

24th International Symposium on Regulatory Peptides

RegPep24

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Welcome note from the IRPS President

Dear members of IRPS and other colleagues and friends,

It is an honor to welcome you to RegPep24, the 24th International Conference on Regulatory Peptides, under the auspices of the International regulatory Peptide Society (IRPS).

RegPep24 is our first in-person meeting in quite a while. We intend that it should be memorable, and the program indicates that it will be. In addition to reports of progress manv areas of peptide physiology, receptor biology. in neuroendocrinology, neuroscience, circadian biology, and behavioral neuroscience. RegPep24 is scheduled to allow very deliberate discussion of the major questions that confront the field, how progress is limited by specific gaps in knowledge or technology, and how recent breakthroughs can be leveraged by the field as a whole to impel more rapid progress in regulatory peptide research and its application to human health. This year, the IRPS has been fortunate to receive support from IBRO. Journal of Neuroendocrinology, the International Neurochemistry Society, and the Chen Institute to achieve two major goals: the participation of early career scientists and the promulgation of our proceedings both through a Special Issue of Journal of Neuroendocrinology and a review of RegPep24 that will be prepared by Chen Science Writer Andrew Gundlach for posting at the Chen Institute's Neuroscience website, which aims to make rapidly available breakthrough information featured at scientific conferences, for rapid dissemination throughout the scientific community.

More importantly, we encourage all participants to take full advantage of the opportunities simply to interact: to debate, discuss and digest new information, to make new connections with students, with mentors, and with peers, to develop fruitful collaborations, and to continue to enjoy our science as a personally fulfilling activity in an interpersonal space.

Welcome!

Limei Zhang, MD, PhD August 1st, 2022

Organizing Committee

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Preface

Seventy-eight speakers and presenters from more than seventeen countries will join together, with other participants, for the 24th International Conference on Regulatory Peptides. For newcomers-and the IRPS is indeed growing-we point out some of the traditions of the biennial RegPep international conference. At each, the Victor Mutt Lectureship is awarded, and the Victor Mutt Lecture given. The list of awardees is now rather long, and is illustrious: each is a mid-career investigator has contributed to the field of regulatory peptide research in a fundamental way, reminiscent of that of Victor Mutt, with the discover of so many important regulatory peptides that is each, today, a 'sub-realm' of research in our field. This year's Mutt Lecturer, Dr. Xiaoke Chen, will be describing the roles of neuropeptides that, within the brain, are important in sculpting the sensory pathways that convey the sensation of pain. The Mutt Lecture will be fittingly book-ended by our closing lecture by Peter Goadsby, who first hypothesized that, outside of the brain, migraine pain might arise in part from neuropeptide neurotransmission within the trigeminal ganglion, leading to the development of CGRP-based treatment for this condition. Plenary lectures by Marion Joëls on the regulation of acute stress, by Patrik Rorsman on peptides regulating glucagon, and by Jerome Swinny on the regulation of neuronal excitability by amyloid peptides all converge on the theme of how peptides work in concert with both classical messengers and other peptides to exert a 'controlling interest' in physiological processes, and therefore can provide therapeutic avenues to correcting pathophysiological ones. The most general theme of RegPep24, and indeed a raison d'etre for RegPep conferences since their inception, is the integration of basic science, from structural studies of peptide-liganded receptors and peptide signaling for information flow in endocrine, neuroendocrine, neuronal and immune systems, and its translation to emergent therapeutics. This year's conference illustrates in many respects that this integration is not unidirectional, but that there is a constant volleying of new basic information, clinical application, barriers to implementation that raise new basic questions, and re-application to therapeutics, leading ultimately to improved public health. RegPep24 includes explicit scheduling that will allow discussion of how progress in each 'peptide-specific' area of inquiry provides key guideposts to progress in others, as the pace of regulatory peptide research accelerates vearly.

Lee E. Eiden Chair, Program committee of RegPep24

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RegPep24: Monday, August 1st, 2022



Inaugural Ceremony Cottrell Lecture Theatre

Welcome (15:00-15:15) Research and public health: The university and the world

> Malcolm Macleod Senior Deputy Principal, University of Stirling

> > 00000

Inaugural Plenary Lecture (15:15-16:00)

Peptidergic regulation of glucagon secretion: implication for diabetes pathophysiology and therapy

Patrik Rorsman Radcliffe Department of Medicine, Oxford University, UK

Musical Interlude (16:00-16:15)

Victor Mutt Lecture (16:15-17:00)

Peptidergic descending control of pain

Xiaoke Chen Department of Biology, Stanford University, CA, USA

Musical Interlude (17:00-17:15)

Lay Lecture (17:15-18:00)

The brain after acute stress

Marian Joëls Faculty of Medical Sciences, Groningen, The Netherlands

Musical Postlude (18:00-18:15)

Welcome Reception and Dinner (18:30-21:30) Abbeycraig Hall

RegPep24: Tuesday, August 2nd, 2022

Keynote symposium 1 (KS1: 08:00-10:00): Chair: Luis de Lecea, USA, Room: Wallace Monument

KS1-1: Tallie Z. Baram, University of California at Irvine, CA, USA:

Plasticity of the CRH stress circuitry to early-life adversity

KS1-2: Limei Zhang, National Autonomous University of Mexico, Mexico

Placing neuropeptide signaling within glutamate/GABA contexts: Extrahypothalamic synapses of magnocellular vasopressin neurons
and their connections to behavioral circuits

KS1-3: Duan Chen, Norwegian University of Science and Technology, Norway

· Peptides and the gut: the unfinished story of gastrin

Coffee and cookies break (10:00-10:30)

Symposium 1: ISN sponsored young investigator symposium: Oxytocin and Vasopressin: Circuits and Behavior Chaired by Vito Hernandez, México

10:30-12:30: Room: Wallace

S1-1 Let Xiao, Fudan University, China: Morpho-electric properties and diversity of oxytocin neurons in mouse paraventricular nucleus of hypothalamus

S1-2 Quirin Krabichler, Heidelberg University, Germany: A novel transgenic arginine vasopressin (AVP)-IRES2-Cre rat line: Characterization, mapping of connectivity, and recording of neuronal activity

S1-3 Amelie Soumier, Center National de la Recherche Scientifique, France: 3D mapping of oxytocin and vasopressin neuronal ontogenesis in the mouse brain

S1-4: Oscar R. Hernández-Pérez, CIATEJ, Jalisco and UNAM, México. Differential activation of arginine-vasopressin receptor subtypes in distinct amygdalold subdivisions modulating emotional responses in the rat by argininevasopressin

S1-5 Mario Zetter, School of Medicine, National Autonomous University of México: Vasopressin acts as a synapse organizer in limbic regions by boosting PSD95 and GluA1 expression Symposium 2. Critical Pathways for Peptide-based Drug Development: Recent Progress Chaired by: Esther Sabban, USA

10:30-12:30: Room Erskine Fintry

S2-1 Esther Sabban, New York Medical College; USA: Preclinical studies in a PTSD model with intranasal NPY in males and females

S2-2 Youssef Anouar, INSERM U1239 & Normandy University Rouen, France: The antioxidant selenoprotein T mimetic, PSELT, exerts a potent neuroprotective effect in PD after intranasal administration.

S2-3 Peter Holzer, Otto Loewi Research Centre, Medical University of Graz, Austria: NPY in gut-brain communication

S2-4. Chun-Mei Zhao, Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Norway: Repurposing peptide-based drugs for treatment of gastric cancer: a proof of concept

Box lunch and posters viewing + DB supported by IBRO+JNE (12:30-13:30) (Room Silver Glen)

Symposium 3 Novel Aspects of Neuropeptides and Behaviour Chaired by: Arpad Dobolyi, Hungary

13:30-15:30: Room: Wallace

S3-1. 1 Árpád Dobolyi, Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary: A new peptidergic thalamo-preoptic pathway promoting positive valence physical contact

S3-2 Tim Viney, Department of Pharmacology, University of Oxford, UK: Neuropeptides and rhythmic neuronal firing in brain networks

S3-3 Chun-Xia Yi, Amsterdam University Medical Centre, Department of Endocrinology and Metabolism, The Netherlands: Regulatory peptides and microglial immunometabolism in hypothalamic regulation of feeding behaviour

S3-4 Kristof Laszlo, Neuroscience Center, University of Pécs, Hungary: Intraamygdaloid oxytocin reduces anxiety in valproate induced autism rat model Symposium 4. Peptide Interactions Chaired by: Greti Aguilera, USA

13:30-15:30: Room Erskine Fintry

S4-1. Marisela Morales, NIDA/NIH, USA: Fast and slow cotransmission and their role in reward and cocaine-seeking behavior

S4-2. Annette D. de Kloet, University of Florida, USA: Angiotensin-vasopressin interactions and blood pressure regulation

S4-3. Jeff Jones, Texas A&M University, USA: Circadian neurons in the paraventricular nucleus entrain and sustain daily rhythms in glucocorticoids

S4-4. Becky Conway-Campbell, Medical School, University of Bristol, UK: Phase-shifting the circadian glucocorticoid profile induces disordered feeding behavior by dysregulating hypothalamic neuropeptide gene expression.

Coffee and cookies break (Wallace & Monument rooms merged) (15:30-16:00)

Theme discussion. Panelists: plenary & keynote speakers. (16:00-17:00)

Chen Institute Plenary Lecture (17:00-18:00): <u>Room: Wallace Monument</u> Inga Neumann, University of Regensburg, Germany: Still more to learn about the brain oxytocin system in the context of socio-emotional behaviour

Free evening

RegPep24: Wednesday, August 3rd, 2022

Keynote Symposium 2 (KS2: 08:00-10:00): Chair: Geert de Vries, USA, Room: Wallace Monument

KS2-1: Andries Kalsbeek, the Netherlands Institute for Neuroscience (NIN), The Netherlands

Vasopressin neurons in the suprachiasmatic nuclei (SCN): critical signalling inside and outside the biological clock

KS2-2: Bice Chini, CNR Neuroscience Institute, Vedano al Lambro, Italy.

• Oxytocin receptor in neurodevelopmental disorders: sex and age-dependent regional distribution and modulation

KS2-3: Patrick Sexton, Monash University, Australia

• Understanding the structure, ligand-binding and function of family B G Protein-coupled receptors

Coffee and cookies break (10:00-10:30)

Symposium 5 Neuropeptides and Regulation of Homeostasis and Allostasis Chair: Robert Millar, South Africa 10:30-12:30: Room: Wallace

S5-1 Dave Grattan, University of Otago, New Zealand: Modulation of complex neuronal circuits by peripherally-derived peptide hormones

S5-2 Aras Petrulis, Georgia State University, USA: Sexually differentiated vasopressin pathways: connective architecture and role in social behavior

S5-3 Carolina Escobar, School of Medicine, UNAM, Mexico: *Peptides involved in food anticipation*

S5-4 **Ruud Buijs**, Institute of Biomedical Research, UNAM, Mexico: Suprachiasmatic nucleus-driven vasopressin release prepares for the inactivity period Symposium 6

Ghrelin and Related Peptides: Crossing Many Barriers Chair: Patrick Sexton, Australia

10:30-12:30: Room Erskine Fintry

S6-1 Mitchell Ringuet, The University of Melbourne, Australia: Ghrelin receptor, GHSR1a: emerging evidence as a GPCR modulator

S6-2 Ki Goosens, Icahn School of Medicine at Mount Sinai, USA: The ghrelin system as a driver of heterogeneity in psychiatric disease

S6-3 Sebastian G.B. Furness, Faculty of Medicine, University of Queensland, Australia: *The physiological role for modulation of transducer coupling at GHSR1a and other peptide-liganded GPCRs*

S6-4 Sarah Melzer Medical University of Vienna. *Neuropeptidergic* modulation of fear memories in the auditory cortex

Box lunch and posters viewing + DB supported by IBRO+JNE (12:30-13:30) (Room Silver Glen)

Symposium 7 The physiology and function of hypothalamic magnocellular neurons Chair: David Murphy, UK 13:30-15:30: Room: Wallace

S7-1 André Mecawi, Federal University of São Paulo, Brazil: Dissecting the molecular profile of the hypothalamic magnocellular neurones: a MultiOMIC journey

S7-2 Tom Cunningham, The University of North Texas Health Science Center at Fort Worth, USA: Sex Differences in Neurohypophyseal Hormone Release in a Model of Dilutional Hyponatremia

S7-3 Ryoichi Teruyama, Louisiana State University, Louisiana, USA: Sexually Dimorphic Expression of Oxytocin Receptors in the CNS

S7-4 Soledad Bárez-López, Medical School, University of Bristol, UK: Shining light into the role of a non-visual opsin in the supraoptic nucleus Symposium 8 PACAP and related peptides in central and peripheral regulation of stress responses Chair: Lee Eiden, USA <u>13:30-15:30: Room:Erskine Fintry</u>

S8-1 Sarah Gray, University of Northern British Columbia, Canada: PACAP expression in central and peripheral neuronal networks regulating adipose tissue

S8-2 Sunny Z. Jiang, NIMH-IRP, NIH, USA: Prefrontal corticohypothalamic and parabrachio-extended anygdalar PACAPergic projections separately control HPA and food intake responses to psychogenic stress

S8-3 Arun Anantharam, University of Toledo, USA: PACAP and acetylcholine regulate distinct calcium responses and secretory outputs in chromaffin cells

S8-4 Vito S. Hernandez, UNAM, México: PACAP co-expression in GABAergic or glutamatergic circuits and its relevance for behavioural adaptation.

Coffee and cookies break (Wallace & Monument rooms merged) (15:30-16:00)

Theme discussion. Panelists: plenary & keynote speakers (16:00-17:00)

Plenary IBRO Sponsored Lecture (17:00-18:00): <u>Room: Wallace Monument</u> Luis de Lecea, Stanford University, USA

Neuropeptide S: Five neuronal clusters, one function?

Free evening

RegPep24: Thursday, August 4th, 2022

Keynote symposium 3 (KS-3: 08:00-10:00) Chair: Javier Stern, USA

- KS3-1: Alan Watts: University of Southern California, USA
 - · Brain neuropeptidergic networks and the control of energy balance
- KS3-2: William Wisden: Imperial College London, UK • Peptides and sleep-promoting circuitry

KS3-3: Francesco Ferraguti: Department of Pharmacology, Medical University of Innsbruck, Austria • Metabotropic glutamate receptors' role in cortical peptide-expressing interneurons

Coffee and cookies break (10:00-10:30)

Symposium 9 Neuropeptides, stress and sex differences Chair: Tallie Z. Baram, USA

10:30-12:30: Room: Wallace

S9-1 Joanna Dabrowska, Rosalind Franklin University of Medicine and Science, USA: It takes three to dance - neuropeptidergic modulation of the BNST activity and fear processing by oxytocin, vasopressin, and CRF

S9-2 Gil Levkowitz, Weizmann Institute of Science, Israel. What makes some individuals fitter than others: The developmental underpinnings of stress resilience

S9-3 Javier Stern, Georgia State University, USA: Novel intercellular communication modalities mediated by hypothalamic neuropeptides in health and disease states

S9-4 Geert de Vries, Georgia State University, USA: Development and function of sex differences in the brain seen from a vasopressin and oxytocin perspective Symposium 10 Vasopressinergic regulation of social behavior Chair: Dora Zelena, Hungary

10:30-12:30: Room Erskine Fintry

S10-1 Matthew Paul, University at Buffalo SUNY, Buffalo, NY, USA: Social Development and Vasopressin: Investigations into the atypical social behavior of Brattleboro rats

S10-2 Alexa Veenema, Michigan State University, USA: Neural circuitry of social play: involvement of oxytocin and vasopressin

S10-3 Dora Zelena, University of Pécs, Hungary: Vasopressinergic influence on disturbed sociability in autism and schizophrenia

S10-4 Abimael Gonzalez-Hernandez, Neurobiology Institute, UNAM, Mexico: The role of oxytocinergic neurotransmission in pain processing at trigeminal level

Box lunch and posters viewing + DB supported by IBRO+JNE (12:30-13:30) (Room Silver Glen)

Symposium 11 Molecular and structural biology of neuropeptides at their cognate GPCRs Chair: John Furness, Australia (13:30-14:30)

13:30-15:30: Room: Wallace

S11-1 Robert P. Millar, University of Pretoria, South Africa: Rescue of function in human mutant peptide GPCRs with cell permeant small molecules: a more viable approach than gene therapy

S11-2 Helene Castel, University of Rouen Normandy, INSERM U1239: Urotensin IL-based local hydrogel trap leads to immune control associated with improved survival and cognitive functions in a mouse model of glioblastoma resection Symposium 12 Neurohypophysial hormone regulation in pathophysiological states Chair: Quentin Pittman, Canada

13:30-15:30: Room: Wallace

S12-1 Andrés Quintanar-Stephano, Universidad Autonoma de Aguascalientes, Mexico: Vasopressin deficiency and Vla-V2 receptors blockade revert liver damage and fibrosis in rats with protracted liver disease: A new therapeutic approach?

S12-2 Margarita Currás-Collazo, University of California Riverside, USA: Maternal 74 supplementation normalizes deficient social behavior and reduced hypothalamic oxytocin content produced by perinatal exposure to PBDE

Visit to Stirling Castle (14:30-18:30)

Conference dinner (19:00-23:00) River House RegPep24: Friday, August 5th, 2022

IRPS General Assembly (09:00-10:00)

Cottrell Lecture Theatre

IBRO Neuroscience Special Lecture (10:00-10:45) Cottrell Lecture Theatre

Interaction of amyloid beta oligomers and alpha3-GABAA receptors in locus coeruleus neuronal excitability and Alzheimer's pathology

> Jerome Swinny University of Portsmouth, UK

Closing Lecture (10:45-11:30)

A long research and translational arc to CGRP-based treatment of migraine

Peter Goadsby

2021 Brain Prize Laureate King's College London, UK

Closing Ceremony (11:30-12:00)

Group Photo at Stirling University Campus

Welcome Lecture

Malcolm MacLeod

University of Stirling, Scotland, UK. Research and public health: The university and the world

Inaugural Plenary Lecture

Patrik Rorsman

University of Oxford, Oxford, UK. Peptidergic regulation of glucagon secretion: implication for diabetes pathophysiology and therapy

Victor Mutt Lecture

Xiaoke Chen

Stanford University, California, USA. Peptidergic descending control of pain

Lay Lecture

Marian Joels

Utrecht University, Utrecht, Netherlands. The brain after acute stress

Welcome Lecture

Research and public health: The university and the world

MacLeod, Malcolm

University of Stirling, Scotland, UK

In his capacity as Senior Deputy Principal, University of Stirling, Professor MacLeod will welcome RegPep24 participants to Stirling, and provide some thoughts about the relationship between basic and applied scientific research and the public health. Professor MacLeod is a Chartered Psychologist and holds a Chair in Experimental Psychology in the Faculty of Natural Sciences. He joined the University of Stirling in January 2015, after serving at the University of St. Andrews in several senior capacities, including Dean of the Faculty of Science, Provost of St. Leonard's College, and Vice Principal for Internationalisation and External Relations. He is an elected Fellow of the Royal Society of Arts and Commerce, an elected Fellow of the British Psychological Society, and a Fellow of the Higher Education Academy. His research focuses on the executive control of memory and particularly the relationship between remembering and forgetting. His research has attracted funding from the ESRC, The British Academy, and The Royal Society of Edinburgh. He also has interests in the civic responsibility of universities, their role in enterprise and entrepreneurship, and as drivers of regional economic growth. Professor MacLeod is the academic lead for industrial-academic collaborations that include a new Institute for Aquaculture and Global Food Security, and Scotland's International Centre for the Environment. Professor MacLeod has primary responsibility for the performance of academic departments, and leads on all aspects of equality, diversity and inclusion across the University, as well as providing institutional leadership on corporate sustainability. He has built up strong connections across the higher education sector, Scottish and UK Governments, business and industry. Professor MacLeod is currently a member of the Scottish Land Commission's Leadership Taskforce on Derelict and Unused Land, and a board member and trustee of various other companies, organisations and trusts. He is a core member of the Clackmannanshire Commission that is tasked to stimulate employment and improve the quality of life for those who live in the region. He has served as Chairman of the Marine Alliance for Science and Technology for Scotland, and MAST-S (2015-2019), and was a member of the Scottish Government's influential review on postgraduate funding.

Inaugural Plenary Lecture

Peptidergic regulation of glucagon secretion: implication for diabetes pathophysiology and therapy

Rorsman, Patrik Radcliffe Department of Medicine, University of Oxford

Diabetes mellitus is associated with impaired insulin secretion, often combined with oversecretion of glucagon. Therapeutic interventions should ideally correct both defects. Glucagon-like peptide 1 (GLP-1) has this profile but exactly how it exerts its glucagonostatic effect remains obscure. We have found that the degradation product GLP-1(9-36) shares the capacity of GLP-1(7-36) to inhibit glucagon secretion evoked by low glucose (IC50 3 pM). GLP-1(9-36) also potently inhibits glucagon secretion stimulated by β -adrenergic stimulation and amino acids. Although circulating GLP-1(9-36) levels (≥30 pM) are sufficient to strongly (70%) lower plasma glucagon in vivo, high exogenous GLP-1(9-36) results in a small additional suppression during insulin-induced hypoglycemia. GLP-1(9-36) retains its glucagonostatic effects after genetic/pharmacological inactivation of the GLP-1 receptor. In HEK293T cells expressing GCGRs and GTP-binding proteins, GLP-1(9-36) specifically leads to the dissociation of Go. In islet α -cells, GLP-1(9-36) leads to Gi/o-dependent inhibition of PKA and depletion of the docked pool of secretory granules, effects that are prevented by the GCRR antagonist REMD2.59, explaining the 280% increase in circulating glucagon produced in vivo and reversal of the GLP-1(9-36)'s inhibitory effect on glucagon secretion in vitro. We conclude that the GLP-1 metabolite GLP-1(9-36), via its glucagonostatic effect mediated by GCGR, plays a previously unrecognized role in systemic metabolism.

