RegPep24

University of Stirling, Scotland, August 1-5, 2023

Meeting report

By Andrew Gundlach
&
Lee Eiden

RegPep24 offered an exciting program consisting of seven plenary lectures, three keynote lecture sessions, and ten symposia. There were also two 'theme discussions' during which recent major advances in the regulatory peptide field were discussed, and important unresolved topics in the field were debated. Two excellent datablitz sessions featured short presentations from early career investigators to introduce their research for subsequent discussion at the poster sessions. The fifteen major lectures of the conference are briefly highlighted below. We thank the speakers for providing their laboratory URL and a graphical abstract of the main point(s) of their presentation, for the benefit of newsroom visitors who wish to delve further into these path-breaking topics in regulatory peptide research and their therapeutic applications. Readers should note that a full commentary on the RegPep24 meeting will accompany a Special Issue of the Journal of Neuroendocrinology to be published in early 2023.

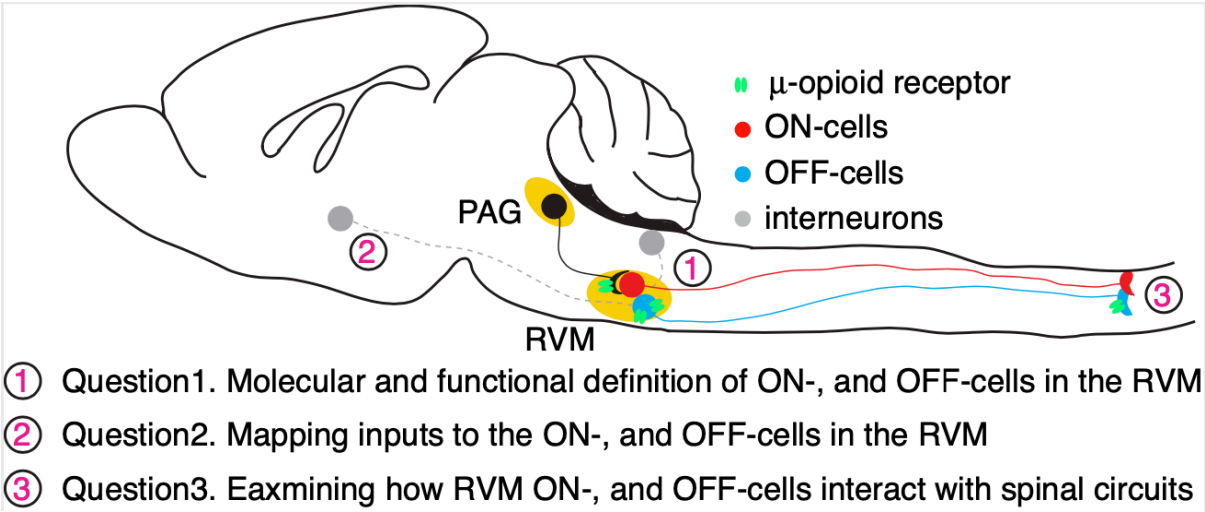
Plenary Lectures

Professor **Patrik Rorsman** (University of Oxford) provided the Inaugural Plenary Lecture on *"Peptidergic regulation of glucagon secretion: Implication for diabetes pathophysiology and therapy"*. His talk highlighted novel approaches to the treatment of diabetes mellitus, which remains one of the foremost public health challenges globally and for which peptide-based therapies continue the tradition of the use of insulin itself to treat this common endocrine disorder, which has a global economic cost of 2.5 trillion dollars/year. Diabetes is often associated with impaired insulin secretion combined with oversecretion of glucagon. Glucagon-like peptide 1 (GLP-1) treatment correct both defects, but its mechanisms of action are unclear. Notably Prof Rorsman discussed his recent studies on the controversial role of a degradation product of GLP-1 in this process. His team discovered that GLP-1(9-36) shares the capacity of GLP-1(7-36) to inhibit glucagon secretion evoked by low glucose and that GLP-1(9-36) also potently inhibits glucagon secretion stimulated by β-adrenergic activation and amino acids. Prof Rorsman also detailed the complex signalling events associated with the actions of GLP-1(9-36) that are independent of actions at the GLP-1 receptor, and involve the glucagon receptor (GCGR).

https://www.rdm.ox.ac.uk/people/patrik-rorsman

Professor **Xiaoke Chen** (Stanford University) was the **2022 Victor Mutt Lectureship** awardee. At each biennial RegPep meeting the International Regulatory Peptide Society recognizes a pioneer in regulatory peptide research in honor of Victor Mutt, the discoverer of multiple gut peptides in the 1960's-80's who set the field on a highly productive course and whose work opened broad new avenues for regulatory peptide research. In his talk on *"Peptidergic descending control of pain"* Dr Chen emphasized that pain perception consists of both input messages to the brain localizing pain in the body, and feedback messages from the brain to the spinal cord that modulate these input signals so that they are alerting, but not distracting, to the enterprise of survival. The peptides involved in the ascending limb are the well-known opioids as well as PACAP and CGRP; while those in the descending pathway described by Dr Chen include a variety of neuropeptides including tachykinins, somatostatin, gastrin-releasing peptide (GRP) and others. Dr Chen's team employed genetic tools including interdiction of the release of peptides from their large dense-core vesicles, versus classical transmitters from smaller vesicles, to demonstrate that neuropeptides work not singly, but as a cohort to modulate pain perception. These findings open the possibilities for combinatorial treatment of conditions such as chronic neuropathic pain, which make many sufferers vulnerable to addiction to opiates, which are the current major treatment for chronic pain.

