KEYNOTE LECTURES

KL 01 | Keynote Lecture 01: Sofia-Iris Bibli

KL 01-01
Endothelial de-CYS-tyl: Impaired cysteine catabolism and the regulation of endothelial dysfunction
S.I. Bibli
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Endothelial cysteine catabolism is tightly regulated from the enzyme cystathionine γ lyase (CSE). CSE is responsible to break down cysteine to other metabolic intermediates, a process which generates the small gaseous signaling molecule hydrogen sulfide (H2S). The latter, has been been proposed to act as a post-translational modifier of proteins in a process termed Persulfidation or S-Sulfhydration. The impact of endothelial H2S generation from intracellular cysteine on vascular homeostasis still remains unknown. Our studies were designed to investigate the impact of endothelial CSE deletion/inhibition on cellular fate decisions focusing on the effects of H2S on protein post-translational marks. By mapping the first endothelial cell S-Sulfhydrdme we were able to show that this oxidative post-translational modification is inflammation sensitive and controls endothelial homeostasis, inhibits atherogenesis and maintains mechanosensing. Such effects were attributed to the inhibitory S-Sulfhydration of the RNA binding protein ELAVL1 and the effects of S-Sulfhydration on disulfide bond rearrangement, extension and activation of the β3 leg of the αvβ3 integrin. Pharmacological application of H2S donors, was able to re-sulfhydrate the aforementioned targets, preserve endothelial fitness and re-sensitize atherosclerotic vessels to blood flow. Taken together our studies propose that targeting cysteine metabolism and S-Sulfhydration might serve as a therapeutic strategy to maintain a healthy vasculature.

References
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[2] Bibli SI*, Hu J*, Loise J., Leisegang MS., Wittig J., Zukunft S., Kapasakalidi A., Fisslthealer B., Tsilimigras D., Zografas G., Filis K., Brandes RP., Papapetropoulos A., Sigala F., Fleming I. Shear stress regulates cystathionine γ lyase expression to preserve endothelial redox balance and reduce membrane lipid peroxidation. Redox Biology 2020;28:101379.

KL 02 | Keynote Lecture 02: Stefan Trapp

KL 02-01
Brain-derived glucagon-like peptide-1: Is it relevant for obesity treatment?
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University College London, Dept of Neuroscience, Physiology & Pharmacology / Centre for Cardiovascular and Metabolic Neuroscience, London, UK

Peripherally-administered GLP-1 receptor agonists (GLP-1RAs) are currently the most efficacious single target treatment for obesity. Whilst their effect is associated with widespread neuronal activation, CNS penetration of these compounds is limited. Consequently, although GLP-1 receptors are found in many parts of the brain, most of these are not accessible for peripherally-administered GLP-1RAs. GLP-1 is produced within the brain by preproglucagon (PPG) neurons located in the lower brainstem. These neurons store GLP-1 in their axon terminals, and there is a good correlation between GLP-1 receptor expression and innervation by PPG neurons in individual brain areas. Selective ablation of PPG neurons in the nucleus of the solitary tract significantly reduces GLP-1 content not only in brainstem, but throughout the CNS. Chemosynthetic activation of PPG neurons acutely and substantially suppresses food intake, consistent with the long-established observation that injection of GLP-1 directly into the brain has the same effect.

Analysis of the inputs to PPG neurons revealed that neither GLP-1 receptor expressing afferent vagal neurons, nor GLP-1 receptor expressing neurons in the area postrema, both of which are accessible to systemic GLP-1RAs, provide significant synaptic input to PPG neurons. Instead, it is oxytocin receptor expressing vagal afferent neurons that project to PPG neurons. These data further separate the PPG neurons from peripherally accessible GLP-1 receptors and explain why GLP-1RAs do not activate PPG neurons. All these findings suggest distinct brain circuitry for GLP-1 neuron-mediated and GLP-1RA-mediated food intake suppression.

