NASCE

SEVENTH BIENNIAL MEETING OF THE NORTH AMERICAN SOCIETY FOR COMPARATIVE ENDOCRINOLOGY

MAY 28– JUNE 1, 2023

Hotel Mision Grand Juriquilla
Juriquilla, Santiago de Queretaro, Mexico

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President's Welcome to the Seventh Meeting of the North American Society for Comparative Endocrinology (NASCE 2023)

We are very honored to welcome you to the Seventh Biennial Conference of the North American Society for Comparative Endocrinology (NASCE 2023), held in Querétaro, México, from May 28 to June 1, 2023. Thanks to the hard work of program committee and the local organizing committee, we have an outstanding program in an excellent venue.

The program covers a wide range of topics in comparative endocrinology, including both invertebrates and vertebrates, with about 50 invited speakers and 40 speakers selected from submitted abstracts, many of whom are trainees and junior investigators. In addition, we have 6 plenary speakers and the Gorbman-Bern Memorial Lecture, given by Dr. Vance Trudeau.

NASCE has flourished since its founding in 2010. We owe our success to the hard work of current and former members of the council, various committees, particular the program and local organizing committees of the biennial meetings, and most importantly, the enduring support from you, our members. To honor the contributions of our members who have made significant contributions to the advancement of general and comparative endocrinology, the NASCE council approved the establishment of NASCE Fellow award in 2022. We will introduce and honor our inaugural and new NASCE Fellows elected in 2022 at this biennial meeting. We encourage all of you to nominate your deserving colleagues for this honor when the next election cycle opens.

This is our first in-person meeting since the start of the long lasting Covid-19 pandemic. We sincerely appreciate your participation. We look forward to meeting you all in Querétaro and hope that you will have a safe, enjoyable, and successful meeting.

Yun-Bo Shi
President, NASCE

LOC's Welcome

The Local Organizing Committee (LOC) warmly welcomes you to the Seventh Meeting of the North American Society for Comparative Endocrinology (NASCE 2023) in Mexico. This is the second time that our institution, the Universidad Nacional Autónoma de México (UNAM), with the support of the Institute of Neurobiology, has the honor of hosting this meeting in the lovely colonial city of Querétaro.

As you recall, the 6th Biennial meeting was originally planned to be held in 2021 in Mexico, but we were forced to run the conference in a virtual format due to the worldwide health security issues imposed by COVID-19 pandemic. We are very excited to meet in person again and to take the fantastic opportunity created by NASCE to grow closer ties between Canada, Mexico and the United States of America through science, technology, culture, and higher education and to continue developing original knowledge and contribute to the formation of the new generations of scientists in the field of comparative endocrinology.

NASCE 2023 includes a thorough scientific program with over 140 presentations from colleagues representing 15 countries. We have no doubt that this will be an excellent opportunity to re-encounter and cultivate old friendships, build up new acquaintances, exchange ideas, discuss results, envision new projects, and promote future interactions to strengthen ties within our vibrant comparative endocrinology community.

So, let us celebrate and enjoy the meeting. Bienvenidos!!

Aurea Orozco, Maricela Luna and Carlos Arámburo
Co-Chairs of the Local Organizing Committee, NASCE 2023.

About the venue

Querétaro is of the most important cities in the country due to its state-of-the-art infrastructure and economic development, but one that embraces its past and proudly shows it to the world. Its Historic Center was founded in 1531 has been a UNESCO World Heritage Site since 1996. In this occasion, the LOC has prepared a tour to the magical town of Bernal in the cultural/social program. This town is part of the "Art, Cheese and Wine Route" of Querétaro and lies at the foot of la Peña, the third largest monolith in the world, after "Peñón de Gibraltar" in Spain and "Pão de Açúcar" in Brazil. La Peña is considered a cosmic symbol of ancient ceremonies and Bernal a small town with beautiful doorways, irregular cobbled streets, inns, restaurants, handicrafts, museums, and colorful houses.

Let yourself be captivated by the magical town of Bernal!

NASCE 2023 Committees

Officers and Council of the North American Society for Comparative Endocrinology (NASCE; La Societé Nord-Americaine d'Endocrinologie Comparée; La Sociedad Norteamericana de Endocrinología Comparada) 2021-2023.

Officers

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NASCE 2023 Program Committee (PC)

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Chair of the NASCE2023 Scientific Program Committee
Vice-President, North American Society for Comparative Endocrinology (NASCE)
Yun-Bo Shi (NICHD, National Institutes of Health (NIH)
President, North American Society for Comparative Endocrinology (NASCE)
José Ávila (Univerisdad Nacional Autónoma de México)
Robert Dores (University of Denver)
Cunming Duan (University of Michigan)
Caren Helbing (University of Victoria)
Andreas Heyland (University of Guelph)
Aurea Orozco Rivas (Universidad Nacional Autónoma de México)
Chair of the NASCE2023 Local Organizing Committee

Awards Committee

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Maricela Luna, UNAM, México. Co-Chair
Carlos Arámburo, UNAM, México. Co-Chair
José Ávila-Mendoza, UNAM, México
Iván Lazcano Sánchez,UNAM, México
Aurora Olvera Vidal, UNAM, México
Santiago Pech Pool, UNAM, México
Rebeca Corona García-Cabral, UNAM, México

María Teresa Morales Guzmán, UNAM, México
J. Elías Pacheco Hernández, UNAM, México
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Anaid Antaramian Salas, UNAM, México
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Universidad Nacional Autónoma de México (UNAM)

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Coordinación de la Investigación Científica (CIC)

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Consejo de Ciencia y Tecnología del Estado de Querétaro (CONCYTEQ)

Secretaría de Turismo del Estado de Querétaro

CIPQUIM
Proveedor de Anticuerpos e Insumos de Alta Tecnología para Investigación Científica en General