https://www.xiaokechenlab.com

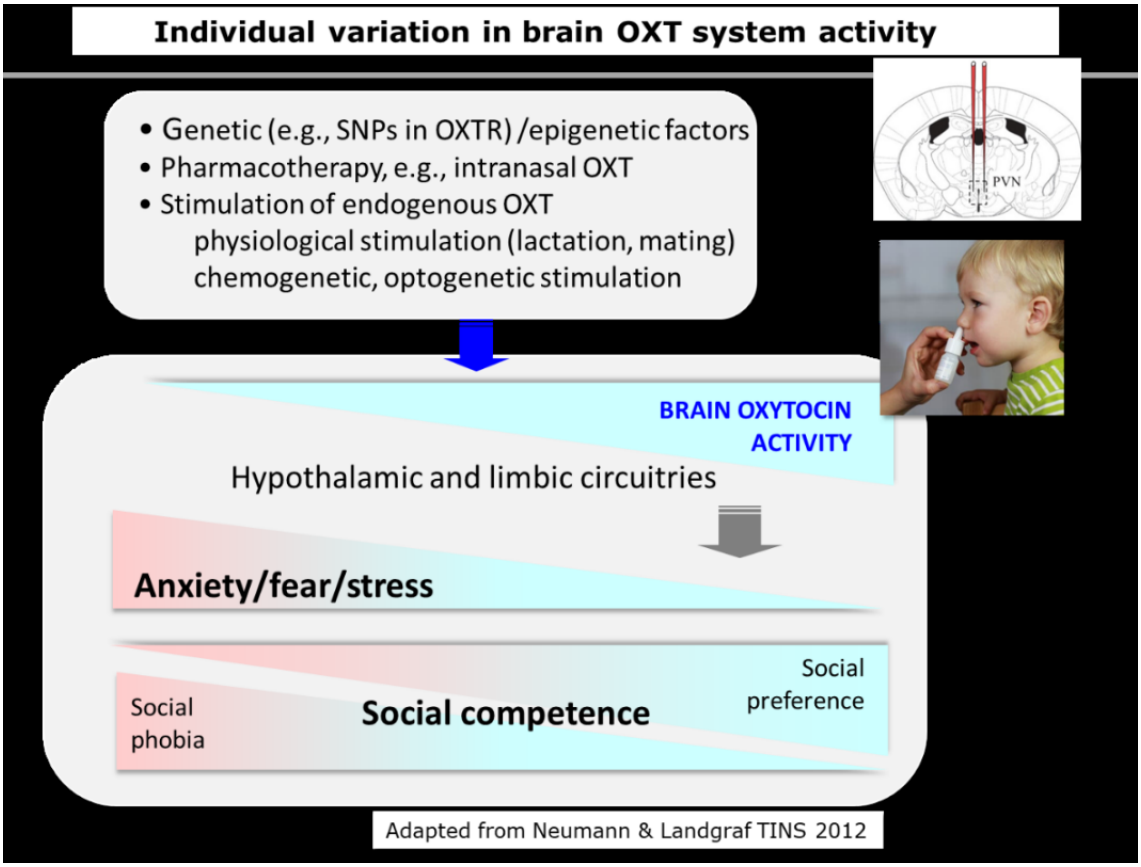


Professor **Marian Jöels** (Utrecht University) provided the concluding lecture of the day, the **Lay Lecture** entitled *"The brain after acute stress"*, in which she summarized the scientific evidence from both clinical and animal studies that resilience to stress is an underexplored area of research that may have significant relevance to human disorders such as anxiety, post-traumatic stress disorder, and affective illnesses such as depression in relation to early life stress. Notably, a large amount of clinical and preclinical research is directed at examining the effect of chronic stress and its suspected detrimental effects, but in important complimentary studies, Prof Jöels' laboratory has obtained substantial data supporting the idea that the acute stress response (with arousal lasting for up to two hours, and the release of catecholamines followed by corticosteroid hormones) can be a helpful process for identifying a correct 'survival'/escape strategy and for promoting advantageous cellular and neuroendocrine signaling over different time scales that assist with long-term survival in similar contexts. In her talk, Prof Jöels described in detail the mosaic of responses that have been discovered using cell-, tissue-, and animal-based, and human studies of key stress peptide, transmitter and hormone actions. These include studies that reveal that cell activity and key neural circuits are very differently affected by often interacting stress mediators on a scale of minutes to hours. In general, directly after stress, arousal is increased through activation of the amygdala, which promotes the use of simple cognitive strategies involving the striatum, and thereafter with a delay of approximately one hour, these systems are suppressed and higher cognitive areas such as the hippocampus and prefrontal cortex become active, which helps to rationalize and contextualize stress-related information.