Victor Mutt Lecture

Peptidergic descending control of pain

Chen, Xiaoke

Department of Biology, Stanford University, CA, USA

Chronic mechanical pain caused by inflammation or nerve injury is a debilitating clinical problem and difficult to treat. Our preliminary studies established the μ -opioid receptor expressing spinal cord projecting neurons in the rostral ventromedial medulla (OPRM1+ RVMSC neurons) as a potent cellular target for treating chronic mechanical pain. Moreover, we found that nerve injury upregulate 6 neuropeptides in these neurons and demonstrated that knocking down 4 of these neuropeptide can restore normal mechanical sensitivity in animal model of neuropathic pain. Furthermore, we identified receptors of these 4 neuropeptides in the spinal cord and found knocking down their receptors can also eliminate chronic mechanical pain. Together, our research revealed important role of neuropeptides in descending modulation of pain.

Lay Lecture

The brain after acute stress

Jöels, Marian

Department of Translational Neuroscience, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands

When a human being or a rodent perceives a potentially threatening situation, a stressor, a highly conserved response is initiated. On the one hand this involves activation of the autonomic nervous system, resulting in exposure of the body and brain to catecholamines like (nor)adrenaline. And on the other hand the hypothalamus-pituitary-adrenal system, which starts with the release of corticotropin releasing hormone, eventually resulting in the secretion of corticosteroid hormones into the circulation, which feed back on the brain. Consequently, brain cells are exposed to consecutive yet overlapping waves of transmitters, peptides and steroids (1). This cocktail of stress mediators changes the activity of cells and circuits such that the individual can directly respond to the situation at hand, promoting survival in the short-term; as well as remember the information in the right context, which helps survival in the long run.

Single cell studies in the rodent brain have shown that cell activity is very differently affected by stress mediators (often in interaction with each other) at a scale of minutes compared to hours. This is also true for the circuits that are sequentially being activated e.g. after a stressful footshock (2). This sequence of circuits that is activated after stress has also been observed in the human brain, with fMRI (3). In general, directly after stress arousal is increased through activation of the amygdala, and individuals revert to simple cognitive strategies involving the striatum. With a delay of approximately one hour, these systems are tuned down in activity and, instead, higher cognitive areas such as the hippocampus and prefrontal cortex become active. The latter help to rationalize and contextualize stress-related information. These two phases of the stress response are mediated by different hormones and receptors. The acute stress response is not written in stone. Genetic background, in interaction with (early) life history, can restrict the range in which the brain can respond to acute stress. By and large, early life stress predisposes the organism's brain to a situation that resembles that seen directly after acute stress: emotional functioning is enhanced whereas higher cognitive functions (like memory of non-emotional information) are suppressed (4). We found in rodents that reversal of this process is still possible in the peripubertal period.

1. Joëls M, Karst H, Sarabdjitsingh RA. The stressed brain of humans and rodents. Acta Physiol (Oxf). 2018 Jun;223(2):e13066.

2. Bonapersona V, Schuler H, Damsteegt R, Adolfs Y, Pasterkamp RJ, van den Heuvel MP, Joëls M, Sarabdjitsingh RA. The mouse brain after foot shock in four dimensions: Temporal dynamics at a single cell resolution. Proc Natl Acad Sci U S A. 2022 Feb 22;119(8):e2114002119.

3. Schwabe L, Hermans EJ, Joëls M, Roozendaal B. Mechanisms of memory under stress. Neuron. 2022 May 4;110(9):1450-1467.

4. Bonapersona V, Kentrop J, Van Lissa CJ, van der Veen R, Joëls M, Sarabdjitsingh RA. The behavioral phenotype of early life adversity: a 3-level meta-analysis of rodent studies. Neurosci Biobehav Rev. 2019 Jul;102:299-307.

Keynote symposium 1 (KS1)

Chair: Luis de Lecea (USA)

Room: Wallace Monument

KS1-1 Tallie Z. Baram

University of California at Irvine, California, USA. Plasticity of the CRH stress circuitry to early-life adversity

KS1-2 Limei Zhang

National Autonomous University of Mexico, Mexico City, México. Placing neuropeptide signaling within glutamate/GABA contexts: Extrahypothalamic synapses of magnocellular vasopressin neurons and their connections to behavioral circuits

KS1-3 Duan Chen

Norwegian University of Science and Technology, Trondheim, Norway.

Peptides and the gut: the unfinished story of gastrin

KS1-1: Plasticity of the CRH stress circuitry to early-life adversity

Birnie, Matt, Bolton, Jessica, Short Annabel K., Baram, Tallie Z. *University of California-Irvine*

Corticotropin releasing hormone (CRH) plays several key evolutionarily conserved roles in the brain, including responses and adaptation to stress: In mammals, CRH release from hypothalamic neurons contributes to the peripheral stress response. In addition, across species, the peptide is expressed in cortical and subcortical brain regions where it plays crucial and context-dependent roles in aspects of emotion and cognition, and their adaptation to stress. Early-life adversity/stress (ELA) is prevalent throughout the world and is associated with vulnerability to mental illness and cognitive problems later in life. In animal models, ELA directly causes aberrant reward- and stress-related behaviors in a sex-specific manner. It is important to uncover the mechanisms by which transient ELA leads to enduring disruption of brain operations because these mechanisms will yield therapeutic targets. CRH neurons and projections, intrinsically sensitive to stress and acting in a neuromodulatory manner, are thus poised to mediate the enduring impact of ELA on brain circuits executing reward, fear and stress-related behaviors. We will discuss two examples of CRH circuit plasticity in the context of ELA, and the profound contribution of this peptide system to the long-lasting outcomes. First, we will focus on the canonic CRH-expressing neurons in the hypothalamus. We capitalize on the power of single-cell transcriptomics to delineate novel subpopulations of CRH neurons that are selectively vulnerable to ELA. We then demonstrate the impact of ELA on microglial pruning of excitatory synapses onto hypothalamic CRH neurons, resulting in an altered stress-circuit and aberrant neuroendocrine and behavioral stress responses.

Second, we will report on the discovery of a novel CRH-expressing amygdalanucleus accumbens projection and demonstrate, using chemo- and optogenetics, that it mediates the depressive phenotype following ELA. In both examples, we capitalize on these mechanistic insights to propose strategies for mitigating the long-lasting impact of ELA on reward and stress-related behavioral and neuroendocrine outcomes associated with human mental illness.

NIH grants MH 096889; MH73136; Hewitt Foundation for Biomedical Research

KS1-2: Placing neuropeptide signaling within glutamate/GABA contexts: Extrahypothalamic synapses of magnocellular vasopressin neurons and their connections to behavioral circuits

Zhang, Limei

Department of Physiology, School of Medicine, National Autonomous University of Mexico

In the current literature of neurobiological research, there are controversies about the mechanisms of action of neuropeptides, small protein molecules composed of 3-100 amino acid residues, in the modulation of neurotransmission. The two currents of understanding can be summarized as follows: A) neuropeptides modulate the processing of neural information exclusively through dendro-somatic release, via autocrine and paracrine mechanisms, at a short distance, to sensitize the neurons and glial, modulating their long term plasticity, without directionality; 2) neuropeptides are released from axonal terminals, over long distances, modulating glutamatergic and GABAergic neurotransmission, on postsynaptic neurons, affecting neurotransmission and short term plasticity. The general objective of this presentation is to challenge the completeness of the paradigms mentioned above and to evaluate the general prediction that the balance/dominance of the neuropeptides modulation modes (the two paradigms long vs short action on neural plasticity) is subsequently modulated by homeostatic states/stress influence of the individual, at both ligand and receptor levels, and that the resulted gen expressions related to the cell excitability (such as potassium channels SK, GIRK and HCN, postsynaptic density proteins PSD 95 and GLUA1, classical neurotransmitter vesicular transporters, VGAT and GLUTs), of the peptidergic neurons and their targeting neurons in a given (known) somatosensory circuit, are modified upon the modified peptide metabolism, resulting the increased or decreased signal vs noise ratios, strengthening or weakening input influences. In this talk I will present a general review of current literature as well as to present our recent studies on extrahypothalamic synapses of magnocellular vasopressin neurons and their connections to behavioral circuits as examples to discuss the above controversy.

Supported by PAPIIT GI200121 and CONACYT CB-283279.

KS1-3: Peptides and the gut: the unfinished story of gastrin

Chen, Duan; Zhao, Chun-Mei

Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

The history of biannual symposia of RepPep reflects the evolution of regulatory peptide discovery from gut hormones, to hypothalamic hypophysiotropic factors, to brain neuropeptides, and finally regulatory encompassing systems physiology as peptides in full (https://iuphar.org/2022/02/09/the-international-regulatory-peptide-society-irps-meeting/). I take the opportunity summarize this history as "Regulatory Peptides: Discovery and Future perspective". Gastrin was named by J. Edkins in 1905, as he found that injection of a pyloric mucous membrane extract resulted in gastric acid secretion in anesthetized cats. This original hypothesis was validated by H.J. Tracy and R.A. Gregory in 1964 by demonstrating physiological properties of a series of synthetic peptides structurally related to gastrin. The gastrin receptor was cloned and characterized by A.S. Kopin and his co-workers in 1992 using isolated canine parietal cells. The gastrin receptor (also called CCK-B or CCK-2 receptor) was also found on the ECL cells and the neck zone proliferating cells of the stomach. Physiologically, gastrin stimulates the acid secretion by acting directly on the parietal cells and/or by mobilizing histamine release from ECL cells, which then induces acid secretion by binding to the histamine (H2) receptors located on parietal cells. Gastrin is also a growth factor, particularly for the oxyntic mucosa of stomach, where an elevated circulating gastrin level (hypergastrinemia) stimulates proliferations of gastric stem cells and ECL cells, resulting in increased parietal- and ECL-cell mass, eventually ECLoma. In clinical setting, Zollinger-Ellison syndrome (ZES) is characterized by gastric acid hypersecretion, hypergastrinemia and ECLoma due to gastrin-producing tumor (gastrinoma) that arrives in the pancreas, ZES patients develop peptic ulcer but not gastric cancer. Treatment of peptic ulcer, particularly with long-term use of proton pump inhibitor (PPI), leads to hypergastrinemia but not gastric cancer. However, in animal models, the insulin-gastrin (INS-GAS) transgenic mice in which the INS-GAS transgene consists of the insulin promoter upstream of the human gastrin coding sequences develop hypergastrinemia, ECLoma and gastric cancer but not peptic ulcer. The mice treated with PPI develop hypergastrinemia and ECLoma but not gastric cancer. Gastrin or gastrin receptor deficient mice exhibit hypochlorhydria and ECL hypoplasia but are more susceptible to chemically-induced gastric cancer compared to wild-type mice. Thus, the unfinished story of gastrin, particularly regarding gastric carcinogenesis, would be completed through the systems approach consisting of three basic components, i.e. elements, processes and analysis. The elements include, at least, amidated gastrin-17, progastrin and the progastrin-derived peptides, gastrin receptors, gastric acid, ECL cells and ECL cell-released molecules (e.g. histamine, chromogranin A and pancreastatin), nerve (vagus nerve) and neurotrophic factors (NGF), H. pylori, IL-8, TFF1 and 2, TGF- α , EGF, in addition to gender difference or sex hormones, dietary cofactors (salt, vitamin A), gut microbiota and difference between humans and mice. The processes include, at least, signaling pathways of Wnt/ β -catenin, mTOR, Hippo/YAP1/TAZ, nuclear factor (NF)-kB, STAT3, Hedgehog, and ERK/MARK, inflammation, and metabolic reprogramming. The analysis in identifying causal relationship should be performed at three layers, i.e., association, intervention and counterfactuals (the so-called The Ladder of Causation).

The Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology (NTNU)

Symposium 1

Oxytocin and Vasopressin: Circuits and Behavior

Chaired by: Vito Hernandez (México)

S1-1 Lei Xiao

Fudan University, Shanghai, China.

Morpho-electric properties and diversity of oxytocin neurons in mouse paraventricular nucleus of hypothalamus

S1-2 Quirin Krabichler

University of Heidelberg, Mannheim, Germany. A novel transgenic arginine vasopressin (AVP)-IRES2-Cre rat line: Characterization, mapping of connectivity, and recording of neuronal activity

S1-3 Amelie Soumier

le Vinatier Hospital and Institute of Cognitive Science Marc Jeannerod, CNRS, Bron, France.

3D mapping of oxytocin and vasopressin neurons ontogenesis in the mouse brain

S1-5 Oscar R. Hernández-Perez

Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. and UNAM, México City, México Differential activation of arginine-vasopressin receptor subtypes in distinct amygdaloid subdivisions modulating emotional responses in the rat

S1-5 Mario Zetter

National Autonomous University of Mexico, Mexico City, México. Vasopressin acts as a synapse organizer by boosting PSD95 and GluA1 expression S1-1: Morpho-electric properties and diversity of oxytocin neurons in mouse paraventricular nucleus of hypothalamus

Chen, Saiyong; Xu, Hao; Dong, Shun; Xiao, Lei

The State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, and the Institutes of Brain Science, Fudan University, Shanghai, China

In addition to its well-known function in labor induction and lactation. oxytocin (OXT) also plays multiple roles in social, feeding, and emotional behaviors via modulating different brain regions. Paraventricular nucleus of hypothalamus (PVN) is one of the major OXT neurons distributed regions. PVN OXT neurons are canonically classified into magnocellular (Magno) and parvocellular (Parvo) subtypes. However, functional and single-cell transcriptomic studies indicate that PVN OXT neurons should be further classified. Meanwhile, morpho-electric properties and the diversity of PVN OXT neurons are not well investigated. In this study, we profiled the morphoelectric properties of PVN OXT neurons by combining transgenic mice, electrophysiological recording. morphologic reconstruction. and unsupervised clustering analyses. Total 224 PVN OXT neurons from 23 mice were recorded and used for analyses, and 29 morpho-electric parameters were measured. Magno and Parvo OXT neurons have prominent differences in their morpho-electric features, but PVN OXT neurons in male and female mice share similar neuronal properties. Some morpho-electric features of PVN OXT neurons, especially Magno neurons, exhibit significant diverse changes along the rostral-caudal axis. Furthermore, we find that PVN OXT neurons are classified into at least six subtypes based on their morpho-electric properties via unsupervised clustering. Only one Magno-Parvo mixed subtype in posterior PVN subregion, but not the other five subtypes, showed significant neuronal activity change in different feeding conditions. Our study supports the diversity of PVN OXT neurons and subtle neuron classification will promote excavating the functions of oxytocinergic system.

This work was supported by National Natural Science Foundation of China Grants 81970727 and 31900738; Shanghai Pujiang Program 19PJ1401800; Shanghai Municipal Science and Technology Major Project 2018SHZDZX01; ZJ Lab; and Shanghai Center for Brain Science S1-2: A novel transgenic arginine vasopressin (AVP)-IRES2-Cre rat line: Characterization, mapping of connectivity, and recording of neuronal activity

Krabichler, Quirin, 1; Lefevre, Arthur, 1,2; Kania, Alan, 1; Hagiwara, Daisuke, 3; Puzowski, Angelika, 1; Schönig, Kai, 4; Bartsch, Dusan, 4; Grinevich, Valery, 1

1 Department of Neuropeptide Research in Psychiatry, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany; 2 Department of Psychology, University of California San Diego, La Jolla, CA, USA; 3 3 Department of Endocrinology and Diabetes, Nagoya University Graduate School of Medicine, Nagoya, Japan; 4 Department of Molecular Biology, Central Institute of Mental Health, University of Heidelberg, Mannheim, Germany.

Arginine vasopressin (AVP) is a neuropeptide with important functions in the regulation of a variety of homeostatic functions in brain and body. In the brain, it is produced by various groups of neurons, including the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. Classical studies rats have revealed many significant insights into how AVP acts on physiology and behaviour, but progress has been limited by the inability to gain specific access to particular AVP subpopulations to study their connectivity and functions. Importantly, whilst a transgenic mouse line to target AVP neurons exists, mice are not necessarily the best suited models to study the AVP system, especially from a translational perspective. Here we present a novel transgenic rat line, AVP-IRES2-Cre knockin rats, generated by CRISPR/Cas9-mediated targeted insertion of the IRES2-Cre transgene in the 3' untranslated region of the AVP gene in Sprague-Dawley rats. This line allows us to specifically target distinct AVP neurons via stereotaxic injections of Cre-dependent viral vectors, to express any genes of interest. We made use of this new ability to first systematically map the output and input connections of PVN and SON AVP neurons throughout the brain, using AAV and recombinant rabies virus strategies. We furthermore tagged AVP neurons by Cre-dependent AAVs expressing EGFP and performed acute slice patchclamp electrophysiology to verify their AVP nature. In conjunction, we performed the in vivo calcium imaging of PVN AVP neurons by fiberphotometry technique, and showed that neuronal activity was drastically increased after acute osmotic challenge (i.p. injection of hypertonic saline). Altogether, our results demonstrate that the novel rat line expresses Cre recombinase exclusively in AVP neurons and hence provides a powerful tool to explore precise anatomy and functions of multiple, largely understudied extrahypothalamic AVP-ergic circuits.

S1-3: 3D mapping of oxytocin and vasopressin neurons ontogenesis in the mouse brain

Soumier, A, 1,2, Habart, M, 2, Lio, G, 1,2, Demily, C, 1,2 and Sirigu, S, 1,2 *liMIND, Center of Excellence for Autism, le Vinatier Hospital, Bron, France. 2Institute of Cognitive Science Marc Jeannerod, CNRS, Bron, France.*

Oxytocin (OT) and its partner Vasopressin (VP) are two essentials neuropeptides involved in the regulation of species- and sex-specific social behaviors. Decades of research in human and in animal models have demonstrated their direct involvement in social recognition and memory, parental behaviors, social bonding, affiliation and cooperation. As such, they play critical roles in typically developing individuals. They are also thought to be implicated in neurodevelopmental disorders including autism, social anxiety and schizophrenia. Given the importance of the balance between OT/VP over social function, it becomes increasingly important to identify the developmental stages that favor the emergence of such relationship. Yet their specific ontogenesis during early postnatal development, which is known to be a sensitive period, is still not clearly defined. Objective and Methods: To specifically address the question of whether neurons co-expressing OT and VP exhibit a unique distribution by region and by age stage, we identified the postnatal developpment using high resolution of cellular 3D-imaging of cleared immunolabeled mice brains over four early postnatal (P) stages, from birth (P0), early life (P3, P7, P14) to young adulthood (Soumier A et al., 2021).

Results: Our 3D atlas-based cellular mapping revealed unique anatomical properties of OT and AP neurons in the developing mice brain, showing dramatic differences between and within the two neural networks during very early development. We also found the number of OT neurons doubles according to unique temporal dynamics in selective hypothalamic regions, namely the periventricular and paraventricular nuclei, and in a novel location we named the antero-lateral preoptic. No changes were observed for VP neurons. Our findings demonstrate the coexistence of innate (antenatal) and plastic (postnatal) OT/VP circuits, that are probably triggered by environmental adaption of the social brain.

S1-4: Differential activation of arginine-vasopressin receptor subtypes in distinct amygdaloid subdivisions modulating emotional responses in the rat by arginine-vasopressin

Oscar René Hernández-Pérez, 1, 2, 3 ¹Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. ²Departamento de fisiología, Facultad de Medicina, UNAM, México ³Instituto de Fisiología Celular, UNAM.

Hypothalamic Vasopressin magnocellular neurons (AVPMNNs) project to limbic regions, influencing learning, motivated behaviour, and fear responses. The mechanism underlying this process has not been fully understood. To address this, the expression of postsynaptic proteins PSD95 and GluA1, two determinants of synaptic strenght were assessed in both in vivo (water deprivation, WD) and ex-vivo (acute hippocampal slices) to determine the effect of vasopressin in their expression by western blot. In vivo, WD increased the amounts of PSD95 and GluA1 at hippocampus, habenula and amygdala, regions which have been previously reported to receive AVPMNNs axon colaterals compared to control animals. With the use of expansion microscopy, the localization of postsynaptic proteins were assessed and quantified at Locus coeruleus, another region that has been reported to receive AVPMNNs axon collaterals. Increased amounts of PSD95 and GluA1 were found in subcellular compartments of TH-positive neurons, in close apposition to AVP immunopositive fibers, this phenomenon was increased in WD animals. Ex vivo, the administration of AVP agonists and antagonists confirmed that AVP could be, at least, one of the hypothalamic factors regulating the expression of excitatory postsynaptic proteins in lymbic strucures through V1a and V1b receptors.

S1-5: Vasopressin acts as a synapse organizer by boosting PSD95 and GluA1 expression

Zetter-Salmón, Mario,1; Hernandez, Vito,1; Padilla, Teresa,1; Campos-Lira, Elba,1; Escobar, Laura,1; Eiden, Lee, 2; Zhang, Limei,1

1 Department of Physiology, School of Medicine, National Autonomous University of Mexico; 2 Section on Molecular Neuroscience, NIH/NIMH, Bethesda MD, USA

Hypothalamic Vasopressin magnocellular neurons (AVPMNNs) project to limbic regions, influencing learning, motivated behaviour, and fear responses. The mechanism underlying this process has not been fully understood. To address this, the expression of postsynaptic proteins PSD95 and GluA1, two determinants of synaptic strenght were assessed in both in vivo (water deprivation, WD) and ex-vivo (acute hippocampal slices) to determine the effect of vasopressin in their expression by western blot. In vivo, WD increased the amounts of PSD95 and GluA1 at hippocampus, habenula and amygdala, regions which have been previously reported to receive AVPMNNs axon colaterals compared to control animals. With the use of expansion microscopy, the localization of postsynaptic proteins were assessed and quantified at Locus coeruleus, another region that has been reported to receive AVPMNNs axon collaterals. Increased amounts of PSD95 and GluA1 were found in subcellular compartments of TH-positive neurons, in close apposition to AVP immunopositive fibers, this phenomenon was increased in WD animals. Ex vivo, the administration of AVP agonists and antagonists confirmed that AVP could be, at least, one of the hypothalamic factors regulating the expression of excitatory postsynaptic proteins in lymbic strucures through V1a and V1b receptors.

