

RegPep2018

Proceedings

*22nd International Symposium on
Regulatory Peptides*

Riviera Diamante Acapulco
September 22nd to 25th, 2018

RegPep2018

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Welcome to RegPep2018

RegPep2018 is the 22nd Symposium of the International Regulatory Peptide Society (IRPS), an international federation of scientists committed to progress in basic research on the biology and physiology of peptides, and translation of that basic research into clinical gains and public health benefit.

RegPep2018 will be a critical meeting in the peptide field, as in previous years, by explicitly uniting basic research and clinical research in regulatory peptides. However, the emphasis on the emergence of peptides as therapeutic agents, for the 2018 conference, will create a synergism between the basic and clinical aspects of the peptide field that will potentially have a strong impact on progress in this area to the benefit of the public health in the coming years. This translational focus will be specially emphasized at RegPep2018, which will be held for the **first time ever in Latin America, on Acapulco Diamante, Mexico, in September 22-25, 2018.**

RegPep past meetings have been held in: Asilomar (USA, 1976), Beito (Norway, 1978), Cambridge (UK, 1980), Stockholm (Sweden, 1982), Rochester (USA, 1984), Vancouver (Canada, 1986), Shizuoka (Japan, 1988), Timmerndorfer Strand (Germany, 1990), Luewen (Belguim 1992), Santa Barbara (USA, 1994), Copenhagen (Denmark, 1996), Mackinac Island (USA, 1998), Cairns (Australia, 2000), Boston (USA, 2002), Toulouse (France, 2004), Hakone (Japan, 2006), Santa Bárbara (USA, 2009), Belfast (Ireland, 2010), Copenhagen (Denmark, 2012), Kyoto (Japan, 2014), Rouen (France, 2016).

Welcome message from host institution

Dear RegPep2018 delegates, visitors to Mexico, members of the Organizing committees of RegPep2018

It is of course a great pleasure to welcome each and every one of you to Acapulco Diamante to participate in the 22nd International Regulatory Peptide Conference. As the Dean of the largest Medical School in Mexico, I see every day, as a clinician, the importance of regulatory peptides in clinical practice, from insulin (discovered in Canada) to incretins (like GLP-1 (discovered in Scandinavia)). I see every day, as a teacher, the importance of basic research aimed at the expanding the range of targets available for therapeutics, including the hypophysiotropic hormones whose structures were thrillingly revealed to the world in the space of just a few short months, after many many years of effort, by Dr. Andrew Schally, our opening lecturer.

A few words about the urgency and importance of your mission here, and our wishes for you as we host your stay here in Mexico. Few things are more obvious to someone in my position as a Medical School Dean than the importance of international cooperation in science at all levels: bureaucratic, academic, scientific, and regulatory. Freedom to communicate across national boundaries is the sine qua non of any enlightened and progressive intellectual enterprise. We are so pleased that you represent 22 countries from every continent except Antarctica, and come here with the understanding that your communication transcends national boundaries to focus on the matters that all of us share as of the ultimate importance: the betterment of human health through scientific knowledge and its rigorous and patient application. Thank you for your hard work in the coming days. It is our sincere hope that you find that this environment facilitates your mission, and that you establish new international cooperations, communications, and collaborations to continue to tackle obesity, heart disease, cancer, addiction, depression and the other diseases that recognize no national borders with the tools and weapons afforded by regulatory peptides and their physiological targets.

Welcome, enjoy your work here in Acapulco, and please inspire our many students in attendance to continue your collective work far into the future.

Dr. German Fajardo
Professor of Medicine and
Dean of Faculty of Medicine (FM)
National Autonomous University of Mexico (UNAM)

Welcome message from International Regulatory Peptide Society

Dear Colleagues,

On behalf of the International Regulatory Peptide Society (IRPS), I am delighted to welcome you in Acapulco Diamante, Mexico for this RegPep2018.

I would like to warmly congratulate Lee E. Eiden and Limei Zhang, Co-Chairs of RegPep2018, and the International Advisory Committee Members for the outstanding scientific program they have established, that encompasses many facets of current research on bioactive peptides from basic to clinical aspects. This program comprises parallel complementary sessions to address, among others, the effect of peptides on stress, pain, social behavior, neuroprotection, tumor development, regulation of energy metabolism, inflammation processes and neuroendocrine functions. Besides those parallel sessions, five keynote speakers, i.e. Andrew Schally, Jean-Louis Charli, Gareth Leng, Suzanne Dickson and Robert Malenka, will give plenary lectures, and five outstanding pioneers of regulatory peptide research, i.e. José Antunes Rodrigues, Sue Carter, Luis de Lecea, John Morris and Robert Millar, will discuss the lessons learned during their scientific careers and address critical questions regarding the future of peptide research. Young investigators will have ample opportunity to learn from these conferences, but also to present their own work either as oral communications or poster presentation.

I want to compliment Laura Bohn for being the 2018 Victor Mutt Awardee and Lecturer. She has been selected by the IRPS Scientific Committee among other outstanding nominees for her prominent work on biased agonists to modulate G protein-coupled receptors function in order to develop new therapies.

I am also most grateful to the members of the Local Organizing Committee who have worked very hard to make this meeting happen and be an unforgettable experience.

Finally, I would like to invite you to participate to the IRPS General Assembly on Tuesday September 25, where we will discuss important issues such as the status of the IRPS and location of our next meeting.

I wish you all an enjoyable meeting and very fruitful discussions.

David Vaudry
President of the International Regulatory Peptide Society (IRPS)

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Preface

The abstracts gathered here represent contributions from the international regulatory peptides research community from more than twenty countries including Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, Hungary, Israel, Italy, Japan, Mexico, Morocco, The Netherlands, New Zealand, Norway, South Africa, Switzerland, the United Kingdom, the United States, and Uruguay.

RegPep2018 has been organized under the auspices of the International Regulatory Peptide Society and the National Autonomous University of México (UNAM) and with the participation of the National Institute of Mental Health, USA and the National Institute of Nutrition “Salvador Zubirán” of México

RegPep2018 represents a continuation of the tradition of the International Regulatory Peptide Society, and features the Society’s Mutt Invited Lectureship, which commemorates the historic contributions of Victor Mutt to the advancement of peptide research, particularly the amidated peptides of the gut including galanin, cholecystokinin, pancreastatin, bombesin, and others. RegPep2018 also inaugurates a rebirth of the goals of the IRPS, however. It is highly significant that Laura Bohn, a pioneer in understanding biased signaling at peptide-liganded GPCRs, is this year’s Victor Mutt Lecturer. Her contributions to how the receptor for the gut and brain mu opiate receptor can be stimulated by ligands that promote the analgesic effects of opiates while minimizing their adverse effects (and receptor desensitization) herald a new age of peptide research, and an increasing awareness that the regulatory peptide field has reached a unique threshold. With the development of actual peptides as therapeutic agents for peripheral GPCR targets, such as the GLP-1 receptor agonist exendin-4 and its analogs for treatment of diabetes, we are on pace to have more peptides as therapeutic agents in the drug pipeline than ever before. At the same time, new methodologies for studying neuropeptide modulation throughout the body have uncovered important roles for regulatory peptides in reward, as described for oxytocin by Plenary Speaker Rob Malenka; new avenues for peptide administration to treat alcoholism, as uncovered by Workshop speaker Mary Lee; and in integration of multiple physiological

drives with motivated behavior, as contemplated by Plenary Speakers Gareth Leng and Suzanne Dickson. The Pioneers session of RegPep2018 emphasizes how the history of regulatory peptides has positioned the field for major gains in understanding physiology, pathophysiology, and therapeutic opportunities for interdiction in disease. Plenary Speaker and Nobel Laureate Andrew Schally reminds us that the progress began with elucidating the structures of regulatory peptides has spiraled back into the cell, where understanding their production from prohormones, and metabolism after secretion, has allowed refinement of controlling peptide metabolism in vivo and developing metabolism-resistant peptoids, a key area summarized by Plenary Speaker Jean-Louis Charli.

In each of these key areas, our speakers have been chosen, and have volunteered presentations, from which the outlines of a new era in regulatory peptides can be discerned: deeper understanding of peptide-modulated physiological and behavioral circuits, more facile screening of agonists and antagonists for peptide-liganded GPCRs; a more precise pharmacology for peptide action so that immune, neuronal, metabolic, and growth-regulatory properties can be precisely dialed up for individual potential therapeutic agents; the use of these tools translationally for clinical benefit and in reverse-translation in animal models from simple to complex, to better understand the basic physiology underlying homeostatic and allostatic function and dysfunction in all complex metazoans who rely on regulatory peptides for intercellular communication.

It is a pleasure to have gathered all of your contributions in this book, in full confidence that they will each contribute concretely to the acceleration of progress in the regulatory peptide field.

Lee E. Eiden and Limei Zhang
Co-Chairs of RegPep2018, July 14, 2018

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List of Travel Awards

Category I - Contributed talks winners (Symposium S11)

Sigal Fleisher-Berkovich
Ben-Gurion University of the Negev, Beer-Sheva, Israel

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Patricia Lagos
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Ai-Min Bao
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Fabien Plisson
CINVESTAV IPN, Irapuato, Mexico

Category II - DataBlitz presentation winners

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Scientific Program

Saturday Sept. 22, 2018

Concurrent symposia (<u>block I: S1-S4</u>)				
	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
14:00 - 16:00	<p>S1. Extrahypothalamic functions of magnocellular neurons: optogenetics, development and behavior Chair: Valery Grinevich (Heidelberg, Germany)</p>	<p>S2. What's new with POMC, TRH, PRL and GH neuroendocrine actions Co-chairs: Patricia Joseph-Bravo (Cuernavaca, México) and Dave Grattan (Dunedin, New Zealand):</p>	<p>S3. Neuropharmacology of vasopressin and oxytocin: physiology and behavior Chair : Maurice Manning (Ohio, USA)</p>	<p>S4. Neuroendocrine peptide GPCRs: from function to therapeutic targets Co-chairs: Hélène Castel (Rouen, France) and Richard Leduc (Sherbrooke, Canada)</p>
14:00 - 14:30	<p>Valery Grinevich (<i>University of Heidelberg, Germany</i>): Oxytocinergic circuits of the amygdala: finding points of intervention for pain and pleasure</p>	<p>Malcolm J. Low (<i>University of Michigan, Ann Arbor, USA</i>): Neuropeptides involved in integrated hypothalamic control of energy homeostasis</p>	<p>Maurice Manning (<i>University of Toledo, USA</i>): Receptor selective oxytocin and vasopressin agonists and antagonists as research tools and therapeutics: a current perspective</p>	<p>Atsuro Miyata (<i>Kagoshima University, Kagoshima, Japan</i>): Astrocyte-neuron lactate shuttle (ANLS) as the major effector of PACAP/PAC1R signaling for CNS functions</p>
14:30 - 15:00	<p>David Murphy (<i>University of Bristol, UK</i>): Linkages between osmoregulation and ingestive behaviors are encoded in vasopressinergic-dynorphinergic projections from hypothalamus to amygdala</p>	<p>Patricia Joseph-Bravo (<i>IBT, UNAM, Mexico</i>): Hypophysiotropic TRH neurons integrate stress and metabolic signals</p>	<p>Gilles Guillon (<i>CNR, Montpellier, France</i>): Selective fluorescent ligands for imaging vasopressin and oxytocin receptors in native tissues</p>	<p>Sunny Z. Jiang (<i>NIMH, Bethesda, USA</i>) PACAP and dopamine signaling in stress: response parcellation by distinct cyclic AMP sensors in neuronal and endocrine cells</p>
15:00 - 15:30	<p>Limei Zhang (<i>Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico</i>): Vasopressin projections to habenula and modulation by sex steroids: control of response to aversive stimuli in mammals</p>	<p>Dave Grattan (<i>University of Otago, Dunedin, New Zealand</i>): Prolactin actions in the maternal brain during pregnancy</p>	<p>Andrés Quintanar-Stephano (<i>UAA, Mexico</i>): Effects of the neuropeptide arginine vasopressin (AVP) deficiency, conivaptan and desmopressin on clinical symptoms, gene expression and blood cytokine levels in rats with experimental autoimmune encephalomyelitis</p>	<p>Laurent Prézeau (<i>INSERM U661 – University of Montpellier, Montpellier, France</i>): GSHR controls YAP phosphorylation via both constitutive and agonist-induced pathway</p>

15:30 - 16:00	Alexa Veenema (Michigan State University, East Lansing, USA): Developmental and sex-specific involvement of vasopressin in the regulation of social behavior	Carlos Arámburo de la Hoz (INB, UNAM, Mexico): Autocrine/paracrine roles of extrapituitary growth hormone in neuroprotection	Nicolas Gilles (IBMM, CNRS, Montpellier, France): Animal toxins for human health, case of the mambaquaretin for the treatment of polycystic kidney disease	Hélène Castel (Inserm U1239, DC2N, Normandie University, Mont-Saint-Aignan, France): Biased signaling of the urotensin II receptor: still a blind spot between direct couplings and brain physiopathology
16:30 - 17:00	<p>Opening ceremony Atlantes Amphitheater</p> <p>Chairs of Organizing Committee & Scientific Committee; National University and Guerrero State Authorities; All delegates and guests.</p> <p>Lay Lay lecture: David Kershenobich: General Director, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ) Mexico The translation of basic research into clinical practice</p> <p>Music interludes: Maestro Carlos Egry (CDMX, México)</p>			
17:40 - 18:30	<p>Opening lectures Atlantes Amphitheater</p> <p>Presentation from Andrew V. Schally (Nobel Prize Laureate, Department of Veterans Affairs and University of Miami, USA)</p> <p>Gareth Leng: (Centre for Discovery in Brain Science, University of Edinburgh, UK) The heart of the brain: the hypothalamus and its hormones</p>			
18:30 - 21:00	<p>Welcome reception (Maya-Mixteca Pool / Ocaen hall)</p>			

Sunday Sept. 23, 2018

8:00 - 9:00	Plenary lecture II <i>Atlantes amphitheater</i> Laura Bohn (Scripps Institute, Florida, USA) 2018 Victor Mutt Awardee and Lecturer Refining opioid receptor signaling to improve the therapeutic index			
Concurrent symposia (<u>block II</u>: S5-S8)				
	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
9:00 - 11:00	S5. Metabolic disorders: central and peripheral mechanisms and therapeutics Chair: Marcia Hiriart (Mexico City, Mexico)	S6. Presentation of self-peptides in the thymus: An essential event of life Chair: Vincent Geenen (Liege, Belgium)	S7. Neuropeptides in headache, inflammation and neuroinflammatory pain: Basic science to clinical trials Chair: James A. Waschek (Los Angeles, USA)	S8. Interaction of hypothalamic peptidergic circuits in the organization of physiology and behavior Chair: Ruud M. Buijs (Mexico City, Mexico)
9:00 - 9:30	Harvey Grill (<i>University of Pennsylvania Perelman School of Medicine, USA</i>): Treating the hyperphagia driving obesity using centrally acting GLP-R agonists	Vincent Geenen (<i>University of Liege, Belgium</i>): Historical introduction to the thymus and concept of immune self-tolerance	James A. Waschek (<i>University of California at Los Angeles, USA</i>): VIP, PACAP and neuroinflammatory disease	Ruud Buijs (<i>IIB, UNAM, Mexico</i>): Interaction between suprachiasmatic and arcuate nuclei is essential for temperature and corticosterone rhythm, roles for vasopressin and alpha-MSH
9:30 - 10:00	Inge Depoortere (<i>University of Leuven, Belgium</i>): Chemosensory signalling mechanisms of enteroendocrine cells in the gut	Georg Holländer (<i>University of Basel, Switzerland and University of Oxford, UK</i>): The thymus: epigenetic control of the molecular mirror of self	Zsuzsanna Helyes (<i>University of Pécs, Hungary</i>): Neuropeptide-mediated sensitization mechanisms in models of trigeminovascular activation: focus on PACAP and hemokinin-1	Charles Bourque (<i>McGill University Health Centre, Montreal, Canada</i>): Suprachiasmatic nucleus vasopressin neurons and the circadian control of fluid homeostasis
10:00 - 10:30	Marcia Hiriart (<i>IFC, UNAM, Mexico</i>): Insulin resistance: physiology and as part of the metabolic syndrome	Hiroyuki Takaba (<i>University of Tokyo, Japan</i>): Distinct features of Fezf2-induced	Ichiro Takasaki (<i>University of Toyama, Japan</i>): Discovery of small-molecule	Valerie Simonneaux (<i>INCI-CNRS, Strasbourg, France</i>): Circuits of kisspeptin and RFRP3 in the

		promiscuous gene expression in the thymus	antagonists of PAC1 receptor for the treatment of neuropathic pain	seasonal control of reproduction and metabolism
10:30 – 11:00	Andrew Gundlach (Melbourne): Relaxin-3/RXFP3 signaling and neuroendocrine function in extrahypothalamic circuits	Jaime Mas-Oliva (Instituto de Fisiología Celular, UNAM, Mexico City, Mexico) Vaccine HB-ATV-8 peptide regulates the metabolism of lipids in the hepatocyte	Leon Martinez-Garcia (Alder Biopharmaceutics, Seattle, WA USA) PACAP inhibition by Alder’s ALD1910 antibody represents a potential non-CGRP redundant new opportunity to treat migraine	Pawel K. Olszewski (University of Waikato, Hamilton, New Zealand): Oxytocin and potential benefits for obesity treatment

11:00 – 11:30	Coffee Break			
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	Concurrent symposia (<u>block III</u>: S9-S12)			
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	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
11:30 - 13:00	S9. Gut peptide: physiology and metabolic syndrome Chair: Duan Chen (Trondheim, Norway)	S10. Peptides and their receptors as oncotargets Chair: Terry Moody (Bethesda, USA)	S11. Symposium with contributed talks Chair: Laura Vivas (Cordoba, Argentina)	S12. Regulatory peptide signaling and circuit logic in controlling concerted behaviors Chair: Lee E. Eiden (Bethesda, USA)
11:30-12:00	Chun-Mei Zhao (Norwegian University of Science and Technology, Norway): Gastric neuroendocrine cells: fine structure and peptide hormones	Gabriela Cesarman (National Institute of Cancer Research, México): The interplay between cancer, it’s microenvironment, and the coagulation system: biomarkers and regulation by peptides.	Sigal Fleisher-Berkovich (Ben-Gurion University of the Negev, Beer-Sheva, Israel): Angiotensin Converting Enzyme Inhibitors Ameliorate Brain Inflammation: Possible Implications for Alzheimer’s Disease	Ben White (NIHH, NIH, Bethesda, USA): The peptide modulome. How neuropeptide circuits modulate neuronal circuits to orchestrate behavior--insights from animal models

			<p>Andrea Godino (INIMEC-CONICET-Universidad Nacional de Córdoba, Argentina): TRPV1 osmosensitive channel involvement in the control of sodium appetite</p>	
12:00 – 12:30	<p>Markus Heimesaat (Charité University, Berlin, Germany): Pituitary Adenyl Cyclase-Activating Polypeptide – a neuropeptide as novel treatment option for intestinal inflammation. Lessons learnt from murine gut inflammation models</p>	<p>Terry Moody (National Cancer Institute, NIH, USA): Bombesin receptors regulate transactivation of receptor tyrosine kinases in lung cancer</p>	<p>Patricia Lagos (Universidad de la República, Montevideo, Uruguay): In vivo and ex vivo studies of the internalization of melanin-concentrating hormone conjugated with rhodamine in hippocampal neurons.</p> <p>Luz Torner (Centro de Investigación Biomédica de Michoacán, Instituto Mexicano Del Seguro Social, Morelia, México): Regulatory role of prolactin on the neuroimmune system of the hippocampus of male rat pups</p>	<p>Esther Sabban (New York Medical College, USA): NPY administration and treatment of behavioral symptoms in a rodent single prolonged stress model for PTSD</p>
12:30-13:00	<p>Duan Chen (Norwegian University of Science and Technology, Norway): Brain-gut axis: Botulinum toxin A treatment for obesity</p>	<p>Matthew Thakur (Thomas Jefferson University, USA): Imaging of prostate cancer using VIP/PACAP analogs</p>	<p>Ai-Min Bao (Zhejiang University, Hangzhou, China): The stress systems in mood disorders: a postmortem study</p> <p>Fabien Plisson (CINVESTAV IPN, Irapuato, Mexico): Constrained GLP-1 mimetics with incretin-like properties</p>	<p>Xiao-Dong Wang (Zhejiang University, China): Neuropeptides and calbindin in stress-related disorders</p>

13:00 - 15:00	<p>Lunch and drinks Poster presentation (P1-P40) Ocean rooms 1-2</p> <p>DataBlitz I P3, P9, P10, P13, P15, P19, P21, P40 Atlantes Amphitheater</p> <p>Editor-in-Chief Lunching: <i>How to publish your research</i> Chair: Robert Millar Atlantes Amphitheater</p> <p>Annal of New York Academy of Sciences, Neuroendocrinology, J. Neuroendocrinology Stress, Journal of Molecular Neuroscience, Frontiers in Neuroendocrine Science</p> <ol style="list-style-type: none"> 1. Introductions by editors 2. Suggestions by participants of areas additional to those listed below which they would like covered 3. Choosing the right journal. Checking the journal scope. Considering flagship journals in the area of your work. The disadvantages of being too ambitious (be ambitious by all means but a reality check is needed) 4. Understanding the review process 5. Why might my manuscript be rejected? (apart from fundamental flaws, things like...inappropriate journal, editorial triage, perceived lack of impact) 6. Features of excellent manuscripts (clear writing, clear figures and tables and clear messages) 7. Responding to reviewer's comments 8. What do impact factors mean? What are appointment and promotion committees looking for in the journals you have published in? 9. Writing reviews 10. Open access may be great but someone has to pay! The pros and cons of these journals. Issues re predatory journals 11. Should reviewers' identity be declared?
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15:00 - 15:50	<p>Plenary lecture III Atlantes amphitheater</p> <p>Jean-Louis Charli (Instituto de Biotecnología, Universidad Nacional Autónoma de México (UNAM), Mexico) Peptide-degrading enzymes and the control of peptide action in vivo</p>
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Concurrent symposia (<u>block IV</u>: S13-S16)				
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	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
16:00 - 18:00	<p>S13: Neuropeptide function in fear circuits Chair: Francesco Ferraguti (Innsbruck, Austria)</p>	<p>S14. Neuropeptides and social behavior Chair: Tallie Z. Baram (Irvine, USA)</p>	<p>S15. Neuropeptides in inflammatory processes Chair: Erika Pintér (Pecs, Hungary)</p>	<p>S16. New insights in the hypothalamic regulation of energy metabolism by neuropeptides Co-chairs: Nicolas Chartrel (Rouen, France) and Carole Rovère (Valbonne, France)</p>

16:00 - 16:30	Ramon Tasan (Medical University of Innsbruck, Austria): Role of neuropeptides in the interaction of fear and hunger	Tallie Z. Baram (University of California at Irvine, USA): CRH and development of the pleasure/reward circuitry	Susan D. Brain (King's College London, UK): CGRP and protective effect in the cardiovascular system; relevance to migraine therapy	Sophie Steculorum (Max Planck Institute for Metabolism Research, München, Germany): Novel regulators of the central control of feeding and systemic insulin sensitivity
16:30 - 17:00	Kay Jüngling (University of Münster, Germany): The impact of the human-relevant NPSR1 polymorphism I107N on anxiety- and fear-related circuits and behavior	Inga D. Neumann (Univ. Regensburg, Germany): Oxytocin and neuropeptide S in social behavior	Soraia Costa (University of Sao Paulo, Brazil): Environmental influence of fumes on TRPA1-induced inflammation	Serguei Fetissov (U1239, Université de Rouen, Normandie, France): Regulation of feeding behavior by a neuropeptide-like protein produced by gut bacteria
17:00 - 17:30	Francisco Sotres-Bayon (IFC, UNAM, Mexico): Neurogenesis regulates fear recovery by recruiting a prefrontal-amygdala-habenula network	Genaro A. Coria-Avila (Centro de Investigaciones Cerebrales, Universidad Veracruzana, México): Oxytocin in conditioned same-sex partner preferences and brain dimorphism	Barbara Kofler (Department of Pediatrics/University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria): Galanin is a versatile modulator of immune cell activation	Nicolas Chartrel (INSERM U1239, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication): 26RFa: a neuropeptide involved in the hypothalamic regulation of energy homeostasis
17:30 - 18:00	Francesco Ferraguti (Medical University of Innsbruck, Austria): Specialized amygdala inhibitory networks for emotional learning	Adi Cymerblit-Sabba (National Institute of Mental Health, NIH, USA): Vasopressin and social behaviors	Erika Pintér (Department of Pharmacology and Pharmacotherapy, University of Pecs, Pecs, Hungary): TRPA1-mediated effect of sulfide compounds in pain and inflammation	Carole Rovère (Institute of Molecular and Cellular Pharmacology, Université Nice Sophia Antipolis, Valbonne, France): Impact of nutritional lipids on glial remodeling and neurons activity in the hypothalamus. Focus on MCH and orexin neurons

Monday Sept. 24, 2018

8:00 - 9:00	Plenary lecture IV <i>Atlantes amphitheater</i> Suzanne Dickson (Institute of Neuroscience and Physiology, University of Gothenburg, Sweden) Impact of peripheral regulators of energy balance on the reward system			
Concurrent symposia (block V: S17-S20)				
	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
9:00- 11:30	S17. Clock mechanisms of mammalian SCN: From peptide to network interactions Chair: Raúl Aguilar-Roblero (Mexico City, Mexico)	S18. Neuropeptide regulation of stress and its consequences Chair: James P. Herman (Cincinnati, Ohio, USA)	S19. Drug design for the apelin receptor across diverse pathophysiological indications: Peptide drug development strategies from bench and clinic to approval Co-Chairs: C. Llorens-Cortes (Paris, France), Eric Marsault and M. Auger-Messier (Sherbrooke, Canada)	S20. Young Investigator Symposium: New investigators embarking on their independent careers sketch out their current progress and plans for future research Co-chairs: Vito S. Hernández (Mexico City, México) and André Mecawi (Rio de Janeiro, Brazil)
9:00 - 9:30	Raúl Aguilar-Roblero (<i>UNAM, Mexico</i>): From the molecular circadian oscillator to the circadian firing pattern in SCN neurons	James P. Herman (<i>University of Cincinnati, USA</i>): Regulation of stress integration by central Glucagon-like Peptide 1 circuits	Catherine Llorens-Cortes (<i>INSERM U1050, Collège de France, France</i>): Development of original metabolically-stable apelin-17 analogs with aquaretic and cardiovascular effects	André Mecawi (<i>Federal Rural University de Rio de Janeiro, Brazil</i>): Electrophysiological effects of ghrelin in the hypothalamic paraventricular nucleus neurons
9:30 - 10:00	Charles N. Allen (<i>Oregon Health & Science University, USA</i>): VIP and vasopressin signaling mechanisms in suprachiasmatic nucleus neurons	Eric G. Krause (<i>University of Florida, USA</i>): Central Angiotensin II and its role in stress responding	Gavin Oudit (<i>University of Alberta, Canada</i>): Enhancing the apelin-apelin receptor axis as a novel therapy for heart failure	Sung Han (<i>Salk Institute for Biological Studies, La Jolla, California, USA</i>): CGRP: the main transmitter of affective pain signals to the amygdala

10:00 - 10:30	Chris Colwell (University of California, Los Angeles, USA): The role of neuropeptides in the photic regulation of the circadian system	Jom Hammack (University of Vermont, USA): Involvement of PACAP in stress-induced behavioral responses	Olivier Lesur (Université de Sherbrooke, Canada): Potential of apelin and ELABELA in the treatment of sepsis	Lorraine Jaimes-Hoy (Instituto de Biotecnología, UNAM, Mexico): Early life stress curtails the hypothalamic-pituitary-thyroid axis cold response in adulthood
10:30 - 11:00	Hugh Piggins (University of Manchester, UK): Intrinsic and Extrinsic Neuropeptide signaling in the suprachiasmatic circadian pacemaker	Jan Deussing (Max Plank Institute for Psychiatry, Munich, Germany): Role of CRH in stress adaptation	Éric Marsault (Université de Sherbrooke, Canada): Understanding and exploiting the structure-signaling relationship of apelin	Zhihua Gao (Zhejiang University School of Medicine, Hangzhou, China): Reconstructing the hypothalamo-neurohypophysis connections by viral tracing.
11:00 - 11:30	Rae Silver (Columbia University, USA): Anticipation in a circadian time frame: Studies of voluntary drug intake in mice	Ki-Ann Goosens (Massachusetts Institute of Technology, Cambridge, USA): Ghrelin and resilience to chronic stress	Hyung Chun (Yale University School of Medicine, New Haven, Connecticut USA): Engaging Apelinergic Pathway for Cardiometabolic Health	Vito S. Hernández (Facultad de Medicina, UNAM, Mexico): Ascending projections of magnocellular vasopressinergic hypothalamic PVN neurons modulate hippocampal oscillatory activity
11:30 - 15:00	<p>Lunch and drinks Poster presentation (P41-P80) Ocean rooms 1-2</p> <p>DataBlitz II P41, P46, P48, P54, P59, P64, P66, P75 Atlantes Amphitheater</p> <p>WORKSHOP Atlantes Amphitheater</p> <p>Peptide-based drug discovery for CNS disorders: Avenues and barriers Co-Chairs: William Z. Potter (Bethesda, USA) and David Vaudry (Rouen, France)</p> <p>Bill Potter (National Institute of Mental Health, NIH, USA): <i>CNS peptide and their receptors as drug targets: creating pre-competitive consortia for target engagement and proof-of-concept for CNS disease targets</i></p> <p>Mary R. Lee (National Institute on Alcohol Abuse and Alcoholism, NIH, USA): <i>Peptide penetration of blood-brain-barrier after administration at olfactory and peripheral sites</i></p>			

	<p>Michael J. Brownstein (Azevan Pharmaceuticals, Bethlehem, PA, USA): <i>Vasopressin: Old dog, new tricks</i></p> <p>David Lovejoy & Dalia Barsyte-Lovejoy (UT, Canada & Protagenic Therapeutics Inc. New York USA): <i>Multiple in vivo peptide delivery approaches with the corticotropin-releasing hormone (CRH) and secretin family-like peptide, teneurin C-terminal associated peptide (TCAP), for energy metabolism and affective disorder treatments</i></p> <p>David Vaudry (INSERM, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Normandy University, Rouen, France): <i>PACAP intranasal delivery represents an efficient approach for the treatment of stroke and Huntington disease</i></p>
15:00 - 17:00	<p style="text-align: center;">Roundtable Atlantes amphitheater</p> <p style="text-align: center;">Pioneers of Regulatory Peptide Research: Drawing inspiration from the past and glimpsing the future</p> <p style="text-align: center;">Chairs: Lee E. Eiden (Bethesda, USA) and Limei Zhang (Mexico City, Mexico)</p> <p>This portion of the RegPep2018 program highlights the unique contributions of four outstanding pioneers of regulatory peptide research who have profoundly altered our understanding of the role of peptides, arising from their prohormone precursors within neurons and endocrine cells (P. Lowry), in orchestrating critical physiological functions in circadian rhythms, fluid and food intake (J. Antunes Rodrigues); social and sexual behavior (S. Carter); and modulation of states of arousal (L. de Lecea) and the implications of this understanding for progress in human health. The speakers will reflect on the lessons learned in their scientific careers that may be valuable to those just embarking on their own research journeys in this incredibly rich and fertile field, and will discuss critical questions about the future of regulatory peptide research, submitted beforehand from RegPep2018 participants.</p> <p style="text-align: center;">José Antunes Rodrigues (Faculty of Medicine, University of Sao Paulo, Brazil) Sue Carter (Kinsey Institute, University of Indiana, USA) Luis de Lecea (Department of Psychiatry, Stanford University, USA) John Morris (University of Oxford, UK) Robert Millar (Centre for Neuroendocrinology, University of Pretoria, South African)</p>
17:00 - 18:00	<p style="text-align: center;">Plenary lecture V Atlantes amphitheater</p> <p style="text-align: center;">Robert C. Malenka (Stanford Neuroscience Institute, Stanford University, USA; Julius Axelrod Prize Laureate) Oxytocinergic gating of social reward</p>
18:30 - 22:00	<p>Conference Dinner (Puerto Hacienda/Condesa Hall II)</p>

Tuesday Sept. 25, 2018 AM

Concurrent symposia (block VI: S21-S24)				
	Princesa Room 1	Princesa Room 2	Princesa Room 3	Princesa Room 4
	<p>S21. Contemporary approaches to studying peptidergic neurons Chairs: Mike Ludwig (Edinburgh, UK)</p>	<p>S22. Neuropeptides and neurodegeneration Co-Chairs: Illana Gozes (Tel Aviv, Israel) and Seiji Shioda (Tokyo, Japan)</p>	<p>S23. Chromogranin- and other protein-derived bioactive peptides: cardiovascular, immune, endocrine and metabolic functions Chair: Sushil Mahata (San Diego, USA)</p>	<p>S24. Nesfatin-1: Update on a pleiotropic peptide Co-chairs Andreas Stengel (Tübingen, Germany) and Yvette Taché (Los Angeles, USA)</p>
8:00 - 8:30	<p>Mike Ludwig (<i>University of Edinburgh, UK</i>): Exploring novel neuronal pathways from the retina to the SCN using transgenic rat models and viral transfection systems</p>	<p>Illana Gozes (<i>Tel Aviv University, Israel</i>): VIP and PACAP to ADNP and NAP: Microtubule neuroprotection in the ADNP syndromic autism</p>	<p>Sushil Mahata (<i>Veterans Administration and UCSD, San Diego, USA</i>): Catestatin regulation of immunometabolism</p>	<p>Tamas Koziacz (<i>Radboud University Nijmegen Medical Centre</i>): Role of nesfatin in emotional processing</p>
8:30 - 9:00	<p>Javier Stern (<i>Georgia State University, USA</i>): Unraveling mechanisms underlying stimulus-secretion coupling at neuronal dendrites using novel cell biosensors</p>	<p>Seiji Shioda (<i>Hoshi University, Japan</i>): PACAP, stem cells and neuroprotection, spinal injury and stroke</p>	<p>Youssef Anouar (<i>Inserm U1239, Université de Rouen, Normandy, France</i>): A selenoprotein-derived peptide with potent in vivo anti-neurodegenerative actions</p>	<p>Suraj Unniappan (<i>Sastatchewan, Canada</i>): Blood glucose homeostatic control by nesfatin-1</p>

9:00 - 9:30	Colin Brown <i>(University of Otago, NZ):</i> Dissecting vasopressin's role in the development of hypertension using transgenic rats	Dora Reglödi <i>(University of Pecs, Hungary):</i> Age-related accelerated systemic amyloidosis in PACAP deficiency	Angelo Corti <i>(IRCCS San Raffaele Scientific Institute, Vita-Salute University, Milan, Italy):</i> Chromogranin A and its fragments in the spatio-temporal regulation of vascular biology and angiogenesis	Andreas Stengel <i>(University of Tübingen, Germany):</i> Role of nesfatin in food intake regulation
9:30 - 10:00	Jeff Tasker <i>(Tulane University, USA):</i> Neuropeptide activation of neuronal-glia circuits	Stephen Salton <i>(Icahn School of Medicine at Mount Sinai, USA):</i> VGF-derived fragment for neuroprotection in Alzheimer's disease	Y. Peng Loh <i>(National Institute of Child Health and Human Development, NIH, Bethesda, USA):</i> Serpinins: tissue distribution and functions	Yvette Taché <i>(University of California-Los Angeles, USA):</i> Gaps in knowledge - what should be addressed next in nesfatin research
10:10- 11:00	IRPS General Assembly <i>Atlantes Amphitheater</i>			
11:00- 11:40	Young Investigator Neuroscience Special lecture (NSL) <i>Atlantes amphitheater</i> Sarah Melzer (Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School) Peptidergic regulation of cortical inhibition			
11:40- 12:00	Conference closure: Organizing committee and conference chairs			

Abstracts

*From
Invited Speakers
In
Chronological order*

S1. Extrahypothalamic functions of magnocellular neurons: optogenetics, development and behavior

Princesa 1

Chair: **Valery Grinevich** (Heidelberg, Germany)

- S1.1 **Valery Grinevich** (University of Heidelberg, Germany):
Oxytocinergic circuits of the amygdala: finding points of intervention for pain and pleasure
- S1.2 **David Murphy** (University of Bristol, UK):
Linkages between osmoregulation and ingestive behaviors are encoded in vasopressinergic-dynorphinergic projections from hypothalamus to amygdala
- S1.3 **Limei Zhang** (Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico):
Vasopressin projections to habenula and modulation by sex steroids: control of response to aversive stimuli in mammals
- S1.4 **Alexa Veenema** (Michigan State University, East Lansing, USA):
Developmental and sex-specific involvement of vasopressin in the regulation of social behavior

S1.1 Oxytocinergic circuits of the amygdala:: finding points of intervention for fear

Valery Grinevich

Division of Neuropeptides (V078), German Cancer Research Center, Central Institute of Mental Health, CellNetworks Cluster of Excellence, University of Heidelberg, Im Neuenheimer Feld 581 (TP4), Office 3.301, D-69120 Heidelberg, Germany

Oxytocin (OT) neurons of the paraventricular (PVN) and supraoptic (SON) nuclei release OT from their distant axons in the central amygdala (CeA) to attenuate the fear response (Knobloch et al., 2012). The big challenge is to reveal the role of OT circuits in fear control and regulation. To address this question, we developed a virus-delivered genetic activity-induced tagging of cell ensembles (vGATE) technique, which is based on c-fos promoter fragment driving rapid and transient expression of the reverse tetracycline (tet) transactivator, controlled by the antibiotic doxycycline. The c-fos-rtTA recombinant adeno-associated virus (rAAV) was complemented by a rAAV carrying a bidirectional tet promoter, to express Cre recombinase and a rAAV with double floxed elements, equipped with the OT promoter to express the genes of interest, allowing labeling and manipulation of OT neurons activated upon fear expression. Applying vGATE, we have identified experience-activated OT neurons in PVN and SON, which are sufficient and necessary in controlling fear behavior. We have further discovered that the central OT system is composed of functionally specialized ensembles of magnOT neurons, which modulate CeA network activity and the subsequent fear expression and extinction in a context-dependent manner. Remarkably, the OT axons providing input to the CeA undergo experience-dependent morphological and neurochemical plasticity, namely a shift from OT to glutamate signalling during associative fear learning. Besides, we have identified a distinct group of parvocellular OT neurons of the PVN (Eliava et al., 2016), which are activated independently from fear context and drive the activity of magnocellular OT neurons of the SON. Indeed, parvocellular OT neurons are crucial for massive OT release from magnocellular SON neurons into the blood stream specifically in a novel fear context. Altogether, contextual fear learning led to tremendous reorganization of the OT system, including neuromodulatory and neuroendocrine responses, essential for behavioral and homeostatic coping with deleterious fear events (Hasan et al., in revision). Furthermore, our results provide mechanistic insight into the pathophysiology of emotional alterations in human patients afflicted with general anxiety syndrome and posttraumatic stress disorder, and support application of OT for treatment of these alterations.

1. Knobloch HS *et al.* Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron*.**73**(3), 553-566 (2012).
2. Eliava, M. *et al.* A New Population of Parvocellular Oxytocin Neurons Controlling Magnocellular Neuron Activity and Inflammatory Pain Processing. *Neuron*.**89**(6), 1291-1304 (2016).
3. Hasan *et al.* Fear memory engram and plasticity of the central oxytocin system (in revision).

S1.2 Linkages between osmoregulation and ingestive behaviors are encoded in vasopressinergic--dynorphinergic projections from hypothalamus to amygdala

David Murphy¹, Olivera Šarenac^{1,2}, Mingkwan Greenwood¹, Michael Greenwood¹ and Nina Japundžić-Žigon²

¹Bristol Medical School: Translational Health Sciences, The Dorothy Hodgkin Building, Whitson Street, Bristol BS1 3NY, England; ²Laboratory for Cardiovascular Pharmacology, Institute of Pharmacology, Clinical Pharmacology and Toxicology, School of Medicine, University of Belgrade, Dr Subotica 1, Belgrade, 11000, Serbia

Integrated neuroendocrine and behavioral mechanisms function to control the excretion and consumption of water and salt in order to maintain the optimal bodily composition required for good health and well-being, and aggressively defend osmotic stability when it is threatened. However, these mechanisms go wrong in old age, and diminished thirst and salt appetite can result in disorders of fluid balance that are a frequent cause of morbidity and mortality in the elderly.

Salt palatability and hence consumption is a balance between hedonic and aversive responses. Given a choice, rats would rather consume 0.9% w/v NaCl over water (appetite), but they prefer to consume water over 2% w/v NaCl (aversion). However, aversion is overcome if 2% w/v NaCl is the only fluid source: after salt loading (SL) for up to 7 days, whereupon the salty solution is avidly consumed. At the same time, integrative hypothalamic structures (the paraventricular nucleus, PVN, and the supraoptic nucleus, SON)) activate the secretion of the antidiuretic neuropeptide hormone arginine vasopressin (VP) from magnocellular neuron (MCN) axon terminals in the posterior pituitary (PP), which acts on the kidney to promote water conservation.

We have identified an mRNA called Giot1 that is robustly up-regulated in the PVN and SON by SL. The Giot1 RNA has an open reading frame that could encode a Kruppel-associated box domain containing zinc finger protein (KRAB-ZFP) transcription factor, and we have shown that this transcript is largely confined to the nucleus of MCNs that express VP and the endogenous opioid peptide dynorphin (Dyn). We have now shown that:

*Giot1 activates salt drinking in SL animals.

*Giot1 regulates the expression of the genes encoding VP and Dyn.

*Blockade of Dyn kappa-opioid receptors (KORs) at the level of the amygdala (Amyg) can inhibit salt intake during SL.

*Expression of Giot1 increases in the PVN and SON with old age in rats.

*MCNs send projections to targets in the Amyg.

We suggest that VP/Dyn MCNs are paradigmatic for the novel concept that neuroendocrine neurons can be involved in the simultaneous dual regulation of homeostasis and allostasis. In response to life-threatening physiological cues, these neurons respond by regulating both PP hormone secretion (homeostatic) and neuronal neurotransmitter secretion (allostatic regulation of preference). In the context of their role in allostasis, we hypothesize that the Giot1 RNA regulates the expression of neurotransmitters that enable salt ingestion, through projections to limbic areas under different physiological conditions. We further hypothesize that these mechanisms become impaired in old age.

S1.3 Vasopressin projections to habenula and modulation by sex steroids:: control of response to aversive stimuli in mammals

Limei Zhang¹, Vito S. Hernández¹, Luis Miguel Garcia-Segura² and Lee E. Eiden³

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The lateral habenula (LHb) plays a key role in integrating a variety of neural circuits associated with reward and aversive behaviors. There is limited information about how the different cell types and neuronal circuits within the LHb coordinate physiological and motivational states. Here, we report a cell type in the medial division of the LHb (LHbM) in male rats that is distinguished by: 1) a molecular signature for GABAergic cells (Slc32a1/VGAT) and estrogen receptor (Esr1/ER α) expression, at both mRNA and protein levels as well as the mRNA for vesicular glutamate transporter Slc17a6/VGLUT2; 2) its axonal projection patterns, identified by *in vivo* juxtacellular labeling, to both local LHb and to midbrain modulatory systems; and 3) its somatic expression of receptors for vasopressin, serotonin and dopamine and mRNA for orexin receptor 2. This cell type, which we term GERN for GABAergic estrogen-responsive neuron, is anatomically located to receive afferents from midbrain reward (dopamine and serotonin) and hypothalamic water and energy homeostasis (vasopressin and orexin) circuits, and these afferents shared in common the expression of estrogen synthase (aromatase) and VGLUT2, both in their somata and axon terminals. We demonstrate dynamic changes in VGAT+ cell density, dependent upon gonadal functional status, that closely correlate with motivational behavior in response to predator and forced swim stressors. The findings suggest that the homeostasis and reward-related glutamatergic convergent projecting pathways to LHbMC employ a localized neurosteroid signaling mechanism via axonal expression of aromatase, to act as a switch for GERN cell excitation/inhibition output prevalence, influencing depressive or motivated behavior.

This work was supported by DGAPA-UNAM-PAPIIT-IN216918, CONACYT-CB-238744 (to L.Z.) and NIMH-IRP-1ZIAMH002386 (to L.E.E.).

S1.4 Sex-- and age-specific involvement of vasopressin in the regulation of social behavior

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The sex difference in vasopressin (AVP) fiber projections from the medial amygdala (MeA) and bed nucleus of the stria terminalis (BNST) to the lateral septum (LS) is one of the best-characterized in the vertebrate brain. Yet, we still know little about its functional consequences. We recently confirmed that this sex difference is already present at juvenile age, suggesting a sex-specific role of the MeA/BNST-to-LS AVP pathway across the lifespan. In support, we showed that the LS-AVP system in rats regulates juvenile social play behavior in sex-specific ways. In detail, pharmacological blockade of AVP 1a receptors (V1aR) enhances social play in males and reduces social play in females. We further demonstrate that dopamine (DA) neurotransmission is involved in the sex-specific regulation of social play behavior by the LS-AVP system. Specifically, an increase in extracellular LS-DA release was observed in response to LS-AVP administration and in response to social play exposure in females, but not in males. Notably, administration of a V1aR antagonist into the LS prevented the social play-induced increase in LS-DA release in females. Furthermore, enhancing LS-DA neurotransmission counteracted the inhibiting effects of LS-V1aR blockade on social play behavior in females.

We further found that the LS-AVP system in rats shows differences across the lifespan: LS-AVP fiber density and LS-V1aR binding density are higher in adults than in juveniles. This may indicate the potential of higher AVP-V1aR signaling in the LS in adults compared to juveniles. This, in turn, may allow for some behaviors to be regulated in an age-specific way. In support, we showed that administration of a V1aR antagonist into the LS impaired social recognition in adult males but not in juvenile males. Furthermore, administration of AVP into the LS improved social recognition in adult males, but not in juvenile males. Finally, the LS-AVP system facilitates intermale aggression in adult males while attenuating social play behavior in juvenile males. These findings suggest that age differences in the LS-AVP system may facilitate the developmental transition from juvenile social play behavior to adult aggression.

Overall, these findings demonstrate a role for the LS-AVP system in facilitating sex-appropriate and age-appropriate social behaviors.

Supported by NIMH R01MH102456 and NSF IOS1253386

S2. What's new with POMC, TRH, PRL and GH neuroendocrine actions

Princesa 2

Co-chairs:

Patricia Joseph-Bravo (Cuernavaca, México) and **Dave Grattan** (Dunedin, New Zealand):

- S2.1 **Malcolm J. Low** (University of Michigan, Ann Arbor, USA):
Neuropeptides involved in integrated hypothalamic control of energy homeostasis
- S2.2 **Patricia Joseph-Bravo** (IBT, UNAM, Mexico):
Hypophysiotropic TRH neurons integrate stress and metabolic signals
- S2.3 **Dave Grattan** (University of Otago, Dunedin, New Zealand):
Prolactin actions in the maternal brain during pregnancy
- S2.4 **Carlos Arámburo de la Hoz** (INB, UNAM, Mexico):
Autocrine/paracrine roles of extrapituitary growth hormone in neuroprotection

S2.1 Neuropeptides involved in the integrated hypothalamic control of energy homeostasis

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A major challenge for obese individuals after dieting is maintenance of reduced body weight. Diet-induced obese humans and mice are hyperleptinemic and resistant to achieving normal body weight when obesogenic conditions are reversed, in part because lowered circulating leptin triggers a reduction in metabolic rate and rebound of hyperphagia that defends the previously elevated body weight set point. Mutant mice with reversible *proopiomelanocortin* (*Pomc*) silencing limited to the arcuate nucleus (*ArcPomc*^{lox/Neo/lox/Neo}) have increased adiposity and leptin levels shortly after weaning and rapidly become morbidly obese eating only low-fat chow (Bumaschny, et al. 2012), with weight gain rate further accelerated by a high fat diet. Meal pattern analyses implicated impaired satiation instead of increased frequency of meal initiation as the principal cause of hyperphagia. The mice also exhibit markedly reduced spontaneous locomotor activity and voluntary wheel running activity. These locomotor deficits are absent in mice with a selective deletion of beta-endorphin, suggesting a predominant role for the absence of melanocortin peptides in the decreased activity of *ArcPomc*^{lox/Neo/lox/Neo} mice. The obese mutant *Pomc* mice are capable of increased locomotor activity, demonstrated by acute responses and locomotor sensitization to repeated morphine administration. Obese *ArcPomc*^{lox/Neo/lox/Neo} mice have much greater bar pressing activity and pellet acquisition in an operant “binge-feeding” paradigm than similarly obese DIO mice, which exhibit decreased pellet acquisition compared to lean wild-type mice.

As hypothalamic POMC neurons are a central leptin target, we investigated whether changes in circulating leptin modify *Pomc* expression to maintain normal energy balance in the genetically predisposed obese mice. We measured body composition, food intake, plasma leptin and leptin sensitivity in *ArcPomc*^{lox/Neo/lox/Neo} mice that were weight-matched to littermate controls by calorie restriction, either from weaning or after developing obesity. Despite chronic calorie restriction to achieve normal body weight, mutant mice remained moderately hyperleptinemic and resistant to exogenous leptin’s effects to reduce weight and food intake. Weight-matching was sufficient to restore normal levels of spontaneous locomotor activity, but not voluntary wheel-running activity, thought to represent an intrinsic rewarding behavior. Furthermore, weight-restricted *ArcPomc*^{lox/Neo/lox/Neo} mice exhibited nearly normal levels of food-anticipatory activity when their access to chow was limited to a 4 hr period each day. *Pomc* reactivation in the Arc by tamoxifen-dependent Cre recombinase transgene expression completely normalized plasma leptin, leptin sensitivity, adiposity, spontaneous locomotor activity and food intake of the weight-matched, but not ad-lib fed obese mutant mice, indicating that appetite- and body weight-set points of the mutant mice were restored to control levels. Preliminary evidence shows that mutant mice infertility can also be reversed by the combined manipulations. In contrast, induction of extreme hyperleptinemia by injection of exogenous PASylated leptin during the five days of tamoxifen administration blocked full restoration of hypothalamic *Pomc* expression in calorie restricted *ArcPomc*^{lox/Neo/lox/Neo} mice. Consequently, those mice regained 30% of their lost body weight and attained a metabolic steady state similar to that of tamoxifen-treated, ad-lib fed obese *ArcPomc*^{lox/Neo/lox/Neo} mice. We conclude that the simultaneous restoration of hypothalamic leptin sensitivity by calorie restriction and hypothalamic *Pomc* expression is necessary for previously obese *ArcPomc*^{lox/Neo/lox/Neo} mice to achieve and sustain normal metabolic homeostasis; whereas deficits in either parameter set a maladaptive allostatic balance that defends increased adiposity and body weight.

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S2.2 Hypophysiotropic TRH neurons integrate stress and metabolic signals

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Paraventricular-hypothalamic (PVN) thyrotropin-releasing hormone (TRH) and corticotropin-releasing hormone (CRH) hypophysiotropic neurons decode metabolic, neuronal and environmental signals, and regulate two neuroendocrine axes: the hypothalamus-pituitary-thyroid (HPT) and the HP-adrenal (HPA) axes. Thyroid hormones and glucocorticoids (Gc) are crucial participants in energy homeostasis. Trh expression and HPT axis are activated by energy demanding situations as cold exposure or exercise, and inhibited by negative energy balance, such as food restriction or fasting, and disease. At the median eminence (ME) level, released TRH may be inactivated, before reaching the thyrotrophs, by the TRH-degrading ectoenzyme expressed in tanycytes and modulated by T3 and by nutritional status. CRH neurons are activated by acute or chronic stress; the Gc receptor (GR) mediates the effects of Gc during stress. Cold rapidly activates Trh expression in the PVN through cAMP response element binding protein (CREB) phosphorylation and Gc or acute stress blunt cold-induced activation of the HPT axis (1,2).

We explored the mechanism of Gc interference on cold-induced activation of Trh expression. In vivo, corticosterone injection prevents cold-induced stimulation of CREB phosphorylation in TRH-PVN neurons, as well as PVN TRH and pituitary thyrotropin synthesis and release. In hypothalamic cells, dexamethasone (Dex, a GR ligand) inhibits cAMP-induced CREB phosphorylation, pCREB and GR binding to response elements of Trh-gene promoter, and Trh mRNA levels, suggesting interference occurs before DNA binding. Furthermore, the catalytic subunit of protein kinase A (PKAc) co-immunoprecipitates with GR and, Dex decreases cAMP-induced nuclear translocation of PKAc. Thus, Gc repress neuronal-induced transcriptional activation of the Trh gene by protein:protein interaction between GR and PKAc (2).

We have recently evaluated the response of HPT axis to several paradigms of chronic stress in adult rats and, in critical periods of development as during lactation and puberty/adolescence. Chronic stress in adult male rats curtails stimulation of the HPT axis provoked by either acute cold (poster A. Gutiérrez-Mata) or voluntary exercise (poster M. Gutiérrez-Mariscal). Deleterious effects of stress insults extend to postnatal stress; maternal separation (MS) causes long-term effects on the activity of the HPT axis in a gender-specific manner (3). HPT axis responses to fasting (3) or cold are partially blunted in male (not female) adults that suffered MS (poster L. Jaimes), a status which could affect their adaptation in conditions of negative energy balance. The HPT axis response to a palatable diet also varies according to previous stress paradigm and sex (Jaimes-Hoy, in preparation). Isolation during puberty-adolescence causes behavioral and endocrine problems that include changes in the HPT response to acute stress, cold or exercise (poster M. Parra). These results confirm that PVN TRH neurons act as energy sensors and demonstrate that they are vulnerable to stress. Stress-induced dysfunction of the HPT axis, needed upon demand, may contribute to development of obesity and metabolic syndrome (*CONACYT 284883, and DGAPA IN204316*).

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2. Sotelo-Rivera I., Cote-Vélez A., Uribe R.M., Charli J.-L. and Joseph-Bravo P. Endocrine 55: 861-871, 2017.
3. Jaimes-Hoy L., Gutiérrez-Mariscal M., Vargas Y., Pérez-Maldonado A., Romero F., Sánchez-Jaramillo E., Charli J.-L. and Joseph-Bravo P.. Endocrinology 157: 3253-3265, 2016.

S2.3 Prolactin actions in the maternal brain during pregnancy

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Prolactin is well known as the hormone responsible for lactation, but it also exerts a broad range of other actions in the body. We have identified widespread prolactin receptor expression throughout the hypothalamus, suggesting that it could influence a number of hypothalamic functions. Our hypothesis is that prolactin actions might coordinate the multiple adaptive changes that occur in the maternal brain during pregnancy and lactation to help the mother adapt to the novel demands of these physiological states. Such changes range from increased appetite and food intake, reduced physical activity, loss of fertility, suppression of the stress axis, and expression of parental behavior. Many of these functions involve prolactin-induced changes in expression of neuropeptides in the brain. Prolactin also acts in peripheral tissues, such as pancreas, gut and bone, to promote adaptive changes that support pregnancy and lactation. Using novel transgenic mouse lines allowing conditional deletion of prolactin receptors from specific cell populations¹ or expression of genetic markers in prolactin receptor expressing cells², we have characterized a number of novel functions of prolactin in the brain and peripheral tissues during pregnancy. Most notably, we have recently demonstrated that prolactin receptor expression in the medial preoptic area is critical to the normal expression of maternal behavior². Together the data support the hypothesis that prolactin plays a major role in the maternal adaptation to pregnancy.

¹Brown RS, Kokay IC, Phillipps HR, Yip SH, Gustafson P, Wyatt A, Larsen CM, Knowles P, Ladyman SR, LeTissier P & Grattan DR. Conditional Deletion of the Prolactin Receptor Reveals Functional Subpopulations of Dopamine Neurons in the Arcuate Nucleus of the Hypothalamus. *J Neurosci* **36**, 9173-9185, (2016).

²Brown RSE, Aoki M, Ladyman SR, Phillipps HR, Wyatt A, Boehm U & Grattan DR. Prolactin action in the medial preoptic area is necessary for postpartum maternal nursing behavior. *Proc Natl Acad Sci U S A* **114**, 10779-10784, (2017).

S2.4 Autocrine/paracrine roles of extrapituitary growth hormone in neuroprotection

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It is now accepted that, besides the pituitary somatotrophs, growth hormone (GH) can be expressed in several extrapituitary locations, such as the nervous, immune and reproductive systems, among others. The brain is a GH target site and GH receptors are expressed widely throughout the central nervous system (CNS). The presence of GH and GH mRNA in neural tissues is well established. There is increasing evidence that GH may be involved in neurotrophic, neuroprotective and neuroregenerative actions. Although systemic (endocrine) GH may exert some of these effects, evidence indicates that locally expressed neural GH, acting through autocrine and/or paracrine mechanisms may also participate in these actions.

We have previously described the local expression of GH and GH receptor (GHR) mRNAs and proteins in various neural tissues of several vertebrate models (mammals, birds, reptiles), and have studied the neurotrophic and neuroprotective actions of GH in response to neural damage provoked by different insults, in the cerebellum (hypoxia/ischemia) and in the neuroretina (excitotoxic damage).

We investigated the possible neuroprotective role of GH in the cerebellum, which is very sensitive to hypoxic/ischemic conditions. Endogenous GH levels increased in the brain and cerebellum (30% and 74%, respectively) of 15-day-old chicken embryos exposed to hypoxia during 24 h compared to normoxia. In primary embryonic cerebellar neuron cultures treated under hypoxia (0.5% O₂) and low glucose (1 g/l) conditions (HLG) for 1 h, GH levels increased 1.16-fold compared to the control. The addition of 1 nM recombinant chicken GH (rcGH) to cultures during HLG increased cell viability (1.7-fold) and the expression of Bcl-2 (1.67-fold); in contrast the caspase-3 activity and the proportion of apoptotic cells decreased (37 and 54%, respectively) compared to HLG. Administration of rcGH activated the PI3K/Akt pathway both under normoxic and HLG conditions, increasing the proportion of phosphorylated Akt (1.7- and 1.4-fold, respectively).

