = 3, p <0.001; Linear mixed-effects model). Swallows generated a brief, near-maximal burst of genioglossus EMG activity during wake (101 ± 35 %, N=5) and during arousal from sleep (92 ± 52 %); in comparison, stable sleep values were significantly lower at 36 ± 21 % (X^2 = 140.34, *df* = 3, p <0.001).

Conclusion: Swallows elicited during arousal from sleep generate maximal EMG and pharyngeal pressures equivalent to wake state. In contrast, swallowing in stable sleep produces a markedly attenuated EMG and pharyngeal pressure.

Investigating resting-state brain activity in lean and overweight/obese individuals using magnetoencephalography

Patel M[1], Braun J[1], Woods W[2], Kameneva T[1,3,4], Keatch C[4] and Lambert E[1,3]

[1]School of Health Sciences
[2]Centre for Mental Health and Brain Sciences
[3]Iverson Health Innovation Research Institute
[4]School of Science, Computing and Engineering Technologies, Swinburne University of Technology, Melbourne, Victoria, Australia

Introduction: Obesity-associated cardiometabolic complications are attributed to chronic activation of the sympathetic nervous system, resulting in altered activation in certain brain regions that are associated with altered reward circuitry and aberrant metabolic pathways. Magnetoencephalography (MEG) was utilised to investigate differences in activity in brain regions during the resting state in two groups, lean and overweight.

Methods: Concurrent recordings of MEG and muscle sympathetic nerve activity (MSNA) were obtained during 10 minutes of resting state in 2 groups: lean (n=7, BMI 18.5-24.9) and overweight/obese (n=5, BMI >25) healthy participants. MEG data was co-registered with structural T1-magnetic resonance imaging (MRI) scans for each participant. The MEG sensor data was projected into a source-space using the Linearly Constrained Minimum Variance Beamforming (LCMV) beamformer model. The power variance in brain activity for each physiological frequency band was compared between the two groups. Similarly, brain activity in known regions of interest, from literature, that are associated with increased MSNA and blood pressure in the higher BMI group is also explored.

Results: Significant differences in mean power variance were observed between the groups in the beta (13-30 Hz) and low gamma (30-80 Hz) bands. Brain regions that showed significant differences included the prefrontal, orbitofrontal and occipital cortices as well as temporal gyrus.

Conclusion: Using the MEG, important cortical structures are shown to have significant differences between lean and overweight/obese groups in the current preliminary analysis. In this study, we also explore regions of interest linked with obesity-related higher sympathetic outflow.

Chronic vagus nerve stimulation reduces muscle sympathetic nerve activity in drug-resistant epilepsy

M Patros, H Simpson, S Sivathamboo, T.J. O'Brien, VG Macefield

Department of Neuroscience, Monash University, Melbourne, Australia

Introduction: Vagus Nerve Stimulation (VNS), delivered via surgically implanted cuff electrodes on the left cervical vagus, is used to treat drug-resistant epilepsy (DRE). VNS is believed to act on the brain to reduce seizures by chronic neuromodulation, but it is not known what effects it has on other systems, including the sympathetic nervous system.

Methods: Here we tested the hypothesis that chronic VNS reduces muscle sympathetic nerve activity (MSNA). Seventeen patients between the ages of 18-66 years with a diagnosis of DRE were recruited from the Alfred Health epilepsy clinics. We obtained MSNA recordings from ten DRE patients with implanted VNS devices who had been stimulated chronically (>6 months stimulation). Recordings were also obtained from five DRE patients yet to receive a VNS implant. In two patients we were unsuccessful in obtaining recordings of spontaneous MSNA.

Results: Baseline recordings were obtained in existing VNS patients, lying supine with their VNS device stimulating at their clinically set parameters (1.125-3.5 mA, 20 Hz, 250μs, duty cycle 10%-35%). Patients without VNS (n=5) had a mean of 29±10 SD bursts/min at rest, while those treated with VNS (n=10) had a mean of 13±8 SD bursts/min, measured between trains of VNS. MSNA was significantly lower in patients with VNS (p=0.002). VNS had no acute effects on MSNA at rest, nor during manoeuvres that increase MSNA, such as passive head-up tilt.

Conclusion: VNS therapy appeared to have no acute effect on MSNA. However, MSNA was reduced in patients with chronic VNS treatment compared to those without. Chronic VNS treatment may have a protective effect on cardiovascular risk by reducing sympathetic activity. This may underly the mechanism behind risk-reduction for sudden unexplained death in epilepsy (SUDEP) in VNS-treated patients (1).