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Symposium 2

Critical Pathways for Peptide-based Drug Development: Recent Progress

Chaired by: Esther Sabban (USA)

S2-1 Esther Sabban

New York Medical College, New York, USA. Preclinical studies in a PTSD model with intranasal NPY in males and females

S2-2 Youssef Anouar

INSERM U1239, University of Rouen Normandy, Mont-Saint-Aignan, France.

The antioxidant selenoprotein T mimetic, PSELT, exerts a potent neuroprotective effect in PD after intranasal administration.

S2-3 Peter Holzer

Medical University of Graz, Graz, Austria. Neuropeptide Y in gut-brain communication

S2-4 Chun-Mei Zhao

Norwegian University of Science and Technology, Trondheim, Norway.

Repurposing peptide-based drugs for treatment of gastric cancer: a proof of concept
S2-1: Preclinical studies in a PTSD model with intranasal NPY in males and females

Sabban, Esther L., Serova, Lidia, Nahvi, Roxanna, J., Tanelian, Arax, Nwokafor, ChisoArax Tanelian, Chiso Nwokafor

Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, New York, USA

Compelling evidence in animals and humans from a variety of approaches demonstrate that NPY in the brain can provide resilience to development of many stress-elicited symptoms. This endogenous, highly conserved, 36 amino acid amidated neuropeptide is abundantly expressed in the brain and in the periphery. Its actions are mediated by at least 4 GPCRs: Y1R, Y2R, Y4R and Y5R, with Y1R requiring an intact C and N-terminus, Intranasal administration of NPY to rats enabled its delivery to the brain. When given shortly before, or immediately after, traumatic stress to male rats in Single Prolonged Stress (SPS) rat model of PTSD, it prevented the development of many PTSD associated neuroendocrine changes and behaviors. These include hyperarousal, depressive/despair behavior, anxiety, impaired social interaction, extinction of fear memory, etc. This was even more pronounced using a selective Y1R agonist, but not Y2R agonist. Early intervention with the Y1R agonist, D-[His26]NPY was sufficient to prevent SPS trigger anxiety, impaired social interaction and depressive-like behavior. It was superior to NPY for prevention of development of development of depressive-like symptoms.

Females have lower expression of NPY in basal and stressed conditions (reviewed in Nahvi and Sabban, 2020, Biomolecules, 10,1248). Intranasal administration of NPY at concentration effective in males, did not alter traumatic stress triggered behaviors in female rats, although 8-fold higher concentration prevented the development of depressive-like behavior. Furthermore, NPY processing may play a role in the higher dose requirement for females Since NPY undergoes removal of the two C-terminal amino acids by DPP4 converting it from a pan-YR to a non-Y1R agonists, we combined low dose intranasal NPY with DPP4 inhibitor omaragliptin. The combined treatment, but neither alone, was sufficient to prevent SPS-triggered depressive-like behavior.

In male rats, intranasal NPY was effective not only to prevent, but also reversed the SPSelicited PTSD associated symptoms. For example, when administered 2 weeks after SPS, when substantial symptoms are observed, intranasal. NPY was able to reverse the SPS triggered, elevation in anxiety/avoidance behavior on the EPM, depressive-like behavior on the FST and hyperarousal as measured by acoustic startle. These reversals were evident several days after intranasal NPY and persisted for up to a week, the longest time tested.

Further studies are needed to translate the preclinical findings to benefit people exposed to traumatic stress. Two small published human clinical trials were performed with intranasal administration of NPY to reverse symptoms of PTSD and depression (Sayed et al, 2018, Mathe et al., 2020). While some encouraging results on anxiety and depression were obtained at the highest dose, they did not reach the maximal tolerated dose.

Taken together, the findings demonstrate that there is great potential for non-invasive intranasal NPY delivery to the brain for prevention and treatment of PTSD and comorbid impairments However, improvements are needed, such as more specific agonist, higher delivery efficiency etc.

US Department of Defense DM102282 and W81XWH-16-1-0016

S2-2: The antioxidant selenoprotein T mimetic, PSELT, exerts a potent neuroprotective effect in PD after intranasal administration

Anouar, Youssef,1; Alsharif, Ifat., 2; Boukhzar, Loubna, 1; Godefroy, David, 1; Eiden, Lee E, 3

1 INSERM U1239, University of Rouen Normandy, Mont-Saint-Aignan, France; 2 Department of Biology, Aljoumoum University, Saudi Arabia; 3 NIMH, NIH, Bethesda, USA

Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor dysfunction for which there is an unmet need for better treatment options. Although oxidative stress is a common feature of neurodegenerative diseases, notably PD, there is currently no efficient therapeutic strategy able to tackle this multi-target pathophysiological process. Based on our previous observations of the potent antioxidant and neuroprotective activity of SELENOT, a vital thioredoxin-like selenoprotein, we designed the small peptide PSELT from its redox active site to evaluate its antioxidant properties in vivo, and its potential polyfunctional activity in PD models. PSELT protects neurotoxin-treated dopaminergic neurons against oxidative stress and cell death, and their fibers against neurotoxic degeneration following intranasal administration in mice. PSELT is cell-permeable and acts in multiple subcellular compartments of dopaminergic neurons that are vulnerable to oxidative stress. In rodent models of PD, this protective activity prevented neurodegeneration, restored phosphorylated tyrosine hydroxylase levels, and led to improved motor skills. Transcriptomic analysis revealed that gene regulation by PSELT after MPP+ treatment negatively correlates with that occurring in PD, and positively correlates with that occurring after resveratrol treatment. Mechanistically, a major impact of PSELT is via nuclear stimulation of the transcription factor EZH2, leading to neuroprotection. Overall, these findings demonstrate the potential of PSELT as a therapeutic candidate for treatment of PD, targeting oxidative stress at multiple intracellular levels.

INSERM, University of Rouen Normandie, Normandy Council, European Union

S2-3: Neuropeptide Y in gut-brain communication

Holzer, Peter; Zenz, Geraldine; Hassan, Ahmed M.; Farzi, Aitak Division of Pharmacology, Otto Loewi Research Centre, Medical University of Graz, Graz, Austria

Neuropeptide Y (NPY) and the related gut hormone peptide YY (PYY) are important messengers along the transmission pathways between the gut and brain. PYY is not only involved in the regulation of food intake and energy homeostasis but also impacts on affective behaviour and even intestinal pain. NPY plays an important role in the cerebral processing of a variety of signals arriving from the gastrointestinal tract. Experimentally induced colitis, for instance, reduces NPY expression in the amygdala and hippocampus and increases Y1 receptor expression in the hippocampus, which may be related to colitis-induced anxiety and other behavioural disturbances. Diet-induced obesity is associated with an increase in depression-like behaviour which is associated with diminished expression of NPY in the hypothalamus and hippocampus. In contrast, antibiotic-induced depletion of the gut microbiota causes upregulation of NPY in the hypothalamus and amygdala along with a diminished expression of Y1 and Y2 receptors in the hippocampus. The downregulation of Y2 receptors in the hippocampus may be relevant to the impairment of learning and memory induced by gut microbiota depletion, because knockout of Y2 receptors likewise results in cognitive impairment. Intestinal translocation of bacterial factors such as lipopolysaccharide (LPS) stimulates the innate immune system and cause behavioural disturbances such (lethargy, anorexia). sickness behaviour Sickness induced as bv intraperitoneal administration of LPS is enhanced in mice deficient in Y2 receptors, which attests to a protective role of the NPY system in the adverse behavioural effects of immune stimulation. This implication is confirmed by intranasal administration of NPY, which prevents LPS-induced sickness behaviour while LPS-induced cytokine induction in the periphery and brain remains unabated. Thus, this neuroprotective effect of NPY takes place in the brain at a neuronal level that converts LPS-induced neuroinflammation into behavioural disturbances.

This work was supported by grants of the Austrian Science Fund and the European Commission.

S2-4: Repurposing peptide-based drugs for treatment of gastric cancer: a proof of concept

Zhao, Chun-Mei; Rabben, Hanne-Line; Wang, Timothy C.; Chen, Duan Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

Autonomic nerves have been shown to regulate stem and progenitor cells. Previously, we have investigated the role of innervation in gastric tumorigenesis using mouse models. Surgical removal of the vagus nerve (vagotomy), which innervates the stomach, during the preneoplastic stage of tumorigenesis diminished tumor incidence and size and attenuated tumor cell proliferation specifically in the denervated portion of the stomach, suggesting that the vagus nerve promotes GC growth. Consistent with this idea, pharmacologic denervation via local administration of botulinum toxin A (Botox) in the gastric wall similarly impaired preneoplastic growth. Furthermore, surgical or pharmacologic denervation at later stages of tumorigenesis suppressed GC progression and augmented the antitumor effect of chemotherapy in tumor-bearing mice, resulting in prolonged survival. This effect was mediated in part by inhibition of WNT signaling; denervation reduced the expression of WNTregulated stem cell markers and decreased the expansion of leucine-rich repeat containing G proteincoupled receptor 5 -positive (LGR5+) stem cells in the gastric mucosa via activation of the muscarinic acetylcholine receptor 3 (M3R) in LGR5+ stem cells. M3R signaling stimulated ligand independent WNT activation and enhanced the growth of gastric organoids in vitro, whereas deletion of M3R or treatment with an M3R inhibitor in combination with chemotherapy suppressed WNT signaling and reduced gastric tumor formation in mice. In patients with GC, WNT signaling was associated with neural pathways and neuronal density was correlated with more advanced tumors. These findings identify nerves as important regulators of gastric stem cell expansion and tumor progression and suggest M3R as a potential therapeutic target in GC.

Furthermore, we have investigated the neural singling in modulating metabolism of GC and developed metabolism-based treatments that prevent and/or inhibit the tumorigenesis and improve the overall survival. Vagotomy reversed the metabolic reprogramming, reflected by metabolic switch from glutaminolysis to OXPHOS/glycolysis and normalization of the energy metabolism in cancer cells and tumor microenvironment via WNT-mTOR signaling pathway. Metabolism-based treatment was developed to pharmacologically target SNAP25, mTOR, PDP1/a-KGDH and glutaminolysis. The efficacy of local Botox treatment (SNAP25 inhibitor) with systemic administration of RAD001 and CPI-613 but not cytotoxic drugs was validated in pre-clinical settings and the feasibility was tested in patients. GC gene expression signature and data/pathway mining revealed 9 molecular targets of ivermectin in both human and mouse GC associated with WNT/β-catenin signaling as well as cell proliferation pathways. The inhibitory effect of ivermectin was validated in silico, in vitro and in vivo. Chemopreventive effect of dietary phenethyl isothiocyanate (PEITC) was validated in vitro and in vivo and synergistic anticancer effect of PEUTC and cisplatin was founded in vitro. PEITC depleted glutathione and induced G2/M cell cycle arrest in GC cells. Thus, GC was glutamine dependent with altered neuronal and metabolic signaling pathways and that SNAP25, WNT/ β -catenin, mTOR, PDP1/ α -KGDH, glutaminolysis, and glutathione were potential drug-targets for GC treatment. These findings point to the importance of neural signaling in modulating the tumor metabolism and provide a proof of concept of the anti-metabolism therapies through drug repurposing.

The Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology (NTNU)

Symposium 3

Novel Aspects of Neuropeptides and Behaviour

Chaired by: Arpad Dobolyi (Hungary)

S3-1 Árpád Dobolyi

Eötvös Loránd University, Budapest, Hungary. A new peptidergic thalamo-preoptic pathway promoting positive valence physical contact

S3-2 Tim Viney

University of Oxford, Oxford, UK. Neuropeptides and rhythmic neuronal firing in brain networks

S3-3 Chun-Xia Yi

Amsterdam University Medical Centers, University of Amsterdam, Amsterdam, The Netherlands.

Regulatory peptides and microglial immunometabolism in hypothalamic regulation of feeding behaviour.

S3-4 Kristof Laszlo

University of Pécs, Pécs, Hungary.

Intraamygdaloid oxytocin reduces anxiety in valproate induced autism rat model

S3-1: A new peptidergic thalamo-preoptic pathway promoting positive valence physical contact

Arpád Dobolyi Eötvös Loránd University, Budapest, Hungary

Social touch is an essential component of communication. Little is known about the underlying pathways and mechanisms. Here, we discovered a novel neuronal pathway from the posterior intralaminar thalamic nucleus (PIL) to the medial preoptic area (MPOA) promoting social grooming and inhibiting aggression. We found that neurons in the PIL and MPOA were naturally activated by physical contact between rats and also by chemogenetic stimulation of PIL neurons. Activity-dependent tagging of PIL neurons was performed in rats experiencing physical social contacts. Chemogenetic activation of these neurons increased social grooming and decreased aggressive behavior between interacting rats as did selective activation of the PIL-MPOA pathway. Neurons projecting from the PIL to the MPOA express the neuropeptide parathyroid hormone 2 (PTH2) and central infusion of its receptor antagonist diminished social grooming. Finally, we showed similarity in the anatomical organization of the PIL-MPOA circuit in the rat and human brain. We propose that the discovered neuronal pathway facilitates physical contacts in both rodents and human.

Grant support: NKFIH-4300-1/2017-NKP_17-00002, OTKA K1342221

S3-2: Neuropeptides and rhythmic neuronal firing in brain networks

Viney, Tim J. 1. Department of Pharmacology, University of Oxford, Oxford, UK

Navigating through familiar environments by recalling the locations of objects, positions of landmarks, and the occurrence of events is a fundamental aspect of animal behaviour. Spatial navigation and episodic memory, collectively referred to as spatial memory, is important for survival as it facilitates exploration of novel environments and the anticipation of new experiences.

The temporal part of the mammalian cerebral cortex containing the hippocampus is required for spatial navigation and episodic memory. The sequential firing of hippocampal place cells is coordinated by rhythmic activity at theta frequency (5 - 12 Hz). This activity is replayed during sleep and is required for memory consolidation. Many investigations into the neurophysiological mechanisms underlying learning and memory focus on the actions of glutamate and GABA. However, glutamatergic pyramidal neurons and GABAergic interneurons also co-release a range of neuropeptides, but the significance in terms of their contribution to network activity is not fully understood. For example, both pyramidal neurons and specific types of GABAergic neurons express the neuropeptides somatostatin and neuropeptide Y, providing high frequency bursts of inhibition across different subcellular domains of pyramidal neurons during each theta cycle, affecting pyramidal neuron firing probability.

The first part of the talk will explore the distributed brain regions required for the encoding and recall of spatial memory. This includes the ascending sensorimotor inputs that reach the cortex via the thalamus, neuromodulatory inputs from the basal forebrain, and their interactions that govern rhythmic cortical neuronal excitability. In the second part of the talk, the diversity of neuropeptide-expressing neurons within the spatial memory network will be addressed, including GABAergic neurons found within different layers of the hippocampus and cortex, their firing dynamics during different behavioural states, and the implications for their influence on spatial memory processes. Finally, the role of subcortical neuromodulators in coordinating cortical neuronal activity will be discussed in relation to rhythmic disinhibition.

S3-3: Regulatory peptides and microglial immunometabolism in hypothalamic regulation of feeding behaviour

Correa-da-Silva, Felipe, 1,2; Wang, Xiao-Lan, 1,2; Gao, Yuanqing, 1,2; Milanova, Irina, 1,2; Kalsbeek, Martin J., 1,2; Korpel, Nikita L., 1,2; La Fleur, Susanne E., 1,2,3; Boutillier, Anne-Laurence, 4; Fliers, Eric, 1; Kalsbeek, Andries, 1,2,3; Yi, Chun-X

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Brain microglia are long-surviving and self-renewing innate immune cells that are crucial for scavenging cell debris and pathogens to maintain brain tissue homeostasis. The hypothalamus contains highly heterogeneous and condensed populations of peptidergic neurons in different regions that control food intake and glucose metabolism. It is reasonable that this region constantly produces cell debris and metabolic waste during different feeding and metabolic states. In order to keep a healthy and clean microenvironment for the hypothalamic neurons to function, the microglial immunometabolic activity in the hypothalamus needs to match the high demands for immune surveillances and debris phagocytosing/clearances. This was demonstrated by the fact that microglia in the mediobasal hypothalamus showed a significantly higher immunometabolic reactivity than in other regions when experimental animals were exposed to high-fat high-sugar (HFHS) diet, and that this occurs rapidly after receiving the HFHS diets. The reactive microglia in the mediobasal hypothalamus upon HFHS diet is not only characterized by increased cytokine production, but also impaired phagocytic capacity, as shown by a downregulation of phagocytic indicator CD68 expression in HFHS diet-fed rats. In microglia, besides governing lipid uptake for fuelling, the lipoprotein lipase (LPL)-gated phospholipid production is also crucial for phagolysosome formation and turnover. In HFHS diet-fed mice that has lack of LPL in microglia, there was a worsened phagocytic capacity and immune response, ultimately associated with lesser anorexigenic pro-opiomelanocortin (POMC) neurons and more vulnerability to diet-induced metabolic disorders. Thus, disrupted microglial immunometabolism has a detrimental effect on POMC neural survival upon diet challenge. Under physiological conditions, the neural activity in the hypothalamus varies during day-night cycle, hypothalamic microglial cells also exert their function in a strict time-of-day manner with higher activity during the dark, active phase, compared to the light, sleep phase. To find out how this intrinsic clock relates to the microglial immunometabolism and phagocytic function, especially in stimulated conditions such as feeding with HFHS diet, we generated mice with microglia-specific knock-down of the core clock gene, Bmal1. Interestingly, we found an increased microglial phagocytosis in mice subjected to HFHS diet-induced metabolic stress. This enhanced microglial phagocytosis was associated with significant retention of POMC expression in the mediobasal hypothalamus, and significantly less body weight gain upon HFHS diet. We conclude that loss of the rigorous control implemented by the intrinsic clock machinery increases the extent to which microglial phagocytosis can be triggered by neighboring peptidergic neurons under metabolic stress during any time of the day. Ultimately, this ensures a healthier microenvironment in the hypothalamus for the neighboring metabolism-regulatory neurons to function and protects animals from diet-induced obesity.

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S3-4: Intraamygdaloid oxytocin reduces anxiety in valproate induced autism rat model

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Intraamygdaloid oxytocin reduces anxiety in valproate induced autism rat model Background: Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder affecting about 1.5 % of children and its prevalence is still increasing. Anxiety is one of the most common comorbid sign of ASD. Despite the increasing prevalence, the pathophysiology of ASD is still poorly understood and its proper treatment has not been solved yet. In order to develop new therapeutic approaches, the valproate (VPA) induced rodent model of autism can be an appropriate tool. Oxytocin (OT), as a prosocial hormone, may ameliorate some symptoms of ASD. Methods: In the present study we investigated the possible anxiolytic effect of

intraamygdaloid OT on VPA treated rats using elevated plus maze test. Results: Our results show that male Wistar rats, prenatally exposed to VPA spent significantly less time in open arms of elevated plus maze apparatus and performed significantly less head dips from open arms. Bilateral OT microinjection to the central nucleus of amygdala increased the time spent in open arms and number of head dips, it reduced the anxiety to the healthy control level. OT receptor antagonist blocked the anxiolytic effects of OT. Antagonist in itself did not influence the time rats spent in the open arms. Conclusions: Therefore, our results show that intraamygdaloid OT has anxiolytic effects on autistic rats.

The project has been supported by the University of Pécs, Medical School, Pécs, Hungary (PTE ÁOK KA-2020-06) and New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-21-5-PTE-1333).

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Symposium 4

Peptide Interactions

Chaired by: Greti Aguilera (USA)

S4-1 Marisela Morales

NIDA, NIH, Baltimore, USA.

Fast and slow co-transmission and their role in reward and cocaine-seeking behavior

S4-2 Annette D. de Kloet

College of Medicine, University of Florida, Florida, USA. Angiotensin-vasopressin interactions and blood pressure regulation

S4-3 Jeff Jones

Texas A&M University and Washington University in St. Louis, USA.

Circadian neurons in the paraventricular nucleus entrain and sustain daily rhythms in glucocorticoids

S4-4 Becky Conway-Campbell

University of Bristol, UK.

Phase-shifting the circadian glucocorticoid profile induces disordered feeding behavior by dysregulating hypothalamic neuropeptide gene expression.