We also found that both GH and GHR are expressed in several layers of the neuroretina, both in the chicken and iguana. The administration of kainic acid (KA), a glutamate receptor agonist, induced retinal excitotoxic damage that provoked a significant decrease of cell density and an increase of apoptotic cells. Under these conditions, both endogenous GH and Insulin-like growth factor I (IGF-I) expression were increased by 70 and 33%, respectively. The addition of exogenous GH significantly prevented the retinal damage produced by the loss of cytoarchitecture and cell density of neuroretina. The co-incubation with a specific anti-GH antibody blocked this effect. The administration of GH in primary neuroretinal cell cultures protected and induced neural outgrowths. Intravitreal injections of GH restored brain derived neurotrophic factor (BDNF) expression in retinas treated with KA. Also, we showed that GH over-expression increased BDNF and neurotrophin-3 (NT3) gene expression in embryonic neuroretinal cells. Our results strongly suggest that these classical neurotrophins are mediators of GHs neuroprotective actions. Thus, a complex cascade of neurotrophins and growth factors, which have been classically related to damage prevention and neuroretinal tissue repair, likely mediates GH neuroprotective actions in neural tissues.

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S3. Neuropharmacology of vasopressin and oxytocin: physiology and behavior

Princesa 3

Chair: **Maurice Manning** (Ohio, USA)

- S3.1 **Maurice Manning** (University of Toledo, USA):
Receptor selective oxytocin and vasopressin agonists and antagonists as research tools and therapeutics: a current perspective
- S3.2 **Gilles Guillon** (CNR, Montpellier, France):
Selective fluorescent ligands for imaging vasopressin and oxytocin receptors in native tissues
- S3.3 **Andrés Quintanar-Stephano** (UAA, Mexico):
Effects of the neuropeptide arginine vasopressin (AVP) deficiency, conivaptan and desmopressin on clinical symptoms, gene expression and blood cytokine levels in rats with experimental autoimmune encephalomyelitis
- S3.3 **Nicolas Gilles** (IBMM, CNRS, Montpellier, France):
Animal toxins for human health, case of the mambaquaretin for the treatment of polycystic kidney disease

S3.1 Receptor Selective Oxytocin and Vasopressin Agonists and Antagonists as Research Tools and Therapeutics: A Current Perspective

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In memory of Svetlana Pancheva, Wilbur H. Sawyer, Serge Jard, Krzysztof Bankowski, Lajos Balásperi

Introduction: As a long-time member of the oxytocin (OT)/vasopressin (VP) field, since my years as a post-doc in the Vincent du Vigneaud laboratory in the early 60's, and a participant in all but two of the World Congresses of Neurohypophysial Peptides (WCNH); from Nasu, Japan (1995) to Rio de Janeiro, Brazil (2017), I am deeply honored to Chair the session on Neuropharmacology of Vasopressin and Oxytocin: Physiology and Behavior at the RegPep 2018 in Mexico. This promises to be a highly interesting session. I relish the opportunity to share with members of the Regulatory Peptide Community some of the key advances in the design of OT and VP ligands, which have resulted from design and synthetic studies carried out in my laboratory in Toledo with long-time collaborators in New York, Montpellier, France, and Milan, Italy and highly-skilled peptide chemists from Poland, Hungary, Bulgaria, England and China. The vibrancy of the OT/VP field is clearly illustrated by the numerous requests for peptides from my laboratory. During the period 1980-2018, over 3,500 samples of OT and VP agonists and antagonists have been, and continue to be, donated to over 750 investigators (some multiple times) in the U.S. and worldwide for use as research tools in their own independent studies; resulting in over 2000 publications in the OT/AVP field. This talk will trace the key factors and findings, some by serendipity, which have led to the discovery of widely used receptor selective peptide and nonpeptide ligands (1). It will also give an update on the striking discovery of bivalent OT agonists (2), coupling selective ligands and OTR selective ligands. The current status of clinical trials on the therapeutic potential of OT for the treatment of neuropsychiatric diseases will be evaluated. The remarkable recent discovery that a VP V_{1b} agonist (d[Cha⁴]AVP) which we reported in 2002 (ref 69 in 1) and a related V_{1b} agonist (d[Leu⁴,Lys⁸]VP (ref 86 in 1) are of therapeutic interest for the treatment of anemia (3) adds a heuristic epilogue to this presentation.

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2. Busnelli et al. *J. Med. Chem.* 2016 59(15): 7152-7166 (Editors choice).
3. Meyer et al. *Sci. Transl. Med.* 2017 9: 1632.

S3.2 Selective fluorescent probes for imaging vasopressin/oxytocin receptors

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The lack of efficient and selective Vasopressin/Oxytocin receptors antibodies and of selective fluorescent VP/OT analogues have hampered their cellular and tissue imaging. To circumvent this difficulty, we designed, synthesized and characterized fluorescent peptides to selectively identify each VP/OT receptors isoforms in native tissues or in primary cultured cells to determine their pattern of expression.

We first added to the selective V_{1B}-selective agonist d(Leu⁴,Lys⁸)VP an Alexa 647 or an Alexa 488 fluorophore in position 8 and tried different spacers to obtain peptides exhibiting good affinities for the V_{1B}-R with a good selectivity versus the other VP/OT receptor isoforms (1). These fluorescent peptides displayed a good efficiency to localize very low amounts of V_{1B}-R, thanks to the excellent brightness of the Alexa probes and represent good tools to precisely map the V_{1B} receptor subtype in the rat brain, especially if AVP-GFP Knockin rats are used to decipher together vasopressin distribution and V_{1B} receptors (2). On the other hand, by starting from the d(Lys⁸)VP and adding Alexa 647 on the Lys in position 8, we also obtained an unexpected OT-selective fluorescent ligand. This was very surprising since dLVP is known to be a non-selective VP/OT-R peptide. This approach presently allows a selective cartography of OT-R in rat brain. Using these 2 selective V_{1B} and OT fluorescent peptides labelled with distinct fluorophores, it is now possible to look for both V_{1B} and OT-R on the same tissue sample.

Promising results concerning the labelling of V₂-R are now in progress using a similar approach. However, we failed so far to generate a selective V_{1A} fluorescent ligand starting either from the recently characterized V_{1A}-selective FE201874 (3) or from other vasopressin derivatives.

Using this series of selective fluorescent V_{1B}/OT-receptor ligands and confocal microscopy, it is now possible to precisely determine the receptor distribution of VP/OT receptor isoforms in the brain and in peripheral tissues with an accurate cellular localization. This opens avenues to better understand the function of Vasopressin and Oxytocin at the cellular level.

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2. M. Corbani et al 2017 (GCE Gen Comp Endocrinol. 2018 Mar 1;258:15-32)
3. R. Marir et al, 2013 (B J Pharm) Br. J. Pharmacol. 2013 Sep; 170(2): 278–292

S3.3 Effects of the Neuropeptide Arginine Vasopressin (AVP) Deficiency, Conivaptan and Desmopressin on Clinical Symptoms, Gene Expression and Blood Cytokine Levels in Rats with Experimental Autoimmune Encephalomyelitis

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Introduction. The experimental autoimmune encephalomyelitis (EAE), the rat paradigm for the human multiple sclerosis (MS), are autoimmune diseases mediated by Th1 and Th17 cells and responsible of the nervous system demyelination and progressive paralysis. It is known that lymphocytes possess AVP receptors and that EAE increases AVP serum levels along the disease. Neurointermediate pituitary lobectomy (NIL) in rats causes AVP and oxytocin permanent low serum levels and decreased humoral and cell-mediated immune responses. Nevertheless the role of AVP as a direct immune regulatory neuropeptide has not been well clarified.

Objective. To investigate the effects of AVP deficiency (by NIL), and the treatment with desmopressin (DP, a synthetic analog of AVP) and conivaptan (CNV, an AVP V1a-V2 receptor antagonist) on the clinical symptoms of EAE, the spleen transcriptional signature associated to the effects of AVP in EAE, and to determine the serum levels of Interleukine-2 (IL-2), IL-4, IL-6, IL-10, IL-17A, INF- γ and TNF- α in the experimental groups.

Methods. Groups of female Lewis rats were divided in: (1) Intact control (Control) (2) Control+EAE, (3) Sham-operated (SHAM), (4) Control+CNV, (5) NIL and (6) NIL+DP. Except the Control the remaining groups were immunized for EAE three weeks post-NIL. DP and CNV administrations started 3 days before EAE immunizations. At 15th day of EAE immunization, animals were sacrificed: spleen tissue sample was subjected to RNA extraction and used for microarray hybridization and analysis. Serum cytokines were analyzed by flow cytometry and EAE clinical symptoms were evaluated by a conventional numerical scale.

Results. A specific transcriptional signature (up-regulated and down-regulated genes) was associated with EAE clinical symptoms. EAE clinical symptoms, TGF- β pathway and associated autophagy genes as well as, INF- γ , TNF- α , and the IL-2, IL-6 and IL-17A were significantly decreased in both NIL and CNV groups. Whereas the serum levels of IL-4 and IL-10 were increased in the CNV group. In NIL+DP group we found a significant increase of EAE clinical symptoms, TGF- β pathway and associated autophagy genes and serum TNF- α , whereas the IL-4 was significantly decreased.

Conclusions. 1) The present clinical and molecular findings demonstrate that AVP deficiency and the block of the AVP receptors decreased immune response, and that DP restores the susceptibility of the NIL animals to develop EAE. 2) The results strongly support that acting directly on the AVP receptors, AVP plays an important immunoregulatory role in the generation and maintenance of the immune-competence, 3) AVP receptors may be therapeutic targets in the treatment of the human MS.

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S3.4 Animal toxins for human health, the case of mambaquaretin for the treatment of polycystic kidney diseases

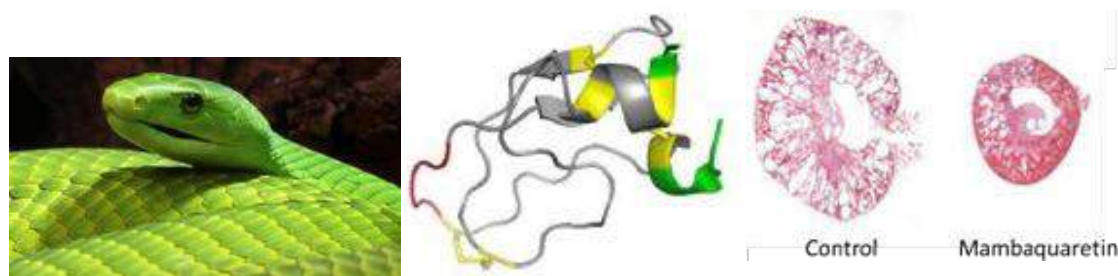
Laura Droctove¹ Justyna Ciolek^a Helen Reinfrank² Gilles Mourier³ Bernard Mouillac³ Christiane Mendre³ Nicolas Floquet⁴ Ralph Witzgall² Denis Servent¹, **Nicolas Gilles¹**

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Autosomal dominant polykystose kidney disease affect 1 over 1,000 peoples and leads to end-stage renal disease. The blockage of the vasopressin type 2 receptor (V2R) is a validated therapeutic line in human by preventing the vasopressin-induced elevation in intracellular cAMP concentration. Currently, only tolvaptan (jirnac) succeeded to reach the market but with many concerns (Torres et al., 2017).

Scorpions, spiders, snakes, conus, insects, miriapodes are often seen as dangerous, frightening and ugly animals. Their venoms are extremely rich in toxins historically identified for their toxicities. We believe that animal toxins are highly valuable in the context of human use and drug development. We optimized a process to identify animal toxins specific to G-Protein Coupled Receptors linked to an unmet therapeutic need. Mambaquaretin was discovered in the green mamba venom by a bioguidage strategy directed against the V2R. This toxin belongs to the Kunitz fold peptide family, and its displays a nanomolar affinity for the V2R. Molecular pharmacological assays demonstrated that mambaquaretin is the most selective V2R antagonist ever identified. Daily injection of 13 µg of mambaquaretin to pcy mice, a model of juvenile recessive kidney polykystose disease, over a period of 99 days, allowed the drug candidate to inhibit cyst growth area by almost 30%. No apparent toxicity was observed in treated animals (Ciolek et al., 2017).

Mambaquaretin is a promising drug candidate with an original mode of action. Molecular modeling and analysis of the structural function make it possible to propose a model of interaction of the mambaquaretin/V2R complex and to understand the molecular basis of its selectivity.



The green mamba

X-ray structure of the mambaquaretin

Representative kidney section from pcy mice

Ciolek, J., Reinfrank, H., Quinton, L., Viengchareun, S., Stura, E.A., Vera, L., et al. (2017). Green mamba peptide targets type-2 vasopressin receptor against polycystic kidney disease. *Proc. Natl. Acad. Sci. U. S. A.* *114*: 7154–7159.

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S4. Neuroendocrine peptide GPCRs: from function to therapeutic targets

Princesa 4

Co-chairs:

Hélène Castel (Rouen, France) and **Richard Leduc** (Sherbrooke, Canada)

S4.1 **Atsuro Miyata** (Kagoshima University, Kagoshima, Japan):

Astrocyte-neuron lactate shuttle (ANLS) as the major effector of PACAP/PAC1R signaling for CNS functions

S4.2 **Sunny Z. Jiang** (NIMH, Bethesda, USA):

PACAP and dopamine signaling in stress: response parcellation by distinct cyclic AMP sensors in neuronal and endocrine cells

S4.3 **Laurent Prézeau** (INSERM U661 – University of Montpellier, Montpellier, France):

GSHR controls YAP phosphorylation via both constitutive and agonist-induced pathways

S4.4 **Hélène Castel** (Inserm U1239, DC2N, Normandie University, Mont-Saint-Aignan, France):

Biased signaling of the urotensin II receptor: still a blind spot between direct couplings and brain physiopathology

S4.1 Astrocyte-neuron lactate shuttle (ANLS) as the major effector of PACAP/PAC1R signaling for CNS functions

Yuki Kambe, Takashi Kurihara, Yu Nakashima and **Atsuro Miyata**

Department of Pharmacology, Graduate School of Medical and Dental Science, Kagoshima University, 8-35-1 Sakaragaoka, Kagoshima 890-8544, Japan

The astrocyte-neuron lactate shuttle (ANLS), in which transfer of lactate from astrocyte to neuron is facilitated when synaptic activity increases, accounts for the coupling between synaptic activity and energy delivery. Many lines of evidence suggest that the ANLS contributes to neuronal activation or synaptic plasticity at the cellular level as well as learning/memory and cocaine addiction at the behavioral level. However, candidate neurotransmitters which evoke ANLS activation are still under discussion. Pituitary adenylyate cyclase-activating polypeptide (PACAP) is widely distributed in the central nervous system, and PAC1R is implicated in a variety of PACAP functions as a PACAP-preferring receptor. Since astrocytic glycogenolysis and lactate secretion are shown to be essential for long-term synaptic facilitation, we attempted to elucidate a possible involvement of spinal astrocyte-neuron lactate shuttle (ANLS) in the development of PACAP/PAC1R-induced pain behaviors. Single intrathecal (i.t.) administration of PACAP induced short-term spontaneous pain behaviors, followed by long lasting mechanical allodynia. These pain behaviors were dose-dependently inhibited by i.t. coinjection of DAB, an inhibitor of glycogenolysis, and this inhibition was reversed by simultaneous application of L-lactate. Furthermore, since microdialysis analysis demonstrated that the intracerebroventricular administration of PACAP evoked the lactate release in hippocampus, we attempted to clarify the involvement of ANLS in the development of PACAP-mediated fear memory acquisition or retrieval. When fear memory was acquired or retrieved, glycogen amount was significantly decreased, but lactate amount was significantly increased in hippocampus of PACAP (+/+) mice, but these responses failed in PACAP (-/-) mice. These findings suggest that PACAP might be a candidate for the endogenous activator of ANLS.

S4.2 PACAP and dopamine signaling in stress: response parcellation by distinct cyclic AMP sensors in neuronal and endocrine cells

Sunny Z. Jiang

Section on Molecular Neuroscience, Laboratory of Cellular and Molecular Regulation, National Institute of Mental Health, Bethesda, MD USA

We have recently identified the neuronally-expressed guanine nucleotide exchange factor NCS-Rapgef2 as a cyclic AMP sensor/effector linking activation of Gs-coupled biogenic amine and neuropeptide receptors (Gs-coupled GPCRs) to specific intracellular consequences of first messenger stimulation mediated through the MAP kinase ERK, specifically associated with stress responding, immediate early gene activation, and long-term plasticity in CNS neurons. In particular, dopamine D1 and PACAP PAC1 receptor expression in heterologous cell lines confers ERK activation in the presence, but not in the absence, of NCS-Rapgef2 [1, 2]. We employed a conditional Rapgef2 knockout mouse line to examine the consequences of abrogation of NCS-Rapgef2 function in stress- and reward-associated cellular and behavioral responses in the mouse central nervous system in vivo.

ERK is activated in the bed nucleus of the stria terminalis, and in the CA3 region of hippocampus, and in the prefrontal cortex in response to 30-60 min restraint stress in wild-type mice, and this response is abrogated in CAMK2a-CRE x floxed Rapgef2 mice. ERK is activated in the nucleus accumbens (NAc) in response to cocaine administration in wild-type mice, and this response is abrogated (as are cocaine-dependent locomotor sensitization and conditioned place preference) in mice in which Rapgef2 expression in NAc is extinguished by stereotaxically specific injection of AAV-Syn-Cre (a tissue-serotyped AAV virus in which the synapsin promoter drives robust Cre expression in neurons). NCS-Rapgef2-dependent activation of ERK by either psychomotor stimulant administration or restraint stress is unaffected in PACAP-deficient mice, indicating that although the PAC1 receptor causes ERK activation in neuroendocrine cell lines in vivo, and functional stress responses in vivo, including depressogenic and endocrine responses to restraint stress and social defeat are PACAP-dependent, these PACAP-dependent stress responses are not driven by ERK activation at the cellular level.

Therefore ERK-dependent stress responses are likely mediated through activation of a Gs-coupled GPCR other than the PAC1 receptor, and PACAP-dependent stress responses are likely mediated, at the cellular level, through a non-ERK-dependent signaling pathway.

Supported by NIMH Intramural Research Program Project MH002386. I thank colleagues Andrew C. Emery, Wen Xu, Sean Sweat, Kate Loane, Gunner Drossel and Lee Eiden for their participation in the experiments reported here.

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S4.3 GSHR controls YAP phosphorylation via both constitutive and agonist-induced pathways

Zindel Diana¹, Mensat Patrick², Vol Claire¹, Charrier-Savournin Fabienne², Trinquet Eric², Banneres Jean-Louis³, Pin Jean-Philippe¹, Pannequin Julie¹, Roux Thomas², Dupuis Elodie², **Prézeau Laurent¹**

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The Hippo pathway originally identified in *Drosophila* is an evolutionary conserved kinase cascade playing essential roles for tissue homeostasis, cellular differentiation, proliferation, and organ size control. Core components of this signaling cascade comprise the Ser/Thr-kinase MST 1/2 which in complex with Sav1 phosphorylates large tumor suppressor kinase LATS 1/2. In turn LATS1/2 deactivates the two downstream effector proteins YAP and TAZ by phosphorylation (notably the Serine 127 on YAP) that leads to their retention in the cytosol and their degradation. Conversely dephosphorylated YAP/TAZ act as transcriptional coactivators, translocate into the nucleus and bind mainly to the TEAD family of transcription factors to induce expression of target genes that inhibit apoptosis and promote proliferation such as connective tissue growth factor (CTGF) or cysteine-rich angiogenic inducer 61 (CYR 61). Numerous upstream regulators have been reported to regulate the activity of the Hippo-YAP/TAZ axis including cell-cell contact inhibition, apical cell polarity complexes, and mechanical signals induced by extracellular matrix (ECM) rigidity and cell shape.

Importantly, G protein-coupled receptors (GPCR) activation has been demonstrated to either activate or inhibit YAP/TAZ activity and promotes the possibility to fine tuning depending on the G-protein that couples to the receptor. While the activation of Gs-coupled GPCRs induces YAP phosphorylation and thereby inactivation of the Hippo pathway, G12/13-coupled GPCRs promote YAP activation via Rho GTPases and inhibition of LATS 1/2 kinases. Interestingly, GPCRs mostly activate YAP through a G12/13 coupling, but constitutively active mutated Gq can also promote YAP activity. Depending on their coupling, GPCR could be related to YAP-promoted cancer formation.

We generated an easy-to-use and High Throughput Screening compatible HTRF® S127-Phosphorylation-YAP assay based on Time-Resolved Förster Resonance Energy Transfer (TR-FRET) signal recording. This assay is rapid and efficient for assessing the effects of drugs and experimental conditions on phosphorylation of the serine 127 of YAP (Commercialized by Cisbio bioassay). With this new assay, and using ghrelin receptor stably expressing cells, we showed that a pure Gq coupling can control YAP activity, without the involvement of G12/13. Moreover, we showed that acting on ghrelin receptor allowed an up-and-down modulation of YAP activity, as activating the receptor increased YAP activity and blocking constitutive activity reduced YAP activity. Our results demonstrate that acting on one GPCR displaying a strong constitutive activity, it is possible to finely up- or down-regulate YAP activity through a pure Gq pathway.

S4.4 Biased signaling of the urotensin II receptor: still a blind spot between direct coupling and brain physiopathology

Hélène Castel

Inserm U1239, DC2N, Normandie University, Mont-Saint-Aignan, France

During the past decade, complex cellular pleiotropic G protein coupled receptor (GPCR) signaling has been unveiled through selectivity/biased agonism effects. Various studies have attempted to link such complex signaling regulation to cellular and physiological behaviors but it remains gaps in the understanding of GPCR activation by biased ligands in pathological situations. The urotensinergic system is currently suspected of being linked to numerous pathological states including atherosclerosis, heart failure, hypertension, pre-eclampsia, diabetes, renal disease, as well as brain vascular dementia. The vasoactive peptide urotensin II (UII) and its paralog urotensin-related peptide (URP) are both endogenous ligands of the GPCR urotensin (UT) receptor, and display nonsystematic identical functional implications. Indeed, we previously demonstrated common and distinct mechanisms of UII and URP in reactive astrocytes, strongly suggesting a strong basis for design of partial agonists and biased ligands, to target brain gliovascular pathologies.

To elucidate the potential differential capacity of UII and URP and the antagonist/biased role of some synthetic UII-analogs at regulating the pleiotropic UT couplings, a solid collaboration with Dr L. Prézeau (IGF, Montpellier) and Pr R. Leduc (University of Sherbrooke, Quebec, Canada) was initiated to investigate on HEK293 cells expressing UT, 6 different signaling pathways (G_q , G_{i1} , $G_{o\alpha}$, G_{13} , and β -arrestins 1 and 2, G_s), downstream events such as ERK1/2, NF κ B phosphorylation and receptor internalization, and cell behaviors. Interestingly, we did not observe any coupling differences for UII and URP, leaving open the question about the differential effects of these peptides in native cells. However, synthetic peptide ligands induced different UT receptor-dependent pathway profiles, not only behaving as full or partial agonists but also displaying biased signaling pathways as illustrated by the analog urantide.

Cerebral vasospasm (VS) is a severe complication of aneurysmal subarachnoid hemorrhage (SAH). A mouse model of SAH was used to study the impact of UII and of urantide on VS and neurological outcome. In our clinical study, UII levels were daily measured in plasma during 9 days and were compared with UII in plasma from healthy volunteers. We demonstrated that the UT biased ligand urantide prevented VS and sensitivomotor deficits in SAH wildtype $UT^{+/+}$ mice while transgenic KO $UT^{-/-}$ mice showed neither VS, microthrombi nor neurological deficits 7 days post-SAH. In addition, we reported that high levels of UII in plasma of SAH patients is a predictive factor of the occurrence of vasospasm; stressing the involvement of the urotensinergic system in SAH.

In conclusion, we have shown that UT antagonism prevents VS and improves neurological outcome after SAH in mice and that higher plasma UII level seems to be associated with cerebral VS consecutive to SAH in humans. We are currently leading studies to establish UII-induced key mechanisms notably endothelial dysfunctions and inflammatory processes in a SAH mouse model and to validate the concept of "pathway targeted drugs" using biased urotensinergic analogs, which would prevent, without affecting other functions, deleterious mechanisms post-SAH.

Supported by Fondation AVC (FRM), Inserm transfert, Université de Rouen Normandie

Lay Lecture:

The translation of basic research into clinical practice

Atlantes Amphitheater

David Kershenobich

Director General, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán" (INCMNSZ), Mexico



Dr. Irwin Arias, physician-scientist founder of the Journal “Hepatology”, who did essential studies in the physiology of bilirubin and bile salts, first introduced me about two decades ago to the concept of bridge-building between medicine and basic science. These are exciting times to be involved in biomedical research, he said. I am the Director General of a National Institute of Mexico whose main task is the quality care of adult internal medicine patients together with a special focus on research and teaching, in an academic setting in which, increasingly, basic and clinical researchers interact. Young residents and senior staff find an ambience that fosters this basic and clinical research interaction. Aside from

research labs in the different departments, we have incorporated new facilities, in collaboration with Universities in Mexico such as the National University of Mexico (UNAM) and the Tecnológico of Monterrey. This includes a research core lab that allows investigators to carry out proteomics, metabolomics and gene sequencing, among other techniques, and a metabolic unit mainly oriented to clinical and basic research on obesity and diabetes mellitus and their complications. Economic and regulatory issues can be daunting barriers for research, and especially for implementation in clinical practice of acquired knowledge. I will be presenting examples of how research at our Institute has had an impact on our clinical practice, and how we have found ways to deliberately enhance this impact through translational practices.

**Letter from Andrew V. Schally (Nobel Prize Laureate,
Department of Veterans Affairs and University of Miami, USA)**

Address to the participants (delegates) to RegPep2018 Symposium in Acapulco:

I deeply regret that I cannot join you in person at this outstanding symposium, but my physicians do not allow me to travel.

A site in Mexico such as beautiful Acapulco deserved to be chosen for this important symposium, in view of the fact that Mexican clinical endocrinologists, primarily Dr. Carlos Gual, were the first to recognize the role and possible clinical importance of hypothalamic peptide hormones and invited me and my collaborators to perform clinical trials in Mexico on TRH and LHRH (GnRH). These investigations on natural and synthetic TRH and LHRH, carried out in Mexico City between 1968 and 1972 and in later years on analogs of LHRH and somatostatin, proceeded all other clinical studies by a wide margin and played a key role in introducing hypothalamic hormones and their analogs into clinical medicine. These pioneering clinical trials established Mexico as an important clinical center in endocrinology. My clinical associates and I made more than 100 visits to Mexico City to carry out endocrine and oncological studies with our Mexican colleagues at the National Institute of Nutrition and the hospitals of Mexican Institute of Social Security (IMSS). In many cases, we were able to spend a weekend of relaxation in Acapulco. More than 100 collaborative publications in US and Mexican journals resulted from these studies. My scientific-medical as well as personal relationship with my Mexican colleagues was truly wonderful over these 30 years of visits to Mexico.

This outstanding symposium has been perfectly organized by Dr. Lee Eiden, Dr. Limei Zhang and others. I wish all the participants/delegates enjoyable and productive participation in scientific and social programs. I hope that you will be as happy in this great country, which I respect and love, as I was in all my preceding visits.

Andrew V. Schally

Miami, August 28th, 2018

Work intended to be presented in RegPep2018:

Hypothalamic hormones; from neuroendocrinology to therapy of cancer and other diseases and conditions

Andrew V. Schally

Nobel Prize Laureate; Veterans Affairs Medical Center Miami, FL, Dept. of Medicine, Dept. of Pathology, and Sylvester Comprehensive Cancer Center, University of Miami Medical School, Miami, FL

About the speaker



Andrzej Viktor ‘Andrew’ Schally is a Polish-born American endocrinologist who received the Nobel Prize in Physiology or Medicine for his pioneering work on the endocrine signaling of the nervous system. He shared the prize with Rosalyn Sussman Yalow and Roger Guillemin. His main study was on the production of peptide hormones that takes place in the brain. His work was centered around the hormones that affect the functions of the pituitary gland such as the ‘thyrotropin-releasing hormone’ or TRH, the ‘gonadotropin-releasing hormone’ or GnRH, also known as the luteinizing hormone-releasing hormone’ or LH-RH and somatostatin. His research helped more studies on reproductive endocrinology and neuro-endocrinology later on and also helped in the study of breast and prostate cancer. He also did a lot of pioneering work in the development of synthetic drugs for the treatment of different types of cancers. He had become interested in medical research at an early age of 23 when he joined the ‘National Institute of Medical Research’ and was influenced in his research by famous scientists like Sir Charles Harington, Dr. D. F. Elliot, Dr. R.R Porter, Dr. A. J. P. Martin and others. Though he joined the institute in a very junior position, his work was appreciated by all the senior scientists. His career in medical research took off from this point onward.

Hypothalamic hormones; from neuroendocrinology to therapy of cancer and other diseases and conditions

Andrew V. Schally

Nobel Prize Laureate; Veterans Affairs Medical Center Miami, FL, Dept. of Medicine, Dept. of Pathology, and Sylvester Comprehensive Cancer Center, University of Miami Medical School, Miami, FL

The discovery, isolation, elucidation of structure, synthesis, and clinical testing of the neuropeptide hypothalamic luteinizing hormone-releasing hormone (LHRH), which regulates reproduction, and of other hypothalamic hormones will be summarized. The synthesis and experimental and clinical testing of agonistic analogs of LHRH will be reviewed focusing on the development of new methods for the treatment of prostate cancer. Subsequent development of antagonistic analogs of LHRH and cytotoxic analogs of LHRH conjugated to doxorubicin will be reviewed, with special emphasis on therapy of prostate cancer. In 1982, we introduced a new hormonal therapy for prostate cancer based of agonists of LH-RH and demonstrated its efficacy in inducing androgen deprivation in men with advanced prostate cancer. Therapy with these agonists of LHRH is still presently the preferred world-wide treatment for men with advanced prostate cancer. Antagonists of Gastrin Releasing Peptide (GRP), which inhibit various tumors, will be also reported.

The synthesis and evaluation of antagonists of growth hormone-releasing hormone (GHRH) for treatment of various cancers will be described. The efficacy of these GHRH antagonists has been demonstrated in models of androgen-independent prostate cancer, pancreatic, colorectal, gastric, renal, and bladder cancer, brain tumors, lung cancer (SCLC and non-SCLC), melanoma and hepatoma. Antitumor effects of GHRH analogs have also been demonstrated *in vivo* in nude mice bearing human breast cancer, including the triple negative variety, and ovarian and endometrial cancer lines, and recently in human acute myeloid leukemia and papillary thyroid cancer. The pathophysiologic basis underlying the response to GHRH antagonist is explained by the presence of GHRH and GHRH receptors in a variety of tumors. Tumor inhibition produced by these GHRH antagonists is associated with suppression in the expression of VEGF, bFGF, pAKT and EGF-/HER receptor family and interference in PKC and MAPK signaling. We determined that GHRH antagonists, LHRH antagonists, and GRP antagonists inhibit Benign Prostatic Hyperplasia (BPH) *in vitro* and *in vivo* and reduce prostate cell volume.

In view of powerful inhibitory action of GHRH antagonists on human cancers, we chose to evaluate the stimulatory and pathophysiologic effects of GHRH agonists. We therefore synthesized new GHRH agonists and tested them. We showed that our earlier GHRH agonists, and new agonists of MR class stimulate cardiac myocytes and accelerate regeneration of the heart in rats and in swine after myocardial infarct. New GHRH agonist MR-409 also stimulated fibroblasts and speeded up wound healing in mice. We demonstrated stimulatory effects of GHRH agonist on pancreatic β -islet cells of rats and diabetic mice before and after transplantation of islets. GHRH agonists exert beneficial effects on diabetes, including an elevation in insulin levels and a lowering of glucose levels. In addition, we are evaluating therapeutic effects of GHRH agonists in eye diseases including diabetic retinopathy. In collaboration, we demonstrated the presence of hypothalamic hormones and their receptors in various structures of the human eye. This may allow the use of peptide analogs for the treatment of eye diseases. Various experimental studies are also continued, on beneficial effects of GHRH antagonists for therapy of Alzheimer's disease.

The overall program has provided extensive new information on the application of hypothalamic peptide analogs for treatment of cancers and other diseases and conditions for which present therapies are inadequate.

Opening lecture (PL1)

The heart of the brain: the hypothalamus and its hormones

Atlantes amphitheater

Gareth Leng

Centre for Discovery in Brain Science, University of Edinburgh, UK

About the speaker



Gareth Leng gained a first class degree in Mathematics from the University of Warwick in 1974, before taking an MSc then a PhD in Physiology from The University of Birmingham. In 1977, he was then recruited by Barry Cross to the Babraham Institute, Cambridge as a project leader in neuroendocrinology. He remained there until 1994, when he was appointed to the Chair of Experimental Physiology at Edinburgh. From 2008 until 2015 he was Head of the School of Biomedical Sciences at the University of Edinburgh, one of the four schools that comprise the College of Medical and Veterinary Sciences at Edinburgh. He has published more than 250 articles and research papers on many aspects of neuroendocrinology, and his work has been cited more than 8,000 times. He is an Honorary member of the British Society for Neuroendocrinology and a Fellow of the Royal Society of Edinburgh, and has served as President of the International Neuroendocrine Federation and as Editor in Chief of the Journal of Neuroendocrinology.

He has published over 250 papers, reviews, book chapters and commentaries, covering diverse aspects of the regulation of vasopressin, oxytocin, growth hormone and appetite regulation. His work has encompassed electrophysiological, neuroanatomical, functional, and behavioural studies and also computational modelling approaches. He has an extensive network of collaborators across the world, including with groups in Japan extending back more than 25 years. In Europe he has been part of large multinational research consortia continuously since 1994, collaborating with neuroendocrinologists in France, Denmark, Sweden, Germany, Spain, Italy, Denmark, the Netherlands and Hungary. He is a member of advisory groups to the Institute of Experimental Medicine in Budapest, the International Center for Biomedical Science at FengHu, China, and the Rowett Institute, Aberdeen

PL1 The Heart of the Brain: the hypothalamus and its hormones

Gareth Leng, Centre for Discovery Brain Sciences, The University of Edinburgh, Edinburgh UK

Most neurons in the hypothalamus make and secrete at least one peptide in addition to a conventional neurotransmitter and other intercellular messengers. Probably the most extensively characterized of these are the oxytocin and vasopressin neurones of the hypothalamus. evolution has been traced back to a single multisensory multifunctional cell type in *Urbilateria*, wormlike marine organisms that are the last common ancestor of vertebrates, flies, and worms. In *Urbilateria*, peptide-secreting cells probably responded to cues from the ancient marine environment. These earliest neurons combined properties that we have thought of as separate properties of endocrine cells and neurons. They used a diversity of signaling mechanisms, made both peptides and neurotransmitters, and were endowed with a wide range of specialized senses. They had not a single role to which they were committed, but multiple behavioral and physiological functions. The neurons of the hypothalamus have retained the multifunctionality of their distant ancestors, and their multitude of sensory abilities (1). Magnocellular oxytocin neurons regulate milk ejection, parturition, and sodium excretion by what they secrete into the blood (2). They also govern reproductive and appetitive behaviors, and these are governed reciprocally, not by the oxytocin that is released into the blood but by oxytocin released from dendrites. They are sensitive to multiple chemical cues from the internal environment—they have receptors for glucocorticoids and gonadal steroids, and for leptin, prolactin, and insulin, as well as for many of the peptides released from the brain itself. But to make sense of how single cell populations can simultaneously regulate diverse functions we have to separate hype from hope (3), sense from nonsense (4), and understand the mechanistic basis of independent regulation of secretion from different neuronal compartments (1, 5).

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3. Leng G, Ludwig M (2016) Intranasal oxytocin: myths and delusions. *BiolPsychiatry* 79:243-50.
4. Leng G, Sabatier N (2016) Measuring oxytocin and vasopressin: bioassays, immunoassays and random numbers. *J Neuroendocrinol*. (10).
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Plenary lecture (PL2)

Harnessing ligand-directed signaling for im(photo)proving opioid receptor therapeutics

Laura Bohn

2018 Victor Mutt Awardee and Lecturer

Supported by Peptides/Elsevier

Department of Molecular Medicine, The Scripps Research Institute,
Jupiter, FL 33458, USA

About the speaker



Laura M. Bohn, Ph.D. is a Professor of Molecular Medicine and Neuroscience at The Scripps Research Institute in Jupiter, FL. She is actively pursuing new therapies for the treatment of pain and addiction by modulating G protein-coupled receptors which are critical to how patients respond to many therapeutics, including opioid analgesics. Dr. Bohn earned degrees in both Chemistry (BA) and Biochemistry (BS) from Virginia Tech and a PhD in Biochemistry and Molecular Biology from St. Louis University School of Medicine. She completed post-doctoral training in the Howard Hughes Medical Institute at the Duke University Medical Center in the laboratory of Dr. Marc Caron in collaboration with Dr. Robert Lefkowitz. Dr. Bohn was a tenured Associate Professor of The Ohio State University College of Medicine in the Departments of Pharmacology and Psychiatry before she joined The Scripps Research Institute in 2009. In recognition of her achievements, she has received Young Investigator Awards from the Society for Neuroscience; the Joseph P. Cochin Award from the College on the Problems of Drug Dependence; and the John J. Abel Award in Pharmacology from the American Society for Pharmacological and Experimental Therapeutics and Pfizer in 2011. Her research program at The Scripps Research Institute is funded by the National Institutes on Drug Abuse.

PL2 Harnessing ligand-directed signaling for im(photo)proving opioid receptor therapeutics

Laura Bohn (2018 Victor Mutt Awardee and Lecturer, Department of Molecular Medicine, The Scripps Research Institute, Jupiter, FL 33458, USA)

Identifying and developing agonists that can drive GPCRs towards engaging with certain signaling pathways over other pathways opens tremendous opportunities for drug development. A major caveat within this approach is that it relies upon the assumption that certain pathways will lead to therapeutically beneficial events while other pathways may lead to adverse effects. The challenge lies in determining first, whether a ligand is truly biasing receptor function in signaling assays and secondly, determining if the bias observed in the cell-based signaling assay is preserved in the endogenous setting. While the first step can be accomplished by establishing mathematical modeling parameters to normalize for assay-dependent variables, the second step relies heavily on the strength of the animal model, the determination of the occupancy of the receptor in the cells that mediates the physiology, and distinguishing between potency, metabolism, bioavailability and relative efficacy in the living system. This presentation will use examples from opioid receptor drug discovery efforts to demonstrate our approach for determining how “biased agonists” can be used to separate distinct physiologies in vivo.

Funding is provided by NIDA 2R01DA031927; 1R01DA038964 and 1R0 DA033073.

S5. Metabolic disorders: central and peripheral mechanisms and therapeutics

Princesa 1

Chair: **Marcia Hiriart** (Mexico City, Mexico)

S5.1 **Harvey Grill** (University of Pennsylvania Perelman School of Medicine, USA):

Treating the hyperphagia driving obesity using centrally acting GLP-R agonists

S5.2 **Inge Depoortere** (University of Leuven, Belgium):

Chemosensory signalling mechanisms of enteroendocrine cells in the gut

S5.3 **Marcia Hiriart** (IFC, UNAM, Mexico):

Insulin resistance: physiology and as part of the metabolic syndrome

S5.4 **Andrew Gundlach** (Melbourne):

Relaxin-3/RXFP3 signaling and neuroendocrine function in extrahypothalamic circuits

S5.1 Treating the hyperphagia driving obesity using centrally acting GLP-1R agonists

Harvey J. Grill

Psychology and Neuroscience, University of Pennsylvania, Philadelphia, PA., USA

Obesity prevalence continues to climb worldwide providing significant challenges to human health. Hyperphagia, the primary driver of this epidemic, results from the activating effects of hedonic sensory features of the food environment on specific, behavior-generating brain circuits. Feeding inhibition results from ingested food triggering GI satiation signals whose effects are conveyed via vagal afferents and processed by n. tractus solitarius (NTS) neurons that are also responsive to leptin and oxytocin. It is interesting to note that GLP-1 released from intestinal cells by food intake excites centrally projecting vagal afferents that in turn excite NTS GLP-1 positive neurons. These NTS neurons send their axons to many anatomically distributed GLP-1R positive neurons resulting in increased brain GLP-1R signaling and feeding inhibition. GLP-1R targeted anti-obesity drug therapy works via brain access engaging endogenous control circuits to reduce feeding and thereby body weight. Among their actions GLP-1R targeting therapies impact neural mediation of hedonic processes involved in food seeking and feeding motivation. GDF-15 is a cytokine whose endogenous levels are elevated in various cancers. GDF-15 acts on the recently discovered GFRAL receptor expressed in NTS and adjacent area postrema and regulates body weight by inhibiting feeding behavior. It remains to be determined which neural circuits and behavioral mechanisms mediate the feeding inhibitory effects of GDF-15. The involvement of a circuit that connects NTS to parabrachial nucleus (PBN) and PBN to central amygdala nucleus will be considered. GDF-15 like compounds are under consideration for obesity while antagonizing its effects in under investigation for anorexia/cachexia treatment. Support from DK-21397

S5.2 Chemosensory signaling mechanisms of enteroendocrine cells in the gut

Inge Depoortere

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

Gustatory processing of the five basic tastes (sweet, salt, umami, bitter and sour) is first achieved through ion channels and G-protein coupled receptors at the level of taste receptor cells clustered in taste buds of the tongue. However, these tastants are also sensed through taste receptors in extra-oral tissues, including the gut. Indeed, enteroendocrine cells in the gut taste the composition of the meal to control the release of gut hormones involved in the regulation of appetite, insulin release and gastrointestinal motility. This will not only result in the assimilation of nutrients but also in the elimination of harmful substances to avoid further food ingestion. Disturbances in this chemosensory signalling mechanisms may contribute to the development or progression of disease. This talk will be focused on the effect and mechanism of action of an aversive taste, bitter, and a pleasant taste, sweet, in the release of the hunger hormone ghrelin. The relevance of targeting extra-oral taste receptors will be put in clinical perspective.

S5.3 Insulin resistance,, physiologic and as part of the metabolic syndrome

Jean Barrera-Sabido, Carmen Sánchez-Soto, Myrian Velasco, Rosa Isela Ortiz-Huidobro, Juan Pablo Chávez and **Marcia Hiriart**

Neuroscience Division, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico

Insulin resistance (IR) is a condition where peripheral tissues do not respond to insulin adequately and result in hyperinsulinism with hyperglycemia. There are two types of IR, one physiological and another that many times is a sign of metabolic syndrome. We have compared both types, in Wistar rats.

We ablated animals on day 20, in the laboratory conditions. We observed in this period physiological insulin resistance, probably associated with a rapid growth period, both in males and females. We also developed a model of metabolic syndrome with 20% sugar in drinking water. After two months rats show central obesity, mild hypertension, triglyceridemia, IR and glucose intolerance, to resume metabolic syndrome. We followed them for six months and understood the cellular mechanisms of hyperinsulinemia and metabolic syndrome that possible leads to exhaustion beta cells in the model of metabolic syndrome.

We have also observed that the extracellular portion of the insulin receptor detaches from the cell membrane in response to hyperinsulinemia by a proteolytic mechanism, leading to a soluble insulin receptor.

We have observed the soluble insulin receptor in Wistar rats, but also in healthy humans and hyperinsulinemic patients. We described that adult animals with metabolic syndrome show higher levels of the soluble insulin receptor. However, in the young animals that are insulin resistant, this mechanism is mild. We characterized two metalloproteases present in the extracellular matrix that could be responsible for the detachment of the receptor in hepatocytes and adipose tissue. We are looking for a specific inhibitor of these proteases for increasing insulin activity.

This work was supported by DGAPA-PAPIIT IN210817, IV-100116, and IN211416 grant and by CONACYT CB-253222

S5.4 Relaxin-3//RXFP3 signalling and neuroendocrine function in extra-hypothalamic circuits

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Complex neural circuits within the hypothalamus govern essential autonomic and neuroendocrine processes, and related complex behaviors, by engaging amino acid, monoamine, peptide, gaseous and lipid transmitters and modulators and various G-protein-coupled receptors (GPCRs) and associated cell-signalling pathways. However, these neural networks are also influenced by extrinsic systems. While the peptide hormone, relaxin, was first discovered in 1926, relaxin-3 is a recently identified neuropeptide (c. 2001), but nonetheless, is highly conserved throughout evolution and is the ancestral member of the relaxin-peptide family. Relaxin-3-expressing GABA neurons in the hindbrain broadly innervate the entire limbic system including the hypothalamus, and relaxin-3 acts via its sole, cognate GPCR, RXFP3. However, exogenous relaxin-3 binds/activates related receptors, RXFP4 and RXFP1, and RXFP3-selective agonist peptides (R3/I5, RXFP3-A2) are preferred for pharmacological studies. Extensive anatomical data in rodents and non-human primates, and regulatory and functional data collected in rats, and wildtype and relaxin-3 or Rxfp3 gene knockout mice, over the last decade, suggest relaxin-3/RXFP3 signalling has a wide range of effects on neuroendocrine systems associated with stress responses, motivation and reward, and social/(sexual) behavior and reproduction [1-3], with some gender-based differences in behavioral responses and associated plasticity of relaxin-3 and RXFP3 expression. Therefore, this presentation will highlight the emerging appreciation of the relaxin-3/ RXFP3 system as an extrinsic regulator of the neuroendocrine axis by briefly reviewing its neuroanatomy and its putative links to arousal-, stress-, and feeding-related behaviors and associated neural substrates and signalling networks. Anatomical studies in rats identified reciprocal interactions between relaxin-3 neurons in nucleus incertus (NI) and CRF, orexin and oxytocin systems, and neurophysiological studies revealed modulation of relaxin-3 neurons by CRF/CRF₁ and orexin/OX₂ signalling. These actions have been associated with the regulation of stress-related alcohol-seeking by studying the effect of CRF₁ and OX₂ antagonist injections into NI, and RXFP3 antagonists on reduction of alcohol-seeking and stress-induced relapse. Relaxin-3/RXFP3 signalling reduces oxytocin and Arg-vasopressin (AVP) neuron activity in magno- and parvocellular PVN neurons of rats, and chronic activation of hypothalamic RXFP3 increases food intake and body weight and reduces hypothalamic oxytocin and AVP expression. Studies are now required to assess the influence of RXFP3 on obesity. Recent studies indicate effects of global RXFP3 manipulation on social interactions in adult male rats and are consistent with a strong co-expression of RXFP3 and oxytocin receptor mRNA observed in social behavior-related areas of the amygdala. These interactions are currently under further investigation. Overall, current experimental, preclinical evidence identifies RXFP3 as a potential therapeutic target for treatment of neuroendocrine disorders and comorbid behavioral dysfunction.

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S6. Presentation of self-peptides in the thymus: An essential event of life

Princesa 2

Chair: **Vincent Geenen** (Liege, Belgium)

- S6.1 **Vincent Geenen** (University of Liege, Belgium):
Historical introduction to the thymus and concept of immune self-tolerance
- S6.2 **Georg Holländer** (University of Basel, Switzerland and University of Oxford, UK):
The thymus: epigenetic control of the molecular mirror of self
- S6.3 **Hiroyuki Takaba** (University of Tokyo, Japan):
Distinct features of Fezf2-induced promiscuous gene expression in the thymus
- S6.4 **Jaime Mas-Oliva** (Instituto de Fisiología Celular, UNAM, Mexico City, Mexico)
Vaccine HB-ATV-8 peptide regulates the metabolism of lipids in the hepatocyte

S6.1 Historical introduction to the thymus and the concept of immune self-tolerance

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The name ‘thymus’ first appeared in Galen’s manuscripts (±160 AD)-named because of its morphological analogy with the leaf of *Thymus cunila*. For centuries this organ was considered to be vestigial, serving as a cushion between the sternum and basal blood vessels, and involuting after puberty. In the late 50’s, JFAP Miller discovered that the thymus was the site for development of a special population of immune cells, the thymo-dependent T lymphocytes. In concordance with P Ehrlich’s early concept of ‘*horror autotoxicus*’, FM Burnet predicted in 1962 that the thymus should play a crucial role in the elimination of developing lymphocytes with potential reactivity against the ‘self’ (‘forbidden’ T cell clones). This theory of intrathymic negative selection of self-reactive T cells was finally demonstrated in late 80’s through the elegant studies in the laboratories of N Le Douarin (Nogent-sur-Marne), P Murrack and J Kappler (Denver), HR Mac Donald (Lausanne) and H von Boehmer (Basel).

At the same time, an important question was raised about the biochemical nature of the ‘self’ expressed in thymic microenvironment. Our laboratory established that thymic epithelial cells (TECs) from different species transcribe prominent neuroendocrine genes (OT for neurohypophysial family, NKA for tachykinins, NT for neuromedins and IGF-2 for insulin family). However, after transcription, neuroendocrine precursors are processed not for classic (neuro)secretion, but for presentation of neuroendocrine self-peptides by proteins of the major histocompatibility complex (MHC) expressed by TECs and thymic dendritic cells. Through this unique process, already during fetal development, the thymus programs central self-tolerance of the adaptive immune system to neuroendocrine functions: an absolute necessity after emergence of this novel form of immunity generating the diversity of antigen recognition some 470 million years ago. The laboratory of the late B Kyewski further demonstrated the central role of the thymus in central immune tolerance to almost all peripheral tissues.

Several laboratories then addressed the logical hypothesis that a defect in the tolerogenic function of the thymus could be a primary event promoting autoimmunity. The question was definitively solved with the identification of the AutoImmuneREgulator gene. *AIRE* controls the level of transcription of many (but not all) tissue specific self-peptides in medullary TECs and *AIRE* mutations (or *Aire* ablation) is associated with the development of autoimmunity tackling many peripheral organs. More recently, the *Fzf2* gene, already known to be involved in neuronal development, was shown to be expressed in TECs and to regulate the transcription level of *Aire*-independent genes also coding for tissue specific self-antigens.

As stated by several authors, the discovery of the central tolerogenic of the thymus revolutionized the whole field of immunology. Undoubtedly, this novel knowledge will pave the way for innovative tolerogenic therapies aiming to prevent and treat autoimmunity, the so heavy tribute paid by all mankind for the extreme diversity and efficiency of adaptive immunity.

Vincent Geenen is research director at F.S.R.-NFSR of Belgium. Supported by F.S.R.-NFSR, Wallonia, the Federation Wallonia-Brussels, European Union, JDRF, and the Fonds Léon Fredericq of Liège University Hospital.

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S6. 2 The thymus: epigenetic control of the molecular mirror of self

Georg A. Holländer

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The thymic microenvironment is unique in its ability to promote the development of naïve T cells with a repertoire purged of vital “Self” specificities and poised to react to injurious “Non-Self”. Thymic epithelial cells (TECs) constitute the major component of the thymic stroma and can be categorized into separate cortical (c) and medullary (m) lineages based on their specific molecular, structural and functional characteristics. cTEC induce the commitment of blood-borne precursor cells to a T cell fate, foster the subsequent maturation and control the positive selection of antigen receptor bearing thymocytes. In contrast, mTEC promote the terminal differentiation of thymocytes which includes the establishment of immunological tolerance to self-antigens via a deletional mechanism and the generation of natural regulatory T cells. In this way, mTEC generate the self-tolerant T cell repertoire in a direct instructive fashion. This essential capacity depends on the mTEC’s promiscuous expression of a large programme of transcripts that encode proteins which are normally only detected in differentiated organs residing in the periphery (a.k.a. tissue restricted self antigens). The molecular regulation of this promiscuous gene expression programme in mature mTEC is in part dependent on the transcriptional AutoImmune REgulator (Aire) and specific epigenetic mechanisms including non-coding RNA and post-translational modifications of histones. Recent insight into the molecular and cellular mechanisms that control mTEC development and the cell’s unique capacity for promiscuous gene expression will be presented and discussed.

S6. 3 Distinct Features of Fezf2-induced Promiscuous Gene Expression in the Thymus

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T cell repertoire selection comprises the positive and negative selection in the thymic cortex and medulla, respectively. A promiscuous expression of a wide array of tissue restricted antigens (TRAs) in the medullary thymic epithelial cells (mTECs) is essential for the negative selection of self-reactive T cells, which is crucial for the establishment of central tolerance. Aire, and more recently identified Fezf2, are key players in inducing this TRA gene expression. Although Aire-dependent TRAs were well characterized so far, Fezf2-dependent TRAs were not thoroughly characterized. Recently, we made a comprehensive list of Fezf2-dependent TRAs by RNA-sequencing analysis using Fezf2-deficient mTECs. Integration of our data and the published next-generation sequencing data revealed several distinct features of Fezf2-dependent TRAs. Interestingly, the number of mTECs expressing Fezf2-dependent genes was higher than that of mTECs expressing Aire-dependent genes. Moreover, Fezf2-dependent TRAs had unique epigenetic features that were clearly different from Aire-dependent TRAs. In this meeting, we would like to show the current findings about TRA expression regulated by Fezf2 and discuss its effects on T cell selection in the thymus.

S6.4 Vaccine HB-ATV-8 peptide regulates the metabolism of lipids in the hepatocyte

Roxana Gutiérrez-Vidal, Blanca Delgado-Coello, Kevin Méndez-Acevedo, Sandra Calixto-Tlacomulco and **Jaime Mas-Oliva**
Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Ciudad de México, México

Background and Aims: Atherosclerosis as an inflammatory disease involved in the etiology of cardiovascular disease worldwide, in our days demands an array of different therapeutic approaches in order to soon be able to visualize its prevention. Based on an immunotherapeutic approach, we designed a non-invasive vaccine (HB-ATV-8) for application in the nasal mucosa, contained in a micellar nanoparticle composed of phosphatidylcholine, lysophosphatidylcholine, lipids from the cell membrane of the archeobacteria *Thermus aquaticus* and a peptide segment (EHLVDFLQSL) derived from the C-terminus of the cholesterol-ester transfer protein (CETP) (1,2). Employing this nanoparticle composition, we have now extended our successful proof of concept from the rabbit to a porcine model (3).

Methods: A preclinical model was developed employing the pig (Large White x Landrace) as the experimental animal. Three groups were studied; a control group fed a standard diet, an experimental group fed a high fat diet (HFD) and a group fed the same HFD treated with vaccine HB-ATV-8 applied nasally every week for up to 7 months. In order to carry out all biochemical and enzymatic analyses, peripheral venous blood was monthly collected. At the end of the experiment, pigs were sacrificed and the thoracic aorta and liver tissue were obtained and examined using optical microscopy to identify atherosclerotic lesions or hepatic damage using conventional histology. Also, two-photon excitation and second harmonic generation microscopy was employed.

Results: The administration of vaccine HB-ATV-8 induced anti-CETP IgG antibodies and reduced atherosclerotic and hepatic lesions induced by the atherogenic diet. In addition, plasma triglyceride levels of vaccine treated pigs fed the HFD were found to be similar to those fed a standard diet, in contrast to the high triglyceride concentration reached with those animals exclusively fed the HFD. **Conclusions.** Vaccine HB-ATV-8 constitutes a promissory preventive treatment useful in the control of atherogenesis (4). The non-invasive characteristics of the vaccine, the simple design employed in its conception and its low production cost, support the novelty of this therapeutic strategy to prevent the process of atherogenesis and control the development of fatty liver disease.

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S7. Neuropeptides in headache, inflammation and neuroinflammatory pain: Basic science to clinical trials

Princesa 3

Chair: **James A. Waschek** (Los Angeles, USA)

- S7.1 James A. Waschek (University of California at Los Angeles, USA):
VIP, PACAP and neuroinflammatory disease
- S7.2 Zsuzsanna Helyes (University of Pécs, Hungary):
Neuropeptide-mediated sensitization mechanisms in models of trigeminovascular activation: focus on PACAP and hemokinin-1
- S7.3 Ichiro Takasaki (University of Toyama, Japan):
Discovery of small-molecule antagonists of PAC1 receptor for the treatment of neuropathic pain
- S7.4 Leon Garcia-Martínez (Alder Biopharmaceutics, Seattle, WA USA):
PACAP inhibition by Alder's ALD1910 antibody represents a potential non-CGRP redundant new opportunity to treat migraine

S7.1 Complexity of PACAP/VIP receptor action in neuroinflammation: Analysis in a mouse model of multiple sclerosis and relationship to migraine..

James A. Waschek, Catalina Abad, Var Tan, Bhavanni Jayaram and Christina Van
University of California at Los Angeles.

PACAP binds to three well-characterized receptors: PAC1, a PACAP-selective receptor, and VPAC1 and VPAC2, which bind PACAP and VIP with equal high affinity. All three receptors are expressed on immune cells and multiple cell types within the brain, including neurons, astrocytes, microglia and oligodendrocytes. Within the context of neuroinflammation, PAC1 receptors are considered to be mainly anti-inflammatory, whereas PAC1 is considered to promote protection and repair. We have examined the actions of these receptors and their ligands in the murine experimental murine autoimmune encephalomyelitis (EAE) model of multiple sclerosis using mice with targeted mutations in the genes encoding these molecules. Consistent with a large body of literature, mice deficient in PACAP and VPAC2 receptors exhibited more severe EAE compared to wild type, with increased numbers of Th1 and Th17 phenotypes, and decreased abundances of Th2 and regulatory T cells. VIP and VPAC1 receptor-deficient (KO) mice also generated an enhanced inflammatory response in the periphery in response to EAE induction. However, the entry of these cells into the brain was impaired and mice were almost completely resistant to EAE. Preliminary studies indicate that PAC1 receptor KO mice develop more severe EAE than wild type mice, but the mechanisms are still under study. These and/or other less characterized receptors may be involved in PACAP actions in migraine headache. Whatever the case, a more precise knowledge of specific PACAP and/or VIP receptor action on immune and neural cells will be important in the development of therapeutic approaches for migraine based on PACAP/VIP signaling.