Meeting Highlights

Plenary Lectures

PL1. Joëlle Rüegg, Uppsala University, Sweden
Impacts of Endocrine Disruptors on the Developing Brain: from Molecular Insights to Novel Test Methods
PL2. Hugo A Barrera-Saldaña, Universidad Autónoma de Nuevo León, México
The lives of growth hormone: news from the past, stories with future.
PL3. David Parichy, University of Virginia, USA
Thyroid Hormone Dependence of Adult Phenotype in Zebrafish
PL4. Patricia Pietrantonio, Texas A&M University, USA.
Comparative endocrinology of bloody vectors towards useful outcomes: probing GPCRS from multifunctional neuropeptides in ticks and mosquitoes
PL5. William Bendena, Queen's University, Canada
Uncovering roles for allatostatin-like receptors in *Caenorhabditis elegans*
PL6. De-Shou Wang, Southwest University, Chongqing, China
Molecular mechanism analysis of sex determination and differentiation of tilapia based on genome editing.
-Gorbman- Bern Memorial Award Lecture. **Vance Trudeau**, University of Ottawa, Canada
Neuroendocrine control of reproduction: is it love or is it just ovulation?

Symposia

S1. State-of-the-art in invertebrate comparative endocrinology
S2. Development of sex in animals
S3. Nothing to stress about: Endocrine and oxidative responses to diet
S4. Endocrinological changes induced by feeding
S5. Thyroid hormone and vertebrate development
S6. Comparative endocrinology of osmoregulation
S7. Growth hormones: from physiological roles to clinical science
S8. Role of the endocrine system in ecological tradeoffs
S9. Trends in the evolution of hormone receptors
S10. Insulin/Insulin-like growth factor peptides
S11. Regulation of salinity tolerance in amphibians
S12. Hormonal control of gonadal development: from biological sex to reproduction
S13. Estrogenic, Androgenic, Thyroidal, and Steroid biosynthetic (EATS) and non-EATS Research Advancements and Testing
S14. Evolution of neuropeptide and hormonal signaling systems
S15. Hormone, Behavior and Reproduction
S16. Hormonal control of regeneration in vertebrates
S17. Emerging topics in comparative endocrinology 1
S18. Emerging topics in comparative endocrinology 2

Panels and Workshops

P1. -Women in Science Panel-

Instructions for NASCE Presentations

1) PLENARIES AND GORBMAN-BERN AWARD LECTURES:

Please prepare your presentation **(45 min presentation and 15 min of questions)** in PowerPoint and send it to the following drive 24 h before your talk: https://drive.google.com/drive/folders/1sogtvHEoiOpTaHSPFVeikM5PsCet5z2Z?usp=sharing.
Please name your file as follows: plenary number_last name
There will be a Speaker Ready Room for you to preview your presentation. Assistants will be on hand to help you.

2) INVITED SYMPOSIUM SPEAKERS

Please prepare your presentation **(15 min presentation and 5 min of questions)** in PowerPoint and send it to the following drive 24 h before your talk. https://drive.google.com/drive/folders/1sogtvHEoiOpTaHSPFVeikM5PsCet5z2Z?usp=sharing:
Please name your file as follows: symposium number_last name
There will be a Speaker Ready Room for you to preview your presentation. Assistants will be on hand to help you.

3) ORAL PRESENTATIONS:

Please prepare your presentation **(10 min presentation and 5 min of questions)** in PowerPoint and send it to the following drive 24 h before your talk: https://drive.google.com/drive/folders/1sogtvHEoiOpTaHSPFVeikM5PsCet5z2Z?usp=sharing:
Please name your file as follows: symposium number_last name
There will be a Speaker Ready Room for you to preview your presentation. Assistants will be on hand to help you.

FOR ALL SPEAKERS: Unfortunately, we cannot allow speakers to present using their own laptops unless there is a compelling reason to do so (e.g. showing movies that only work on Mac) and prior approval has been requested.

4) POSTER PRESENTATIONS:

Poster boards for NASCE 2023 are **170 cm WIDE x 120 cm HIGH (5.57w x 3.9h ft).** Posters must fit within this space. Poster can be displayed in portrait or landscape. Pushpins will be provided. You do not need to include the poster number on your poster. All posters should be set up on display on May 29 and will remain for view.