S4-1: Fast and slow co-transmission and their role in reward and cocaine-seeking behavior

Barbano, Flavia; Qi, Jia; Wang, Huiling; Morales, Marisela NIDA, NIH, Baltimore, MD, USA

Findings from clinical studies and animal models support a crucial role for dopamine neurons from the Ventral Tegmental Area (VTA) in the relapse to seek drugs of abuse, such as cocaine. It is unclear how different inputs to VTA participate in regulating the activity of VTA-dopamine neurons and reinstatement of cocaine seeking behavior. We previously found that DR-glutamatergic neurons (expressing vesicular glutamate transporter type 3, VGluT3) and dual DR-serotonergic-glutamatergic neurons (expressing VGluT3 and serotonergic markers) induced activation of VTA dopamine neurons, resulting in dopamine release in the nucleus accumbens, and mouse preference for a place associated with the activation of this pathway; suggesting that both DR-glutamatergic and dual DR-serotonergic-glutamatergic neurons regulate the function of VTA dopamine neurons. We also determined the extent to which DR inputs to VTA participate in the reinstatement of cocaine-seeking behavior, measured by a conditioned place preference (CCP) task. We used VGluT3-Cre mice in which we injected a viral vector for the selective expression of Channelrhodopsin tethered to eYFP in DR-VGluT3-glutamatergic neurons and a control cohort for the expression of eYFP without Channelrhodopsin. To induce the release of glutamate from the DR by VTA photostimulation, we implanted an optic fiber in the VTA. VTA release of glutamate from DR-VGluT3-glutamatergic fibers induced reinstatement of cocaine-seeking behavior. We next determined the extent to which DR-serotonergic inputs to the VTA play a role in cocaine-seeking behavior. We used serotonin transporter (SERT)-Cre mice to induce the expression of Channelrhodopsin in the total population of DR-serotonergic neurons. In contrast to VTA glutamate release from DR-VGluT3-fibers, VTA-serotonin release from DR-serotonergic fibers did not induce reinstatement of cocaine-seeking behavior, although VTA release of glutamate or serotonin from DR-fibers induced reward. We have previously demonstrated that many DR neurons projecting to the VTA are dual glutamatergic-serotonergic neurons expressing both VGluT3 and SERT. So far, the use of VGluT3-Cre transgenic mice has allowed us to access the total population of DR-VGluT3 neurons including those that corelease glutamate and serotonin. To specifically access the population of DR-VGluT3 neurons that releases glutamate without serotonin, we generated double recombinase VGluT3::Cre/SERT::Flip mice, and in the DR of these mice injected "intronic recombinase sites enabling combinatorial targeting viral vectors" (intersectional vectors) to access specific DR neuronal subpopulations. We injected in the DR of dual VGluT3::Cre/SERT::Flip mice an intersectional Cre-on/Flip-off vector to drive the expressing of ChR2 in DR VGluT3-only neurons (those lacking serotonin): VTA release of glutamate alone (in the absence of serotonin co-release) from DR VGluT3glutamatergic fibers reinstated cocaine-seeking behavior. In summary, while VTA release of glutamate or serotonin from DR induces reward, VTA release of glutamate from DR neurons glutamate from DR neurons, but not release of serotonin, induces reinstatement of cocaine-seeking. Supported by NIDA-IRP, NIH

S4-2: Angiotensin-vasopressin interactions and blood pressure regulation

de Kloet, Annette D.,

Department of Physiology and Functional Genomics, College of Medicine, University of Florida, USA

Blood pressure is controlled by endocrine, autonomic, and behavioral responses that maintain blood volume and perfusion pressure at levels optimal for survival. Although it is clear that central angiotensin receptors influence these processes, the neuronal circuits mediating these effects are incompletely understood. The present studies characterize the structure and function of specific populations of angiotensin type 1a receptor (AT1aR) and type 2 receptor (AT2R) containing neurons that impact blood pressure by way of their interactions with vasopressin-synthesizing and/or preautonomic neurons of the paraventricular nucleus of the hypothalamus (PVN). A particular emphasis is placed on angiotensin receptor-expressing neurons within the lamina terminalis (containing the median preoptic nucleus and organum vasculosum of the lamina terminalis). Using male AT1aR-Cre or AT2R-Cre mice, neuroanatomical studies reveal that AT1aR neurons in the lamina terminalis are largely glutamatergic and send projections to the PVN that appear to synapse onto vasopressin-synthesizing neurons, while AT2R neurons of the area are of a mixed neurotransmitter phenotype. To evaluate the functionality of lamina terminalis AT1aR neurons, in particular, we virally-delivered light-sensitive opsins and then optogenetically excited or inhibited the neurons while evaluating cardiovascular parameters or fluid intake. Optogenetic excitation robustly elevated blood pressure, water intake, and sodium intake, while optogenetic inhibition produced the opposite effects. Intriguingly, optogenetic excitation of these AT1aR neurons of the lamina terminalis also resulted in c-Fos induction in vasopressin neurons within the PVN and supraoptic nucleus. Further, within the PVN, selective optogenetic stimulation of afferents that arise from these lamina terminalis AT1aR neurons induced glutamate release onto magnocellular neurons and was sufficient to increase blood pressure. These cardiovascular effects were attenuated by systemic pretreatment with a vasopressin-1a-receptor antagonist. Similar studies were conducted to evaluate the functionality of AT2R neurons of the lamina terminalis and revealed that their excitation exerts depressor actions. Collectively, these data indicate that excitation of lamina terminalis AT1aR neurons induces neuroendocrine and behavioral responses that increase blood pressure, and that these effects are opposed by excitation of AT2R neurons within the same area. The overall implication is that coordinated targeting of interactions among angiotensin receptor-containing neurons, neuroendocrine PVN neurons and preautonomic PVN neurons may serve as an avenue to alleviate high blood pressure.

NIH/NHLBI - R01HL145028; NIH/NHLBI -R01HL136595; NIH/NHLBI - R35HL150750

S4-3: Circadian neurons in the paraventricular nucleus entrain and sustain daily rhythms in glucocorticoids

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The central circadian pacemaker, the suprachiasmatic nucleus (SCN), controls the daily timing of most, if not all, hormone release. However, we do not know how the SCN regulates different hormones, such as corticosterone, so that they each peak at different times of day. Here, we hypothesized that circadian signals from the SCN entrain and sustain daily rhythms in the hypothalamic paraventricular nucleus (PVN) to time the daily release of corticosterone to around waking. In vivo recording over >10 days from freelybehaving mice revealed a critical circuit from SCN vasoactive intestinal peptide (SCN VIP)-producing neurons to PVN corticotropin-releasing hormone (PVN CRH) neurons. In a light cycle, PVN CRH neurons peak in Period2 clock gene expression around midday and peak in calcium activity a few hours later. These rhythms persist in constant darkness and dampen in constant light. Loss of the core clock gene BMAL1 in CRH neurons results in arrhythmic PVN CRH calcium activity and dramatically reduces the amplitude and precision of the daily rhythm in corticosterone release. Discrete activation or inactivation of SCN VIP activity reliably alters the peak amplitude and timing of corticosterone release and PVN CRH calcium activity, and daily SCN VIP activation entrains PVN clock gene rhythms by inhibiting PVN CRH neurons. Together, these results demonstrate that the amplitude and phase of the daily corticosterone surge depends on coordinated clock gene expression and neuronal activity rhythms in both SCN VIP and PVN CRH neurons.

S4-4: Phase-shifting the circadian glucocorticoid profile induces disordered feeding behavior by dysregulating hypothalamic neuropeptide gene expression

Yoshimura, Mitsuhiro, 1,2; Flynn, Benjamin, P., 1; Kershaw, Yvonne, M., 1; Zhao, Zidong, 1; Ueta, Yoichi, 2; Lightman, Stafford L., 1; Conway-Campbell, Becky L., 1. ITranslational Health Sciences, Bristol Medical School, University of Bristol. UK; 2 Department of Physiology, University of Occupational and Environmental Health, Japan.

We demonstrate, in rodents, how feeding behaviour becomes disordered when circulating glucocorticoid rhythms are dissociated from circadian light/dark cues, a phenomenon most commonly associated with shift-work and transmeridian travel 'jetlag'. Adrenalectomized rats were infused with physiological patterns of corticosterone modelled on the endogenous adrenal secretory profile, either in-phase, or out-of-phase with circadian lighting cues. For the in-phase group, food intake was significantly greater during the rats' active period (during lights-off) compared to their inactive period (during lights-on); a feeding pattern similar to adrenal-intact sham-operated control rats. In contrast, the feeding pattern of the out-of-phase group was dysregulated, with rats ingesting significantly more food during their inactive period compared to both in-phase and control rats. Consistent with a direct hypothalamic modulation of feeding behaviour, we found that the altered appetitive timing was accompanied by altered anorexigenic and orexigenic neuropeptide gene expression. In situ hybridization histochemistry (ISHH) was performed on brains taken from rats in each of the three groups at Zeitgeber Time (ZT) 1 or 13 on the final day of the five-day infusion experiment. The gene expression of feeding regulating neuropeptides, neuropeptide Y (NPY), agouti-related peptide (AgRP), proopiomelanocortin (POMC), and cocaine-and-amphetamine-regulated transcript (CART) in the arcuate nucleus (ARC) and melanin concentrating hormone (MCH) in the lateral hypothalamic area (LHA) were analyzed. The orexigenic neuropeptides NPY, AgRP and MCH were significantly increased at ZT13 compared to ZT1, for both the in-phase and control groups. This pattern was reversed in the out-of-phase group. The anorexigenic neuropeptides POMC and CART exhibited opposing expression patterns to the orexigenic neuropeptides, hence were significantly decreased at ZT13 compared to ZT1 for both the in-phase and control groups. Again, the reverse pattern was seen for the out-of-phase group, with POMC and CART markedly increased at ZT13 compared to ZT1. The HPA axis was also assessed. For both in-phase and control groups, corticotrophin releasing hormone (CRH), arginine vasopressin (AVP) and oxytocin (OXT) in the paraventricular nucleus (PVN) and POMC in the anterior pituitary were significantly higher at ZT1 than ZT13. This pattern was reversed for the out-of-phase group. In the supraoptic nucleus (SON), both OXT and AVP transcripts were also significantly higher at ZT1 than ZT13 for both in-phase and control groups, and this pattern

were also significantly higher at ZT1 than ZT13 for both in-phase and control groups, and this pattern was reversed in the out-of-phase group. Locomotor activity, along with clock genes in the suprachiasmatic nucleus (SCN) and orexin in the LHA were unaffected by out-of-phase corticosterone infusion, confirming their exclusive entrainment by light/dark cues. Interestingly, water intake profiles were similar among the groups, even during the disordered feeding behavior of the out-of-phase group. The dissociation of the two closely related behaviors of eating and drinking seems counterintuitive, and is not easily explained. We can only conclude that corticosterone was involved in feeding but not in drinking behavior, in the present study design at least. In conclusion, our data indicate the adverse behavioural outcome that arises when an extra-SCN oscillator is out of synch with the master clock, in this case impacting on a process as fundamental to health as feeding behaviour.

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Chen Institute Plenary Lecture

Still more to learn about the brain oxytocin system in the context of socio-emotional behaviour

Neumann, Inga D.

Department of Behavioral and Molecular Neurobiology, University of Regensburg, Germany

The nonapeptide oxytocin (OXT) currently attracts enormous scientific attention due to its prominent prosocial, anxiolytic and anti-stress effects demonstrated both in rodents and humans. However, there still exist open questions regarding fundamental mechanisms of action, which need to be deciphered, before OXT can be considered a safe treatment option for various psychopathologies associated with social and emotional dysfunctions. The role of endogenous, brain OXT in balancing socio-emotional behaviours can be studied using complementary approaches including physiological models of naturally occurring elevated brain OXT system activity, such as lactation in females, or sexual stimulation and mating in males, in combination with neuropsychopharmacological pharmacogenetics approaches, and optogenetics. For example, brain OXT was found to be essential for naturally occurring social preference behaviour, for social fear extinction studied in a mouse model of social fear-conditioning, and for the fine-tuned regulation of inter-female aggression. However, with respect to its therapeutic implications it is important to note that OXT effects are dose- and duration-dependent, as opposite effects on anxiety- related and social behaviours have been found after chronic treatment. The underlying molecular mechanisms of the anxiogenic effect of chronic OXT involve alternative splicing of the hypothalamic CRF receptor 2a into its anxiogenic and soluble variant. These data may challenge the concept of OXT as a chronic treatment option of psychiatric disorders.

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Keynote symposium 2 (KS2)

Chair: Geert de Vries (USA)

Room: Wallace Monument

KS-2-1 Andries Kalsbeek

Amsterdam University Medical Centers and Netherlands Institute for Neuroscience, Amsterdam, The Netherlands. Vasopressin neurons in the suprachiasmatic nuclei (SCN): Critical signaling inside and outside the biological clock

KS-2-2 Bice Chini

CNR Neuroscience Institute, Vedano al Lambro, Italy. Oxytocin receptor in neurodevelopmental disorders: sex and agedependent regional distribution and modulation

KS-2-3 Patrick Sexton

Monash University, Victoria, Australia. Understanding the structure, ligand-binding and function of family B G protein-coupled receptors KS-2-1: Vasopressin neurons in the suprachiasmatic nuclei (SCN): Critical signaling inside and outside the biological clock

Kalsbeek, Andries, 1,2

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The discovery of the hypothalamic suprachiasmatic nuclei (SCN) as the seat of the central biological clock in 1972 was made almost at the same time as the discovery of the neurotransmitter function of neuropeptides in general and of vasopressin in particular. Only a few years after its discovery it was demonstrated, in 1975, that next to the well-known vasopressin production sites in the paraventricular and supraoptic nuclei (PVN and SON), also the suprachiasmatic nuclei (SCN) contained a prominent population of vasopressin-producing neurons. In the first 10-15 years after its discovery in the SCN vasopressin mainly served as an SCN marker, as the vasopressin-containing subpopulation turned out to be a characteristic feature of the SCN in many species, including humans. However, in this presentation I will show that in the years after the vasopressin neurons in the SCN have been at the heart of the deciphering and understanding of the mechanism of the circadian timing system. As the activity of the vasopressin neurons in the SCN had been found to show a pronounced daily variation in its activity that even could be demonstrated in human post-mortem brains, the next 10-15 years concentrated on the significance of the SCN vasopressin neurons for the functional output of the biological clock. Animal experiments showed an important role for SCN-derived vasopressin in the control of neuroendocrine day/night rhythms such as that of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes. The remarkable correlation between a diminished presence of vasopressin in the SCN and a deterioration of sleep/wake rhythms during ageing and depression make it likely that, also in humans, the vasopressin neurons contribute considerably to the rhythmic output of the SCN. More recently attention has shifted to the role of vasopressin signaling within the SCN. Indeed a high proportion of the SCN neurons (>40%) can be excited through the vasopressin V1a receptor. Moreover, microdialysis studies have clearly proven that vasopressin is also released within the SCN. By now a number of studies have demonstrated that the vasopressin neurons in the SCN are critical for interneuronal coupling within the SCN. As a consequence mice that lack vasopressin V1a and V1b receptors are resistant to jet lag, i.e., these mutant mice shift almost instantaneously to a new environmental light/dark cycle. More physiological models indicate that an absence of rhythmic intra SCN vasopressin signaling may allow animals to be active during their usual sleep period, when necessary.

KS-2-2: Oxytocin receptor in neurodevelopmental disorders: sex and agedependent regional distribution and modulation

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The neuropeptide oxytocin (OT) has been firmly established as a master regulator of the social brain. This has led to propose OT as a drug to ameliorate social deficits in a number of neuropsychiatric conditions in adults. OT has been also shown to regulate key neurodevelopmental events, suggesting that this peptide can modify the onset and progression of these conditions if administered postnatally and/or in early childhood. To fully exploit the potential therapeutic effects of OT in neurodevelopmental disorder, we are working to identify the molecular targets and time window of action of OT in the developing brain. We performed a scrutiny of OT receptor (OTR) expression in different mice models of neurodevelopmental disorders such as Magel2 (Bertoni et al Mol Psychiatry 2021), Dysbindin2 (Ferretti et al Curr Biol 2019) and Oprm1 (Gigliucci et al Front Pediatr 2014). In addition, we have tested the action of OT and other neurotrophic factors (IGF1) in a Rett syndrome mouse model (Gigliucci et al Cerebral Cortex 2022). Our data indicate that the postnatal administration of oxytocin in different mouse model of neurodevelopmental disorders modifies the expression level of OTR in region-dependent and sex-dependent manner. Here, we will focus on the mouse model bearing the specific loss of the Magel2 gene, which, in humans, causes a Prader Willi-like disease, identified as Schaaf Yang Syndrome (SYS; OMIM 615547), characterized by a higher prevalence of autism. Magel2 KO mice show a reduction in hypothalamic OT, and an early postnatal OT treatment was demonstrated to rescue neonatal lethality and to prevent the appearance of social and learning deficits in adult Magel2 KO mice (Meziane et al, Biol Psy 2015; Schaller et al, Hom Mol Genetics 2010), providing strong preclinical evidence for pilot studies of OT treatment in PWS and SYS infants (Tauber et al Pediatrics 2017). Our data indicate that male and female Magel2 KO mice at PND8 already display a significant reduction in OTR expression levels in all regions analyzed as compared to WT animals, indicating a major defect in OTR expression trajectories at this very early post-natal developmental age. Quite surprisingly, a post-natal administration of OT has no acute effects on OTR expression levels in the Magel2 KO neonate brain. On the contrary, a post-natal OT treatment restored OTR levels in several brain regions of the adult brain, consistent with the hypothesis that region-specific effects underlie the behavioral rescue effects observed in Magel2 animals treated at birth with OT (Bertoni et al Mol Psychiatry 2021). In adult Magel2 KO females, an unexpected reduction in OTR levels was observed in several areas of the brain, a particularly relevant finding given that Magel2 KO females did not show impairment in social behavior when tested in the 3 chamber test and in the open field arena, nor in any other behavioral or learning test. The investigation of OTR trajectories in the Magel2 KO females could be particularly relevant to understand specific compensatory mechanisms preventing the expression of autistic phenotypes, thus representing a crucial step for a translational approach.

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KS-2-3: Understanding the structure, ligand-binding and function of family B G protein-coupled receptors

Sexton, Patrick M.; Zhang, Xin; Cao, Jianjun; Piper, Sarah J.; Johnson, Rachel J.; Belousoff, Matthew J.; Wootten, D.

Drug Discovery Biology and ARC Centre for Cryo-electron Microscopy of Membrane Proteins, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia

G protein-coupled receptors (GPCRs) are the largest superfamily of cell surface receptor proteins and a major target class for drug development. GPCRs are inherently flexible proteins that have evolved to allosterically communicate external signals to modulation of cellular function through recruitment and activation of transducer proteins, particularly G proteins. Technological evolution in cryo-EM combined with continuing advances in biochemical approaches for the stabilisation of active-state complexes of GPCRs with different transducer proteins is now enabling structural interrogation of receptor activation and transducer engagement. Moreover, cryo-EM can access conformational ensembles of GPCR complexes that are present during vitrification, which can provide a window into the dynamics of these complexes. Using exemplar class B peptide hormone GPCRs, I will discuss how we are using cryo-EM to provide insight into ligand binding and GPCR activation by different agonists, and mechanisms of differential transducer coupling. I will also discuss how analysis of conformational dynamics of different agonist-GPCR-transducer complexes can contribute to mechanistic understanding of GPCR pharmacology.

DW is a Senior Research Fellow (grant no: 1155302), and PMS a Senior Principal Research Fellow (grant no: 1154434) of the National Health and Medical Research Council of Australia. PMS is Director of the Australian Research Council Industrial Transformati

Symposium 5

Neuropeptides and Regulation of Homeostasis and Allostasis

Chair: Robert Millar (South Africa)

S5-1 Dave Grattan

University of Otago, New Zealand Modulation of complex neuronal circuits by peripherally-derived peptide hormones

S5-2 Aras Petrulis

Georgia State University, Atlanta, USA. Sexually differentiated vasopressin pathways: connective architecture and role in social behavior

S5-3 Carolina Escobar

National Autonomous University of Mexico, Mexico City, México. Peptides involved in food anticipation

S5-4 Ruud Buijs

National Autonomous University of Mexico, Mexico City, México. Suprachiasmatic nucleus-driven vasopressin release prepares for the inactivity period

S5-1: Modulation of complex neuronal circuits by peripherally-derived peptide hormones

Grattan, David R.

Centre for Neuroendocrinology, University of Otago, New Zealand

Neuropeptides are typically thought of as peptide signals that are produced by neurons and are secreted to influence neuronal circuits in the brain. Such actions might be restricted to synaptic release points, but there is also evidence of much wider release from dendrites and cell bodies, potentially acting through volume transmission at sites distant from the source of the peptide. Neuronally-derived peptides can also be secreted outside of the nervous system, and act to influence peripheral organs. In this talk, I plan to discuss how peptide and protein hormones derived from peripheral endocrine tissues and circulate via the bloodstream, can be transported across the blood-brain-barrier and act in the brain through receptors expressed on neurons. Such hormones are not derived from neural tissue, but function analogously to neuropeptides to modulate complex neuronal circuits. Examples include insulin, leptin and growth hormone, but I will primarily use the anterior pituitary hormone prolactin to illustrate this mode of action. The requirement for transport into the brain provides a mechanism to control signalling, and for some factors, may limit central actions to circumventricular organs. However, many hormones are rapidly transported into the brain, in a saturable manner, and can then exert actions at multiple sites. Some hormones, such as leptin, show differential time-course of action at different sites in the brain, acting rapidly at circumventricular sites, and then over a longer time-course (presumably because of delays in transport or differential transport mechanisms) at other sites in the brain. While there is some evidence for very rapid actions of prolactin in the median eminence and area postrema, potentially from the hormone diffusing into these "leaky" regions of the brain, we have found that peripherally administered prolactin can exert rapid actions throughout the brain essentially simultaneously. Importantly, endocrine-derived peptides are not limited anatomically, and can access a neural circuit at multiple sites. The major control of action is through the expression of the hormone receptor. Such a mode of action is ideally suited to wide-scale adaptive changes. Here, I will discuss the example of prolactin, which, together with its pregnancy-specific homologue placental lactogen, modulates multiple functions in the maternal brain. These hormones reach extremely high levels in the blood during pregnancy, and then act on prolactin receptors that are widespread in the brain, influencing distinct neuronal circuits that control a range of physiological functions, including maternal behaviour, physical activity, fertility, thermoregulation, appetite and food intake. Rather than thinking about this as multiple different actions of a single pleiotropic hormone, from a system perspective, these hormones can be thought of as providing specific interoceptive sensory information that the mother has moved to a new physiological state. To cope with this state, multiple homeostatic systems need to be adjusted. Hence, these peripherally derived hormones serve as global signals to help complex neural circuits adapt their function to changing physiological conditions. Despite the fact that they are not derived from neurons, these neuroendocrine factors should rightly be considered part of the complex category of neuropeptides, or perhaps better, a neuroactive class of regulatory peptides! Health Research Council of New Zealand

S5-2: Sexually differentiated vasopressin pathways: connective architecture and role in social behavior

Petrulis, Aras

Neuroscience Institute. Georgia State University, Atlanta, USA

The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication, often sex-specifically, but the sources of AVP release relevant for behavior has not been precisely determined. AVP cells in the bed nucleus of the stria terminalis (BNST) are a major source of sexually differentiated AVP innervation in brain regions associated with social behavior (males > females). However, other sources, mostly hypothalamic, and more similar in the sexes, release AVP synaptically as well as non-synaptically to potentially modulate behavior as well. Consequently, to define the behaviorally relevant sources of AVP, we have used targeted genetic approaches (specific ablation, RNA-interference, optogenetic) to demonstrate that AVP cells within the BNST regulate male social investigation of male competitors and certain aspects of male-typical communicative behavior. Possible downstream structures responding to this BNST AVP signal will be discussed. In addition, using viral vector-based tracing, we established that BNST AVP cells receive strong inputs from areas known to regulate social behaviors as well, such as the preoptic area, and/or are enriched with V1aR or AVP. Our findings indicate that AVP cells in the BNST play a larger role in controlling social behavior in males than in females. This work suggests that sex differences in the neurochemical underpinnings of behavior may contribute to sex differences in disorders of social behavior and communication.