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Using faecal microbiota transplant to investigate the effects of Huntington's disease gut microbiota on brain and gut function in mice

Qin W[1,2], Masson BA[1], Renoir T[1], Guber Ct[1], Hannan AJ[1,2]

[1]Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria, Australia
[2]Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia

Introduction: Huntington's disease (HD) is a fatal neurodegenerative disease that is characterised by psychiatric, motor, and cognitive impairments. It is caused by a mutation in the huntingtin gene, which is expressed throughout the brain and peripheral tissues – including the gastrointestinal tract. Previously, research has focused on the brain to explain the symptoms of HD. A whole-body approach has uncovered the presence of gut microbial disruption in preclinical and clinical HD. We were the first to show that faecal microbiota transplant (FMT) from wild-type (WT) into HD mice improved cognitive outcomes.

Methods: We assessed whether FMT from HD into WT mice can transfer any motor, cognitive, or gastrointestinal outcomes. Antibiotics (ABX) were given prior to FMT to deplete the microbiota so donor bacteria can proliferate in a less competitive environment. To control for the effects of ABX and FMT processes, separate groups of mice were given their respective vehicles (water and glycerol).

Results: No difference was found in motor, cognitive, and gastrointestinal outcomes across the groups ($p>0.05$). Despite no significant differences in behaviour, ABX treatment alone decreased brain mass ($p<0.05$), which was rescued by FMT (irrespective of donor genotype).

Conclusion: Results suggest that gut microbiota alone is not enough to induce the HD endophenotypes measured in this study. However, there may be a lack of pathogenic HD-associated microbiota engraftment, as the healthy gut physiology of WT mice can constrain types of bacteria that can proliferate. Microbiota profiling of faecal samples is needed to elucidate the extent of engraftment of donor microbiota.

The effect of long-term recurrent intermittent hypercapnia on central respiratory chemoreflex function

Kim D[1], Nedoboy P[2], Oh A[1], Farnham M[2], Kumar NN[1].

[1]Department of Pharmacology, School of Biomedical Sciences, UNSW Sydney, Kensington, NSW
[2]Heart Research Institute, Newtown, NSW

Introduction: The homeostatic central respiratory chemoreflex responds to extracellular carbon dioxide (CO2) levels which are directly sensed by central respiratory chemoreceptors (CRCs), to regulate breath-by-breath ventilation. The intrinsically acid-sensing CRC neurons within the retrotrapezoid nucleus (RTN) are highly active during hypercapnia. Concurrent intermittent hypoxia (IHx) and hypercapnia (IHc) are hallmarks of respiratory disorders, yet previous investigations have predominantly focused on the effects of IHx alone. Both stimuli converge on the same brain regions, therefore studying these regions is important to understand the brain response to IHc. This study aimed to investigate the effects of long-term recurrent IHc on chemoreflex chemoresponsiveness of RTN neurons to an acute respiratory stressor (acute hypercapnia challenge; AHC), RTN neuroplasticity, and plasma corticosterone (CORT) levels in adult mice.

Methods: The 7-day IHc paradigm consisted of recurrent hypercapnic exposures (8% CO2, 18x5-minute cycles) followed by AHC (10% CO2, 1 hour). Brainstem tissue sections were assayed for paired-like homeobox 2b (Phox2b), tyrosine hydroxylase (TH), and cFos or ΔFosB immunohistochemistry (IHC) to allow quantification of RTN chemoresponsiveness or neuroplasticity, respectively. Body weight and plasma CORT were measured to determine the impact of IHc on psychophysiological stress levels.

Results: Results showed that long-term recurrent IHc had no effect on RTN cFos expression following AHC and plasma CORT levels, and it increased ΔFosB expression.

Conclusion: Taken together, this study revealed that RTN neurons retain their chemoresponsiveness yet exhibits neuroplasticity without increasing stress in response to long-term recurrent IHc. This conclusion suggests that RTN neurons undergo neuronal adaptation to retain their chemosensitivity during long-term respiratory stress.