https://www.rug.nl/staff/m.joels/

The **Chen Institute Plenary Lecture** by Professor **Inga Neumann** (University of Regensburg) entitled *"Still more to learn about the brain oxytocin system in the context of socio-emotional behavior"* reiterated the Day 1 Theme Discussion notion that not only do "peptides work in cohorts" to meet physiological drives like hunger, thirst, reproduction, etc., but individual regulatory peptides also have broad functions in coordinating

related behaviors. Thus, oxytocin acts throughout the brain, at distinct synapses and loci, to mediate a complex suite of responses that have in common the regulation of social behavior. The intense evolutionary specialization of oxytocin to interorganismic interaction (social behavior), from voles to primates, indicates that we still have much to learn about the actions of oxytocin and other neuropeptides based on the 'Rosetta stone' of conserved function within complex organisms and their behaviors.
In her lecture Prof Neumann briefly reviewed the long history of oxytocin research highlighting the seminal contributions of Nobel Laureate Vincent du Vigneaud and Bruce Merrifield for the discoveries of the structure and the synthesis, respectively, of oxytocin, and other prominent neuroendocrinologists, physiologists and peptide chemists (including De Wied, Young, Russell, Leng, Pittman and Manning). She reviewed the major findings of her own preclinical research on the importance of oxytocin levels and signalling for the control of anxiety and social interaction and its likely involvement in the pathology of social withdrawal, phobia/fear and excessive aggression. She also discussed the role of oxytocin release in priming of mating and related anxiolytic actions that are associated with activation of the oxytocin receptor within the paraventricular nucleus of the hypothalamus. Prof Neumann highlighted the huge increase in the number of human studies with oxytocin over recent years and described her own examination of the dynamics of saliva oxytocin levels in a range of human subjects including choir and solo singers in relation to socio-emotional behavior. Notably, Prof Neumann examined the complex biology associated with acute vs chronic oxytocin treatment and links to reduced and increased anxiety, respectively, and the need for further studies of this key regulatory peptide and its physiological and therapeutic actions.

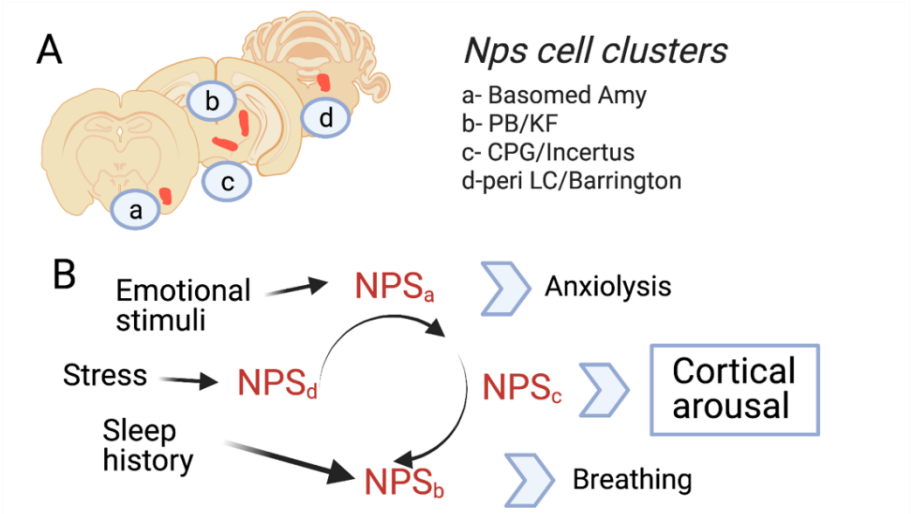


Prof Neumann is exploring the hypothesis that the activity of the brain oxytocin system, which can be modified by various genetic, epigenetic factors, pharmacotherapy or the physiological state, determines the individual level of state anxiety, fear and stress, but also of social competence, which can span from social preference behavior in rodents to social fear.

https://www.uni-regensburg.de/biologie-vorklinische-medizin/neurobiologie-tierphysiologie/team-mitarbeiter/prof-dr-inga-d-neumann/index.html

Professor **Luis de Lecea** (Stanford University) spoke about research conducted mainly by postdoctoral fellow Chris Angelakos in his laboratory on *"Neuropeptide S: Five neuronal clusters, one function?"* Prof de Lecea reviewed the importance of neuropeptides, including hypocretin/(orexin), as the orchestrators of sleep, and the nature of the brain "arousal spectrum" spanning sleep and baseline and enhanced arousal, as well as hyperarousal observed during severe stress and addiction. He discussed how his and others landmark

studies to understand the role of the hypocretin/Hcrt1/2 receptor system in arousal/sleep and narcolepsy provided a template for a similar comprehensive characterization of the NPS/NPSR system. Notably, Prof de Lecea described recent studies using a novel transgenic, NPS Cre knock-in mouse line that has enabled his team to identify and characterize prominent clusters of NPS neurons in the brainstem peri-locus coeruleus and Kölliker-Fuse nuclei, and in the dorsomedial thalamus. He reported that in vivo monitoring of NPS neuron populations revealed an increase in their activity immediately preceding transitions to wakefulness, and a robust decrease in activity during REM sleep. He also highlighted that sustained chemogenetic activation of the Kölliker-Fuse NPS neurons significantly reduced REM sleep time and altered several respiratory parameters indicative of increased breathing rate. Finally some exciting new data were presented on the use of focused ultrasound as a non-invasive way to alter the activity of brain circuits, which should be a valuable method for future studies of key arousal systems, including NPS.