National Institutes of Health grant R01 MH121603

S5-3: Peptides involved in food anticipation

Escobar, Carolina; Boy Waxman S., Setien F. Department of Anatomy, Universidad nacional Autónoma de México, México

The predictable conditions imposed by the day-night cycle allow living organisms to prepare and anticipate to regular events. When the time of access is scheduled to similar daily hours, animals can anticipate and prepare for the coming meal event. This anticipation is observed at the behavioral level and also at the metabolic and digestive level for which important preparatory changes occur. In the brain regions in the hypothalamus and corticolimbic system participate in the regulation of metabolic and feeding responses. A series of studies have reported that in such region at the cellular level, cyclic responses occur in anticipation to the meal event as well as a response after feeding. In this response, NPY is released from the arcuate nucleus to the paraventricular nucleus (PVN) during food anticipation; also, a strong activation of orexigenic cells was seen. Interestingly, oxytocin (OT) cells participate selectively in this response, while in the PVN anterior OT cells respond to food intake, posterior PNV-OT cells anticipate the schedules meal suggesting a differential and regional function of OT cells. In this presentation the possible role of different hypothalamic and corticolimbic cells in food anticipatory behavior will be discussed. DGAPA-PAPIIT IG201-321

S5-4: Suprachiasmatic nucleus-driven vasopressin release prepares for the inactivity period

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Vasopressin (VP) is an important hormone produced in the Supraoptic (SON) and Paraventricular nucleus (PVN) released from the neural lobe into the circulation with antidiuretic and vasoconstrictor functions. As one of the first discovered peptide hormones, VP was also shown to act as a neurotransmitter, whereby VP is produced and released under the influence of various stimuli. In neurons of the biological clock, the Suprachiasmatic Nucleus (SCN) VP production is influenced by the rhythm of clock genes and the light-dark cycle. We will primarily discuss the different functions of the circadian output and the release of VP from SCN terminals, one of the core signals via which the biological clock imposes its rhythm on its target structures. This is contrasted with VP production and release influenced by gonadal hormones in the bed nucleus of the stria terminalis and medial amygdala and with VP, originating from the PVN reaching central targets. VP arising from the SCN is critical in the circadian regulation of corticosterone secretion, reproductive cycle, locomotor activity, and metabolism in rodents. These circadian variables, such as corticosterone and glucose levels, are regulated within very narrow boundaries at a specific time of the day, whereby the day-to-day variation is less than 5% at a particular hour. However, the circadian peak values can be ten times higher than the circadian trough values, indicating the need for an elaborate feedback system to inform the SCN and other participating nuclei about the actual levels reached during the circadian cycle. Here we will discuss that the circadian variation in SCN output is vital for any given rhythm. The interplay between SCN output and peripheral feedback to the SCN is essential for the adequate organization of circadian rhythms in physiology and behavior.

This work was supported by the Dirección General de Asuntos del Personal Académico Grant DGAPA IG-20132

Symposium 6

Ghrelin and Related Peptides: Crossing Many Barriers

Chair: Patrick Sexton (Australia)

S6-1 Mitchell Ringuet

The University of Melbourne, Victoria, Australia. Ghrelin receptor, GHSR: emerging evidence as a GPCR modulator

S6-2 Ki Goosens

Icahn School of Medicine at Mount Sinai, New York, USA. The ghrelin system as a driver of heterogeneity in psychiatric disorders

S6-3 Sebastian Furness

University of Queensland, Queensland, Australia. The physiological role for modulation of transducer coupling at GHSR1a and other peptide-liganded GPCRs

S6-4 Sarah Melzer

Medical University of Vienna Neuropeptidergic modulation of fear memories in the auditory cortex

S6-1: Ghrelin receptor, GHSR: emerging evidence as a GPCR modulator

Ringuet, Mitchell T.

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Background:. Ghrelin is not found in the central nervous system (CNS), but its receptor is distributed widely, has identified functions and is conserved between mammals. Consistent with this, Dopamine, but not Ghrelin, is found in nerve terminals in the defecation centres, where GPCRs, Dopamine D2 receptor (DRD2) and Ghrelin receptor (GHSR) transcripts have been identified. A new hypothesis, for which there is mounting evidence, is that GHSR alters signalling pathways of other GPCRs, and its role may be independent of ghrelin. Our studies investigated whether altered signaling/ GPCR interaction is observed in native cells of the defecation centre, as well as in transfected cells in vitro.

Methods: DRD2 and GHSR transcripts were located in rat spinal cord using RNAscope. For electrophysiology recordings, neonatal rats were i.p. injected with Dil to label PGNs. After 3-7 days of transport, animals were euthanised, spinal cords removed by dorsal laminectomy and lumbosacral spinal cord cut into 300μ M slices in ice-cold sucrose aCSF, followed by incubation in NMDG hepesbuffered recovery solution for 10 minutes at 32° C. Tissue was left in aCSF at RT for 1 hour before recording at 32° C. All recordings were made in open, whole cell patch configuration under voltage clamp using an Axopatch 200A or Multiclamp 700B amplifier. Dopamine hydrochloride (DA, 30μ M), Capromorelin (10nM), and GHSR receptor blockers, JMV2959 (1 μ M), YIL781 (100nM), PLC β inhibitor (U73122) and Thapsigargin (TG) were washed onto slices (3-5mL/ min) and current (pA) changes were assessed relative to baseline. Parallel experiments were conducted using HEK293 and CHO cells transfected with GHSR, DRD2 or both.In vivo colonic motility experiments were conducted in anaesthetized rats.

Results: A DRD2 agonist, quinpirole, applied directly to the defecation centres of rats in vivo, stimulated colorectal propulsion that was antagonized by systemic ghrelin receptor antagonist, YIL781, indicating that activation of DRD2 of the defecation pathway requires interaction with an agonistactivatable GHSR. In situ hybridization revealed Ghsr in neurons of the defecation centres, but not in down-stream neurons. Moreover, 80% of neurons in the centres that expressed Drd2 also expressed Ghsr. Both Capromorelin and DA caused an inward current and increased excitability of a subpopulation of Dil labelled PGNs. Pre-incubation with GHSR antagonist, JMV2959, and inverse agonist, YIL781, for 5-10 minutes did not change the excitability of PGNs, suggesting that baseline constitutive activity of GHSR is tissue specific, not occurring in these neurons. Furthermore, the presence of GHSR blockers significantly reduced the excitability of the neurons to DA. Repeat DA applications in separately recorded PGNs did not show a comparable decrease in response. Additionally, U73122 and TG pre-incubation blocked excitation caused by DA. Thus, our results using pharmacological techniques in native spinal cord slices suggests that DRD2 signalling can be switched from inhibition to excitation, dependent on interaction with GHSR. Evidence for similar switching was obtained from HEK and CHO cells. In these cells, the additional presence of GHSR switched agonism at DRD2 from inhibition of adenyl cyclase to mobilisation of Ca.

Conclusions: DRD2 and GHSR are in the same neurons in the CNS and are functionally involved in defecation control pathways. Our pharmacological and localisation studies are consistent with these receptors interacting and with DA being the physiological activator. The complex can be activated by both Dopamine and Ghrelin, but only Dopamine has a physiological role, which is dependent on GHSR-mediated modulation of DRD2 signalling.

NHMRC - 2021/GNT2012657

S6-2: The ghrelin system as a driver of heterogeneity in psychiatric disorders

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Chronic stress exposure elevates the risk of developing psychiatric disorders years after the primary stressor ends. Our laboratory has demonstrated that a peptide hormone called acyl-ghrelin, made by the stomach and released into the bloodstream, is roughly doubled after a chronic stressor, and that this elevation persists for months in rodents and years in humans. Moreover, we have also shown that this elevation of acyl-ghrelin plays a causal role in driving stress-induced vulnerability to elevated fear in a rodent model of posttraumatic stress disorder. In humans, we have found that acyl-ghrelin levels correlate with both the risk and severity of posttraumatic stress disorder, a stress-induced psychiatric disorder. Despite this strong correlation, it is unlikely that a single peptide causes PTSD. Recent work has revealed that multiple proteins in the bloodstream can impact the signaling of acylghrelin at its receptor. These include butyrylcholinesterase (BChE), which is thought to be a primary mechanism by which acyl-ghrelin is metabolized, and liver-expressed antimicrobial peptide (LEAP2), which acts as an inverse agonist at the ghrelin receptor. We hypothesized that individual variation in BChE activity and LEAP2 may modulate the ability of acyl-ghrelin to drive stress-induced vulnerability to psychiatric disorders. I will discuss our recent efforts to characterize levels of individual variation, as well as the stresssensitivity, of these proteins, and our efforts to link these proteins to stresssensitive psychiatric disorders.

S6-3: The physiological role for modulation of transducer coupling at GHSR1a and other peptide-liganded GPCRs

Furness, Sebastian G.B.

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Receptor:transducer coupling is central to the function of G protein-coupled receptors and changes in receptor:transducer interactions underlie a variety of physiologically important phenomenon. Moreover, probing these interactions can provide new therapeutic leads. Modifiable interactions include some well-characterized examples of ligand dependent differential efficacy (which also underlies biased signaling), changes induced by different membrane environments and those resulting from the presence of modulating proteins, including other G protein-coupled receptors. We are seeking to define the molecular bases for these phenomenon in the physiological context of the gutbrain axis. Our work utilizes a variety of biophysical, advanced imaging, and cell signaling techniques, which we translate to physiologically relevant settings. I will discuss our work to analyse the biophysical basis for differential efficacy at the calcitonin receptor, our work on understanding cholesterol dependent uncoupling of the cholecystokinin 1 receptor and work to reveal how dopamine D2 receptor coupling is modified by the ghrelin receptor.

ARC Future Fellowship FT180100543, NHMRC Ideas 2012657

S6-4: Neuropeptidergic modulation of fear memories in the auditory cortex

Sarah Melzer Medical University of Vienna

Inhibitory neurons throughout the mammalian cortex are powerful regulators of circuit excitability and plasticity, thus controlling cortical functions such as learning, memory and perception. All major inhibitory cortical cell types are marked by differential expression of neuropeptide receptors, suggesting highly diverse context- and experience-dependent peptidergic modulation of cortical processing.

We found that the bombesin-like neuropeptide gastrin-releasing peptide (GRP) serves as an important regulator of cortical memory formation through selective targeting of one specific inhibitory neuronal cell type. Using in vivo imaging, CRISPR/Cas9-mediated knockout and a combination of molecular and electrophysiological techniques, we deciphered underlying signaling mechanisms. Our data establish peptidergic regulation of cortical disinhibitory microcircuits as a mechanism to regulate auditory fear memory.

Symposium 7

The physiology and function of hypothalamic magnocellular neurons

Chair: David Murphy (UK)

S7-1 Andre Mecawi

Federal University of São Paulo, São Paulo, Brazil. Dissecting the molecular profile of the hypothalamic magnocellular neurones: a MultiOMIC journey

S7-2 Tom Cunningham

UNT Health Science Center, Fort Worth, USA. Sex Differences in Neurohypophyseal Hormone Release in a Model of Dilutional Hyponatremia

S7-3 Ryoichi Teruyama

Louisiana State University, Baton Rouge, USA. Sexually dimorphic expression of oxytocin receptors in the CNS

S7-4 Soledad Bárez-López

University of Bristol, Dorothy Hodgkin Building, Bristol, UK. Shining light into the role of a non-visual opsin in the supraoptic nucleus S7-1: Dissecting the molecular profile of the hypothalamic magnocellular neurones: a MultiOMIC journey

André S. Mecawi

Laboratory of Molecular Neuroendocrinology, Department of Biophysics, Paulista School of Medicine, Federal University of São Paulo, São Paulo – Brazil.

The magnocellular neurons (MCNs) of both supraoptic (SON) and paraventricular (PVN) hypothalamic nuclei produce and secrete vasopressin (AVP) and oxytocin (OXT) through the neurohypophysis to the circulation. Those neuropeptides are the main hormones controlling renal water and sodium excretion. The MCNs are intrinsically osmosensitive and respond to changes as low as 1% in osmolality by modifying the frequency and/or the discharge pattern of action potentials. While the SON has a very homogenous neuronal population (the vast majority are MCNs), sends axonal projections mainly to the neurohypophysis, and produces AVP and OXT primarily, the PVN is more complex with several subsets of parvocellular neurons which produce other neuropeptides. In recent years we and others have used the OMICs approach to comprehensively describe the molecular profile of the MCNs cell bodies and axon terminal and its associated glial cells in the hypothalamus and the neurohypophysis, respectively. We took advantage of this recently published data to integrate the bulk transcriptome (microarrays and RNAseq), proteome, and phosphoproteome into the single-cell/nucleus RNA signature of the MCNs to uncover the molecular profile of the MCNs and the associated glia under basal and stimulated conditions. Financial Support: FAPESP (#2019/27581-0).

S7-2: Sex Differences in Neurohypophyseal Hormone Release in a Model of Dilutional Hyponatremia

Cunningham, J. T., 1; Balapattabi, K., 2; Nguyen, D. H., 1:; Little, J.T.; 1

1 Physiology and Anatomy, UNT Health Science Center, Fort Worth, USA; 2 Department of Physiology, Medical Collège of Wisconsin, Milwaukee, USA

Hyponatremia resulting from increased circulating vasopressin (AVP) increases morbidity and mortality in patients with cirrhosis and heart failure. Our laboratory has been using = an animal model of cirrhosis caused by ligation of the common bile duct (BDL) to study the central mechanisms related to pathogenesis of inappropriate AVP release. More recently, we have investigated possible sex differences in this model. In studies of adult male and female rats, all the BDL rats had significant increases in liver to body weight ratio compared to sham rats indicating liver failure. Male BDL rats demonstrated hyponatremia along with significant increases in plasma copeptin (an AVP surrogate) and FosB expression in Supraoptic AVP neurons compared to male shams (all p<0.05; n=5-7). Unlike male BDL rats, the female BDL rats did not become hyponatremic and did not demonstrate Supraoptic AVP neuron activation or increased copeptin secretion as compared to sham operated females. Plasma oxytocin concentration was significantly higher in female BDL rats compared to female sham controls (p<0.05; n=6-10). This increase was not observed in male BDL rats.

Ovariectomy significantly decreased plasma estradiol concentration in shams compared to intact female sham (p < 0.05;6-10). However, circulating estradiol concentration was significantly elevated in ovariectomized BDL (OVX BDL) rats compared to the ovariectomized sham (OVX sham) and female sham rats (p < 0.05;6-10). To identify the source of estradiol contributing to the observed increase in OVX BDL rats, adrenal glands were collected at the end of protocol. Adrenal gland steroids were extracted to measure estradiol and its precursors, testosterone and DHEA concentration. The OVX BDL rats had significantly increased adrenal estradiol along with significant decrease in adrenal testosterone and DHEA compared to OVX sham rats (all p < 0.05;6-7). Female OVX BDL rats did not hyponatremia or increased copeptin secretion compared to female OVX sham rats. It is possible that the increase in adrenal estradiol compensated for the lack of ovarian estrogens in OVX BDL rats.

To test the role of estrogen receptors in the sex difference in AVP release, we chronically infused female sham and BDL rats with ICI 182,780 (0.6 ug/day) into the cerebral ventricles. The ICI infusions significantly increased plasma copeptin and decreased plasma osmolality in female BDL rats (all p<0.05; n=6-8). These data suggest that estrogen receptors could protect females from increased copeptin release. In a follow up study, the contribution of G-protein coupled estrogen receptors (GPER) was tested by infusing sham and BDL female rats with a GPER antagonist alone (G15 40 μ g/day sc) and with ICI. The results show a trend for lower plasma osmolality in the BDL ICI group compared to the respective sham group as previously observed; but this trend was not present in the BDL G15+ICI and Sham G15+ICI groups (n=6-8). Lower hematocrit was observed in BDL G15+ICI Vehicle group compared to respective sham group. These data, although still preliminary, suggest the effects of ICI on plasma osmolality in female BDL rats could be due to GPER activation. The GPER effects on hematocrit may involve a different mechanism.

R01 HL119458, AHA 18PRE34060035, F30 HL158145

S7-3: Sexually dimorphic expression of oxytocin receptors in the CNS

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Sex differences in the expression of oxytocin receptors (OXTR) in the central nervous system (CNS) of Venus mice were assessed using the OXTR reporter, OXTR Venus, where a part of the OXTR gene was replaced with Venus (a variant of the green fluorescent protein) cDNA. Recognizable sex differences in the expression pattern were found in the medial preoptic area (MPOA), the perinuclear zone (PNZ) immediately dorsal to the supraoptic nucleus (SON), and in the retina. The expression of OXTR in these areas are estrogen dependent, because ovariectomy blocked the expression of OXTR-Venus whereas an estradiol implant restored the expression. Within the MPOA, numerous OXTR-Venus neurons were found in the anteroventral periventricular nucleus (AVPV) of females, but not in males. Specific inactivation of the OXTR neurons in the AVPV using a chemogenetic approach specifically impaired pup retrieval behavior. These results demonstrated that the sexually dimorphic expression of OXTR in the AVPV is necessary for induction of maternal behavior. In the PNZ, there was no sex difference in the number of OXTR-Venus neurons between virgin females and males. However, these neurons significantly increased during lactation and returned to the level of virgin females after weaning. Using a double transgenic mouse line reporting GABA and OXTR, the majority of the OXTR cells in the PNZ were found to be GABAergic and send direct projections to oxytocin neurons in the SON. A bath application of the selective OXTR agonist, [Thr4,Gly7]-oxytocin (TGOT), caused a massive increase in the frequency of GABAA receptor mediated synaptic currents in oxytocin neurons in the hypothalamic slices from lactating females, but not from non-lactating females. These findings suggest that the release of GABA from OXTR cells in the PNZ to oxytocin cells in the SON play a role in the autoregulatory mechanism of the hypothalamic oxytocin system in response to physiological demands for oxytocin during lactation. In the retina of female mice, the expression of OXTR-Venus was found in large and small amacrine cells in the inner nuclear layer and ganglion cells in the ganglion cell layer. A series of immunocytochemical experiments indicated that OXTR-Venus expressing large and small amacrine cells are dopaminergic and glycinergic, respectively. Furthermore, the OXTR-Venus expressing ganglion cells were not immunoreactive to melanopsin suggesting they are not the intrinsically photosensitive retinal ganglion cells (ipRGCs). In the male retina, OXTR-Venus expression was found only in the large amacrine cells and ganglion cells, but not in the small amacrine cells. To assess the projection sites of OXTR ganglion cells, a Cre-dependent, anterograde viral tracer was injected into the subretinal space of OXTR-Cre mice. The anterograde tracing found projections to the suprachiasmatic (SCN) and SON. These findings suggest the involvement of retinal OXTR cells in the regulation of the circadian rhythm and oxytocin neurons, although the function of the female specific OXTR in glycinergic amacrine cells is unknown. NIH R21MH119609, NIH R21HD097565

S7-4: Shining light into the role of a non-visual opsin in the supraoptic nucleus

Bárez-López, Soledad, 1; Elsamad, Ghadir, 1; Bishop, Paul, 1; Murphy, David, 1; Greenwood, Michael, P., 1

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In response to osmotic stimulation the supraoptic nucleus (SON) undergoes a plethora of activity-dependent adaptations, including dramatic changes in gene expression. In previous work, we have identified Opsin3 as one of the most responsive genes to osmotic hypo- and hypertonic stress in the SON. OPSIN3 (OPN3), the protein encoding Opsin3, is a light-sensitive protein present throughout the mammalian brain, although its function in this organ is still unknown. The potential role of a light sensitive protein in SON is highly intriguing, therefore we have sought to address the role of OPN3 in the SON. By in situ hybridization, we have demonstrated that Opn3 is expressed in neurones expressing both arginine vasopressin (AVP) and oxytocin (OXT) in the rat SON, suggesting that OPN3 mediates a function in both neuronal types. Gene profiling analysis by RNAseq suggested that neuropeptide production is impaired when knocking down Opn3 in the rat SON. Among these, genes encoding the peptides AVP (Avp), OXT (Oxt), Prodynorphin (Pdyn), Cocaine- and amphetamine-regulated transcript protein (Cartpt) and Galanin (Gal) were downregulated. The genes encoding Caprin family member 2 (Caprin2), that increases Avp and Oxt transcript stability, and rasrelated dexamethasone induced 1 (Rasd1), that controls the transcriptional response to osmotic stress were also downregulated. Moreover, knocking down Opn3 in the rat SON led to changes in physiological parameters including water intake, body temperature and motor activity. Altogether the data indicates that OPN3 in the SON is involved in the regulation of several neuropeptides and additional proteins that participate in water homeostasis, body temperature, motor activity, and possibly sleep.

Symposium 8

PACAP and related peptides in central and peripheral regulation of stress responses

Chair: Lee Eiden (USA)

S8-1 Sarah Gray

University of Northern British Columbia, Prince George, BC, Canada.