S7.2 Neuropeptide-mediated sensitization mechanisms in models of trigeminovascular activation:: focus on PACAP and hemokinin-1

Zsuzsanna Helves^{1,2}, Éva Szőke^{1,2}, József Kun^{1,2}, Tímea Aczél^{1,2}, Maja Mayrits^{1,2}, Tibor Rauch⁴, Sini Junttilla⁵, Attila Gyenesi^{2,5}, Adrienn Markovics^{1,2}, Viktória Kormos^{1,2}, Balázs Gaszner³, Sándor Farkas^{1,2}, Dóra Reglődi³ and Kata Bölcskei^{1,2}

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Headache disorders including migraine are among the most debilitating pain conditions, which are still unmet medical needs. Their pathophysiology is diverse, but sensitization of trigeminal primary afferents is a common mechanism. Pituitary adenylate cyclase-activating polypeptide (PACAP) and its receptors (PAC1, VPAC) are present in primary sensory neurons of the trigeminal ganglia (TRG) and vascular smooth muscle. PACAP infusion triggers migraine-like headache in humans and exerts central pro-nociceptive functions, but the mechanisms and target remain unknown. The other excitatory peptide, the tachykinin hemokinin-1 (HK-1) has substance P (SP)-like structure, but distinct actions independent of the NK1 tachykinin receptor.

Gene expression changes were analyzed in the TRG, trigeminal nucleus caudalis (TNC) and peripheral blood mononuclear cells (PBMC) evoked by Complete Freund's Adjuvant (CFA)-induced orofacial inflammation in rats. Microarray analysis revealed 512 differentially expressed genes between the ipsi- and contralateral TRGs 7 days later. Time-dependent upregulation of PACAP and HK-1, changes of G-protein coupled receptor 39, kisspeptin-1 receptor, kisspeptin, as well as synaptic plasticity-associated *Lkaear1*, *Neurod2* mRNA were detected. The greatest alterations were observed on day 3 ipsilaterally, when orofacial mechanical allodynia reached its maximum. This corresponded well with patterns of neuronal (*Fosb*), microglia (*Iba1*), and astrocyte (*Gfap*) markers in both TRG and TNC, and in PBMCs.

The effects of PACAP, related peptides and fragments, and HK-1 were investigated on primary TRG cultures by Ca-imaging. Slowly increasing $[Ca^{2+}]_i$ was detected after PACAP1-38, PACAP1-27, vasoactive intestinal polypeptide (VIP) and the selective PAC1 receptor agonist maxadilan, but interestingly, PACAP6-38, VIP6-28 and the PAC1 receptor antagonist M65 also caused similar activation. The VPAC2 receptor agonist induced similar activation, while the VPAC1 receptor agonist had no effect. PACAP6-38, M65 and VIP6-28 described as antagonists in several models acted as agonists on primary sensory neurons. HK-1, but not SP also caused slow $[Ca^{2+}]_i$ in TRG neurons of wildtype and NK1-deleted mice. Nitroglycerol (NTG)-induced trigeminovascular activation was investigated using PACAP gene-deleted (PACAP^{-/-}) mice. Photophobia both in the early and late phases due to direct vasodilation and trigeminal sensitization, respectively, meningeal blood flow, and the number of c-Fos expressing cells referring to neuronal activation in the TRG and TNC were significantly reduced in PACAP^{-/-} mice.

This is the first description of orofacial inflammation-induced transcriptional alterations including PACAP and HK-1 upregulation both in primary and secondary sensory neurons of the trigeminovascular system and also in the PBMCs similarly to the neuronal changes. We provide the first evidence for HK-1-induced activation independently of the NK1 receptor, as well as for PACAP being a pivotal mediator of activation/sensitization and meningeal vasodilation related to migraine presumably via an unknown receptor or PAC1 splice variant linked to distinct signal transduction pathways.

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S7.3 Discovery of small-molecule antagonists of PAC1 receptor for the treatment of neuropathic pain

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¹ Graduate School of Science and Engineering, University of Toyama, Japan. ² Graduate School of Innovative Life Science, University of Toyama, Japan. ³ Graduate School of Medical and Dental Sciences, Kagoshima University, Japan. ⁴ School of Pharmacy, Showa University, Japan.

Previously, we found that intrathecal injection of PACAP or maxadilan, a selective PAC1 receptor agonist, induces long-lasting mechanical allodynia in mice, suggesting that PAC1 receptor is involved in chronic pain and that the selective PAC1 antagonist may become a new analgesic. Although several PAC1 antagonists such as PACAP 6-38 have been reported, all of them are peptide compounds. Here we identified new small-molecule antagonists of the PAC1 receptor by computer-based *in silico* screening and *in vitro/vivo* pharmacological assays ⁽¹⁾.

The identified small-molecule compounds, named PA-8 and PA-9, dose-dependently inhibited the phosphorylation of CREB and cAMP elevation induced by PACAP in PAC1-, but not VPAC₁- or VPAC₂-receptor-expressing CHO cells. *In vivo* pharmacological assays showed that i.t. injection of these compounds blocked the induction of PACAP-induced aversive responses and mechanical allodynia in mice. In contrast, the compounds when administered alone exerted neither agonistic nor analgesic actions in the *in vitro/vivo* assays. Systemic administration of PA-8 exerted analgesic effects in some mouse models of pain, such as spinal nerve ligation-induced neuropathic pain model, chemotherapy-induced peripheral neuropathy, and nitroglycerin-induced migraine-like pain model.

The compounds we found in this study are new small-molecule antagonists of the PAC1 receptor and may become new analgesics in the treatment of various types of pain.

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S7.4 PACAP inhibition by Alder's ALD1910 antibody represents a potential non-CGRP redundant new opportunity to treat migraine

Leon Garcia-Martínez

Alder Biopharmaceutics, Seattle, WA USA

Migraine is a highly prevalent neurological disorder that poses a substantial socio-economic, personal and individual burden. Current approved abortive and therapeutic treatment options do not provide the desired outcome for most patients. Recently, anti-CGRP antibodies (such as eptinezumab) have emerged as novel therapeutic agents that, in clinical trials, provide improved pharmacological control of migraines. The clinical development of these antibodies has also highlighted the potential for multiple etiologies for migraines since not all migraineurs respond to anti-CGRP therapies. The pituitary adenylate cyclase-activating polypeptide (PACAP) has been identified as a potential new target for treatment of migraines. PACAP plasma levels are elevated during migraine attacks, its infusion in migraineurs can lead to a migraine, and PACAP-driven migraines are associated with symptoms such as nausea, photophobia, and phonophobia. ALD1910 is an anti-PACAP antibody that effectively antagonizes PACAP38 signaling in-vitro, it is pharmacologically active in vivo, and represents an attractive potential therapeutic modality for migraine prevention. A summary of preclinical data of ALD1910 will be presented along with data supporting non-redundancy between CGRP and PACAP driven biology in preclinical models.

S8. Interaction of hypothalamic peptidergic circuits in the organization of physiology and behavior

Princesa 4

Chair: **Ruud M. Buijs** (Mexico City, Mexico)

S8.1 **Ruud Buijs** (IIB, UNAM, Mexico):

Interaction between suprachiasmatic and arcuate nuclei is essential for temperature and corticosterone rhythm, roles for vasopressin and alpha-MSH

S8.2 **Charles Bourque** (McGill University Health Centre, Montreal, Canada):

Suprachiasmatic nucleus vasopressin neurons and the circadian control of fluid homeostasis

S8.2 **Valerie Simonneaux** (INCI-CNRS, Strasbourg, France):

Circuits of kisspeptin and RFRP3 in the seasonal control of reproduction and metabolism

S8.3 **Pawel K. Olszewski** (University of Waikato, Hamilton, New Zealand):

Oxytocin as a potential pharmacological tool to curb overeating

S8.1 Interaction between suprachiasmatic and arcuate nucleus is essential for temperature and corticosterone rhythm, a role for vasopressin and α MSH

Ruud M Buijs, Luis León-Mercado, Frederik N Buijs, Mara Guzmán-Ruiz
Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico

The suprachiasmatic nucleus (SCN) is generally considered the master clock, independently driving all circadian rhythms. However micro cuts effectively removing SCN-arcuate nucleus (ARC) interconnectivity in Wistar rats result in a loss of rhythmicity in locomotor activity, corticosterone levels, and body temperature (Tb) in constant dark (DD) conditions. We will show that for the expression of physiological rhythms this reciprocal SCN-ARC interaction is essential. For example, while normally i.v. glucose infusions induce changes in neuronal activity of the SCN in disconnected animals, SCN c-Fos expression is unaltered demonstrating the importance of the ARC as a metabolic modulator of SCN neuronal activity. Consequently the SCN is more than an autonomous clock, it forms an essential component of a larger network controlling homeostasis. This is illustrated by further investigating the role of SCN-ARC interaction in the control of body temperature. In mammals, daily changes in body temperature (Tb) depend on the integrity of the SCN. Fasting strongly decreases Tb in the resting period which is dependent on the SCN. However, the origin of this circadian/metabolic influence is unknown. We will show that, not only the SCN but also ARC, are involved in the Tb setting through afferents to the thermoregulatory median preoptic nucleus (MnPO). Vasopressin release from the SCN decreases the temperature just before light onset, whereas α MSH release, especially at the end of the dark period, maintains high temperature. Both peptides have opposite effects on the brown adipose tissue activity through thermoregulatory nuclei such as the dorsomedial nucleus of the hypothalamus and the dorsal raphe nucleus. The present study indicates that the coordination between circadian and metabolic signaling within the hypothalamus is essential for an adequate temperature control.

S8.2 Suprachiasmatic nucleus vasopressin neurons and the circadian control of fluid homeostasis

Claire Gizowski, Cristian Zaelzer, Eric Trudel and **Charles W. Bourque**
Centre for Research in Neuroscience and McGill University Health Centre, Montreal, Canada

Acute changes in extracellular fluid osmolality (ECF, total solute concentration) can provoke neurological symptoms due to shrinking or swelling of the brain within a rigid cranium. Consequently, mammals have evolved sophisticated behavioral and physiological mechanisms that oppose perturbations in ECF osmolality via negative feedback. For example, ECF hyperosmolality increases the perception of thirst to motivate water intake, and causes release of vasopressin (VP, antidiuretic hormone) from the neurohypophysis to promote water reabsorption by the kidney. Conversely, ECF hypotonicity suppresses thirst and VP secretion to cause a net loss of body water and restore isotonicity. These negative feedback responses are triggered by when changes in ECF osmolality are detected by osmoreceptor neurons in the organum vasculosum lamina terminalis (OVLT) which project to thirst promoting centers and VP-releasing neurons in the hypothalamus. Osmoreceptor neurons show changes in firing rate that vary in proportion with fluid osmolality and cause similar changes in electrical activity in homeostatic effector neurons via the release of glutamate.

Studies in our laboratory have revealed that neurons in the suprachiasmatic nucleus (SCN) can promote feed forward changes in thirst (1, 2) and VP release (3) that anticipate physiological requirements for fluid conservation associated with the absence of fluid intake during sleep. Notably, clock neurons mediate such effects via postsynaptic modulation of firing by Thirst-promoting neurons, and via circadian modulation of synaptic transmission between osmoreceptor and baroreceptor inputs to VP neurons. In both cases, the effects appear to be mediated by the activity dependent release of VP from the axon terminals of SCN neurons (4). Here, Dr. Bourque will review and update recent findings which clarify how the electrical activity of SCN clock neurons generates adaptive changes in thirst or VP release. Supported by the Canadian Institutes of Health Research.

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S8.3 Circuits of kisspeptin and RFRP3 in the seasonal control of reproduction and metabolism

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Animals living in the wild experience marked seasonal changes in their environment. To overcome these environmental challenges, thus increasing their chances of survival, they display seasonal changes in several aspects of their physiology, such as reproduction, metabolism, and behavior. Most species use the annual change in day length (photoperiod) to time their physiology to the up-coming seasons. In mammals, the annual change in photoperiod is converted into seasonal change in the nighttime secretion of melatonin from the pineal gland. Recent studies have demonstrated that this endocrine signal is decoded at the *pars tuberalis* into seasonal changes in the production of thyroid stimulating hormone (TSH) which further acts on the tanocytes to control thyroid hormone (T3) concentration in the basal hypothalamus, so that T3 is higher in summer long days as compared to winter short days. One of the current challenges is to unveil how seasonal changes in hypothalamic T3 synchronize biological functions, notably reproduction and metabolism. Here we will show evidence indicating that two hypothalamic RFamide peptides, kisspeptin and RFRP-3, may link seasonal cues to the hypothalamic networks controlling reproduction and metabolism. Indeed, both peptides display marked seasonal changes in expression, although with species-specific differences, and both peptides are able to regulate reproductive and metabolic parameters. Finally we will discuss the hypothesis that kisspeptin and RFRP-3 may be used to connect reproduction and metabolism in a seasonal context.

S8.4 Oxytocin as a potential pharmacological tool to curb overeating

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The past three decades of extensive research have defined the neuropeptide oxytocin (OT) as a potent anorexigenic molecule. It has been shown that the release of OT and activation of hypothalamic OT neurons coincide with meal termination. Plasma hyperosmolality and excessive stomach distension—typically arising from avid consumption of large food loads—also result in OT secretion. Central and peripheral administration of OT decreases meal size in deprived animals as well as in free-feeding individuals injected just prior to the onset of the nocturnal feeding phase. Reduced food consumption has been shown after OT treatment in humans. Importantly, OT appears to be particularly effective in reducing consumption of carbohydrates and sweet tastants. Yet, despite these promising basic research data, attempts to use OT in the clinical setting to combat obesity and overeating have generated somewhat mixed results. Here, we present studies that shed light on the nature of the limitations as well as on the benefits of OT as a therapeutic molecule. We focus on findings in humans and laboratory animals that delineate effectiveness of OT in decreasing a drive to eat in the hunger-satiation continuum. We also point to a relationship between the magnitude of OT's anorexigenic action and the composition of ingested diet, its energy density, and the current (patho)physiological state of an individual.

S9. Gut peptide: physiology and metabolic syndrome

Princesa 1

Chair: **Duan Chen** (Trondheim, Norway)

- S9.1 **Chun-Mei Zhao** (Norwegian University of Science and Technology, Norway):
Functional morphology of peptide hormone-producing ECL cells and A-like cells in stomach
- S9.2 **Markus Heimesaat** (Charité - Universitätsmedizin Berlin
Institut für Mikrobiologie und Infektionsimmunologie, Berlin, Germany):
Pituitary Adenyl Cyclase-Activating Polypeptide – a neuropeptide as novel treatment option for intestinal inflammation. Lessons learnt from murine gut inflammation models
- S9.3 **Duan Chen** (Norwegian University of Science and Technology, Norway):
Brain-gut axis: Intragastric injection of botulinum neurotoxin A as a weight-loss treatment for obesity and rescue treatment for weight regain following sleeve gastrectomy. Botulinum toxin A treatment for obesity

S9.1 Functional morphology of peptide hormone-producing ECL cells and A-like cells in stomach

Chun-Mei Zhao

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The term enterochromaffin-like cells was introduced to describe endocrine, non-enterochromaffin cells in the gut epithelium in the late 60s. Subsequently, it was shown by electron microscopy that enterochromaffin-like cells comprise many different cell populations, e.g. ECL cells and A-like cells in the stomach.

The ECL cells can be defined as a small, irregularly shaped endocrine cell present in large numbers basally in the oxyntic mucosa, displaying typical ultrastructure, usually with numerous large electron-lucent cytoplasmic vesicles, a few electron-dense granules and a few macrovesicles. The granules and secretory vesicles form part of a secretory pathway. The process of exocytosis is coupled with endocytosis, resulting in the formation of endocytotic microvesicles. When the ECL cells are excessively stimulated, a crinophagic pathway takes place, which is manifested by formation of both vacuoles and lipofuscin bodies. The functional significance of the ECL cells can be expected to reflect the nature/bioactivity of the secreted products. The ECL cells are known to produce histamine and respond to gastrin with activation of histamine synthesis and release. The gastrin-ECL cell-parietal cell axis is one of the pathways in regulation of gastric acid secretion. The ECL cells also contain chromogranin A-derived peptides (e.g. pancreastatin) and other peptide hormones (e.g. the blood calcium-lowering peptide *Pthlh*).

The term A-like cells derives from the fact that the ultrastructure appearance of the secretory granules is reminiscent of that of the pancreatic glucagon (A) cell granules. The granular organelles develop from the trans-Golgi network and are loaded with cargo (e.g., PC1/3, chromogranin A, and preproghrelin). As a result of protein condensation, the granules become highly electron dense. When the granules embark upon their journey toward the periphery of the cell, they start to take up amine from the cytosol by means of VMAT1 or VMAT2 located in the granule membrane. The continued accumulation of amine is associated with the formation of a halo between the dense core and granule membrane. As a consequence of the stimulation of the cell, these secretory granules fuse with the cell membrane and ultimately release ghrelin by exocytosis.

S9.2 Pituitary Adenylate Cyclase-Activating Polypeptide – a neuropeptide as novel treatment option for intestinal inflammation. Lessons learnt from murine gut inflammation models

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The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) plays pivotal roles in immunity and inflammation. Data regarding anti-inflammatory properties of PACAP within the intestinal tract are scarce, however. We therefore addressed whether PACAP treatment could alleviate experimental acute and/or subacute small intestinal inflammation. To address this, mice were perorally infected with different cyst numbers of the parasite *Toxoplasma gondii* to induce ileitis. Mice were then either prophylactically or therapeutically treated with synthetic PACAP38 (1.5 mg per kg body weight per day) or placebo (PLC) once daily via the intraperitoneal route. Whereas placebo treated mice suffered from full-blown disease, intestinal immunopathology was ameliorated following PACAP application in a time-of-treatment dependent manner. Remarkably, PACAP-mediated anti-inflammatory effects were not restricted to the intestinal tract, but could also be observed in extra-intestinal compartments such as liver, lungs and kidneys and, strikingly, also systemically as indicated by reduced pro-inflammatory mediator secretion in spleen and serum as compared to placebo controls. In summary, synthetic PACAP ameliorated small intestinal inflammation by down-regulating Th1-type immunopathology, reducing oxidative stress and up-regulating anti-inflammatory cytokine responses. We conclude that synthetic PACAP might open novel treatment options for intestinal inflammation including inflammatory bowel diseases.

Key Words: Pituitary adenylate cyclase-activating polypeptide (PACAP), *Toxoplasma gondii*, experimental ileitis, Th1-type immunopathology, anti-inflammatory effects, gut-brain axis

S9.3 Intra-gastric injection of botulinum neurotoxin A as a weight-loss treatment for obesity and rescue treatment for weight regain following sleeve gastrectomy. Botulinum toxin A treatment for obesity

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Previously we reported that the mechanism of action of "vagal blocking therapy for obesity (VBLOC)" might involve leptin and CCKb receptors, interleukin-1 β , tumor necrosis factor, and transforming growth factor β 1 in the brainstem and NPY, AgRP, and Foxa2 in the hypothalamus (1). VBLOC is induced by an electrical device surgically implanted around gastric vagal trunks. In the present study, we applied Botulinum neurotoxin A (BtxA) injection to block the vagal nerve terminals in order to develop a new treatment for obesity. High-fat diet-induced obese (DIO) rats (male Sprague-Dawley) were used. BtxA was injected into the gastric wall in the region of the antrum in rats. No sign of gastroparesis was observed. In response to BtxA treatment (injected 6 weeks apart), DIO rats had body weight loss, i.e., 24% and 33% reduction after 1st and 2nd injection, respectively. This was associated with decreased food intake and increased energy expenditure (measured by CLASM). Gene expression in the brain and gut hormones were analyzed by *in situ* hybridization, tagman array gene analysis and radioimmunoassay. At 48h post injection of BtxA, expression of energy balance-linked genes in hypothalamus and brainstem, and concentrations of gut hormones were unchanged. However, 8 weeks after BtxA, hypothalamic gene expression for NPY and AgRP was increased, whereas POMC was decreased, and plasma concentrations of CCK, gastrin, and PYY were reduced. Furthermore, we wanted to study whether BtxA treatment could be used as rescue treatment for sleeve gastrectomy (SG), as SG is a commonly used bariatric surgery but with a high rate of body weight regain (75.6% at 6 years)(2). DIO rats underwent SG and, 6 weeks later, received BtxA treatment. During the first 6 weeks, DIO rats with SG had reduced body weight and food intake by 10% and 20%, respectively. After receiving BtxA treatment, the body weight and food intake were reduced by 25% and 31% at 10 weeks, respectively. We then analyzed the hypothalamus, dorsal vagal complex of the brainstem and cingulate cortex for expression of 48 genes, including those directly linked to energy balance control, inflammation, and dopamine, GABA and glutamate signaling, and did not detect any changes in the gene expression with the exception of POMC which was increased. In conclusion, intra-gastric injection of BtxA can be a new weight-loss treatment, probably acting by blocking the vagal nerve function in the brain-gut axis (resulting in compensatorily increased NPY and reduced POMC in the hypothalamus). Given that POMC is an anorexigenic peptide, its elevation in response to the combined SG and BtxA could be important for the weight loss outcome.

1: Johannessen H, et al. *Obes Surg.* 2017;27:177-85

2: Lauti M, et al. *Obes Surg.* 2016 ;26 :1326-34

S10. Peptides and their receptors as oncotargets

Princesa 2

Chair: **Terry Moody** (Bethesda, USA)

S10.1 **Gabriela Cesarman** (National Institute of Cancer Research, CDMX, Mexico):

The interplay between cancer, its microenvironment, and the coagulation system: biomarkers and regulation by peptides

S10.2 **Terry Moody** (National Cancer Institute, NIH, USA):

Bombesin receptors regulate transactivation of receptor tyrosine kinases in lung cancer

S10.3 **Matthew Thakur** (Thomas Jefferson University, USA):

Imaging of prostate cancer using VIP/PACAP analogs

S10.1 The interplay between cancer, it's microenvironment, and the coagulation system: biomarkers and regulation by peptides

Gabriela Cesarman

National Institute of Cancer Research, México

Biomarkers for cancer can be direct or indirect; can contribute via imaging to identification of tumor burden and location; can provide information about tumor stage and type, guiding prognosis and therapy; and can become themselves targets for therapeutic intervention, in some cases even when within tumor stroma or helper cells rather than the transformed cells or cell masses themselves. A sometimes overlooked function of biomarkers is the monitoring of physiological support by other tissues and effects of chemotherapy upon them, as well as assessment of availability of tumor via its existing vasculature. Here, I will provide some specific examples of coagulation-dependent of tumor progression and the role of proteins such as tissue factor as biomarkers and promoters of the malignant phenotype. We will discuss the potential role of peptides and protein fragments or specific antibodies that target coagulation. Personalization of biomarker technology can also provide support for corresponding personalized medical treatment plans for individual patients. In many cases, biomarkers evolve from a single role in cancer treatment to multiple ones, including as potential oncotargets themselves.

S10.2 Bombesin--like peptides cause transactivation of the EGFR and HER2 in lung cancer cells

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Mammalian bombesin (BB)-like peptides include gastrin releasing peptide (GRP) and neuromedin (NM) B. GRP and BB bind with high affinity to the BB₂ receptor (R) whereas NMB binds with high affinity to the BB₁R. Bombesin receptor subtype (BRS) 3 is an orphan receptor which does not bind GRP, BB or NMB with high affinity. The BB₁R, BB₂R and BRS3 are type 1 GPCRs which interact with G_q and cause phosphatidyl inositol (PI) turnover. GRP and NMB function as autocrine growth factors in lung cancer cells stimulating lung cancer proliferation whereas the antagonists PD168368 and PD176252 inhibit growth. Some of the growth effects regulated by the B₁R and BB₂R result from transactivation of receptor tyrosine kinases (RTK). The epidermal growth factor (EGF) R and HER2 are overexpressed in many lung cancer cells. Addition of GRP or NMB to lung cancer cells increased tyrosine phosphorylation of the EGFR (Tyr¹⁰⁶⁸) 4-fold and HER2 (Tyr¹²⁴⁸) 3-fold. GRP or NMB increased release of transforming growth factor α , which binds with high affinity to the EGFR, however, no ligand is known for HER2. Gefitinib, an EGFR tyrosine kinase inhibitor (TKI), blocked the increase in EGFR and HER2 tyrosine phosphorylation caused by GRP or NMB. The formation of EGFR homodimers as well as EGFR/HER2 heterodimers caused by NMB addition to lung cancer cells was blocked by PD168368. The ability of NMB to increase tyrosine phosphorylation of the EGFR or HER2 was impaired by gefitinib, lapatinib (EGFR and HER2 TKI), N-acetylcysteine (ROS inhibitor), DPI (Nox and Duox inhibitor), PP2 (Src inhibitor) and GM6001 (MMP inhibitor). PD168368 inhibited the proliferation of lung cancer cells and potentiated the cytotoxicity of gefitinib. The results indicate that the BB₁R and BB₂R regulate the formation of EGFR homodimers and EGFR-HER2 heterodimers in lung cancer cells.

S10.2 Imaging Prostate cancer using VIP/PACAP analogs

Mathew Thakur

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Prostate cancer (PCa) is the most common non-skin cancer in men. The risk of having prostate cancer in men older than sixty years of age is estimated to be 17%. As the life span continues to increase, it is estimated that, in 2020, there will be one million new cases of PCa in the USA alone

For early and accurate diagnosis of PCa, histologic examination which requires prostate tissue extraction, remains a gold standard. Not only is the prostate tissue extraction an invasive and a morbid procedure but in more than 66% of the times, it also finds benign pathology at the expense of millions of healthcare dollars.

Increased understanding of diseases at molecular and cellular levels has paved the way for the development of innovative approaches for diagnosis and therapy of PCa. Our approach consists of targeting VPAC receptors known to express in high density on PCa cells. We have designed, synthesized and validated a 27 amino acid VIP/PACAP analogue that has a high affinity ($K_d = 3.1 \times 10^{-9}$ M) for VPAC. When labeled with a radionuclide Cu-64, and administered intravenously the biomolecule (Cu-64-TP3805) allows us to detect pCa (N=44) and its metastatic bone lesions and malignant lymph nodes non-invasively with >95% accuracy. Furthermore the biomolecule when labeled with a near infrared fluorophore (NIR) allows us to detect shed prostate cancer cells in voided urine (N= ~300), also with > 98 % sensitivity.

Data, although limited, suggest that targeting VPAC receptors for early and accurate imaging of PCa noninvasively, is a highly promising approach which may minimize a number of unnecessary biopsies and thereby reduce patient morbidity and contain health care cost.

I thank NIH, my colleagues and those who have contributed so much to the Regulatory Peptides which have made this work possible.

S11. Symposium with contributed talks

Princesa 3

Chair: **Laura Vivas** (Cordoba, Argentina)

S11.1 **Sigal Fleisher-Berkovich** (Ben-Gurion University of the Negev, Beer-Sheva, Israel):

Angiotensin Converting Enzyme Inhibitors Ameliorate Brain Inflammation: Possible Implications for Alzheimer's Disease

S11.2 **Andrea Godino** (INIMEC-CONICET-Universidad Nacional de Córdoba, Argentina):

TRPV1 osmosensitive channel involvement in the control of sodium appetite

S11.3 **Patricia Lagos** (Universidad de la República, Montevideo, Uruguay)

In vivo and ex vivo studies of the internalization of melanin-concentrating hormone conjugated with rhodamine in hippocampal neurons.

S11.4 **Luz Torner** (Centro de Investigación Biomédica de Michoacán, Instituto Mexicano Del Seguro Social, Morelia, México)

Regulatory role of prolactin on the neuroimmune system of the hippocampus of male rat pups

S11.5 **Ai-Min Bao** (Zhejiang University, Hangzhou, China):

The stress systems in mood disorders: a postmortem study

S11.6 **Fabien Plisson** (CINVESTAV IPN, Irapuato, Mexico)

Constrained GLP-1 mimetics with incretin-like properties

S11.1 Angiotensin converting enzyme inhibitors ameliorate brain inflammation: possible implications for Alzheimer's disease

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The renin-angiotensin system (RAS) is an important peripheral system involved in homeostasis modulation, with angiotensin II (Ang II) serving as the main effector hormone. The main enzyme involved in Ang II formation is angiotensin-converting enzyme (ACE). ACE inhibitors (ACEIs) such as captopril are predominantly used for the management of hypertension. All of the components of the RAS have also been identified in brain. Centrally located hormones such as Ang II can induce glial inflammation. Moreover, in Alzheimer's disease (AD) models, where glial inflammation occurs and is thought to contribute to the propagation of the disease, increased levels of Ang II and ACE have been detected. However, the specific effects of captopril on glial inflammation and AD remain obscure. In the present study, we investigated the effect of captopril, given at a wide concentration range, on inflammatory mediators released by lipopolysaccharide (LPS)-induced glia. In the current study, both primary glial cells and the BV2 microglial cell line were used. Captopril decreased LPS-induced nitric oxide release from primary mixed glial cells as well as regulating inducible nitric oxide synthase expression, nitric oxide, tumor necrosis factor- α and interleukin-10 production by BV2 microglia. We further obtained data regarding intranasal effects of captopril on cortical amyloid β (A β) and CD11b expression in 5XFAD cortex over three different times. Interestingly, we noted decreases in A β in captopril-treated mice over time which was paralleled by increased microglial activation. Reduced A β deposition, alongside microglial activation by angiotensin-related drugs, had already been shown to potentially improve cognitive performance in AD mice. These results thus shed light on the neuroprotective role of captopril in AD which might be related to modulation of microglial activation.

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S11.2 TRPV1 osmosensitive channel involvement in the control of sodium appetite

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Changes in body water/sodium balance are tightly controlled by central and peripheral osmo-sodium receptors among others, which trigger the activity of a central network to release vasopressin and/or aldosterone, increase renal sympathetic nerve activity, and induce thirst and sodium appetite. The main central osmoreceptive neurons are in the circumventricular organs of the lamina terminalis, i.e. the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO) and along the hypothalamic magnocellular cells. It has been postulated that their intrinsic osmosensitivity is mediated in part by different types of channels, TRPV1, TRPV4, NaX and EnaC. The function of TRPV1 channel has been previously analyzed using knockout (KO) TRPV1 mice; however its functional role after different types of hyperosmotic thirst remains controversial (Ciura and Bourque 2006; Kinsman et al., 2014). The TRPV1 channel involvement in the control of sodium appetite (SA) induced by sodium depletion (SD) has not been yet analyzed. The aim of the present work was to explore the TRPV1 channel involvement in the genesis of the SA that prompts a new role for TRPV1 channel. We used the TRPV1 KO mouse model to analyze basal and induced SA by SD. We studied the renal responses and the TRPV4, NaX and angiotensin type 1 receptor (AT1a) RNAm expression within the SFO and OVLT. We also recorded sodium and water intake and the renal excretion at 2 h and 24 h after SD induced by furosemide (50mg/kg) in combination with low sodium diet in wild type (WT) and KO mice.

After SD, the KO animals showed an increase in the sodium preference ($F=8.49$; $p=0.006$) and consumed a higher hypertonic cocktail ($F=8.49$; $p=0.0059$) in relation to WT animals, independent of the time after SD. These data suggest that KO animals, when stimulated to drink water and sodium, make a hypertonic cocktail instead of the isotonic one usually made by the control animals. The urinary volume ($F=5.45$; $p=0.0003$) and sodium excretion ($F=3.99$; $p=0.028$) induced by Furosemide at 30 minutes were both reduced in KO animals in comparison to WT. There was no change in plasma osmolality between the groups 2 h and 24 h after SD. Interestingly, 2 hs after SD, the WT animals, showed an increased expression of TRPV4 channels along the SFO and in comparison to KO mice ($F = 5.11$, $p < 0.05$).

In sum, these data suggest that TRPV1 KO animals have a differential renal and behavioral response after sodium depletion. Besides, in contrast to WT animals, they did not show any changes in the SFO TRPV4 expression early after sodium depletion. In conclusion our results suggest that the TRPV1 channels are involved in the osmoregulatory processes after an acute body sodium depletion possibly to modulate renal and intake responses

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S11.3 In vivo and ex vivo studies of the internalization of melanin-concentrating hormone conjugated with rhodamine in hippocampal neurons

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Introduction. Melanin-concentrating hormone (MCH) is a 19-amino acid neuropeptide synthesized in neurons localized principally in the hypothalamus in mammals. Several lines of evidence suggest a role of MCH in learning and memory (Monzon et al., 1999; Adamantidis et al., 2005). The hippocampus, a structure important to such functions, is densely innervated with MCHergic fibers and has a dense distribution of MCHR-1 mRNA. MCHR-1 is one of the two G-protein coupled receptors (GPCR) for MCH, and the only functional receptor expressed in rats (Bittencourt et al., 1992). A clear cellular localization of MCHR-1 protein in hippocampal cells has not yet been described.

Objective. The aim of this work was to provide evidence for specific MCH-receptive cells in the hippocampus of the rat, employing immunohistochemical detection of MCH conjugated with rhodamine (MCH-ROD), in *in vivo* and *ex vivo* experiments. We based our experimental approach on previous finding with several other peptidergic GPCRs: that ligand activation of the receptor causes intracellular internalization of the ligand-receptor complex, probably in a clathrin-dependent manner.

Methods and Results. Adult male rats were intracerebroventricullarly injected with MCH-ROD or rhodamine as control. Twenty (T20) and sixty (T60) minutes later, the brains were processed to perform immunofluorescence anti-NeuN, GFAP, GAD-67 and MCH in hippocampus. At T20, another set of animals was pre-treated with ATC-0175 (MCHR-1 antagonist), or phenilarsine oxide (PAO, a clathrin-dependent-endocytosis inhibitor). In these experiments, more hippocampal cells internalize MCH-ROD at T20 than at T60 (17±2 MCH-ROD (+) cells vs 14±1; mean ± SEM, respectively), in a 0.3 mm² of area analyzed in CA1 and CA2 regions (n=8 rats/group; 8 coronal sections/rat). Rhodamine-injected controls showed a general and disperse signal through several structures. MCH-ROD (+) cells were NeuN (+) and GFAP (-), and several were GAD-67 (+). In some areas of hippocampus, MCHergic fibers were close to MCH-ROD (+) neurons. ATC-175 and PAO pre-treatment significantly diminished the number of MCH-ROD (+) cells at T20. With the aim of exploring the mechanism of MCH-ROD internalization and its effects, we are validating an *ex vivo* approach with cultured hippocampal neurons from E18 rat embryos. After eleven days *in culture*, the cells were incubated with MCH-ROD and the incorporation of the labeled molecule was evaluated after five (T5), ten (T10) and thirty (T30) minutes in α -tubulin (+) cells. We observed a time-dependent increase in the percentage of area occupied by MCH-ROD in hippocampal cultured neurons.

Conclusions. We conclude that MCH-ROD internalizes in hippocampal neurons of adult rats in a receptor- and clathrin-dependent manner. MCH-ROD seems to be an adequate experimental tool to evaluate in a simple manner native MCH-receptive neurons in brain tissue. Further experiments must be done, both *in vivo* and in cultured neurons, to study in depth the effects of MCH on hippocampal neurons.

S11.4 Regulatory role of prolactin on the neuroimmune system of the hippocampus of male rat pups

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Increased concentrations of hormones and neuropeptides early in life lead to physiologic alterations and increases the risk to suffer psychiatric illness. Neonatal administration of prolactin (PRL) was shown to reduce hippocampal neurogenesis on postnatal day 15. This reduction was later associated with a depressive behavior in adulthood. PRL is considered also a cytokine, thus PRL might exert its actions regulating cytokine expression and through the action of neuroimmune cells in the hippocampus. Here we will show the effects of PRL on the neuroimmune system. Neonatal administration of PRL induced a significant decrease in the astrocyte population of the hippocampal hilus. However, no change in microglial activation was observed. Further, neonatal PRL affected cytokine expression of TNF- α , IL-1 β , and IL-6 in the hippocampus in response to a stressful stimulus of male rat pups at PN15. We conclude that PRL contributes to modulate the morphology and reactivity of the neuroimmune system, and extends its actions as a regulator of the immune response within the brain.

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S11.5 The stress systems in mood disorders: a postmortem study

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Our hypothesis for the heterogeneous pathogenesis of mood disorders is that factors including polymorphisms in stress-related genes, gender, age, developmental history and environmental stressors make different parts of the stress-related brain systems vulnerable to different stressful life events, causing different alterations in a network mediated by neurotransmitters and neuromodulators, which finally make individuals in a personal way at risk for mood disorders. The hypothalamo-pituitary-adrenal (HPA) axis has a prominent position in this network. With postmortem human brain material that is clinically and neuropathologically well-characterized, animal models mimicking depression and molecular mechanistic work on cell lines, we try to elucidate the specific changes and the interaction in these molecular systems in relation to special subtypes of mood disorders. The final aim is to set up tailor-made treatment for mood disorder patients on his/her particular vulnerability in particular stress systems. This presentation reviews our findings of changes in systems of sex steroids, corticotrophin-releasing hormone, oxytocin, and orexin in the etiology of mood disorders, in relation to HPA activity and sex differences.

S11.6 Constrained GLP-1 mimetics with incretin-like properties

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Type 2 diabetes mellitus (T2DM) is a chronic disorder characterized by hyperglycemia from insulin resistance and defective insulin secretion. Glucagon-like peptide 1 (GLP-1) is a 30-residue peptide that activates GLP-1 receptor (GLP-1R) in pancreatic β -cells stimulating glucose-dependent insulin secretion, insulin gene transcription and β -cell proliferation. Pharmacological treatment of type 2 diabetic patients with longer-lived, proteolysis-resistant GLP-1R agonists like ByettaTM (Exenatide) and VictozaTM (Liraglutide) are gaining use for treatment of T2DM [1]. Such injectable peptides are the only class of glucose-lowering agents resulting in body weight reduction. Both academia and industry seek oral GLP-1R agonists to improve compliance while maintaining or improving efficacy and tolerability [2]. A better understanding of how GLP-1 interacts with its receptor may better facilitate the identification of orally bioavailable therapeutic agents. This talk will illustrate our design of conformationally constrained GLP-1 analogues in collaboration with the pharmaceutical company Pfizer. Our results led to a series of highly helical analogues that exhibited potent receptor activation, greater insulin secretion and insights on receptor-ligands interactions. [3]

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S12. Regulatory peptide signaling and circuit logic in controlling concerted behaviors

Princesa 4

Chair: **Lee E. Eiden** (Bethesda, USA)

S12.1 **Ben White** (NIHH, NIH, Bethesda, USA):

The peptide modulome. How neuropeptide circuits modulate neuronal circuits to orchestrate behavior--insights from animal models

S12.2 **Esther Sabban** (New York Medical College, USA):

NPY administration and treatment of behavioral symptoms in a rodent single prolonged stress model for PTSD

S12.3 **Xiao-Dong Wang** (Zhejiang University, China):

Neuropeptides and calbindin in stress-related disorders

S12.1 The Broad Reach of Neuromodulators in the Control of Behavior

Feici Diao, Amicia D. Elliott, Adama Berndt, Fengqiu Diao, Sarav Shah, and **Benjamin H. White**

Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD USA

Species-specific behavioral patterns required for survival, reproduction, and development often rely on the action of hormones and neuromodulators. These factors are thought to act broadly in the brain to orchestrate coordinated responses to particular environmental circumstances and somatic needs, but knowledge of where in the sensorimotor circuitry they act to achieve this coordination is fragmentary for most behaviors. This is due in part to the difficulty of tracking neuromodulator action throughout the brain. Taking advantage of the small nervous system of the fruit fly, *Drosophila melanogaster*, and of genetic tools for selectively targeting neurons that express particular neuromodulator receptors, we have shown that a small complement of neuromodulators acts coordinately all levels of a hierarchically organized neural circuit to coordinate the behavioral sequence required for molting at the pupal stage. This sequence (called the “pupal ecdysis sequence”) consists of three distinct motor programs and its progression depends on the staggered release of several highly conserved factors, which act somatically as hormones and within the brain as neuromodulators. The receptors for these factors are expressed in functionally distinct layers of the pupal ecdysis circuit, including an input layer that processes the initiating signal, a multifunctional central pattern generating layer, and a layer consisting of motor neurons and a small subset of muscles. Our evidence indicates that the neuromodulators governing the pupal ecdysis sequence coordinately mediate circuit-wide changes to induce motor program transitions. Using whole-brain Ca^{++} imaging, our goal is to follow neuromodulator action at cellular resolution throughout the circuit.

S12.2 Potential for intranasal neuropeptide Y and/or melanocortin 4 receptor antagonist for preventing or treating PTSD

Esther L. Sabban, Xiaoping Liu, Robert Camp and Lidia I Serova

Department of Biochemistry and Molecular Biology, New York Medical College Valhalla, New York 10595 USA

There is a great need for effective treatment options for posttraumatic stress disorder (PTSD). Neuropeptide Y (NPY) is associated with resilience to traumatic stress. Melanocortin 4 receptor (MC4R) antagonists, such as HS014, also reduce response to stress. Both regulate stress responsive systems—the hypothalamic pituitary axis (HPA) and the noradrenergic nervous system and their associated behaviors. Therefore, we examined if their intranasal delivery to brain could attenuate development of PTSD related symptoms in a single prolonged stress (SPS) rodent PTSD model. We demonstrated widespread delivery to the brain 30 min after intranasal infusion. Three regimens of intranasal delivery were used: (1) prophylactic treatment 30 minutes before SPS stressors; (2) early intervention right after SPS stressors; (3) therapeutic treatment when PTSD behaviors manifested one week or more after the traumatic stress. A week or more after SPS stressors, the animals displayed behavioral, neuroendocrine and biochemical impairments. Animals given vehicle had elevated anxiety, depressive-like behavior, hyperarousal and defects in retention of extinguished fear memories. In contrast, these behaviors were similar to unstressed controls with early intervention or prophylactic NPY treatment (regiment 1 or 2) with intranasal NPY (100-150 µg/rat). The side effects were minimal. Intranasal NPY did not change the stress-triggered effects on body weight and had a very transient effect on feeding behavior. There were no major side effects on the cardiovascular system, with possible benefit from transient amelioration of the SPS-triggered elevation in heart rate. Prophylactic or early intervention with intranasal NPY reduced dysregulation of HPA axis. The levels of hypothalamic CRF mRNA and GR in the ventral hippocampus were significantly induced in the vehicle-, but not NPY-treated group. NPY also prevented the SPS elicited changes in gene expression in the locus coeruleus (LC), hypersensitivity of LC/NE system to novel mild stressor and induction of CRF in amygdala. Some of these impairments were also reduced with HS014, alone or together with NPY. Low concentrations of NPY or HS014 which by themselves were ineffective, prevented depressive-like behaviors when combined. When given after symptoms manifested (regiment 3) intranasal NPY could still reverse the anxiety, depressive behaviors and impaired social interactions. A higher dose was required to reverse the more severe symptoms manifested at longer times following the traumatic stress. The results demonstrate strong preclinical proof of concept for intranasal NPY, and perhaps MC4R antagonists, for non-invasive early pharmacological interventions for PTSD and comorbid disorders and possibly also as therapeutic strategy.

S12.3 Neuropeptides and calbindin in stress--related disorders

Xiao-Dong Wang

Department of Neurobiology, Zhejiang University School of Medicine

As a Ca^{2+} buffer, sensor and transporter, calbindin critically modulates synaptic plasticity. Reduced hippocampal calbindin levels have been implicated in cognitive disorders, including those induced by early-life stress. However, it is unclear how early-life stress modulates calbindin expression in distinct hippocampal neurons and the contribution of each calbindin-expressing neuronal population to memory. Here, we report that hippocampal excitatory and inhibitory calbindin neurons modulate the susceptibility and resilience to early-life stress-induced spatial memory deficits respectively. Stress exposure during early postnatal period lastingly reduced calbindin levels in all CA1 and DG neurons. Reduced calbindin levels in CA1 or DG excitatory neurons, but not CA1 interneurons, strongly correlated with spatial memory impairments. Accordingly, selective knockdown of calbindin in CA1 or DG excitatory neurons mimicked postnatal stress-induced memory deficits in adulthood. By contrast, calbindin knockdown in CA1 interneurons preserved memory both under basal conditions and after an acute stress challenge. Moreover, calbindin expression levels were suppressed by early-life stress through the cell adhesion molecule nectin3, and in turn reduced IMPase levels. Our findings highlight calbindin as a key molecule for the reprogramming effects of early-life stress on cognition, and exemplify how distinct neurons sharing a common molecule confer the susceptibility or resilience to stress.

Editors-in-chief luncheon

How to publish your research: guidelines for young investigators

Chair: **Robert Millar**, Editor, Neuroendocrinology

Participants: Illana Gozes (Editor, Journal of Molecular Neuroscience); Dave Grattan (Fmr Ed-in-chief, J. Neuroendocrinology); James Herman (Editor, Stress)

Agenda for the gathering:

- Introductions by editors
- Suggestions by participants of areas additional to those listed below which they would like covered
- Choosing the right journal. Checking the journal scope. Considering flagship journals in the area of your work. The disadvantages of being too ambitious (be ambitious by all means but a reality check is needed)
- Understanding the review process
- Why might my manuscript be rejected? (apart from fundamental flaws, things like...inappropriate journal, editorial triage, perceived lack of impact)
- Features of excellent manuscripts (clear writing, clear figures and tables and clear messages)
- Responding to reviewer's comments
- What do impact factors mean? What are appointment and promotion committees looking for in the journals you have published in?
- Writing reviews
- Open access may be great but someone has to pay! The pros and cons of these journals
Issues re predatory journals
- Should reviewers' identities be declared?

Plenary lecture III

Atlantes amphitheater

Peptide-degrading enzymes and the control of peptide action in vivo

Jean-Louis Charli

Instituto de Biotecnología, Universidad Nacional Autónoma de México
(UNAM), Mexico

About the speaker



Dr. Jean-Louis Charli is a professor at the Institute of Biotechnology (IBT) of the National University of Mexico (UNAM). He was formerly Dean of the Postgraduate Studies in Biochemistry, Chairman of the Department of Biochemistry, and Chairman of the Department of Developmental Genetics and Molecular Physiology at IBT. A member of the Mexican Academy of Sciences, he is the author of over 100 manuscripts, book chapters and reviews and the tutor of numerous graduate students. Dr

Charli's research centers on the role of peptides in intercellular communication in brain and hypophysis. He studies the influence that ectopeptidases exert on peptide actions; he has extensively studied the properties of thyrotropin-releasing hormone degrading ecto-peptidase. Charli has received the highest awards of the Mexican Academy of Sciences (Natural Sciences Award), the National System of Research and the National University of Mexico (Program for Academic Excellence)

PL3 Tanycyte thyrotropin-releasing hormone degrading ectoenzyme and the control of the thyroid axis

Jean-Louis Charli

Institute of Biotechnology, UNAM, MEXICO

The biological activities of peptides are controlled by activating and inactivating peptidases. Many extracellular (ecto)peptidases hydrolyze multiple peptides, making the identification of their roles quite complex in the nervous system. Some peptidases have a narrow specificity, and it may be easier to pinpoint their physiological role. One of them, the thyrotropin-releasing hormone degrading ectoenzyme (TRH-DE) is predominantly localized to the brain. Tanycytes are glial cells that line the ventral wall of the third ventricle; they express Trhde. The $\beta 2$ subtype sends cytoplasmic projections into the external layer of the median eminence, in the vicinity of the nerve terminals of the hypophysiotropic TRH neurons. I will describe the evidence that suggests tanycyte TRH-DE controls the output of TRH into the hypothalamus-pituitary portal capillaries and the thyroid axis. I will contrast these data with ectopeptidases functions in other neuroendocrine axes.

S13: Neuropeptide function in fear circuits

Princesa 1

Chair: Francesco Ferraguti (Innsbruck, Austria)

S13.1 **Ramon Tasan** (Medical University of Innsbruck, Austria):

Role of neuropeptides in the interaction of fear and hunger

S13.2 **Kay Jüngling** (University of Münster, Germany):

The impact of the human-relevant NPSR1 polymorphism I107N on anxiety- and fear-related circuits and behavior

S13.3 **Francisco Sotres-Bayon** (IFC, UNAM, Mexico):

Neurogenesis regulates fear recovery by recruiting a prefrontal-amygdala-habenula network

S13.4 **Francesco Ferraguti** (Medical University of Innsbruck, Austria):

Specialized amygdala inhibitory networks for emotional learning

S13.1 Neuropeptides at the crossroads of fear and hunger

D. Verma¹, G. Lacha, J. Wood¹, L. Comerasa, B. Hoermer¹, H. Herzog² & **R. Tasan**¹

¹Department of Pharmacology, Medical University Innsbruck, Innsbruck, Austria;

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Physiologically, emotions control evolutionarily conserved behavior that is central to survival in a natural environment, while imbalance within these circuitries may result in malfunctioning and manifestation of anxiety disorders. We recently focused on the question of how metabolic processes, such as hunger, may influence emotional-affective behavior. Especially, neuropeptides, which are central for metabolic and emotional balance seem to play a pivotal role. For instance, Neuropeptide Y (NPY) is highly expressed in the arcuate nucleus of the hypothalamus, where it promotes food intake when energy supply is low, while NPY in limbic brain areas, such as amygdala and hippocampus, reduces fear memory, promotes fear extinction and acts as an anxiolytic neuromodulator. Importantly, we also found that short-term fasting inhibits fear memory consolidation, and promotes fear extinction. Thus, hunger may drive food seeking by reducing inborn and learned aversion to environmental threats. Since NPY neurons are activated by caloric deficiency, NPY-release could be crucial for hunger induced suppression of fear. We investigated the role of selected NPY receptors on food intake, fear related behaviors and in particular fear extinction. Furthermore, we identified several brain areas and synaptic correlates underlying hunger-induced inhibition of fear and facilitation of extinction learning. Our data suggest that fear and extinction memories are differentially regulated by hunger and that both phenomena are fundamentally controlled by neuropeptides. Thus, we propose neuropeptide receptors as promising drug targets for treating emotional-affective as well as metabolic disorders.

Funded by Austrian Science Fund, FWF, P 29952.

S13.2 The impact of the human-relevant NPSR1 polymorphism I107N on anxiety- and fear-related circuits and behavior

Kay Jüngling

University of Münster, Germany

The neuropeptide S system, consisting of the peptide neuropeptide S (NPS) and its G-protein coupled receptor (NPSR1), is involved in a variety of autonomous and cognitive functions (e.g. stress, fear and anxiety). In humans, a single-nucleotide polymorphism (SNP) in the NPSR1 gene has been linked to panic disorders and increased stress levels. We hypothesize that introduction of the human-relevant SNP in mice will impact NPSR1 functionality, fear-related circuits and behavior due to differential efficacy of the NPS system.

We use a novel mouse model in which an amino acid substitution of isoleucine (I) by arginine (N) at position 107 in the NPSR1 protein was induced by CRISPR/Cas9-mediated gene editing, mimicking the human SNP. To prove our hypothesis, we performed electrophysiological recordings from principal-neurons (PN) of the anterior-basal amygdala (BAa). Furthermore, we conducted standard anxiety tests and utilized a Pavlovian fear conditioning paradigm to test for differences in anxiety- and fear-like behavior.

Patch-clamp recordings from PN of the BAa show that NPSR1-signaling and PN depolarization is strongly decreased in the NPSR1-N107 variant as compared to the -I107 variant, resulting in a hypoactivation of the amygdalar excitatory network. Behavioral results show that male NPSR1-N107 mice exhibit reduced freezing responses to a neutral tone presentation during fear memory retrieval. Furthermore, female NPSR1-N107 mice display facilitated extinction learning as compared to I107 females.

S13.3 Adult hippocampal neurogenesis regulates fear recovery by recruiting a prefrontal-amygdala-habenula network.

Francisco Sotres-Bayon

Instituto de Fisiología Celular-Neurociencias, Universidad Nacional Autónoma de México, Ciudad de México, MEXICO

Learning to extinguish defensive responses to threats (fear) in a place, leads to the formation of a new memory that inhibits a previously acquired fear memory to that context. However, fear responses often return with the simple passage of time (spontaneous recovery). Given that contextual fear and extinction memories are hippocampus-dependent and hippocampal neurogenesis has been reported to modify preexisting memories, we hypothesized that neurogenesis-mediated modification of a preexisting extinction memory would modify fear spontaneous recovery levels. To test this, rats underwent contextual fear conditioning followed by extinction in the same context. Subsequently, we exposed the rats to an enriched environment (EE) or X-irradiation to enhance or ablate neurogenesis, respectively. One month after these manipulations, rats were tested to evaluate spontaneous fear recovery. We found that increasing neurogenesis after, but not before, extinction prevented fear recovery. In contrast, neurogenesis ablation after, but not before, extinction promoted spontaneous fear retrieval. Notably, using the neural activity marker c-Fos,, we identified the brain regions recruited in these opposing neurogenesis-mediated changes in fear recovery levels. Together, our findings suggest that neurogenesis manipulation after extinction learning modifies fear recovery by recruiting brain network activity that mediates the expression of preexisting contextual fear and extinction memories.

S13.4 Specialized amygdala inhibitory networks for emotional learning

Sabine Krabbe¹, Enrica Paradiso², Thomas Rhomberg², Laura Rovira-Esteban³, Yael Bitterman¹, Chun Xu¹, Milica Markovic¹, Attila Viktor³, Jan Gründemann¹, Norbert Hajos³, Francesco **Ferraguti**² and Andreas Lüthi¹

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Learning and memory are fundamental neuronal processes that are essential for adaptive behavior. Memory formation is shaped by dynamic changes in the balance between excitatory and inhibitory neuronal circuit elements. Although local inhibitory interneurons represent a minority of the cells in cortical brain areas, they tightly regulate the activity and plasticity of large populations of projection neurons in a spatially and temporally precise manner. To date, however, little is known about how different subtypes of interneurons contribute to memory formation. Interneurons can be classified into several functionally distinct subtypes based on several parameters including firing activity, axonal projections and molecular fingerprints such as expression of neuropeptides. A subgroup, which expresses the vasoactive intestinal polypeptide (VIP), has been postulated to have as its primary post-synaptic target other interneurons.

Using fear conditioning as a model system for associative learning, we show that interactions between VIP interneurons and other interneuron subtypes of the basolateral amygdala (BLA) can gate behaviorally relevant stimuli to instruct plastic changes in neighbouring glutamatergic pyramidal neurons (PNs). We used a multidisciplinary approach to characterize at first the morphological and functional properties of VIP interneurons as well as their intrinsic connectivity. We then explored their long-range connectivity by means of a rabies virus-based retrograde mono-transsynaptic tracing approach. Finally, using a combination of deep brain calcium imaging and optogenetic manipulations in freely behaving mice, we have analysed how the disinhibitory role of VIP interneurons could affect plastic changes of BLA PN and thus gate fear memory formation.

S14. Neuropeptides and social behavior

Princesa 3

Chair: **Tallie Z. Baram** (Irvine, USA)

S14.1 Tallie Z. Baram (University of California at Irvine, USA):

CRH and development of the pleasure/reward circuitry

S14.2 Inga D. Neumann (Univ. Regensburg, Germany):

Oxytocin and neuropeptide S in social behavior

S14.3 Genaro A. Coria-Avila (Centro de Investigaciones Cerebrales, Universidad Veracruzana, México):

Oxytocin in conditioned same-sex partner preferences and brain dimorphism

S14.4 Adi Simerblit-Sabba and W. Scott Young III (National Institute of Mental Health, NIH, USA):

Vasopressin and social behaviors

S14.1 CRH and development of the pleasure / reward circuitry

Tallie Z. Baram, A. K. Short, J. Bolton and Y. Chen
University of California at Irvine, USA

Rationale: The stress neuropeptide CRH is expressed in and acts on numerous brain circuits, with the overarching role of modulating brain functions in response to stress/adversity. CRH expression in regions such as the hypothalamus, hippocampus and amygdala is commonly influenced by stress. The reward circuit underlies fundamental processes of pleasure, reward and happiness. Disruption of the functions of this circuit contributes to serious health problems including anhedonia (reduced ability to experience pleasure), a predictor of addiction, alcohol abuse and depression. The nucleus accumbens is a critical node of the pleasure/reward circuit, influenced by inputs from many brain regions including amygdala. Excessive amygdalar inputs (presumably related to fear/anxiety) can disrupt circuit function, leading to anhedonia and depression.

Results: (1) We find that early-life adversity/stress provokes anhedonia in adolescent rodents, via disruption of the function of the reward circuitry. (2) CRH expression is increased in the amygdala central nucleus (ACe) of early-life stressed rats. (3) Silencing CRH in the ACe reverses several measures of anhedonia in early-life stressed rats. (4) Finally, viral tracing and brain clearing in mice is beginning to demonstrate aberrant CRH expression and function also in the nucleus accumbens and amygdala regions.

Conclusion: CRH modulates the functions of the pleasure reward circuit. Early-life experiences exert long-lasting consequences on this circuit by influencing CRH expression, thus promoting anhedonia and vulnerability to mental illness including depression.

Supported by MH73136; MH096889

S14.2 Oxytocin and neuropeptide S (inter)actions for the sake of socio-emotional balance

Inga D. Neumann

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

The neuropeptides oxytocin (OXT), neuropeptide S (NPS), vasopressin and corticotrophin releasing factor are important modulators of social and emotional behaviors with differential and partly even opposite effects on anxiety, stress coping and social interactions¹. Selective targeting of these neuropeptidergic systems has appeared to be a promising option for the treatment of psychopathologies, which are mostly accompanied by socio-emotional dysfunctions. Not only OXT, but also NPS promotes social interactions and social preference behavior in rats and mice, particularly under conditions of social stress. For example, social defeat-induced social avoidance of conspecifics is attenuated or even completely reversed by central application of each of these neuropeptides in a dose-dependent manner both in rats² and mice³. In a mouse model of social fear conditioning (SFC), both synthetic OXT and NPS reverse social fear and reinstate social preference behavior. Specifically, the social fear-attenuating effects of OXT have been localized within the lateral septum⁴, which receives major OXT projections from the hypothalamic supraoptic nucleus (SON). Physiological activation of the OXT system as seen in lactation or during mating in males prevents or strongly inhibits SFC-induced social fear. As revealed by pharmacological and chemogenetic approaches enhanced OXT signaling within the lateral septum of lactating mice is directly associated with this effect, making lactating animals more resilient to trauma.

As both OXT and NPS share many behavioral effects, including general anxiety-related behavior, we tested their possible interactions. NPS neurons of the brainstem target OXT neurons of the hypothalamic paraventricular nucleus (PVN). These OXT neurons are activated by NPS as reflected by increased Calcium signaling and stimulated local OXT release. Importantly, both NPS and OXT exert an anxiolytic effect directly within the PVN, but in case of NPS, this local effect essentially requires OXT activity: If local OXT receptor-mediated effects were blocked pharmacologically, or if the reactivity of OXT neurons was prevented by chemogenetic silencing of PVN-OXT neurons the anxiolytic effect of NPS was prevented.⁵ Thus, the multiple neuropeptide effects on socio-emotional behaviors are likely to rely on their complex brain region and context -specific interactions. Supported by DFG, BMBF and EU.