Arguably the most intriguing remaining unanswered question about PPG neurons is that about their precise physiological function. Selective ablation and chemogenetic inactivation experiments aimed at revealing the physiological role of PPG neurons found no clear role in primary satiation in ad libitum fed mice. These experiments did however reveal the importance of PPG neurons in restraint-stress-induced hypophagia and the termination of large volume meals and it seems likely that this list of secondary satiation effects will grow.

For now, the capacity to strongly reduce food intake by chemogenetic activation makes PPG neurons an exciting potential target for anti-obesity medication, and their separation from the circuits mediating GLP-1RA hypophagia, suggests that successful combination therapy with GLP-1RAs should be feasible. In support of this concept, we demonstrated that combination of the systemic administration of the GLP-1RA semaglutide with chemogenetic or pharmacological activation of PPG neurons suppresses food intake to a greater extent than either treatment alone. Future advances in the understanding of the precise physiology of these neurons are likely to add more therapeutic opportunities linked to stress, reward or eating disorders.
KL 03 | Keynote Lecture 03: Bernd Fakler

The Interactome of AMPA-receptors - Protein-coding of Excitatory Neurotransmission and its Plasticity
B. Fakler
University of Freiburg, Institute of Physiology II, Freiburg, Germany

Processing, propagation and storage of information in the brain fundamentally rely on fast excitatory neurotransmission and its context-dependent dynamics that are both mediated by AMPA-type glutamate receptors (AMPARs). These ligand-gated ion channels conduct the depolarizing currents required for point-to-point transmission and adjust their number to the degree of synaptic activity thereby endowing synapses with the plasticity required for memory formation. Investigation of the processes and mechanisms underlying the complex cell physiology of AMPARs has received a strong boost when proteomic research uncovered their interactome, the ensemble of protein building blocks of the native receptor channels. The interactome constitutes, roughly 40 distinct proteins, determine subunit architecture(s), functional properties, biogenesis and trafficking, as well as positioning and stabilization of the AMPARs in the mammalian brain under normal and pathological conditions.

I will discuss the molecular and cellular physiology of the AMPARs on the background of their interactome including latest results on newly identified extracellular networks of proteins and discuss their significance for memory formation in rodents and humans.

KL 04 | The Physiological Society Michael de Burgh Daly Prize Lecture: Merry L. Lindsey

Cardiac Wound Healing Following Myocardial Infarction
M. L. Lindsey

In response to myocardial infarction (MI), the left ventricle (LV) undergoes a series of cardiac wound healing responses involving both the stimulation of robust inflammation to clear necrotic myocytes and tissue debris and the induction of extracellular matrix (ECM) protein synthesis to generate an infarct scar. For wound healing to be optimal, appropriate communication must occur. Cell constituents need to come in at the right time, be activated at the right time in the right amount, and exit at the right time. When optimal healing occurs, a new homeostasis is obtained within the infarct, such that infarct scar size and quality are sufficient to maintain LV size and shape. In reality, the ideal scenario does not always occur. Often, miscommunication can occur within and between infarct and remote spaces, across the temporal wound healing spectrum, and across organs. When there is miscommunication, adverse remodeling can progress to heart failure. Matrix metalloproteinases (MMPs) are key regulators of LV remodeling after MI, through direct effects on ECM turnover as well as indirect effects on the regulation of the major cell types that coordinate cardiac wound healing—namely the infiltrating leukocytes and the cardiac fibroblasts. This keynote lecture will discuss current knowledge and recent developments to identify biomarker indicators that reflect the status of each component to better predict outcomes.

KL 05 | Acta Physiologica Prize Lecture

This year we have two awardees for the Acta Physiologica Award.