PACAP expression in central and peripheral neuronal networks regulating adipose tissue

S8-2 Sunny Z. Jiang

NIH-National Institute of mental Health, MD, USA. Prefrontal cortico-hypothalamic and parabrachio-extended amygdalar PACAPergic projections separately control HPA and food intake responses to psychogenic stress

S8-3 Arun Anantharam

University of Toledo, OH, USA. PACAP and acetylcholine regulate distinct Ca2+ responses and secretory outputs in chromaffin cells

S8-4 Vito Hernández

National Autonomous University of Mexico, Mexico City, México. PACAP co-expression in GABAergic or glutamatergic circuits and its relevance for behavioural adaptation.

S8-1: PACAP expression in central and peripheral neuronal networks regulating adipose tissue

Gray, Sarah L.

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Adipose tissue plays a key role in regulating energy homeostasis, contributing to energy availability when exogenous sources are lacking, storing energy in times of plenty and burning energy in response to thermal stress. The neuropeptide pituitary adenylate cyclaseactivating polypeptide (PACAP), is well established as an overarching regulator of the stress response 1,2, including in the maintenance of energy homeostasis. A recent focus of my lab has been to understand if PACAP acts centrally and/or peripherally to modulate sympathetic regulation of adipose tissue function in response to metabolic stress, including cold stress. Previous work in PACAP null mice shows PACAP regulates adaptive thermogenesis in response to cold stress, yet the site of PACAP expression influencing energy expenditure is not well understood. Centrally, PACAP is expressed in the preoptic area where it is involved in initiating an efferent response to afferent cold and warm sensing neurons. As well, it is highly expressed in structures of the tuberal hypothalamus well known to regulate energy expenditure such as the paraventricular nucleus (PVH), the ventromedial nucleus (VMH) and the dorsomedial nucleus (DMH) of the hypothalamus. To determine if PACAPinergic neurons in such nuclei of the tuberal hypothalamus interact directly with sympathetic outflow tracts that innervate intrascapular brown adipose tissue, we infected postganglionic neurons innervating intrascapular brown adipose tissue with the retrograde neural tracer, pseudorabies virus expressing β-galactosidase (β-gal, PRV-BaBlu) in PACAP-eGFP transgenic mice. Neurons co-expressing eGFP and β -gal immunoreactivity were identified in several structures in the tuberal hypothalamus (anterior nucleus, VMH, arcuate nucleus, PVH, DMH, lateral hypothalamus and zona incerta) providing neuroanatomical evidence that small populations of PACAPinergic neurons are part of sympathetic outflow tracts to intrascapular brown adjpose tissue. Further work aimed at understanding if PACAP functionally modulates the efferent pathways of thermogenesis within these specific structures of the tuberal hypothalamus is ongoing.

Further along the sympathetic outflow tracts to brown adipose tissue are the pre-ganglionic nerves of the sympathetic nervous system. PACAP is expressed in the splanchnic nerve innervating the adrenal medulla, modulating catecholamine synthesis and release in response to both psychogenic and physiological stress. PACAP regulation of catecholamine synthesis and release in postganglionic nerves innervating adipose tissues has not been studied. Recently, we have identified VPAC1 receptors and PAC1 receptor variants to be expressed in the stellate ganglia (from which postganglionic nerves innervate intrascapular brown adipose tissue) with sex-specific, differential gene expression based on housing temperature and have begun to examine the effect of PACAP ablation on postganglionic nerve gene expression in response to both acute and chronic cold stress. In summary, we continue to strive to elucidate the complexity by which PACAP coordinates the sympathetic response to acute and chronic stress which broadly influences an array of tissues and physiological systems, including adipose tissue and metabolism.

NSERC Discovery Grant (RGPIN-2021-04040)
S8-2: Prefrontal cortico-hypothalamic and parabrachio-extended amygdalar PACAPergic projections separately control HPA and food intake responses to psychogenic stress

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The neuropeptide PACAP is a master regulator of central and peripheral stress responses, and PACAP/PAC1 receptor signaling has been implicated in the pathophysiology of neuropsychiatric disorders related to stress (Jiang & Eiden 2016a). PACAP neurons are abundantly distributed in cortical and limbic areas, hypothalamus and brain stem, including the external lateral parabrachial nucleus (elPBn) (Zhang et al 2021). PACAPergic projections from these areas have been implicated in the regulation of stress coping and defensive behaviors. It is not clear yet how specific PACAPergic projections regulate stress responses, and how PACAP itself, as a neuropeptide neurotransmitter, contributes to neurotransmission at these synapses. Recently we have identified distinct PACAPergic circuits separately controlling endocrine and behavioral responses to stress, using an acute restraint stress paradigm combined with intersectional genetics (Jiang & Eiden 2016b; 2021). PACAP in extrahypothalamic areas, especially prefontal cortex, contributes to resiliency of hypothalamo-pituitary adrenal (HPA) axis activation in stress, through augmentation of CRH biosynthesis in the paraventricular nucleus of the hypothalamus (PVN) during periods of stress, thus controlling an endocrine response to stress. A PACAPergic projection from elPBn to PKCdelta neurons in extended amygdala (including CeA and BNSTov) contributes to acute stress-induced hypophagia, thus controlling a behavioral response to stress. Further studies on these PACAPergic circuitries will help us understand better how PACAP integrates into the neurotransmission events required for endocrine and behavioral responses to stress. It may also open new avenues for intervention in stress-related neuropsychiatric disorders.

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National Institute of Mental Health Intramural Research Program, Project MH002386 to L.E.E.

S8-3: PACAP and acetylcholine regulate distinct Ca2+ responses and secretory outputs in chromaffin cells

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The adrenal chromaffin cell transduces chemical messages into outputs that regulate end organ function throughout the periphery. Two important neurotransmitters are released by preganglionic neurons onto chromaffin cells - acetylcholine (ACh) and pituitary adenylate cyclase activating polypeptide (PACAP). Unlike ACh, the mechanisms coupling PACAP stimulation to exocytosis are poorly understood. Our goal was to address this knowledge gap. We show that PACAP stimulates a Galpha-s-coupled pathway that signals through PLCepsilon to drive Ca2+ entry and exocytosis. PACAP stimulation causes a complex pattern of Ca2+ signals, leading to a sustained secretory response that is kinetically distinct from the form stimulated by ACh. PACAP and ACh are also associated with fusion modes that differentially impact the release of granule cargos. Importantly, only the secretory response to PACAP, not ACh, is eliminated in cells lacking PLCepsilon expression. The data show that the actions of ACh and PACAP on the chromaffin cell enable nuanced and variable outputs, rather than stereotyped responses, to synaptic stimulation.

Support was provided by NIH grants R37DA0333987 to JRT, R35GM127303 to AVS, R01GM111997/NS122534 to AA, and the American Heart Association 17GRNT33661156 to KC.

S8-4: PACAP co-expression in GABAergic or glutamatergic circuits and its relevance for behavioural adaptation.

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PACAP is widely expressed in the brain and has been associated to multiple biological actions within the brain including the regulation of circadian rhythms, modulation food intake, activating the vasopressinergic magnocellular neurons, activating the HPA axis, among others. Although the location of PACAPergic neurons and their innervation patterns, as well as that of its main receptors PAC1, VPAC1 and VPAC2 have been previously characterized, its specific distribution within GABAergic or glutamatergic neurons of defined circuits has not been well described. In this work we develop a systematic and extensive whole brain mapping of the mouse neurons expressing the mRNA for PACAP or PAC1 and its colocalization with vGLUT1 and vGLUT2, the vesicular transporters for glutamate type 1 and 2, or with VGAT, the vesicular GABA transporter. We tested the effects of PACAP deficiency using a constitutive PACAP knock-out mouse, and a predator odour behavioural test, KO mice displayed less awareness of the predator odour, and FOS analysis showed a decreased activation of targets of PACAP neurons in extended amygdala. Also blunted VGLUT and VGAT expression was observed in KO mice. These results shed light of the role of PACAP signalling on modulating excitatory or inhibitory circuits that embody a successful behavioural adaptation.

Plenary IBRO Sponsored Lecture 3:

Neuropeptide S: Five neuronal clusters, one function?

Luis de Lecea Stanford University, USA

Neuropeptide S (NPS) is a highly conserved peptide found in all tetrapods which functions in the brain to powerfully promote heightened arousal. The broad distribution of NPS receptor expression suggests the possibility for multifactorial inputs to regulate behavior; however, the function of independent NPS circuits remains unknown. Here we generate a novel NPS Cre knock-in mouse to identify prominent NPS clusters in the Kölliker-Fuse nucleus, dorsomedial thalamus, and the peri-locus coeruleus. In vivo Calcium recordings from individual NPS subpopulations reveals a relative increase in NPS cellular activity immediately preceding transitions to wakefulness, as well as a robust decrease in Calcium activity during REM sleep. Across these subpopulations, NPS+ cells in the Kölliker-Fuse nucleus receive a substantially larger proportion of their connections from anxiety-associated regions such as the central amygdala and bed nucleus of the stria terminalis, which may contribute to the arousal-promoting effects of NPS. Chemogenetic activation of Kölliker-Fuse NPS+ neurons significantly reduces REM sleep time and alters several respiratory parameters indictive of increased breathing rate. Together, our data suggests that NPS neurons in the Kölliker-Fuse represent a novel subpopulation regulating breathing and are sufficient to recapitulate the sleep/wake phenotypes observed with broad NPS+ cellular activation.

Keynote symposium 3 (KS3)

Chair: Javier Stern (USA)

KS-3-1 Alan Watts

University of Southern California, Los Angeles, USA. Brain neuropeptidergic networks and the control of energy balance

KS-3-2 William Wisden

Imperial College London, UK. Peptides and sleep-promoting circuitry.

KS-3-3 Francesco Ferraguti

Medical University of Innsbruck, Austria. Metabotropic glutamate receptors' role in cortical peptide expressing interneurons

KS-3-1: Brain neuropeptidergic networks and the control of energy balance

Watts, Alan G

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Energy balance describes the state of the relationship between the origins and destinations of the ATP derived from oxidizable fuels. In this way, ATP is the apical regulated variable in energy homeostasis. ATP availability-as indicated by the ATP/ADP ratio—is very tightly maintained at about 10:1 in all cells. Failing to maintain ATP availability very rapidly leads to the death of cells and potentially the organism. Maintaining energy homeostasis relies on a series of mechanisms that control: 1), eating behavior (nutrient acquisition); 2), the various destinations of nutrients after being absorbed from the gut (nutrient partitioning); and 3), which of the many and varied cellular and organismal functions are the targets of the energy released by ATP hydrolysis at a particular time (energy expenditure). A series of interacting neuronal networks in the brain are central components that enable all three sets of these control mechanisms. In this talk I will discuss the structure/function relationships of an exemplar control network that is defined by catecholaminergic/glutamatergic/ neuropeptidergic projections from the medulla to a set of interconnected gray matter regions in the forebrain. These include the paraventricular, dorsomedial (DMH), and arcuate (ARH) nuclei in the hypothalamus, together with the paraventricular thalamic nucleus (PVT), and parts of the bed nuclei of the terminal stria. Ablating these medullary projections to this network disrupts how its various outputs influence the control of energy balance during both short-term and long-term metabolic challenges. The fact that the DMH, PVT, and ARH also contribute to a second network that controls the distribution of circadian timing information helps illustrate how inter-network connectivity can enable the way that different networks interact to generate a variety of appropriate motor responses at a particular time. The fact that some hypothalamic nuclei appear to be nodes that are common to more than one functional network offers a way to apply formal network analytical methods to reveal the functional significance of these different connectomes.

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KS-3-2: Peptides and sleep-promoting circuitry

Wisden, William

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Neurons that control sleep are scattered throughout the central nervous system. In addition to using the small molecule neurotransmitters such as glutamate, GABA and amines, and gaseous transmitters such as nitric oxide, many of these neurons, depending on location, express and presumably release peptides such as somatostatin, galanin, parvalbumin and neurotensin. As with much recent work on dissecting neural circuits for all kinds of behaviours in neuroscience, the majority of the sleep field has tended to focus on this peptide expression as a tool to mark out the neurons for genetic manipulation rather than study the function of the peptides themselves in relation to sleep. Nevertheless, it could well be that many of these peptides are playing essential roles in regulating sleep. In this lecture, I will give a brief overview of this circuitry that governs sleep. I will then focus on our recent work showing how we discovered that somatostatin 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. We also found that after stress, these VTA somatostatin neurons inhibit corticotropin-releasing factor (CRF) release in the paraventricular hypothalamus. We propose that a specific circuit allows animals to restore mental and body functions by sleeping, potentially providing a refined route for treating anxiety disorders.

Wellcome Trust and UK Dementia Research Institute

KS-3-3: Metabotropic glutamate receptors' role in cortical peptide expressing interneurons

Ramos-Prats, Arnau, 1; Matulewicz, Pawel, 1; Edenhofer, Marie, 2; Wang, Kay-Yi, 3; Yeh, Chia-Wei, 3; Kress, Michaela, 2; Lien, Cheng-Chang, 3; Kummer, Kai, 2; Ferraguti, Francesco, 1

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Metabotropic glutamate subtype 5 (mGlu5) receptors are known to play an important role in regulating cognitive, social as well as positive and negative valence domains. However, it remains largely unknown at which neural circuits and neuronal classes mGlu5 receptors act to influence specific behaviors. Here, we examined the contribution of these receptors in somatostatin-expressing (SST+) neuron focusing on their role in regulating activity function, plasticity, brain synchrony and behavior. Localization of mGlu5 receptors in SST neurons was primarily observed in cortical interneurons, whereas in the lateral septum, hypothalamus and central amygdala no coexistence could be detected.

By crossing mGlu5loxP/loxP animals with the SST-ires-Cre driver mouse line, we obtained the conditional ablation of the mGlu5 gene selectively from SST+ interneurons. Loss of mGlu5 receptors in SST+ interneurons produced excitatory synaptic dysfunction in a region and sex-specific manner and a range of emotional imbalances, which included diminished social novelty preference, fear responses and anxiety-like behavior. During fear memory retrieval, the lack of mGlu5 receptors in SST+ interneurons impaired the generation of theta frequency in the medial prefrontal cortex and ventral hippocampus. In female mice, brain rhythmic activity during fear memory retrieval was dependent on the estrous stage. Altogether these findings reveal a critical role of mGlu5 receptors in regulating SST+ interneuron excitability necessary for emotional behavior homeostasis.

Austrian Science Fund grant W012060-10 to F.F.

Symposium 9

Neuropeptides, stress and sex differences

Chair: Tallie Z. Baram (USA)

S9-1 Joanna Dabrowska

Chicago Medical School, Rosalind Franklin University of Medicine and Science, Chicago, USA.

It takes three to dance - neuropeptidergic modulation of the BNST activity and fear processing by oxytocin, vasopressin, and CRF

S9-2 Gil Levkowitz

Weizmann Institute of Science, Rehovot, Israel. What makes some individuals fitter than others: The developmental underpinnings of stress resilience

S9-3 Javier Stern

Georgia State University, Atlanta, GA, USA. Novel intercellular communication modalities mediated by hypothalamic neuropeptides in health and disease states

S9-4 Geert de Vries

Georgia State University, Atlanta, GA, USA. Development and function of sex differences in the brain seen from a vasopressin and oxytocin perspective

S9-1: It takes three to dance - neuropeptidergic modulation of the BNST activity and fear processing by oxytocin, vasopressin, and CRF

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The dorsolateral bed nucleus of the stria terminalis (BNSTDL) is heavily innervated by peptidergic fibers, including fibers expressing oxytocin (OT), vasopressin (AVP) and corticotropin-releasing factor (CRF). The BNSTDL contains GABA-ergic neurons classified based on intrinsic membrane properties and firing pattern into three types (Type I-III neurons). Using in vitro patch-clamp recordings in male adult rats, we recently demonstrated that OT, via oxytocin receptor (OTR), affects activity of all three types of BNSTDL neurons in a distinct fashion. Specifically, OT selectively excites and increases spontaneous firing of Type I BNSTDL interneurons. As a result, OT increases the frequency, but not amplitude, of spontaneous inhibitory post-synaptic currents (sIPSCs) selectively in Type II neurons and reduces spontaneous firing of Type II neurons. As the majority of Type II BNSTDL neurons were shown projecting to the central amygdala (CeA), we showed that OT indirectly inhibits these Type II BNST→CeA projection neurons (Francesconi et al., 2021). In contrast, CRF has been previously shown to excite Type II BNSTDL neurons via CRF receptor type 1 (CRFR1). Therefore, we recorded from the retrogradely labeled BNST \rightarrow CeA projection neurons after CRF application and we show that CRF directly excites these BNST \rightarrow CeA output neurons. These findings suggest that OT and CRF have opposite, inhibitory and excitatory, effects on the BNSTDL output. In Type III BNSTDL neurons, OT reduced the amplitude, but not frequency, of both sIPSCs and evoked IPSCs via a postsynaptic mechanism without changing their intrinsic excitability (Francesconi et al., 2021). Our new findings show that AVP as well as a potent OTR agonist [Thr4,Gly7]-oxytocin (TGOT), but not OT, directly excite Type III BNSTDL neurons via a post-synaptic, OTR-dependent mechanism. As Type III neurons are putative CRF-producing neurons of the BNSTDL, we also recorded from fluorescent BNSTDL neurons after CRF-Cre transgenic rats (Cre-recombinase expression under CRF promoter) were injected with Cre-dependent AAV driving mCherry expression. We show that all CRF-mCherry-BNSTDL neurons in male and female CRF-Cre rats are classified as Type III and AVP directly excites these CRF-BNSTDL neurons via a direct, postsynaptic mechanism. Our results show that OTR and CRFR1 activation have opposite, inhibitory and excitatory, effects on Type II BNST-CeA output neurons, whereas OTR activation has a direct excitatory effect on Type III/CRF neurons and might therefore control local CRF release in the BNSTDL. As the BNSTDL has emerged as a key brain region translating an exposure to unpredictable threats into sustained fear and anxiety-like behavior, our findings suggest that OT, CRF, and AVP play an integrated role in the modulation of BNSTDL output and BNSTDL-dependent fear and anxiety-like behaviors. NIMH, R01 MH113007

S9-2: What makes some individuals fitter than others: The developmental underpinnings of stress resilience

Swaminathan, Amrutha, #; Gliksberg, Michael, #; Anbalagan, Savani, #; Wigoda, Noa, #; Levkowitz, Gil, #

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Individuals in a population respond differently to stressful situations. While resilient individuals recover efficiently, others are susceptible to the same stressors. However, it remains challenging to identify resilience in mammalian embryos to determine if stress resilience is established as a trait during development or acquired later in life. Using a new behavioural paradigm in zebrafish larvae, we show that resilience is a stable trait, which is determined and exhibited early in life and is passed on to the next generation. Resilient larvae showed higher expression of resilienceassociated genes such as neuropeptide Y and miR218, and larvae with mutations in these factors were significantly under-represented in the resilient population. Unbiased transcriptome analysis revealed that multiple factors of the innate immune complement cascade were downregulated in resilient larvae in response to stressors. Pharmacological inhibition and genetic knockouts of critical complement factors led to an increase in resilience. We conclude that resilience is established as a stable trait early during development, and that neuropeptides and the complement pathway play positive and negative roles in determining resilience respectively.

S9-3: Novel intercellular communication modalities mediated by hypothalamic neuropeptides in health and disease states

Stern, JE Neuroscience Institute, Georgia State University, Atlanta, GA, USA

Hypothalamic neuropeptides play critical roles in the maintenance of bodily homeostasis, acting both peripherally and centrally. Within the brain, neuropeptides can be released and exert actions in a synaptic (axonal, localized) and non-synaptic (somatodendritic, diffusible) manner. Most of our current knowledge on precise mechanisms regulating release and actions of neuropeptides has been obtained from studies evaluating axonal release. Thus, much less is known about mechanisms and actions involving neuropeptides as diffusible signals. In this presentation, I will use vasopressin (VP) as a neuropeptide model to highlight recent studies from our group and our collaborators, which provide novel insights into VP release and actions as a diffusible signal. Within this context, I will summarize recent studies that support that unique firing activity modalities can differentially evoke axonal vs. dendritic release of VP. I will also present recent findings supporting diffusible VP to mediate an unconventional form of salt-evoked neurovascular coupling within the supraoptic nucleus. Finally, I will introduce and discuss recent data supporting a novel hypothalamic portal system that communicates the suprachiasmatic nucleus with the OVLT, which stands as an alternative route for secreted neuropeptides to act in a diffusible manner within the hypothalamus.

S9-4: Development and function of sex differences in the brain seen from a vasopressin and oxytocin perspective

De Vries, G. J.

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Research over the past half century has revealed myriads of sex differences in the brain in almost every aspect studied, e.g., in the volume of brain areas, number of cells, content of specific neurotransmitters, etc. Attempts to link these sex differences to sex differences in overt functions and behavior have been surprisingly difficult. One reason for that may be that most of these sex differences are merely adaptations, geared to make male neural circuits work most optimally in male bodies, and the same for females. In other words, in most cases sex differences may be compensatory in nature and are meant to avoid undesirable sex differences. Recent advances in genomic analysis have added thousands of newly discovered differentially expressed genes and equally intimidating numbers of differentially expressed epigenomic marks. Interestingly, in cases where it has been studied, these sex differences do not necessarily cause sex differences in the systems they control. For example, sex differences in epigenetic marks do not predict well differences in expression levels and, likewise, functions regulated by genes that show differences in expression do not necessarily differ between males in females. Given their sheer number, it is difficult to envision a study that tries to identify the function of each gene that shows sexually differentiated expression. Lessons can be learned, however, from genes that have been known for a long time to be expressed sexually differentiated way. For example, vasopressin (AVP) innervation of the brain shows some of the most consistently found neural sex differences among vertebrates, with males having denser AVP projections from, the bed nucleus of the stria terminalis (BNST) and medial amygdaloid nucleus than do females. Although sex differences have been found in other AVP cell groups as well, e.g., in the suprachiasmatic, paraventricular (PVN), and supraoptic nucleus, these differences are not nearly as extreme. Recently, we have begun to directly test the function of these sexually dimorphic cells, by using AVP Cre mice and Cre-dependent viral vectors to specifically manipulate AVP-expressing cells. We find, for example, that ablating BNST AVP cells affects social and anxiety-related behavior stronger in males than in females. Interestingly, ablating PVN AVP cells, which do not show marked sex differences, have more pronounced effects in females. Possible significance of these different effects at the circuit level will be discussed.