A. Clusters of NPS cell bodies identified by reporter expression in NPS-cre-tdTomato mice.

B. Schematic of the possible configuration of NPS clusters regulating anxiety, cortical arousal and breathing in response to arousing stimuli.

https://med.stanford.edu/delecea/home.html

Professor **Gil Levkowitz** (Weizmann Institute of Science) discussed *"What makes some individuals fitter than others: The developmental underpinnings of stress resilience"*, Prof Levkowitz described seminal work in zebrafish, which, like mammals, possess a complex neuroendocrine system that guides behavior, but which is much more genetically tractable for systematic genetic studies of the underpinnings of the stress response. His talk featured the identification of a suite of inflammatory genes that are induced by stress during development, and that can later affect behaviors in the adult.
Prof Levkowitz reminded the audience of the historical definition of stress attributed to Hans Selye (1936) and the nature of the major HPA neuroendocrine axis activated by stress; and then described the characteristic "bottom-diving" and "darting" responses of zebrafish to stress. He then defined resilience as the ability to rebound effectively from stressful situations and raised the question of whether resilience is established as a trait during development or acquired later in life. Notably, Prof Levkowitz described a series of innovative behavioral assays used to assess responses to different stressors such as netting and osmotic stress (50% seawater), including 96-well formats for high-throughput. His studies suggest that resilience is an inheritable and persistent trait that can passed on to subsequent generations; and his team's molecular studies revealed transcriptome changes in resilient zebrafish that demonstrate a specific profile of neuropeptides expression in resilient larvae, and that the innate immune complement cascade was downregulated in resilient larvae in response to stressors. Lastly, he demonstrated how pharmacological inhibition and genetic deletion of critical complement factors led to an increase in resilience.

https://www.weizmann.ac.il/mcb/GLevkowitz/home

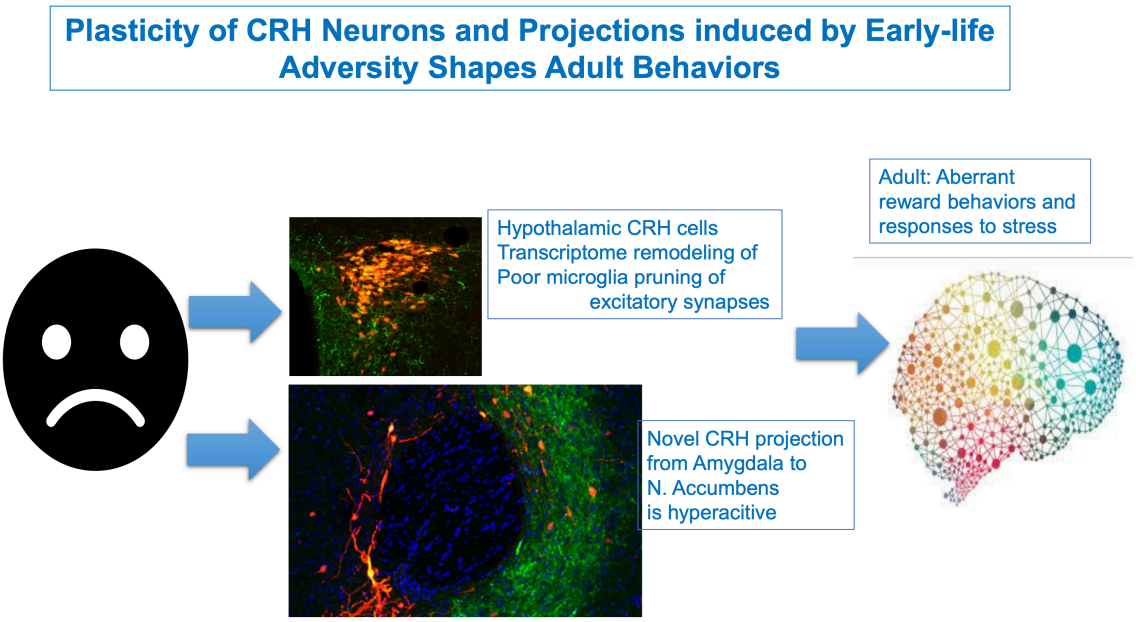
Professor **Peter Goadsby** (Kings College London), the 2021 Brain Prize Laureate for his research on CGRP as a treatment for migraine spoke on *"A long research and translational arc to CGRP-based treatment of migraine"*. Prof Goadsby described the timeline of how both small-molecule drugs, and antibody-based

treatments have brought significant relief for migraine sufferers through a decades-long process of understanding the role of regulatory peptides such as CGRP in the etiology and pathology of migraine, a debilitating cerebrovascular condition affecting many millions worldwide. He illustrated the application of this understanding to practical treatments with drugs and biosimilars that are used both preventively and prophylactically for this disorder. Prof Goadsby's talk highlighted an important theme of RegPep24 that the 'long research and translational arc' to the development of effective treatments is not always a unidirectional one, but involves applying basic knowledge to clinical practice, and then 'reverse engineering' the most effective treatments in additional rounds of basic research in order to more precisely treat the disorder with effective treatments and minimal side-effects.