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S14.3 Oxytocin in conditioned same-sex partner preferences and brain dimorphism

Genaro A. Coria-Avila

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Learned social preferences may develop when a conditioned stimulus (CS) is associated in contingency with an unconditioned stimulus (UCS) that functions as reinforcer. Consequently, an individual may display a learned preference for a partner that bears that specific CS. There are many types of reinforcers, and depending on critical periods of development they may be more or less effective. Partner preferences are formed, strengthened, or weakened because reinforcers exert an effect on the dynamics of certain brain neurotransmitters such as dopamine, opioids, oxytocin (OT) and vasopressin. These neurotransmitters modulate attention, prediction, expectation, reward, and trust, which may be considered the emotional substrates for partner preference. For example, conditioned same-sex partner preference (learned) can develop in male rats that undergo cohabitation under the effects of systemic treatment with D2-type dopamine agonists (QNP) or OT. In one of our studies male rats were treated with: nothing, saline, QNP, OT, or QNP+OT during cohabitation with another male (+) or single-caged (-). This resulted in the following groups: 1) Intact-, 2) Saline+, 3) QNP-, 4) OT-, 5) QNP+, 6) OT+ and 7) QNP/OT+. Males cohabited during 24 h with a male partner that bore almond scent on the back as conditioned stimulus. This was repeated every 4 days for a total of three trials. Social and sexual preference were assessed four days after the last conditioning trial in a drug-free test (without treatment of QNP or OT) in which experimental males chose between the scented familiar male and a novel sexually receptive female. We found that males from groups Intact-, Saline+, QNP- and OT- displayed a clear preference for the female (heterosexual), whereas groups QNP+, OT+ and QNP/OT+ displayed socio/sexual preference for the male partner (same-sex). Accordingly, it was not the pharmacological treatment with QNP or OT that modified the sexual preference, but the cohabitation under the effects of those neurochemicals that served as reinforcers, facilitating learning. In a second experiment we assessed changes in sexual dimorphism (as observed with Nissl dye) in the brains of those males. Specifically, we measured the area size of the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) and the supraoptic nucleus (SON) and compared it between groups. The SDN-POA was smaller in males from groups OT-, OT+ and QNP/OT+ (indicating an effect of OT in nucleus size), whereas the SON was larger in groups QNP+ and QNP/OT+. Accordingly, conditioned same-sex social/sexual partner preference can develop during cohabitation under enhanced D2 or OT activity (which function as reinforcers) but such preference does not depend on the area size of those sexually dimorphic nuclei.

S14.4 Vasopressin and social memory

Adi Cymerblit-Sabba and W Scott Young

Section on Neural Gene Expression| National, Institutes of Health, USA

Since David De Wied first reported the influence of lysine vasopressin on the maintenance of a conditioned avoidance response in rats after the removal of the posterior pituitary (1), the role of vasopressin in memory has been the subject of many studies. Social memory or recognition has been of particular interest and has been studied and found to be deficient in both spontaneous deletions of vasopressin (Brattleboro rats) and engineered deletions of its CNS receptors, the Avpr1a and Avpr1b. Our research has focused on the CA2 subfield of the hippocampus because Avpr1b is predominantly expressed there and its knockout results in impaired social recognition (2). We proposed that the CA2 area was important for social recognition which has been confirmed by a series of experiments. We will present some of this historical background as well as some recent work examining the role of vasopressin in the hippocampal CA2 area.

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S15. Neuropeptides in inflammatory processes

Princesa 3

Chair: **Erika Pintér** (Pecs, Hungary)

S15.1 **Susan D. Brain** (King's College London, UK):

CGRP and protective effect in the cardiovascular system; relevance to migraine therapy

S15.2 **Soraia Costa** (University of Sao Paulo, Brazil):

Environmental influence of fumes on TRPA1-induced inflammation

S15.2 **Barbara Kofler** (Department of Pediatrics/University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria):

Galanin is a versatile modulator of immune cell activation

S15.4 **Erika Pintér** (Department of Pharmacology and Pharmacotherapy, University of Pecs, Pecs, Hungary):

TRPA1-mediated effect of sulfide compounds in pain and inflammation

S15.1 CGRP and protective effect in the cardiovascular system; relevance to migraine therapy

Susan D. Brain

School of Cardiovascular Medicine & Sciences, BHF Centre of Cardiovascular Excellence, King's College London, SE1 9NH, UK.

Sensory nerves contain and release the highly potent vasodilator α -calcitonin gene-related peptide (α -CGRP). This peptide is a potent microvascular dilator, especially in the cutaneous microcirculation (1). However, blocking the action of CGRP does not influence cardiovascular regulation in healthy individuals. This has been shown in clinical trials with CGRP antagonists and antibodies, which are being developed for use in the treatment of migraine. This leads to the suggestion that CGRP does not play a physiological role in the regulation of blood pressure. We believe that this is because insufficient CGRP is released in healthy conditions for a functionally important role.

This is in comparison with knowledge that deletion or blockade of CGRP can worsen cardiovascular diseases in various animal models (1,2). Moreover, cold-induced vasoconstriction with the subsequent restoration of blood flow involves an active CGRP-mediated response. These results highlight the potential of the CGRP pathway in ameliorating disease.

We now have evidence that a CGRP agonist protects against hypertension and heart failure in mice. We therefore support the viewpoint that, while CGRP may not have a role in core cardiovascular regulation, it remains important in the periphery (e.g. skin) and that addition of CGRP may be of therapeutic benefit in cardiovascular disease.

These studies are supported by the British Heart Foundation in association with Novo Nordisk and the MRC and BBSRC.

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S15.2 Influence of environmental fume contaminants on TRPA1-induced Inflammation

Soraia K. Costa

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According to the World Health Organization (WHO, 2016), early exposure to ambient pollutants (AP), such as diesel exhaust particles (DEP), can significantly affect later exacerbation of respiratory diseases, such as asthma, which often display a degree of sex bias (1). Compelling evidence showed an important correlation between DEP-induced adverse respiratory effects and activation of transient receptor potential (TRPV1, TRPA1), which in turn sensitizes C-fibers, leading to airway irritation and inflammation due to neuropeptide release and generation of reactive oxygen species (ROS) [2, 3]. Besides there being studies showing the role played by TRP in the deleterious effects of DEP, the impact of the electrophilic pollutant 1,2-naphthoquinone (1,2-NQ), found in DEP, in asthma and non-cardiovascular comorbidity such as non-alcoholic fatty liver disease (e.g. steatosis) is elusive. Therefore, a close follow-up on the role of TRPA1 in the postnatal effects of 1,2-NQ is required to understand its participation in asthma and steatosis development. Male and female C57Bl/6 mice (2-5 g; local animal licence 48/2016 CEUA) were nebulized with 1,2-NQ (100 nM) or vehicle for 3 alternate days. Four weeks later, mice were sensitized with ovalbumin (OVA; 10 µg 0.2/ml PBS; s.c.) and challenged with OVA (1%) at 40, 41 and 42 days old. A separate set of mice was treated concomitantly with the TRPA1 receptor antagonist HC030031 (50 mg/kg, i.p., -2 h). After 24 hours, mice were submitted to airways responsiveness testing (Penh) and euthanized. The bronchoalveolar lavage (BAL), lung and liver were obtained from mice and submitted to inflammatory / histological analysis. Data are presented as mean ± SEM, and statistical analysis were performed by ANOVA plus Bonferroni's test. Changes related to body weight were not detected among groups, but the allergic insult in male, but not female, mice prior-exposed to 1,2-NQ led to a marked eosinophilia in the BAL and peripheral blood compared to the allergic group. This response in male mice was accompanied with increased Th2 cytokines concentration, but only female lungs showed augmented catalase, glutathione peroxidase, reductase and S-transferase activities, as well as increased Nrf2 and TRPA1 mRNA expression. Exposure to 1,2-NQ did not evoke changes in metabolic parameters or liver weight, but it evoked increased ion [Ca²⁺] fluxes in dorsal root ganglion (DRG) cells in culture. Treatment with TRPA1 antagonist, HC030031 reduced 1,2-NQ-induced exacerbated eosinophilia in vivo and reduced 1,2-NQ-induced augmented [Ca²⁺] in cultured cells. In conclusion, exposure to 1,2-NQ during postnatal development leads to differential sex susceptibility to asthma, in which male infants are at greater risk than female infants. TRPA1 signaling pathway is involved in 1,2-NQ-induced asthma susceptibility, and the protective effect seen in female mice is linked to higher antioxidant defenses possibly due to greater pulmonary maturity.

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S15.3 Galanin is a versatile modulator of immune cell activation

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The regulatory peptide galanin is broadly distributed in the central and peripheral nervous systems and in non-neuronal tissues, and exerts its diverse physiological functions via three G-protein coupled receptors (GAL1-3-R). Some regulatory peptides are important players in the cross-communication between the nervous and immune systems and are in focus as new therapeutics for diverse inflammatory diseases. Various studies on inflammatory animal models and immune cells revealed both pro- and anti-inflammatory functions of galanin, suggesting complex galanin signaling in a tissue- and cell type-dependent manner. The microenvironment of tissues upon an immune challenge is dynamic and depends on the type of infiltrating immune cells as well as the type of stimuli, and might determine the specific role of galanin during inflammation. Accordingly, galanin and GAL1-R and GAL2-R mRNA were expressed in a range of immune cells. Especially, macrophages displayed a differentiation and polarization dependent expression of galanin and its receptors. Our data revealed that galanin can be regarded as an immunomodulatory peptide as it can sensitize neutrophils and natural killer cells towards proinflammatory cytokines.

Galanin also affected the cytokine/chemokine expression profile of macrophages, depending on differentiation and polarization, and mainly modulated the expression of chemokines (CCL2, CCL3, CCL5 and CXCL8) and anti-inflammatory cytokines (TGF- β , IL-10 and IL-1Ra) especially in type 1 macrophages. Cytokine/chemokine expression of IFN γ - and lipopolysaccharide-polarized macrophages were upregulated, whereas cytokine/chemokine expression levels of unpolarized macrophages were downregulated, upon galanin treatment after 20 hours. Our studies indicate a comprehensive regulation by galanin of the expression of important cytokines/chemokines and modulation of cytokine responses in different types of immune cells.

This work was supported by the Austrian Research Promotion Agency [FFG; 822782/THERAEP] and Paracelsus Medical University Salzburg [PMU-FFF: R-17/01/086-KOL].

S15.4 TRPA1--mediated effect of sulphide compounds in pain and inflammation

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The gaseous mediator hydrogen sulfide (H₂S) and polysulfides (H₂Sn) activate the Transient Receptor Potential Ankyrin 1 (TRPA1) non-selective ligand-gated cation channels expressed in primary sensory neurons. Proinflammatory (e.g. SP, CGRP), as well as anti-inflammatory (e.g. somatostatin) neuropeptides are released from the activated nerve endings and influence pain sensation and inflammatory processes. In the present study we investigated the effects of H₂S donors (NaHS, GYY4137) and a polysulfide compound (dimethyl trisulfide, DMTS, inorganic sodium polysulfide (POLY) in mild heat injury-induced hyperalgesia model, experimental arthritis and carrageenan-evoked hind paw inflammation in mice focusing on the role TRPA1 and somatostatin sst4 receptors. Mechanical hyperalgesia, plasma extravasation, oedema formation, as well as leukocyte accumulation were measured in TRPA1 and sst4 KO mice and wild-type controls. DMTS had analgesic action in mild heat injury-induced mechanical hyperalgesia in wild-type mice, but it had no inhibitory action in TRPA1 or sst4 knockouts. Slow H₂S donor GYY4137 inhibited the arthritic changes in wild-type mice but it did not act in TRPA1 KO animals. POLY decreased carrageenan-evoked mechanical hyperalgesia in a TRPA1 and sst4 receptor-dependent manner. DMTS reduced all examined inflammatory parameters. Mitigation of mechanical hyperalgesia and paw swelling by DMTS were mediated by sst4 receptors. Our study presented evidence that TRPA1 as well as sst4 receptors play role in mediation of antinociceptive and anti-inflammatory effects of H₂S and polysulfides.

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S16. New insights in the hypothalamic regulation of energy metabolism by neuropeptides

Princesa 4

Co-chairs:

Nicolas Chartrel (Rouen, France) and **Carole Rovère** (Valbonne, France)

S16.1 Sophie Steculorum (Max Planck Institute for Metabolism Research, München, Germany):

Novel regulators of the central control of feeding and systemic insulin sensitivity

S16.2 Serguei Fetissov (U1239, Université de Rouen, Normandie, France):

Regulation of feeding behavior by a neuropeptide-like protein produced by gut bacteria

S16.3 Nicolas Chartrel (INSERM U1239, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication):

26RFa: a neuropeptide involved in the hypothalamic regulation of energy homeostasis

S16.4 Carole Rovère (Institute of Molecular and Cellular Pharmacology, Université Nice Sophia Antipolis, Valbonne, France):

Impact of nutritional lipids on glial remodeling and neurons activity in the hypothalamus. Focus on MCH and orexin neurons

S16.1 Novel regulators of the central control of feeding and systemic insulin sensitivity

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Activation of orexigenic AgRP--expressing neurons in the arcuate nucleus of the hypothalamus potently promotes feeding. We discovered a new regulator of orexigenic AgRP-neurons by demonstrating that they express the purinergic receptor 6 (P2Y6) and that activation of P2Y6 by its endogenous ligand uridine-diphosphate (UDP) increases AgRP-neuron's action potential firing and promotes feeding in mice. We further show that hypothalamic UDP content is elevated in obese animals as a consequence of increased circulating uridine concentrations.

Taken together, our experiments reveal that, in obesity, circulating uridine concentrations are increased, providing enhanced substrate availability for hypothalamic UDP synthesis and ultimately promoting feeding via UDP-induced P2Y6 signaling in AgRP-neurons. We further demonstrated that central injection of UDP acutely promotes feeding in diet-induced obese mice and that acute pharmacological blocking of P2Y6 receptors reduces food intake. Importantly, mice with AgRP-neuron-restricted inactivation of P2Y6 exhibit reduced food intake and fat mass as well as improved systemic insulin sensitivity with improved insulin action in liver. Our results reveal that P2Y6 signaling in AgRP neurons is involved in the onset of obesity-associated hyperphagia and systemic insulin resistance. Collectively, these experiments define P2Y6 as a potential target to pharmacologically restrict both feeding and systemic insulin resistance in obesity.

S16.2 Regulation of feeding behavior by a neuropeptide-like protein produced by gut bacteria

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Humans and animals naturally display autoantibodies reactive with α -melanocyte-stimulating hormone (α -MSH), an anorexigenic melanocortin neuropeptide¹. Based on the concept of molecular mimicry explaining the origin of cross-reactive autoantibodies, the microbial origin of α -MSH-reactive autoantibodies was confirmed by identification of an α -MSH-like autoantigen in *Escherichia coli*. Caseinolytic protease B (ClpB) homologue protein of 86 KDa was shown as the antigen responsible in *E.coli* for production of α -MSH-cross-reactive autoantibodies². Beside its antigenic action, ClpB was shown to have a direct satietogenic effect in mice when it was naturally produced by *E.coli* delivered to the mouse gut². Using an *in silico* approach it was shown that the unique discontinuous α -MSH-like epitope of ClpB is selectively present in all commensal and pathogenic species of the *Enterobacteriaceae* family of bacteria and contained the amino acid from the melanocortin pharmacophore sequence necessary for the activation of melanocortin receptors (MCR). Indeed, *in vitro* tests revealed that a 14 amino acid peptide fragment of *E.coli* ClpB containing α -MSH-like epitope has a full agonist activity on the MC1R. Furthermore, ClpB protein was detected in plasma in direct proportion to ClpB DNA in gut bacteria and its application on the hypothalamic slices increased electrical activity of anorexigenic proopiomelanocortin neurons known to express both MC3R and MC4R³. Thus, the natural presence of ClpB in the gut and plasma support the physiological role of ClpB produced by gut *Enterobacteriaceae* in the regulation of short-term and long-term satiety, respectively. These data open the possibility to improve satiety signaling and modulate altered feeding behavior by developing ClpB-containing probiotics.

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S16.3 26RFa: a neuropeptide involved in the hypothalamic regulation of energy homeostasis

Nicolas Chartrel¹, Mouna El Mehdi¹, Saloua Cherifi¹, Arnaud Arabo¹, Julie Maucotel¹, Hind Berrhamoune^{1,2}, Alexandre Benani³, Emmanuelle Nedelec³, Jérôme Leprince¹, Youssef Anouar¹, Gaëtan Prévost^{1,2} and Marie Picot¹

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The neuropeptide 26RFa and its receptor, GPR103, form a hypothalamic system known to strongly stimulate food intake. We recently showed that this system regulates glucose homeostasis at the periphery and that 26RFa acts as an incretin. As it is now well accepted that the hypothalamus is also involved in the control of glucose homeostasis, we investigated whether 26RFa may play a role in the hypothalamic regulation of glucose homeostasis. For this, we performed a glucose challenge concurrently with a central administration of 26RFa and we found that hypothalamic 26RFa exerts an anti-hyperglycemic effect similar to that observed peripherally, that is associated with an insulinotropic activity of the neuropeptide. In addition, this central anti-hyperglycemic effect of 26RFa is partially abolished by a central administration of a GPR103 antagonist and in 26RFa knock out mice. To understand how the 26RFa/GPR103 peptidergic system is involved in the central glycaemic regulation, we examined whether the expression and the secretion of 26RFa by hypothalamic neurons may be regulated by factors known to control glucose homeostasis. Using mouse hypothalamic explants, we showed that insulin strongly stimulates 26RFa secretion by hypothalamic neurons. We also found that hypothalamic 26RFa-neurons express the insulin receptor, that insulin induced c-fos expression in these neurons and that the central anti-hyperglycemic effect of insulin is partially abolished by the GPR103 antagonist and in 26RFa knock out mice. Together, these data reveal, for the first time, that the hypothalamic 26RFa/GPR103 system plays a pivotal role in the hypothalamic regulation of glucose homeostasis, notably by acting as a relay of insulin signaling in the brain.

S16.4 Impact of nutritional lipids on glial remodeling and neurons activity in the hypothalamus. Focus on MCH and orexin neurons

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Energy balance is finely regulated by the central nervous system (CNS): it integrates peripheral signals reflecting the energy status of the organism and in turn adapts food intake and energy expenditure in order to maintain a stable weight throughout adult life. The hypothalamus (HT) is one of the cerebral structures having a major role in the integration of those signals. Several studies show that obesity induced by a high fat diet (HFD) leads to inflammation at the level of HT which could cause obesity. Moreover, lipids contained in HFD might be directly responsible for the onset of the inflammatory response. At the cellular level, this inflammation is in part characterized by an activation of microglia cells and astrocytes in the HT. In rodent, recent studies show that hypothalamic proliferation of microglia cells and astrocytes is observed in the first 24 hours of consumption of HFD, well before the development of obesity, and seems to be reversible. We therefore assume that early glial activation would be an adaptive mechanism involved in the physiological regulation of energy balance and that overexposure to nutritional lipids could deregulate this inflammatory response and lead to obesity. In our study we observed an increase in the expression of the astrocytes and microglial cells markers (GFAP and Iba1 respectively) in the HT after 1 h of HFD consumption. Moreover we observed morphological modifications of microglial cells in the HT after 3h of HFD consumption. This remodeling is associated with differential activation of specific inflammatory markers and hypothalamic peptides involved in energy balance regulation. Our results suggest that inflammation induced by HFD consumption is a very early phenomenon which might be involved in the central regulation of energy balance. In the future, this glial remodeling will modulate using pharmacogenetic tools in order to establish the cascade of molecular and cellular events at the origin of CNS perturbations associated with obesity.

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Plenary lecture IV

Atlantes amphitheater

Impact of peripheral regulators of energy balance on the reward system

Suzanne L Dickson

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The Sahlgrenska Academy at the University of Gothenburg, Sweden

About the speaker



Suzanne L Dickson is Professor of Physiology /Neuroendocrinology at the Sahlgrenska Academy at the University of Gothenburg, Sweden (2004-). She graduated first from the University of Edinburgh with a BSc (Honours) in Pharmacology (1993) and then from the University of Cambridge (UK) with a PhD in Neuroendocrinology (1996). Prior to her current appointment, she held a tenured position as Senior Lecturer in Physiology at the University of Cambridge, UK (1996-2004). Her current research focus is the role of the endocrine gut-brain axis in appetite control. This work builds upon almost 20 years research exploring the central actions of ghrelin and, before its discovery, growth hormone secretagogues (now known to be ghrelin mimetics). Her work was first to show that these ghrelin mimetics act in the brain, that they target an orexigenic system (the neuropeptide Y cells in the hypothalamus), that they are important for food intake and fat accumulation and, more recently, target brain reward pathways involved in feeding control. She has coordinated three European Union large integrated projects, one of which (NeuroFAST) currently addresses the integrated neurobiology of food intake, reward and stress, involving 13 European research groups.

PL2 Impact of peripheral regulators of energy balance on the reward system

Suzanne L Dickson

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The brain's reward system is engaged in food intake, no matter whether this is driven by energy deficit or by the anticipated pleasure of a palatable meal. Human functional resonance imaging studies have revealed that brain pathways involved in (visual) food reward processing are regulated by dietary, hormonal and potentially other energy metabolic signals. The neural substrates engaged include the ventral striatum and rodent studies have shown that the ventral tegmental area is an important target for adiposity signals (such as leptin and insulin) and gut-derived hormones (such as ghrelin, PYY₍₃₋₃₆₎ and GLP-1). We have shown that the orexigenic hormone ghrelin engages the mesoaccumbal dopamine pathway [1] involved in incentive salience and that this is important for its effects on food motivated behavior [2, 3]. Ghrelin also alters food choice [4], food anticipatory [5] and other behaviors in ways that would lead us to question whether it is only a hunger hormone (for which its release and effects might be expected to be limited to a state of negative energy balance) or whether we should instead be considered an "appetite-stimulating" hormone. Although obesity is associated with reduced sensitivity/resistance to certain circulating hormones, we recently discovered the existence of a novel body weight sensing mechanism that appears to be independent of leptin and other circulating hormones and labelled it the "gravitostat", revealed through loading studies in rodents (i.e. implantation of weighted capsules)[6]. Loading is effective for reducing body weight in obese animals. The mechanism appears to include a weight sensing mechanism in bone and we are currently exploring the mechanisms, including neural circuits involved.

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S17. Clock mechanisms of mammalian SCN: From peptide to network interactions

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Princesa 1

Chair: **Raúl Aguilar-Roblero** (Mexico City, Mexico)

S17.1 Raúl Aguilar-Roblero (UNAM, Mexico):

From the molecular circadian oscillator to the circadian firing pattern in SCN neurons

S17.2 Charles N. Allen (Oregon Health & Science University, USA):

VIP and vasopressin signaling mechanisms in suprachiasmatic nucleus neurons

S17.3 Chris Colwell (University of California, Los Angeles, USA):

The role of neuropeptides in the photic regulation of the circadian system

S17.4 Hugh Piggins (University of Manchester, UK):

Intrinsic and Extrinsic Neuropeptide signaling in the suprachiasmatic circadian pacemaker

S17.5 Rae Silver (Columbia University, USA):

Re-visiting the core-shell connectome of the brain's clock in the hypothalamic suprachiasmatic nucleus

S17.1 From the molecular circadian oscillator to the circadian firing pattern in SCN neurons

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The suprachiasmatic nuclei (SCN) in the hypothalamus contain a primary circadian clock responsible for driving circadian rhythms in mammals. Neuronal firing rate in the SCN encodes information used by the clock to control different effector systems. The circadian SCN firing frequency varies from about 10 Hz during the subjective day to about 1 Hz during the subjective night. Molecular genetics studies have shown that transcriptional-translational feedback loops (TTFL) generate a molecular circadian rhythm controlling physiological and behavioral rhythmicity. Therefore, the molecular circadian oscillation must eventually be translated into a neuronal firing pattern in order to transmit a meaningful signal to other tissues and organs in the animal. This translation from molecular to electrical signal within SCN neurons is the beginning of the output pathway from the circadian clock to its effectors at all levels of the organism. Intracellular Ca^{2+} signaling through ryanodine receptor type 2 (RyR-2) are part of the clock output pathway. RyR-2 mRNA mouse SCN neurons shows a circadian rhythm with highest levels during the day. Pharmacological manipulation of RyR in mice or rat SCN neurons alters the free $[\text{Ca}^{2+}]_i$ in the cytoplasm and the spontaneous firing: activating RyRs with 100 nM ryanodine during the midnight caused a release of Ca^{2+} from the ER to the cytoplasm and increased spontaneous firing frequency in SCN neurons; on the other hand, closing of RyRs during midday by administration of dantrolene or 80 μM ryanodine decreased free cytoplasmic Ca^{2+} while reducing the spontaneous firing rate. In spite of these effects on intracellular Ca^{2+} and firing rate, in mice these RyR manipulations did not affect the molecular clock mechanism, that is neither activation nor closing the RyR had major effects on the period, phase or amplitude of PER2::LUC expression. The increase in firing rate by intracellular Ca^{2+} release does not likely involve calcium modulated potassium channels, because its main effect on neuronal SCN excitability is to reduce neuronal firing frequency by lengthening the interspike interval. On the other hand, Calcium regulated chloride channels (CaCCs) Anoctamin-1 and Anoctamin-2 has been recently identified in neurons. In neurons which express the $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ co-transporter (NKCC1) activation of CaCCs increase firing frequency, by reversion between the Cl^- equilibrium potential and the membrane resting potential leading to membrane depolarization by Cl^- extrusion from the cell. NKCC1 is present in SCN neurons where it has been shown to be involved in the excitatory effects of GABA. Immunohistochemistry and western blots studies from rat SCN indicate the presence of the CaCCs Anoctamin-1. This was confirmed by RT-qPCR which shows the expression of Anoctamin-1 mRNA in the SCN. These results clearly indicate the presence of this CaCC in the rat SCN. Thus, Intracellular Ca^{2+} mobilization from the endoplasmic reticulum convey a circadian signal from the molecular clock to the membrane in SCN neurons to modulate its firing rate. The mechanisms involved in this process are not yet clear, but Anoctamine-1 channels could be the membrane target of intracellular Ca^{2+} release to the increase in firing rate in SCN neurons during the day.

S17.2 Neuropeptide modulation of the intracellular calcium levels in suprachiasmatic nucleus neurons

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The neural network of suprachiasmatic nucleus (SCN) neurons play a critical role in the generation and entrainment of circadian rhythms. SCN neurons express a variety of neuroactive peptides including vasoactive intestinal peptide (VIP), vasopressin (AVP) and nociceptin/orphanin FQ (OFQ). VIP and AVP are expressed rhythmically within populations of SCN neurons. Modulation of the intracellular calcium concentration ($[Ca^{2+}]_i$) in SCN neurons is an important signal mediating photic entrainment and is an essential component of the feedback loops that generate circadian rhythms. Changes of $[Ca^{2+}]_i$ are a critical link coupling synaptic neurotransmission to changes of gene expression. Neuropeptides may regulate the $[Ca^{2+}]_i$ by multiple mechanisms, including activating Ca^{2+} permeable channels, membrane hyperpolarization and depolarization with activation of voltage-gated Ca^{2+} channels, and sequestration or release of Ca^{2+} from intracellular stores. We hypothesize that VIP, AVP, and OFQ regulation of Ca^{2+} homeostasis in SCN neurons may be necessary for synchronizing the phase of SCN neurons. However, very little is known of the role that Ca^{2+} plays in the neuropeptide regulation of the circadian clock. We, therefore, examined the effect of VIP, AVP, and OFQ on $[Ca^{2+}]_i$ in SCN neurons, by imaging multiple SCN neurons maintained in hypothalamic slices. VIP reduced the $[Ca^{2+}]_i$ in SCN neurons during the day but had little effect at night. During the day, VIP lowered the $[Ca^{2+}]_i$ to near nighttime levels while AVP elevated $[Ca^{2+}]_i$ during both the day and night. These data indicate that the VIP effects on $[Ca^{2+}]_i$ was dependent, and the AVP effects independent of the action potential firing activity state of the neuron. Like VIP, OFQ appeared to reduce the excitability of SCN neurons during the day with a reduction of the $[Ca^{2+}]_i$ to levels and variances more typical of neurons recorded during the night, and this was consistent with an inhibition of action potential firing by SCN neurons. Stimulation of the retinohypothalamic tract at frequencies that mimic environmental light input signaling evoked transient $[Ca^{2+}]_i$ elevations that were not altered by VIP suggesting that an SCN neuron inhibited by VIP can still respond to photic input as occurs following light applied during the night. AVP slowly elevated the $[Ca^{2+}]_i$ during both the day and night, without an apparent dependence on the firing activity state of the neuron. These data are consistent with the induction of Ca^{2+} -mediated transcriptional and translational changes to the clock, being at least in part dependent on the activity state of the neuron and its ability to rapidly alter $[Ca^{2+}]_i$, and modulation by neuropeptides via both external and internal sources of Ca^{2+} .

S17.3 The role of neuropeptides in the photic regulation of the circadian system

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The neuropeptide vasoactive intestinal peptide (VIP) is expressed at high levels in the neurons of the suprachiasmatic nucleus (SCN). While VIP is known to be important to the input and output pathways from the SCN, the physiological effects of VIP on electrical activity of SCN neurons are not well known. Here, the impact of the loss of VIP on SCN neurons was investigated in mouse slice cultures using several techniques. We found that the loss of VIP disrupted the rhythm in neural activity recorded in the SCN with less activity during the day and more action potentials during the night. The regularity of action potential generation was also disrupted as was the normal day/night rhythm in resting membrane potential. Whole cell patch clamp recording techniques were used to measure some key potassium currents with the magnitude of both the fast delayed rectifier and the IA currents found to be altered in the VIP deficient mice. Intracellular pathways were impacted by the loss of VIP with the day/night difference in both resting calcium and p-CREB lost in the mutant mice. Finally, measurements of rhythm in PER2:LUC bioluminescence indicates that the loss of VIP KO reduces the amplitude of these molecular rhythms as well as spatiotemporal organization of SCN circuit.

S17.4 Voluntary exercise stabilizes circadian rhythms in neuropeptide signaling deficient mice

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The suprachiasmatic nuclei of the hypothalamus (SCN) contain the brain's main circadian pacemaker that serves to generate and organize near 24h rhythms throughout the brain and body. Individual cells of the SCN contain an intracellular molecular clock and use GABA as well as neuropeptides to synchronize their internal timekeepers. Of the intrinsic SCN neuropeptides, vasoactive intestinal polypeptide (VIP) acting via its cognate receptor, VPAC₂, is arguably the most important. For example, genetically targeted loss of VIP or VPAC₂ (*Vip*^{-/-} and *Vipr2*^{-/-} mice respectively) grossly disrupts cell-cell communication in the SCN, leading to arrhythmia or aberrant non-24h activity in molecular, neuronal, behavioral and physiological processes. Animals with a neurochemically intact SCN are entrained to the external world by recurrent environmental cues such as varying levels of ambient light as well as by stimuli that evoke internal arousal. However, *Vip*^{-/-} and *Vipr2*^{-/-} mice have abnormal responses to light and exhibit pronounced alterations in their alignment to the external light-dark cycle. Therefore, we evaluated whether an arousal-promoting stimulus, scheduled voluntary exercise (SVE) in a running-wheel, could restore circadian rhythms in neuropeptide signaling deficient animals. Male C57Bl6j, *Vip*^{-/-}, *Vipr2*^{-/-}, and *Vip*^{-/-}*Vipr2*^{-/-} mice were individually housed in cages in which a computer controlled when they could voluntarily exercise in running-wheels. When running-wheels were freely accessible in constant dark (DD) conditions, all C57Bl6j mice exhibited near 24h (~23.6h) rhythms in feeding and drinking activity, whereas ~24h rhythms were very rarely observed in the different strains of neuropeptide signaling deficient mice. Subsequent exposure to 6h SVE per 24h rapidly re-organized the drinking and feeding rhythms of neuropeptide signaling deficient mice such that within 5-7 days of the onset of SVE, most of these mice showed 24h rhythms in these behaviors. Interestingly, when given the opportunity to freely exercise in the running wheels after 21 days of SVE, ~70% of *Vipr2*^{-/-} mice sustained ~24h rhythms in behavior, while only ~40% of *Vip*^{-/-} and *Vip*^{-/-}*Vipr2*^{-/-} mice did so. C57Bl6j mice did not synchronize to SVE over 21 days and showed minimal changes in circadian period post-SVE. To evaluate if and how exercise influenced electrical activity, we compared neuronal measures in SCN brain slices of *Vipr2*^{-/-} and C57Bl6j mice pre- and post-SVE. Neurophysiological assessment using voltage-clamp recordings and multielectrode arrays indicated that SVE reduced SCN GABAergic activity in both genotypes. Further examination of molecular timekeeping using mice crossed onto clock gene/protein reporter lines revealed that SVE stabilized 24h molecular rhythms and increased synchrony among the cell autonomous oscillators of the *Vipr2*^{-/-} SCN. No overt SVE-associated changes were detected in similar recordings of the molecular clock in corresponding wild-type SCNs. However in both genotypes, blockade of GABAergic signaling had fewer effects on SCN molecular rhythms post-SVE. These results indicate that voluntary exercise can down-regulate GABAergic signaling to improve and stabilize circadian rhythms in neuropeptide signaling deficient mice. Since a reduction in VIP expression is observed in the aged human SCN, this raises the possibility that regular physical exercise will have considerable utility as an intervention in age-related deterioration of human circadian rhythms.

S17.5 Re-visiting the core-shell connectome of the brain's clock in the hypothalamic suprachiasmatic nucleus

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A brain clock, constituted of ~20,000 peptidergically heterogeneous neurons, is located in the hypothalamic suprachiasmatic nucleus (SCN). While many peptidergic cell types have been identified, little is known about the connections among these neurons, and about their distinct functions. We first identified the precise localization of the major peptides in the mouse SCN, including arginine vasopressin (AVP) calretinin (CALR) met-enkephalin (ENK) gastrin-releasing peptide (GRP), vasoactive intestinal polypeptide (VIP). Next we sought to identify contacts among major peptidergic cell types in the SCN using triple-label fluorescent immunocytochemistry. To this end, contacts among VIP, GRP, and CALR cells of the core, and AVP and ENK cells of the shell were analyzed. We discovered that some core-to-shell and shell-to-core communications are specialized. Specifically, in wild-type mice, AVP fibers make extremely sparse contacts onto VIP neurons but contacts in the reverse direction are numerous. In contrast, AVP fibers make more contacts onto GRP neurons than conversely. All other cell types examined made reciprocal connections. We also explored the SCN in VIP deficient (VIP-KO) mice. Here, there are a reduced number of AVP-positive neurons in the SCN, but numbers are not altered in the paraventricular and supraoptic nuclei. Surprisingly, in VIP-KO mice, the number of AVP appositions onto other peptidergic cell types is not reduced. Colchicine administration restored numbers of AVP neurons to that of wild-type littermates supporting the hypothesis that AVP synthesis is reduced but not eliminated in neurons of VIP-KO animals. In summary, VIP has an important role in modulating AVP expression levels in the SCN. Importantly, there are peptidergic cell type specific communications between core and shell neurons in the SCN.

S18. Neuropeptide regulation of stress and its consequences

Princesa 2

Chair: James P. Herman (Cincinnati, Ohio, USA)

S18.1 **James P. Herman** (University of Cincinnati, USA):

Evidence of a role for glucagon-like peptide 1 in coordination of stress

S18.2 **Eric G. Krause** (University of Florida, USA):

Central Angiotensin II and its role in stress responding

S18.3 **Jom Hammack** (University of Vermont, USA):

Involvement of PACAP in stress-induced behavioral responses

S18.4 **Jan Deussing** (Max Plank Institute for Psychiatry, Munich, Germany):

Role of CRH in stress adaptation

S18.5 **Ki-Ann Goosens** (Massachusetts Institute of Technology, Cambridge, USA):

Ghrelin and resilience to chronic stress

S18.1 Evidence of a role for glucagon-like peptide 1 in coordination of stress

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Glucagon-like peptide-1 (GLP-1) is important in regulation of glucose homeostasis in the brain and periphery[1]. Within the CNS, GLP-1 is known to mediate anorectic processes, and is a key component of systems signaling visceral illness [2]. In brain, expression of the gene encoding the GLP-1 precursor protein proglucagon (ppg) is largely limited to neurons in the nucleus of the solitary tract and mediolateral medulla, regions that are involved in processing taste, ingestion, cardiovascular homeostasis and HPA axis stress responses, suggesting a major role in integration of stress and anorexia[1]. Consistent with this notion, GLP-1 infusion promotes cardiovascular and hypothalamo-pituitary-adrenocortical (HPA) responses to psychogenic, systemic and visceral stressors[1, 3, 4]. The importance of GLP-1 in stress integration is underscored by rapid, glucocorticoid-feedback dependent depletion of ppg mRNA and central GLP-1 peptide stores following stress[5, 6], suggesting a role for GLP-1 inactivation in stress recovery. These data suggest that hormonal cues (i.e., glucocorticoids) resulting from either metabolic or stressful stimuli are able to limit activation of GLP-1 signaling. Deletion of the GLP-1 receptor (glp1r) in the paraventricular nucleus of the hypothalamus (PVN) is able to attenuate the effects of stress on HPA axis output, cardiovascular reactivity and anxiety-related behavior, indicating a role of GLP-1 in coordinating systemic and psychological responses to adversity. Notably, loss of PVN glp1r also attenuates the impact of chronic stress on the organism, invoking GLP-1 as a contributing factor to chronic stress pathologies [4]. Overall, the data suggest that over the course of evolution, GLP-1 neurons originally tasked with generation of food aversion have been coopted to act in generalized whole-organism stress activation. The data lend additional evidence to support a role for peptides in parallel functional integration of biological functions across multiple organ systems, including the brain.

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S18.2 Central Angiotensin II and its role in stress responding: Shedding light on brain angiotensin receptor signaling to understand and alleviate stress-related disease

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Affective and cardiovascular disorders are highly co-morbid and stressful life events are predictors of their onset. While augmented renin-angiotensin-system activity is known to promote cardiovascular disorders, there is increasing interests in how angiotensin signaling in the brain may contribute to the onset of affective disorders, like anxiety, depression and PTSD. This series of experiments uses genetically modified mice to characterize a unique set of 'angiotensin sensitive' neurons within the paraventricular nucleus of the hypothalamus that coordinate cardiovascular, endocrine and behavioral responses to psychogenic stress. The implication is that angiotensin signaling within the paraventricular nucleus of the hypothalamus may determine susceptibility or resiliency to stress-related diseases, like affective and cardiovascular disorders.

S18.3 Pituitary adenylate cyclase activating polypeptide (PACAP)) in the bed nucleus of the stria terminalis (BNST): A nexus for stress and emotion

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²Department of Neurological Sciences, University of Vermont, Burlington VT USA

Exposure to stressful stimuli has been argued to play an important role in the etiology of anxiety disorders. Consistent with this role, increases in anxiety-like behavior are often observed in rodents repeatedly exposed to environmental stressors. Several brain nuclei have been implicated in coordinating the autonomic, endocrine and behavioral response to stressor exposure. In particular, the bed nucleus of the stria terminalis (BNST) has been argued to mediate anxiety-like behavioral responding to long-duration anxiogenic stimuli, coordinate autonomic and endocrine stress responses, and also play a critical role in stress-related drug relapse. Hence, the BNST may be a critical nodal structure whereby chronic stressor exposure produces an anxiogenic behavioral profile and alters stress responding. We have shown that chronic stress substantially and selectively increases pituitary adenylate cyclase-activating peptide (PACAP) and its cognate PAC1 receptor in the BNST oval nucleus. PACAP increases the excitability of BNST neurons and may also enhance indices of BNST neuroplasticity. PACAP infusion into the BNST produces many of the consequences of chronic stress in male and female rats, and BNST PACAP receptor inhibition during chronic stress prevents many stress-related behavioral consequences. These data corroborate gene association studies showing PACAP dysregulation in several stress-related disorders, including post-traumatic stress disorder, and suggest that the BNST may be a critical brain region mediating these effects. Hence, BNST PACAP expression may represent a critical nexus by which chronic stress alters emotional responding and stress-related psychopathology.

S18.4 Dissecting CRH/CRHR1 circuits modulating anxiety-related behavior and stress vulnerability

Jan M. Deussing

Max Plank Institute for Psychiatry, Munich, Germany

The role of hypothalamic corticotropin-releasing hormone (CRH) as an indispensable initiator of the hypothalamic-pituitary-adrenal axis is well defined. However, we are just starting to comprehend the function of extrahypothalamic CRH with regards to emotionality and behavioral stress responses. In this respect, we could previously demonstrate that anxiety-related behavior is bidirectionally modulated by CRH receptor type 1 (CRHR1)-controlled anxiogenic glutamatergic and anxiolytic dopaminergic circuits. Interestingly, the identity of CRH-releasing neurons and sites of CRH action that modulate anxiolytic behavioral responses have not been fully established yet. Using neurochemical and mouse genetic tools we identified that cortical and limbic CRH is primarily expressed in GABAergic neurons, which exhibited distinct morphologies depending on the brain region. Anterograde tracing studies of forebrain limbic CRH neurons revealed GABAergic long-range projecting axons, which innervated distant brain regions including the ventral tegmental area, which harbors the majority of CRHR1-expressing dopaminergic neurons. We found that deletion of CRH from these GABAergic long-range projection neurons enhanced anxiety and fear memory expression, implicating that this specific CRH circuit is required under physiological conditions to maintain a positive emotional state. Assessment of dopaminergic neurotransmission in mice lacking CRH in GABAergic projection neurons revealed reduced baseline dopamine release in the PFC, suggesting that a subset of CRH-expressing GABAergic projection neurons in the limbic forebrain target CRHR1 on dopaminergic neurons to modulate emotional behavior by regulating dopaminergic neurotransmission. In conclusion, our results reveal a previously unidentified anxiety-suppressing CRH circuit which regulates DA release to ultimately modulate anxiety-related behavior.

S18.5 Ghrelin as a stress hormone

Ki Goosens

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Although prolonged stress exposure is a risk factor for psychiatric illnesses such as post-traumatic stress disorder (PTSD), it is not clear what drives this maladaptive consequence of stress. Here, I will present a series of experiments showing that fear circuits are exquisitely tuned by ghrelin, a peripheral peptide hormone, and that chronic stress-induced changes in ghrelin drive the vulnerability to excessive fear that typifies PTSD. I will discuss our findings that show that endogenous peripheral acyl-ghrelin robustly inhibits fear memory consolidation in rodents through actions in the amygdala and, surprisingly, accounts for virtually all inter-individual variability in long-term fear memory strength. In contrast, I will show that rodents exposed to chronic stress display chronically elevated acyl-ghrelin and that this promotes the overconsolidation of fear memories by inducing central ghrelin resistance, a novel form of metabolic resistance. I will also discuss our results showing that this stress pathway exists in humans. Together, these data reveal intimate links between ghrelin and fear, and establish a novel mechanism by which chronic stress leads to disinhibition within fear circuits.

S19. Drug design for the apelin receptor across diverse pathophysiological indications: Peptide drug development strategies from bench and clinic to approval

Supported by AMGEN

Princesa 3

Co-Chairs: **C. Llorens-Cortes** (Paris, France), **Marsault and M. Auger-Messier** (Sherbrooke, Canada), **Éric Marsault** (Université de Sherbrooke, Canada):

S19. 1 **Catherine Llorens-Cortes** (INSERM U1050, Collège de France, France):

Development of original metabolically-stable apelin-17 analogs with aquaretic and cardiovascular effects

S19. 2 **Gavin Oudit** (University of Alberta, Canada):

Enhancing the apelin-apelin receptor axis as a novel therapy for heart failure

S19. 3 **Olivier Lesur** (Université de Sherbrooke, Canada):

Potential of apelin and ELABELA in the treatment of sepsis

S19.4 **Éric Marsault** (Université de Sherbrooke, Canada):

Understanding and exploiting the structure-signaling relationship of apelin

S19.5 **Hyung Chun** (Yale University School of Medicine, New Haven, Connecticut USA):

Engaging Apelinergic Pathway for Cardiometabolic Health

S19.1 Development of original metabolically stable apelin-17 analogs with aquaretic and cardiovascular effects

Adrien Flahault¹, Romain Gerbier¹, Xavier Iturrioz¹, Nadia De Mota¹, Rodrigo Alvear-Perez¹, Jean-Francois Margathe², Lucie Esteouille², Hubert Dabire³, Alain Berdeaux³, Marcel Hibert², Dominique Bonnet² and **C. Llorens-Cortes¹**

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The search for a G protein coupled receptors (GPCR) specific for angiotensin III led us to isolate the murine homolog of the human orphan receptor, APJ, that shares 31% sequence identity with the angiotensin II receptor type 1. Apelin is a neuro-vasoactive peptide which inhibits vasopressin release, thus increasing aqueous diuresis, decreases blood pressure (BP), improves cardiac contractility and reduces cardiac loading. Thus, apelin plays a key role in maintaining body fluid homeostasis and cardiovascular functions, and its receptor is an interesting potential new target for therapeutic research and drug design.

Since the half-life of apelin in the blood circulation is in the minute range, we therefore developed metabolically stable apelin-17 (K17F) analogs. We generated LIT01-196 by the original addition of a fluorocarbon chain to the N-terminal part of K17F. This analog was much more stable in plasma (half-life > 24 h) versus K17F (4.6 min), displayed a subnanomolar affinity for the apelin receptor and behaved as a full agonist with regard to cAMP production, ERK phosphorylation and apelin receptor internalization. *Ex vivo*, this compound induced vasorelaxation of rat aortas and glomerular arterioles, respectively, precontracted with norepinephrine and angiotensin II. *In vivo*, following intracerebroventricular administration in water-deprived mice, LIT01-196 was 160 times more efficient than K17F at inhibiting systemic vasopressin release. Given systemically (nmol/kg range) by subcutaneous route in alert normotensive rats, LIT01-196 potently increased urine output and induced a profound and sustained decrease in BP. The beneficial effects of LIT01-196 were also investigated in a model of hyponatremia induced by AVP and in an experimental model of hypertension, the deoxycorticosterone acetate (DOCA)-salt hypertensive rat.

This new compound, which favours aqueous diuresis and decreases BP, represents a promising candidate for the treatment of hypertension and water retention/hyponatremic disorders.

S19.2 Role of apelin/apelin receptor axis in heart disease: Therapeutic role of apelin analogues

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The apelin peptide system is widely distributed throughout the human body and is a critical mediator of cardiovascular homeostasis. Activation of the apelin receptor by its cognate peptide ligand, apelin, induces a wide range of physiological effects, including vasodilation, cardiac contractility, angiogenesis, and regulation of energy metabolism and fluid homeostasis. The apelin/apelin receptor is also implicated in pathologies including atherosclerosis, hypertension, coronary heart disease, heart failure, diabetes, obesity, and cancer, making it a promising therapeutic target. Considering the potential therapeutic effects by modulation of the apelin/apelin receptor system, research is expanding to develop novel therapies that inhibit degradation of endogenous apelin peptides and augment stable agonists and antagonists to more efficiently interfere in the apelin/apelin receptor system. Given the role of apelin/apelin receptor in cardiovascular diseases, an increased understanding of the cardiovascular actions of apelin/apelin receptor system will help to develop novel therapeutic interventions for cardiovascular diseases. The apelin/apelin receptor signaling represent a relatively new therapeutic axis for the potential treatment of cardiovascular disease. Angiotensin converting enzyme 2 (ACE2) and neutral endopeptidase (NEP) are two key proteases which cleaves and inactivates apelin peptides. The ability of NEP inhibition to can explain the therapeutic benefits of the newly approved heart failure therapy, LCZ696 (EntrestoTM), which includes the NEP inhibitor, sacubitril. We have designed and synthesized novel and potent apelin analogues which are resistant to degradation by proteases as potential drugs for cardiovascular diseases.

S19.3 Potential of apelin and ELABELA in the treatment of sepsis

Olivier Lesur

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Apelin-13 was recently proposed as an alternative to the recommended β -adrenergic drugs for supporting endotoxin-induced myocardial dysfunction. Since apelin-13 signals through its receptor (APJ) to exert singular inotropic/vasotropic actions and to optimize body fluid balance, this candidate pathway might benefit septic shock management. Whether the newly-discovered ELABELA, a second endogenous ligand of the APJ receptor highly expressed in the kidney, further improves cardio-renal impairment remains unknown.

Polymicrobial sepsis-induced cardiac dysfunction was produced by cecal ligation puncture (CLP) in adult rats to assess hemodynamic efficacy, cardioprotection and biomechanics under acute or continuous infusions of the apelinergic agonists ELABELA or apelin-13 vs. normal saline. In this context of experimental sepsis, breakdown ACE2 and neprilysin (NEP) enzymatic activities were also measured in heart & renal tissues and in plasma, as well as the apelinergic peptide contents in bloodstream. Apelinergic peptide's infusion offers original and distinctive impacts with added-value in protecting the cardio-renal axis in sepsis.

S19.4 Understanding and exploiting the structure-activity relationship of apelin

Éric Marsault

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Apelin is the endogenous ligand of the APJ receptor, a class A G protein-coupled receptor. It represents a promising target for the treatment of various pathophysiological conditions, particularly those associated with cardiovascular dysregulations in acute or chronic settings. In an effort to decipher the structure-activity relationship of apelin-13, we replaced several positions with with unnatural amino acids to enhance both affinity and to better understand the structural determinants of signaling. Additionally, modifications to the peptide's conformation via macrocyclization led to a better understanding of the required topology for receptor interaction. These modifications have led to low pM agonists of the APJ receptor, with greatly improved plasma stability. Finally, we explored how these modifications impact vascular pressure and cardiac performance in rats, using *in vivo* measurements of pressure, *ex vivo* measurements of inotropy in the Langendorff model, as well as their efficacy in septic shock. These new ligands represent very promising pharmacological tools to link the intracellular signaling signatures to desired physiological responses.

S19.5 Engaging the apelinergic pathway for cardiometabolic health

Hyung Chun

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Cardiovascular complications of diabetes continue to remain a huge burden on the health care system. Novel therapeutic strategies that can couple improvements in glycemic control with reduction of cardiovascular complications would represent a significant advancement in how we manage these challenging patient population. Investigations of the apelin-APLNR signaling pathway have identified this to be a highly metabolic and vasculo-protective pathway, with much of its impact being driven by the endothelial cells. Recent findings related to the downstream signaling mechanisms of apelin-APLNR signaling, as well as future directions towards therapeutic application of this pathway, will be discussed in detail.

S20. Young Investigator Symposium: New investigators embarking on their independent careers sketch out their current progress and plans for future research

Princesa 4

Co-chairs:

Vito S. Hernández (Mexico City, México) and **André Mecawi** (Rio de Janeiro, Brazil)

S20.1 **André Mecawi** (Federal Rural University de Rio de Janeiro, Brazil):

Ghrelin effects on the paraventricular nucleus and basolateral amygdala neurons

S20.2 **Sung Han** (Salk Institute for Biological Studies, La Jolla, California, USA):

CGRP: the main transmitter of affective pain signals to the amygdala

S20.3 **Lorraine Jaimes-Hoy** (Instituto de Biotecnología, UNAM, Mexico):

Early life stress curtails the hypothalamic-pituitary-thyroid axis cold response in adulthood

S20.4 **Zhihua Gao** (Zhejiang University School of Medicine, Hangzhou, China):

Reconstructing the hypothalamo-neurohypophysis connections by viral tracing.

S20.5 **Vito S. Hernández** (Facultad de Medicina, UNAM, Mexico):

Extra-neurohypophyseal axonal projections from individual vasopressin-containing magnocellular neurons in rat hypothalamus

S20.1 Ghrelin effects on the paraventricular nucleus and basolateral amygdala neurons

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For an animal to expose itself and search for food, the drive to eat has to surpass the drive to stay safe. Therefore, hunger decreases anxiety. The mechanisms of this response have not been completely elucidated. Ghrelin is an orexigenic peptide secreted by the stomach at negative energy balances; however, functions of ghrelin aside from the control of food intake have been demonstrated. In this regard, ghrelin has been shown to affect anxiety-like behavior in rats. For such, ghrelin has to influence the brain; however, mechanisms of central ghrelin actions are controversial. Therefore, evaluate the effects of food deprivation, a physiological stimulus that increases plasma ghrelin, and intraperitoneal administration of ghrelin on exploratory and anxiety behavior in rats. Food deprivation did not affect exploratory activity, but increased time spent in open arms in the elevated plus maze, therefore demonstrating a decreased anxiety-like behavior, independent of exploratory activity. Peripheral ghrelin was not capable of changing performance in the EPM in sated rats. Thus, food deprivation shows an anxiolytic effect that is independent of peripheral ghrelin. Then, we assessed the effects of ghrelin in nuclei related to the control of food intake and anxiety-like behavior. We used extracellular and whole cell patch clamp to assess the influence of ghrelin on the excitability of paraventricular nucleus neurons. After intracellular recordings, cells were collected and used in RT-PCR to assess the presence of mRNA for four target genes: vasopressin (AVP), oxytocin (OT), thyrotropin (TRH) and corticotropin (CRH) releasing hormones. Ghrelin induced responses on most PVN neurons (91/136 on extracellular and 62/95 on intracellular recordings) with both depolarisations and hyperpolarisations. When the different PVN neuronal populations were analyzed, neuroendocrine neurons showed mixed responses, magnocellular neurons showed mostly unresponsive neurons (14/28) and pre-autonomic neurons showed a majority of depolarisations (11/18). The single-cell RT-PCR showed that the majority of TRH-expressing (4/5) and CRH-expressing (3/4) neurons are hyperpolarized in response to ghrelin and that AVP mRNA is expressed in most (30/45) neurons in the PVN. BLA neurons were assessed on whole-cell patch-clamp and depolarize in response to ghrelin (15/31). Thus, our data shows that ghrelin is able to change both PVN and BLA neurons activity, probably contributing to the food intake control and the related behavioral responses.

S20.2 CGRP: the main transmitter of affective pain signals to the amygdala

Sung Han

Salk Institute for Biological Studies, La Jolla, CA.

Learning to avoid painful situations is critical for survival of all organisms. Association of a neutral stimulus (e.g., a tone) with a painful stimulus (e.g., a foot shock) results in a stable memory such that the tone alone will elicit a defensive response (immobility or freezing). In mammals, this form of associative aversive learning requires the amygdala. Although the pain is the main driver of aversive learning, the circuit-based mechanism by which pain information is conveyed and processed to the amygdala is not well-understood. We found that neurons expressing calcitonin gene-related peptide (CGRP) in the brainstem and the thalamus are critical for relaying pain signals to the amygdala during aversive learning. Genetic silencing of these CGRP neurons attenuated pain responses and memory formation, and optogenetic stimulation of CGRP neurons produced robust aversive behaviors. These results provide compelling evidence that CGRP conveys pain signals to the amygdala through brainstem and thalamus during aversive learning.

S20.3 Early life stress curtails the hypothalamic-pituitary-thyroid axis cold response in adulthood

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In most mammals, their early life is spent in close contact with the mother. Early maternal separation causes significant stress in the pups, and shapes their behavioral and neurochemical phenotype in adulthood. The consequences of this stress, such as the hyperreactivity of the hypothalamic-pituitary-adrenal axis are expressed in adulthood and persist for life (1). Effects of maternal separation in adulthood extends to the hypothalamic-pituitary-thyroid axis (HPT), early life stress reduces the fasting-induced inhibition of the HPT axis activity compared to non-handled male rats (2).

Basal and the cold-induced activity of the HPT axis is inhibited by a previous stress experience in adult male rats (3). In the present study we evaluated if maternal separation during the lactation period alters the HPT axis response to a metabolic challenge such as cold exposure. Wistar dams were divided into a non-handled (NH) or a maternal separation (MS) group. From PD 2-21, male pups in the MS group underwent maternal separation for 3h daily. At PD 23, rats were housed 4/cage and fed ad libitum until adulthood. At PD60, rats were housed in pairs, and half of the N and MS rats were exposed for 1, 2 or 4 h to 4°C. Coronal brain sections were cut through the rostrocaudal extent of the paraventricular nucleus of the hypothalamus (PVN) for quantitative *in situ* hybridization of levels of pro*Trh* mRNA. Since cold induces the thermogenic activity in brown adipose tissue (BAT), we quantified the expression of genes involved in the activity of BAT in response to cold exposure, by RT-PCR. Serum hormones were analyzed by radioimmunoanalysis or ELISA.

NH rats exposed to 1 h of cold showed increased pro*Trh* mRNA levels in medial PVN and serum TSH and corticosterone concentration as reported; while TSH remained increased after 4 h, and T3 increased at 2 and 4 h. In contrast, MS rats did not show an increase in the activity of the HPT axis, suggesting a blunted response to cold in animals submitted to maternal separation. In BAT, 1 h of cold exposure increased the expression of deiodinase type 2 and the specific marker of thermogenesis, UCP1, in NH rats while in MS rats, cold-induced expression of UCP1 was delayed to 4 h. In conclusion, maternal separation alters the HPT axis response to an acute stress stimulus such as cold exposure in adulthood.