Supported by NIH R01 grant MH121603

Symposium 10

Vasopressinergic regulation of social behavior

Chair: Dora Zelena (Hungary)

S10-1 Matthew Paul

University at Buffalo SUNY, New York, USA. Social Development and Vasopressin: Investigations into the atypical social behavior of Brattleboro rats

S10-2 Alexa Veenema

Michigan State University, USA. Neural circuitry of social play: Involvement of oxytocin and vasopressin

S10-3 Dora Zelena

University of Pécs, Pécs, Hungary.

Vasopressinergic influence on disturbed sociability in autismus and schizophrenia

S10-4 Abimael Gonzalez-Hernandez

National Autonomous University of Mexico, Mexico City, México. The role of oxytocinergic neurotransmission in pain processing at the trigeminal level S10-1: Social Development and Vasopressin: Investigations into the atypical social behavior of Brattleboro rats

Paul, Matthew J., 1 1 Department of Psychology, University at Buffalo SUNY, Buffalo, NY, USA

Vasopressin has been implicated in a variety of social behaviors in many species and across all stages of development. It is often assumed that vasopressin regulates these behaviors through actions on social motivation, but this hypothesis is rarely tested. Alternative hypotheses have proposed that vasopressin influences social behavior via its actions on sensorimotor processing, stress, autonomic function, and behavioral state. In this talk, I will present data from experiments that have begun to address this question within a developmental framework using Brattleboro rats, which lack vasopressin due to a spontaneous mutation in the vasopressin gene. We have found that male and female juvenile Brattleboro rats exhibit an atypical social behavior profile characterized by decreased social play and vocal communication, but increased huddling behavior. This atypical social behavior is associated with a hyppoaroused, low anxiety-like phenotype, which is consistent with the hypothesis that vasopressin influences social behavior through its regulation of behavioral state. The hypoaroused phenotype persisted after viral-rescue of vasopressin function within magnocellular cells of the paraventricular nucleus of the hypothalamus, suggesting that these effects are mediated by central actions of vasopressin. Initial tests using operant conditioning, social approach, and social preference testing paradigms have found little support for the social motivation hypothesis within this animal model. These findings suggest that vasopressin plays a developmental role in regulating behavioral states such as arousal. Further studies are needed to directly test whether these effects on arousal account for vasopressin actions on social development. Uncovering the mechanism by which vasopressin regulates social development will guide the development of vasopressin treatments for disorders of social development such as Autism Spectrum Disorders. National Science Foundation IOS-1754878

S10-2: Neural circuitry of social play: Involvement of oxytocin and vasopressin

Veenema, Alexa H.; Lee, Jessica D.A.; Bowden, Samantha M.; Bredewold, R.

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Playing with peers in children and young animals is essential for the development of lifelong social competence and social skills. Children with autism spectrum disorder (ASD) have difficulties with this social play which contributes to their lifelong social dysfunction. ASD is more prevalent in males than in females, suggesting sex differences in the etiology of ASD. Developing effective means to restore social play in ASD children is expected to improve their lifelong social functioning, but this requires understanding of the neural basis of social play and potential sex differences herein. In this talk I will discuss the neurobiological mechanisms driving social play behavior with specific emphasis on the involvement of the neuropeptides vasopressin and oxytocin. Our research demonstrates that vasopressin originating in the medial amygdala and bed nucleus of the stria terminalis and acting on vasopressin 1a receptors in the lateral septum and ventral pallidum has opposing effects on the expression of social play behavior in male and female juvenile rats. We further demonstrated that oxytocin originating in the paraventricular nucleus and acting on oxytocin receptors in the nucleus accumbens facilitates social play behavior in both sexes, but it more potent in males than in females. These findings may have implications for the sexspecific use of therapeutic drugs targeting the vasopressin and/or oxytocin systems in aiming to improve social play engagement in ASD children. Supported by NSF IOS 1735934, NIH R01MH102456, NIH R01MH125806 to AHV and NSF GRFP DGE-1848739 to JDAL

S10-3: Vasopressinergic influence on disturbed sociability in autismus and schizophrenia

Zelena, Dóra Department of Physiology, Medical School, University of Pécs

Social behaviour plays a pivotal role both in everyday life as well as in pathological conditions, among others in autism and schizophrenia. Different forms can be distinguished from social interest through amiable social behaviour till aggression. Vasopressin, a well-known antidiuretic hormone, was long implicated in social behaviour. In relation to autism we confirmed in pups that vasopressin deficiency may reduce maternal-separation-induced ultrasound vocalization, which can be reversed by antipsychotic treatment. This effect went through the V1a and V1b receptors. In adult rats the vasopressin-deficient Brattleboro rats showed several schizophrenia-like symptoms including social disturbances, which was also reversed by acute as well as chronic antipsychotic treatment. In relation to aggression we found that the condition of the subject (e.g. sexually experienced, naive male or lactating female) may deeply influence the role of vasopressin. Our recent work confirmed previous studies that the effect of vasopressin is dependent on the brain region and the imbalance in the vasopressinergic system may unmask region-specific effects. Some epigenetic changes were detected as possible background mechanism. All in all, modification of the vasopressinergic system may have beneficial effect in autism and schizophrenia, however, several details need to be clarified e.g. sex dependency.

S10-4: The role of oxytocinergic neurotransmission in pain processing at the trigeminal level

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Apart from the well-known functions of oxytocin in parturition, lactation, and social behavior, this neuropeptide plays a relevant role as a modulator of pain transmission. Current data, using nociceptive, inflammatory, and neuropathic pain models, consistently show that this molecule inhibits pain transmission at the spinal cord level by activating its receptor, the OTR. Interestingly, little is known about the role of oxytocinergic transmission modulating nociception at the trigeminal level, a primary neuroanatomic site involved in the modulation of craniofacial pain. In anesthetized male Wistar rats (290-320 g) and using in vivo unitary extracellular recording of second-order wide dynamic range cells located in the trigeminocervical complex (TCC; Sp5c – C2 region), we showed that topical oxytocin administration inhibits the periorbital nociceptive-evoked responses of the first branch (V1: ophthalmic) of the trigeminal nerve. The antinociceptive action of oxytocin (0.2 -20 nmol) was reversed by spinal pretreatment with a selective OTR antagonist (L-368,899) and remained unaffected by a selective V1A vasopressin receptor antagonist (SR-49059). Furthermore, using a similar approach but recording a second-order neuron receiving simultaneous input from the periorbital and meningeal afferents, 20 nmol oxytocin administration at TCC diminishes the electrically evoked neuronal responses of meningeal nociceptive afferents probably by OTR activation. Since exogenous oxytocin elicits inhibition of pain transmission at the trigeminal level, we tested the role of hypothalamic paraventricular nuclei (PVN) stimulation on the trigeminal nociceptive activity, and we found that PVN stimuli block nociceptive transmission at trigeminal level via OTR. Coupled with the immunohistochemical evidence showing that OTR receptors are expressed at the trigeminal level and in the meningeal tissue near CGRP-positive fibers, these data strongly support the contention that enhancement of oxytocinergic transmission could be used as a potential therapy to treat headaches. Part of these data has been previously published in Neuropharmacology (2018; 129:109-117) and Experimental Neurology (2020; 113079).

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Symposium 11

Molecular and structural biology of neuropeptides at their cognate GPCRs

Chair: John Furness (Australia)

S11-1 Robert P. Millar

University of Pretoria and University of Cape Town, South Africa. Rescue of function in human mutant peptide GPCRs with cell permeant small molecules: a more viable approach than gene therapy

S11-2 Helene Castel

Normandie Univ, UNIROUEN, INSERM U1245, Rouen, France. Urotensin II-based local hydrogel trap leads to immune control associated with improved survival and cognitive functions in a mouse model of glioblastoma resection S11-1: Rescue of function in human mutant peptide GPCRs with cell permeant small molecules: a more viable approach than gene therapy

Robert P Millar1,2, Claire L Newton1, Ross C Anderson1

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G-protein coupled receptors (GPCRs) convey 80% of signalling across cell membranes and hence inactivating mutations give rise to diverse pathologies. Reproduction in vertebrates is driven by hypothalamic peptides, kisspeptin and neurokinin B which stimulate gonadotropin releasing hormone (GnRH), which in turn stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) which regulate testis and ovarian function. Inactivating mutations in G-protein coupled receptors (GPCRs) at all levels of this axis give rise to incomplete reproductive development and adult infertility. The majority of the mutations in these GPCRs cause misfolding of the receptor and a failure to traffic to the cell surface. We have therefore sought for cell permeant small molecules which can bind orthosterically or allosterically to stabilize the nascent GPCR in the endoplasmic reticulum and chaperone the mutant GPCR to the cell membrane.

We have successfully identified cell-permeant small molecules targeting receptors at all levels of the axis and demonstrated rescue of cell surface expression and restoration of function for NKB, GnRH, LH and FSH receptors. Moreover we have also been able to allosterically activate binding deficient and signalling deficient LHR with an LHR small molecule

These discoveries represent an advance towards personalized medicine for GPCR inactivating mutations in the human reproductive hormone axis. As GPCRs constitute 80% of signalling in humans, inactivating mutations are likely to be a major contributor of disease and hence targets for small molecule rescue of function. The existence of vast numbers of GPCR-targeted small molecules from pharma data bases, many of which have already entered the clinic but abandoned, provides a rich source for treating GPCR human mutations and is more viable than a gene repair approach.

S11-2: Urotensin II-based local hydrogel trap leads to immune control associated with improved survival and cognitive functions in a mouse model of glioblastoma resection

Castel, Hélène1,2

INormandie Univ, UNIROUEN, INSERM U1245, CBG, Genetics, Biology and Plasticity of Brain Tumors, 76000 Rouen, France, 2Institute for Research and Innovation in Biomedicine (IRIB), 76000 Rouen, France

Background: Glioblastoma (GB) is the most aggressive brain primary tumor. The prognosis remains poor despite extensive resection, radio and/or chemotherapy. More than 95% of GBs recur in the margin of the resection cavity, an area in which invasive tumor cells are found acting as a tumor reservoir. This invasiveness associated with the radio and/or chemoresistance of these cells, the presence of a blood-brain barrier that limits the delivery of anti-neoplastic agents, and the immunosuppressive microenvironment justify the development of original therapeutic strategies. We have previously demonstrated that the peptide Urotensin-II (UII) as well as its receptor UT are systematically expressed in human glioblastomas (GB) stimulating the infiltration of healthy brain parenchyma by chemotaxis, as well as angiogenesis and immune infiltration. Here, we propose to use a local delivery system based on a biocompatible hydrogel5 containing the chemopeptide urotensin II (hUII) or a biased synthetic analog DAB8-hUII, to "trap" GB cells, and/or to control immune cells expressing its G protein-coupled receptor UT, leading to tumor regression and neurological benefit, in a mouse model of GB resection. Material and Methods: In vitro, invasion towards UII/analog across different hydrogels or glue of human or murine GB-GFP cell lines was evaluated in Boyden chamber and cloning ring assays. In vivo GB cells were intrastriatally xenografted, then resected while hydrogel- or glue-containing UII/analog was injected in the cavity resection. Behavioral tests, brain immunohistochemical analyses and mouse survival were then investigated.

Results: In vitro, invasive capacity of human U87 and 42MG or murine GL261 and CT2A GB cells was stimulated by UII loaded into hydrogel-based hyaluronic acid supplemented with collagen or other chemicals, PNIPAAm-PEG, or thrombin-fibrin glue. In vivo, injection of UII- or DAB8-hUII-loaded glue into the cavity resection of GL261 and CT2A GB in C57BL/6 mice significantly improved survival compared with tumor and resected experimental conditions. Neurological status was also tested before and after GB resection. We found that GL261 and CT2A cell-bearing mice expressed altered spontaneous activity, emotion and cognitive functions. Intracavity injection of the glue improved resignation and anxiety and increased motor activity and cognition with a best cognitive recovery with hUII and DAB-8-hUII-loaded glue groups. Ex vivo brain analyses revealed high expression of UT and UII in some GB GFP-positive cells and macrophages within GB core and at the interface with the normal brain, GB cells expressing UT migrating along tortuous podocalyxin+ vascular components. In brains bearing hydrogel/hUII glue, vascularization appears modified and GFAP+ astrocytes and F4/80+ macrophages were highly recruited in the border of the cavity, compared with the other conditions. Conclusion: A local glue containing UII may trap GB cells and remodel the tumor microenvironment responsible for survival and cognitive improvements, providing new option in the therapeutic arsenal of GB.

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Symposium 12

Neurohypophysial hormone regulation in pathophysiological states

Chair: Quentin Pittman (Canada)

S12-1 Andres Quintanar-Stephano

Vasopressin deficiency and V1a-V2 receptors blockade revert liver damage and fibrosis in rats with protracted liver disease: A new therapeutic approach?

S12-2 Margarita Currás-Collazo

University of California, Riverside, California, USA. Maternal T4 supplementation normalizes deficient social behavior and reduced hypothalamic oxytocin content produced by perinatal exposure to PBDE

S12-1: Vasopressin deficiency and V1a-V2 receptors blockade revert liver damage and fibrosis in rats with protracted liver disease: A new therapeutic approach?

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Liver diseases account two million annual deaths worldwide approximately and pushing researchers to find effective treatments for liver fibrosis. Portocaval anastomosis (PCA) and carbon tetrachloride (CCl4) administration are proved models of liver inflammation, failure and cirrhosis. Arginine vasopressin (AVP) is an important player in immune regulation, inflammation and fibrotic processes. Here we present our studies on molecular and cell mechanisms through AVP deficiency or blocking the V1a-V2 AVP improve/revert liver damage. Neurointermediate pituitary lobectomy (NIL) in rats induces significant-permanent decrease in AVP and oxytocin in serum levels. In cirrhotic+NIL hamster AVP deficiency restored liver functions and histophysiology: restoration of alkaline phosphatase serum levels, type I collagen and TIMP-2 gene expression, increased deposition of type III collagen, increased MMP-13 gene expression, and increased in number and size of regeneration nodules. In other study in PCA and NIL+PCA rats, AVP deficiency restored liver failure and fibrosis, thus, results showed that in the PCA+NIL animals, damaged liver function (bilirubin, protein serum, and liver glycogen content) were restored back to normal. Cell and molecular variables also indicated restoration of the liver functions (gene expression of IL-1 and COLL-I [proinflammatory profile] were decreased, whereas the IL-10, TGF-β, and MMP-13 [anti-inflammatory profile markers] were increased. Histopathology of this PCA+NIL group also showed significantly decreased signs of liver damage with lower extent of collagen deposition and fibrosis. We speculate that that low AVP serum levels are not enough to fully activate the AVP receptors of the immune cells and thus resulting in a decreased activation of the cell signaling pathways associated with inflammatory-profibrotic responses, while simultaneously the activation of anti-inflammatory-fibrotic signaling pathways are responsible of the liver improvement. The success of the AVP deficiency strategy suggests that blocking AVP receptors may be therapeutically useful to treat inflammatory-profibrotic liver diseases. To test this hypothesis, we compared the effects of AVP deficiency and the effects of conivaptan (CV), a V1a-V2 AVP receptors antagonist to determine if the AVP receptors blockers may restore the liver damage in rats with chronic PCA. Results showed that NIL and CV in PCA animals caused normalization of liver functional tests (AST, bilirubin and albumin serum levels) and liver glycogen content. In addition, the histopathological findings showed a diminishment of fibrotic septa, increased number of regenerative nodules, and decreased collagen content. In summary, present results strongly suggest that AVP deficiency in chronic liver disease significantly improves liver functions and fibrosis, whereas at the doses used of CV, caused a significant decrease in liver fibrosis and recovery of some liver functions. We conclude that the protracted decrease of AVP serum levels and chronic CV administration could be used as an alternative therapy in the treatment of liver disease and fibrosis. Of course, more experiments are needed to test this possibility.

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S12-2: Maternal T4 supplementation normalizes deficient social behavior and reduced hypothalamic oxytocin content produced by perinatal exposure to DE-71

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Introduction: The prevalence of neurodevelopmental disorders (NDDs) is rising at an alarming rate[1]. NDD etiology is multifactorial, resulting from sex-specific genetic susceptibilities that may interact with environmental exposures during critical developmental periods. Polybrominated diphenyl ethers (PBDEs) are anthropogenic persistent organic pollutants (POPs) that have been widely used commercially as flame retardants in common household products. Even though PBDEs have been partially banned in Western countries, large amounts in stock, and continuous emissions and waste recycling still pose challenges on human health. In humans, PBDEs are endocrine-disrupting chemicals (EDCs) with neurotoxic effects including altered socioemotional development and hyperactivity[2],[3], as well as endocrine disruption of steroidal hormone systems such as the thyroid system. The latter may have consequences on their regulated neuroendocrine systems critical for social behavior. Interestingly, transcription of the OXT gene (Oxt) is regulated by thyroid hormones (TH) that are critical for nervous system development[4]. We have previously shown that PBDE-exposed F1 female progeny display autism-relevant characteristics such as deficient social novelty preference, social recognition memory (SRM), exaggerated repetitive behavior and altered social odor discrimination[5]. These phenotypes are concomitant with altered plasma and brain TH species[8]. In this study, we tested the hypothesis that developmental exposure to PBDEs can reduce hypothalamic OXT in exposed offspring in a TH-dependent manner. Materials and Methods: C57BL/6N dams were exposed to the commercial PBDE mixture, DE-71, at 0.1 mg/kg/d (L-DE-71), 0.4 mg/kg/d (H-DE-71) or with corn oil vehicle control (VEH/CON) for 10 wks (pre-conception: 4 wk, gestation: 3 wk, lactation: 3 wk). A subset of dams in each exposure group was treated with levothyroxine, a synthetic analogue of thyroxine (T4) (8 µg/100 g bw) in drinking water containing 0.01% bovine serum albumin (BSA), during gestation and lactation (GD 12-PND 21)[6]. Another group of dams were administered the thyroid synthesis inhibitor, 6-propyl-2-thiouracil (PTU; 50 mg/L) in drinking water at GD 14-PND 21[7]. Social and repetitive behavior, hypothalamic immunochemical OXT content and thyroid responsive gene expression were examined in offspring. Results: Compared to VEH/CON, PBDE exposure did not alter dam plasma T4 at PND 0 or

21. T4 supplementation resulted in elevated plasma T4 only in L-DE-71 group, suggesting recovery of an altered TH system in L-DE-71. Early maternal attachment of exposed pups was normal compared to VEH/CON. In adulthood, L-DE-71 male and female offspring showed deficient social memory recognition (24 hr memory) at PND 30 and social novelty preference (30 min retention memory) at PND 100 indicating persistence of deficient social recognition ability. Interestingly, maternal T4 supplementation rescued normal social behavior only in females. In contrast, T4 supplementation was equally effective at reversing exaggerated repetitive behavior in L-DE-71 male and female offspring. DE-71 reduced OXT immunofluorescence density in male (L- and H-DE-71) and female (L-DE-71) paraventricular (PVN) and male (L-DE-71) supraoptic nuclei (SON) of the hypothalamus. T4 supplementation normalized OXT content in L-DE-71 female and H-DE-71 male PVN. For plasma OXT, all dam exposure groups showed normal levels and these were elevated by T4 supplementation in VEH/CON and H-DE-71. Maternal T4 supplementation increased plasma OXT in VEH/CON female and L-DE-71 male offspring, RT-oPCR analysis showed possible mechanisms for L-DE-71 reduced brain T4[8], reduced hypothalamic OXT and deficient social behavior, i.e., downregulated hypothalamic Trh and cortical Oatp1c1 and Dio3 in females and upregulated hypothalamic Dio2 in males. H-DE-71 males show elevated hypothalamic mRNA transcript for Mct8 and Dio2 which may lead to excess inactivation of T4 and T3 and reduced PVN OXT. While PTU produces sex-dependent alterations like DE-71, its actions do not mimic exactly suggesting more varied TH system targets of PBDEs. Conclusions: These results provide novel insight into neuroendocrine disruption of the central oxytocin system and possible TH targets produced by maternal transfer of PBDEs and its possible contribution to neurodevelopmental disorders such as autism. Further, maternal T4 supplementation may provide a novel therapy for autistic-like phenotypes, although sex-dependent effects are possible.

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IRPS General Assembly

Cottrell Lecture Theatre (09:00-10:00)

IBRO Neuroscience Special Lecture:

Interaction of amyloid beta oligomers and alpha3-GABA A receptors in locus coeruleus neuronal excitability and Alzheimer's pathology

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Background and Aims:

Amyloid β oligomers (A β O) are potent modulators of Alzheimer's pathology, yet their impact on one of the earliest brain regions to exhibit signs of the condition, the locus coeruleus (LC), remains to be determined. Of particular importance is whether A β O impact the spontaneous excitability of LC neurons. This parameter determines brain-wide noradrenaline (NA) release, and thus NA-mediated brain functions, including cognition, emotion and immune function, which are all compromised in Alzheimer's. Therefore, the aim of this research was to determine the expression profile of A β O in the LC of Alzheimer's patients and to probe their potential impact on the molecular and functional correlates of LC excitability, using a mouse model of increased A β production (APP-PSEN1).

Methods Immunohistochemistry and confocal microscopy were used to provide correlative A β O expression analyses in samples from Alzheimer's patients and APP-PSEN1 mice. Patch clamp electrophysiology was used to assess the impact of native A β O on LC neuronal excitability and neurotransmitter receptor function.