28th May	29th May	30th May	31th May	1th June
Registration (9:00 - 14:30)	Registration (8:00 - 9:00)	Registration (8:00 - 9:00)	Registration (8:00 - 9:00)	Registration (8:00 - 9:00)
	Plenary 2 Hugo A Barrera-Saldaña (9:00 - 10:00)	Plenary 4 Patricia Pietrantonio (9:00 - 10:00)	Plenary 5 William Bendena (9:00 - 10:00)	Plenary 6 De-Shou Wang (9:00 - 10:00)
	Coffee Break(10:00 - 10:30)	Coffee Break(10:00 - 10:30)	Coffee Break (10:00 - 10:30)	Coffee Break (10:00 - 10:30)
	Symposium 1 State-of-the-art in invertebrate comparative endocrinology. (10:30 - 12:00) / Symposium 2 Development of sex in animals. (10:30 - 12:00) / Symposium 3 Nothing to stress about: Endocrine and oxidative responses to diet. (10:30 - 12:00)	Symposium 7 Growth hormones: from physiological roles to clinical science. (10:30 - 12:00) / Symposium 8 Role of the endocrine system in ecological tradeoffs. (10:30 - 12:00) / Symposium 9 Trends in the evolution of hormone receptors. (10:30 - 12:00)	Symposium 13 Estrogenic, androgenic, thyroidal, and steroid biosynthetic (EATS) and non-EATS research advancements and testing. (10:30 - 12:00) / Symposium 14 Evolution of neuropeptide and hormonal signaling systems. (10:30 - 12:00) / Symposium 15 Hormones, behavior and reproduction. (10:30 - 12:00)	Symposium 16 Hormonal control of regeneration in vertebrates. (10:30 - 12:00) / Symposium 17 Emerging topics in comparative endocrinology I. (10:30 - 12:00) / Symposium 18 Emerging topics comparative endocrinology II. (10:30 - 12:00)
	Coffee Break (12:00 - 12:30)	Coffee Break (12:00 - 12:30)		Coffee Break (12:00 - 12:30)
	Plenary 3 David Parichy (12:30 - 13:30)	Gorbman- Bern Lecture (12:30 - 13:30)		Women in Science (12:30 - 13:30)
	Lunch (13:30 - 14:30)	Lunch (13:30 - 14:30)		Lunch (13:30 - 14:30)
NASCE Council Meeting 1 (13:30 - 15:30)	Symposium 4 Endocrinological changes induced by feeding. (14:30 - 16:00) / Symposium 5 Thyroid hormone and vertebrate development. (14:30 - 16:00) / Symposium 6 Comparative endocrinology of osmoregulation. (14:30 - 16:00)	Symposium 10 Insulin/Insulin-like growth factor peptides. (14:30 - 16:00) / Symposium 11 Regulation of salinity tolerance in amphibians. (14:30 - 16:00) / Symposium 12 Hormonal control of gonadal development: from biological sex to reproduction. (14:30 - 16:00)	Excursion/free afternoon (12:00 - 18:00)	NASCE Council Meeting 2 (14:30 - 16:30)
Opening Ceremony (16:00 -16:30)				
Plenary 1 Joëlle Rüegg (16:30 -17:30)	Poster Session 1 (16:00 - 18:00)	Poster Session 2 (16:00 - 18:00)		
Welcome Reception (17:30 - 19:30)				Closing Ceremony and Student Awards Presentation (18:00 - 19:00)
				Closing Banquet 19:00...

NASCE 2023 Meeting Schedule

Sunday, May 28th

-Registration-
Misión Juriquilla Hotel
Lobby
(Starting at 8:00)

-NASCE Council Meeting 1-
(13:30 - 15:30)
Salón Gobernador-Misión Juriquilla Hotel

-NASCE 2023 Opening Ceremony-
(16:00 - 16:30)
Cultural and Academic Center (CAC)
Auditorium "Dr. Flavio M. Mena Jara "
Campus UNAM Juriquilla

-Plenary Session 1-
Joëlle Rüegg
Uppsala University, Sweden
IMPACTS OF ENDOCRINE DISRUPTORS ON THE DEVELOPING BRAIN: FROM MOLECULAR INSIGHTS TO NOVEL TEST METHODS
(16:30 - 17:30)
CAC, Campus UNAM Juriquilla

-Welcome Reception-
(17:30 - 19:30)
Instituto de Neurobiología
Campus UNAM Juriquilla

Monday, May 29th

-Registration-
Misión Juriquilla Hotel
Lobby
(Starting at 8:00)

-Plenary 2-
Hugo A. Barrera-Saldaña
Universidad Autónoma de Nuevo León, México
THE LIVES OF GROWTH HORMONE: NEWS FROM THE PAST, STORIES WITH FUTURE
Salón Juárez- Misión Juriquilla Hotel
(9:00 - 10:00)

-Coffee Break-
(10:00 - 10:30)

-Morning Symposia (S1-S3)-
(10:30 - 12:00)

	Symposium 1 State-of-the-art in invertebrate comparative endocrinology Co-Chairs: **Jean-Paul Paluzzi and Meet Zandawala** *-Salón Juárez-*		**Symposium 2** Development of sex in animals Co-Chairs: **Chun Peng and Yong Zhu** *-Claustro II-*		**Symposium 3** Nothing to stress about: Endocrine and oxidative responses to diet Co-Chairs: **Pierre Deviche and Karen Sweazea** *-Claustro III-*
Symposium Speakers					
S1-1	**Luis Yanez Guerra** *Reconstructing the evolution of neuropeptide signaling in non-bilaterian metazoa*	**S2-1**	**Kataaki Okubo** *Sex steroid regulation of sexually dimorphic behavioral responses to conspecifics in medaka*	**S3-1**	**Clara Cooper-Mullin** *How birds during migration maintain (oxidative) balance*
S1-2	**Farwa Sajadi** *An anti-diuretic signaling system in the female mosquito, Aedes aegypti*	**S2-2**	**Bon-chu Chung** *Single-cell analysis of zebrafish germ cell lineage and sex development*	**S3-2**	**Jose Pablo Vazquez-Medina** *Prolonged fasting stimulates endogenous antioxidant production in elephant seals*
S1-3	**Meet Zandawala** *Visualizing context-dependent modulation in-vivo using TANGO-MAP MkII neuropeptide activity sensors*	**S2-3**	**Wei Ge** *Genetic analysis for roles of gonadotropins in zebrafish sex differentiation, gonadal development, and function*	**S3-3**	**Karen Sweazea** *Acute metformin induces hyperglycemia in healthy adult mourning doves, Zenaida macroura?*
Oral Presentations					
OR1-1	**Areej N. Al-Dailami** *Glycoprotein hormone (GPA2/GPB5) and corticotropin-releasing factor (CRF) signaling are involved in reproduction in adult female Rhodnius prolixus*	**OR2-1**	**John Postlethwait** *Gonadal development in zebrafish with chromosomal sex determination*	**OR3-1**	**Juan R. Riesgo-Escovar** *Moderate activation of NRF2 in insulin-signaling impaired Drosophila melanogaster improves resistance to oxidative stress*
OR1-2	**Elias Taylor** *Thyroid Hormones Signaling Mechanisms in Sea Urchins and Other Echinoderms*	**OR2-2**	**Yong Zhu** *Adamts9 modulates gonad sex in juvenile zebrafish*	**OR3-2**	**Elizabeth Brammer-Robbins** *Do healthy manatees from a poor-quality habitat exhibit evidence physiological of stress?*