Recipient 1:
The circadian clock regulates the diurnal levels of microbial short-chain fatty acids and their rhythmic effects on colon contractility in mice.
Segers A, Desmet L, Thijs T, Verbeke K, Tack J, Depoortere I. Acta Physiol (Oxf). 2019 Mar;225(3):e13193. doi: 10.1111/apha.13193. Epub 2018 Oct 22. PMID: 30269420

Anneleen Segers, Louis Desmet, Theo Thijs, Kristin Verbeke, Jan Tack and Inge Depoortere from the Translational Research Center for Gastrointestinal Disorders, Leuven, Belgium

Recipient 2:
Intact vagal gut-brain signalling prevents hyperphagia and excessive weight gain in response to high-fat high-sugar diet.
Molly McDougle, Danielle Quinn, Charlene Diepenbroek, Arashdeep Singh, Claire de la Serre, Guillaume de Lartigue

University of Florida, Gainesville, USA; The John B. Pierce Laboratory, New Haven, USA; Yale Medical School, New Haven, USA and the University of Georgia, USA

Laudatio
P. B. Persson
Acta Physiologica, editor in chief, Germany

KL 05-02
Cross-talk between circadian clocks and nutrient sensing pathways in the gut
I. Depoortere

University of Leuven, Gut Peptide Research Lab, Translational Research Center for Gastrointestinal Disorders (Targid), Leuven, Belgium

The circadian system enables organisms to optimally adapt their physiology and behavior to the correct time of the day. The master clock located in the hypothalamus is synchronized by the light-dark cycle and synchronizes clocks present in peripheral tissues. Misalignment between the light-dark cycle and feeding/fasting cycle, as occurs for example in shiftwork and chronic jetlag, will result in desynchronization between rhythms of central and peripheral clock systems and will affect rhythmicity in several biological processes. We showed that chronic jetlag in mice, enhanced body weight gain and abolished the day/night food intake pattern. As a result, the rhythm in faecal short-chain fatty acid levels was abolished and this was paralleled by a
phase delay in clock gene expression and clock-controlled genes in the gut mucosa. During this talk, I will show how foods become the dominant zeitgeber for peripheral clocks during chronodisruption in the gut and how time-restricted feeding can contribute to the resynchronization and rhythmic activation of the peripheral circadian clocks to restore gut epithelial homeostasis and body weight.

**KL 05-03**
**Re-evaluating the role of the vagal gut-brain axis in obesity**

G. de Lartigue, M. McDougle, D. Quinit, A. Singh, C. Diepenbroek, C. de La Serre
Monell Chemical Senses Center and the University of Pennsylvania, Philadelphia, USA

Obesity is a big problem and the mechanisms for the onset are poorly understood. Foods rich in Fat and Sugar are major driver of weight gain so understanding the mechanisms by which fats and sugars are sensed and communicated to the brain may provide novel insights into treating obesity. In this presentation I will discuss evidence that fats and sugars are individually sensed by separate vagal sensory populations. Each of these separate vagal populations are necessary and sufficient to control food choice in a nutrient specific manner. This provides a mechanism by which foods that combine fats and sugars activate vagally mediated reward circuits that increases motivation to overeat obesogenic diets. Simultaneously gut innervating vagal sensory neurons recruit satiety signals that serve to defend against uncontrolled overeating of high fat diets. These data suggest that vagal sensory neurons play a role in a number of previously unsuspected functions in higher order control of food intake. Under normal physiological conditions the balance of homeostatic and hedonic inputs from the gut help to maintain a healthy body weight. I will provide evidence that overtime, continued consumption of high fat high sugar diets, impairs vagally mediated satiety promoting a higher body weight. Therefore, efforts to restore vagal satiety may be an effective strategy for treating obesity.

**KL 06 | Du Bois-Reymond Prize Lecture**

*The Du Bois-Reymond Prize is the most prestigious distinction for junior investigators in German-speaking Physiology. The prize is provided by the Erwin Riesch Foundation, Tübingen. With this prize, the German Physiological Society honors an outstanding and independent scientific work in a current field of physiology.*