https://www.kcl.ac.uk/people/peter-goadsby

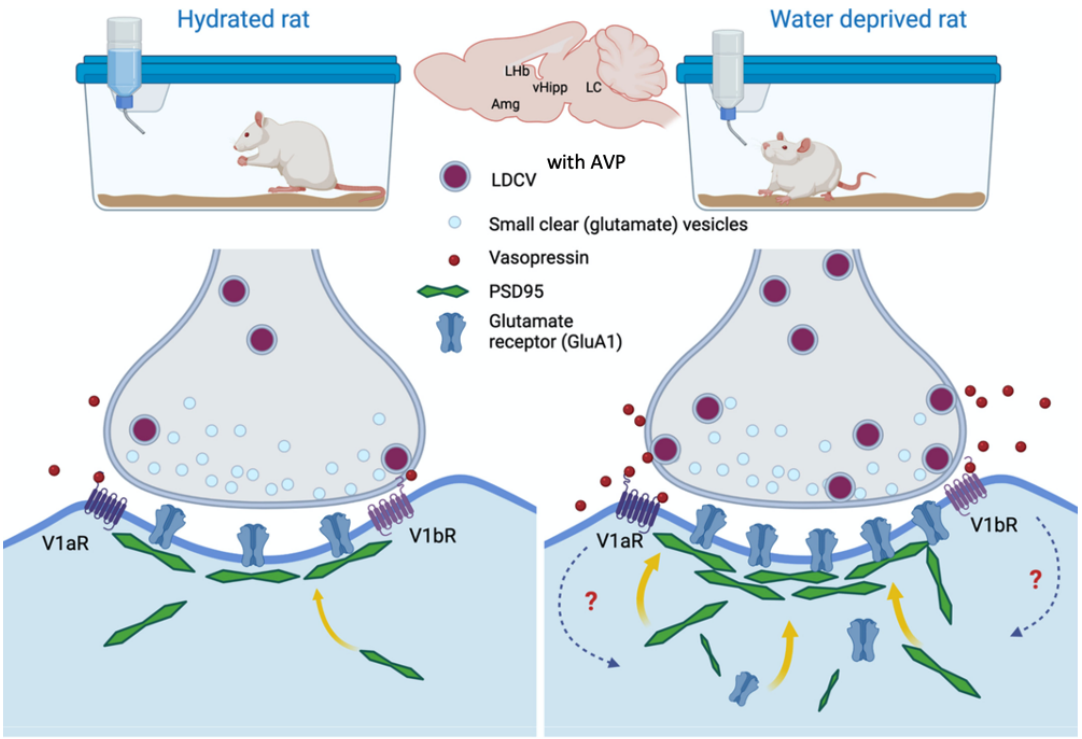
Keynote Lectures

Professor **Tallie Baram** (University of California Irvine), speaking on *"Plasticity of the CRH stress circuitry to early-life adversity"*, emphasized the effects of early-life adversity on adult stress responding, using a model in which maternal care of mouse pups is fragmented by a chronic lack of bedding materials. This seemingly 'mild' stressor is conveyed to pups, despite their growing to otherwise apparently healthy adults, in changes in stress circuitry during development that profoundly affects later responses to stress, eroding stress resiliency. The issue of stress and later resilience or vulnerability based on the level/degree of environmental stress, is particularly relevant to the public health sphere in modern life, and Prof Baram's work underscores how a deeper understanding of neuroendocrine stress mechanisms can generate both markers to identify at-risk populations, and potential therapeutics for treatment of stress-vulnerable individuals.



https://contecenter.uci.edu/

Professor **Limei Zhang** (National Autonomous University of Mexico) spoke about *"Placing neuropeptide signaling within glutamate/GABA contexts"*, highlighting that neuropeptides are invariably co-secreted, as hormones and neurotransmitters, by neurons and neurosecretory cells that also release the classical transmitters, GABA and glutamate. Thus, a full accounting of how neuropeptides act in the brain must be accompanied by an understanding of whether they are contained in so-called 'excitatory' (glutamatergic) or 'inhibitory' (GABAergic) neurons. This theme was illustrated by a description of vasopressin magnocellular neurons, co-expressing glutamate, and PACAP neurons also co-expressing glutamate, that function together in a neural circuit mediating thirst-induced changes in social behavior involving a newly identified pathway from the supraoptic nucleus of the hypothalamus to a 'social control hub' within the more rostral nucleus of the lateral olfactory tract. Prof Zhang also emphasized that by their ability to control gene expression, neuropeptides such as vasopressin can act as long-term 'synaptic organizers' within specific projection areas of the brain, and well as acting as hormones and paracrine factors elsewhere.