-Coffee Break-
(12:00 - 12:30)

-Plenary 3-
David Parichy
University of Virginia, USA
THYROID HORMONE DEPENDENCE OF ADULT PHENOTYPE IN ZEBRAFISH
Salón Juárez- Misión Juriquilla Hotel
(12:30 - 13:30)

-Lunch-
Misión Juriquilla Hotel
(13:30 - 14:30)

-Afternoon Symposia (S4-S6)-
(14:30 - 16:00)

Symposium 4 Endocrinological changes induced by feeding Co-Chairs: **Ian Orchard and Angela Lange** *-Salón Juárez-*		**Symposium 5** Thyroid hormone and vertebrate development. Co-Chairs: **Yun-bo Shi and Aurea Orozco Rivas** *-Claustro II-*		**Symposium 6** Comparative endocrinology of osmoregulation. Co-Chairs: **Jason P. Breves and Stephen D. McCormick** *-Claustro III-*	
Symposium Speakers					
S4-1	**Mark Sheridan** *Integration of feeding with the control of growth and metabolism: insights from studies in fish*	**S5-1**	**Sheue-Yann Cheng** *Thyroid Hormone Receptor alpha 1 in uterus functions*	**S6-1**	**Nicholas Bernier** *Central and peripheral contributions of the corticotropin-releasing factor (CRF) system to smoltification and seawater acclimation in Atlantic salmon*
S4-2	**Jimena Leyria** *Insulin like peptides as multitaskers for successful reproduction in Rhodnius prolixus, a vector of chagas disease*	**S5-2**	**Yuki Shibata** *Genetic analysis of thyroid hormone regulation of intestinal remodeling in Xenopus*	**S6-2**	**Ningping Gong** *Strategies of anadromous sea lamprey for coping with salinity challenges by regulating hormone signaling systems and ion-transporters in the gill*
S4-3	**Monika Gulia-Nuss** *Regulation of blood digestion in lyme disease vector tick, Ixodes scapularis*	**S5-3**	**Laurent Sachs** *Thyroid hormone and glucocorticoid effects on Transcriptome and Methylome in Xenopus tropicalis tadpoles*	**S6-3**	**Andre Seale** *The prolactin cell as a nexus for the integration of osmotic and thermal sensory modalities?*
Oral Presentations					
OR4-1	**Valeria Urban** *Effects of fasting upon the somatotropic axis and metabolism in the green iguana*	**OR5-1**	**Hui Zhao** *Disruption of thyroid hormone receptor alpha a (THRaa) promotes heart regeneration in zebrafish*	**OR6-1**	**Britney Picinic** *The influence of diuretic and anti-diuretic factors on aquaporin expression in female A. aegypti*
OR4-2	**Dina Iathzil Vázquez Carrillo** *Sulpiride reduces hyperglycemia and insulin resistance in diet-induced obese female and male mice*	**OR5-2**	**Aurora Olvera Vidal** *Specific temporal windows of T3 action in oligodendrogenesis. Lessons from the zebrafish.*	**OR6-2**	**Ciaran Shaughness** *Regulation of the hypothalamus-pituitary-interrenal (HPI) axis in Atlantic sturgeon (Acipenser oxyrinchus) during salinity acclimation and acute stress?*

-Poster Session 1-Even numbered Posters-

Portal Capilla

Misión Juriquilla Hotel

(16:00 - 18:00)

Tuesday, May 30th

-Registration-
Misión Juriquilla Hotel
(Starting at 8:00)

-Plenary 4-
Patricia Pietrantonio
Texas A&M University, USA
COMPARATIVE ENDOCRINOLOGY OF BLOODY VECTORS TOWARDS USEFUL OUTCOMES: PROBING GPCRS FROM MULTIFUNCTIONAL NEUROPEPTIDES IN TICKS AND MOSQUITOES
Salón Juárez- Misión Juriquilla Hotel
(9:00 - 10:00)

-Coffee Break-
(10:00 - 10:30)

-Morning Symposia (S7-S9)-
(10:30 - 12:00)

Symposium 7 Growth hormones: from physiological roles to clinical science. Co-Chairs: **Farwa Sajadi and Santiago Pech-Pool** *-Salón Juárez-*		**Symposium 8** Role of the endocrine system in ecological tradeoffs. Co-Chairs: **James Carr and Breanna Harris** *-Claustro II-*		**Symposium 9** Trends in the evolution of hormone receptors. Chair: **Robert Dores** *-Claustro III-*	
Symposium Speakers					
S7-1	**Stephanie Thebault** *Current insights into the spontaneous activity of the retina: from basics to clinics*	**S8-1**	**James A. Carr** *Visual system neuropeptides modulate predator/prey tradeoffs*	**S9-1**	**Ciaran A. Shaughnessy** *Applying ancestral sequence reconstruction to resolve the functional evolution of melanocortin 2 receptor (MC2R)*
S7-2	**Carlos Guillermo Martínez Moreno** *Neural growth hormone (GH): from basic to applied research*	**S8-2**	**Breanna Harris** *Stress and Ecological Tradeoffs: Predictions, perseverance, and the pacing of pandemic productivity*	**S9-2**	**Mathilakath Vijayan** *Functional evolution of the corticosteroid receptors in a piscine model*
S7-3	**John Chang** *PI3K and ERK signaling in goldfish acute basal and agonist-stimulated GH secretion, and long-term basal hormone release and availability in vitro*	**S8-3**	**Gabriela González-Mariscal** *A comparative approach to the study of mammalian maternal behavior: nest-building in rabbits, sows, and rats*	**S9-3**	**Femilarani Antomagesh** *Both GR and MR are important for the increased metabolic flux of glucose during stress in the zebrafish brain*
Oral Presentations					
OR7-1	**Juan David Olivares Hernández** *Neuroprotective and regenerative effects of GH in the embryonic chicken cerebral pallium exposed to hypoxic-ischemic (hi) injury*	**OR8-1**	**Alexander Baugh** *Sex is stressy. Is stress sexy? Glucocorticoids and mate choosiness in Cope's gray treefrogs*	**OR9-1**	**Laura Cristina Berumen** *Ace-2 differential expression in A549 type II alveolar cell line*
OR7-2	**María Magdalena Zamora Corona** *Vasoinhibin acts directly on cancer cells to inhibit thrombin-induced proliferation and invasion*	**OR8-2**	**Ya-Xiong Tao** *Unique pharmacology of fish neural melanocortin receptors and origin of melanocortin receptors*	**OR9-2**	**Jinghan Tan** *Characterization and insight into the physiological role of the CCHamides in the yellow fever mosquito, Aedes aegypti*