**Laudatio**

I. Hanganu-Opatz
Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

**KL 06-02**
**Early activity shapes the development of the prefrontal cortex**

S. H. Bitzenhofer
Universitätsklinikum Hamburg-Eppendorf, Zentrum für Molekulare Neurobiologie (ZMNH), Hamburg, Germany

Disturbed neuronal activity in neuropsychiatric pathologies emerges during development and might cause multifocal neuronal dysfunction by interfering with apoptosis, dendritic growth, and synapse formation. However, how altered electrical activity early in life impacts neuronal function and behavior of adults is unknown. We addressed this question by transiently increasing the coordinated activity of layer 2/3 pyramidal neurons in the medial prefrontal cortex of neonatal mice and monitoring long-term functional and behavioral consequences. We found that increased activity during early development causes premature maturation of pyramidal neurons and alters interneuron density. Consequently, reduced altered inhibitory feedback by fast-spiking interneurons and altered excitation/inhibition ratio in prefrontal circuits of young adults results in weaker evoked synchronization in gamma frequency. These structural and functional changes ultimately lead to poorer mnemonic and social abilities. Thus, prefrontal activity during early development actively controls the cognitive performance of adults and might be critical for cognitive symptoms of in neuropsychiatric diseases.

**KL 07 | The Physiological Society Bayliss-Starling Prize Lecture:**

**Maria Fitzgerald**

**KL 07-01**
**Pain and the infant brain - it all starts here**

M. Fitzgerald
UCL, Department of Neuroscience, Physiology and Pharmacology, London, UK

**Introduction**

This lecture will focus upon our understanding of how pain is processed by the developing nervous system. I will present our recent research on the functional maturation of the connections underlying pain experience in the young mammalian brain, drawing on data from human infants and
laboratory rodent pups, and highlighting fundamental differences between pain processing in the young and adult nervous system.

Methods
We use a wide range of neurophysiological and behavioural recording methods in the laboratory and the clinical setting. Our philosophy is to translate results from laboratory rodent models into a better understanding of human infant pain and then to use our human data to guide future laboratory research.

Results
Newborn mammals display robust responses to noxious or tissue-damaging stimulation. These nociceptive or “pain” responses arise from neural activity at different levels of the central nervous system. Protective reflex movements and physiological reactions mediated by spinal cord and brainstem circuits are essential for the preservation of life and well-being but should not be equated with pain awareness. The unique sensation of pain and its unpleasant, threatening quality requires activity in the cortical and subcortical regions of the brain.

Our data shows that nociceptive maps in the human cortex are widespread in the newborn and undergo a series of critical network changes in early life, suggesting that infants do not feel pain in the same way as we do. Furthermore, our laboratory research shows that injury in early life alters pain connectivity in the adult brain, both within and between cortical areas involved in the sensory and affective dimensions of pain. This data provides the first direct evidence that the cortical pathways underlying adult pain experience are shaped by events in infancy.

Conclusion
Pain is learned in infancy, and understanding the processes by which this happens is relevant to all who are interested in somatosensory and pain perception, in both health and disease, throughout life.

This suggests that myokines may be useful biomarkers for monitoring exercise prescription for people with, for example, cancer, diabetes, or neurodegenerative diseases. The talk will include suggestions about how to translate basic research to clinical praxis and political decisions.

KL 09 | Keynote Lecture 09: Walter Boron

KL 09-01
The substrates of the electrogenic Na/HCO₃ cotransporter NBCe1: making an unambiguous distinction among simple mechanisms of HCO₃⁻ vs. CO₂ vs. H⁺ transport.

W. F. Boron, S.-X. Lee, R. Occhipinti, F. J. Moss
Case Western Reserve University School of Medicine, Department of Physiology and Biophysics, Cleveland, USA