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S20.4 Reconstructing the hypothalamo-neurohypophysis connections

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The hypothalamo-neurohypophysis (HP-NH) constitutes one of the most important components in the neuroendocrine system. It is well-known that the hypothalamic magnocellular neurons in the paraventricular hypothalamic nucleus (PVH), supraoptic nucleus (SON)) project to the NH, where they release vasopressin (AVP) and oxytocin (OXT) into the bloodstream. Although studies have shown that scattered magnocellular neurons in other hypothalamic areas also project to the NH, a clear overview on the HP-NH connection is missing. Using recently developed virus-based retrograde (AAV-Retro-GFP) and anterograde (AAV-GFP) tracing tools, we systematically reconstructed the comprehensive rat atlas of the neuroendocrine system originating from the HP to the NH. Bidirectional tracing revealed that multiple hypothalamic regions including the lateral hypothalamus area (LHA), the bed nucleus of the stria terminalis (BST), the preoptic area (POA) and several accessory nuclei (AN) directly projected to the NH. These neurons were also labelled by peripherally administered Fluoro-Gold, which was selectively taken-up by neurons projecting beyond the blood brain barrier, verifying their neuroendocrine features. The heterogeneity of the nuclei was revealed by distinctive soma volume distribution patterns and further confirmed by immunostaining analysis with AVP and OXT antibodies. The neuropeptide identity was further investigated with fluorescent *in situ* hybridization (FISH). Quantification showed the AN accounted for more than one third of the total magnocellular population, consisting of the nucleus of anterior commissure (AC), the anterior fornix nucleus (AF), the circular nucleus (CiN), the posterior fornix nucleus (PoF), the nucleus of medial forebrain bundle (Nmfb) and the retrochiasmatic part of supraoptic nucleus (RCN). We also traced the fiber-projection of single cells with Amira software to illustrate a complete HP-NP network in a three-dimensional atlas. Together, our study provides a mesoscopic level mapping of the HP-NH connections and new insights into further understanding the neuroendocrine regulation.

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S20.5 Extra-neurohypophyseal axonal projections from individual vasopressin-containing magnocellular neurons in rat hypothalamus

Vito S. Hernández and Limei Zhang

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Conventional neuroanatomical, immunohistochemical techniques, and electrophysiological recording, as well as in vitro labeling methods may fail to detect long range extra-neurohypophyseal-projecting axons from vasopressin (AVP)-containing magnocellular neurons (magnocells) in the hypothalamic paraventricular nucleus (PVN). Here, we used in vivo extracellular recording, juxtacellular labeling, post-hoc anatomo-immunohistochemical analysis and camera lucida reconstruction to address this question. We demonstrate that all well-labeled AVP immunopositive neurons inside the PVN possess main axons joining the tract of Greving and multi-axon-like processes, as well as axonal collaterals branching very near to the somata, which project to extra-neurohypophyseal regions. The detected regions in this study include the medial and lateral preoptical area, suprachiasmatic nucleus (SCN), lateral habenula (LHb), medial and central amygdala and the conducting systems, such as stria medullaris, the fornix and the internal capsule. Expression of vesicular glutamate transporter 2 was observed in axon-collaterals. These results, in congruency with several previous reports in the literature, provided unequivocal evidence that AVP magnocells have an uncommon feature of possessing multiple axon-like processes emanating from somata or proximal dendrites. Furthermore, the long-range non-neurohypophyseal projections are more common than an "occasional" phenomenon as previously thought.

Workshop

Peptide-based drug discovery for CNS disorders: Avenues and barriers

Co-Chairs:

William Z. Potter (Bethesda, USA) and **David Vaudry** (Rouen, France)

W1 William Z. Potter (National Institute of Mental Health, NIH, USA):

CNS peptide and their receptors as drug targets: creating pre-competitive consortia for target engagement and proof-of-concept for CNS disease targets

W2 Mary R. Lee (National Institute on Alcohol Abuse and Alcoholism, NIH, USA):

Peptide penetration of blood-brain-barrier after administration at olfactory and peripheral sites

W3 Michael J. Brownstein (Azevan Pharmaceuticals, Bethlehem, PA, USA):

Vasopressin: Old dog, new tricks

W4 David Lovejoy & Dalia Barsyte-Lovejoy (UT, Canada & Protagenic Therapeutics Inc. New York USA):

Multiple in vivo peptide delivery approaches with the corticotropin-releasing hormone (CRH) and secretin family-like peptide, teneurin C-terminal associated peptide (TCAP), for energy metabolism and affective disorder treatments

W5 David Vaudry (INSERM, Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Normandy University, Rouen, France):

PACAP intranasal delivery represents an efficient approach for the treatment of stroke and Huntington disease

W1 CNS peptides and their receptors as drug targets: creating pre-competitive consortia for target engagement and proof of concept for CNS disease targets

William Z Potter

National Institute of Mental Health, Rockville, MD, USA

The development of novel drugs for CNS disorders has been much more difficult than anticipated given the enormous progress in neuroscience with the maturation of the field of molecular biology. This has been especially true with regard to translating basic science on the many roles of peptides and their receptors in the brain into useful therapeutic agents. Even in such cases as exploring the potential of CRF-1 antagonists, the field has been frustrated with ruling in or out the potential of this mechanism. The underlying problem has been the gaps in our ability to know whether or not a particular compound sufficiently engages the molecular target in question to be sure of testing a hypothesis of action. Although such tools as PET ligands can relatively easily be made for orthosteric antagonists of many GPCRs adequate ones for the peptide receptors of greatest interest have eluded even large pharmaceutical companies with teams of skilled synthetic chemists.

There are a growing number of pre-competitive consortia involving shared investment in generating drug development tools that can benefit all stakeholders without jeopardizing the business potential of proprietary patented compounds. These range from standardization of preclinical testing paradigms through analogous measures of brain function in animals and humans (e.g. fMRI) to activation of domains of cognitive function that are generally viewed as only testable in humans and perhaps non-human primates.

To date, no consortia are specifically focused on refining the different tools for assessing aspects of brain function (PET, fMRI, MRS, DTI, EEG and MEG) to increase their ability to detect effects of compounds that affect peptide function in the brain at the level of their respective receptors. There are, however, several consortia focused on the potential of these measures as biomarkers of disease state. This talk will highlight how the work in ongoing or planned biomarker consortia might be leveraged to provide needed tools for detecting effects of peptidergic compounds in human brain, especially fMRI and EEG/MEG.

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Brady LS, Potter WZ. Public-private partnerships to revitalize psychiatric drug discovery. *Expert Opin Drug Discov.* 2014 Jan;9(1):1-8. doi: 10.1517/17460441.2014.867944. Epub 2013 Dec 6.

W2 Peptide penetration of blood-brain-barrier after administration at olfactory and peripheral sites

Mary R. Lee and Lorenzo Leggio

Section on Clinical Psychoneuroendocrinology and Neuropsychopharmacology, National Institute on Alcohol Abuse and Alcoholism, NIH, USA

The peptides oxytocin (OT) and ghrelin interact with dopamine in the mesocorticolimbic reward system to modulate motivated behaviors [1, 2]. As such, currently their receptors are targets for medication development for various neuropsychiatric disorders, including obesity and addiction. Accordingly, we have investigated the CNS penetrance of OT [3] as well as a novel ghrelin receptor inverse agonist, PF-5190457[4] as potential medications for alcohol use disorder. In a nonhuman primate study, we administered deuterated (d5) OT (80 IU) by the intravenous (IV) and, the clinically important, intranasal (IN) routes of administration. We used a highly sensitive and specific quantitative liquid chromatography-mass spectrometry-tandem mass spectrometry assay (LC-MS/MS) that measures endogenous (d0) and administered deuterated (d5) OT in the cerebrospinal fluid (CSF) and plasma. D5 OT administered via the IN and IV routes reaches the CSF. Endogenous CSF OT concentrations are unaffected following peripheral administration of d5OT. The IN compared to the IV route is not a privileged pathway to the CSF as assessed by pharmacokinetic parameters, C_{max}, T_{max}, and AUC for blood and CSF sampling over 1 hour post-d5 OT (80IU) administration. In preparation for a translational study examining the safety and tolerability of the ghrelin receptor inverse agonist, PF-5190457 administered with alcohol, we determined the doses and pre-treatment times for PF-5190457 in a rodent study. Wistar rats were injected with PF-5190457 given at 5 doses (0.3, 1, 3, 10 and 30 mg/kg i.p.). At 15 and 60 min post administration, brains were perfused with saline to remove any residual blood. Receptor occupancy was indirectly estimated from: 1) PF-5190457 concentrations in plasma and brain tissue measured by ultra-performance liquid chromatography-mass spectrometry-tandem mass spectrometry (UPLC-MS/MS) assay[5]; and 2) IC₅₀ value from rat GHS-R1a filter study and unbound fraction of PF, measured using equilibrium dialysis.

Supported by NIAAA/NIDA Intramural Research Programs

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W3 Vasopressin: Old Dog. New Tricks

Michael J. Brownstein

Azevan Pharmaceuticals, Bethlehem, Pennsylvania, USA and AARDVARx Research and Development, Rockville, Maryland, USA

Arginine vasopressin (AVP) exerts its effects through three distinct receptors: V1a, V1b, and V2. Well known actions include regulation of water homeostasis through activation of V2 receptors in the kidney and effects on blood pressure and ACTH secretion mediated by V1a and V1b receptors, respectively. Furthermore, in the past 20 years, the peptide's behavioral effects have received considerable attention. Azevan Pharmaceuticals has developed first-in-class, orally available, CNS-active V1a receptor antagonists. In animal models, these compounds inhibit anxiety, aggressive behavior, conditioned fear responses, and “depression”. In humans, they appear to attenuate the response of vasopressin-sensitive circuits that are activated by angry faces (1). On the strength of these studies, we have advanced one of our compounds, SRX246, into Phase 2 clinical trials in patients with Intermittent Explosive Disorder, PTSD, and Huntington's disease (2-4).

Recently we have found another potential use for SRX246 as a novel intervention for Traumatic Brain Injury. This potential use is based on results showing that after moderate-intensity closed head trauma, treatment of rats with a V1a antagonist for 5 days blocked edema formation, prevented cognitive decline, and enhanced resting state functional connectivity in hippocampal circuits.

Targeting the V1b receptor may also be useful. When we discovered vasopressin receptor mRNAs and their protein products in hematopoietic stem cells (HSCs), we wondered if AVP might stimulate red blood cell production (following hemorrhage, for example) at the same time that it drives conservation of water. This appears to be the case, and the V1b receptor, which is the most abundant subtype on HSCs, is likely to mediate the action of AVP on erythropoiesis. Thus, a V1b agonist might be used to correct anemia, especially in patients who cannot take Erythropoietin.

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W4 Multiple in vivo peptide delivery approaches with the corticotropin-releasing hormone (CRH) and secretin family-like peptide, teneurin C-terminal associated peptide (TCAP), for energy metabolism and affective disorder treatments.

David A. Lovejoy^{1,3} and Dalia Barsyte-Lovejoy^{2,3}

¹Department of Cell and Systems Biology

²Structural Genomics Consortium, University of Toronto, Toronto, Ontario Canada

³Protogenic Therapeutics Inc. New York USA

The secretin family of peptides includes closely related peptides such as vasoactive intestinal peptide, (VIP), glucagon, growth hormone-releasing hormone (GHRP) and pituitary adenylate cyclase-activating peptide (PACAP) but also encompasses the calcitonin and CRH family of peptides as evidenced by the structural similarity of their respective receptors. Current studies indicate that the teneurin-C-terminal associated receptors (TCAP) and their putative ligands, latrophilins, are ancestral members of the secretin ligand-receptor family. TCAP, a 41-mer peptide, is highly mobile in vivo and is efficacious for the regulation of glucose metabolism and control of affective disorder models in rodents. This soluble peptide can be easily prepared for analytical- and clinical-compatible solutions via intravenous, (IV) subcutaneous (SC) and potentially nasal administration routes. Pharmacokinetic studies indicate that the plasma half-life of the peptide is about 10 minutes with respect to SC administration, yet single doses at nanomolar concentrations induce significant decreases in plasma insulin and glucose after one week. Similarly, fPET studies of equivalent concentrations show a significant increase in ¹⁸F-deoxyglucose uptake into the cortex and diencephalic regions after 3 days. IV and nasal administration shows uptake of ¹²⁵I-TCAP into limbic structures. Together, these studies indicate that TCAP is a highly mobile peptide in tissues in plasma and its function may provide insight into the energy-associated neurological applications of the secretin, CRH and calcitonin family of peptides.

W5 PACAP intranasal delivery is efficient for the treatment of stroke and Huntington Disease

David Vaudry^{1,2}, N. Cabezas-Llobet,³ A. Cherait¹, L. Vidal-Sancho^{3,4}, M. Masana³, A. Fournier⁵, J. Leprince^{1,2}, J. Alberch⁴ and X. Xifró^{3,4}

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Many studies have shown that in the brain PACAP exerts neuroprotective and neurotrophic activities. However PACAP and its three receptors are also widely expressed in peripheral tissues where they exert many biological functions. Thus, administration methods which target the brain without affecting other organs can avoid the occurrence of deleterious side effects. In this context, intranasal PACAP administration could represent an interesting method of treatment. Indeed, in an acute model of neuronal degeneration, it has been shown that a single intranasal administration of low quantity of PACAP protects the brain from middle cerebral artery occlusion. PACAP remains efficient when administered up to 6 hours after stroke, and functional studies highlight that a beneficial effect is still observed 8 days after the ischemia. Besides stroke, which induces a massive cell death in a short period of time, chronic intranasal administration of PACAP also appeared efficient to enhance hippocampal synaptic plasticity and improve memory performance in an Huntington's disease model. Taken together, these data highlight the potential of intranasal delivery of PACAP for neuroprotection in various pathological situations.

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Round table (RT): Pioneers of Regulatory Peptide Research:

Drawing inspiration from the past and glimpsing the future

Co-Chairs:

Lee E. Eiden (Bethesda, USA) and **Limei Zhang** (Mexico City, Mexico)

This portion of the RegPep2018 program highlights the unique contributions of four outstanding pioneers of regulatory peptide research who have profoundly altered our understanding of the role of peptides, arising from their prohormone precursors within neurons and endocrine cells (P. Lowry), in orchestrating critical physiological functions in circadian rhythms, fluid and food intake (J. Antunes Rodrigues); social and sexual behavior (S. Carter); and modulation of states of arousal (L. de Lecea) and the implications of this understanding for progress in human health. The speakers will reflect on the lessons learned in their scientific careers that may be valuable to those just embarking on their own research journeys in this incredibly rich and fertile field, and will discuss critical questions about the future of regulatory peptide research, submitted beforehand from RegPep2018 participants.

RT1 José Antunes Rodrigues (Faculty of Medicine, University of Sao Paulo, Brazil)

RT2 Sue Carter (Kinsey Institute, University of Indiana, USA)

RT3 Luis de Lecea (Department of Psychiatry, Stanford University, USA)

RT4 John Morris (University of Oxford, UK)

RT5 Robert Millar (Centre for Neuroendocrinology, University of Pretoria, South African)

About the panel members



Professor Jose Antunes-Rodrigues is emeritus Professor at University of Sao Paulo, Ribeirao Preto Medical School and has contributed to the teaching of medical undergraduate students since 1960. During these decades he has taught Physiology to more than 4500 medical students. His devotion to teaching was recognized by the medical students who dedicated several honors and awards to Professor Antunes. He has robust contributions on the role of CNS in the control of hydromineral balance. His research team demonstrated the role of oxytocin in the ANP secretion from the heart. He has trained more than 40 Ph students and supervised several (15) Post-Doc young

investigators. He has more than 350 publications and 4500 citations. He is member of Brazilian Academy of Science and his significant contributions to the Science can be verified by the Awards he has received from Brazilian Ministry of Science and Technology (National Order of Scientific Merit).



Dr. Sue Carter is currently Director of the Kinsey Institute and Rudy, Professor of Biology at Indiana University, a position she accepted in 2014. Prior to her current position she held Professorships at the University of Illinois, Champaign-Urbana and the University of Illinois at Chicago, Department of Psychiatry, as well as the University of Maryland and the University of North Carolina. Dr. Carter is a neurobiologist, known for her research on the biology of social bonding and the development of the prairie vole as a model for studying the behavioral and physiological actions of peptide hormones. Research originated by Dr. Carter has shown that oxytocin is at the biological heart of positive emotions, including love. Oxytocin and its receptors can facilitate a sense of safety, which in turn allows social cognition, social bonding, social support, growth and restoration. The oxytocin system also plays a major role in early life and is epigenetically influenced by

experience. The capacity of oxytocin to regulate the brain and behavior across the lifespan helps to explain the adaptive consequences of social bonds and attachments for emotional and physical health, especially in the face of fear and anxiety. More details on Dr. Carter's work and a full cv are available at the Kinsey Institute website. <https://kinseyinstitute.org/about/profiles/cscarter.php>



Professor Luis de Lecea is a Professor in the Department of Psychiatry and Behavioral Sciences at Stanford University School of Medicine. Prof. de Lecea trained in Barcelona, Brussels and San Diego before starting his laboratory at The Scripps Research Institute in 1996, where he discovered two neurotransmitters critical for the regulation of sleep and wakefulness. He joined Stanford in 2006 and his work now focuses on the role of neuromodulators in mammalian behavior, especially behaviors related to sleep, reward, stress, and learning/memory.



Professor John Morris has worked in what is now the Department of Physiology, Anatomy & Genetics (DPAG) since 1977, teaching medical and science students at St Hugh's and other colleges. From 2004-2010 he was director of Preclinical Studies and since becoming emeritus in 2010 he continues to be engaged in research with the DPAG and in undergraduate teaching. John is currently Emeritus Fellow of St Hugh's College, and previously Wellcome-Franks Fellow in Medicine, was the recipient of a **Lifetime Achievement Award for Teaching Excellence by the Medical Sciences Division**. The award is intended to acknowledge recipients for high quality and sustained commitment to education demonstrated throughout their career at Oxford.



Professor Robert P Millar is Director of the Centre for Neuroendocrinology at the University of Pretoria, Senior Research Fellow at UCT, Research Fellow at the Centre of Integrative Physiology at the University of Edinburgh and Professor emeritus at the university of St Andrews.

Previously he was Director of the MRC Human Reproductive Sciences Unit in Edinburgh which comprised over 100 researchers. He secured over 70 million pounds research funding during his tenure. The Unit research focused on pathologies of female and male reproductive tissues (eg prostate, breast and ovarian cancers). He was founder of the reproductive health company, Ardana Biosciences which raised 73 million pounds, was listed on the LSE, and took three drugs into the market. He has an H-index of 70, published >400 papers which received >20,000 citations, and filed 18 patents.

Bob's research focuses on peptide regulators of reproductive hormones. He pioneered the discovery of the GnRH prohormone, novel GnRH structures, and the first cloning of the GnRH I and GnRH II receptors. His laboratory delineated GnRH binding sites and the molecular mechanisms underlying receptor activation and coupling. He has participated in, and led, a number of programs developing GnRH analogues for use in a wide range of clinical pathologies. His group's research on direct antiproliferative effects of selective GnRH analogues on tumour cells has revealed the novel concept of ligand-induced-selective-signalling by GnRH analogues which has implications for improved selectivity in the development of new GnRH therapeutics and other GPCR targets. His research encompasses a continuum of basic through to clinical and he has been involved in taking ten neuropeptides and analogues into humans.

Recently he has focused on novel upstream GPCRs regulating GnRH, kisspeptin and neurokinin B. He developed kisspeptin and NKB antagonists as potential therapeutics in hormone-dependent diseases. The functional 'rescue' of human GPCRs with inactivating mutations by small molecules is a new interest.

RT1 Neuroendocrine control of hydromineral balance:: lessons from hypothalamic lesions to neural and molecular circuitries

José Antunes Rodrigues

Faculty of Medicine, University of Sao Paolo, Brazil)

Early systematic studies using electrolytic lesions and electrical/chemical stimulation in rats demonstrated the participation of several CNS structures in the selective intake of water or NaCl. According to these data, it was possible to demonstrate the existence of a neural circuitry (involving the septal area, anteroventral region of the third ventricle (AV3V), the amygdaloid complex, the hypothalamus, the olfactory bulb, the hippocampus, and the sensory and motor cortices) that controls sodium intake and/or excretion. However, at that time it was not clear the mechanism by which the CNS controls the salt balance. The mechanisms involved in the regulation of hydroelectrolytic balance are complex, extremely sensitive and accurate, involving central, cardiovascular, endocrine and renal responses. Afferent inputs are represented by (i) mechanoreceptors (baroreceptors and volume receptors) in the cardiovascular system and (ii) osmoreceptors and Na⁺ receptors in the periphery and in the CNS. Inputs performed by these sensory systems are conveyed to specific areas of the CNS, with the hypothalamic neurohypophyseal system the final common route for these integrative circuitries. Simultaneously, behavioral responses are modulated; the selective stimulation or inhibition of motivational and locomotor aspects directly affects the search for and acquisition of water and/or sodium. Finally, the primary systemic effectors targeting renal management of fluid and electrolytes are composed of the sympathetic autonomic system and hormones (AVP, OXT, NPs, and RAAS which are released by a wide variety of endocrine cells). More recently, gaseous modulators have been included in the increasing list of substances with effects on neuronal activity. Soluble gases are signaling molecules with a short half-life that exert their actions in an autocrine or paracrine manner. The most well-known gaseous neuromodulator is NO, which is synthesized from L-Arginine by the enzyme nitric oxide synthase (NOS) and rapidly diffuses to the extracellular space. In 1998, the discovery of the vasodilatory properties of NO in the vascular smooth muscle cells mediated by the endothelial isoform of the NOS was awarded with the Nobel Prize in Physiology and Medicine, and since then, many physiological and pathological effects have been attributed to NO, such as involvement in the control of neuroendocrine function. The expression of the neuronal isoform of NOS (nNOS) is increased by dehydration in the magnocellular neurons of the PVN and SON. The intracerebroventricular injection of L-NAME, which is a nonselective NOS inhibitor, leads to an increased secretion of both AVP and OXT, as well as increased water and sodium intake. With the development of molecular biology, global expression analysis (genomes, proteomes, and transcriptomes), bioinformatics, epigenetics, the ability to control gene expression, and genetic engineering, our perspectives concerning the study of hydromineral balance have been consistently broadened in recent decades. It is now possible to establish extremely convincing and complementary hypotheses concerning the mechanisms underlying water and sodium management based on upcoming functional results (obtained by the use of advanced methodological strategies), as well as on behavioral clues provided by the pioneer lesion-based studies conducted in the early 1960s. However, the major challenge for the future in this field is to match a deeper understanding of the local mechanisms (types and properties of receptors, ion channels, and signaling cascades) with the overview regarding how an intact organism (considering gender- and age-specific mechanisms) maintains a homeostatic balance of body fluids when exposed or re-exposed to different challenges.

RT2 A peptide cocktail for monogamy

C. Sue Carter

The Kinsey Institute and Department of Biology,
Indiana University, Bloomington, IN USA

Social interactions are linked to the ability to mate, survive and thrive within an always changing environment. Of particular value to our current understanding of the biology of mammalian social behavior, as well as the behavioral effects of two major neuropeptides - oxytocin and vasopressin - have been studies in the socially monogamous prairie vole. At the level of a species sexual monogamy is rare. However, the features of social monogamy in mammals cluster as a “syndrome,” including the capacity for selective pair bond formation, paternal and alloparental behavior, reproductive suppression of the young, and reductions in anatomical sexual dimorphism¹. Components of this syndrome have evolved independently at least 60 times in unrelated species. The major traits of social monogamy, including *selective* social relationships, are supported by patterns of hormonal function originating in the neurobiology of maternity, including interactions between oxytocin and vasopressin pathways.

Based on research in prairie voles, it also seems likely that reduced sensitivity to gonadal androgens (especially in early life), and a concurrent increased reliance on vasopressin and oxytocin play a central role in the emergence of social monogamy. Oxytocin allows immobility without fear in the presence of offspring or partners and is essential in the formation of social bonds between mothers and offspring and between adults. Oxytocin is also necessary for paternal behavior. A more ancient neuropeptide, vasopressin, in dynamic interplay with oxytocin, also regulates selective social bonds, paternal behavior, defensive behaviors, and supports active coping strategies. Research on oxytocin is complicated by the fact that many of the effects of oxytocin are through actions on vasopressin receptors². Both males and females are affected by oxytocin and vasopressin, although males may be more dependent on vasopressin, which is regulated centrally by androgens (converted locally to estrogens).

The processes associated with sexual differentiation, and specifically masculinization, appear to differ between socially monogamous versus nonmonogamous species of mammals. For example, recent evidence from our work with prairie voles indicates that perinatal oxytocin exposure reduces sex differences in the nervous system in males, with little effect on females. In addition, as in monogamous New World monkeys, prairie voles have high levels of glucocorticoids, which also may reduce the effects of androgens and influence sexual dimorphism. We can further hypothesize that mutations or epigenetic changes in genes for the androgen receptor and/or a reduction in dihydrotestosterone (possible due to changes in 5alpha reductase) might contribute to the relative absence of sexually dimorphic traits, and increased sociality, seen in social monogamy. Data from a variety of mammalian species suggest that it is unlikely that there is single recipe for social monogamy. However, there is consistency in the necessary ingredients, and among these are oxytocin and vasopressin.

Supported by NICHD P01 HD 075750.

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RT3 Orexin/hypocretin and modulation of states of arousal: Twenty years of Hypocretins/orexins

Luis de Lecea

Department of Psychiatry, Stanford University, USA

How the brain controls vigilance state transitions remains to be fully understood. The discovery of hypocretins, also known as orexins, and their link to narcolepsy two decades ago has allowed us to advance our knowledge on key mechanisms controlling the boundaries and transitions between sleep and wakefulness. Lack of function of hypocretin neurons (a relatively simple and non-redundant neuronal system) results in inappropriate control of sleep states without affecting the total amount of sleep or homeostatic mechanisms. Anatomical and functional evidence shows that the hypothalamic neurons that produce hypocretins/orexins project widely throughout the entire brain and interact with major neuromodulator systems in order to regulate physiological processes underlying wakefulness, attention, and emotions. In the panel, I will review the role of hypocretins/orexins in arousal state transitions, and discuss possible mechanisms by which such a relatively small population of neurons controls fundamental brain state dynamics.

RT4 Insights from a lifetime in neuroendocrine ultrastructure

John Morris

Emeritus Professor, Department of Physiology, Anatomy & Genetics, University of Oxford OX1 3QX, UK

It is difficult to remember that, when I started research on hypothalamic magnocellular neurons in 1969, the current hypothesis for the release of peptides was ‘molecular dispersion’ because tissue preparation for electron microscopy at that time had shown apparently full and partially empty dense-cored vesicles. It was in fact a lab error using what was considered an inappropriately acidic fixative that revealed uniformly dense vesicles and thus indicated exocytosis of entire vesicles – one can learn valuable lessons from mistakes! The lack of quantitation in many ultrastructural studies was also striking; no hormone assays would be accepted without numerical data. Careful quantitation of vesicle numbers coupled with colleagues’ assays allowed determination of the molecular content of vasopressin and oxytocin in vesicles which has proved valuable in subsequent studies. Sadly, a recent literature scan shows that the molecular content of most peptidergic vesicles is still unknown. Learning about techniques used for invertebrate ultrastructure allowed us to capture individual exocytoses in the posterior pituitary and show that exocytosis could occur, not only the nerve endings, but also the larger swellings (Herring bodies) and undilated axons. This led on, with talented students, to the demonstration that the peptidergic vesicles in the dendrites of magnocellular neurons were released by exocytosis and has been shown to account for the local release of vasopressin and oxytocin in the hypothalamus, where both have subsequently been shown to have important physiological and behavioral effects. Young researchers may be encouraged to know that when this first direct evidence for dendritic secretion was submitted to ‘Nature’ it was declined as being ‘of no general interest’ although it now seems clear that, throughout the CNS, peptides can be released from many different parts of neurons and have profound consequences far distant from their axonal terminals. Indeed, others have recently shown that, for GnRH neurons the apparent distinction between axon and dendrite is untenable and their processes should be referred to as ‘dendrons’. Quantitative ultrastructure in combination with many other techniques still has a great deal to offer to our understanding of the multiple roles of regulatory peptides.

RT5 Hormones from prohormones--the biosynthesis of regulatory peptides: Making discoveries through recognizing what has gone before

Robert Millar Centre for Neuroendocrinology, University of Pretoria, South African

I started my scientific career by working on a relative of the elephant, the rock hyrax, a small rabbit-sized subungulate which has a long gestation and intra-abdominal testes like the elephant. The mechanism whereby these testicond mammals achieve functional spermatogenesis remains an unresolved conundrum. Hyrax are highly seasonal in their breeding – testis size increases from a small marble to the size of a lemon in just a few weeks. I found that you could achieve this growth at any time of the year by increasing then reducing artificial light with a simple light bulb by about an hour. Geoffrey Harris had produced evidence that the changes in photoperiod which drive reproduction were mediated through factors in the hypothalamus, so I set about showing that simple acid extracts could do this.

Soon after, Andrew Schally isolated and determined the sequence of GnRH (called LHRH by him). Because it was a small peptide of only 10 amino acids, and such small peptides had been shown to be synthesized in bacteria by a non-ribosomal enzyme template mechanism (eg gramicidin S) by my brother in law, Wieland Gevers, in Lippman's laboratory at the Rockefeller it was presumed that GnRH was synthesized in the same way and a number of papers published in support of this. However, these researchers failed to recognize a number of fairly obvious facts. Firstly, the small peptides synthesized on enzyme templates, such as gramicidin S, had unusual D-amino acids and fatty acids in their sequence which GnRH and TRH (discovered by Guillemin) did not. They had conventional D-amino acids suggesting they were synthesized on ribosomes! Secondly, the related neurohypophyseal hormones had been shown to be synthesized ribosomally. Thirdly, the demonstration that TRH and GnRH could be synthesized in a non-ribosomal setting by Jeff McKelvy using tritiated amino acids and showing incorporation in the correct position in simple chromatography was clearly flawed as many more steps had been needed to isolate pure GnRH and TRH. We therefore reasoned that GnRH was elaborated as a polypeptide precursor which was processed to the GnRH decapeptide and demonstrated this by gel chromatography of sheep hypothalamic extracts and detection of GnRH immuno-activity using an antiserum we produced. Moreover, the putative precursor could be reduced to the size of GnRH by trypsin as had been described for other peptide precursors. I used this discovery to demonstrate my credibility as a serious scientist and had the temerity to write to Andrew Schally asking if I could do a sabbatical in his lab – which he agreed to – thus giving me a wonderful opportunity which helped launch my career.

The lesson learned were that one should pay attention to previous science and not shoe-horn a hypothesis into a convenient phenomenon and ignore more cogent explanations. The other lesson was don't be afraid to make contact with the international leaders in the field.

The second example of this came from the claim that the GnRH sequence was conserved in all vertebrates. We felt that this was unlikely since the related mammalian neurohypophyseal hormones, oxytocin and vasopressin, were represented by structural variants in non-mammalian vertebrates. To demonstrate this we subjected crude hypothalamic extracts to ion exchange chromatography and showed that while amphibians had an immunoreactive form with identical charge to mammalian GnRH, the forms in fish, birds and reptiles were much more acidic and eluted much earlier on columns. We proposed that the arginine in position 8 of mammalian GnRH was substituted by a neural amino acid. As a group in South Africa there was skepticism that such insightful work could emerge from a developing country and our work had been rejected on many occasions. However, on this occasion Science accepted the article and subsequently published four more Science articles. **The lesson was again that one needs to pay**

attention to past work and the logic of science. We also learned that even if you are in a backwater if you do good science you will bridge the credibility gap.

The advances we had made demonstrating the existence of novel forms of GnRH in vertebrates was all very well but definitive proof was necessary so we set about establishing what the sequence changes were. Initially we did this by indirect means on crude extracts of chicken hypothalamic. We assembled a library of antisera that recognized different regions of GnRH and showed that it was arginine in position eight that was changed in chicken GnRH. This was confirmed by specific chemical modification of each amino acid in GnRH and demonstrating that arginine was absent in chicken GnRH and had been replaced by a neutral amino acid. Since the only single nucleotide substitution which would accommodate a change of arginine to produce a neutral amino acid was glutamine this was the most likely substitution we synthesized glutamine eight GnRH and showed that it had identical ionic, reverse phase HPLC and LH releasing properties to the impure chicken GnRH. This indirect identification of the chicken GnRH sequence was confirmed by purification and amino acid sequence analysis from 600,000 chicken hypothalamic. This set the scene for the discovery of a large number of GnRH structural variants in vertebrates and a second mid brain form (GnRH II) conserved from teleost fish to humans which is involved in reproductive behaviours. These structures also revealed the functionally important domains in GnRH conserved over 500 million years of evolution which aided in the development of GnRH analogs which have become major therapeutics for hormone-dependent diseases such as prostatic cancer and the mainstay of IVF. **The lesson from these studies was that the “experiments of nature” can provide instructive insight into structure/activity relations more effectively than the empirical systematic substitution of amino acids.**

All of these advances had been made in the absence of knowledge about the GnRH receptor. Thus cloning of this receptor became the next major challenge which was achieved in collaboration with Stuart Sealfon’s lab. This provided considerable insight into the physiological regulation of reproduction through up a down regulation of the GnRH receptor and the detailed delineation of GnRH binding and activation of its cognate receptor. Moreover, the cloned receptor facilitated the discovery of small molecule orally active GnRH analogs such as Elagolix which is being used to treat endometriosis. **The lesson was that a systematic and complete study of all aspects of peptide biology and its receptor are required to advance the field.**

The next phase of advance in the field came with the discovery of novel upstream peptide regulators of GnRH, kisspeptin and Neurokinin B through identification of inactivating mutations in the genes encoding these peptides and their receptors. I realized the enormous potential of investigating these peptides as more subtle regulators of reproduction and set about developing peptide analogs of kisspeptin and the development of small molecule antagonists with Graeme Fraser. This new generation of analogs have provided further understanding of the regulation of reproduction and already found application in IVF and the treatment of polycystic ovarian syndrome and post menopausal hot flushes (flashes). **The lesson is that one should be aware that there is frequently an even higher level of physiological regulation to be pursued.**

PL5 Oxytocinergic gating of social reward

Atlantes amphitheater

Robert C. Malenka

**Society for Neuroscience/NIMH Julius Axelrod Prize Laureate
(Stanford Neuroscience Institute, Stanford University, USA)**

About the speaker



Robert C. Malenka is the Pritzker Professor of Psychiatry, Director of the Pritzker Laboratory and Deputy Director of the Stanford Neurosciences Institute at Stanford University. He graduated from Harvard summa cum laude and Phi Beta Kappa in biology. He then received an M.D. and a Ph.D. in neuroscience at Stanford University. Over the ensuing 6 years he completed residency training in psychiatry at Stanford and postdoctoral research at the University of California, San Francisco (UCSF). In 1989, he was appointed Assistant Professor of Psychiatry and Physiology at UCSF at which he reached the rank of Full Professor in 1996. At UCSF he was also the founding Director of the Center for the Neurobiology of Addiction and Associate Director of the Center for Neurobiology and Psychiatry. He returned to Stanford University School of Medicine in 1999. His many contributions over the last 3 decades have laid the groundwork for a more sophisticated understanding of the adaptations in synaptic communication that underlie many forms of normal and

pathological behavior. He is a member of the National Academy of Sciences and the National Academy of Medicine and a fellow of the American Academy of Arts and Sciences and the American Association for the Advancement of Science. He has served on the National Advisory Council on Drug Abuse and as a Councilor for the Society for Neuroscience and the American College of Neuropsychopharmacology. He is co-author of “Molecular Neuropharmacology: A Foundation for Clinical Neuroscience”.

PL5 Oxytocinergic gating of social reward

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Positive prosocial interactions contribute to the development and maintenance of a range of adaptive, cooperative behaviors. Conversely, inability to participate in normal social interactions is a debilitating symptom of several prominent neuropsychiatric disorders. Although the role of neuromodulators in social behaviors, in particular oxytocin, is an active area of investigation, relatively little is known about the detailed neural mechanisms that influence sociability. This talk will review evidence that modulation of reward circuitry by oxytocin, dopamine, and serotonin all play a role in the reinforcing components of conspecific social behavior. Evidence will be presented that oxytocin acts in both the nucleus accumbens and ventral tegmental area to promote social reward. In the nucleus accumbens, oxytocin appears to act by stimulating the release of serotonin. Consistent with this hypothesis, direct release of serotonin in the nucleus accumbens via optogenetics enhances prosocial behavior while optogenetic inhibition of serotonin release decreases social interactions. Oxytocin action in the ventral tegmental area is also required for social reward. Genetic deletion of oxytocin receptors in the ventral tegmental area impairs social reward while stimulating its release using optogenetics promotes prosocial behavior in a context specific manner. Electrophysiological recordings in acute slices reveal that oxytocin promotes the firing of dopamine neurons that project to the nucleus accumbens. These findings demonstrate that the key nodes of classic mesolimbic reward circuitry, the nucleus accumbens and ventral tegmental area, are subject to multiple types of modulation by oxytocin and other neuromodulators, each of which is important for promoting prosocial behavior.

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S21. Contemporary approaches to studying peptidergic neurons

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Journal of Neuroendocrinology*

Princesa 1

Chairs: **Mike Ludwig** (Edinburgh, UK)

S21.1 Mike Ludwig (University of Edinburgh, UK):

Exploring novel neuronal pathways from the retina to the SCN using transgenic rat models and viral transfection systems

S21.2 Javier Stern (Georgia State University, USA):

Unraveling mechanisms underlying stimulus-secretion coupling at neuronal dendrites using novel cell biosensors

S21.3 Colin Brown (University of Otago, NZ):

Dissecting vasopressin's role in the development of hypertension using transgenic rats

S21.4 Jeff Tasker (Tulane University, USA):

Neuropeptide activation of neuronal-glia circuits

S21.1 Exploring novel neuronal pathways from the retina to the SCN using transgenic rat models and viral transfection systems

Mike Ludwig

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In all animals, the transition between night and day engages a host of physiological and behavioral rhythms. Retinal ganglion cells (RGCs) detect the ambient light level in the environment and these project to the suprachiasmatic nucleus (SCN) of the hypothalamus to entrain circadian rhythms that are generated within the SCN. Using transgenic rat lines, immunohistochemistry, tracer injection and viral transfection systems, we show here that vasopressin (VP) is expressed in many retinal cells that project to the SCN¹. Using triple immunohistochemistry, we found that VP-RGCs co-expressed the vesicle glutamate transporter 2, and that 74% of VIP positive cells and 66% of GRP positive cells in the SCN were apposed by boutons from VP-RGCs fibres. VP-RGCs were often closely juxtaposed to immunoreactive melanopsin expressing cells and 25% co-expressed melanopsin. *In vitro* patch-clamp recordings from RGCs showed that 75% of these cells were transiently excited by light, and the other 25% were inhibited. Recording of the spike activity of single SCN neurons in urethane-anesthetised rats *in vivo* showed that two thirds of light-responsive cells were excited by light and one third were inhibited². In a number cells excited by light, the light-induced activation was reduced by 30% after an icv injection of a vasopressin V1a antagonist. Microdialysis experiments have shown that either retino-hypothalamic tract stimulation or light evokes vasopressin release in the SCN. Light-induced vasopressin release also enhances expression of the immediate early gene product Fos in the SCN, which is critical for photic entrainment of circadian rhythms. Thus, these newly discovered vasopressin cells in the retina are a major, light-activated pathway that has a key role in regulating important circadian rhythms. The previously reported association of vasopressin with jet-lag raises the interesting possibility that interventions in vasopressin signalling from the retina may have important therapeutic benefits.

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S21.2 Unraveling mechanisms underlying stimulus- secretion coupling at neuronal dendrites using novel cell biosensors

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Dendrites are now recognized to be active transmitting neuronal compartments subserving complex brain functions, including motor behaviors and homeostatic neurohumoral responses, among others. Still, the precise mechanisms underlying activity-dependent release of dendritic signals, and how dendritic release can be regulated independently from axonal release of signals, remain largely unknown. We developed “sniffer” biosensor cells to enable the measurement and study of activity-dependent dendritic release of vasopressin (VP) from hypothalamic neurons. Dendritic release of VP was strengthened by clustered firing, compared to continuous irregular activity. Moreover, release evoked at any given frequency was robustly potentiated when firing was triggered by NMDA receptor (NMDAR) activation. Differently from axonal release, NMDAR activation was necessary for dendritic release to occur at physiological firing frequencies, acting thus as a potential gating mechanism by which activity-dependent release from these two neuronal compartments could be differentially regulated. The NMDAR-mediated potentiation of dendritic release was independent of a particular action potential waveform or firing pattern evoked, but correlated with higher dendritic Ca^{2+} levels. Overall, our studies provide fundamental novel information regarding stimulus-secretion coupling at neuronal dendrites.

S21.3 Dissecting the role of vasopressin in the development of hypertension using transgenic rats

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Despite advances in our understanding of the pathology of hypertension, the mechanisms that underpin the development of hypertension remain to be fully elucidated. This problem is, in part, due to limitations of established animal models of hypertension. Here, we highlight the use of genetically-modified Cyp11a1-Ren2 rats, in which the onset and severity of angiotensin II-dependent hypertension is tightly controlled by dietary administration of indole-3-carbinol, to determine the mechanisms that contribute to the development of hypertension.

Diet containing 0.225% w/w indole-3-carbinol caused progressive induction of moderate hypertension over seven days in Cyp11a1-Ren2 rats, which was accompanied by increased sympathetic tone (estimated using spectral analysis of heart rate variability) and mesenteric artery eNOS expression, but no gross morphological remodelling of the heart or vasculature.

Concurrent peripheral administration of a vasopressin V₁ receptor antagonist prevented blood pressure increasing from days 3 – 7. Vasopressin is secreted from the posterior pituitary gland by hypothalamic magnocellular neurons to inhibit diuresis and promote vasoconstriction. Induction of moderate hypertension for seven days increased the basal firing rate of vasopressin neurons. Circulating angiotensin II excites vasopressin neurons via subfornical organ inputs. However, the hypertension-induced increase in vasopressin neuron firing rate was not blocked by intra-subfornical organ infusion of an angiotensin AT₁ receptor antagonist. Microglial activation and blood-brain barrier breakdown help maintain established hypertension, but hypothalamic microglial ionised calcium-binding adapter molecule-1 staining and endothelial transferrin receptor staining were unchanged on day 7 of hypertension.

Intravenous α_1 -adrenoreceptor agonist injection transiently increased blood pressure to induce baroreflex inhibition of vasopressin neuron firing rate in non-hypertensive rats. By contrast, baroreflex activation did not inhibit vasopressin neurons in hypertensive rats, despite a similar increase in blood pressure. The baroreflex normally inhibits vasopressin neurons via GABAergic inputs. Local administration of a GABA_A receptor antagonist excited vasopressin neurons from non-hypertensive rats but inhibited vasopressin neurons from hypertensive rats.

We conclude that the Cyp11a1-Ren2 rat is an effective model for investigating the development of angiotensin-dependent hypertension and that vasopressin exacerbates the increase in blood pressure evident early in the development of hypertension due to blunted baroreflex inhibition of vasopressin neurons by an excitatory shift in their response to GABA.

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S21.4 Neuropeptide activation of neuronal-glia circuits

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Astrocytes regulate neurotransmission at the synapse via local bidirectional signaling with neurons. Astrocytes also can signal distally via elaborate processes and electrotonic coupling, providing a possible substrate for an alternate mechanism of neuronal communication via intercalated astrocytes. Here, we demonstrate a novel retrograde signaling mechanism in corticotropin-releasing hormone (CRH) neurons that uses the dendritic release of vasopressin to stimulate astrocytes to activate local recurrent excitatory synaptic circuits. Noradrenergic afferents excite CRH neurons in the hypothalamus to activate the hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Noradrenergic activation of the HPA axis has long been known, but the cellular mechanisms of the noradrenergic excitation of CRH neurons have been elusive. We found that norepinephrine (NE) activates postsynaptic $\alpha 1$ receptors in paraventricular nucleus CRH neurons to trigger the dendritic release of vasopressin, which activates V1a receptors and a calcium response in neighboring astrocytes in the PVN. The activated astrocytes then release ATP and activate P2X receptors to stimulate upstream glutamate neurons. This results in the activation of recurrent excitatory synaptic inputs to the CRH neurons, which causes a robust increase in CRH neuron spiking. The NE excitation of CRH neurons is strengthened by simultaneous presynaptic $\alpha 2$ receptor-mediated suppression of GABA release, but is dampened at higher NE concentrations by activation of upstream GABAergic circuits via the same postsynaptic $\alpha 1$ receptor-triggered retrograde neuronal-glia signaling mechanism, albeit with a higher threshold for activation. The NE activation of local excitatory circuits in the PVN is recapitulated by optogenetic stimulation of noradrenergic inputs from the solitary tract nucleus and locus coeruleus, although there appears also to be direct excitation via co-release of glutamate from the noradrenergic afferents. Thus, the NE stimulation of CRH neurons in the PVN is mediated by a novel retrograde signaling mechanism that enlists a trans-neuronal-astroglial circuit to activate presynaptic glutamate and GABA neurons. This retrograde neuronal-glia circuit activation reveals a novel mechanism to amplify the volume neurotransmission mediated by dendritic release of vasopressin.

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S22. Neuropeptides and neurodegeneration

Princesa 2

Co-Chairs:

Illana Gozes (Tel Aviv, Israel) and **Seiji Shioda** (Tokyo, Japan)

S22.1 **Illana Gozes** (Tel Aviv University, Israel):

The VIP- PACAP regulated ADNP is an alcohol-responsive gene and negative regulator of alcohol consumption in female mice

S22.2 **Seiji Shioda** (Hoshi University, Japan):

PACAP, stem cells and neuroprotection, spinal injury and stroke

S22.3 **Dora Reglödi** (University of Pecs, Hungary):

Age-related accelerated systemic amyloidosis in PACAP deficiency

S22.4 **Stephen Salton** (Icahn School of Medicine at Mount Sinai, USA):

VGF-derived fragment for neuroprotection in Alzheimer's disease

S22.1 The VIP-PACAP regulated, ADNP is an alcohol--responsive gene and negative regulator of alcohol consumption in female mice

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Regulated by vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide, activity-dependent neuroprotective protein (ADNP) is crucial for brain development, and is implicated in neural plasticity in adulthood (1). Neuroadaptations in the brain reward system caused by excessive alcohol intake, lead to drinking escalation and alcohol use disorder phenotypes. Given the involvement of ADNP in neuronal plasticity and neuroprotection, we asked if ADNP is involved in adult alcohol use disorder. We discovered that alcohol exposure regulates *Adnp* expression in the mesolimbic system, and that *Adnp* keeps alcohol drinking in moderation, in a sex-dependent manner. Specifically, Sub-chronic alcohol treatment (2.5 g/kg/day for 7 days) increased *Adnp* mRNA levels in the dorsal hippocampus in both sexes, and in the nucleus accumbens of female mice, 24 h after the last alcohol injection. Long-term voluntary excessive alcohol consumption (~10-15 g/kg/24 h, 5 weeks) increased *Adnp* mRNA in the hippocampus of male mice immediately after an alcohol-drinking session, but the level returned to baseline after 24 h of withdrawal. In contrast, excessive alcohol consumption in females led to long-lasting reduction in hippocampal *Adnp* expression. We further tested the regulatory role of *Adnp* in alcohol consumption, using the *Adnp* haploinsufficient mouse model (2). We found that *Adnp* haploinsufficient female mice showed higher alcohol consumption and preference, compared to *Adnp* intact females, whereas no genotype difference was observed in males. Interestingly, daily nasal administration of the ADNP snippet drug candidate NAP normalized alcohol consumption in the female mice. Finally, female *Adnp* haploinsufficient mice showed a sharp increase in alcohol intake after abstinence, suggesting that *Adnp* protects against relapse in females. The current data suggest that ADNP is a potential novel biomarker and negative regulator of alcohol drinking-behaviors. Furthermore, NAP administration to females suffering from *Adnp* haploinsufficiency prevents escalation in alcohol drinking in mice (3). Together, our studies set a genetic basis to excessive alcohol consumption and offer means for precision brain protective medicine.

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S22.2 PACAP plays an important role for neurogenesis and nerve regeneration after brain ischemia and spinal cord injury

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PACAP (Pituitary Adenylate Cyclase-Activating Polypeptide) is a neuroprotective peptide expressed in the central/peripheral nervous system and other peripheral organs and tissues. PACAP is shown to exert a wide range of effects on neural stem cells (NSCs) during ontogenetic development, neuroregeneration after brain ischemia and spinal cord injury. We have shown that PACAP increases bromodeoxyuridine (BrdU) positive-proliferative cells in PACAP gene deficient animals. Moreover, the BrdU positive cells are immunopositive against SOX2, a marker of NSCs, and differentiated into NeuN-positive mature neurons after brain ischemia. Thus, PACAP may contribute to stimulate proliferation of NSCs and may be associated with recovery after brain injury.

In the spinal cord injury (SCI) model animals, expression levels of mRNA for collapsin response mediator protein 2 (CRMP2), a factor related to axonal regeneration, were increased in the PACAP-treated group as compared with control one. A significantly increased number of CRMP2-positive cells were observed around the injury lesion in the PACAP-treated animals, while CRMP2 was co-expressed in neurons and oligodendrocytes in the spinal cord. Two weeks after SCI, anterograde tracing revealed that a significantly increased number of nerve fibers were observed in the spinal cord, suggesting that PACAP stimulated functional motor recovery after SCI through axonal regeneration mediated by CRMP2.

Finally, we will present a schematic and hypothetical model showing the genes potentially involved behind neuroprotective function of PACAP in the penumbra early on and a link to the previously identified CRMP2 protein that has a role in neuronal development in mice.

S22.3 Age-related accelerated systemic amyloidosis in PACAP deficiency

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a potent cytoprotective peptide that provides an endogenous control against a variety of tissue-damaging stimuli. Dysregulation of neuropeptides may play an important role in aging-induced impairments. We hypothesized that the progressive decline of PACAP throughout life, the increased vulnerability to various stressors of animals partially or totally lacking PACAP and the well-known general cytoprotective effects of PACAP lead to age-related pathophysiological changes in PACAP deficiency. Using young and aging CD1 PACAP knockout mice, we found pre-senile appearance of amyloidosis in young PACAP knockout (KO) animals and showed that senile amyloidosis in mice lacking endogenous PACAP appeared accelerated, more generalized, more severe and affected more individuals. Histopathological analysis showed a type of age-related systemic amyloidosis with mainly kidney, spleen, liver, skin, thyroid, intestinal, tracheal and esophageal involvement. Mass spectrometry-based proteomic analysis, re-confirmed with immunohistochemistry, revealed that apolipoprotein A-IV was the main amyloid protein in the amyloid deposits together with several other accompanying proteins. Although the local amyloidogenic protein expression is disturbed in the KO animals, no difference was found in lipid laboratory parameters, suggesting a complex pathway leading to increased age-related degeneration with amyloid deposit in the lack of PACAP. In summary, here we describe accelerated systemic senile amyloidosis in PACAP gene deficient mice, which might indicate an early aging phenomenon in this mouse strain. Thus, PACAP KO mice could serve also as a model of accelerated aging, with human relevance.

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S22.4 VGF and its peptides have pro-cognitive and antidepressant efficacy

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Our lab has investigated function of the BDNF-inducible neuronal peptide precursor VGF (non-acronymic) in major depressive disorder (MDD) and neurodegenerative disorders, including Alzheimer's disease (AD). We find that hippocampal and cortical VGF expression is required for efficacy of the rapid-acting antidepressant ketamine, and that administration of the VGF-derived peptide TLQP-62 (named by N-terminal 4 amino acids and length), into either hippocampus (dHc) or ventromedial prefrontal cortex (vmPFC), has rapid-onset, prolonged antidepressant effects that are BDNF-dependent, and in dHc are blocked by rapamycin and NBQX (thus, mTOR- and AMPA receptor-dependent), like ketamine ¹. VGF also has pro-cognitive efficacy ², and high-resolution proteomics have identified VGF as a strong candidate biomarker of AD progression ³. Recent integrative genomic efforts (AMP-AD consortium) that map networks underlying the onset and progression of human AD have converged on VGF, showing that reduced VGF expression significantly correlates with mean amyloid plaque density, CDR, CERAD, and Braak scores, and that VGF, the critical 'driver', is part of a network underlying AD pathogenesis and progression. We have further determined that germline VGF overexpression, chronic icv TLQP-62 or TLQP-21 peptide infusion, and AAV-mediated VGF overexpression in dorsal hippocampus, reduce amyloid load, microgliosis, and astrogliosis in the 5xFAD amyloid mouse model of AD, in the CNS, in a region-specific manner. Mechanisms likely include modulation of neurogenesis, synaptic plasticity, and neuroprotection (via TLQP-62/BDNF pathways), and microglial-mediated inflammation and amyloid uptake (via TLQP-21/C3aR1 signaling), which are currently under investigation *in vivo* and *in vitro* along with the underlying RNA and protein networks and drivers.

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S23. Chromogranin- and other protein-derived bioactive peptides: cardiovascular, immune, endocrine and metabolic functions

Princesa 3

Chair: **Sushil Mahata** (San Diego, USA)

S23.1 **Sushil Mahata** (Veterans Administration and UCSD, San Diego, USA):

Catestatin regulation of immunometabolism

S23.2 **Youssef Anouar** (Inserm U1239, Université de Rouen, Normandy, France):

A selenoprotein-derived peptide with potent in vivo anti-neurodegenerative actions

S23.3 **Angelo Corti** (IRCCS San Raffaele Scientific Institute, Vita-Salute University, Milan, Italy):

Chromogranin A and its fragments in the spatio-temporal regulation of vascular biology and angiogenesis

S23.4 **Y. Peng Loh** (National Institute of Child Health and Human Development, NIH, Bethesda, USA):

Serpinins: tissue distribution and functions

S23.1 Catestatin regulation of immunometabolism

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Obesity and Type 2 diabetes (T2D) affect both the innate and adaptive immune system, as inflammation plays a key role in the pathogenesis of T2D. The activation of Kupffer cells (KCs) and monocyte (Mc)-derived recruited macrophages (McMΦs) in the liver contributes to obesity-induced insulin resistance (IR) and T2D. Although the last 15 years has witnessed a renaissance in the fields of immunology and metabolism, immunometabolism is still a young field with many questions to be answered. Here, we sought to determine whether the Chromogranin A (CgA) peptide Catestatin (CST: human CgA₃₅₂₋₃₇₂) regulates recruitment of macrophages in liver and modulates metabolism in insulin-resistant diet-induced obese (DIO) mice.

DIO mice treated with CST (5 μg/g body weight for 15 days) showed decreased hepatic/plasma lipids and plasma insulin, diminished expression of gluconeogenic genes, attenuated expression of pro-inflammatory genes, increased expression of anti-inflammatory genes in McMΦs, and inhibition of the infiltration of McMΦs, leading to an improvement of insulin sensitivity. Systemic CST knockout (CST-KO) mice on normal chow diet (NCD) ate more food, gained weight, and displayed elevated blood glucose and insulin levels. Supplementation of CST to NCD-fed CST-KO mice normalized glucose and insulin levels. To verify that the CST deficiency caused macrophages to be very pro-inflammatory in CST-KO-NCD mice and produced glucose intolerance, we tested the effects of FACS-sorted F4/80⁺Ly6C⁻ cells (representing KCs) and F4/80⁺Ly6C⁺ cells (representing McMΦs) on hepatic glucose production (HGP). Administering CST had no effect on insulin or glucose tolerance in control lean mice, showing that the effect of CST is restricted to obese animals. This difference may be explained by the reduced levels of normal CST in obese mice compared to the lean control animals. Both basal and glucagon-induced HGP was markedly increased in hepatocytes co-cultured with KCs and McMΦs from NCD-fed CST-KO mice, and the effect was abrogated upon pre-treatment of CST-KO-MΦs with CST. The anti-inflammatory effect of CST in DIO mice was not due to decreased adiposity, as food intake and epididymal white adipose tissue weights were not reduced in obese mice by CST treatment. In CST-KO-DIO mice, absence of CST did increase food intake and body weight that was normalized after CST treatment so endogenous CST may help maintain body weight by suppressing food intake and enhancing glucose tolerance especially under conditions of nutrient excess. Furthermore, exogenous CST can compensate for the decreased level of endogenous CST in DIO, partially reverse the hepatic steatosis, and improve both glucose and insulin tolerance.

We conclude that (i) CST can directly suppress glucose production from hepatocytes and can indirectly (ii) suppress lipid accumulation in liver as well as (iii) macrophage mediated inflammation in obese mice. The net results are improved glucose tolerance and insulin sensitivity in obese mice. Thus, the present studies provide a novel pathway for suppression of HGP through CST-mediated inhibition of macrophage infiltration and function.

S23.2 A selenoprotein-derived peptide with potent *in vivo* anti-neurodegenerative actions

Youssef Anouar, Ifat Alsharif, Benjamin Lefranc, Jérôme Leprince, David Maltête and Loubna Boukhzar
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Parkinson's disease (PD) is characterized by selective and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc), resulting in dopamine production failure (DA) and motor deficit. Although several treatments are currently available for PD, these treatments do not cure the disease and do not stop neurodegeneration. It is therefore necessary to develop other therapeutic strategies that can improve the management of patients. Numerous studies have demonstrated the important role of oxidative stress in the degeneration of dopaminergic neurons, suggesting that proteins that play a role in protecting neurons against the effects of oxidative stress may represent attractive therapeutic targets. It has been shown that cells maintain the redox equilibrium by recruiting several redox enzymes. Among these, selenoproteins such as Selenoprotein T (SelT), a new selenoprotein stimulated by the neuropeptide PACAP, are plausible candidates. Results obtained *in vitro* and *in vivo* showed that SelT exerts an antioxidant activity and is neuroprotective in models of PD. We have thus developed a peptide derived from the active site of SelT, which was able to protect dopaminergic cells in culture after treatment with a neurotoxin, by inhibiting oxidative stress and caspase 3 activity, suggesting that this peptide could stop neurodegeneration in PD. In a mouse model of PD, intranasal administration of this same peptide protected dopaminergic neurons and improved motor skills. The SelT peptide reaches the SNc, reduces oxidative stress and inhibits apoptosis of dopaminergic neurons. Our findings revealed that this SelT-derived peptide may represent a new potential cure for PD.