-Coffee Break-
(12:00 - 12:30)

-Gorbman – Bern Lecture-
Vance Trudeau
University of Ottawa, Canada
NEUROENDOCRINE CONTROL OF REPRODUCTION: IS IT LOVE OR IS IT JUST OVULATION?
Salón Juárez- Misión Juriquilla Hotel
(12:30 - 13:30)

-Lunch-
Misión Juriquilla Hotel
(13:30 - 14:30)

-Afternoon Symposia (S10 – S12) -
(14:30 - 16:00)

Symposium 10 Insulin/Insulin-like growth factor peptides Chair: **Cunming Duan** *-Salón Juárez-*		**Symposium 11** Regulation of salinity tolerance in amphibians Co-Chairs: **Daniel Buchholz and Erica Crespi** *-Claustro II-*		**Symposium 12** Hormonal control of gonadal development: from biological sex to reproduction Co-Chairs: **Juan I Fernandino and Diana Castañeda-Cortés** *-Claustro III-*	
Symposium Speakers					
S10-1	**Nicolas Rohner** *Insulin resistance and metabolic adaptation in cavefish*	**S11-1**	**Molly Albecker** *Salt-regulation in anurans: What do we know?*	**S12-1**	**Juan I. Fernandino** *Thyroid axis participates in heat temperature-induced male sex reversal through its activation by the stress response*
S10-2	**Andrew Nuss** *Insulin-mediated nutrient partitioning in a non-model insect: the western tarnished plant bug, Lygus hesperus*	**S11-2**	**Brian Tornabene** *Determining whether adrenal steroids mediate phenotypic and physiologic effects of elevated salinity on larval amphibians*	**S12-2**	**Diana Castañeda-Cortés** *Role of steroid-5α* *-reductase type 2 on development and reproduction in amphibians*
S10-3	**Cunming Duan** *Regulation of cell plasticity by IGF signaling: Lessons from fish ionocytes*	**S11-3**	**Myra Hughey** *Integrating the gut microbiota into our understanding of amphibian responses to salinity and pathogen stress*	**S12-3**	**Maya Zanardini** *Role of arginine vasotocin in the regulation of zebrafish spermatogenesis*
Oral Presentations					
OR10-1	**Yudong Jia** *Characterization of IGF3 in turbot and its expression patterns during ovarian and embryonic development*	**OR11-1**	**Erica Crespi** *Salinity increases differentiation of mucus secreting cells and secretion of mucus in amphibian embryos: a potential role for leptin?*	**OR12-1**	**Verónica Angélica Alves** *Gonadal development in the frog Silurana tropicalis: does thyroid hormone signaling play a role?*

OR10-2	**Aurelien Chuard** *Functional Characterization of Viral Insulin/IGF-1 like peptides in host-pathogen interactions*	OR11-2	**Daniel R. Buchholz** *Role of mineralocorticoid receptor in tadpoles of Xenopus tropicalis.*	OR12-2	**Gaganpreet Sidhu** *NDR3 in zebrafish sex differentiation and fertility regulation*

-Poster Session 2-Odd numbered Posters-

Portal Capilla

Misión Juriquilla Hotel

(16:00 - 18:00)

Wednesday, May 31th

-Registration-
Misión Juriquilla Hotel
(Starting at 8:00)

-Plenary 5-
William Bendena
Queen's University, Canada
UNCOVERING ROLES FOR ALLATOSTATIN-LIKE RECEPTORS IN *Caenorhabditis elegans*
Salón Juárez- Misión Juriquilla Hotel
(9:00 - 10:00)

-Coffee Break-
(10:00 - 10:30)
-Morning Symposia (S13-S15)-
(10:30 - 12:00)