The electrogenic Na/HCO₃ cotransporter NBCe1 (SLC4A4) plays important roles in organ systems throughout the body. In the renal proximal tubule, for example, NBCe1-A provides the main pathway for the exit across the basolateral membrane of the cotransporter’s namesake, a solute that appears to be bicarbonate (“HCO₃⁻”). NBCe1 is also a major pathway for “HCO₃⁻” uptake across the basolateral membrane of pancreatic ducts, and plays a key role in the regulation of intracellular pH (pH) in numerous cell types throughout the body. Investigators generally agree that, as expressed in Xenopus oocytes, NBCe1 appears to mediate the influx of 1 Na⁺ and 2 “HCO₃⁻”. (Note: one can reverse the electrochemical gradient and force NBCe1 to mediate a net efflux.) However, despite speculation and numerous studies taken as supporting one mechanism or another, it has been impossible to distinguish (1) the uptake of 1 Na⁺ + 2 HCO₃⁻ ions per se, (2a) the uptake of 1 Na⁺ + 1 CO₂ ions or (2b) the NaCO₃ ion pair, and (3) the CO₂/HCO₃⁻-dependent exchange of extracellular Na⁺ for 2 H⁺.

We now combine the power of surface chemistry, electrophysiology, and mathematical simulations (based on the reaction-diffusion model of Somersalo et al) to distinguish, unambiguously, among #1 vs. #2ab vs. #3. The principle is that the predicted amount of H⁺ appearing at the cell surface (after reactions involving the CO₂/HCO₃⁻ under our experimental conditions, but not other buffers) per unit charge (e⁻) transported (1 in the case of NBCe1) differs markedly among the three models: H⁺/e⁻ = 0.14 protons/charge for #1, ≤0.68 for #2ab, and ≤1.2 for #3. We measure the amount of H⁺ appearing on the cell surface with a pH electrode, which records the ΔpH produced as we use a 2-electrode voltage clamp to shift NBCe1-A from an equilibrium state to either net-inward or net-outward transport. The voltage clamp reports the amount of charge transported. Thus, our key experimentally determined parameter is ΔpH/ΔIₑ (analogous to H⁺/e⁻). For NBCe1-A, we find that ΔpH/ΔIₑ = 0.09 pH units/µA. To calibrate our ΔpH/ΔIₑ, we express the voltage-gated H⁺ channel H₁-1. According to our simulations, H⁺/e⁻ = 0.6 protons/charge (i.e., exactly half that of mechanism #3). According to our experimental measurements of oocytes expressing H₁, ΔpH/ΔIₑ = 0.08 pH units/µA. Using this value as a calibration, we find that our observed (ΔpH/ΔIₑ) / (ΔpH/ΔIₑ(cal)) = 1.13 is nearly the same as that predicted in our mathematical simulations, in which (H⁺/e⁻)cal / (H⁺/e⁻)exp = 1.14, and quite different for the predictions for mechanisms #1 (~0.23) or #3 (~2.00).
More complex stoichiometries (e.g., 2 Na\(^+\) + 2 CO\(_3^{2-}\) + 1 HCO\(_3^-\)) than we have considered do not comport with known structures of the SLCA4 family. Thus, we conclude that NBCe1-A must transport 1 Na\(^+\) with 1 CO\(_3^{2-}\) (or 1 NaCO\(_3\)), and we can definitively rule out HCO\(_3^-\) and H\(^+\) as possible substrates in either direction of net transport. In negative-control experiments, we show that AE1 is indeed a CI-HCO\(_3^-\) exchanger, as long suspected, and not 2Cl-CO\(_3^-\) exchanger. Once could apply the approaches outlined here to virtually any acid-base transporter to distinguish among HCO\(_3^-\), CO\(_3^{2-}\), and H\(^+\) as potential substrates.

**KL 10 | The Physiological Society’s Otto Hutter Physiology Teaching Prize Lecture: Dee U. Silverthorn**

**KL 10-01**

**Teaching in the time of COVID – and beyond**

D. U. Silverthorn

*University of Texas at Austin, Dept of Medical Education, Austin, USA*

In March 2020 academic education as we have known it came to an abrupt halt with the declaration of a global pandemic due to the SARS-CoV2 virus. In-person classes and practicals ceased, and instructors had almost no time to convert their teaching to virtual platforms. It has been a challenging two and a half years for teachers and students alike as we all struggled with adapting to remote instruction. Now, as another academic year begins with a return to in-person instruction, this talk will reflect back on what we learned from pandemic teaching and ask what we should carry forward and what is best left behind.