The association between grey matter volume, mean blood pressure, and muscle sympathetic nerve activity in hypertensive humans

Rim D[1], Fatouleh R[2], Schlaich M[3], Dawood T[4], Henderson L[5], Macefield VG[1]

1. Department of Neuroscience, Central Clinical School, Monash University, VIC, Australia, 2. School of Medicine, Western Sydney University, NSW, Australia 3. Dobney Hypertension Centre, Medical School – Royal Perth Hospital Unit, The University Western Australia, WA, Australia, 4. Baker Heart and Diabetes Institute, VIC, Australia 5. School of Medical Sciences (Neuroscience), The Brain and Mind Centre, University of Sydney, NSW, Australia

Introduction: Neurogenic hypertension is primarily driven by increases in sympathetic nerve activity (1). Besides the cardiovascular and autonomic changes with hypertension, significant structural changes in the brain occur (2-4). However, whether these structural brain changes are linked to cardiovascular (measured by mean blood pressure, MBP) or sympathetic changes (measured by recording muscle sympathetic nerve activity, MSNA) remains to be investigated. The aim of this study was to determine the relationship between regional grey matter (GM) volume, MSNA and MBP in neurogenic hypertension relative to healthy normotensive controls.

Methods: T1-weighted structural MRI (3T MRI, Siemens) of the brain were acquired from 20 hypertensives and 16 normotensive controls. Region-wise volumetric comparisons of grey matter among hypertensives and healthy controls were performed by voxel-based morphometry analysis (controlling sex, age, and total brain volume). Spontaneous bursts of MSNA were recorded from the right common peroneal nerve via tungsten microelectrode immediately prior to structural MRI scanning.

Results: In hypertensives, increased GM volume ($p<0.01$(uncorrected)) was observed in 23 different brain regions, and decreased GM volume ($p<0.01$(uncorrected)) was observed in 11 different brain regions. 10 out of 23 regions, including the left superior occipital gyrus, left superior parietal lobule, left superior frontal gyrus, bilateral precuneus, bilateral postcentral gyrus, right central operculum and right middle frontal gyrus, showed an inverse relationship with either MSNA frequency (bursts/min) or incidence (bursts/100 heartbeats). 3 out of the 10 identified regions showed negative correlations with MBP and DBP, which includes the left superior parietal lobule, right postcentral gyrus, and right central operculum. Additionally, the left occipital gyrus, right superior parietal lobule, and right middle frontal gyrus were shown to correlate with DBP.

Conclusion: Given that MSNA contributes to elevated MBP in neurogenic hypertension, it is imperative to investigate the central sources of the increased MSNA to understand the underlying mechanism and discover novel therapeutics. Our finding suggests that GM volume alterations in specific brain regions may contribute to the dysregulation of MSNA and BP.

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Functional differentiation of enteric neurons and glia from human iPSCs

Rowland E, Daniszewski M, Yildiz GS, Bergner AJ, Hao MM, Stamp LA

Department of Anatomy and Physiology, University of Melbourne, Australia

Introduction: The enteric nervous system (ENS) is essential for many key functions of the gastrointestinal tract, including water and nutrient absorption, hormone secretion and motility. Perturbations in the ENS can result in debilitating disease. Understanding the development, function, and communication of this complex neural network will be essential to developing new therapies to treat these diseases. The development of enteric neurons and glia can now be mimicked in vitro, by the sequential and guided differentiation of human induced pluripotent stem cells (hiPSC) towards an enteric neuronal and glial fate. However greater insight into the developmental and functional properties of hiPSC-derived ENS cells is critically needed to validate their potential as disease models and for cell therapy.

Methods: In this study, we investigated the functional activity of hiPSC-derived enteric neurons and glia during early time points post-differentiation. Using an established method, hiPSCs were differentiated into enteric neurons and glia. Live calcium imaging was performed to examine neuronal and glial activity at differentiation day (D)22, D29, D36, D43, D50 and D57. Additionally, we investigated the impact of different growth factors on directing neuronal vs glial differentiation, including endothelin-3 and delta like ligand 1.

Results: Ca^{2+} transients were observed in response to high K^{+} application, as well as the neurotransmitter receptor agonists DMPP (10μM) and ATP (10μM). Further, spontaneous Ca^{2+} transients were observed. Maturation of responses were identified along the cells' developmental trajectory.

Conclusions: This is the first study to characterise the functional maturation of neuronal and glial hiPSC-derived ENS cells.