	Symposium 13 Estrogenic, androgenic, thyroidal, and steroid biosynthetic (EATS) and non-EATS research advancements and testing Co-Chairs: **Vicki Marlatt and Valerie Langlois** *-Salón Juárez-*		**Symposium 14** Evolution of neuropeptide and hormonal signaling systems Chair: **Andreas Heyland** *-Claustro II-*		**Symposium 15** Hormones, behavior and reproduction Co-Chairs: **Rebeca Corona and Maricela Luna** -Claustro III-
Symposium Speakers					
S13-1	**Celia Marti** *Identification of molecular markers on zebrafish embryo for thyroid disruption by transcriptomic analysis*	**S14-1**	**Andreas Heyland** *Function and evolution of thyroid hormone signaling in Sea Urchin embryonic and post-embryonic development*	**S15-1**	**Wendy Portillo Martínez** *Adult neurogenesis induced by socio-sexual behavior in the prairie vole, a social monogamous mammal*
S13-2	**Christopher Martyniuk** *Knock(in) knock(out): who's there? Gene editing approaches for developing? non-eats? Zebrafish screens.*	**S14-2**	**Joao CR Cardoso** *Neuropeptide regulation of shell biomineralization in bivalves*	**S15-2**	**Angela Nava Bolaños** *Reproductive isolation barriers in Argia damselflies*
S13-3	**Emmanuelle Monniez** *Rapid screening tool for disruption of endocrine stress responses linked to the developmental origins of disease*	**S14-3**	**María Fernanda Vergara Martínez** *Oxytocin/vasopressin-related neuropeptide distribution in ovaries of Pogonomyrmex barbatus ant*	**S15-3**	**Rebeca Corona** *Prolactin modulates the olfactory system response to reproductive chemosignals?*
Oral Presentations					
OR13-1	**Michael McKay** *Copper impacts on endocrine processes in the liver of developing rainbow trout (Oncorhynchus mykiss)*	**OR14-1**	**Chunyu Lu** *Simultaneous extraction and detection of neuropeptides, steroids, and proteins in small tissue samples*	**OR15-1**	**Enezi Khalid** *Small GTPase involvement in basal and GNRH-dependent luteinizing hormone and growth hormone secretion from dispersed goldfish pituitary cells*
OR13-2	**Paisley Thomson** *Emerging evidence that the synthetic progestin, melengestrol acetate, disregulates three of the*	**OR14-2**	**Robin Warne** *Microbiome mediation of animal life histories via*	**OR15-2**	**Melissa Pamela Lozano Staines** *Adiponectin, resistin and chemerin as obesity associated biomarkers in a murine model*

	major endocrine pathways in Silurana tropicalis		*metabolites and insulin-like signaling.*		*supplemented with probiotics and prebiotics*

-Excursion/free afternoon-
Trip to Bernal, Querétaro
(12:00 - 18:00)

Thursday, June 1st

-Registration-
Misión Juriquilla Hotel
(Starting at 8:00)

-Plenary 6-
De-Shou Wang
Southwest University, Chongqing, China
MOLECULAR MECHANISM ANALYSIS OF SEX DETERMINATION AND DIFFERENTIATION OF TILAPIA BASED ON GENOME EDITING
Salón Juárez- Misión Juriquilla Hotel
(9:00 - 10:00 am)
-Coffee Break-
(10:00 - 10:30)

-Morning Symposia (S16-S18)-
(10:30 - 12:00)

Symposium 16 Hormonal control of regeneration in vertebrates. Chair: **José Ávila-Mendoza** *-Salón Juárez-*		**Symposium 17** Emerging topics in comparative endocrinology I. Chair: **Christopher Martyniuk** *-Claustro II-*		**Symposium 18** Emerging topics in comparative endocrinology II. Co-Chairs: **Aurora Olvera Vidal and Ivan Lazcano** *-Claustro III-*	
Symposium Speakers		**Oral Presentations**			
S16-1	**José Luis Quintanar Stephano** *Neuronal regeneration in spinal cord injury by gonadotropin-releasing hormone*	**OR17-1**	**Tracy Larson** *Seasonal plasticity in songbirds as novel model for uncovering mechanisms that limit neural degeneration and regeneration*	**OR18-1**	**Glen Van der Kraak** *Ammonia inhibits oocyte maturation, ovulation and spawning in zebrafish*
S16-2	**Robyn Reeve** *Pleiotrophic roles of leptin signaling in xenopus tail tip regeneration*	**OR17-2**	**Robert M. Dores** *Re-evaluation the evolution of the POMC gene: a study on melanocortin peptides and melanocortin receptors of the hagfish, Eptatretus stoutii*	**OR18-2**	**Yuta Tanizaki** *Thyroid hormone regulates iron transport via ferroportin to stimulate erythropoiesis during postembryonic development*
S16-3	**José Ávila Mendoza** *Comparative analysis of KLF transcription factors in axonal regeneration: insights from fish and mammalian studies*	**OR17-3**	**Jessica Paloma Alvarez-Rendón** *Characterization of behavioral changes in insulin-signaling impaired males and females in Drosophila Melanogaster.*	**OR18-3**	**Tharindu Malintha Gardi** *Hewage Salinity-dependent endocrine pathways in tilapia gill and kidney*
Oral Presentations		**Oral Presentations**			
OR16-1	**David Epardo** *Neuroprotective effects of growth hormone in an optic nerve crush model as an experimental model for glaucoma*	**OR17-4**	**Andre Barany** *In vitro and in vivo studies on the function and osmoregulatory action of neurohypophysial hormones and receptors in the sea lamprey (Petromyzon marinus)*	**OR18-4**	**Rosario Baltazar-Lara** *Effect of growth hormone (GH) on neuroinflammation in the postnatal cerebellum of rats subjected to hypoxia*

OR16-2	**Ana Luisa Ocampo-Ruíz** *Prolactin receptor deficiency promotes hypomyelination during central nervous system maturation of suckling and prepubertal mice*	OR17-5	**Sajid Alvi** *The role of NDR1 and NDR2 in regulating zebrafish ovarian function*	OR18-5	**Yorgui Santiago Andres** *The contribution of the communication systems in the hypothalamus-pituitary unit is plastic among vertebrates*
		OR17-6	**Liezhen Fu** *Disruption of T3-induced epigenetic regulator mbd3 leads to growth inhibition and development retardation in Xenopus*	OR18-6	**Brianna Raven** *Effects of kisspeptins on gene expression in the hypothalamo-pituitary-gonadal axis of the female western clawed frog, Silurana tropicalis*

-Coffee break-
Misión Juriquilla Hotel
(12:00 - 12:30)

-Women in Science Panel-
Dra. Veerle Darras, Dra. Angela Lange, Dra. Aurea Orozco
Salón Juárez- Misión Juriquilla Hotel
(12:30 - 13:30)

-Lunch-
Misión Juriquilla Hotel
(13:30 - 14:30)

-NASCE Council Meeting 2-
Salón Gobernador- Misión Juriquilla Hotel
(14:30 - 16:30)