S23.3 Chromogranin A and its fragments in the spatio-temporal regulation of tumor vascular biology and angiogenesis

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Chromogranin A (CgA), a secretory protein released in the blood by neuroendocrine cells and neurons, is the precursor of various bioactive fragments involved in the regulation of the cardiovascular system, metabolism, and innate immunity. Several studies have shown that CgA can be present at abnormal levels in the blood of patients with various neoplastic, cardiovascular, gastrointestinal, and inflammatory diseases. We have found that physiological levels of full-length chromogranin A (CgA₁₋₄₃₉) and its N-terminal fragment CgA₁₋₇₆ can inhibit angiogenesis, whereas the truncated form CgA₁₋₃₇₃ lacking the C-terminal region is pro-angiogenic. While CgA₁₋₃₇₃ is almost absent in the blood of normal subjects, this and other fragments are increased in patients with non-neuroendocrine tumors, such as multiple myeloma and pancreatic adenocarcinomas, with important prognostic implications. Studies in animal models of non-neuroendocrine tumors have shown that tumor progression is associated with increased cleavage of the C-terminal region of circulating CgA, tipping the balance toward a proangiogenic state. Mechanistic studies have shown that full-length CgA can induce protease-nexin-1, an anti-angiogenic molecule, whereas the fragment CgA₁₋₃₇₃ can induce FGF-2, a pro-angiogenic factor. Receptor studies have shown that CgA₁₋₃₇₃, but not CgA₁₋₄₃₉, interacts with neuropilin-1 on endothelial cells, and that this interaction is crucial for tumor angiogenesis and growth in various animal models on non-neuroendocrine tumors. Finally, we have found the C-terminal residue R₃₇₃, crucial for binding, is rapidly cleaved in plasma to generate CgA₁₋₃₇₂, with consequent loss of neuropilin-1 binding and gain of potent anti-angiogenic activity.

Overall, these results suggest that cleavage of CgA at the R₃₇₃R₃₇₄ dibasic site in tumors and the subsequent removal of R₃₇₃ in plasma represents an important “off/on/off” switch for the spatio-temporal regulation of angiogenesis in tumors, thereby representing a novel therapeutic target.

S23.4 Serpinins: : tissue distribution and functions

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Serpinins are a family of peptides derived from proteolytic processing at paired basic residues at the C-terminus of chromogranin A, followed by aminopeptidase activity to trim the N-terminus of the liberated peptides and pyroglutamination. Three serpinin peptides have been identified that are released from the mouse endocrine pituitary cell line, AtT20. These include serpinin, pyroglutaminated serpinin and C-terminal extended serpinin, serpinin-RRG. Each of these peptides have been found in different amounts in various tissues such as, adrenal medulla, heart, trigeminal ganglion, retina and brain. Serpinin has also been found in human dental pulp. Cellular localization and secretion studies of these peptides indicate that they are packaged in secretory granules and secreted in a regulated (stimulated) manner. Thus serpinin can function extracellularly as signaling molecules. Serpinin and pGlu-serpinin play an important role in up-regulating secretory granule biogenesis in endocrine cells. In the heart, endocrine cells and neurons, serpinin and pGlu-serpinin have been found to protect these cells against cell death under oxidative or ischemic stress. Serpinin and pGlu-serpinin also act as positive cardiac β -adrenergic-like inotropes with a powerful effect on enhancing myocardial contractility. The various localization of these peptides suggests that they may have many physiological functions, possibly as neurotransmitters and neuroodulators

S24. Nesfatin-1: Update on a pleiotropic peptide

Princesa 4

Co-chairs:

Andreas Stengel (Tübingen, Germany) and **Yvette Taché** (Los Angeles, USA)

S24.1 **Tamas Kozicz** (Radboud University Nijmegen Medical Centre, The Netherlands):

Role of nesfatin in emotional processing

S24.2 **Suraj Unniappan** (Sastatchewan, Canada):

Blood glucose homeostatic control by nesfatin-1

S24.3 **Andreas Stengel** (University of Tübingen, Germany):

Role of nesfatin in food intake regulation

S24.4 **Yvette Taché** (University of California-Los Angeles, USA):

Gaps in knowledge - what should be addressed next in nesfatin research

S24.1 Role of nesfatin-1 in emotional processing

Tamas Kozicz

Department of Clinical Genomics, Mayo Clinic, Rochester, MN, USA

Nesfatin-1, derived from an 82-amino-acid peptide precursor protein nucleobindin-2 (NUCB2), is a highly conserved peptide across mammalian species. Nesfatin-1 has initially been described as a novel satiety molecule in the central nervous system. Subsequent studies, however, revealed an unsuspected role for nesfatin-1 mediating emotional processing during stress. In my talk, I will first outline neuroanatomical evidence supporting nesfatin-1's role in the stress adaptation response. Next I will present clinical and preclinical evidence that has significantly broadened our understanding of the biological significance of nesfatin-1 as a novel, integral neuropeptide mediating the impact of stress on mental health. Finally, I will integrate this knowledge to propose that nesfatin-1 may participate in elicitation of different multifaceted internal states such as anxiety/depression and food intake.

S24.2 Nesfatin-1 Regulation of Glucose Homeostasis

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Nesfatin-1 is a multifunctional orphan ligand encoded in the precursor peptide, nucleobindin-2 (NUCB2). NUCB2 mRNA expression and NUCB2/nesfatin-1-like immunoreactivity have been identified in the pancreatic islet beta cells of rats, mice, humans, pigs and dogs, while the immunoreactivity is absent in cats. No nesfatin-1 immunoreactivity was detected in non-beta endocrine cells, and thus, insulin is the only peptide co-localized with nesfatin-1 in the pancreatic islets. Nesfatin-1 stimulated insulin secretion *in vitro* from mouse islets and islet beta cell lines and *in vivo* in the serum of rats. While nesfatin-1 was found to elevate glucagon secretion *in vitro*, serum glucagon was suppressed by nesfatin-1 *in vivo* in rats. During an oral glucose challenge, nesfatin-1 elevated serum insulin levels without affecting glucose in circulation. Nesfatin-1 stimulates the expression and secretion of incretins glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) *in vitro* and *in vivo*. Nesfatin-1 has tissue specific effects on glucose uptake and metabolism in the brain, liver, muscle and adipose tissue. Nesfatin-1 modulates the cellular machinery that regulates insulin sensitivity and glucose production (including gluconeogenesis) in the liver. We recently reported a nesfatin-1-like peptide (NLP) that co-localizes insulin in the pancreatic islets of mice. Similar to nesfatin-1, NLP is insulinotropic. Whether NLP directly regulates glucose homeostasis is currently unknown. In summary, nesfatin-1 acts a glucoregulatory hormone by eliciting insulinotropic effects, and by the direct actions on glucose utilizing and/or storage tissues. The mechanism of action of nesfatin-1 is slowly emerging, while its receptor still remains elusive. Nesfatin-1 binding sites were detected in the pancreas and intestine. Endogenous nesfatin-1 is altered in both type 1, and type 2 diabetes. This lecture will provide a comprehensive discussion on nesfatin-1 and its glucoregulatory effects, and its implications in health and disease.

Acknowledgments: Our research on nesfatin-1 is generously funded by the Canadian Institutes of Health Research (CIHR), Saskatchewan Health Research Foundation (SHRF) and the Canada Foundation for Innovation (CFI).

S24.3 Role of nesfatin-1 in food intake regulation

Andreas Stengel

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Early on, the anorexigenic effect of nesfatin-1 has been described. This effect was suggested to be physiologically relevant as the blockade of endogenous nesfatin-1 led to a stimulation of food intake and subsequently body weight gain. Since then, converging evidence corroborated the food intake-inhibitory action of nesfatin-1. Subsequent studies also investigated the underlying food intake microstructure along with downstream signaling pathways involved. The discovery of nesfatin-1 in X/A-like cells of the stomach together with the only known peripherally produced and centrally acting peptide, ghrelin, attracted a lot of attention and led to the hypothesis of a bidirectional modulation of food intake by this gastric cell: stimulation *via* ghrelin and inhibition *via* nesfatin-1. However, it is to note that the anorexigenic effect of nesfatin-1 is robustly observed after injection into the brain, while after peripheral application conflicting data exist. The present talk will provide an overview on the state of knowledge on nesfatin-1's role in the modulation of food intake and also discuss potential clinical implications.

Acknowledgements: This work was supported by funding of the German Research Foundation (STE 1765/3-2) and Charité University Funding (UFF 89/441-176, A.S.).

S24.4 The interaction between stress and NUCB2/nesfatin-1—what gaps in knowledge should be filled?

Yvette Taché

Vatche and Tamar Manoukian Division of Digestive Diseases, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA and CURE: Digestive Diseases Research Center, VA GLA HS, Los Angeles, CA, USA

In 2006, Oh et al. identified a new 82-residue polypeptide derived from nucleobindin2 (NUCB2) in rodents that was named nesfatin-1 based on its satiety action in the hypothalamus. Since then, 437 articles have been published on nesfatin-1. In the central nervous system, convergent studies quickly established nesfatin-1 as a novel regulator of appetite. Subsequently, growing evidence demonstrated pleiotropic actions in the brain to influence emotion, anxiety-related, fear-like and cognitive behaviors, sleep, reproduction and visceral functions. There is also growing evidence that various acute and chronic stressors alter NUCB2/nesfatin-1 in specific brain nuclei. We will discuss the functional consequences and the interaction between nesfatin-1 and the corticotropin-releasing factor stress system and its involvement in mediating specific biological actions of centrally injected nesfatin-1.

However, several challenges remain in the characterization of the biological actions and the role of nesfatin-1 in health and disease. Important gaps need to be filled related to the identification of the receptor, although binding sites have been detected in the hypothalamus and cortex. The absence of selective antagonists to NUCB2/nesfatin-1 also hampers the further characterization of the functional consequences of blocked nesfatin-1 signaling. In most tissue studied either full length NUCB2 is present or studies did not distinguish between NUCB2 and processed nesfatin-1. The processing of NUCB2 may take place physiologically, however, it has not been proven *in vivo* or *in vitro*. In Chinese hamster ovary cells, overexpressing the relevant genes (*Nucb2*, *Pc1*, and *Pc2*) failed to produce nesfatin-1. Which other enzyme may be involved? At the cellular level in the paraventricular nucleus of the hypothalamus, NUCB2/nesfatin-1 is mainly localized in secretory vesicles in perikarya near the Golgi apparatus and not in axon terminals, suggesting dendritic release and autocrine or paracrine actions yet to be established.

Neuroscience Young Investigator Special Lecture

Peptidergic regulation of cortical inhibition

Atlantes amphitheater

Sarah Melzer

Department of Neurobiology, Howard Hughes Medical Institute, Harvard Medical School

About the speaker



Sarah Melzer is a postdoctoral fellow in Bernardo Sabatini's lab in the department of Neurobiology at Harvard Medical School in Boston. Her research focuses on peptidergic regulation of inhibitory neurons in the cortex. She uses approaches to study cellular, network and behavioral effects and collaborates with several research groups to develop new techniques for the investigation of peptides.

Sarah received a Bachelor's degree in Biosciences from Westfaelische Wilhelms-University Muenster (Germany) and Rijksuniversiteit Groningen (Netherlands), and a Master's and PhD degree in Molecular Biosciences and Neurosciences at Heidelberg University (Germany). Her graduate research in Hannah Monyer's lab focused on local and long-range cortical inhibitory neurons and oxytocinergic regulation of neuronal activity. Her work was published in *Science*, *Nature Neuroscience*, *Neuron* and *Cell Reports*. She has performed research at RIKEN Brain Science Institute (Japan) and at Newcastle University (UK). She joined Bernardo Sabatini's lab in 2015 and has received several fellowships since then (EMBO Long-term fellowship, DFG fellowship, Brooks fellowship, Gordon fellowship).

SL: Peptidergic regulation of cortical inhibition

Sarah Melzer, Adrienne Philson, Bernardo L. Sabatini

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The cerebral cortex underlies sensory processing, sensorimotor learning and control of a wide range of behaviors. Its function is maintained by a network of excitatory principal cells that are under tight control by several types of inhibitory neurons that regulate cortical activity and plasticity. Recent work has demonstrated that enhancing or suppressing the activity of subpopulations of these neurons alters sensory processing, motor control, sensorimotor learning, neuropathic pain and adult functional plasticity. Investigating how their activity is controlled is thus crucial to understand cortical processing and function and to reveal new therapeutic targets for the treatment of traumatic brain injury, ischemia, neurodegenerative and neuropsychiatric diseases.

All inhibitory neurons in the cortex are marked by a rich repertoire of neuromodulator receptors, including neuropeptidergic receptors. Neuropeptides are often released in a state-dependent manner and can thus lead to adaptive changes in cortical activity and behavior. The function of most cortical peptides remain largely unknown.

Our aim is to gain detailed insights into cortical neuropeptide and neuromodulator function at a cellular, circuit and behavioral level. I will talk about previous and current studies that highlight the importance of inhibition and neuromodulation in the cortex and point out challenges and new techniques that can help to tackle them.

Abstracts

*From
Free contributions
In
Alphabetic order of the presenting author*

P1. Vasoactive intestinal peptide localization in the developing chicken ovary

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The follicle assembly during chicken ovary development is a complex process in which germ cells are associated with somatic cells. This process is regulated by hormones, and growth factors, among other signals (1). In the ovary of mammals, the presence of peptidergic nerve fibers has been described. These are not only responsible for the release of neurotransmitters and regulation of blood flow, but participate in the regulation of steroidogenesis. Among other neurotrophins, the vasoactive intestinal peptide (VIP) has been detected in the ovary associated with preantral and antral follicles of mammalian species (2).

Studies in the chicken ovary have identified the presence of the receptor for VIP (cVIP) in the cells of the granulosa layer of the follicles, so its participation in follicular maturation, ovulation and possibly in the regulation of steroidogenic secretion is suggested (3). The presence and function of neuropeptides in chicken gonadal development has not been described, so we investigated the location of VIP in the ovary in stages around the hatching day. At 14 days of incubation, VIP is very scarce in the deep ovarian medulla, among the steroidogenic cells cords of and surrounding the lacunar channels and is present in the surface epithelium. Between 15 and 19 days of incubation the VIP-positive innervation in the medulla and deep medulla becomes more evident near steroidogenic cell cords and lacunar channels. From the time of hatching, VIP is observed in nerve fiber of the subcortical medulla and between the oocyte nests. Subsequently, the presence of VIP positive nerve fibers throughout the ovary increases progressively, among the oocyte nests and the incipient follicles, VIP localization remains faintly in the surface epithelium between posthatching days 8 and 21.

The presence of VIPergic nerve fibers is observed already integrated within the layers of cells that will form the theca of the ovarian follicles. VIP immunolabeling could be topographically associated with steroidogenic function and its participation in follicle maturing. We propose that VIP could act as a modulator of gonadal histodifferentiation and steroidogenic activity.

We appreciate the technical support of Verónica Rodríguez Mata and the laboratory of Rosa Guadalupe Fera Segura.

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P2. Food motivation, food reward and anhedonia are unaffected in a new model of altered body weight homeostasis in rats

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Most strategies to reduce body weight are largely ineffective because they do not reduce the so-called set point for body weight homeostasis. Such strategies do not decrease body weight because there is a compensatory hyperphagia. A new model of weight loss was recently developed in rodents (1), which is caused by a reduction in food intake and could indicate a lowering of the set point. We have explored the mechanism in this model and sought to determine whether it is largely homeostatic or whether it also impacts on behaviors important for food reward and motivation. Male Sprague-Dawley rats were intraperitoneally implanted with capsules, either loaded (15% of body weight) or empty (1.3% of body weight), and then subjected to the following tests: (i) Consumption of chow; (ii) Food choice paradigm consisting of chow, lard and sucrose pellets; (iii) Conditioned place preference (CPP) with chocolate as the reinforcer; (iv) Progressive ratio (PR) lever pressing for a sucrose reward; (v) Two-bottle preference test with sucrose solution; and (vi) Pica test. Increased loading in rats leads to a decrease in body weight and chow consumption. When a food choice is offered, the consumption of all foods is decreased and the food preference is therefore unchanged. However loading did not alter food reward in the CPP test or food motivation during PR lever pressing for sucrose. Furthermore, loading did not result in anhedonia or malaise, as shown in the two-bottle choice or pica tests, respectively. Our data demonstrate that behaviors linked to food reward and motivation were largely unaffected by increased loading. Given that such behaviors are expected in situations of negative energy balance, we repeated the PR experiment with an additional group pair-fed to the loaded group (about 70 to 80% restriction). However a 50% restriction was needed to increase food motivation. We conclude that the weight loss model of loading may involve a lowering of the set point for body weight homeostasis, without reaching the threshold for an increased food motivation.

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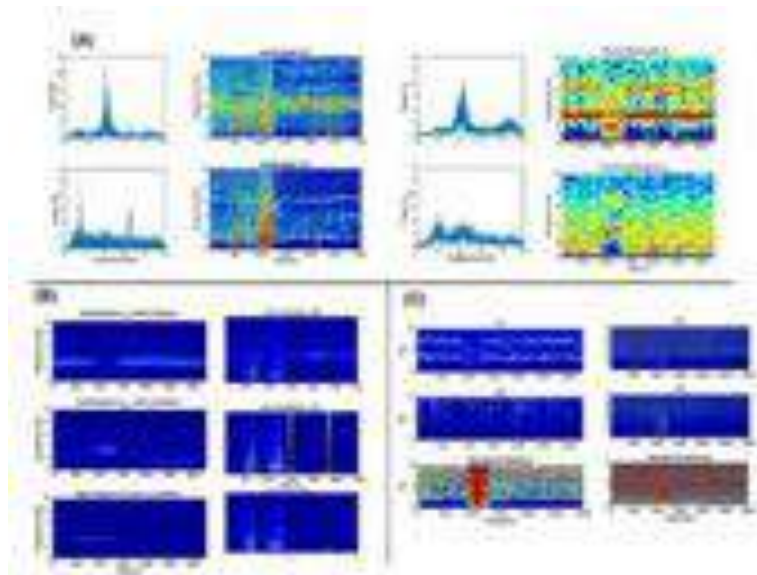
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P3. Analysis and modeling of low frequency local field oscillations in a hippocampal circuit under osmotic stimuli

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Time series analysis is known to be a useful tool in the study of the dynamics and characteristics of complex systems, such as neural interactions. This study explores the behavior of different analytical methods for measurements made within two regions of interest of the hippocampus, dorsal and ventral CA2 regions, of adult male Wistar rats. A 2% body weight volume of a 900 mM NaCl (hyperosmotic) solution, was intraperitoneally injected to perturb the hypothalamus activity and measurements were obtained using electrodes inserted in the hippocampus to observe the local field potential (LFP). The resulting data was analyzed using Fourier Transformations and Wavelet Analysis. In all the methods it was observed that there was a modification in the delta (0.1 - 4.0 Hz) and an increase in theta (4.0 -8.0 Hz) oscillations after the solution injection into the rat. A comparison between the signals was then done to determine the quality of the data and correlation methods were used to determine if there was any coherence between the signals due to the perturbation, under the hypothesis that the strength of the functional connections between the vasopressinergic hypothalamic magnocellular neurons and their targets in the hippocampus is affected by the hyperosmotic solution. Results showed an increased coherence for these regions which would suggest that increased activity in the hypothalamus provides neuromodulatory input to the hippocampus, as well as enhancing functional coupling between the theta oscillations. Based on the hypothesis of these connections, a model is then developed to emulate the behavior of the signals observed. On the figure provided for this abstract we can see an example of the experimental and theoretical results obtained in this work showing comparison of (A) Analysis of the Fourier transform, (B) the correlation analysis and (C) the Wavelet analysis. The results of the theoretical model and those of the experiment show a similar behavior which reinforces the hypothesis concerning connections between the hypothalamus and the hippocampus proposed in this study.



P4. Superoxide-Dependent Redox Signaling in the Supraoptic Nucleus is Associated with the Neuroendocrine Response to Water and Electrolyte Imbalance

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In models of altered osmolality and water balance, including two-day salt loading and water deprivation, previous studies have suggested that intracellular redox signaling in the supraoptic nucleus (SON) contributes to the brain neuroendocrine response. However, important molecular components of the redox-dependent signaling pathways, including the specific reactive oxygen species (ROS), involved in this response are not yet delineated. Here, we tested the hypothesis that water deprivation increases specific ROS, particularly superoxide, and stimulates the redox-sensitive transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) in the SON, and that these responses are dependent on the duration of water restriction. We used rodents deprived of water for 24 and 48 hours (24h WD and 48h WD, respectively) as the model animal to test this hypothesis.

We observed an increase in Nrf2 mRNA in the SON of mice subjected to 24h WD (Control: 0.84 ± 0.08 vs 24h WD: 1.16 ± 0.09 arbitrary units) suggesting the activation of redox signaling during dehydration. In addition, at 48h WD, levels of hemeoxygenase-1 (HO-1), a Nrf2 target gene, were elevated in the SON (Control: 0.98 ± 0.1 vs 48h WD: 1.46 ± 0.1 arbitrary units). HO-1 activity was also increased at both time points of water restriction ($F_{2,34} = 7.718$, $p < 0.05$). To determine if levels of specific ROS are altered during water deprivation, we used electron paramagnetic resonance (EPR) spectroscopy, the gold standard for measuring free radicals. After 24h or 48h of water deprivation in C57BL/6 mice, fresh non-fixed brains were collected and the SON was harvested via micropunch. Superoxide levels were measured in the SON micropunches by EPR. We also measured plasma osmolality and volume. We observed a dehydration period-dependent increase in superoxide levels (reported as EPR spectra amplitude in arbitrary units) in the SON (Control: 7150 ± 3856 ; 24h WD: 14240 ± 9931 ; 48h WD: 15795 ± 7400 arbitrary units/mg of tissue) along with plasma osmolality (Control: 317 ± 2.0 ; 24h WD: 326.8 ± 1.3 ; 48h WD: 330.7 ± 1.3 mOsm/kg H₂O); protein (Control: 3.56 ± 0.16 ; 24h WD: 4.36 ± 0.16 ; 48h WD: 3.94 ± 0.14 g/dL plasma); and hematocrit (Control: 35.8 ± 5.1 ; 24h WD: 42.4 ± 0.9 ; 48h WD: 45.7 ± 0.9 %).

Together, these data suggest that the neuroendocrine system is associated with superoxide-dependent redox signaling and activation of Nrf2 in the SON to cope with dehydration.

P5. Interaction of chronic unpredictable stress and brain trauma on depressive-like behaviors, cognitive performance, and markers of neurotransmitter signaling and immune function

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Traumatic brain injury (TBI) survivors endure cognitive impairments and are vulnerable to neuropsychiatric disorders. Chronic unpredictable stress (CUS) is a risk factor for many psychopathologies, thus we began to assess cognitive and anxiety-like dimensions sensitive to both TBI and CUS. We hypothesized that a controlled cortical impact (CCI) injury in conjunction with CUS will cause cognitive impairments in rats in an attentional set-shifting test (AST), reduced sucrose preference, open field exploration, blunted weight gain, and increased inflammatory markers. Adult male rats were subjected to CCI (2.8 mm depth) or sham injury, and were assigned to receive CUS (21 days) or handling (CTRL). Rats were then tested for anxiety and anhedonia, as well as on AST, which involves increasingly difficult tasks for food reward. A separate cohort was sacrificed post-stress for serum corticosterone (CORT) and cytokine analyses, as well as brain monoamine protein markers. CUS resulted in 5-10% weight reduction compared to CTRL, yet the combination of TBI and CUS did not negatively impact the open field test or sucrose preference, albeit it produced a near-significance raise in CORT ($p=0.056$). TBI and CUS alone induced cognitive deficits on AST, but not when given together, suggesting a resilience effect or compensatory interaction. Similarly, proinflammatory markers such as IL-2, IFN- γ or TNF- α were decreased in the combined TBI + CUS group. Current analyses include markers of neurotransmission life cycle in various discrete brain regions mediating anxiety-like responses and cognitive function. This project provides outcomes pertaining to cognition, anxiety and depression following overlapping chronic stress and the recovery phase of TBI, with future directions involving environmental enrichment as a preclinical model of neurorehabilitation.

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P6. Identification of a novel PACAP--stimulated essential thioredoxin, selenoprotein T, which protects dopaminergic neurons in mouse models of Parkinson's disease

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Oxidative stress is central to the pathogenesis of Parkinson's disease, but the mechanisms involved in the control of this stress in dopaminergic neurons have not been fully elucidated. Here we demonstrate that selenoprotein T (SelT), a novel PACAP-stimulated thioredoxin-like protein, has potent oxidoreductase activity essential for embryonic development and dopaminergic neuron survival and function. Analysis of human brain samples showed that SelT is highly expressed in the striatum of Parkinson's disease patients compared to controls. Conditional disruption of the SelT gene in the mouse brain provoked a reduction in the size and dopamine content of the striatum. Treatment with a Parkinson's disease-inducing neurotoxin such as 1-methyl-1-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or rotenone triggered SelT expression in the nigrostriatal pathway of wild-type mice, but provoked rapid and severe parkinsonian-like motor defects in conditional brain SelT-deficient mice. This motor impairment was associated with marked oxidative stress and neurodegeneration, and decreased tyrosine hydroxylase activity and dopamine levels in the nigrostriatal system of brain-specific SelT deficient mice compared to wild-type littermates. These findings revealed a hitherto unidentified PACAP-stimulated thioredoxin enzyme that protects dopaminergic neurons and prevents early and severe movement impairment, providing clues for the understanding of the molecular underpinnings of oxidative stress in Parkinson's disease.

P7. Vasopressin in the lateral septum sex-dependently alters neurotransmitter release: Implications for sex--specific regulation of social play

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Social play is a rewarding behavior displayed by nearly all mammals and peaks during the juvenile period. We recently showed that arginine vasopressin (AVP) in the lateral septum (LS) regulates social play in opposite directions in male and female juvenile rats. Here, we sought to determine whether and how the LS-AVP system modulates the release of a wide array of neurotransmitters (NTs) in the LS.

We used microdialysis with and without retrodialysis to quantify extracellular NT release in the LS of freely moving juvenile rats, while 1) AVP was applied into the LS, 2) rats were exposed to social play or 3) a vasopressin V1aR antagonist was administered into the LS.

We observed a variety of dynamic release patterns of NTs that were sex- and condition-specific. LS application of AVP caused an increase in the glutamate and dopamine release in females, while no change was seen in males. Other NTs did not change in sex-specific ways. Exposure to social play resulted in an increase in the release of all NTs in females, while in males, dopamine and norepinephrine remained unchanged. Finally, application of a V1aR antagonist in the LS caused increased release of glutamate and norepinephrine, with a greater effect on release in females than in males. Interestingly, the sex differences in glutamate and dopamine release were eliminated with V1aR antagonist administration.

These findings suggest a differential involvement of NTs in the LS of male and female juvenile rats exposed to social play, with potential roles of glutamate and dopamine in the sex-specific regulation of social play by the LS-AVP system.

P8. Characterisation of periventricular nucleus kisspeptin neuron projections to oxytocin neurons in pregnancy and lactation

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Oxytocin induces uterine contractions during birth. Oxytocin is secreted from the posterior pituitary gland by hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus (SON) neurons. The trigger for increased oxytocin secretion is largely unknown. However, we have shown that kisspeptin increases the firing rate of oxytocin neurons in late-pregnant rat but not in non-pregnant rats. Here, we tested whether kisspeptin fibre density changes around oxytocin neurons in pregnant mice and determined the origin of these kisspeptin fibres. To determine whether kisspeptin projections to oxytocin neurons are changed in pregnancy, kisspeptin and oxytocin double-label immunohistochemistry was performed on brain slices from non-pregnant ($n = 7$), day 7, 14 and 19 ($n = 8/\text{group}$) pregnant and day 7 lactating ($n = 7$) mice. Sections were photographed on a confocal microscope and the fraction of kisspeptin-positive voxels in each area was analysed using FIJI. PVN kisspeptin fibre density was similar in non-pregnant, day 7 and day 19 pregnant mice but was lower on day 14 of pregnancy and day 7 of lactation than in non-pregnant mice ($P < 0.05$). A similar pattern of kisspeptin fibre density was evident in the SON. Close appositions of kisspeptin fibres with oxytocin cell bodies and dendrites are currently being analysed. To determine the origin of the kisspeptin projection to oxytocin neurons, retrograde label was injected into the PVN of non-pregnant and pregnant mice. Retrograde label was only found in the periventricular nucleus (PeN), with a higher proportion of retrogradely-labelled PeN kisspeptin neurons in day 18 pregnant mice than in non-pregnant mice. We conclude that the PeN kisspeptin projection to oxytocin neurons is more prominent at the end of pregnancy.

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P9. The suprachiasmatic nuclei are involved on the generation of neural signals required for the regulation of ovulation during each stage of the estrous cycle

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Several species of mammals, including primates and rodents, ovulate one or more oocytes spontaneously during each reproductive cycle. Ovulation is regulated by the pituitary gonadotropins, which secretion is in turn regulated by the gonadotropin releasing hormone (GnRH), a hypothalamic neuropeptide. The fluctuating levels of estradiol exert inhibitory and stimulant feedback at the pituitary and hypothalamus determining the pattern of GnRH release. It has been shown that in addition to high estradiol levels, neural signals are required to initiate the preovulatory surge of GnRH. Such signals occur between 14:00 and 16:00 h in every stage of the estrous cycle. This circadian periodicity, along with the impairment of estrous cycles and ovulation that accompanies the prolonged exposure to constant light or darkness leads to the conclusion that the circadian pacemaker residing at the suprachiasmatic nucleus (SCN) is involved in the timing of GnRH release. There is anatomical evidence linking the SCN with the GnRH-neurons at the preoptic area. In addition, lesions of the SCN result in blockade of ovulation. We studied the possibility that the SCN regulates ovulation by means of temporal inactivation of the SCN electrical activity, which does not modify its endogenous period. Female rats were implanted with guide bilateral cannulas aimed to the SCN. After full recovery of estrous cycles animals were connected to a micro-infusion system and injected with either tetrodotoxin (TTX) or saline while freely moving in their cages. Injections were performed at 14:00 h of each stage of the estrous cycle and animals were euthanized on the next predicted estrous. The number of ova shed was counted and intact estrous-rats were used as absolute control. All intact animals ovulated (7/7). Saline-treated rats displayed an inhibitory effect that depends on the stage of the estrous cycle when the treatment was performed (proestrous 7/7, metestrous 6/9, diestrous 2/7*, estrous 2/9*; * $p \leq 0.05$ Fisher test vs intact). On the other hand, TTX blocked the ovulation irrespectively of the stage of the cycle (proestrous 0/5*, metestrous 1/4*, diestrous 0/7*, estrous 1/4*; * $p \leq 0.05$ Fisher test vs intact). These results may be explained on the basis of inhibition of stimulant signals arising at the SCN and targeting the GnRH neurons directly or indirectly. In the first case, inhibition may occur via VIP-containing projection to POA, and in the second case via the AVP-containing projection to RP3V kisspeptin-neurons. Experiments designed to disclose the deleterious effect of saline solution, as well as the replacement of the VIP and AVP signals, are in progress.

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P10. Dynamic modulation of mouse locus coeruleus neurons by the vasopressin-1b receptor system

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The locus coeruleus (LC) nucleus is the main source of noradrenaline (NA) throughout central nervous system (CNS) and modulates a range of brain functions, such as arousal and cognition, in response to stress. Neurochemically diverse inputs from many brain regions regulate the level of LC neuronal activity in a behavior-dependent manner. One such afferent input contains the neuro-hormone arginine-vasopressin (AVP). The overall aim of the project was to characterise the involvement of specific AVP receptor subtypes on activation on the spontaneous firing rates (FRs) of the mouse LC, using AVP receptor agonists and antagonist together with a patch clamp electrophysiology in acute brain slices of mouse.

A total of 50 cells were recorded from 15 animals. Application of the V1b and V2 agonist desmopressin (200 μ M) has contrasting effects on LC spontaneous FR (n = 20) with 45% of cells responding with increased FR (1.7 to 2.8 Hz) and 55% showing a significant decrease in FR (1.4 to 0.8 Hz). The application of a V1a antagonist ((d(CH₂)₅1, Tyr(Me)₂, Arg₈)-Vasopressin, 30 nM) (n=16) also has contrasting effects on LC FR with 62% of cells exhibiting an increase in FR (1.6 to 2.1 Hz) and 38% a decrease (2 to 1.4 Hz). For the V1b receptor antagonist (TASP 039325, 20 nM) (n = 14), 57% responded with an increased FR (2.2 to 3.2 Hz); 43% showed a decrease in activity (1.7 to 1.4 Hz). The diverse responses to LC-AVP receptor modulation suggest that various populations of LC neurons express distinct receptor profiles.

Neurochemically diverse inputs set the tone for locus coeruleus (LC) neuronal activity, which in turn modulates adaptive physiological and behavioural responses essential for survival. We provide the first demonstration of the molecular and functional characteristics of one such LC afferent system, arginine-vasopressin (AVP), by characterising its receptor-specific modulation of identified LC neurons and plasticity in response to stress. AVP receptor 1b (V1b) was expressed on plasma membranes of LC neurons adjacent to GABAergic and glutamatergic synapses. V1b activation either increased or decreased the activity of different LC neuronal populations. V1b blockade significantly altered LC neuronal activity, demonstrating that endogenous AVP sets the basal LC neuronal firing rates. Finally, exposing animals to acute stress significantly increased V1b expression. The study reveals the AVP-V1b system as a considerable component of the LC molecular architecture and regulator of LC activity, thereby serving as a novel modulator of LC-mediated homeostatic responses.

P11. Adult male rats subjected to chronic variable stress present a response of Hypothalamus-Pituitary-Thyroid (HPT) axis to cold exposure

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The hypothalamus-pituitary-thyroid (HPT) axis is involved in energy homeostasis. It is activated by a drop in external temperature and inhibited by stress; a previous stress exposure or corticosterone injection blunts cold-induced activation of the HPT axis. Certain types of chronic stress also inhibit the HPT axis, but their effects on the axis response to acute energy-demanding stimuli are unknown (1, 2). Animals may show habituation if exposed to the same type of chronic stress, but not if it varies (i.e, restraint, tilted cage, elevated platform, cart transport, stroboscopic light, white noise, etc). The aim of this work is to evaluate whether or not chronic variable stress exposure interferes with the response of the HPT axis to cold.

Wistar male rats were subjected to 15 days of Chronic Variable Stress (CVS) at 75 days of age. On day 16, at 10:00 am, animals were exposed for 1h to 4° or 22°C. Compared to naïve animals, CVS decreased food intake: experimental controls were therefore pair-fed. Variations in levels of hormones or mRNA were evaluated by immunoassays or RT-PCR, respectively. Corticosterone serum concentration was quantified at day 1, 7 and 14 of CVS; elevation of corticosterone concentration increased up to the final day, showing no habituation. Cold exposure increased paraventricular hypothalamic nucleus thyrotropin-releasing hormone, and corticotropin-releasing hormone receptor 1 expression in controls, but not in animals exposed to both cold and CVS. Serum concentration of thyrotropin increased only in controls, but, as published (3), at this short time neither T3 or T4 changed. In contrast, T3 serum concentration decreased in CVS rats exposed to RT or cold. The thermogenic organ, brown adipose tissue (BAT), showed increased expression of uncoupling protein-1 (UCP-1) in cold-exposed controls but not in CVS. These results corroborate the finding of hypothalamus-pituitary-adrenal hyperactivity after CVS, and support the inhibitory effect of stress on HPT response to cold stimulation. Dysfunction of the HPT axis response may contribute to altered energy homeostasis.

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P12. *Aedes aegypti* salivary gland extract alleviates acute itching by blocking TRP channels

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The escalating incidence of arboviruses such as dengue during the blood feeding of the vector female mosquito *Aedes aegypti* (*A. aegypti*) is now regarded as Latin America's most important public health problem [1]. In parallel, it has been shown that the female *A. aegypti* salivary gland extract (SGE) contains a variety of immunomodulatory active substances, including peptides (31 kDa - 56 kDa) with anti-inflammatory actions [2]. Interestingly, primary exposure of humans to *A. aegypti* first feeding did not evoke itch [3]. This study aimed to investigate the effects of the SGE toward sensitive function, such as itch. Acute pruritus (and skin oedema) was evoked by the intradermal (i.d.) injection of compound 48/80 (C48/80 10 µg/site), PAR-2 receptor agonist SLIGRL (40 nmol/site), Mrgrp receptor agonist chloroquine (100 µg/site) or TRPA1 receptor agonist allyl isothiocyanate (AITC, 1 µmol/site). The dorsal skin scratching or oedema to the injections site was assessed in the absence or presence of SGE. The concentration of intracellular Ca²⁺ ions on cultured dorsal root ganglia (DRG) neurons or transfected HEK293t lineage (TRPA1) was measured using the Ca²⁺ imaging method. By recording bouts of scratching, we show that i.d. injection of *A. aegypti* SGE (0.3 to 3 µg/site) inhibited itch, skin oedema and neutrophil influx evoked by C48/80, but it did not protect against mast cell degranulation *in vivo* or *in cellula*. SGE partially reduced SLIGRL, chloroquine and AITC-induced pruritus and capsaicin-induced neurogenic oedema *in vivo*, suggesting that SGE affect pruriceptive nerve firing independently of histaminergic pathway. Activation of TRPA1 results in a strong increase of intracellular Ca²⁺ both in transfected HEK293T lineage (TRPA1) and DRG neurons, and this was reduced by SGE. We show, for the first time, that conserved protein/peptides present in the SGE inhibit sensitive responses (itch) to histaminergic and non-histaminergic pathways (TRPA1 channels). EGS contents may represent a potential tool to treat itch.

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P13. Differential effect of acute stress on hypothalamic and brainstem expression of orexigenic and anorexigenic neuropeptides genes in rats.

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Several lines of evidence suggest that stress applied acutely or chronically leads to dysfunction of many central and peripheral functions including food intake and regulation of metabolism. Centrally, the hypothalamus and a particular zone in the brainstem, the dorsal vagal complex (DVC), play an important role in monitoring and modulating body energy balance and food intake involving orexigenic and anorexigenic peptides. We analyzed the long term effects of a single exposure to immobilization stress (IS) (1 h) on the expression of anorexigenic (Pro-opiomelanocortin (POMC) and cocaine amphetamine related transcript (CART)) and orexigenic (neuropeptide Y (NPY), Agouti related peptide (AgRP)) factors in hypothalamus and DVC in the rat. We showed that in the hypothalamus, the expression of POMC and CART were up-regulated at the end of IS and for up to 24 h. This up-regulation persists until 48-72 h after IS for CART only. In the DVC, peptide elevation peaks significantly at 24 h post stress and declines afterwards; CART expression is down-regulated after 48 h post stress. Concerning the orexigenic factors, the expression of NPY and AgRP show a gradual increase just after the end of IS. The up-regulation is significant only at 24 h after stress for AgRP but remains significantly higher for NPY compared to controls. In DVC, the expression of the two factors shows generally a similar post-stress pattern. Thus, a significant increase just after the end of IS persists up to 24 h. The levels tend then to reach the basal levels although, they were slightly but significantly higher up to 72 h after stress for NPY.

These results indicate that stress differentially affects orexigenic and anorexigenic neuropeptide gene expression in whole rat hypothalamus and DVC. They show also the presence of a parallelism between dynamics of regulation of POMC and AgRP, compared to that of CART and NPY, when each brain region (hypothalamus and DVC) is considered separately in accordance with their different but complementary roles in food intake regulation. The latter effects of orexigenic peptides tend to attenuate the anorexigenic effects of anorexigenic peptides, and consequently to abolish the anorexic state generated by stress.

P14. Do dopamine DA1 receptors modulate GnRH release necessary for ovulation?

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Ovulation is one of the final steps of follicular development, which begins with the activation of primordial follicles and culminates with the release of a mature oocyte capable of being fertilized, along with formation of the corpus luteum. This process is regulated by the neuroendocrine hypothalamus-pituitary-ovary (HPO) axis within which the hypothalamus secretes the gonadotropin releasing hormone (GnRH). This decapeptide is the major stimulus for gonadotropin secretion by the hypophysis. The gonadotropins, follicle stimulating hormone and luteinizing hormone, regulate the ovarian functions such as the hormonal secretion, follicular development and ovulation. The secretion of GnRH is regulated by a variety of neurotransmitters through a complex network of afferent inputs. One of these inputs is the dopamine (DA) system, although its participation is controversial. There are experimental results suggesting that its participation is either stimulatory or inhibitory or that it is without HPO effect. There are five different DA receptors classified in two families (DA1 and DA2) with opposite signaling properties, perhaps contributing to controversy about DA's role in HPO axis function. The unilateral implantation of haloperidol (a non-selective DA receptor blocker) crystals in the anterior hypothalamic-preoptic area (POA-AHA) region, performed at estrus or diestrus-1, blocked ovulation, while the same treatment performed at diestrus-2 or proestrus had smaller effects.

In the present study we analyzed the possibility that the controversial participation of DA in the regulation of ovulation depend on the kind of DA receptor analyzed in a given study. To determine if the DA1 receptors (RDA1) present in neurons of the POA are involved in the regulation of ovulation, a batch of rats was unilaterally implanted with a permanent cannula directed to the right or left POA. Animals were allowed to recover and then microinjected with SCH-23390 (blocker of the RDA1) or vehicle at 09:00 h of diestrus-1, diestrus-2, proestrus or estrus. All groups were sacrificed by an overdose of sodium barbiturate at the next expected day of estrous; the oviducts were dissected and ova shed counted using a dissecting microscope. The microinjection of the vehicle did not modify neither the ovulation rate nor the number of ova shed in comparison with the intact group, except for a higher number of ova shed by the vehicle microinjection in the left POA made in estrus (13.0 ± 0.5 vs. 9.2 ± 0.8 , $p =$).

Taken together, the results show that no differences were observed in ovulation rate between rats microinjected with the vehicle or with the RDA1 blocker ($41/41$ vs. $35/35$), while the number of ova shed by the animals with blockade of the RDA1 was higher than in those rats injected with the vehicle (11.7 ± 0.4 vs. 10.1 ± 0.4 , $p = 0.01$, Mann-Whitney two-tailed test). The current results suggest that the RDA1 present in the POA participates in an inhibitory way on the release of GnRH and ovulation and varies throughout the estrous cycle.

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P15. Thyrotropin-releasing hormone-degrading ectoenzyme null male mice are resistant to diet-induced obesity

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Thyrotropin-releasing hormone (TRH, pGlu-His-Pro-NH₂) is expressed in the brain as well as in a few peripheral tissues. It is a short-lived intercellular signaling molecule, that is hydrolyzed by the thyrotropin-releasing hormone-degrading ectoenzyme (TRH-DE), a narrow specificity peptidase whose only known biological substrate is TRH. TRH-DE is expressed in various brain regions, including at the base of the third ventricle, in β 2 tanycytes whose cytoplasmic extensions contact TRH terminals in the external layer of the median eminence (Joseph-Bravo et al, 2016). Functional evidence suggests that median eminence TRH-DE controls the turnover of TRH before entry into the portal capillaries that connect the hypothalamus with the anterior pituitary and indicate that the peptidase controls the levels of thyrotropin in the circulation (Rodríguez Rodríguez et al, 2018). The activity of the enzyme in tanycytes is sensitive to energy balance cues and may contribute to adjusting the thyroid axis to changing energy needs. To test whether the enzyme is indeed relevant for energy balance, we characterized a line of mice generated in the B6129S5 background in which exon was 2 deleted (Tang et al, 2010). TRH-DE activity was eliminated in the KO animals. The mutation was backcrossed for 11 generations on the C57BL/6NJ background. On a standard diet, genotype-dependent effects were generally not significant. 70-75 days old male mice, either wild type (WT), or heterozygous (HZ) or homozygous (KO) for the mutation, were switched from a standard diet to a high fat (45% Kcal from lard fat) and high fructose (10% in water) diet (HFFD) for 9 weeks. The genotype did not change the amount of food (kcal) ingested on the HFFD. The body weight of HFFD WT and HT mice was much higher than that of KO mice. Bio-impedance data indicated a lower fat mass in KO mice, compared to WT or HT mice. The body mass index was also lower, and glucose tolerance higher in KO mice than in WT or HT mice. The data suggest that ablation of TRH-DE produces metabolic alterations that reduce some of the effects of diet-induced obesity. Further studies are required to clarify whether it is the central or the peripheral suppression of Trhde expression that is responsible for the distinct phenotype.

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P16. Thyme essential oil inhalation decreases endotoxin-induced acute airway inflammation in a mouse model via TRPA1/TRPA1/V1 ion channels

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Inflammatory lung diseases affect a large population worldwide. Essential oils can easily reach the respiratory tract via inhalation. Their effects in inflammation are poorly studied and still controversial *in vivo*. There are cell culture data for the anti-inflammatory and antibacterial activities of thyme oil (TO), but its *in vivo* actions and mechanisms have not been investigated. Transient Receptor Potential Vanilloid1 (TRPV1) and Ankyrin1 (TRPA1) ion channels are co-expressed on sensory nerves and epithelial cells of the airways, activated by a variety of inflammatory mediators and inhaled irritants, and play a role in sensory-immune interactions. Therefore, we examined 1) the role of TRPA1/V1 in the pathogenesis of lipopolysaccharide (LPS)-induced airway inflammation; 2) the effects of TO inhalation in this model, and 3) the involvement of TRPA1/V1 ion channels in mediating TO effects.

The chemical composition of TO was determined by GC-MS. Lung inflammation was evoked by intratracheal administration of 60 µL LPS (*E. coli* 083: LPS) in female TRPA1/V1^{+/+} (WT) and TRPA1/V1^{-/-} (KO) mice (n=7-10/group). TO or the control paraffin oil was inhaled 3 times for 30 min during the 24-h period. Airway responsiveness was measured in awake, spontaneously breathing animals by unrestrained whole body plethysmography. Lung myeloperoxidase (MPO) activity was assessed by spectrophotometry, histopathological alterations by semiquantitative scoring.

The main component (46.3%) of TO was thymol. LPS significantly decreased breathing frequency, minute ventilation, and increased inspiratory/expiratory time, peak expiratory flow and airway hyperreactivity in both WT and KO mice. However, TO inhalation significantly alleviated airway hyperreactivity in WT, but aggravated it in KO mice. Histological evaluation revealed prominent perivascular/peribronchial edema formation with neutrophil and macrophage inflammatory infiltration upon LPS administration, which was not reduced by TO inhalation in either WT or KO mice. LPS treatment induced a remarkably increased MPO activity. This was significantly reduced by TO inhalation in WT, but not in KO mice.

Although TRPA1/V1 ion channels are not involved in LPS-induced interstitial pneumonitis, but they mediate the inhibitory effects of TO on inflammatory hyperreactivity and neutrophil/macrophage activation. TO can be considered to be an effective treatment for acute airway inflammation.

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P17. Hemorphins released from bovine haemoglobin gastrointestinal digestion: Dual effects on intestinal hormones and DPP-IV regulations

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Hemorphins are a group of peptides encrypted within the beta chain of bovine and human haemoglobins with “morphine-like” properties, i.e. exhibiting interaction with opioid receptors. Although hemorphins are atypical opioid peptides, no specific roles in food intake and body weight regulation have been reported. Indeed, opioid peptides are classically associated with many aspects of food intake regulation and energy homeostasis. Multiple experiments in animals and humans have shown that oral administration of synthetic opioid receptor antagonists reduce appetite and food intake, whereas agonists, albeit less clearly demonstrated, increase food intake. These effects were long described as solely central effects until recent work (*Duraffourd et al., 2012*) proposed a gut-brain loop initiated by food-derived opioid receptor antagonists interacting with portal vein opioid receptors which would sustain the well-recognised protein induced satiety. Thus, opioid peptides and especially food-derived ones could exert some of their effects peripherally. However, no particular antagonist opioid sequence and specifically no hemorphin has evidenced a role in appetite and food intake so far. Since natural exogenous hemorphins come from food and given the recent importance of obesity research, we found it relevant to investigate whether hemorphins display bioactivities in the intestine related to food intake regulation.

Using an in vitro controlled digestion (*Caron et al., 2016*), five hemorphins were identified by LC- MS/MS in a 2 h-intestinal digestate. These food-derived hemorphins showed secretagogue properties for the anorexigenic hormones cholecystokinin (CCK) and glucagon-like peptide 1 (GLP- 1) when in contact with murine enteroendocrine STC-1 cells. They did not show significant modulatory effect on the corresponding prohormone mRNA levels. However, they downregulated the enzyme PC1 that processes proglucagon into GLP-1 specifically in the intestine. Moreover, three of them were potent inhibitors of the GLP-1 inactivating dipeptidyl-peptidase IV (DPP-IV) and transiently upregulated its mRNA in human Caco-2 cells. These results suggest that food-derived hemorphins display dual luminal effects that participate at different levels in food intake and glycaemia regulation and add to the known roles of the special class of food-derived opioid peptides, the hemorphins.

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P18. G proteins as key regulators of chemokine peptide receptors regulating proliferation and migration of high-grade glioma cells

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Gliomas (IDHwt, about 90% of cases) mainly known as glioblastoma multiform (GBM), are the most common malignant brain tumors that, despite therapeutic improvements combining radiotherapy and chemotherapy (Stupp protocol), often recur in few months, with life expectancy of about 15 months for GBM patients. Therefore, improved strategies for an effective treatment are urgently needed. Recently, G-protein-coupled receptors (GPCRs) were shown to be involved in oncogenic cell signaling and are therefore attractive as targets for therapeutic peptide development. Our recent The Cancer Genome Atlas (TCGA) database analysis of over 200 putative GPCR members pointed that more than 10 peptide GPCRs belong to the most expressed GPCRs receptors in GBM. In this context, we recently demonstrated that the vasoactive peptide urotensin II (UII), activating the GPCR named UT, stimulates glioma cell invasion through $G\alpha_{13}$ and $G\alpha_i$ coupling pathways, and growth of GBM cells in culture, through angiogenic processes, suggesting a key role of this peptide GPCR and some G proteins in glioma progression.

The redundancy of expression of chemotactic GPCRs raises the issue of studying "signaling nodes" common to all these GPCRs. These nodes, represented by G proteins that couple these GPCRs and relay the secondary effectors, play a central role in the regulation of glioma development. We analyzed the expression of the 31 subunits (15α , 5β and 11γ) of G proteins from the TCGA database and showed that $G\alpha_{12}$, $G\alpha_{13}$, $G\alpha_i2$, $G\alpha_i3$, $G\beta_2$, $G\gamma_5$, $G\gamma_{11}$ and $G\gamma_{12}$ subunits, were overexpressed in GBM. The mRNAs encoding the $G\alpha_{15}$, $G\alpha_i3$, $G\beta_2$ and $G\gamma_5$ subunits were indeed overexpressed in six GBM cell lines, and in eight GBM patient samples. To study their potential role in GBM, we stably down-regulated $G\alpha_{15}$, $G\alpha_i3$, $G\beta_2$ and $G\gamma_5$ subunits in the U87 and 42MG GBM cell lines by pGIPZ-shRNA infection and showed that the level of these G proteins control cell migration and proliferation in unstimulated conditions. A bioinformatics analysis identified the $G\alpha_{15}$ subunit as being specifically expressed in the most aggressive mesenchymal-type group of GBMs. We confirmed the key role of this $G\alpha_{15}$ subunit in UT-induced recruitment of the polyphosphoinositides (FRET-HTRF), as well as the shift of UT- $G\alpha_q$ coupling (BRET). By means of Crispr-Cas9 technology to stably inactivate GNA15 gene expression in U87 and 8MG GBM cell lines, GNA15-KO GBM clones were shown to produce a major alteration of cell proliferation associated with a decrease of the mesenchymal transition marker N-cadherin expression.

These results suggest that some uncommon G protein subunits, if overexpressed in GBM, could couple peptide GPCRs such as UT, and participate in the process of glioma malignancy and the acquisition of a mesenchymal GBM phenotype. Intrastriatal orthotopic xenografts of two U87-KO-GNA15 clones in nude mice are in progress to determine the importance of this G protein in GBM growth and invasiveness *in vivo*.

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P19. Salt loading induces labile Ca-permeable AMPA receptor synaptic plasticity in hypothalamic magnocellular neurons

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Magnocellular neuroendocrine cells of the hypothalamus play an important role in the regulation of fluid and electrolyte homeostasis. They undergo a dramatic structural plasticity under sustained physiological activation, including an increase in glutamatergic synaptic innervation. We tested for plasticity in the glutamate AMPA receptor expression in magnocellular neurons of the hypothalamic supraoptic nucleus (SON) induced by sustained activation by chronic salt loading-induced dehydration. We found that salt loading by administration of 2% saline drinking water for 5-7 days resulted in a significant selective increase in GluA1 protein expression in the rat SON, while no change in the mRNA expression or membrane localization of any of the AMPA receptor subunits was observed, suggesting a post-transcriptional up regulation of GluA1 at excitatory synapses on SON neurons. We performed whole-cell recordings of excitatory postsynaptic currents (EPSCs) to determine the contribution of Ca²⁺-permeable AMPA receptors (CP-AMPA receptors) to synaptic transmission by the EPSC rectification index and the sensitivity of EPSCs to the selective antagonist of CP-AMPA receptors, 1-naphthyl-acetyl-spermine (NAS). We found that neurons from the salt-loaded group showed a significantly greater inward rectification and an increased sensitivity to NAS compared to the control group. To further determine the role of rapid protein turnover in the AMPAR plasticity, we preincubated slices for over 60 min in the translational inhibitor cycloheximide to block local protein synthesis. Blocking protein synthesis reduced the EPSC frequency and EPSC rectification in SON neurons from the salt-loaded group to the levels of controls.

These findings in aggregate suggest that salt loading induces highly labile glutamate synapses in magnocellular neurons that are comprised almost exclusively of CP-AMPA receptors, and indicate an essential role for rapid protein synthesis in the maintenance of the new synapses. This glutamate receptor plasticity is predicted to result in an increase in glutamate-induced calcium influx, which could play a key role in the activity-dependent neuronal-glia remodeling of the magnocellular neurons that occurs during chronic dehydration.

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P20. Gut-brain axis: could food-derived hemorphins cross intestinal and blood-brain barriers?

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Hemorphins are a group of opioid peptides encrypted in the beta chain of hemoglobin, in a region conserved between bovine and human hemoglobins. Hemorphins, from endogenous or food-derived haemoglobin, are found in many different tissues and species. Several types of bioactivity have been uncovered for these hemorphins, notably in blood pressure regulation and cognitive functions. They were first shown to interact with opioid receptors (OR), hence the name “hemorphin”, and their activities may explain aspects of intestinal peristalsis, bladder contraction, inflammation and pain modulation. Our laboratory is interested in the fate of alimentary or digested hemoglobin protein, and particularly in bioactive peptides derived from them, in relation to food intake regulation. We have previously identified five hemorphins from the simulated gastrointestinal digestion of hemoglobin and studied their effects at the intestinal lumen level in relation to gut hormones synthesis and release and DPP-IV (CD26) regulation (Domenger et al., 2017). Among these five hemorphins, three have an N-terminal extension, LLVV-, LVV- and VV-, one has a C-terminal extension, -QRF, and one has both an N-terminal, VV- and C-terminal -QRF extension, on the tetrapeptide core YPWT that has been demonstrated to bind to OR. OR are involved in many aspects of food intake regulation via central effects. Recently, peripheral OR have also been involved in food intake regulation, and a gut-brain loop has been described with a crucial role of portal vein mu-opioid receptors. It was thus proposed that opioid peptides originating from the digestion and absorption of dietary proteins would interact with the OR located in the portal vein and trigger a gut-brain loop mediating high-protein diet-induced satiety. In this work, a qualitative study is presented in which the main question is whether or not food-derived hemorphins, i.e. originating from digested alimentary haemoglobin, could pass the intestinal barrier and/or the blood-brain barrier (BBB). Hemorphins that were previously identified (Caron et al., 2016) in the 120 min digest resulting from the simulated gastrointestinal digestion of hemoglobin were synthesised to be tested in cell culture models of passage of IB and BBB and followed by LC-MS/MS analyses. We further provide preliminary results regarding the effects of hemorphins on tight junction proteins, in particular claudin-4, which are involved in paracellular permeability.

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P21. Electrophysiological effects of ghrelin in the hypothalamic paraventricular nucleus neurons

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We used a 64 multielectrode array to examine the effects of ghrelin administration on extracellular spike frequency in paraventricular nucleus (PVN) neurons recorded in brain slices obtained from male Sprague-Dawley rats. Bath administration of 10 nM ghrelin increased (52/135, 38%) or decreased (44/135, 33%) spike frequency in PVN neurons. GABAA and glutamate receptor antagonists abolish the decrease in spike frequency without changes in the proportion of increases in spike frequency (23/53, 43%) induced by ghrelin. The results indicate a direct effect of ghrelin to increase PVN neuronal activity and an indirect effect to decrease PVN neuronal activity. The patch clamp recordings showed similar proportions of PVN neurons influenced by 10 nM ghrelin (33/95, 35% depolarised; 29/95, 30% hyperpolarised). Using electrophysiological fingerprints to identify specific subpopulations of PVN neurons we observed that the majority of pre-autonomic neurons (11/18 -61%) were depolarized by ghrelin, while both neuroendocrine (29% depolarisations, 40% hyperpolarisations), and magnocellular neurons (29% depolarisations, 21% hyperpolarisations) showed mixed responses. Finally, to correlate the electrophysiological response and the neurochemical phenotype of PVN neurons, cell cytoplasm was collected after recordings and RT-PCR performed to assess the presence of mRNA for vasopressin, oxytocin, thyrotropin (TRH) and corticotropin (CRH) releasing hormones. The single-cell RT-PCR showed that most TRH-expressing (4/5) and CRH-expressing (3/4) neurons are hyperpolarised in response to ghrelin. In conclusion, ghrelin either directly increases or indirectly decreases the activity of PVN neurons. This suggests that ghrelin acts on inhibitory PVN neurons that, in turn, decrease the activity of TRH-expressing and CRH-expressing neurons in the PVN.

P22. Family B neuropeptide GPCRs couple cyclic AMP to MAP kinase (ERK) signaling through the neuron-specific guanine nucleotide exchange factor NCS-Rapgef2

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The 15-member secretin family of G-protein coupled receptors (GPCRs) have in common liganding by peptides, and coupling through Gs to cause elevation of intracellular cyclic AMP (cAMP). Recently, a novel cAMP sensor/effector, NCS-Rapgef2, has been characterized that i) links cAMP elevation to activation of the MAP kinase ERK; and ii) is expressed in a neuroendocrine-specific manner in adult mammals [1-3]. We have surveyed several family B receptors for activation of NCS-Rapgef2, protein kinase A (PKA), and Epac2, the three cAMP sensor/effectors presence in the neuroendocrine cell line NS-1. The PAC1, VPAC1, VPAC2 and GLP-1 receptors all cause activation of all three cAMP sensor/effectors, and each leads to a distinct downstream cellular action (these are neuritogenesis, neuron-specific gene expression/survival, and growth arrest, for NCS-Rapgef2, PKA and Epac, respectively). More rapidly-desensitizing Gs-coupled receptors in the rhodopsin family, such as the adrenergic beta receptor type 2 (ADBR2) activate both Epac- and PKA-dependent cAMP signaling, but not NCS-Rapgef2-dependent signaling, presumably due to the requirement for sustained ERK activation for expression of the latter.