Neurovascular transduction and baroreflex sensitivity in the context of severe COPD

Sesa-Ashton G[1,2], Macefield VG[2]

[1]Human Neurotransmitters Laboratory, Baker Heart and Diabetes Institute, Melbourne, VIC, 3004
[2]Human Autonomic Neurophysiology, Department of Neuroscience, Monash University Central Clinical School, Melbourne, VIC, 3004

Introduction: Chronic Obstructive Pulmonary Disease is an obstructive lung disease often secondary to long-term tobacco exposure as well as other noxious respiratory irritants. The condition is defined by the presence of emphysema and chronic bronchitis alongside dyspnoea, fatigue and recurrent respiratory infections. Alongside these respiratory sequelae, the disease is associated with marked sympathoexcitation and significantly elevated cardiovascular risk. While many studies have confirmed this sympathoexcitation measured by muscle sympathetic nerve activity (MSNA), neurovascular transduction – the capacity for the resistance vasculature to respond to increases in sympathetic drive – has not yet been characterised in this patient cohort.

Methods: 16 COPD patients and 10 age-matched controls were recruited. COPD patients received arterial blood gases to confirm hypoxaemia and hypercapnia. All participants underwent sympathetic microneurography measuring MSNA co-recorded with ECG, blood pressure, O_2 saturation and expired CO_2 fraction. Burst frequency, burst incidence, spontaneous baroreflex sensitivity and neurovascular transduction were calculated. Neurovascular transduction was correlated against arterial partial pressure of CO_2.

Results: MSNA burst frequency ($p=0.003$) as well as burst incidence ($p=0.0382$) were significantly higher in COPD patients relative to age-matched controls. Both baroreflex gain ($p=0.53$) and neurovascular transduction ($p=0.97$) were not significantly different between COPD patients and controls. However, neurovascular transduction significantly, positively correlated with $PaCO_2$ ($p=0.015$, $r=0.73$). Moreover, spontaneous baroreflex gain decreased relative to increasing PaCO2 ($p=0.0091$, $r=0.74$).

Conclusion: Neurovascular transduction in COPD patients remains consistent with age-matched controls despite the marked sympathetic drive. Severity of hypercapnia did positively scale with neurovascular transduction. Given these findings, it appears the degree of neurovascular transduction is, at least partially, driven by hypercapnia-associated vasodilatation.

Development of a fully-implantable, wireless battery-free optogenetic device for control of the enteric nervous system and gut-brain axis in freely-moving animals

Spencer, NJ[1], Efimov A[2], Kim J[2], Travis J[1], Vázquez-Guardado A[3], Hibberd T[1], Sorensen J[4], Hu H[5], Rogers JA[2]

[1]College of Medicine, Flinders University, South Australia
[2]Department of Biomedical Engineering, Northwestern University, USA
[3]University of North Carolina
[4]Cyber Sensing and Shaping, Cyber and Electronic Warfare Division, Defence, Science and Technology Group, Edinburgh, South Australia
[5]Icahn School of Medicine at Mount Sinai, New York, USA

Introduction: Wireless activation of the enteric nervous system (ENS) in freely moving animals using optogenetic technologies offers a unique and exciting opportunity to selectively control gastrointestinal (GI) transit *in vivo*, including the gut-brain axis. Applying current commercially available wireless optogenetic technology to the GI-tract, however, poses many challenges, not encountered with current optogenetic devices applied to the central nervous system (CNS).

Results: We report the development of an implantable wireless battery-free device specifically designed for the GI-tract, capable of generating sufficient light to robustly activate the ENS, potently inducing colonic motility *ex vivo* and increased propulsion *in vivo*. Deployment through *in vivo* studies reveals unique patterns of stimulation that increase expulsion of colonic content, likely mediated in part by activation of an extrinsic brain-gut motor pathway, via pelvic nerves.

Conclusion: The technology overcomes major limitations of conventional wireless optogenetic hardware designed for the CNS, providing targeted control of specific neurochemical classes of neurons in the ENS and brain-gut axis, for direct modulation of GI-transit and behaviour in freely moving animals.

Symptoms associated with exercise intolerance and resting heart rate following mild traumatic brain injury in adults: results from the prospective observational CREST concussion recovery study

Thorne J[1,2,3], HellewellSC[2,3], Cowen G[3,4], Ring A[1,5], Jefferson A[4], Chih HJ[6] Gozt AK[2,3], Buhagiar F[7], Thomas E[6,8], Papini M[2,3], Bynevelt M[10], Celenza A[11,12], Xu D[4,6,13], Honeybul S[14], Pestell CF[3,7], Fatovich D[15,16] and Fitzgerald M[2,3]