**KL 11 | Keynote Lecture 10: Claudine Blin**

**KL 11-01**

**The innate immune function and diversity of osteoclasts**

C. Blin

*CNRS, Universite Cote d’Azur, LP2M, Nice, France*

**Introduction**

Osteoclasts are the cells responsible for bone resorption in steady state and bone destruction in chronic inflammatory diseases and osteoporosis. Up to recently, they have been considered just as a single population of bone-resorbing cells whose differentiation and activity are increasing in pathologies associated with bone destruction. However, recent data demonstrated that besides bone resorption, osteoclasts are innate immune cells. In particular, they are antigen-presenting cells activating T cell responses towards tolerance in steady state. In addition, they are also able to stimulate inflammatory T cells in the context of chronic inflammation.

**Methods**

We setup new tools to investigate mature osteoclasts. Combining RNAseq and in vitro functional assays with in vivo models of bone destruction, we explored the phenotype and function of osteoclasts sub-populations.

**Results**

Using RNAseq on purified mature osteoclasts, we showed demonstrated the existence of phenotypically and functionally distinct subsets of osteoclasts. We could identified specific characteristics of osteoclasts associated with bone destruction, such as in osteoporosis or in chronic colitis. In particular, we showed that while some osteoclast populations promote immunosuppression, others are clearly inflammatory and can participate in chronic inflammation. Based on these characteristics and in particular their different capacity to respond to danger signals arising from gut dysbiosis, we could specifically block the differentiation of inflammatory osteoclasts and reduced bone destruction in ovariectomized mice.

**Conclusion**

Our results revealed that bone destruction not only relies on an increased number of osteoclasts but also on the implication of different osteoclast subsets having opposite immune outcomes. These new data on the diversity and innate immune function of osteoclasts open very novel perspectives for fighting against inflammatory bone destruction.

**KL 12 | Keynote Lecture 11: Alain Lacampagne**

**KL 12-01**

**Role of type 2 ryanodine receptors in dilated cardiomyopathy in patients with Duchenne muscular dystrophy**

A. Lacampagne

*Montpellier University, PhyMedExp, Montpellier, France*

**Introduction**

Duchenne muscular dystrophy (DMD) is a genetic disease where an X-linked mutation in the DMD gene initiates pathogenic development caused by the absence of dystrophin. Patients with DMD have progressive dilated cardiomyopathy associated with fatal cardiac arrhythmias. Electrical and functional abnormalities have been attributed to cardiac fibrosis. However, electrical abnormalities may occur in the absence of overt cardiac histopathology. Over the past fifteen years, we have endeavored to demonstrate the role of the cardiac sarcoplasmic reticulum (SR) Ca\(^{2+}\) release channel/ryanodine receptor (RyR2) as a pathophysiological target in this disease.

**Methods**

To address our hypotheses, we used both animal models of DMD (mdx mice and GRMD dogs) as well as ventricular cardiomyocytes derived from hiPS cells. On these models we applied several methodological procedures, in vivo (electrocardiogram, echocardiography, speckle tracking analysis), at the cellular level (electrophysiology, Ca\(^{2+}\)-imaging) and at the molecular level (electrophysiology and biochemistry).

**Results**

In all models studied, RyR2 were depleted of calstabin2 (FKBP12.6), resulting in “leaky” RyR2 channels and a diastolic SR Ca\(^{2+}\) leak responsible for cardiac arrhythmias, decrease of global LV longitudinal strain and presence of and the presence of hypokinetiic segments. Stabilizing RyR2 with a small molecule Rycal, abolished SR mediated Ca\(^{2+}\) leak, arrhythmias and reduced LV strain and the number of hypokinetic segments.

**Conclusion**

All of this work has made it possible to highlight the major role of RyR2 in the onset of dilated cardiomyopathy and opens up important therapeutic prospects for slowing the progression of this disease, which is today the main cause of death in DMD patients.