In NS-1 and PC-12 cells, challenge with PACAP in cells expressing PAC1, or with VIP in cells expressing VPAC1 or VPAC2, leads to ERK-dependent neuritogenesis that is blocked by a specific NCS-Rapgef2 inhibitor, EL1101 [4], and to PKA-dependent CREB and Epac2-dependent p38 phosphorylation, which are not. In INS-1 cells, exendin-4-induced GLP-1 receptor activation causes ERK phosphorylation which is attenuated by treatment of cells with siRNA targeting NCS-Rapgef2.

We postulate that family B neuropeptide signaling in brain and peripheral endocrine tissues mediated through ERK requires activation of NCS-Rapgef2, and that this signaling pathway, and therefore its downstream cellular effects, may be pharmacologically distinguished from those of other cAMP effectors, including PKA and Epac, activated by family B neuropeptide ligands.

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P23. Neuroglobin immunoreactivity changes after orexin administration in the rat brain

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Neuroglobin (Ngb) is a protein member of the globin family, expressed mainly in the central and peripheral nervous system. It is involved in the transport of oxygen, in the response to hypoxic/ischemic and oxidative stress-related insults. Recently, we showed that sleep deprivation reduces the number of Ngb-positive (Ngb⁺) cells in brain areas related to sleep [1]. Currently, it is poorly understood whether Ngb expression depends on waking promoting factors. It is well documented that prolonged wakefulness induces an increase in orexin levels in the cerebrospinal fluid [2]. We hypothesize that the decreased number of Ngb⁺ cells seen after 24-h sleep deprivation may be the result of high orexin levels induced by prolonged wakefulness. Hence, the aim of this study was to determine whether orexin administration reduces the number of Ngb⁺ cells in areas related to sleep regulation. For this purpose, non-sleep-deprived male Wistar rats (n=6) received an injection of orexin-A into the left lateral ventricle. Three hours post-injection, rats were euthanized, and their brains processed for Ngb immunohistochemistry. The injection of orexin-A increased the number of Ngb⁺ cells in areas associated with sleep-wake regulation, suggesting that orexin modulates Ngb expression.

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P24. The onset of sodium appetite: role of oxytocinergic and serotonergic central systems

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A temporal dissociation exists between sodium depletion (SD) and the appearance of sodium appetite behavior (SA), and an inhibitory modulation can be postulated. Previous studies demonstrated the inhibitory participation of serotonergic (5-HT) and oxytocinergic (OT) neurons in SA induced by SD (Godino et al., 2007). We recently found the serotonin 2C receptor (5HT2C) involved in the appearance of SA modulation (Porcari et al., 2017). Our aim was to evaluate, during the delay of SA appearance after SD, gene expression changes of different components of central oxytocinergic (OT) and serotonergic (5HT) systems previously involved in SA regulation.

Wistar rats were sodium depleted using furosemide combined with low sodium diet, and then 2h or 24h later the animals were sacrificed by decapitation. Specific brain areas [dorsal raphe nucleus (DRN), subfornical organ (SFO), lateral parabrachial (LPBN) and anteroventral area of third ventricle (includes the organum vasculosum of the lamina terminalis and the ventral part of median preoptic nucleus), plus supraoptic nucleus (AV3V + SON)], were submitted to RT-PCR quantification of oxytocin receptor (OTR), serotonin 2A receptor (5HT2A), tryptophan hydroxylase 2 (TPH2) and serotonin transporter (SERT).

OTR mRNA expression significantly increases ($p=0.045$) early at 2 hs after SD along AV3V + SON in comparison to control and 24 h SD groups. The opposite pattern was observed in the OTR mRNA expression along the DRN, decreasing (but not significantly; $p=0.06$) 24 h after SD in relation to control and 2h SD groups. No significant changes in the SERT and TPH2 mRNA expression along the DRN or the 5HT2A mRNA expression in the LPBN and SFO were observed.

In sum, our results suggest that an oxytocinergic circuit, acting through its receptors within nuclei previously implicated in the regulation of sodium appetite (areas involved in the genesis and the inhibition of SA), may modulate SA appearance.

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P25. Oxytocin in the trigeminocervical complex modulates meningeal nociceptive traffic: implications for migraine

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Migraine is a complex brain disorder where abnormal activation of the trigeminocervical complex (TCC) neurons play a key role. Recently, oxytocin has emerged as an interesting molecule with analgesic properties. At spinal cord level, oxytocin (endogenous or exogenous) selectively inhibits the neuronal nociceptive activity mediated by A δ - and C-fibers but not A β -fibers, pointing out the relevance of oxytocinergic mechanisms modulating nociception. In this regard, in humans with tension type headache or migraine, intranasal oxytocin seems to have analgesic effects, but the mechanisms/receptors involved remain unknown. The present study aims to determine in a well-established migraine model, the effect of oxytocin (at TCC or intranasal) on TCC neuronal activity evoked by the supraorbital or dural trigeminovascular (meningeal) stimulation. Furthermore, the role of oxytocin receptor (OTR) was explored.

We performed *in vivo* electrophysiology in anesthetized male Wistar (280-310g) rats. A craniotomy to expose the middle meningeal artery (MMA) region was made and unitary extracellular recordings of wide-dynamic-range (WDR) neurons of TCC were performed (quartz-insulated Pt/W microelectrodes, 4-9 M Ω). The nociceptive neuronal responses were evoked by supraorbital electrical or meningeal electrical stimulation (20 electrical pulses at 0.5 Hz with 1-msec pulse duration at 0.1-3 mA) to evoke the neuronal response of TCC neurons. The effect of local spinal (at TCC) or intranasal oxytocin on the evoked neuronal activity was analyzed with peri-stimulus time histograms that allowed characterization of the WDR activity by their response latencies (A δ -fibers: 3-25 msec; and C-fibers: 25-80 msec). Furthermore, the role of OTR in the oxytocin effect was tested using a highly selective and potent antagonist, the L-368,899.

Oxytocin dose-dependently inhibited the peripheral-evoked activity in the TCC nociceptive transmission in response to supraorbital or meningeal electrical stimulation. This inhibition was associated with a blockade of neuronal activity of nociceptive fibers. The antinociceptive effect of oxytocin was abolished by pretreatment (in the TCC) with the OTR antagonist.

This study demonstrates that oxytocin inhibits the peripheral electrically-induced neuronal activity of TCC nociceptive neurons by directly activating OTR and provides a mechanistic rationale for the observed antimigraine effects. In conclusion, our results suggest that OTR activation may represent a new potential drug target to treat migraine.

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P26. The cold-induced activation of the thyroid axis is inhibited by chronic stress

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The activity of the hypothalamus-pituitary-thyroid (HPT) axis is important for energy homeostasis. It is activated by energy demands such as cold exposure or physical activity, and inhibited by acute stress or corticosterone injection. Chronic stress, depending on type and duration, also inhibit the HPT axis [1]. The response to cold is blunted by a previous stress exposure or corticosterone injection [2]. In the present work we evaluated the effect of chronic stress on the response of HPT axis to acute energy-demanding stimuli (1h of cold exposure). A well characterized animal model of psychogenic stress is restraint (RES), which if applied during various days produces habituation but, if animals are exposed to a heterotypic stressor, hyper-reactivity of HP-adrenal axis is observed [3].

Wistar male rats were introduced into a restraint apparatus and an equivalent number were placed in individual cages (controls), every day during 30 min, for 14 days. On day 15th at 9.30 AM animals were introduced, in clean individual cages, either into the cold room (5°C), or into a nearby room at room temperature (RT). Parameters of HPT axis function, thyrotrophin-releasing hormone-TRH, thyrotrophin-TSH, and thyroxine-T₄, were evaluated, as well as those representing the activity of the HPA axis (CRH, corticosterone). Hormones were measured using immunoassays, and gene expression by RT-PCR.

Compared to controls, RES decreased body weight gain and serum TSH serum, whereas corticosterone serum concentration was decreased. Cold stimulation diminished *Crh* mRNA, and increased *Trh* mRNA in the paraventricular nucleus of the hypothalamus (PVN) in controls, but not in RES animals. At the median eminence level, where hypophysiotropic terminals are concentrated, levels of processed TRH were similar in RT-control or RT-RES groups but were lower in both groups exposed to cold, suggesting cold-induced hormone release. In contrast, serum TSH concentration was augmented only in the cold-exposed control group. Corticosterone was increased 8.5 fold by cold exposure in controls and by 18 fold in RES. Brown adipose tissue (BAT) is the thermogenic organ that in response to cold-induced adrenergic stimulation activates deiodinase 2 (D2) increasing T₃ intracellular levels that stimulate the uncoupling protein UCP-1. Cold increased D2 and UCP1 expression in controls but not in RES animals. These results corroborate the hyperactivity of the HPA axis to a heterotypic stimulus (cold) in the chronically stressed group (RES) and demonstrate that a chronically stressed animal presents a blunted HPT axis response to cold. Dysfunction of the HPT axis response may contribute to altered energy homeostasis.

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P27. The octapeptide NAP alleviates intestinal and extra-intestinal anti-inflammatory sequelae of acute experimental colitis

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Background: The octapeptide NAP has been shown to exert neuroprotective actions. For the first time we here investigated potential anti-inflammatory effects of NAP in acute murine colitis.

Methodology: C57BL/6j mice were treated with 3.5% dextran sulfate sodium (DSS) from day 0 until day 6 and subjected either to intraperitoneal NAP (1.0 mg per kg body weight per day) or placebo (NaCl 0.9%) treatment from day 1 until day 6 post-induction (p.i.).

Results: Whereas NAP application did not alleviate macroscopic (i.e. clinical) sequelae of colitis, lower numbers of apoptotic, but higher counts of proliferating/regenerating colonic epithelial cells could be observed in NAP as compared to placebo treated mice at day 7 p.i. Furthermore, lower numbers of adaptive immune cells such as T lymphocytes and regulatory T cells were abundant in the colonic mucosa and lamina propria upon NAP versus placebo treatment that were accompanied by less colonic secretion of pro-inflammatory mediators including IFN- γ and nitric oxide at day 7 p.i. In mesenteric lymph nodes, pro-inflammatory IFN- γ , TNF and IL-6 concentrations were increased in placebo, but not NAP treated mice at day 7 p.i., whereas elevated anti-inflammatory IL-10 levels could be observed in NAP treated mice only. The assessed anti-inflammatory properties of NAP were not restricted to the intestinal tract, given that in extra-intestinal compartments such as the kidneys, IFN- γ levels increased in placebo, but not NAP-treated mice upon colitis induction. NAP-induced effects were accompanied by distinct changes in intestinal microbiota composition: colonic luminal loads of bifidobacteria, regarded as anti-inflammatory, "health-promoting" commensal species, were two orders of magnitude higher in NAP as compared to placebo treated mice and even naive controls.

Conclusion: NAP alleviates intestinal and extra-intestinal anti-inflammatory sequelae of acute experimental colitis.

Key Words: NAP; activity-dependent neuroprotective protein (ADNP); acute colitis; anti-inflammatory effects; immunomodulatory properties; bifidobacteria; gut-brain axis

P28. Anti-inflammatory properties of NAP in acute *Toxoplasma gondii* induced ileitis in mice

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Background: The octapeptide NAP has been shown to exert neuroprotective properties. Here, we investigated potential anti-inflammatory effects of NAP in an acute ileitis model.

Methodology: To address this, C57BL/6j mice were perorally infected with *Toxoplasma gondii* (day 0).

Results: Within one week postinfection (p.i.), placebo (PLC) treated mice developed acute ileitis due to Th1-type immune responses. Mice subjected to intraperitoneal NAP treatment (1.0 mg per kg body weight per day) from day 1 until day 6 p.i., however, developed less distinct macroscopic and microscopic disease as indicated by less body weight loss, less distinct histopathological ileal changes, lower ileal apoptotic, but higher proliferating cell numbers, less abundance of neutrophils, macrophages, monocytes and T lymphocytes, but higher numbers of regulatory T cells in the ileal mucosa and lamina propria, and lower concentrations of pro-inflammatory mediators in the ilea as compared to PLC controls at day 7 p.i.. Remarkably, NAP-mediated anti-inflammatory effects could also be observed in extra-intestinal compartments including liver and spleen. Lower MCP-1, TNF and IL-12p70 serum concentrations in NAP as compared to PLC treated mice at day 7 p.i. indicate a pronounced systemic anti-inflammatory effect of NAP in acute ileitis.

Conclusion: These findings provide first evidence for NAP as a potential novel treatment option in intestinal inflammation.

Key Words: NAP; activity-dependent neuroprotective protein (ADNP); gut-brain axis; Th1-type immunopathology; *Toxoplasma gondii*; acute ileitis; anti-inflammatory effects; immunomodulatory properties

P29. Pituitary Adenyl Cyclase-Activating Polypeptide—a neuropeptide as novel treatment option for subacute ileitis in mice harboring a human gut microbiota

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Background: The neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is well known for its important functions in immunity and inflammation. Data regarding anti-inflammatory properties of PACAP in the intestinal tract are limited, however. We here investigated whether PACAP treatment could alleviate experimental subacute ileitis.

Methodology: Human microbiota associated (hma) mice were generated following human fecal microbiota transplantation of secondary abiotic mice. On day 0, subacute ileitis was induced by peroral low-dose (i.e. 1 cyst) *Toxoplasma gondii* infection. From day 3 until day 8 post infection (p.i.), mice were either treated with synthetic PACAP38 (1.5 mg per kg body weight per day) or placebo (PLC) intraperitoneally once daily.

Results: At day 9 p.i., PLC, but not PACAP treated mice exhibited overt macroscopic sequelae of intestinal immunopathology as indicated by significant body weight loss and shortening of intestinal lengths. PACAP treatment of hma mice further resulted in less distinct apoptotic responses in ileal and also colonic epithelia that were accompanied by lower T cell numbers in the mucosa and lamina propria and less secretion of pro-inflammatory cytokines in intestinal *ex vivo* biopsies at day 9 p.i. Notably, ileitis-associated gut microbiota shifts were less distinct in PACAP as compared to PLC treated mice. Anti-inflammatory effects of PACAP were not restricted to the intestines, but could also be observed in extra-intestinal including systemic compartments as indicated by lower apoptotic cell counts and less pro-inflammatory cytokine secretion in liver and lung taken from PACAP as compared to PLC mice at day 9 p.i., which also held true for markedly lower serum TNF and IL-6 concentrations in the former as compared to the latter.

Conclusion: We conclude that synthetic PACAP alleviates subacute ileitis and extra-intestinal including systemic sequelae of T cell-driven immunopathology. These findings further support PACAP as a novel treatment option for intestinal inflammation including inflammatory bowel diseases.

Key Words: Pituitary adenylate cyclase-activating polypeptide (PACAP), *Toxoplasma gondii*, intestinal microbiota shifts, subacute ileitis, Th1-type immunopathology, human fecal transplantation, anti-inflammatory and anti-apoptotic effects, gut-brain axis

P30. Prenatal ethanol exposure induces selective changes in neural Met-enkephalin expression in both infant and adolescent rats

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Opioid peptides play a major role in alcohol (ethanol) reinforcement and reward. Ethanol induces important alterations in opioidergic transmission in brain areas of the reward circuit (i.e., dopaminergic mesocorticolimbic system). Numerous studies suggest that prenatal ethanol exposure (PEE) facilitates alcohol intake. Opioid systems may play a key role in the neurobiological mechanisms underlying this process. Ethanol-induced changes in opioidergic transmission have been extensively studied in adult organisms. However, the impact of ethanol exposure at low or moderate doses during early ontogeny has been barely explored. The aim of this work was to study the effect of prenatal ethanol exposure on Methionine-enkephalin (Met-enk) expression in several brain regions of rat offspring.

Pregnant rats were treated with ethanol (2 g/kg ig) or water during gestational days (GDs) 17–20. Changes in Met-enk content were investigated in infant or adolescent rats at 15- or 30-postnatal days (PD15, PD30) after an ethanol intake test (0, 5 or 10%) (PD15) or a challenge dose of the drug (1 g/kg ip) (PD30). Met-enk content was quantitated by radioimmunoassay in the following brain regions: ventral tegmental area (VTA), nucleus accumbens (NAcc), prefrontal cortex (PFC), substantia nigra (SN), caudate-putamen (CP), amygdala, hypothalamus and hippocampus. PEE increased Met-enk content in mesocorticolimbic regions (PFC and NAcc) of infants that consume higher levels of alcohol than control rats. Conversely, Met-enk levels in the VTA were reduced by this treatment. PEE also increased Met-enk concentration in the medial-posterior zone of the CP, and strongly augmented peptide content in the hippocampus and hypothalamus. On the other hand, PEE increased Met-enk content in the PFC, CP, hypothalamus and hippocampus of ethanol-challenged animals at PD30, but did not alter peptide levels in the amygdala, VTA and NAcc. These findings show that PEE induces selective changes in Met-enk levels in regions of the meso-accumbens, mesocortical and nigrostriatal systems, the hypothalamus and hippocampus of both infant and adolescent rats.

Our results support the role of mesocorticolimbic enkephalins in ethanol reinforcement in offspring, as has been reported in adults. Ethanol-induced activation of specific neural enkephalinergic pathways may play a relevant role in these drug actions.

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P31. Basolateral amygdala,, but not orbitofrontal cortex, is necessary for motivational conflict responses guided by previous experiences.

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Animals foraging for food are often challenged with a conflict between opposing motivations driven by previous experiences, such as facing a cue that predicts threat, to approach another cue that predicts food. Selecting the appropriate behavioral response to execute during such motivational conflicts, guided by biologically significant memories, is critical for survival. The basolateral amygdala (BLA) has been implicated in the regulation of defensive responses to threats (fear) as well as reward-seeking signaling. The orbitofrontal cortex (OFC) has been involved in action selection based on the relative value of cues learned by positive and negative experiences. It is not clear, however, whether these structures are necessary when opposing cues compete during a motivational conflict.

To address this issue, we performed pharmacological inactivations of BLA or OFC in rats trained to face a threat (crossing an electrified grid floor signaled by white noise) to obtain a reward (press a bar to receive food signaled by light). We found that BLA inactivation (with a high but not low dose of GABA agonists muscimol and baclofen) decreased the time it takes (latency) for rats to cross the predicted threat area to obtain food. This result suggests that the BLA is necessary to promote fear-related responses when competing with reward-seeking behaviors. Consistent with previous studies, we found that BLA inactivation impaired the retrieval of a threat memory in the absence of available reward in fear conditioning, but did not affect the latency to obtain food in the absence of threat during no-conflict trials.

These results support the predominant notion that the BLA is key to execute behavioral responses triggered by cues associated with threats, but not by cues associated with rewards. Surprisingly, we found that OFC inactivation did not affect any of the motivational conflict responses including fear-related responses and reward-seeking behaviors. Together, our findings suggest that the BLA, but not the OFC, is necessary to promote fear-related behaviors when an animal is challenged to face potential threats to approach a reward guided by previous experiences.

Keywords: amygdala, orbitofrontal cortex, fear, reward, motivation, conditioning

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P32. Gonadal status modifies the number of GABA-expressing neurons in limbic areas of the rat brain

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Gonadal steroids act upon classical nuclear receptors to alter the function of many brain areas, including the limbic system, involved in affective and cognitive processing. However, how the sexual steroids modulate the activity of the neurons that constitute the limbic system is not known. Previously we have reported the existence in the medial habenula of a population of neurons that receive inputs from hypothalamic homeostatic nuclei and may be induced to change to a GABAergic phenotype by local (synaptic) conversion of the gonadal steroid testosterone to estrogen via aromatase contained in nerve terminals.

In this work we used the RNAscope technique to evaluate at light microscopy level, the density of mRNA for GABA expression in neurons of limbic related areas that express the steroid receptors for estrogen (ERa and ERb), testosterone (AR) and the enzyme aromatase. We compared male rats under four different gonadal status (castrated "GNX", sexually inactive "SI", sexually active "SA" and Testosterone administration). Preliminary results show that GNX rats have diminished number of GABA expressing neurons compared to SA rats in the following limbic structures: anterior cingulate cortex (64% of that in SA rats); anterior olfactory nucleus (44%); accumbens (56%); lateral septum (72%); oval nucleus of stria terminalis (72%). The possible implications of this modulation of GABAergic transmission by sexual steroids in limbic areas will be discussed.

P33. Is peripheral thyrotropin-releasing hormone-degrading ectoenzyme a therapeutic target for diet-induced obesity?

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Obesity is a major health problem, leading to diabetes type II and cardiovascular diseases, which are among the principal causes of mortality in Mexico, and world-wide. Among many other systems, energy related clues control the output of the hypophysiotropic neurons of the paraventricular nucleus (PVN) that project to the median eminence (ME) and secrete thyrotropin releasing hormone (TRH) into the portal capillaries. These neurons positively regulate the secretion of thyrotropin (TSH) by the anterior pituitary. In turn, TSH promotes the synthesis and secretion of thyroid hormones (TH), which control metabolism and thermogenesis, and can be associated with body weight (BW) changes. Once TRH is released into the ME extracellular space, it can be hydrolyzed by thyrotropin-releasing hormone-degrading ectoenzyme (TRH-DE), which is present in tanycytes of the ME (Sanchez et al, 2009), but also in other brain regions, and is secreted by the liver. Previous studies in the laboratory have shown that tanycyte TRH-DE controls TSH secretion in rats (Rodriguez et al, unpublished). Furthermore, the body weight of male TRH-DE KO mice on a high fat diet is lower than control mice (Cote et al, unpublished). The intravenous injection of a phosphinic analogue of TRH (GlpΨ[P(O)(OH)]HisProNH₂; P-TRH) (Matziari et al, 2008) specifically inhibits PPII activity in the ME and in serum, but not in the brain (Charli et al, 2016), in rats.

We tested the hypothesis that chronic partial inhibition of TRH-DE activity by P-TRH in ME and periphery produces a discrete enhancement of HPT axis activity sufficient to lead to increased metabolism and decreased BW in a mouse (C57BL/6NJ male young adult) model of obesity induced by a high fat and 20% fructose diet (HFFD). At 30 days, BW was increased in HFFD mice compared to mice on a standard diet. At this time point, we initiated a 28 days systemic treatment with P-TRH (80μg/day) or vehicle (saline 0.9%) administered through osmotic pumps (ALZET) connected to an intraperitoneal catheter. P-TRH treatment induced a partial and significant inhibition of PPII activity in serum of HFFD mice compared with HFFD mice treated with vehicle. There was no significant effect of peripheral TRH-DE inhibition on BW. The status of the HPT axis and of peripheral metabolism is under study.

Tentatively, these results suggest that the peripheral inhibition of TRH-DE activity may be insufficient to reverse BW changes induced by HFFD.

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P34. Differential activation of vasopressin receptor subtypes in the amygdaloid modulation of anxiety in the rat

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Vasopressin is a peptide synthesized in the paraventricular nucleus (PVN) and supraoptic (SON) of the hypothalamus. Three different receptors have been so far identified for vasopressin effects (V1a, V1b and V2 receptors). The central vasopressin release contributes to behavioral regulation, in emotional states such as fear, depression and anxiety, through the V1a and V1b receptors. The effects of vasopressin on anxiety have been shown using vasopressin-deficient strains, knockout mice and drug administrations. The amygdala is a key structure in processing anxiety because sensory information integrates and implements responses to aversive stimuli. In the central nucleus of the amygdala (CeA) the existence of V1a and V1b vasopressin receptors have been reported in the rat. Various behavioral studies have shown the involvement of V1a and V1b receptors in anxiety using different behavioral paradigms of anxiety. The effects of vasopressin on anxiety seem to be clear, however there is no information about whether differential activation of the V1a and V1b receptors present in the central nucleus of the amygdala mediate the effects. The aim of this study was to study the involvement of vasopressinergic neurotransmission in the amygdaloid modulation of unconditioned anxiety and to ascertain whether or not AVP receptor subtypes have a differential role in this modulation.

Anxious behavior was evaluated both in the Shock-Probe Burying Test and Light-Dark Box following the bilateral micro-infusion of AVP alone or AVP together with either AVP 1a or AVP 1b receptor antagonists into CeA. AVP micro-infusion elicited at low (1ng/side) but not at high doses (10 ng/side) anxiogenic-like responses in the Shock-Probe Burying Test but not in the Light-Dark Box. SSR149415, an AVP 1b antagonist, but not Manning compound, an AVP 1a antagonist fully prevented AVP effects in the Shock-Probe Burying Test when it was administered together with AVP. No effects of any AVP antagonist by itself were observed in either anxiety paradigms.

Our results indicate that AVP 1b receptors contribute to the amygdaloid modulation of anxiety. It remains for future to ascertain whether AVP receptor subtypes have indeed differential actions in the modulation of specific features of unconditioned anxiety

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P35. Regulatory role of peripheral peptidergic sensory nerves in the proteoglycan-induced chronic autoimmune arthritis model of the mouse

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Objective: The immunological aspects of rheumatoid arthritis are well-known, but there is significantly less information about the role of sensory-immune interactions in this condition. Capsaicin-sensitive peptidergic sensory nerves densely innervating the synovium and the joint capsule release pro- and anti-inflammatory neuropeptides, which have important regulatory functions in various diseases associated with pain and inflammation. Therefore, we investigated the role of these sensory nerves in a translational mouse model of chronic autoimmune arthritis.

Methods: Peptidergic afferents were inactivated by resiniferatoxin (RTX) pretreatment in one group of BALB/c mice (desensitization). Arthritis was induced by i.p. proteoglycan aggrecan obtained from human osteoarthritic cartilage (PGIA). Hind paw volume, arthritis severity, grasping ability and mechanonociceptive threshold were monitored during the 4-month experiment. Neutrophil myeloperoxidase activity, vascular leakage and bone turnover were evaluated by *in vivo* optical imaging. Bone morphology was assessed using micro-CT, the intertarsal small joints were processed for histopathological analysis.

Results: Following desensitization of the capsaicin-sensitive afferents, ankle edema, arthritis severity and mechanical hyperalgesia were markedly diminished. Myeloperoxidase activity was lower in the early, but increased in the late phase, whilst plasma leakage and bone turnover were not altered. Desensitized mice displayed similar bone spurs and erosions, but increased trabecular thickness of the tibia and bony ankylosis of the spine. Intertarsal cartilage thickness was not altered in the model, but RTX-pretreatment increased this parameter in both the non-arthritic and arthritic groups.

Conclusion: This is the first complex *in vivo* functional and morphological characterization of the PGIA mouse model, where peptidergic afferents have an important regulatory function. Their overall effect is proinflammatory by increasing acute inflammation, immune cell activity and pain. Meanwhile, their activation decreases spinal ankylosis, arthritis-induced altered trabecularity, and cartilage thickness in small joints.

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P36 The participation of the muscarinic M1 receptors at the suprachiasmatic nucleus in the regulation of ovulation varies during the estrous cycle and is asymmetric

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The suprachiasmatic nucleus (SCN) is the master clock in vertebrates which synchronizes many physiological processes vital for the organisms. The SCN is synchronized with the light-dark cycle and in addition its activity is modulated by a wide variety of neurotransmitters. Acetylcholine modulates the electrical activity of the SCN-neurons through the activation of muscarinic receptors. This, in turn, affects several circadian regulated-processes like reproduction. In females, ovulation is regulated by the pituitarygonadotropins: luteinizing hormone and follicle stimulating hormone. The secretion of these hormones is regulated by the gonadotropin releasing hormone (GnRH) a decapeptide synthesized by neurons located mainly at the preoptic area of the hypothalamus. Lesion of the SCN inhibits the secretion of gonadotropins and blocks the ovulation; a similar result is observed in animals whose cholinergic system is blocked by injectingatropine, a muscarinic antagonist. These results support the idea that the cholinergic system of the SCN participates in the circadian mechanisms regulating ovulation.

In order to study the specific role of the cholinergic receptors on this process, we analyzed the effects of the blockade of M1 receptors present in the SCN on spontaneous ovulation. We implanted a unilateral guide cannula aimed to the left or rightSCN. After two weeks of recovery post-surgery, animals were microinjected with either 200pg ofpirenzepine (PZP), a specific antagonist of M1 receptors, or 0.9% saline solution as vehicle (VH). Microinjection was performed at 09:00 h of the each day of the estrous cycle. Animals were sacrificed at the next predicted estrous and the number of oocytes shed was counted, in addition, blood was collected for hormone analysis. The mean±s.e.m. of the number of ova shed by rats microinjected in the left SCN was: at proestrousVH 9.6 ± 0.24 vs PZP 12.4 ± 1.03 , $p=0.0357$; at metaestrous VH 4.28 ± 1.72 vs PZP 12 ± 0.58 , $p=0.0167$. Microinjection into the right SCN at metaestrous VH 12.5 ± 1.19 vs PZP 1.25 ± 1.25 ; $p=0.0286$ (Mann-Whitney).

Differential participation of the M1 receptors in the left and right SCN in the regulation of ovulation support the idea of the existence of a hypothalamic asymmetry in the mechanisms that regulate GnRH secretion and hence ovulation.

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P37. PACAP and dopamine signaling in stress: response parcellation by distinct cyclic AMP sensors in neuronal and endocrine cells

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We have recently identified the neuronally-expressed guanine nucleotide exchange factor NCS-Rapgef2 as a cyclic AMP sensor/effector linking activation of Gs-coupled biogenic amine and neuropeptide receptors (Gs-coupled GPCRs) to specific intracellular consequences of first messenger stimulation mediated through the MAP kinase ERK, specifically associated with stress responding, immediate early gene activation, and long-term plasticity in CNS neurons. In particular, dopamine D1 and PACAP PAC1 receptor expression in heterologous cell lines confers ERK activation in the presence, but not in the absence, of NCS-Rapgef2 [1, 2]. We employed a conditional Rapgef2 knockout mouse line to examine the consequences of abrogation of NCS-Rapgef2 function in stress- and reward-associated cellular and behavioral responses in the mouse central nervous system in vivo.

ERK is activated in the bed nucleus of the stria terminalis, and in the CA3 region of hippocampus, and in the prefrontal cortex in response to 30-60 min restraint stress in wild-type mice, and this response is abrogated in CAMK2a-CRE x floxed Rapgef2 mice. ERK is activated in the nucleus accumbens (NAc) in response to cocaine administration in wild-type mice, and this response is abrogated (as are cocaine-dependent locomotor sensitization and conditioned place preference) in mice in which Rapgef2 expression in NAc is extinguished by stereotaxically specific injection of AAV-Syn-Cre (a tissue-serotyped AAV virus in which the synapsin promoter drives robust Cre expression in neurons). NCS-Rapgef2-dependent activation of ERK by either psychomotor stimulant administration or restraint stress is unaffected in PACAP-deficient mice, indicating that although the PAC1 receptor causes ERK activation in neuroendocrine cell lines in vivo, and functional stress responses in vivo, including depressogenic and endocrine responses to restraint stress and social defeat are PACAP-dependent, these PACAP-dependent stress responses are not driven by ERK activation at the cellular level.

Therefore ERK-dependent stress responses are likely mediated through activation of a Gs-coupled GPCR other than the PAC1 receptor, and PACAP-dependent stress responses are likely mediated, at the cellular level, through a non-ERK-dependent signaling pathway.

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P38. Early life stress increases the vulnerability to traumatic brain injury

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Exposure to stress during the early life period decreases hippocampal neurogenesis, increases brain inflammation and affects cognitive performance. Child abuse, a form of chronic early life stress (ELS), is one of the leading causes of traumatic brain injury (TBI) in the pediatric population; however most of the studies do not consider the increased vulnerability caused by chronic exposure to stress. Therefore, the aim of the study was to test the hypothesis that ELS increases the vulnerability to TBI.

Control and maternally separated (3 hours / day from day 1 to 21) rats underwent a sham or a mild controlled cortical impact surgery (1.2 mm tissue deformation at 4 m/sec) at postnatal day (P) 21 and were left to recover for two weeks. Rats were injected with bromodeoxyuridine (BrdU, 300 mg/kg, 12 hr apart) at P31 and further evaluated in the MWM. Ten days after BrdU labeling, the rats were euthanized and we evaluated the density of BrdU+ cells in the hippocampus as an indicator of neurogenesis and Iba1+ cells as a marker of inflammation. Our results show that MS and TBI alone did not affect cognitive performance in the MWM; however MS + TBI caused a significant increase in the time to locate the escape platform and the distance traveled to the platform. Moreover; in the probe trial, MS-TBI rats show a decrease in the time and distance traveled in the target quadrant. MS + TBI, but not MS or TBI alone, decreased BrdU+ nuclei density in the ipsilateral hippocampus. An increase in Iba1+ cells density of both the ipsilateral and contralateral hippocampus was observed in the MS + TBI group.

In conclusion, our results indicate that exposure to adverse environment during early life increases the vulnerability to traumatic brain injury.

P39. Neuropeptide processing enzyme carboxypeptidase E and a derived peptide fragment mediate neuroprotection via ERK-FGF2 signaling

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Carboxypeptidase E (CPE) is highly expressed in the endocrine and nervous systems and functions as a prohormone and neuropeptide processing enzyme. Mutations of the CPE gene have been linked to diseases in humans such as obesity, diabetes, infertility and learning disability. Recently, in vitro studies using recombinant CPE and a mouse model expressing a non-enzymatic form of CPE showed that it plays a novel role extracellularly, as a trophic factor, independent of its enzymatic activity, to mediate neuroprotection, and anti-depression-like activity. These neurotrophic actions of CPE are mediated through ERK signaling and up-regulation of FGF2 and BCL2. Thus CPE could be a potential therapeutic agent for neurodegenerative diseases and depression. To this end we have identified a peptide fragment derived from CPE that is able to activate ERK signaling, up-regulate FGF2 expression and mediate neuroprotection in Neuro2a cells and hippocampal neurons. This peptide is being explored as a potential therapeutic agent for neuroprotection.

P40. Vasopressin V1a receptors in the paraventricular nucleus of the hypothalamus mediate anxiety-related behavior

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It has been shown that the central vasopressinergic system contributes to the development of anxiety disorders. We have sought to investigate the role of vasopressin V1a receptors (V1aR) in the paraventricular nucleus of the hypothalamus (PVN) in anxiety-related behavior in rats using elevated plus maze (EPM), marble burying test and grooming microstructure analysis. In the first set of experiments, acute infusions of increasing doses (0, 3, 10, 30 ng) of highly selective V1aR antagonist were bilaterally applied twenty minutes prior to behavioral testing into the PVN of male Wistar rats. Another set of experiments was performed in rats subjected to *in vivo* gene transfer of adenoviral vectors (Ads) engineered to induce expression of eGFP, or to induce overexpression of V1aR along with eGFP, in the PVN. Behavioral tests were performed seven days following transfections, when the peak of gene expression was expected. Our results demonstrate that acute blockade of paraventricular V1aR induced dose-dependent decrease in marble burying behavior and increased percentages of both time spent in the open arms and entries into the open arms of the EPM, while the overexpression of V1aR in the PVN had the opposite effects, significantly increasing the occurrence of anxiety-related behavior. In addition, rats with the higher density of V1aR in the PVN, determined by the immunostaining, showed an increased number of grooming patterns and bouts, increased time spent grooming and a greater number of episodes of caudal grooming compared to controls. Our results suggest a possible role of hypothalamic V1aR in the pathogenesis of anxiety disorders.

P41. Catestatin modulation of vesicular quanta upon cholinergic and peptidergic (or PACAPergic) secretagogue stimulation in PC12 cells

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We have previously shown that the chromogranin A (CgA)-derived peptide catestatin (CST: human CgA₃₅₂₋₃₇₂) inhibits nicotine-induced secretion of catecholamines from adrenal medulla and cultured chromaffin cells. In the present study, we sought to determine whether CST affects secretion via modulation of dense core vesicle (DCV) quanta, comprised mainly of catecholamines, CgA, chromogranin B (CgB) and neuropeptides, in response to either cholinergic (nicotine) or peptidergic [pituitary adenylyl cyclase activating polypeptide (PACAP)] secretagogue stimulation in PC12 cells.

CST (2 μ M) reversed depletion of norepinephrine (NE) from PC12 cells treated with either nicotine (60 μ M) or PACAP (0.1 μ M) promptly (within 30 min of stimulation) and this was reflected in NE content 24 hours later as well. CST elicited corresponding changes in the appearance of DCVs and their dense cores (DCs) after nicotine or PACAP stimulation. Thus, decreases in both diameter and area of both DCVs and DCs, elicited by either nicotine or PACAP, were reversed by co-treatment with CST, with no effects on vesicle morphology of CST alone. Nicotine or CST alone increased expression of CgA protein (but not CgB), and in combination elicited an additional increase in CgA protein, implying that nicotine and CST utilize separate signaling pathways to activate the expression of CgA. In contrast, PACAP increased expression of CgB protein (but not CgA), indicating differential regulation of CgA and CgB proteins by cholinergic and peptidergic stimulation. Although CST augmented expression of tyrosine hydroxylase (TH), it did not induce synthesis of catecholamines, presumably due to its inability to cause post-translation activation of TH via serine phosphorylation. Increased area and diameter of DCVs, and their DCs after CST, CST+nicotine and CST+PACAP are consistent with increased quantal size. We suggest that decreased quantal size is mediated by increased synthesis of granulogenic proteins: CgA after CST treatment; CgA after CST+nicotine treatment; and CgB after CST+PACAP treatment.

We propose that at the adrenal medulla *in vivo*, CST likely acts in a paracrine fashion after its release from chromaffin cells in response to cholinergic stimulation under basal conditions, and to PACAPergic and cholinergic stimulation under conditions of stress, to regulate DCV quanta (catecholamines and chromogranins).

P42. CETPI functions as a novel plasma LPS-binding protein with implications in the treatment of septic shock.

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Background: The cholesteryl-ester transfer protein isoform is exclusively expressed in the small intestine (CETPI) and present in human plasma (1). Unlike the cholesteryl-ester transfer protein (CETP), CETPI does not present exon 16 and 54 bases contained in intron 15 form part of the new mRNA replacing the 24 C-terminus residues present in CETP with a sequence of 18 residues containing a high concentration of prolines and positively charged amino acids (1). The present study introduces CETPI as a new protein with the potential capability to recognize, bind and neutralize lipopolysaccharides (LPS) in circulation during sepsis and septic shock (2).

Results: Peptide VSAK derived from the last 18 residues of CETPI protects against the cytotoxic effect of LPS upon macrophages, hepatocytes and microglial cells, does not show cytotoxicity by itself, and prevents against the expression of pro-inflammatory cytokines and the generation of oxidative stress (2). Despite efforts made to counteract what it is known as the inflammatory disequilibrium syndrome and overall the outcome of septic shock, current therapeutic possibilities, mainly involving inflammation and metabolic control and antibiotic treatment, have not changed for many years. Therefore, since CETPI efficiently binds LPS in vitro, is overexpressed in small intestine cell cultures, and proved to be present in human plasma, the efficacy of the administration of peptide VSAK against LPS cytotoxicity was studied in vivo. Employing a septic shock model in rabbits, we demonstrate that peptide VSAK protects against the deleterious effects of LPS and reduces TNF α levels in plasma (2, 3).

Conclusions: CETPI is a new protein that undoubtedly will advance the possibilities to better understand and treat the dangerous effects of LPS present during the treatment of sepsis caused by Gram-negative bacteria and its common consequence, a septic shock condition.

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P43. Leptin acts on the hypothalamus to modulate circadian temperature rhythm

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The suprachiasmatic nucleus of the hypothalamus, as the master clock, interacts with several hypothalamic regions to coordinate circadian rhythmicity. The interaction between the suprachiasmatic nucleus and the arcuate nucleus is important in modulating several circadian rhythms such as temperature. These nuclei regulate temperature according to the time of the day. Thus, while α -MSH release from the arcuate to the MnPO promotes temperature increase during the night, AVP release from the suprachiasmatic nucleus to both the arcuate and the MnPO promotes the temperature decrease during the day. This temperature rhythm can be affected by conditions such as fasting, in which circulating levels of leptin (an adipokine that classically acts as a feedback signal of satiety) are decreased. We hypothesized that leptin could act on the arcuate and the suprachiasmatic nuclei to regulate temperature in a time-dependent fashion and thus modulate the circadian temperature rhythm according to food availability. We therefore analyzed the activity of α -MSH and AVP neurons after leptin administration at different time points and observed that the suprachiasmatic nucleus, via the arcuate, appears to modulate the temperature changes induced by leptin. These data could contribute to the better understanding of leptin's role in physiological regulation dependent upon circadian rhythm.

P44. Prolactin: protective actions in neurotoxin-hippocampal injury

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Prolactin (PRL) is a pituitary hormone with a variety of physiological actions, including those within the CNS, in which its effects relate to reproduction, metabolism, emotional response, neurogenesis, and neuroprotection. In neuronal tissue, PRL can function as a protective or repair agent after a lesion, as we have documented in the hippocampus of both females and males. By taking advantage of the experimental neurotoxin lesion model we have demonstrated that during lactation, a hyperprolactinemic reproductive state, the hippocampus of the dam is less sensitive to kainic acid (KA) lesion compared to that of diestrus-virgin rats. Such diminished sensitivity has been proven by systemic or intracerebral administration of KA, at different time-points after the lesion, it prevails even 48h after weaning, and has been detected by apoptosis, glial, and neurodegeneration markers.

In virgin female rats, the pre- or post-lesion treatment with PRL diminishes damaging effects of the excitotoxic injury. Pre-treatment with ovine-PRL or human-PRL and its phosphorylated mimic S179D-PRL diminishes KA-damage to pyramidal neurons in the hippocampus, which correlates with less progression of the behavioral manifestations of epilepsy activity. Additionally, PRL applied after the KA-lesion diminishes the cell loss in CA1 subfield of the hippocampus as well as the glial response and attenuates the cognitive deficit in a novel-object recognition test.

Protective effects of PRL have been documented in male mice, in which pre-treatment with this hormone decreases neuronal loss and neurodegeneration. Paternity has protective actions in this KA-lesion model, and currently we are investigating whether PRL is involved in this phenomenon. PRL receptor presence in the hippocampus has been controversial, but we have detected it in fixed brain tissue or neuronal culture. This symposium presentation will further discuss candidate mechanisms of PRL actions.

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P45. GPCR-induced repression of autophagy in glioblastoma cells: impact on lamellipodium expansion and chemotactic migration

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Grade IV gliomas, called glioblastomas (GBM), are the most aggressive primary brain tumors in adults. They are characterized by massive invasion of the healthy brain parenchyma. This process is controlled by endogenous factors, including chemotactic cytokines (chemokines), the majority of which bind to cell surface receptors that belong to the GPCR superfamily. Although autophagy has been shown to be involved in cancer cell invasion, the functional connection between these processes is still elusive, and the impact of chemotactic GPCRs on the autophagy machinery remains largely unexplored. Work already done by our team has demonstrated that activation of two chemotactic receptors highly expressed in GBM, UT (urotensin II receptor) and CXCR4, induce a marked inhibition of autophagosome biogenesis from its precursor structure, the phagophore [1]. Experiments done in parallel on HEK-293 cells and GBM cells further demonstrated that chemotactic GPCRs exert their anti-autophagic effects by preventing the formation of a specific population of endosomes, the “preautophagic endosomes”, which normally deliver phospholipids for the expansion of the phagophore. We then made the hypothesis that activation of chemotactic GPCRs may trigger a “trafficking switch” favouring, at the expense of phagophore expansion, the formation of VAMP3-positive recycling endosomes which are known to play a key role in chemotactic migration [2,3].

Interrogation of databases (*The Cancer Genome Atlas, Rembrandt*) from glioma patients allowed us to demonstrate that a high expression of VAMP3 correlates with a very poor prognosis. The median survival from the time of initial diagnosis of grade I-III glioma patients with high VAMP3 expression was less than 10 months, whereas patients with a low expression of VAMP3 exhibited a median survival of 40 months. Moreover, in GBM, the highest expression of VAMP3 was found in the mesenchymal subtype, which is associated with the most aggressive phenotype. By performing experiments on GBM cell lines (8MG, U87MG), we have demonstrated that activation of CXCR4 or UT by their respective ligands induces an increase in VAMP3 protein levels, as well as an increase in the number of VAMP3-positive endosomes. By the use of interfering RNA, we have shown that depletion of the pool of VAMP3 protein totally abrogates chemotactic migration of GBM cells induced by CXCR4 or UT, as well as the formation of cell-matrix adhesions. Altogether, these data suggest that activation of chemotactic receptors in glioma cells may, by preventing the formation of preautophagic endosomes, stimulate the production of VAMP3-positive endosomes and their targeting at the cell front in order to initiate lamellipodium expansion and invasive processes.

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P46. Re-expression of the neurodevelopmental protein filamin A in glioblastoma via signaling switch of the urotensin II vasoactive peptide receptor

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Filamin A (FlnA) is a platform protein of actin anchorage widely expressed during development. The key role of FlnA in many cancers was recently suggested. FlnA directly controls cytoskeleton dynamics, transcription factors and G protein-coupled receptor (GPCR) trafficking and activity. Our bioinformatic analysis of The Cancer Genome Atlas (TCGA) database of the most common GPCRs, highlighted that a number of peptide GPCRs including apelin, endothelin, neurotensin and somatostatin receptors, showed the highest expression levels (in the 3rd percentile). Our team previously demonstrated that the vasoactive peptide urotensin II (UII) and its GPCR, UT, are systematically expressed in human glioblastoma (GBM), and activate chemotactic invasion and tumor angiogenesis. A two-hybrid screening of human cDNA isolated FlnA as a protein partner of the C terminus tail of UT, suggesting a potential involvement of FlnA in UT signaling pathways in GBM.

Immunohistochemistry of patient's biopsies (Rouen Hospital, France) revealed re-expression of FlnA in high grade gliomas, showing the highest level in IDH non-mutated gliomas (most often GBM). In high grade gliomas, FlnA, and the UII receptor UT were strongly expressed in perinecrotic areas. TCGA database analysis mRNA expression level of FlnA confirmed these results: FlnA expression level (>5000 rsem) is correlated to a bad prognosis and a lower survival rate. We demonstrated that UII-induced chemotactic migration of a GBM cell line *via* formation of dynamic focal adhesions (increased p-paxillin and vinculin stainings) is abolished when FlnA expression is downregulated by shRNA transfection or upon transient expression of a plasmid vector encoding the UT-C-terminus, suggesting that interaction of FlnA with UT mediates migration. When FlnA expression is genetically altered by CRISPR/Cas9 (U87-KOF), GBM chemotactic migration is significantly decreased (Boyden chamber assay, video-tracking, and wound healing assay) compared with U87 (U87-wt), showing 51% reduced migration speed. Morphometric analysis of U87 and U87-KOF cell lines highlighted complete loss of lamellipodium protrusion (41% in U87 vs 0% in KOF), stress fiber actin suppression (Phalloïdin-rhodamin staining), drastic inhibition of focal adhesions and significant decrease of cell cytoplasmic area in the absence of FlnA, all impacting chemotactic migration. *In vivo*, brain striatal injections of U87 and U87-KOF cells in nude mice led to animal median survival of 66 days (U87) and 39 days (U87-KOF), a significant difference mainly attributed to inhibition of invasion and increase of the cerebral mass-effect in KOF mice.

Our data suggest that FlnA likely plays a key role in the invasive properties of high grade gliomas relayed by the UII vasoactive peptide receptor, responsible for high tumor recurrence. Interacting peptidomimetics able to counteract the specific UT/FlnA interaction would constitute new potential therapeutic tools for treatment of aggressive GBM.

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P47. Developmental pattern of peptidergic systems in the human hypothalamus

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The brains of mammals, including humans, are not mature at birth. The maturation continues for many months during the postnatal period. Growth and brain development are influenced by the hormonal state in which the hypothalamus plays the major regulatory role. The maturation of the hormonal patterns leads to the physiological establishment of chronological variations. It has been established that hypothalamic peptide variations are regulated by hormonal feed-back and amine systems, with the maturation of the latter also being dependent upon the whole functional maturation of the brain. Though these systems have been studied in the rat, very little information is currently available with regard to the human brain. For this, we have investigated the developmental pattern of principal peptidergic systems: somatostatin-releasing inhibiting factor (SRIF), thyrotropin-releasing hormone (TRH), luteinizing hormone-releasing hormone (LHRH), vasoactive intestinal polypeptide (VIP) and neurotensin in human hypothalamus notably during the first postnatal year, a highly dynamic period of neurogenesis and neuronal growth. Globally, receptors of these peptides were widely distributed throughout the rostrocaudal extent of the hypothalamus and were generally low in the fetal and neonatal periods with a tendency in increasing densities observed during postnatal development. The age comparison of binding density indicates variations in several hypothalamic structures. Thus, the densities were higher in older infants in the preoptic area, lamina terminalis, and infundibular nucleus. Of note these receptors show another developmental profile particularly in posterior hypothalamus. On the other hand, the low levels of NT binding sites observed in posterior hypothalamus did not vary during the first postnatal year contrasting in that with the very high levels we in adult. These differences suggest the implication of these peptide receptors in the development of this brain structure and the maintenance of its various functions.

P48. Sex differences in the response of the thyroid axis to individual housing and exercise

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The activity of the hypothalamus-pituitary-thyroid (HPT) axis is inhibited by acute and some types of chronic stress, and by energy deficit. HPT is activated by energy demands such as cold exposure or exercise but this may be blunted by previous stress¹. Stress history of the animal may program the response of the HPT axis as has been demonstrated in rats deprived of maternal care that modifies the activity of the HP-adrenal (HPA) axis in adulthood². Another critical period is adolescence when the HPA axis attains maturity and stressful situations may cause long-term neuroendocrine dysfunction.

We evaluated the response of the HPT axis to acute restraint or to voluntary exercise, in male and female rats isolated since postnatal day (PND) 20 or 30. Wistar female or male rats were divided into 2 groups, one where rats were kept two per cage (controls, C) and the other, individually housed (Iso). In experiment 1, rats were introduced to a restraining apparatus at PND 90 for 1h; a blood aliquot was obtained from the tail at 0, 30 and 60 min to measure corticosterone serum concentration. Iso males had a higher increase at 30 min than controls returning both to basal levels by 60 min whereas females showed higher levels only at 60 min and the area under the curve showed a stronger stress response in Iso males than females. These results corroborated the long-term effects of individual housing on stress response. In experiment 2, groups of isolated (at PND 30) or control rats were further divided in 2 groups at PND 63, one placed in a cage with a running wheel (EX) on 10 alternated nights, the other kept in a nearby cage (SED). EX decreases food intake thus it was measured every night, the same amount offered to SED (pair-fed). Hormones and mRNA levels of parameters related to HPT activity were quantified by immunoassay or RT-PCR. Isolated-sedentary males had higher body weight gain and *Crh* expression in the paraventricular hypothalamus (PVN) than controls, and lower that of *Ucp1* y *Adrb3* in brown adipose tissue; exercise did not alter adipose tissue mass, *Trh* PVN-expression, nor TSH, T4 or T3 serum levels as reported³ but increased mRNA levels of deiodinase 2 (*Dio2*) in mediobasal hypothalamus (MBH) of controls only. The stress of isolation in females lowered TSH and T4 serum concentration, *Dio2* and *Trhde* expression in MBH. Females ran 4x more than males. Exercise decreased WAT mass in controls which had higher serum levels of TSH than the sedentary group. Results showed higher stress response in isolated males but this did not alter the effect of exercise in contrast to isolated females that had a blunted response to exercise compared to controls.

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P49. Mexico's peptide diversity: discover, design and development

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Summary: Naturally occurring peptides play crucial roles in human physiology targeting growth factors, ion channels, protein receptors or enzymes.^[1] Peptides are recognized for being selective and efficacious signaling molecules that bind specifically to larger protein targets.^[2] Consequently, peptide-based drugs are used to treat a variety of disorders, including pain, autoimmune disease, stroke, and cancer. With respect to biotechnology applications, the widespread use of small molecule agrochemicals has resulted in genetic selection pressure leading to insecticide-resistant arthropods. Peptides like spider toxins have proven to be a rich source of insecticidal agents that cause paralysis or lethality through modulation of ion channels and receptors.^[2]

Among the diversity of bioactive peptide scaffolds, cysteine-rich peptides (<50 amino acid residues) are the most abundant class of molecules found in the secretions of venomous species (e.g. conotoxins) and plants (e.g. defensins). These peptides have been identified from diverse taxa such as marine invertebrates (cone snails, sea anemones, stingrays, octopus), arthropods (centipedes, hymenopterans, spiders, scorpions), reptiles (snakes, lizards), mammals (platypus) and plants (e.g. Rubiaceae, Violaceae, Cucurbitaceae, Fabaceae and Poaceae families). Yet, the great majority of well-studied cysteine-rich scaffolds results from just a few venomous eumetazoans. Mexico has proven to be a valuable source for pharmacologically active disulfide-rich peptides ranging from marine snails to scorpions and is home to the first-ever reported cave-dwelling venomous crustacean. Some of these peptides have demonstrated good physicochemical and pharmacokinetic properties i.e. metabolic stability and membrane permeability, all considered desirable properties for development of peptides as drug treatments.^[3]

This poster will present research directions intended to harness the Mexican rich peptide diversity for potential applications as biological control agents and drug treatments by (i) discovering novel cysteine-rich peptides from Mexican venomous species and plants using both transcriptomic and proteomic technologies, (ii) designing peptide analogues using computational algorithms (structure-based drug design, de novo design) followed by traditional solid-phase peptide synthesis or recombinant synthesis in *E.coli* and (iii) developing novel and potent protein target modulators with potential applications against agricultural pests and unmet medical disorders.

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P50. Constrained GLP-1 mimetics with incretin-like properties

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Keywords: Glucagon like peptide 1, Diabetes, cAMP, -arrestin 2, Insulin-release, -helix

Summary: Type 2 diabetes mellitus (T2DM) is a chronic disorder characterized by hyperglycaemia from insulin resistance and defective insulin secretion. Glucagon-like peptide 1 (GLP-1) is a 30-residue peptide that activates GLP-1 receptor (GLP-1R) in pancreatic β -cells stimulating glucose-dependent insulin secretion, insulin gene transcription and β -cell proliferation. Pharmacological treatment of type 2 diabetic patients with longer-lived, proteolysis-resistant GLP-1R agonists like ByettaTM (Exenatide) and VictozaTM (Liraglutide) are gaining use for treatment of T2DM ^[1] Such injectable peptides are the only class of glucose-lowering agents resulting in body weight reduction. Both academia and industry seek oral GLP-1R agonists to improve compliance while maintaining or improving efficacy and tolerability ^[2] A better understanding of how GLP-1 interacts with its receptor may better facilitate the identification of orally bioavailable therapeutic agents. This talk will illustrate conceptualization, design and synthesis of novel conformationally constrained GLP-1 analogues. Our results led to a series of highly helical analogues that exhibited potent receptor activation, greater insulin secretion and insights into receptor-ligand interactions leading to receptor activation. ^[3]

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P51. Permeability of blood-hypothalamus barrier changes throughout the day

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The suprachiasmatic nucleus (SCN), the master clock in mammals, regulates metabolism and hormonal levels according to the time of the day, by imposing circadian tone over many other nuclei including the arcuate nucleus (ARC). The ARC is a major nucleus for energy regulation and for hormone secretion. It is known that ARC and SCN sustain a tight and bidirectional communication that it is essential for the expression of circadian rhythms. To accomplish its functions, ARC depends on information received from the periphery through the circulation whose access is restricted by the blood-hypothalamic barrier (BHB). We investigated whether access of the ARC to chemical information from peripheral blood is also circadian, and if the SCN controls this. Through permeability assays using Evans Blue (EB) in adult intrajugular-cannulated male Wistar rats, we compared the amount of EB that penetrated into the ARC at different time points correlated with circulating glucose levels (ZT2, ZT11, ZT14, ZT22). in animals with unilateral and bilateral electrolytic lesions of the SCN. We found that the penetrability of ARC/ME is highest at Zeitgeber Time (ZT) 2, and lowest at ZT11. Permeability at ZT14 is intermediate between ZT11 and ZT22. The penetration of EB to the ARC at ZT2 is prevented with a bilateral lesion of the SCN, while unilateral lesions of the SCN induce ipsilateral closing of the ARC/ME barrier. The permeability of the ME itself also changes throughout the day, and is diminished by the bilateral lesions at ZT2. Also, through light pulses during the dark phase of the animal, we evaluated the speed at which the BHB can change. We found that BHB permeability changes required at least 30 min.

Our findings indicate that there is a daily rhythm in the permeability of the BHB which is controlled neurally by the SCN. Because of their circadian profiles of expression and their strong input into the ARC/ME we hypothesize that certain peptides produced by the SCN, such as VIP and AVP, are regulating the circadian changes of BHB permeability. The understanding of the communication between the periphery and the hypothalamus could help us to better comprehend the consequences of a defective crosstalk between the brain and periphery, manifested in diseases such as obesity and type 2 diabetes.

KEY WORDS: Blood-hypothalamus barrier, suprachiasmatic nucleus, metabolism, arcuate nucleus, arginine vasopressin, vasoactive intestinal peptide.