-Closing Ceremony and Student Awards Presentation-
Salón Juárez- Misión Juriquilla Hotel
(18:00 - 19:00)

-Closing Banquet-
Salón Juárez- Misión Juriquilla Hotel
(19:00 - ∞)

Plenary Lecture Abstracts		
PL-1	Joëlle Rüegg Uppsala University, Sweden	*Impacts of endocrine disruptors on the developing brain: from molecular insights to novel test methods*
PL-2	Hugo A. Barrera-Saldaña Universidad Autónoma de Nuevo León, México	*The lives of growth hormone: news from the past, stories with future*
PL-3	David Parichy University of Virginia, USA	*Thyroid hormone dependence of adult phenotype in zebrafish*
PL-4	Patricia Pietrantonio Texas A&M University, USA	*Comparative endocrinology of bloody vectors towards useful outcomes: probing GPCRs from multifunctional neuropeptides in ticks and mosquitoes*
PL-5	William Bendena Queen's University, Canada	*Uncovering roles for Allatostatin-like receptors in Caenorhabditis elegans*
PL-6	De-Shou Wang Southwest University, Chongqing, China	*Molecular mechanism analysis of sex determination and differentiation of tilapia based on genome editing*

Gorbman-Bern Award Lecture		
GBMAL	Vance Trudeau University of Ottawa, Canada	*Neuroendocrine control of reproduction: is it love or is it just ovulation?*

Invited Symposium Speaker Abstracts		
S1-1	Luis Yanez Guerra. University of Exeter, United Kingdom (UK)	*Reconstructing the evolution of neuropeptide signaling in non-bilaterian metazoa*
S1-2	Farwa Sajadi York University, Canada	*An anti-diuretic signaling system in the female mosquito, Aedes aegypti*
S1-3	Meet Zandawala Julius-Maximilians-University of Würzburg, Germany	*Visualizing context-dependent modulation in-vivo using TANGO-MAP MkII neuropeptide activity sensors*
S2-1	Kataaki Okubo The University of Tokyo, Japan	*Sex steroid regulation of sexually dimorphic behavioral responses to conspecifics in medaka*
S2-2	Bon-chu Chung Academia Sinica, Taiwan	*Single-cell analysis of zebrafish germ cell lineage and sex development*
S2-3	Wei Ge University of Macau, Taipa, Macao Special Administrative Region, China	*Genetic analysis for roles of gonadotropins in zebrafish sex differentiation, gonadal development, and function*
S3-1	Clara Cooper-Mullin University of Rhode Island, USA	*How Birds During Migration Maintain (Oxidative) Balance*
S3-2	Jose Pablo Vazquez-Medina University of California, Berkeley, USA	*Prolonged fasting stimulates endogenous antioxidant production in elephant seals*

S3-3	Karen Sweazea Arizona State University, USA	*Acute metformin induces hyperglycemia in healthy adult mourning doves, Zenaida macroura?*
S4-1	Mark Sheridan Texas Tech University, USA	*Integration of feeding with the control of growth and metabolism: insights from studies in fish*
S4-2	Jimena Leyria University of Toronto Mississauga, Canada	*Insulin like peptides as multitaskers for successful reproduction in Rhodnius prolixus, a vector of chagas disease*
S4-3	Monika Gulia-Nuss University of Nevada, Reno, USA	*Regulation of blood digestion in lyme disease vector tick, Ixodes scapularis*
S5-1	Sheue-Yann Cheng National Cancer Institute, NIH, USA	*Thyroid hormone receptor alpha 1 in uterus functions*
S5-2	Yuki Shibata Department of Biology, Nippon Medical School, Japan	*Genetic analysis of thyroid hormone regulation of intestinal remodeling in Xenopus*
S5-3	Laurent Sachs Muséum National d'Histoire Naturelle, Paris, France	*Thyroid hormone and glucocorticoid effects on Transcriptome and Methylome in Xenopus tropicalis tadpoles*
S6-1	Nicholas Bernier University of Guelph, Canada	*Central and peripheral contributions of the corticotropin-releasing factor (CRF) system to smoltification and seawater acclimation in Atlantic salmon*
S6-2	Ningping Gong Texas Tech University, USA	*Strategies of anadromous sea lamprey for coping with salinity challenges by regulating hormone signaling systems and ion-transporters in the gill*
S6-3	Andre Seale University of Hawaii, USA	*The prolactin cell as a nexus for the integration of osmotic and thermal sensory modalities?*
S7-1	Stephanie Thebault Instituto de Neurobiología, UNAM, México	*Current insights into the spontaneous activity of the retina: from basics to clinics*
S7-2	Carlos Guillermo Martínez Moreno *Instituto de Neurobiología, UNAM, México*	*Neural growth hormone (GH): from basic to applied research*
S7-3	John Chang University of Alberta, Canada	*PI3K and ERK signaling in goldfish acute basal and agonist-stimulated GH secretion, and long-term basal hormone release and availability in vitro*
S8-1	James A. Carr Texas Tech University, USA	*Visual system neuropeptides modulate predator/prey tradeoffs*
S8-2	Breanna Harris Texas Tech University, USA	*Visual system neuropeptides modulate predator/prey tradeoffs*
S8-3	Gabriela González-Mariscal CINVESTAV- Universidad Autónoma de Tlaxcala, México	*A comparative approach to the study of mammalian maternal behavior: nest-building in rabbits, sows, and rats*
S9-1	Ciaran A. Shaughnessy University of Denver, USA	*Applying ancestral sequence reconstruction to resolve the functional evolution of melanocortin 2 receptor (MC2R)*
S9-2	Mathilakath Vijayan University of Calgary, Canada	*Functional evolution of the corticosteroid receptors in a piscine model*