Results: LC ABO immunoreactivity was located both intraneuronally and extracellularly in both Alzheimer's and APP-PSEN1 samples. APP-PSEN1 LC neuronal hyperexcitability accompanied this ABO expression profile, arising from a diminished inhibitory effect of GABA, due to impaired expression and function of the GABA-A receptor (GABAAR) α3 subunit. This altered LC α3-GABAAR expression profile overlapped with ABO expression in samples from both APP-PSEN1 mice and Alzheimer's patients. Aged a3-GABAAR knockout mice in turn exhibited increased LC ABO expression, impaired recognition memory, neuroinflammation and decreased brain volume, suggesting a direct interaction between α3-GABAAR, AβO pathways and Alzheimer's-associated brain pathology. Finally, strychnine-sensitive glycine receptors (GlyRs) remained resilient to Aβinduced changes and their activation reversed APP-PSEN1 LC hyperexcitability. Conclusions: The data suggest a close association between ABO and a3-GABAARs in the LC of Alzheimer's patients, and their potential to dysregulate LC activity, thereby contributing to the spectrum of pathology of the LC-NA system in this condition.

The Alzheimer's Society, UK

Closing Lecture:

A long research and translational arc to CGRP-based treatment of migraine

Goadsby, Peter J

NIHR King's Clinical Research Facility, King's College London, UK

Migraine is a common, disabling neurological disorder of the brain (1). Forty years ago products of alternative splicing of the RNA from the expression of calcitonin gene recognized calcitonin generelated peptide (CGRP) (2). It had been known branches of the trigeminal nerve were implicated in migraine (3), that fact had not been exploited in clinical practice. After a presentation on the protective effects of the trigeminal innervation on the cerebral circulation (4), Lars Edvinsson and I formulated an hypothesis that neuropeptides in the trigeminovascular system may be targets for migraine therapeutics. We showed direct stimulation of the trigeminal ganglion in cat and humans caused cranial release of CGRP and substance P (5), while stimulation of the pain-producing structure, the superior sagittal sinus, only resulted in CGRP and not substance P release (6). We then showed that CGRP and not substance P was elevated in the cranial circulation during migraine (7). This work predicted the failure of substance P receptor antagonists. We reported the triptan, serotonin 5-HT1B/1D receptor agonist, sumatriptan reversed the CGRP effect and controlled migraine (8), predicting the outcome of the proofof-principle study that showed olcegepant, a CGRP receptor antagonist, was effective in acute migraine (9). CGRP pathway blockers: monoclonal antibodies and gepants, have now been used successfully in more than thirty countries and in some millions of patients to reduce disease burden in migraine. References

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Datablitz

Effects of pituitary hormones deficiency from the specific pituitary lobes on learning, memory and survival behavior in the Wistar rat

Adrian Limón-Mendoza1, Martín López-Rico1, José N Muñoz-Tabares1, Ángel H Hernández-Gómez1; Norma Ramírez-Rojas1, Andrés Quintanar-Stephano1

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The role of the different adenohypophyseal hormones (GH, PRL, ACTH, TSH, LH and FSH) and neurohypophyseal (AVP and OXY) hormones on animal behavior, memory and learning have been stablished, however the role of lobe-specific hormones on these cerebral functions has been not completely dilucidated. Here we study the specific pituitary hormone deficiencies in hypophysectomized (HYPOX), with anterior lobectomized (AL) and neurointermediate lobectomized (NIL) rats, subject to novel object recognition (NOR), forced swim (FS), electric avoidance chamber (EC), damsel in distress (DIS) and prepulse inhibition (PPI) tests. For comparisons the intact control (IC) and sham operated (SHAM) groups were also included. The NOR test indicates that in the NIL animals the AVP and OXT are required to maintain the ability to learn in short and long-term. The FS shows that in the NIL and HYPOX groups the AVP and OXT deficiency are more important than the anterior pituitary hormones in the motivation and survival behavior. In the IPP test the AL group showed an inability to respond to a harmful stimuli; indicating a deficiency in acquiring long-term memory, thus suggesting that the anterior pituitary hormones are required to maintain the alert state. In the EC test HYPOX animals had an inability to recognize longterm dangerous situations. The results of the DIS test showed that despite the several pituitary hormones deficiencies, all groups remained with the same innate motivation and empathy to save the damsel-in-distress. This and previous experiments indicate that specific hormones have direct effects on some brain functions associated to individual behavioral patterns. Present results support that models of pituitary lobe specific hormones deficiencies would be a tool to sift the role of every specific pituitary hormones on behavioral.

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Phenotypes associated with constitutive versus conditional PACAP knock out in mice: Implications for understanding neuropeptide function via genetic manipulation

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We recently identified (Bakalar et. al., Psychoneuroendocrinology 135: 105447, 2022), we revealed transcriptomic effects of PACAP deficiency that occur in the basal/quiescent state (in unstressed mice), and transcriptomic effects that emerge in response to stress in wild-type mice but are absent in constitutively PACAP-deficient C57Bl6/N mice. We have provisionally attributed the former to putative developmental effects of PACAP (these are not phenocopied in PAC1 knockout mice), and the latter to putative direct effects of adult PACAP neurotransmission (these are phenocopied in PAC1 knockout mice). These reflect the changing functions and mechanisms of action of neuropeptides across the lifespan.

To further exploit mouse genetics to distinguish PACAP's developmental effects from its actions as a neurotransmitter in the adult nervous system, we focused on repetitive jumping, a well-documented phenotype in constitutive PACAP knockout mice. Repetitive jumping is not phenocopied in PAC1 knockout mice, indicating that it is not driven by acute loss of peptide-receptor signaling. We showed that repetitive jumping does not occur in $CamK2\alpha$ -Cre::PACAPfl/fl mice, in which PACAP knockout occurs predominantly in excitatory neurons during late development, in VGAT-Cre:: PACAPfl/fl, in which PACAP knockout occurs in inhibitory neurons during late development. We now demonstrate that repetitive jumping does not occur in estrogen-dependent Cre:: PACAPfl/fl mice in which we have induced PACAP knockout by treatment with tamoxifen during early postnatal or adult life. These results suggest an early developmental contribution of PACAP deficiency on induction of repetitive jumping. We have further characterized repetitive jumping as part of a hyperlocomotive phenotype which emerges postnatally, between p21 and p36, and can be mediated by environment. The introduction of a running wheel into the cages of group housed PACAP knockout mice from weaning until 4 weeks later ameliorates repetitive jumping, but not increased overall locomotion, in animals tested 4 weeks after wheels were removed. It is anecdotally reported that PACAP knockout in some strains, such as C57Bl6/J, does not cause repetitive jumping. We are therefore currently attempting to positionally clone a gene or genes potentially associated with repetitive jumping caused by PACAP deficiency in C57Bl6/N by moving the defective (knockout) PACAP gene onto the C57Bl6/J background, and monitoring loss of repetitive jumping, loss of 'benchmark' C57Bl6/N-specific genes, and loss of regulation of 'sentinel' genes whose suppression and induction is associated with repetitive jumping in C57Bl6/N PACAP-deficient mice. These data underscore the hazards associated with the use of constitutive neuropeptide knock-out mice for drawing inferences about neuropeptide neurotransmitter function in adult mice, and the opportunities associated with tandem examination of constitutive and conditional neuropeptide knock-out mice to explore the role(s) of regulatory peptides in mammalian developmental neurobiology. National Institute of Mental Health Intramural Research Program, Project 1ZIAMH002386

3D distribution of the Oxytocin and Vasopressin cells in the developing mice brain

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Oxytocin and vasopressin are two neuropeptides involved in social behavior. Although the anatomy of these systems is known in the adult mouse brain, few studies have focused on their development. We implemented a 3D developmental atlas based on 3D imaging of marked and –cleared mouse brains at 5 postnatal ages (P0, P3, P7, P14, P56). This mapping showed that the number of oxytocin cells doubles during development while vasopressin cells remain stable. This increase in cell number is expressed in selective regions such as the paraventricular nucleus, the periventricular nucleus and a specific newly referenced oxytocin region named antero-lateral preoptic. More precisely, our analyses based on cell density in the PVN revealed the existence of two oxytocinergic clusters, one postero-dorsal present from birth and the other antero-ventral appearing later. Our results show the coexistence of two neural networks, one acquired, and the other experience dependent.

Antagonists at the PACAP type 1 (PAC1) receptor: In cellula methods for functional analysis

1; Xu, Wenqin, 1; Emery, Andrew C., 2; Ye, Wenjuan, 2; Hu, Xin, Henderson, 2; Mark, 2; Ferrer, Marc, and 1; Eiden, Lee E.

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The pituitary adenylate cyclase-activating polypeptide (PACAP) type 1 receptor PAC1 is one of fifteen GPCRs in the secretin receptor family. PACAP and PAC1 mRNAs are highly expressed in central nervous system and the PACAP-PAC1 signaling pathway is implicated in human disorders such as posttraumatic stress disorder. We wished to identify antagonists specifically targeting PAC1 and not other receptors of family B including the VPAC1 and VPAC2 receptors with which PACAP also interacts. To this end, we created a battery of HEK293 cell lines, expressing human PAC1 or other GPCRs including VPAC1, VPAC2, ADBR1, ADBR2, HR2 and GLPR1, and responding via a cyclic AMP biosensor (CBS) uniting a split luciferase in the presence of cAMP, to allow intracellular detection of cAMP elevation. We also created a corresponding high-content cell battery (the neuroendocrine cell line NS-1) for tracing the effect and selectivity of PAC1 antagonists on PACAP-initiated cellular processes including neuritogenesis for downstream screening of potential hits. The accuracy and sensitivity of these batteries for high-throughput drug screening was validated using the LOPAC1280 Library of Pharmacologically Active Compounds from Sigma as control ligand probes. Specific hits were identified for HEK293 cell lines expressing ADBR1, ADBR2, HR2 in this library, as expected, without appreciable false positive or false negative results. A high-throughput screen of >150,000 compounds did not produce hits for PAC1 antagonism. Since this assay system did not demonstrate inhibitory effects of the well-known PACAP antagonists PACAP6-38 and maxadilan-based M65 on PACAP signaling, we have re-tooled it using human neuroblastoma SH-SY5Y cells expressing CBS (SY5Y CBS), in which PACAP action at PAC1 is appropriately blocked by both PACAP6-38 and the M65. We tested in addition the putative PACAP antagonist PA-8, which was without effect on PAC1 activation by PACAP in HEK293 or SY5Y CBS cells. We intend to use the fully validated SY5Y CBS cell line to identify valid hits for PAC1 antagonism, and establish these as potential lead compounds using the NS-1 CBS cell line. NIMH-IRP-MH002386 to LEE

The role of the Caprin2 RNA binding protein in the supraoptic nucleus (SON) function.

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The expression of the gene encoding the RNA binding protein Caprin2 is robustly upregulated by dehydration in the hypothalamic supraoptic nucleus (SON). Caprin2 has previously been reported to play a key role in the osmotic defense response by binding to the vasopressin (Avp) mRNA and modulating the length of its poly(A) tail [1]. Increased polyadenylation is thought to promote transcript stability and to control translation. In order to define the network of genes regulated by Caprin2 in the SON, we used virally delivered shRNAs to knock down Caprin2 expression in the SON of both euhydrated and dehydrated rats. RNA sequencing was then used to identify global alterations in gene expression consequential to Caprin2 knockdown. We found that many genes known to be upregulated in the SON as a consequence of dehydration [2] were significantly downregulated by Caprin2 knockdown. To validate these findings, we conducted immunohistochemistry on the brain slices to map several neuropeptides known to be secreted from the SON-pituitary axis. We examine AVP, oxytocin (OXT), VGF, and cellular markers for the endoplasmic reticulum (ER) and Golgi apparatus (GA) compartments responsible for neuropeptide trafficking. It was observed that as we knocked down Caprin2, higher accumulation of AVP precursor protein was observed in the SON. In contrast, the neuropeptides OXT and VGF decrease within the SON following Caprin2 knockdown. Caprin2 knockdown resulted in compromised increase of soma size, ER and GA which are normally observed as a consequence of dehydration. In the intact SON, dehydration evokes enhanced soma-somatic membrane apposition as a consequence of astrocytic retraction. However, when Caprin2 was knocked down, this glial retraction was compromised. Previous studies have suggested a vital role for SON RNA binding protein Caprin2 in osmotic defense. Here we show that knockdown of Caprin2 expression massively alters the SON transcriptome under both euhydrated and dehydrated conditions. Our data suggest that Caprin2 is vital for the preservation of overall SON function during osmotic stress.

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Transcriptomic plasticity of the hypothalamic osmoregulatory control centre of the Arabian dromedary camel

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To live in arid environments places enormous evolutionary pressure on water conservation mechanisms. These are epitomised by the numerous adaptations manifested in those mammals, such as the dromedary camel (Camelus dromedarius), that thrive in the scorching heat of the Arabian deserts. At the level of the kidney, the dromedary produces low volumes of highly concentrated urine, more so when water is scarce, to conserve body water. Two hormones, arginine vasopressin (AVP) and oxytocin (OXT), both produced in the supraoptic nucleus (SON), the core hypothalamic osmoregulatory control centre, are vital for this adaptive process. Studies on rats (Pauža et al. 2021) have shown that the rat SON transcriptome undergoes dramatic function-related plasticity during water deprivation (WD), but the mechanisms that enable the camel SON to cope with osmotic stress are not known. Thus, to investigate the central control of water homeostasis in the camel, we have performed RNAseq transcriptome studies on the SON under control (water ad libitum) and WD conditions. We first used multiplex fluorescence in situ hybridization (RNAscope) to build three dimensional models of the camel SON based on the expression of the AVP and OXT mRNAs in order to facilitate sampling. We then compared the transcriptomes of the SON under control and WD conditions and identified genes that change in expression due to hyperosmotic stress. By comparing camel and rat datasets, we have identified common elements of the WD transcriptomic response network, as well as elements that appear to be unique to the dromedary camel and essential for adaptations necessary for life in the desert. (Reference: Pauža A, Mecawi A, Paterson A, Hindmarch C, Greenwood M, Murphy D, Greenwood MP. 2021. Osmoregulation of the transcriptome of the hypothalamic supraoptic nucleus: a resource for the community. J Neuroendocrinol. e13007. https://doi.org/10.1111/jne.13007)

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Characterization of hypothalamic transcription factor Giot1 in osmoregulatory gene expression pathways

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Introduction/Aim:

The neural regulation of fluid and sodium balance represents a dynamic example of functional neural plasticity driven by the induction of rapid changes in gene expression. Furthermore, it is intrinsically linked to the cardiovascular system via the synthesis and secretion of hormones which regulate blood pressure and volume. The transcript encoding the putative Kruppel-type zinc finger domain transcription factor Giot1 is robustly up regulated in response to osmotic challenge in the rat hypothalamic supraoptic nucleus (SON). Examination of Giot1 expression in vivo and in vitro demonstrated the localisation of this RNA in the nucleus, suggesting that it may function as a non-coding RNA. We have shown that AAV-mediated shRNA knockdown of Giot1 expression dramatically inhibits fluid consumption in rats challenged by salt loading. In this study we have characterised the molecular function of the Giot1 gene in regulating pathways underlying osmoregulatory homeostasis in the hypothalamus. Methods/Results:

We knocked-down expression of the Giot1 RNA in one rat SON by AAV-mediated delivery of a specific shRNA (shGiot1), a control virus expressing a scrambled shRNA (scCntrl) was delivered to the contralateral SON. RNAseq and differential gene expression analysis was performed to compare global transcriptional changes in the SON between shGiot1- knockdown and scCntrl conditions in euhydrated (n=5) and 2 days dehydrated (n=5) rats. A strong inverse correlation (Pearson correlation r=-0.82) was observed between differentially expressed genes in response to shGiot1 knockdown in dehydrated animals and the normal dehydration response (Pauža et al, 2021), indicating a central role for Giot1 in regulating an essential transcriptome profile in the SON.

Conclusions: This study provides evidence that Giot1 regulates a key hypothalamic transcriptional profile in the rat SON.
Rasd1: A small G protein with a big role in the neuroendocrine control of salt and water balance

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RASD1 is a member of the Ras family of monomeric G proteins which acts as a signaling molecule that inhibits adenylyl cyclase (AC) activity. We have previously shown that Rasd1 is expressed in vasopressin (AVP) magnocellular neurons (MCNs) of the supraoptic nucleus (SON) and paraventricular nucleus (PVN), and its transcript levels are upregulated during osmotic stress. To explore the cellular signalling events being controlled by Rasd1 to further understand how these highly specialised secretory cells co-ordinate changes to AVP synthesis and secretion in response to hydromineral challenges, we knockdown (KD) Rasd1 specifically in AVP MCNs. The polyadenylated transcriptome was assessed using RNA sequencing (RNA seq) and mined to generate comprehensive catalogues of functional classes of genes, expressed in this nucleus in the euhydrated state following Rasd1 KD. These transcript changes were also analysed to describe enriched gene ontology (GO) categories, KEGG, and Reactome pathways. We have identified the gene nitric oxide synthase 1 adaptor protein (NOS1AP) and extracellular signal regulated kinase (ERK) to be differentially expressed in the KD SON and validated the interaction of RASD1, NOS1AP, and nNOS in the SON using coimmunoprecipitation and immunofluorescence. We propose that RASD1 induction mechanism in the hypothalamus is through complexing with NOS1AP and nNOS to modulate the cAMP-PKA signalling pathway in the hypothalamus.

Dehydration effects on hypothalamic paraventricular nucleus and kidney gene expression in the spontaneously hypertensive rat

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Hypertension is a multisystem medical condition that affects around 1.13 billion people worldwide according to the World Health Organization. The spontaneous hypertensive rat (SHR) develops high blood pressure with aging, similarly to humans, and is a well-stabilised animal model for studying hypertension. The hypothalamo-neurohypophysial system (HNS) consists of the magnocellular neurons (MCNs) located at the hypothalamic supraoptic and the paraventricular nucleus (PVN) and its axonal projections to the posterior pituitary (PP). The antidiuretic hormone vasopressin (AVP) and natriuretic hormone oxytocin (OXT) are produced by the MCNs and secreted by the PP, regulating the extracellular liquid volume, osmolality, and vascular resistance, directly impacting the blood pressure control. Since the PVN activity and AVP secretion are intrinsically involved in the physiopathology of hypertension, in this work we studied how the SHRs respond to gene expression changes in the PVN when challenged by 24h water deprivation (WD). We used qPCR to analyse the expression of plastic genes previously demonstrated to be increased by water deprivation in the MCNs and some classical neuropeptides produced at the PVN level. Additionally, we have also analysed the expression of AVP and OXT receptors and transporters in the kidneys. As expected, WD increase the expression of hnAVP, Slc12a1, Giot1, Creb3L1, and Caprin2 in the PVN. The SHRs showed an increased expression of Trh and hnAVP, and reduced expression of CrebL1 and Slc12a1 compared to Wistar rats. No significant differences were observed in Rasd1, Cartpt, Pdyn, hnOXT, and Crh. The renal cortex showed an increased expression of Aqp2, Aqp3, and Avpr1a in response to WD, while the SHRs showed a reduced expression of Oxtr and Slc12a1. The Slc12a1 gene expression response to WD was blunted in the SHRs compared to Wistar rats. Regarding the kidney medulla, we found that Aqp7, Oxtr and Slc12a1 expression were decreased in SHRs. The Slc12a1 is an important cotransporter of sodium, potassium, and chloride in the kidneys. Mutations in this gene were reported to protect against hypertension development in humans. Thus, the Slc12a1 gene responsiveness might be one marker for decreased capacity of SHRs to cope with osmotic challenges at the renal level. ECR IRPS

Inputs and outputs of vasopressin neurons in the bed nucleus of the stria terminalis and medial amygdala

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The neuropeptide arginine-vasopressin (AVP) has long been implicated in the regulation of social behavior and communication. Ablations and optogenetic stimulation of sexually dimorphic AVP-expressing cells within the extended amygdala (i.e., the bed nucleus of the stria terminalis (BNST)) result in sex-different effects on social approach and communicative behaviors. Despite the substantial body of work implicating AVP in social behavior, evidence for inputs and outputs of BNST and medial amygdala (MeA) AVP cells is largely circumstantial. Here, we use a Cre-dependent, modified rabies virus (RV; for retrograde tracing) and a synaptophysin-tagged virus (for anterograde tracing) in combination with AVP-iCre (or iCre negative control) mice to map out monosynaptic inputs and outputs of BNST and MeA AVP cells. Adult male and female mice received unilateral injections of adeno-associated viruses (AAVs) expressing either Cre-dependent, TVA-eGFP (for driving cell infection by RV) and glycoprotein (for transsynaptic retrograde infection), or GFP-2A Synaptophysin-mRuby into the BNST and MeA. For RV tracing, mice also received an injection of RV following a 10-day incubation period. Preliminary results indicate that BNST AVP cells in male mice show strong reciprocal connections with the lateral septum, ventral pallidum, medial amygdala, lateral and medial preoptic area, and premammillary nucleus, all regions known to be innervated by male-biased, sex-different AVP fibers. Whereas BNST AVP cells receive inputs from nucleus accumbens and the paraventricular nucleus of the hypothalamus, they do not project to these regions. In addition, BNST AVP cells also receive strong inputs from, but project moderately to, the lateral, anterior, and ventromedial hypothalamic areas. Conversely, BNST AVP cells receive few inputs from, but strongly project to, the lateral habenula, periaqueductal gray, dorsal raphe, piriform cortex, and anterior olfactory nucleus. Lastly, BNST AVP cells send moderate outputs to, but do not receive inputs from, the horizontal diagonal band and entorhinal cortex. Collectively, we establish that brain regions previously shown to contain sex-different AVP innervation receive projections from BNST AVP cells, while these areas (i.e., lateral septum, ventral pallidum) also provide input to drive these cells. The BNST and medial amygdala also display profound reciprocal connections, and therefore may function as a system for the regulation of sexually different social behavior. This information will be foundational for understanding sexually different function of AVP circuitry and future studies will differentiate inputs/outputs of extended amygdala AVP cells in both sexes.

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