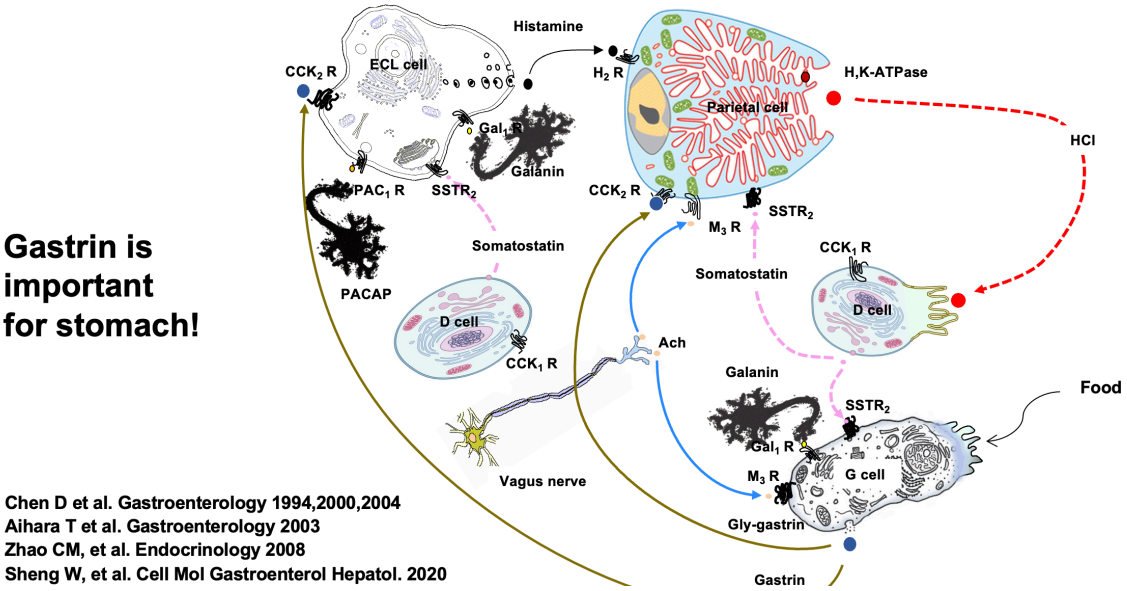


Water deprivation *in vivo* and vasopressin application *ex vivo*, increase the expression of postsynaptic density proteins PSD95 and GluA1 in limbic regions

https://fisiologia.facmed.unam.mx/index.php/pagina-zhang-ji-limei/

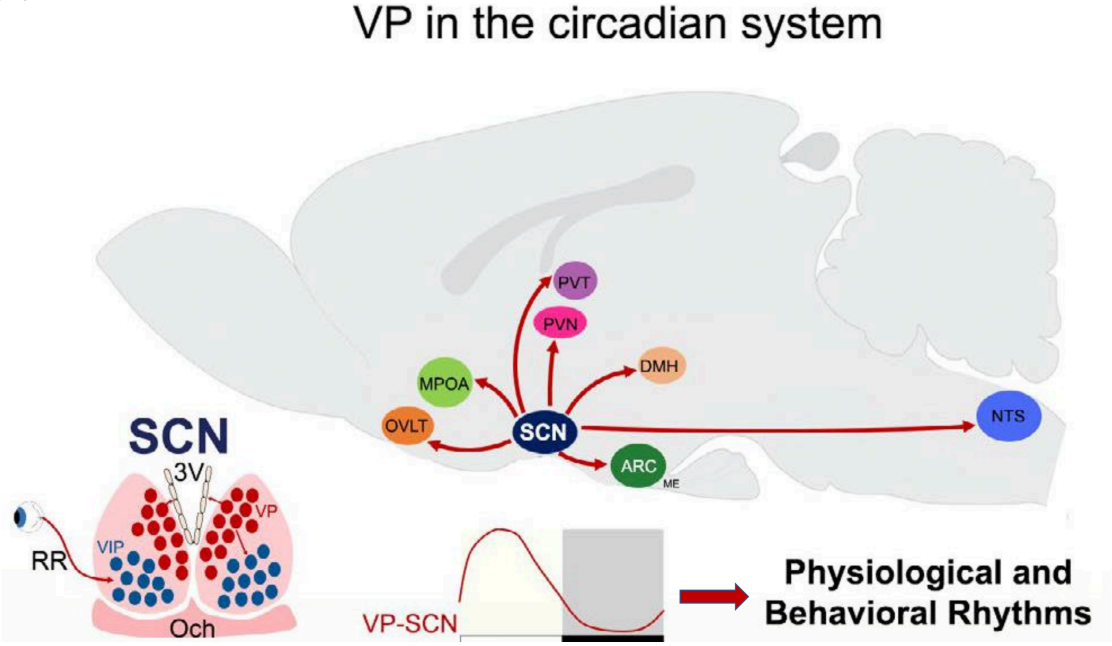
Professor **Duan Chen** (Norwegian University of Science and Technology), concluded the first session of keynote lectures with his talk on *"Peptides and the gut: the unfinished story of gastrin"*, a retrospective on gastrin, first discovered as a gut hormone regulating gastric acid secretion, and later revealed to be a pleiotropic hormone/paracrine factor affecting a wide variety of physiological functions. In this respect, gastrin is a paradigm for the emerging picture of regulatory peptides having multiple functions at multiple loci in vertebrate physiology. This underlines the challenge, in human peptide-based therapy, to find specificity for the treatment of organ-specific disease among pleiotropic regulatory peptides, by parsing the specificity of cell signaling, receptor subtypes, differential pharmacodynamics and pharmacokinetics, and biased signaling at regulatory peptide receptors.