[1]School of Allied Health, Faculty of Health Sciences, Curtin University, WA
[2]Perron Institute for Neurological and Translational Science, WA
[3]Curtin Health Innovation Research Institute, Curtin University, WA
[4]Curtin Medical School, Faculty of Health Sciences, Curtin University, WA
[5] Institute for Immunology and Infectious Diseases, Murdoch University, WA
[6] School of Population Health, Curtin University, WA
[7] School of Psychological Science, UWA, WA
[8]Centre for Clinical Research Excellence, School of Population Health, Curtin University
[9]Division of Surgery, School of Medicine, UWA
[10] Neurological Intervention & Imaging Service of WA at Sir Charles Gairdner Hospital, Nedlands, WA
[11]Emergency Department, Sir Charles Gairdner Hospital, WA
[12]Division of Emergency Medicine, School of Medicine, UWA
[13] The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China
[14]Sir Charles Gairdner, Royal Perth and Fiona Stanley Hospitals, Perth, WA
[15]Emergency Medicine, Royal Perth Hospital, UWA, Australia
[16]Centre for Clinical Research in Emergency Medicine, Harry Perkins Institute of Medical Research

Introduction: People may experience a range of symptoms after mild traumatic brain injury (mTBI), but the relationship between symptoms and objective assessments is poorly characterised. This study investigated the association between symptoms, resting heart rate (HR), exercise intolerance and recovery in individuals following mTBI.

Methods: Prospective observational study of adults aged 18-65 years within 7 days of mTBI. Symptoms were assessed using the Post-Concussion Symptom Scale[1], HR measured at rest and exercise tolerance assessed using the Buffalo Concussion Bike Test[2]. Symptom burden and symptom-based clinical profiles (physiological, vestibular-oculomotor, mood, autonomic) were examined with respect to exercise tolerance, resting HR and recovery.

Results: 32 participants were assessed (mean age 36.5±12.6 years, 41% female, 5.7±1.1 days since injury). Number of symptoms (p=0.002) and symptom severity (p=0.004) were associated with exercise intolerance. Physiological and vestibular-ocular clinical profiles were associated with exercise intolerance (p=0.001 and p=0.014 respectively), with exercise intolerant individuals having higher mean number of symptoms in each profile. Mood-related and autonomic clinical profiles were associated with higher resting HR (greater than 80bpm) (p=0.048 and p=0.028 respectively), suggesting altered autonomic response for participants with these symptoms. After adjusting for age and mechanism of injury, higher initial mood-related clinical profile scores were associated with persisting symptoms at three months post-injury (aOR=2.08; 95%CI=1.11-3.90; p=0.013).

Conclusion: Symptom-based clinical profiles, in conjunction with objective measures such as resting HR and exercise tolerance, are important aspects of clinical care for those having sustained mTBI. These results provide preliminary support for the concept that specific symptoms are indicative of autonomic dysfunction following mTBI.

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How space dust settles our mind: discovery of the cell and receptor target of the mood stabiliser lithium

Thorpe DW, Peterson RA, Humenick A, Ootsuka Y, Blessing WW, Keating DJ

Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University

Introduction: Lithium has been a first-line treatment for the manic phase of bipolar disorder for over 70 years. Its primary mechanism, presumed to be in the brain itself, remains unknown. We now present evidence that lithium alters brain function via a primary action in the gut, with modulation of brain function via the gut brain axis.

Methods: In vitro studies measured vagal afferent activity, secretion from and activation of gut endocrine cells and single cell gene expression data to identify the gastrointestinal mechanism of action of lithium and its receptor target. Pharmacological and transgenic mouse [1] approaches confirmed the site of action of lithium and the gut transmitters responsible for its action. Measures of mouse behaviour were also undertaken.

Results: We find that lithium activates a subset of afferent nerves innervating the gut and this action requires a cell intermediary lining the gut wall. Genes linked to bipolar disorder are highly enriched in these gut cells. Lithium activates these cells dose-dependently, in the human therapeutic range. A cell ablation transgenic mouse model confirms that the cells are the primary target of lithium, and pharmacological studies demonstrate release of multiple signalling molecules, including serotonin, known to activate vagal afferents and area postrema neurons. Using pharmacological and genetic approaches, we identified the cell receptor target by which lithium activates these gut cells and their role in vivo.

Conclusion: Our data suggest that lithium's therapeutic action in bipolar disorder occurs via a previously undiscovered gut-brain axis pathway.