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P52. Expression of arginine-vasopressin and corticotrophin releasing hormone in hypothalamus and hippocampus after early life stress in rats

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Arginine-vasopressin (AVP) and corticotropin releasing hormone (CRH) are neuropeptides involved in the control of social behavior, learning, memory and stress responses in adult animals. Maternal separation (MS) in rodents is a potent stressor that programs the hypothalamus-pituitary-adrenal (HPA) axis and affects CRH and AVP expression in the adult; however the effects of MS during the early life period are poorly understood. Therefore, we tested the hypothesis that early life stress increases the expression of AVP and CRH during early life. Control and MS (3 hour / daily from day 1 to 14) male rat pups were sacrificed at postnatal day (PD) 6, PD12, PD15, DP21 or as adults. The hippocampus and hypothalamus were dissected, and AVP and CRH expression were analyzed by real-time PCR (qPCR). Different groups were used to evaluate AVP and CRH expression by in situ hybridization at PND15. Our results show that MS increases hypothalamic expression of AVP during the first two weeks of life, but not in adulthood. We demonstrate for the first time that AVP expression in hippocampal extracts is already detectable on PD6; however, AVP expression quantified in the entire hippocampal formation by qPCR was not affected by MS. On the other hand, we observed that MS caused an increase in hypothalamic CRH expression during the first two weeks that prevails until adulthood. Hippocampal CRH expression was unchanged at all ages. When analyzing data of in situ hybridization we observed that MS increased AVP and CRH expression in the PVN at PD15. In the hippocampus, MS selectively increased AVP expression in the CA3 region. Basal corticosterone plasma levels were not affected by MS. These results indicate that MS alters the expression of both AVP and CRH in the hypothalamus during the first two weeks of life, but increases AVP expression only in the CA3 region of the hippocampus, suggesting a role for these neuropeptides in the maturation of the HPA axis during this period.

P53. Recolonization with native microbiota reverses the effects of early life stress on depressive-like behavior and metabolic risk

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Early life stress (ELS) decreases hippocampal neurogenesis, increases depressive-like behavior and metabolic vulnerability, and causes intestinal dysbiosis. It has been proposed that the gut microbiota could be involved in the modulation of the stress response; however, the relationship between the gut-brain axis and ELS-induced alterations is poorly understood. Here, we propose that restoring intestinal dysbiosis could reverse some of the long term effects of ELS. Therefore, the aim of the study was to evaluate if depletion of gut microbiota and posterior recolonization with native microbiota from control animals reverses the effects of ELS on behavior, hippocampal neurogenesis and metabolic risk.

Control and maternally separated (MS, 3 hours / day from day 1 to 14) adult (3-4 months) Sprague Dawley male rats were treated with an antibiotic cocktail (ampicillin 1 g/L, vancomycin 500 mg/L, neomycin sulfate 1g/L and metronidazole 1g/L) from postnatal day (P) 60 to P90 and placed in cages with donor control or MS animals for one month (P90-120). We evaluated depressive-like behavior on the forced swimming test and glucose tolerance immediately after microbiota ablation, and after one month of recolonization. Our results show that MS causes a passive coping strategy in the forced swimming test, affects glucose homeostasis, increases body weight and decreases hippocampal neurogenesis. Microbiota ablation with antibiotics caused similar effects to MS on behavior and hippocampal neurogenesis, however, it does not affect the glucose homeostasis or body weight. Recolonization of control rats with microbiota from donor MS animals caused an increase in depressive-like behavior, and affected glucose homeostasis and body weight. Recolonization of MS rats with microbiota from control rats reverses the effects of MS on behavior, glucose homeostasis and body weight but not on hippocampal neurogenesis. These results indicate that brain-gut axis alterations play a central role in mediating the effects of ELS exposure, and that reversing ELS-induced dysbiosis could be a potential therapeutic strategy to reverse those effects.

P54. New mechanistic insights into the role of the sodium ion binding site in the allosteric modulation of the APJ receptor binding, signaling and function

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The APJ receptor, a class A G protein-coupled receptor (GPCR), is involved in various key physiological processes such as energy metabolism regulation, angiogenesis and cardiovascular functions. Recent studies have highlighted the crucial role played by sodium ions as endogenous allosteric modulators on agonist binding and activation of several GPCRs. Using mutagenesis and competitive radioligand binding experiments, we identified the sodium binding site of the APJ receptor and demonstrated by Bioluminescence Resonance Energy Transfer (BRET) assay that the sodium ion functions as a negative allosteric modulator on agonist-mediated APJ signal transduction. We also showed that increasing concentrations of sodium differently affect the ability of apelinergic endogenous ligands to bind to APJ. In addition, we were able to demonstrate using the BRET-based assay that some mutations led to the constitutive activation of the APJ receptor. To investigate further the potential involvement of sodium in the physiological action of the apelinergic system, we examined the impact of sustained release of different APJ endogenous ligands on the cardiovascular and renal functions in Spontaneously Hypertensive Rats (SHRs) fed with either normal or high salt diet (8% sodium). Interestingly, we found that treatment with endogenous ligands confers protective effects against cardiac and renal injury to SHR receiving high sodium diet, compared to SHR on normal diet. These results thus suggest that the allosteric modulation of the APJ receptor by sodium ions observed in *in cellula* studies could translate into beneficial effects *in vivo*, improving both cardiovascular and renal functions in SHR fed with sodium-enriched diet. We further demonstrated that the apelin peptides used in the *in vivo* studies behave differently in SHR on high-salt diet. Altogether, these results should accelerate drug development strategies targeting the apelinergic system for the improvement of cardiovascular and renal diseases.

P55. Efferent projections of thyrotropin-releasing hormone-synthesizing neurons from the tuberal region of the lateral hypothalamus impinge on histaminergic neurons of the tuberomammillary nuclei.

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Thyrotropin-releasing hormone (TRH) is a tripeptide widely distributed in the mammalian brain. Besides its critical role in the central regulation of the hypothalamic-pituitary-thyroid axis, TRH has been proposed as a metabolic sensor that regulates appetite and energy homeostasis. Hypophysiotropic and non-hypophysiotropic TRH neurons originating from the hypothalamic paraventricular (PVN) nucleus respond to highly demanding conditions as such food deprivation and cold exposure. However, little is known about other hypothalamic cells that synthesize TRH and may also be involved in eating behavior. Central administration of TRH decreases food intake but increases histamine in the tuberomammillary nucleus (TMN), where all the histaminergic neurons of the brain reside. As TMN histamine neurons are densely innervated by TRH fibers from an unknown origin (Sarvari et al., 2012), we mapped the TRH afferents using two complementary tracers.

The retrograde tracer, Cholera toxin B subunit (CTB, 0.5%) was injected by iontophoresis under stereotaxic guidance from a glass micropipette placed into the TMN E1-E2 and E4 subdivisions of 8 week old male Sprague Dawley rats (n=7). After 1 week of transport interval, animals were perfused and brain sections were prepared for double-labeling immunofluorescence using an antibody directed against pro-TRH (178-199) and CTB. The origin of CTB afferents to the TMN included the septum, medial, central and lateral preoptic area, bed nucleus of the stria terminalis, perifornical area, anterior parvocellular PVN, lateral, anterior and ventromedial hypothalamus, peduncular part of the lateral hypothalamus, suprachiasmatic nucleus, medial amygdala and tuberal lateral hypothalamus (TuHL). However, double-labeled neurons were found only in the TuLH suggesting that TRH neurons innervating histaminergic neurons in the TMN originate in the TuLH. To confirm the specificity of the retrograde tract-tracing result, we administered iontophoretically the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHAL, 2.5%) in the TuLH of 8 week old male Sprague Dawley rats (n=7). PHAL injections hit rostral, mid or caudal TuHL. After a 10 day transport interval, we identified double-labeled PHAL- and pro-TRH- immunofluorescence cells in the TMN. We are currently determining the number of PHAL/pro-TRH-ir containing axon terminals and *en passant* boutons in the E1-E4 subnuclei of the TMN. The data suggest, therefore, that TuLH TRH neurons may function as an important metabolic sensor in the brain to regulate the effects of neuronal histamine on energy homeostasis and thermogenesis.

Keywords: TRH; Histamine; retrograde tracer; anterograde tracer.

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P56. Leptin resistance induced by endotoxin tolerance is associated with increased expression of counter-regulatory phosphatases in the hypothalamus

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Lipopolysaccharide (LPS) is a cell-wall component of gram negative bacteria which acts as an activator of immune responses such as the IKKB/NF- κ B pathway through Toll-like 4 receptors (TLR4). We have previously shown that a single dose of LPS induces hypophagia and weight loss, while repeated exposure induces a desensitization of these effects. Obesity is associated with low grade chronic inflammation, an increase in serum LPS levels and resistance to leptin, a hormone that acts in the hypothalamus to reduce food intake. Leptin signaling pathways are regulated by phosphatases like TCPTP and PTP1B, via dephosphorylation of downstream signaling molecules. Several studies have demonstrated a pivotal role of these phosphatases in the leptin resistance phenotype, therefore it is relevant to study their role during chronic inflammation. The aim of this study was to assess the role of counter-regulatory phosphatases in the leptin resistance induced by endotoxin tolerance. All experimental protocols were approved by the Ethics Committee for Animal Use of the Ribeirão Preto Medical School (063/2015).

Male C57Bl6 mice were assigned to three different groups: 7 injections of saline (CT); 6 injections of saline + 1 injection of LPS (1LPS) and 7 injections of LPS (7LPS) (5 μ g/animal, ip, n=8). During the experimental period, food intake and body weight were monitored. To evaluate the leptin sensitivity, exogenous leptin (5mg/kg ip) or vehicle was injected intraperitoneally after treatment with LPS (1LPS or 7LPS) or saline. To investigate the phosphatase expression, mice were euthanized by decapitation and the brains were collected for Western blotting analyses (n=5). One-way analysis of variance (ANOVA), followed by Newman-Keuls post hoc test, was used to analyze the experiments with single or repeated LPS treatment. In the leptin protocols, two-way ANOVA followed by Newman-Keuls post hoc test was used. After the first injection, LPS reduced food intake (CT 3.5 \pm 0.5g; 1LPS 1 \pm 0.9g) and body weight (CT Δ 0.2 \pm 0.1 1LPS Δ -1.6 \pm 0.8g). However, after the third application, the 7LPS animals did not show the same hypophagic effect, revealing a desensitization to endotoxin (CT 3.7 \pm 0.4g; 7LPS 3.5 \pm 0.4g). Leptin injection reduced food intake in the control group but not in the 7LPS group (CT VEH 4.3 \pm 0.7; CT LEP 2.3 \pm 0.6; 7LPS VEH 4.3 \pm 1.3; 7LPS; LEP 4.3 \pm 0.3g). Similarly, leptin increased STAT3 phosphorylation in the control but not in the 7LPS group (CT: LEP 196.8 \pm 48; 7LPS: VEH 98.8 \pm 0.4; 7LPS: LEP 102.4 \pm 16.5 % of control). In the 1LPS, exogenous leptin had no additional effect on LPS-induced hypophagia (1LPS VEH 0.87 \pm 0.3g; 1LPS LEP 0.88 \pm 0.4g) however, 1LPS showed significant STAT3 phosphorylation after leptin (1LPS VEH 127 \pm ; 1LPS LEP 295.8 \pm 55 % of control). Also, we observed that SOCS3 (7LPS 262 \pm 52 % of control), PTP1B (7LPS 242 \pm 50.4 % of control) and TCPTP (7LPS 231 \pm 68 % of control) expression were increased in the 7LPS group compared to control, but not in the 1LPS group. These data indicate that resistance to leptin action in the hypothalamus after repeated exposure to LPS is associated with increased expression of leptin counter-regulatory signaling molecules.

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P57. Neural basis of methamphetamine anticipation in a non-invasive voluntary self-administration paradigm

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The ability to sense time and anticipate events is critical for survival. Learned responses that allow anticipation of the availability of food or water have been intensively studied. While anticipatory behaviors also occur prior to presentation of regularly available rewards, there has been relatively little work on anticipation of drugs of abuse, specifically methamphetamine (MA). In the present study, we used a protocol that avoided possible CNS effects of handling or surgery stress, by testing anticipation of MA availability in animals living in their home cages, with daily voluntary access to the drug at a fixed time of day. Anticipation was operationalized as the amount of wheel running prior to MA availability. Mice were divided into four groups given access to either nebulized MA or water, in early or late day. Animals with access to MA, but not water controls, showed anticipatory activity, with more anticipation in early compared to late day and significant interaction effects. Next, we explored the neural basis of the MA anticipation, using c-FOS expression, in animals euthanized at the usual time of nebulization access. In the dorsomedial hypothalamus and orbitofrontal cortex, the pattern of c-FOS expression paralleled that of anticipatory behavior, with significant main and interaction effects of treatment and time of day. The results for the lateral septum were significant for main effects and marginally significant for interaction effects. These studies suggest that anticipation of MA is associated with activation of brain regions important in circadian timing, emotional regulation and decision-making. In ongoing studies, we are examining the possibility of reducing anticipation and voluntary intake under various treatment regimens.

P58. A pharmacological approach on how TRP channels and traffic-related environmental pollution impairs cardiovascular function

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Diesel exhaust particles (DEP) and its chemical contaminant 1,2-naphthoquinone (1,2-NQ) are among the harmful components of traffic-related air pollution and may represent risk factors affecting airways and cardiovascular systems of humans living in highly populated urban areas (1). Studies, including ours, demonstrate the role played by the transient receptor potential (TRP) or neuropeptides, e.g. substance P (and its receptors NK1 and NK2) on the effects of DEP and 1,2-NQ on the pulmonary system (1, 2, 3). However, the health impact of the early exposure to 1,2-NQ in the cardiovascular system is elusive.

We investigated effects of airway exposure at days 6, 8 and 10, of 1,2-NQ (100 nM, 15 min) on *in vivo* and *ex vivo* cardiovascular parameters in young C57bl/6 mice (43 days of age), and the protective effect of TRPV1 channels. Mice exposed to 1,2-NQ had reduced autonomic function in both *ex vivo* right atria (RA, bpm) and pulmonary artery (PA), and significant changes *in vivo* in the electrocardiographic (ECG) parameters, characterized by an increase in the low (LF) and high-frequency (HF) rate (LF/HF: 0.128 ± 0.016 and $0.342 \pm 0.069^*$ for control and 1,2-NQ-treated mice, respectively, n= 7) and reduction in both RR (100 ± 3.4 and $91 \pm 1.3^*$ ms, for control and 1,2-NQ-treated mice, respectively, n= 7) and SDRR (4.15 ± 0.56 and $2.43 \pm 0.19^*$ ms, for control and 1,2-NQ-treated mice, respectively, n= 7)-wave duration. The TRPV1 blocker capsazepine (50 mg/kg, i.p.) reversed the 1,2-NQ-induced reduction in atria function (bpm) but aggravated PA responsiveness. In young male mice prior exposed to 1,2-NQ, the cardiovascular changes occurred along with lung inflammation. Pretreatment with TRPV1 antagonist prevented 1,2-NQ-induced RA changes, but it did not appear to protect against the vascular disorders.

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P59. Circadian gating of spinal inflammatory input from the liver shapes the diurnal pattern of TNF α release by the liver and spleen

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The autonomic nervous system (ANS) regulates the intensity of the inflammatory process in response to endotoxin, however, whether this is parasympathetic or sympathetically mediated is not yet clear. Moreover, how the brain is informed about the presence of a peripheral immune challenge has remained elusive. We hypothesized that the sensory part of the autonomic nervous system is responsible for the immediate sensing of lipopolysaccharide (LPS), in order to modify its output to the immune system and mount an adequate response to the inflammatory stimulus. We show that neurons in the dorsal horn (DH) of the spinal cord become activated by prostaglandin after an LPS challenge. Sympathetic and sensory afferent denervation of the liver abolished the LPS-induced neuronal activation and increased the inflammatory response. Surprisingly, the cytokine production was higher in the spleen than in the liver, thus suggesting that the liver controls sensing of LPS, the immune message is integrated in the brain, and the ANS is then able to modify its output to the spleen, the final effector organ.

The circadian system, which strongly influences the ANS, imposes rhythmicity on this circuit, modulating the sensitivity of the DH to LPS and thus, the subsequent autonomic output to the liver and spleen. During the active phase of the animal, the sensitivity of DH neurons to LPS is decreased and allows the spleen and liver to release all their TNF α content into circulation, ultimately resulting in high plasma

TNF α at night. This process is reversed during the resting phase, allowing the neurons in the DH to respond to the inflammatory stimulus, which then results in a high retention of TNF α in the organs, and thus, a diminished plasma TNF α concentration at this time point.

This study unravels the circuit used to transmit a peripheral LPS signal back to the brain, which involves the sensory innervation of the liver as well as the motor autonomic innervation of the spleen. This circuit, strongly driven by the circadian system, determines the strength of the inflammatory response by controlling the release dynamics of TNF α from the spleen and liver in a time dependent manner, mounting an efficient inflammatory response when it is most likely needed.

P60. Nesfatin-1₃₀₋₅₉ injected intracerebroventricularly increases anxiety, depressiveness and anhedonia in normal weight but not in diet-induced obese rats

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The anorexigenic peptide nesfatin-1 is well known for its effect in inducing satiety and satiation. As food intake-regulating peptides often also impact behavior, this was investigated for nesfatin-1 as well, and nesfatin-1 showed an anxiogenic effect following intracerebroventricular (icv) injection. In humans, recent studies demonstrated an association between nesfatin-1 plasma levels and body weight, with levels positively associated with body mass index (BMI) described in most studies. However, it remains to be established whether the effects of nesfatin-1 differ under conditions of chronically altered body weight. The aim of the present study was to investigate the effect of nesfatin-1₃₀₋₅₉ (the active core of nesfatin-1) on depressiveness and anxiety in normal weight and in diet-induced (DIO) obese rats.

ICV cannulated male normal weight or DIO rats fed *ad libitum* were injected with nesfatin-1₃₀₋₅₉ (0.1, 0.3, or 0.9 nmol/rat) or vehicle icv 30 min prior to testing. Depressive and anhedonic behavior was evaluated by the means of the sucrose preference test and the novelty-induced hypophagia test. To detect explorative and anxiety-related behaviors the open field test, elevated zero maze and dark/light box were used. In normal weight rats nesfatin-1₃₀₋₅₉ at a dose of 0.3 nmol decreased the amount of sucrose consumed in the sucrose preference test, resulting in a decreased preference ratio compared to vehicle (-33%, $p < 0.05$). As this was the most effective dose, 0.3 nmol was used for all subsequent experiments. Nesfatin-1₃₀₋₅₉ also induced a reduction of palatable snack consumption in the novelty-induced hypophagia test in normal weight rats compared to vehicle (-62%, $p < 0.05$). Additionally, in the open field test nesfatin-1₃₀₋₅₉ reduced the number of entries into the center zone (-45%, $p < 0.01$) and the number of entries into the open arms of the elevated zero maze compared to vehicle (-39%, $p < 0.01$). In the dark-light box no significant differences were observed after nesfatin-1₃₀₋₅₉ injection ($p > 0.05$). In rats that developed DIO an icv injection of nesfatin-1₃₀₋₅₉ did not result in an alteration of anxiety and depressiveness ($p > 0.05$). These results lead to the assumption that nesfatin-1₃₀₋₅₉ takes part in the mediation of anhedonia, depressiveness and anxiety under normal weight conditions, whereas in DIO nesfatin-1₃₀₋₅₉ possibly induces a desensitization.

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P61. NUCB2/nesfatin-1 is associated with the severity of eating disorder symptoms in female obese patients

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Nesfatin-1 is a potent anorexigenic neuropeptide derived from nucleobindin 2 (NUCB2). Besides its role in the regulation of hunger and satiety in rodents, recent studies point towards the involvement of nesfatin-1 in the modulation of emotional processes. In a previous study we reported a negative association of NUCB2/nesfatin-1 with anxiety in obese men and positive associations with anxiety, depression and perceived stress in obese women indicating a sex-specific regulation of nesfatin-1. Apparently, emotions and food intake mutually influence each other, so that emotional dysfunction can affect appetite and body weight. Since the role of nesfatin-1 in food intake has not been extensively examined in humans so far, the aim of the study was to investigate a possible association of NUCB2/nesfatin-1 with disordered eating habits in a mixed-sex study population displaying a wide range of body weight.

We enrolled 243 female and male inpatients who received medical treatment for anorexia nervosa ($n = 66$), obesity ($n = 143$) and associated somatic and mental comorbidities. Patients with normal weight ($n = 33$) were hospitalized due to somatoform, depressive and anxiety disorders. Blood samples were taken within three days after hospital admission and eating disorder symptoms were psychometrically assessed using the Eating Disorder Inventory-2 (EDI-2, range 0 - 100). NUCB2/nesfatin-1 plasma levels were measured using a commercial ELISA. With a mean EDI-2 total score of 42.7 ± 14.6 (ranging from 5 to 80), the whole study population displayed a distinct prevalence of eating disorder symptoms. Women exhibited significantly higher EDI-2 scores (44.7 ± 14.8) than men (37.7 ± 12.8 , $p < 0.001$). While women with anorexia nervosa (45.5 ± 17.3 vs. 25.6 ± 10.7) and obesity (47.9 ± 10.5 vs. 25.6 ± 10.7) reported significantly higher EDI-2 scores than normal weight females ($p < 0.001$), no differences were observed in the male body mass index (BMI) subgroups. Interestingly, we found a significant positive correlation between NUCB2/nesfatin-1 plasma levels and EDI-2 total scores in obese female patients ($r = 0.293$, $p = 0.013$). However, no associations were detected between NUCB2/nesfatin-1 and EDI-2 total scores in male populations or in females from other BMI subgroups. In conclusion, NUCB2/nesfatin-1 plasma levels were positively correlated with EDI-2 total scores in obese women, whereas no association was apparent in men. Therefore, our data might point towards a sex-specific regulation of eating behavior in obese patients. It has to be further investigated whether NUCB2/nesfatin-1 is selectively involved in eating behavior in women. Moreover, longitudinal studies will be helpful to examine alterations of circulating NUCB2/nesfatin-1 levels after treatment of eating disorders in relation to changes in EDI-2 total scores.

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P62. Evidence for the role of lipid rafts in Ca²⁺-gating of the Transient Receptor Potential channels in sensory neurons and the analgesic effect of sphingomyelinase

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Background and aims Transient Receptor Potential ion channels, such as TRP Vanilloid 1 and Ankyrin repeat domain 1 (TRPV1 and TRPA1) are expressed in nociceptive primary sensory neurones. TRPV1 can be activated by capsaicin, resiniferatoxin (RTX), low pH and noxious heat. Irritant molecules such as allyl isothiocyanate (AITC), and formalin activates TRPA1 receptors. TRPM8 and TRPM3 belong to the „melastatin” TRP family and can be activated by cold temperatures (18-24°C), menthol and icilin, or pregnenolon-sulfate, respectively. Lipid rafts are defined as liquid ordered plasma membrane microdomains rich in cholesterol, sphingomyelin and gangliosides. Our previous finding have revealed that cholesterol depletion by methyl-β-cyclodextrin inhibits the function of TRP receptors. Sphingomyelinase (SMase) decreases membrane sphingomyelin. The aim of the present study is to analyze the effects of lipid raft disruption by SMase on responses evoked by TRPV1, TRPA1, TRPM8 and TRPM3 agonists. We examine the potential analgesic effect of SMase in *in vivo* mouse models.

Methods Intracellular calcium measurement with the fluorescent indicator fura-2-AM on cultured trigeminal cells and radioactive ⁴⁵Ca-uptake experiments in TRPV1-expressing CHO cells were performed. The effect of SMase on formalin (2.5%)-induced mechanical hyperalgesia and RTX-induced thermal hyperalgesia was monitored, and capsaicin-evoked acute nocifensive response (“eye-wiping”) was detected.

Results In TRPV1-expressing CHO cells SMase (30 mUN) decreased significantly the capsaicin-induced (330 nM) intracellular calcium influx. Calcium influx decreased significantly in the proportion of cells responding to capsaicin and RTX after 30 mUN SMase incubation. SMase (30 mUN) also diminished the TRPA1 activation by AITC (200 μM) or formalin (0.01%) on cultured trigeminal neurons. Significant decrease in TRPM8 activation was observed after 30 mUN SMase incubation. In contrast, SMase treatment had no effect on pregnenolon-sulfate (50 μM)-induced TRPM3 receptor activation. Ceramide and sphingosine had no effect on TRPV1 activation. Calcium release from intracellular stores evoked by thapsigargin (1 μM) or calcium influx elicited by KCl (50 mM) were not changed by SMase treatment. A significant drop of the mechanonociceptive and heat threshold was observed in response to intraplantar resiniferatoxin (0.01 μg/ml) or formalin administration which was diminished in SMase-treated group. SMase treatment abolished the capsaicin-evoked acute nocifensive response.

Conclusions The present findings provide the first evidence that disruption of lipid rafts by SMase results in analgesic effect in *in vivo* mouse models. We suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels. Therefore, targeting this interaction might be a promising tool for drug developmental purposes.

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P63. Plasma concentrations of soluble (pro)renin receptor are elevated in obstructive sleep apnea patients with morbid obesity, and decreased by bariatric surgery

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(Pro)renin receptor ((P)RR), a receptor for renin and prorenin, activates the renin-angiotensin system via the activation of prorenin, and stimulates intracellular MAPK signaling, when prorenin binds to it. Moreover, (P)RR forms a functional complex with vacuolar H⁺ ATPase, which plays an essential role in maintaining the acidic environment of intracellular compartments. Plasma concentrations of soluble (P)RR, which consists of the extracellular domain of (P)RR, are elevated in patients with obstructive sleep apnea (OSA) (Nishijima et al. *Peptides* 2014; Nishijima et al. *Tohoku J Exp Med* 2016). The aim of the present study is to establish the range of plasma soluble (P)RR concentrations in OSA patients with morbid obesity, and effects of bariatric surgery on its plasma concentrations. Plasma soluble (P)RR concentrations were studied in 23 OSA patients complicated by morbid obesity (10 men and 13 women; BMI 40.7 ± 6.16 kg/m², mean \pm SD) without chronic kidney disease. Overnight polysomnography was performed before surgery, and 4 and 24 weeks after surgery. Pre-operative plasma soluble (P)RR concentrations were elevated in these 23 patients compared to non-OSA subjects, and showed significant positive correlations with arousal index ($p < 0.01$), apnea hypopnea index ($p < 0.05$), apnea index ($p < 0.005$), desaturation index ($p < 0.05$) and serum creatinine ($p < 0.05$), and a significant inverse correlation with an estimated glomerular filtration rate ($p < 0.05$). No significant association was found between plasma soluble (P)RR concentrations and body mass index, or plasma leptin levels. With the improvement of these polysomnography parameters by bariatric surgery, plasma soluble (P)RR concentrations significantly decreased from 15.3 ± 3.6 ng/mL to 12.5 ± 2.7 ng/mL (4 weeks after surgery) and 11.4 ± 2.4 ng/mL (24 weeks after surgery). The association between plasma soluble (P)RR levels and the polysomnography parameters disappeared after surgery. In conclusion, elevated plasma soluble (P)RR levels were decreased by bariatric surgery in OSA patients with morbid obesity. The most highly associated factor for these changes was the severity of OSA, represented by arousal index, apnea hypopnea index, apnea index and desaturation index.

P64. Pro-depressive effect induced by melanin-concentrating hormone microinjected into the locus coeruleus: relevance of stress in this effect.

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The hypothalamic neuropeptide melanin-concentrating hormone (MCH), has been implicated in the regulation of mood; however, the underlying mechanisms of its role remain unknown. MCH neurons project to dorsal raphe nucleus (DRN), and we previously reported that intra-DRN MCH microinjections induced a pro-depressive response in rats, which was prevented by fluoxetine and nortriptyline, serotonergic and noradrenergic antidepressants, respectively. This result pointed to a potential role of the MCHergic system, classically related to control of energy homeostasis, in the induction of depressive-like behaviors via modulation of a brain area (the DRN) related to mood disorders. Also, our results are in accordance with the antidepressant and anxiolytic actions described for MCH receptor 1 antagonists in rodents. Considering that MCH neurons also project to the locus coeruleus (LC), a brain area intimately related with stress response and depression, the present study was designed to further investigate the role of MCH in the LC.

We first characterized the behavioral effects induced by unilateral microinjections of MCH (100 and 200 ng) into the LC in rats using the forced swimming test (FST) and the learned helplessness model (LH). Secondly, the neurochemical effects on extracellular levels of noradrenaline (NA) were assessed in the ipsilateral medial prefrontal cortex (mPFC) after the microinjection of MCH intra-LC by in vivo microdialysis in awake animals. Results showed that MCH induced a dose-dependent depressive-like effect in the FST (i.e., significantly increased the immobility and decreased climbing time), without changes in locomotor activity evaluated in an open field. In the LH, MCH only at the 100 ng dose elicited a pro-depressive effect in those animals previously stressed (i.e., significantly increased the number of escape failures). This response was not evident in non-stressed rats. Moreover, NA levels were decreased in the mPFC, suggesting an inhibitory action of MCH on the LC-NAergic neurons.

Our data demonstrate that, in addition to DRN, LC is a neural substrate for MCH pro-depressive actions and also show that the effect of MCH is potentiated by stress. All of these results support the hypothesis that MCH acts as a depression-promoting factor.

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P65. Impact of high fat diet-induced obesity development on leptin signaling and thyrotropin releasing hormone biosynthesis in the paraventricular nucleus of the hypothalamus in male rats

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Hypothalamus-pituitary-thyroid (HPT) axis activity plays an important role in energy homeostasis. The axis is inhibited by fasting, chronic stress and stimulated by cold and obesity. Thyrotropin-releasing hormone (TRH) neurons of the paraventricular nucleus of hypothalamus (PVN) have a pivotal role in control of HPT axis activity. These neurons project to the median eminence, where TRH is released into the portal blood capillaries. TRH stimulates the secretion of thyrotropin (TSH) from the pituitary, which activates the synthesis and release of thyroid hormones (T_4 and T_3), critical regulators of metabolism and thermogenesis. Orexigenic and anorexigenic signals modulate HPT activity in opposite ways, in part through regulation of TRH mRNA levels in the PVN neurons, a proxy for TRH neuron activity. Neuronal terminals from the arcuate nucleus and lateral hypothalamus contact PVN TRH-synthesizing neurons and control their activity. Furthermore, leptin is a peripheral anorexigenic signal that increases PVN TRH mRNA levels directly or by activation of α -melanotropin (α MSH) neurons from the arcuate nucleus. Established diet-induced obesity is associated with changes in thyroid function, but the early phase events are unknown.

We hypothesized that an increase in serum leptin concentration associated with a positive energy balance would increase PVN TRH mRNA levels. 60 days old Wistar male rats were fed with a regular diet (RD; 18% kcal from fat) or a high fat diet (HFD; 45% kcal from lard fat) during 3 or 30 days. At day 3, HFD animals body weight (BW) and serum TSH, T_4 , T_3 and glucose concentrations were not different from those of RD rats; however, serum leptin and insulin concentrations, as well as TRH mRNA levels in the PVN area, were higher than in RD animals. At day 30, while serum TSH, T_4 , T_3 and glucose concentrations did not differ between RD and HFD animals, leptin and insulin serum concentrations, BW and weight of white fat depots were higher in HFD compared to RD rats. In rats fed with HFD for 30 days, semi-quantitative RT-PCR revealed higher TRH mRNA levels in the PVN area than in RD rats, a finding corroborated by in situ hybridization. Furthermore, the number of anterior and medial TRH neurons positive for phosphorylated signal transducer and activator of transcription 3 (pSTAT3) was higher in HFD than in RD rats. Thus, in HFD rats, leptin or another pSTAT3 regulator, exerts a direct effect on TRH neurons. In contrast, we did not observe differences in the number of TRHergic neurons that co-localize with phosphorylated cyclic-AMP response element binding protein, suggesting the balance of the activity of inputs from the arcuate nucleus (including α MSH and neuropeptide Y/Agouti-related protein neurons) and lateral hypothalamus onto TRH neurons may be unaltered by 1-month HFD. In conclusion, the data suggest that the early increase in PVN TRH mRNA levels induced by positive energy balance is mediated by a direct action of leptin, which may predominate over the influence of the arcuate nucleus. This effect may be the initial driver of HPT axis activation.

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P66. PACAP/PAC1 modulation of thermogenesis, metabolism, and inflammation via catecholaminergic neurons in the sympathetic nervous system

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Dysregulation of the sympathetic nervous system (SNS) underlies a vast array of diseases, including cardiovascular diseases, metabolic syndrome, autoimmune diseases, and psychiatric diseases associated with stress. The SNS is a critical regulator in diseases with some of the highest disease burdens to society. According to the United States Center for Disease Control, cardiovascular disease is the leading cause of death in the U.S., accounting for approximately 1 in 4 deaths, and more deaths than all types of cancer combined. In addition, the number of people in the U.S. suffering from diabetes has increased by 20 million people in the past fifty years. Moreover, in recent years, it has become increasingly clear that the SNS also plays a key role in many inflammatory diseases as well¹. Currently the only approaches to pharmacologically modify sympathetic function is to target catecholamine signaling, for example, with drugs that alter catecholamine metabolism or uptake, or that interact specifically with catecholamine receptors. However, several studies have implicated that the neuropeptide PACAP (pituitary adenylyl cyclase activating peptide) and its PAC1 receptor are critical regulators of SNS function under different types of stress^{2,3}. Our studies using genetically-modified mice indicate that selective loss of PAC1 receptors in catecholaminergic neurons results in significant alterations in inflammation and thermogenesis. In an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, we found that deletion of PAC1 receptors from the postganglionic neurons in the SNS lead to a deficiency in regulatory T-cell expansion during disease. Furthermore, there are changes in expression of transcription factors and cytokines in Th1 and Th17 CD4+ helper T-cells in the lymph nodes and spleen. These results indicate that PACAP plays a role in regulating inflammation during EAE through the PAC1 receptors in the SNS. In addition, deletion of PAC1 receptors from the catecholaminergic neurons in the SNS also increases expression of thermogenic genes during mild cold stress and prevents age-related lipid accumulation in the liver. These results indicate that PACAP also acts through the PAC1 receptor to regulate metabolism. These findings implicate PAC1 as a target for manipulate SNS function to correct pathological inflammation and dysregulated metabolic processes.

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P67. A peptidomic approach to characterize peptides involved in cerebellar development leads to the identification of the neurotrophic effects of nociceptin

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The cerebellum is a brain structure involved in motor and cognitive functions. The development of the cerebellar cortex (the external part of the cerebellum) is under the control of numerous factors. Among these factors, neuropeptides including PACAP or somatostatin modulate the survival, migration and/or differentiation of cerebellar granule cells. Interestingly, such peptides contributing to cerebellar ontogenesis usually exhibit a specific transient expression profile with a low abundance at birth, a high expression level during the developmental processes, which take place within the first two postnatal weeks in rodents, and a gradual decline toward adulthood. Thus, to identify new peptides transiently expressed in the cerebellum during development, rat cerebella were sampled from birth to adulthood, and analyzed by a semi-quantitative peptidomic approach. A total of 33 peptides were found to be expressed in the cerebellum. Among these 33 peptides, 8 had a clear differential expression pattern during development, 4 of them i.e. cerebellin 2, nociceptin, somatostatin and VGF [353-372], exhibiting a high expression level during the first two postnatal weeks followed by a significative decrease at adulthood. A focus on nociception, by a genomic approach, confirmed that the precursor mRNA is transiently expressed during the first week of life in granule neurons within the internal granule cell layer of the cerebellum, and showed that the nociceptin receptor is also actively expressed between P8 and P16 by the same neurons. Finally, functional studies revealed a new role for nociceptin, acting as a neurotrophic peptide able to promote the survival and differentiation of developing cerebellar granule neurons.

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P68. Habenula is necessary to promote expression of fear memory competing against a safety memory

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Although the habenula has been implicated in the regulation of emotional behaviors, it is not clear *when* it is necessary to regulate defensive responses to a threat (fear). During auditory fear conditioning, animals learn the tone-shock association, which leads to the formation of a fear memory. During extinction training, animals learn that the tone no longer predicts shock, which forms a “safety memory”. Thereby, it has been suggested that after extinction training, fear memory and safety memory coexist in the brain and compete for control of behavior. To evaluate the contribution of the habenula in the different phases of learned fear regulation, we performed pharmacological inactivations of the habenula at specific time points. On day 1, rats associated the presence of tones with foot shock delivery (fear learning). On day 2, rats learned to extinguish fear responses by presenting tones in the absence of foot shocks (safety learning). On day 3, rats were presented with tones alone to test for fear against safety memory retrieval. We found that habenula inactivation before fear acquisition or before extinction acquisition had no effect, indicating that this structure is not necessary for: fear learning, fear memory expression nor extinction learning. Surprisingly, however, we found that habenula inactivation before the memory retrieval test decreased fear responses, suggesting its role in regulating competing fear and safety memories. To further test the idea that habenula is necessary when fear and safety memories compete, we switched the order of fear and safety learning by using a latent inhibition protocol. Thus, on day 1, rats acquired safety learning (tones, no shocks). On day 2, rats acquired fear learning (tones paired with foot shocks). On day 3, rats were presented with tones alone to test for safety memory vs fear memory retrieval. Indeed, we found again that habenula inactivation before retrieval test of safety memory against fear memory decreased fear responses. Together our findings suggest that the habenula is necessary to promote the expression of fear memory when it competes against a safety memory.

Keywords: habenula, conditioning, extinction, latent inhibition

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P69. Sex chromosome complement (SCC) involvement in the sexually dimorphic vasopressinergic system

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Numerous studies indicate differences between males and females in the regulation of blood pressure and hydroelectrolyte balance control. This study aimed to explore the role of the sex chromosomes (XX/XY) and/or the organizational hormonal effects of gonadal steroids in the vasopressinergic sexual dimorphic response, analyzing the effect of desmopressin administration, a vasopressin V2 receptor (V2R) agonist, on urinary osmolarity and the blood pressure regulation in response to systemic vasopressin infusion. To carry out these experiments we used gonadectomized male (XX and XY) and female (XX and XY) mice of the "four core genotypes" model, in which the effect of gonadal sex and sex chromosome complement (SCC) is dissociated, allowing comparisons of sexually dimorphic traits among XX and XY females, as well as in XX and XY males.

Forty two days post gonadectomy, mice were subcutaneously injected with vehicle solution and desmopressin (1 mg / kg) and after a four hour period (with no access to water or food) urine samples were obtained for subsequent determination of osmolarity (Vapro Osmometer). The statistical analysis of desmopressin infusion showed a significant effect of the interaction of the treatment and sex factors { $F(1,35) = 5.0650$, $p = 0.03080$ }, since male mice showed irrespectively of the SCC (XX-male and XY-male mice) a significant increase in urinary osmolarity when compared to females (both XX-females and XY-females). Furthermore, in anesthetized gonadectomized transgenic mice we evaluated changes in blood pressure in response to a 30 minute vasopressin infusion (0,2 UI/ml, infusion volume 100 μ l). The blood pressure results reveal a SCC modulatory effect on vasopressinergic pressor response; regardless of sex (male or female) XX-SCC mice showed a greater increase in blood pressure when compared to XY-SCC mice (XY-male and XY-female).

In sum this evidence may indicate that in the absence of the activating hormonal effect, the organizational hormonal factor would define the sexually dimorphic urinary osmotic phenotype, while sex differences in the pressor response to vasopressin infusion may be driven by the sex chromosomal complement factor.

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P70. VPAC1 null mice are resistant to weight gain in a high fat diet-induced obesity model and have altered energy expenditure

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Introduction: Vasoactive intestinal peptide (VIP) and its VPACs receptors are expressed both centrally in the hypothalamus and peripherally in the GI tract, adipose and liver tissues. VIP and its VPACs receptors have been implicated in the development of obesity according to pathway-based genome-wide association studies. Our group has previously shown in VIP knockout mice (VIP^{-/-}) that VIP is a key regulator of body weight, mass composition and metabolic hormones. Other authors have observed that VIP^{-/-} and VPAC2^{-/-} mice have disrupted patterns of circadian feeding behavior resulting in an abolished regular nocturnal/diurnal feeding and lower total energy expenditure. However, the role of the VIP/VPAC1 pathway on energy intake/expenditure and metabolism has not been fully defined.

Aims: To investigate in VPAC1^{-/-} mice the effects of a 45% high fat inducing obesity diet on body phenotype, energy intake/expenditure and metabolic hormones.

Materials and Methods: VPAC1^{-/-} and wild type (WT) mice, age- and sex-matched, were fed either a 45% high fat diet (HFD) or an isocaloric standard diet (SD) for 12 weeks. Body weight and mass composition were assessed bi-weekly by using an EchoMRI system for rodents. At the end of the study, energy intake and expenditure were analyzed using a Promethion metabolic system for mice. Plasma levels of active-ghrelin, GLP-1, PYY, insulin, glucagon, and leptin were measured in fasting and postprandial conditions in all experimental animals.

Results: HFD-fed VPAC1^{-/-} mice had lower body weight and fat mass accumulation and higher lean mass, starting at week 8 until the end of the 12 week diet study, in such a way showing a significant resistance to HFD induced obesity as compared to HFD-fed WT mice. Also, VPAC1^{-/-} mice had a significantly reduced energy expenditure rate with lower levels of VO₂, VCO₂, RQ and TEE (total energy expenditure) during both dark and light phase. In addition, VPAC1^{-/-} mice, fed either HFD or SD, had higher fasting and postprandial levels of GLP1^{-/-}, glucagon and PYY, however only HFD-fed VPAC1^{-/-} had lower levels of leptin in both fasting and postprandial conditions.

Conclusions: VPAC1^{-/-} mice fed a 45% HFD for 12 weeks showed a significant resistance to weight gain and fat mass accumulation and no alteration in energy intake, but a significant reduction in energy expenditure compared to HFD-fed WT controls. These metabolic changes observed in VPAC1^{-/-} mice were associated with increased plasma levels of GLP-1, glucagon, leptin, and PYY. Therefore, we conclude that the VIP/VPAC1 pathway is critical for the regulation of body mass phenotype and metabolism and could be a potential target for a future treatment of obesity.

P71. Vasoactive Intestinal Peptide (VIP) regulates adipogenesis through PPAR γ , C/EBP α , C/EBP β , C/EBP δ and SIRT1 expression

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Introduction: Vasoactive intestinal peptide (VIP) is a 28-amino acid neuropeptide that is expressed centrally in the hypothalamus and peripherally in the GI tract and adipose tissue. VIP knockout (-/-) mice have lower body weight, mass composition and metabolic hormone levels. Furthermore, VIP and its VPAC1 receptors have been implicated in the development of obesity by pathway-based genome-wide association studies. Obesity is a condition characterized by excessive fat accumulation in the adipose tissue that results from an altered energy balance. Therefore, this study is aimed at defining the molecular mechanisms linking VIP and VPAC1R to the regulation of body metabolism and adipogenesis.

Materials and Methods: Male VIP^{-/-} (n=8) and WT mice (n=8), (6-8 weeks of age, backcrossed >12 generations to C57BL/6J mice) were genotyped and fed either a 45% high fat diet (HFD) or a standard diet (SD) for 12 weeks. At the end of the study, epididimal fat depots were extracted from each experimental animal and the adipose tissue morphology was examined. VIP's role in adipogenesis was studied in NIH3T3-L1 preadipocytes by inducing differentiation into mature cells in the presence or absence of the VIP antagonist (VIP-Hybrid) in a dose-dependent manner. Cells were harvested at the end of the study for Oil-o-red (ORO) staining, RNA extraction, qRT-PCR and Western-blot analysis. Finally, VIP and VPAC1R correlation to adipogenic markers was studied by using the METSIM, a cross-sectional and follow-up study design including deep phenotyping of 10,197 healthy Finnish men, collecting subcutaneous adipose tissue samples from 770 participants and using global expression arrays. To analyze VIP and VPAC1R correlation across adipogenic markers, heatmaps and gene-by-gene correlations were constructed using the bicor coefficient of the VIP and VPAC1R genes in the METSIM population and the top 50 transcripts from adipose tissue expression arrays.

Results: In 3T3-L1 pre-adipocytes, the expression of VIP and VPAC1R was shown and quantified by qRT-PCR. VIP stimulation of 3T3-L1 pre-adipocytes dose-dependently increased cAMP responses. VIP-Hybrid dose-dependently inhibited adipogenesis, as quantified by ORO staining and gene expression of PPAR γ , C/EBP α , C/EBP β , C/EBP δ , while increasing SIRT1 expression in a time-dependent manner as demonstrated by qRT-PCR and protein analysis by Western-blotting. The METSIM database showed positive correlations between VIP, VPAC1R and key genetic drivers of adipogenesis. Scatterplots of the most statistically significant gene versus gene correlations demonstrated a positive association between the expression of VIP, VPAC1R and integral genetic drivers of adipogenesis: PPAR γ (p= 1.0 x 10⁻¹¹), C/EBP α (p= 1.5 x10⁻⁵), C/EBP γ (p= 3.4 x10⁻⁶), and LXR α (p= 6.8 x10⁻⁴).

Conclusions: VIP binding to its VPAC1R on adipocytes upregulated adipogenic transcription factors and activators of PPAR γ , as shown in mouse by qRT-PCR and Western blotting, and in human by METSIM studies. The VIP antagonist, VIP-Hybrid, at the initial stage of adipogenesis inhibited the activation of C/EBP β and C/EBP δ thus suppressing PPAR γ and C/EBP α expression, and thereby suggesting a positive feedback loop with these two transcription factors that can activate the full mechanism of adipogenesis. Our data demonstrate that the VIP/VPAC1R pathway is very significantly involved in the activation of adipogenesis, thus indicating VIP and VPAC1R as novel key targets for the development of future therapeutic strategies to reduce obesity.

P72. The molecular features of the mechanisms of biological action of certain regulatory peptides

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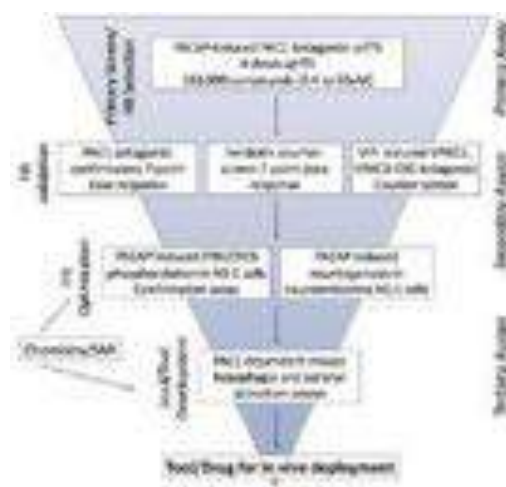
Regulatory peptides belong to a unique class of universal chemical regulators, whose influence extends from the control of cell functioning stability to the management of entire systems and organs, including behavior and cognitivity. Certain pharmacologically important peptides as Semax, Selank and Progylprol have long been used in medicine as therapeutic and prophylactic agents, but the detailed description of their action mechanism does not exist. The key points of molecular mechanism may conceivably be a proteolysis of peptide, which leads to the formation of tissue-specific synacton (biologically active putative products of N- and C-terminal proteolysis of the initial peptide), as well as specific interactions of peptides on brain target cell plasma membranes. Understanding the molecular aspects of the above processes will not only reveal the basics of the biological activity of synthetic neuropeptides, but also approach the understanding of the mechanism of regulatory peptides in general. Based on the method of radioligand receptor analysis, a system for assessing the molecular-functional activity of pharmacologically important peptides was developed. The influence of separate regulatory peptides on specific interactions of labeled analogs of endogenous effector molecules with their targets on plasma membranes of rat brain cells, as well as the joint action of molecules in the peptide + non-peptide allosteric modulator system was characterized. The modulatory effect of pico - and nano-molar concentrations of the peptides was demonstrated. The obtained results suggest that the neuropeptides Semax, Selank and Progylprol have an important role in the occurrence of direct and indirect changes in the basic parameters of the specific ligand-receptor interactions of endogenous neuroregulation systems in the brain. The peptides we studied are apparently allosteric modulators of wide range of neuroreceptors. So, the intermolecular interactions of these peptides with plasma membranes of neuronal brain targets are probably not limited by specific binding at their orthosteric sites. The spectrum of possible specific interactions of Selank, Semax and Progylprol with their targets on plasma membranes of nerve cells is dose-dependent and may also include the modulation of receptors of other types. This work was supported by the Program of Basic Research of Presidium of RAS "Molecular and cell biology and post-genomic technologies".

P73. Development of *in cellula* assays for screening of biased and unbiased PACAP antagonists for neuronal cells

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Specific antagonists and agonists at the PAC1 receptor for the neuropeptide PACAP are sought as tools for drug discovery as well as potential therapeutic agents, given the importance of PACAP in the pathophysiology of migraine, depression, PTSD, ischemic injury, and atherosclerosis. As some of these potential targets are neuronal, we have developed a set of cell-based assays that reflect specificity of signaling through the PAC1 receptor in neuroendocrine cells. The schematic below illustrates the development of a HEK293 cell line expressing the human PAC1 receptor at physiologically meaningful levels, and a split-luciferase-based luminescent detection system for cyclic AMP elevation and its use in screening large compound libraries for their ability to inhibit PACAP-induced cAMP elevation. We counter-screened selected compounds for their ability to differentially affect PACAP signaling through the three cAMP effectors that are activated by PACAP engagement of the PAC1 receptor (PKA, Epac and NCS-Rapgef2) in neuroendocrine (NS-1) cells. The ability of various compounds, including newly developed PAC1 antagonists reported in the literature, are compared to screened compounds for their selectivity for PAC1 activation by PACAP and VIP, compared to VPAC1 and VPAC2 activation by PACAP and VIP. Implications for the development of neuron-specific compounds acting at PAC1 will be considered.



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P74. Vasopressin is produced in lung and participates in immunopathology during experimental pulmonary tuberculosis.

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Background: Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), produces approximately 1.5 million human deaths per year worldwide. An important component of the mechanisms underlying this disease is the immunoendocrine response of the host, which, if inappropriate, will generate immunopathology, lung damage and contribute to death. Several signaling molecules such as cytokines, hormones and neuropeptides are involved in the modulation of the response against the bacillus. However, when the infection persists, these same molecules cause tissue damage. Vasopressin (AVP), a hypothalamic osmoregulatory and stress-related peptide, is also a regulator of peripheral inflammation, apoptosis and the process of tissue healing. There is evidence of inappropriate production of AVP in individuals with active TB, however, the association between this finding and its relationship with pathogenesis had not been studied.

Materials and methods: Male Balb/c mice were infected intratracheally with *Mycobacterium tuberculosis* of strain type H37Rv, to study the kinetics of synthesis of AVP and its receptors during the disease. Subsequently, groups of mice infected in the same way were treated with the synthetic agonist desmopressin (DdAVP) and the non-selective antagonist Conivaptan (CVP). To further investigate the role of AVP in phagocytosis of Mtb, infected or non-infected murine alveolar macrophages (MH-S) were treated with different doses of desmopressin and CVP. Unity forming units were counted 1 hour and one day post-infection.

Results: The infected mice developed active disease. In the hypothalamus, the amount of AVP mRNA progressively increased during the first 21 days of the disease, to subsequently decrease by day 60 after infection. In the lungs, the presence of AVP was detected by immunohistochemistry in granulomas and foamy macrophages in the late phase of the disease, which coincided with the amount of mRNA in the lung, suggesting its local production, independent of the hypothalamus. On the other hand, at the transcriptional level, the expression of the V2 receptor in the lung decreased at the end of the disease. Treatment with the DdAVP agonist resulted in a greater number of bacilli in the lungs of infected mice, in addition to causing a significant increase in pulmonary fibrosis. In contrast, blockade of the receptors with the CVP antagonist resulted in a reduction of the bacillary load. Finally, studies in cell culture showed that DdAVP affects macrophages in their bacillary elimination capacity.

Conclusions: Together, these results confirm the production of AVP in the lung during the course of experimental Tb mainly by macrophages. Results of pharmacological manipulation suggest that AVP has a local anti-inflammatory/immunosuppressive effect and promotes healing in the lung. When the concentration of AVP is excessive, this effect is deleterious because it favors bacterial growth and fibrosis. Thus, modulating vasopressin activity could represent an endocrine-immunotherapy strategy in active TB.

P75. Reconstructing the hypothalamo-neurohypophysis connections

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The hypothalamo-neurohypophysis (HP-NH) constitutes one of the most important components in the neuroendocrine system. It is well-known that the hypothalamic magnocellular neurons in the paraventricular hypothalamic nucleus (PVH), supraoptic nucleus (SON) project to the NH, where they release vasopressin (AVP) and oxytocin (OXT) into the bloodstream. Although studies have shown that scattered magnocellular neurons in other hypothalamic areas also project to the NH, the detailed microanatomy of the HP-NH connection is incomplete. Using recently developed virus-based retrograde (AAV-Retro-GFP) and anterograde (AAV-GFP) tracing tools, we systematically reconstructed the comprehensive rat atlas of the neuroendocrine system originating from the HP and projecting to the NH. Bidirectional tracing revealed that multiple hypothalamic regions including the lateral hypothalamus area (LHA), the bed nucleus of the stria terminalis (BST), the preoptic area (POA) and several accessory nuclei (AN) directly project to the NH. These neurons were also labelled by peripherally administered Fluoro-Gold, which was selectively taken up by neurons projecting beyond the blood brain barrier, verifying their neuroendocrine features. The heterogeneity of the nuclei was revealed by distinctive soma volume distribution patterns and further confirmed by immunostaining analysis with AVP and OXT antibodies. The neuropeptide identity was further investigated with fluorescent *in situ* hybridization (FISH). Quantification showed the AN accounted for more than one third of the total magnocellular population, consisting of the nucleus of anterior commissure (AC), the anterior fornix nucleus (AF), the circular nucleus (CiN), the posterior fornix nucleus (PoF), the nucleus of medial forebrain bundle (Nmfb) and the retrochiasmatic part of supraoptic nucleus (RCN). We also traced the fiber projection of single cells with Amira software to illustrate a complete HP-NP network in a three-dimensional atlas. Together, our study provides a mesoscopic level mapping of the HP-NH connections and new insights into further understanding the neuroendocrine regulation.

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P76. A new approach for pancreatic cancer biomarker discovery using the peptidome

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Pancreatic cancer is expected to become the second leading cause of cancer-related death by 2030. More than 8 out of 10 exocrine pancreatic cancers are adenocarcinomas. Nearly all of these (90%) are pancreatic ductal adenocarcinoma (PDAC) with a 5-year survival rate of 5-8%. PDAC is the only cancer that has not seen an improvement in mortality figures for both men and women during the past 20 years. Because of lack of early diagnostic biomarkers, the majority of patients of PDAC are diagnosed at advanced-stage, either locally advanced cancer (30%) with a median survival of 6-10 months, or metastasis (primarily to liver and lung)(50%) with 3-5 months survival. Only a minority (10-15%) of patients with localized tumor can undergo potentially curative surgical resection. Recently, the circulatory proteome, termed the “peptidome”, has been developed (1-3). The aim of the present study was to develop a novel approach for PDAC biomarker discovery using the peptidome. The peptidome was identified by mass spectrometry (electrospray ionization). The study design was *i*) determination of peptides in the cultivated media of PDAC cells which were derived from a genetically engineered mouse model of PDAC (KrasG12D;Pdx1-Cre); and *ii*) determination of peptides in the sera of mice that were implanted orthotopically (in pancreas) with the PDAC cells. We found 152 peptides in the cultivated media and 126 peptides in the sera. Based on sequence annotation, the identified proteins are involved in signaling pathways in angiogenesis, neurogenesis, inflammation, acute phase response signaling, LXR/RXR activation, FXR/RXR activation, intrinsic and extrinsic prothrombin activation pathway, antigen presentation pathway. Furthermore, we found many unique peptides/proteins, e.g. talin-2, NALP2, eukaryotic translation initiation factor 3 subunit A, cadherin-6, prosaposin, and fibulin-2. Currently, we plan to verify these peptidome markers in patient-derived xenograft mouse model of PDAC and in patients with PDAC (comparison between before and after resection of total tumor). In conclusion, measuring panels of peptidome markers might be a potential approach for early diagnosis of PDAC.

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P77. Median eminence thyrotropin releasing hormone-degrading ectoenzyme controls serum thyrotropin concentration

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The median eminence is an interface between the general circulation and the hypothalamus, a portion of the brain that controls energy intake and expenditure, stress responses, growth and reproductive behavior. Lining the floor of the third ventricle a group of specialized glial cells, $\beta 2$ tanycytes, with long apical processes directed to the portal vessels that connects the median eminence to the anterior pituitary, are critical in the regulation of the hypothalamus-pituitary-thyroid (HPT) axis. They express high levels of thyroid hormone (TH) transporters, deiodinase II, and the thyrotropin-releasing hormone (TRH)-degrading ectoenzyme (TRH-DE). Since $\beta 2$ tanycyte processes establish synaptoid contacts with terminals of hypophysiotrophic neurons that release TRH, and median eminence TRH-DE activity is regulated by TRH, TH and fasting, TRH-DE from $\beta 2$ tanycytes may regulate TRH concentration before its entrance into portal vessels, and thus thyrotropin (TSH) secretion from the anterior pituitary. Consistent with this hypothesis, the systemic injection of a TRH-DE inhibitor enhances cold-induced serum TSH concentration; however, this effect could also be attributed to inhibition of TRH-DE activity in other brain regions, or in the anterior pituitary, or in blood; notably, both anterior pituitary and blood TRH-DE activities are also up-regulated by TH.

To test the hypothesis that TRH-DE from $\beta 2$ tanycytes controls TSH secretion, we developed an experimental strategy to modify specifically $\beta 2$ tanycyte TRH-DE activity *in vivo*. Serotype-1 adeno-associated virus (AAV1) expressing GFP injected into the third ventricle of male adult rats transduce median eminence tanycytes but do not affect TSH secretion. Median eminence TRH-DE activity was raised in rats that received an AAV1 expressing TRH-DE, two or three weeks after virus administration. Overexpression of TRH-DE in the median eminence decreased serum TSH concentration and enhanced food intake. In another experiment we used the truncated isoform of TRH-DE (TRH-DE*), a dominant negative isoform that reduces TRH-DE activity. The injection of AAV1 expressing TRH-DE* tended to decrease median eminence TRH-DE activity, increased serum TSH concentration and lowered food intake, two weeks after virus administration. Injection of AAV1 expressing TRH-DE or TRH-DE* didn't change body and fat weights, serum TH, prolactin or growth hormone concentrations, nor the activity of the anterior pituitary or circulating form of TRH-DE. These observations support the hypothesis that after release from hypophysiotrophic neurons, TRH concentration is controlled by $\beta 2$ -tanycyte TRH-DE activity, which limits its entrance into the portal vessels and thus regulates TSH release from the anterior pituitary. Thus, in addition to the activity of the hypophysiotrophic TRH neurons, the post-secretory hydrolysis of TRH by $\beta 2$ -tanycyte TRH-DE likely shapes, in response to median eminence clues, TSH secretion.

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