Prof Chen provided an informative history of gastrin biology which began in 1905 with Edkins and was expanded in the 1960's by Tracy and Gregory, who demonstrated physiological properties of synthetic peptides structurally related to gastrin, and by the identification of the gastrin receptor (also called the CCK-B or CCK-2 receptor), which was cloned and characterized by Kopin and colleagues in 1992. Prof Chen emphasized the importance of gastrin for stomach function via gastrin receptors on ECL cells and neck-zone proliferating stomach cells, and the ability of gastrin to directly (and indirectly) stimulate acid secretion. He also briefly discussed the possible role of gastrin in bone, and discussed in detail the complex biology and clinical evidence that suggests that gastrin is a potential oncopeptide. He noted that the unfinished story regarding gastric carcinogenesis, should be completed through the systems approach consisting of three components, i.e., elements (peptides, receptors and cells, etc.), processes (signaling pathways and metabolic reprogramming) and analysis (by association, intervention and counterfactuals to identify a causal relationship).



https://www.ntnu.edu/ikom/pharmasurg#/view/about

Professor **Andries Kalsbeek** (Netherlands Institute for Neuroscience/University of Amsterdam) spoke on *"Vasopressin neurons in the suprachiasmatic nuclei (SCN): critical signalling inside and outside the biological clock.* Circadian rhythms are a major theme of regulatory peptide actions, their uncanny dual functions in mediating precise hormonal homeostatic functions (e.g., vasopressin regulation of the osmoskeleton) and simultaneously participating in whole-organism allostasis, including cycles of arousal and sleep, and their coordination with anticipatory eating/drinking behaviors.
Prof Kalsbeek provided an excellent background on the anatomical projections of AVP neurons in the suprachiasmatic nucleus (SCN) and the nature of their rhythmic activity during the day/night cycle. He detailed actions of these key neurons on levels of cortisol/ corticosterone via actions within the PVN region, and the LH surge via effects on kisspeptin (not GnRH) neurons in the medial preoptic area. He also detailed studies that have identified how AVP influences body temperature via interactions with α-MSH in the arcuate nucleus, and 'anticipatory thirst' prior to sleep. Prof Kalsbeek reported on studies in whole-of-life and conditional receptor knockout mice to identify the role of specific vasopressin receptors in regulating circadian rhythms and the expression of 'jetlag'. Lastly, he described the impact of cold and hunger on rhythms and AVP neurons in the SCN, highlighting the flexibility of the biological clock under different environmental conditions. (Note, the topic of Prof Kalsbeek's lecture was different to that described in the Conference Proceedings).



From Buijs et al., JNE, 2021

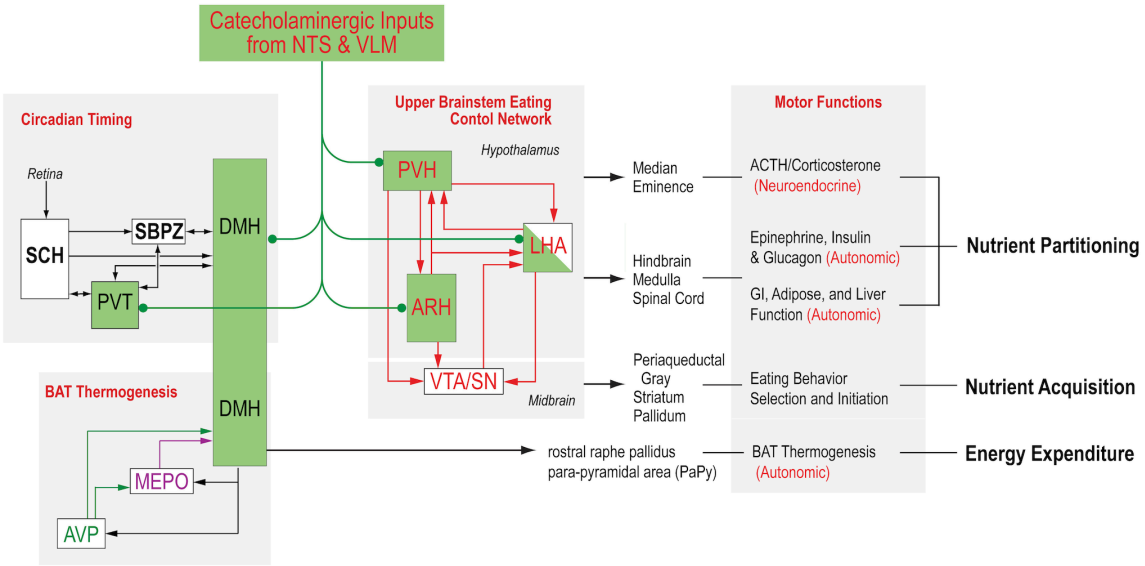
https://www.amsterdamumc.org/en/research/researchers/andries-kalsbeek.htm

Professor **Patrick Sexton** (Monash University) spoke on *"Understanding the structure, ligand-binding and function of family B G protein-coupled receptors"*. Prof Sexton's talk focused on the family-B G-protein-coupled receptors for calcitonin/CGRP/amylin, glucagon/GLP-1 and PACAP/VIP, and the structural basis for their specificity of both ligand recognition, and signaling. A key message was that static views of receptor-ligand conformations derived from x-ray crystallography and more recently, cryo-EM, need to be supplemented by more dynamic depictions of the processivity of ligand-receptor interactions leading to receptor activation and G-protein engagement and activation. The initial expectation that structural analysis would reveal exactly where ligands bind to receptors, and hence provide insights into how to design small molecules for precise placement at key receptor locations, has had to be modified in light of recent findings. Thus, while detailed structural analysis has been quite useful for explaining where existing small-molecule drugs are likely to position themselves to affect receptor activation or inhibition, it has been less useful in the design and prediction of new drugs. This may be in part because of the inherent dynamism of ligand-receptor binding in particular of large, flexible peptides to progressively available docking sites on their receptors, followed by stepwise/concerted additional mutual conformational adjustments. Thus designing and building activators and inhibitors for peptide receptors is a more complex process than previously envisaged.