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The mood stabiliser lithium affects autonomic and behavioural functions via peripheral mechanisms of actions

Ootsuka Y, Blessing WW, Thorpe DW, Humenick A, Jones L, Peterson R and Keating DJ

Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University

Introduction: Lithium has been a treatment for the manic phase of bipolar disorder for over 70 years. A direct effect of lithium on the brain has been assumed. We propose that lithium has a primary action on the brain via vagal afferents and the area postrema, the gut-brain axis. Lithium reduces body temperature and causes conditioned taste aversion (CTA). Our study determined whether these actions depend on specific gut cells.

Methods: We compared transgenic mice with specific gut cells ablated by tamoxifen (10mg/kg, i.p.) induction of diphtheria toxin [1] and control mice with intact cells. We tested the effect of lithium chloride (LiCl, 2 mEq/kg, i.p.) on body temperature, behavioural activity and CTA. Sucrose water was used as a conditioned stimulus.

Results: In control mice (n=5), LiCl reduced body temperature (1.7±0.5˚C, $p<0.01$) and behavioural activity by 2877±721 (arbitrary, $P<0.05$) and induced CTA. Such reductions and CTA were not observed in our ablation mice (n=6). Thus, prior ablation of a specific subtype of gut cells substantially reduces lithium induced brain-mediated physiological and behavioural/psychological actions.

Conclusion: We suggest that the therapeutic (approximately 0.6 mEq/kg) dose of lithium causes a “pre-nausea” CTA state in humans, reducing engagement with the external environment, a key component of the anti-manic action of lithium. Neural pathways activated by vagal and area postrema inputs to the brain include the amygdala and insula and prefrontal cortices, key regulators of emotional function. Our new gut-brain axis framework suggests testable hypotheses for therapeutic mechanism of lithium in mania.

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

Unnikrishnan N, Korim WS, Yao ST

Department of Anatomy and Physiology, The University of Melbourne, VIC 3010

Introduction: Although essential hypertension was first recognised several decades ago, there still is no identifiable cause. Significant progress has been made in linking neuroinflammation, sympathetic nerve activity (SNA), 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. 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.

Methods: This study was designed to determine whether: 1) Microinjections of SDF-1 into the RVLM of normotensive rats increases BP; blockade of the two receptors, CXCR4 and CXCR7, is sufficient to 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 increased in the RVLM neurons of hypertensive rats.

Results: Injecting 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.

Conclusion: 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.

Targeted stimulation of Npff neurons induces a torpor-like state in mice

Zhang L

Neuroscience Division, Garvan Institute of Medical Research, St Vincent's Hospital

Introduction: Neuropeptide FF (NPFF) belongs to the evolutionarily conserved RF-amide peptides family, with NPFF receptor 2 (NPFFR2) considered to be its cognate receptor. Although best known for its modulatory role in pain response, increasing evidence suggest that the NPFF system also plays important roles in other physiological processes including energy and glucose metabolism. In keeping with this, strongest NPFF expression is found in the sub postrema area immediate next to the circumventricular organ area postrema, and its expression alters according to energy status.

Results: Brainstem stimulation of NPFF neurons via DREADDs led to significant reduction in energy expenditure, that occurred within 30 min upon CNO injection and lasted ~10 hours. This hypometabolism following NPFF neuronal stimulation was associated with significant reduction in respiratory exchange ratio, - indicative of a shift towards lipid as fuel source, reduction in locomotion due to increased time being still, and reductions in food and water intake. Moreover, rectal temperature and BAT thermogenesis were also significantly decreased, indicating hypothermia is induced following NPFF neuronal stimulation. These effects of NPFF neuronal stimulation were seen at both 22^{o}C and 28^{o}C ambient temperatures. Importantly, the increases in energy expenditure and BAT thermogenesis expected from decreasing ambient temperature from 28^{o}C to 22^{o}C were also seen with CNO injection, indicating a functional thermoregulatory system in NPFF stimulation-induced hypometabolic and hypothermic state, - a hall mark of torpor.

Conclusion: These results show that stimulation of NPFF neurons induces a torpor-like state associated with the regulated hypometabolism and hypothermia. Targeting these neurons may have therapeutic potential for the induction of surgical hypothermia.

Integration of autonomic reflexes in the nucleus of the solitary tract

Zhu H[1,2], Furuya W[1], Bassi J[2], Connely-Huf A[2], Allen A[2] & McDougall S[1]

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

Introduction: 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.

Methods: 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.

Results: 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.

Conclusion: These data indicate reflexes are mostly mediated by dedicated populations of neurons in the initial phase of information integration.