https://research.monash.edu/en/persons/patrick-sexton

Professor **Alan Watts** (University of Southern California) discussed "*Brain neuropeptidergic networks and the control of energy balance"* in particular the role of hypothalamic systems in controlling energy balance through the complex interplay between the control of feeding (hunger, satiety, food reward, anticipatory feeding) and metabolism (temperature regulation, glucose homeostasis, lipolysis, etc.). Unraveling multi-peptide control of overlapping homeostatic and allostatic mechanisms driving the 'caloric economy' clearly will form the basis for treatment of obesity, cachexia and other feeding disorders, much as promising multi-peptide approaches are emerging for treatment of diabetes, as discussed by Prof Rorsman in his lecture.

Brain Neuropeptidergic Networks and the Control of Energy Balance

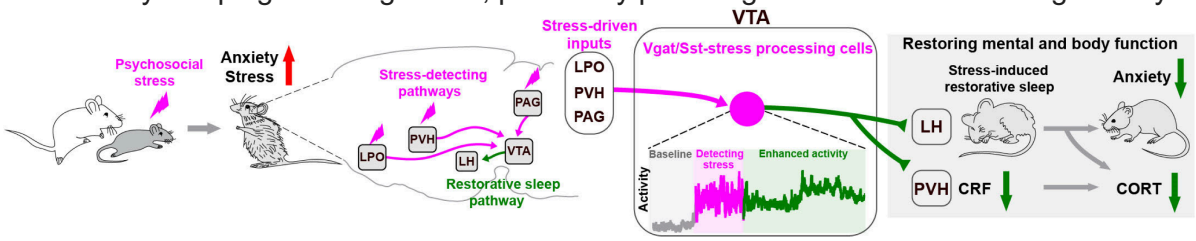


ARH, arcuate nucleus; AVP, anteroventral preoptic nucleus; DMH, dorsomedial nucleus; LHA, lateral hypothalamic area; MEPO, median preoptic nucleus; PVH, paraventricular hypothalamic nucleus; PVT, paraventricular thalamic nucleus; SCH, suprachiasmatic nucleus; SBPZ, sub-paraventricular zone; VTA/SN, ventral tegmental area/substantia nigra.

https://dornsife.usc.edu/cf/faculty-and-staff/faculty.cfm?pid=1003813

Professor **William Wisden** (Imperial College London) spoke about *"Peptides and sleep-promoting circuitry"*, providing a brief overview of the brain circuitry that governs sleep and noting that neurons that control sleep are scattered throughout the brain and that they contain a large number of small molecule neurotransmitters including glutamate, GABA and various amines and gaseous transmitters (nitric oxide), and that many of these neurons, express and release peptides such as somatostatin, galanin and neurotensin. He also highlighted a point common to many research areas involving peptides that the majority of the sleep field has

tended to focus on peptide expression as a tool for genetic manipulation of these neurons, rather than study the potentially essential roles of the peptides themselves in the control of sleep. Prof Wisden then outlined his team's recent innovative research that enabled them to discover that a population of somatostatin/GABA neurons in the ventral tegmental area (VTA) of mice are responsible for inducing a specific type of sleep after social defeat stress, and that this sleep aids recovery from stress. He also described how these VTA somatostatin neurons inhibit release of the stress-related peptide, corticotropin-releasing factor (CRF) in the paraventricular hypothalamic nucleus. These novel findings, using an innovative combination of genetic, molecular and physiological methods suggest that a specific circuit allows animals to restore mental and body functions by sleeping following stress, potentially providing a new route for treating anxiety disorders.



https://www.imperial.ac.uk/people/w.wisden

Professor **Francesco Ferraguti** (Medical University of Innsbruck) spoke about *"Metabotropic glutamate receptors' role in cortical peptide-expressing interneurons"*, focusing initially on the role of specific metabotropic glutamatergic receptors in modulation of the tone and function of inhibitory neurons that ultimately control activity of pyramidal and other excitatory projection neurons whose output results in neuronal excitation mediated directly by ionotropic glutamatergic receptors. Neuropeptides are currently principally used as 'markers' to gain genetic access to these neuronal subpopulations. Prof. Ferraguti went on describing the critical role that one of these receptors co-expressed with the neuropeptide somatostatin has on emotional behavior homeostasis. In particular, he reported dysfunctions in the negative valence domain (e.g. anxiety) that were region and sex-specific. How and when, during development and in the adult, the neuromodulatory function of neuropeptides function is influenced by blocking the signaling of these metabotropic glutamate receptors to alter behavior is clearly a research area requiring further investigation.

https://www.i-med.ac.at/pharmakologie/forschung/research_ferraguti.html