S9-3	Femilarani Antomagesh University of Calgary, Canada	*Both GR and MR are important for the increased metabolic flux of glucose during stress in the zebrafish brain*
S10-1	Nicolas Rohner Stowers Institute for Medical Research, USA	*Insulin resistance and metabolic adaptation in cavefish*
S10-2	Andrew Nuss University of Nevada, Reno, USA	*Insulin-mediated nutrient partitioning in a non-model insect: the western tarnished plant bug, Lygus hesperus*
S10-3	Cunming Duan University of Michigan, Ann Arbor, USA	*Regulation of cell plasticity by IGF signaling: Lessons from fish ionocytes*
S11-1	Molly Albecker University of Houston, USA	*Salt-regulation in anurans: What do we know?*
S11-2	Brian Tornabene University of Montana, USA	*Determining whether adrenal steroids mediate phenotypic and physiologic effects of elevated salinity on larval amphibians*
S11-3	Myra Hughey Vassar College, USA	*Integrating the gut microbiota into our understanding of amphibian responses to salinity and pathogen stress*
S12-1	Juan I. Fernandino INTECH (CONICET-UNSAM), Argentina	*Thyroid axis participates in heat temperature-induced male sex reversal through its activation by the stress response*
S12-2	Diana Castañeda-Cortés Institut national de la recherche scientifique, Canada	*Role of steroid-5α-reductase type 2 on development and reproduction in amphibians*
S12-3	Maya Zanardini University of Calgary, Canada	*Role of arginine vasotocin in the regulation of zebrafish spermatogenesis*
S13-1	Celia Marti UMR 7221 CNRS, France	*Identification of molecular markers on zebrafish embryo for thyroid disruption by transcriptomic analysis*
S13-2	Chris Martyniuk University of Florida, USA	*Knock(in) knock(out): who's there? Gene editing approaches for developing? non-eats? Zebrafish screens.*
S13-3	Emmanuelle Monniez University of Ottawa, Canada	*Rapid screening tool for disruption of endocrine stress responses linked to the developmental origins of disease*
S14-1	Andreas Heyland University of Guelph, Canada	*Function and evolution of thyroid hormone signaling in Sea Urchin embryonic and post-embryonic development*
S14-2	Joao CR Cardoso Centre of Marine Sciences, Portugal	*Neuropeptide regulation of shell biomineralization in bivalves*
S14-3	María Fernanda Vergara Martínez Instituto de Investigaciones Biomédicas, UNAM, México	*Oxytocin/vasopressin-related neuropeptide distribution in ovaries of Pogonomyrmex barbatus ant*
S15-1	Wendy Portillo Martínez Instituto de Neurobiología, UNAM, México	*Adult neurogenesis induced by socio-sexual behavior in the prairie vole, a social monogamous mammal*
S15-2	Angela Nava Bolaños UMDI-Juriquilla, Facultad de Ciencias, UNAM, México	*Reproductive isolation barriers in Argia damselflies*

S15-3	Rebeca Corona Instituto de Neurobiología, UNAM, México	*Prolactin modulates the olfactory system response to reproductive chemosignals?*
S16-1	José Luis Quintanar Stephano Universidad Autónoma de Aguascalientes, México	*Neuronal regeneration in spinal cord injury by gonadotropin-releasing hormone*
S16-2	Robyn Reeve Washington State University, USA	*Pleiotropic roles of leptin signaling in xenopus tail tip regeneration*
S16-3	José Ávila Mendoza Instituto de Neurobiología, UNAM	*Comparative analysis of KLF transcription factors in axonal regeneration: insights from fish and mammalian studies*

Oral Presentation Abstracts

OR-1-1	Areej N. Al-Dailami University of Toronto Mississauga, Canada	*Glycoprotein hormone (GPA2/GPB5) and corticotropin-releasing factor (CRF) signaling are involved in reproduction in adult female Rhodnius prolixus*
OR-1-2	Elias Taylor University of Guelph, Canada	*Thyroid hormones signaling mechanisms in Sea Urchins and other echinoderms*
OR2-1	John Postlethwait University of Oregon, USA	*Gonadal development in zebrafish with chromosomal sex determination*
OR2-2	Yong Zhu East Carolina University, USA	*Adamts9 modulates gonad sex in juvenile zebrafish*
OR3-1	Juan R. Riesgo-Escovar Instituto de Neurobiología, UNAM, México	*Moderate activation of NRF2 in insulin-signaling impaired drosophila melanogaster improves resistance to oxidative stress*
OR3-2	Elizabeth Brammer-Robbins University of Florida, USA	*Do healthy manatees from a poor-quality habitat exhibit evidence physiological of stress?*
OR4-1	Valeria Urban Instituto de Neurobiología, UNAM, México	*Effects of fasting upon the somatotropic axis and metabolism in the green iguana*
OR4-2	Dina Iathzil Vázquez Carrillo Instituto de Neurobiología, UNAM, México	*Sulpiride reduces hyperglycemia and insulin resistance in diet-induced obese female and male mice*
OR5-1	Hui Zhao Chinese University of Hong Kong	*Disruption of thyroid hormone receptor alpha a (thraa) promotes heart regeneration in zebrafish*
OR5-2	Aurora Olvera Vidal Instituto de Neurobiología, UNAM, México	*Specific temporal windows of T3 action in oligodendrogenesis. Lessons from the zebrafish*
OR6-1	Britney Picinic York University, Canada	*The influence of diuretic and anti-diuretic factors on aquaporin expression in female A. aegypti*
OR6-2	Ciaran Shaughness University of Denver, USA	*Regulation of the hypothalamus-pituitary-interrenal (HPI) axis in Atlantic sturgeon (Acipenser oxyrinchus) during salinity acclimation and acute stress?*
OR7-1	Juan David Olivares Hernández Instituto de Neurobiología, UNAM, México	*Neuroprotective and regenerative effects of GH in the embryonic chicken cerebral pallium exposed to hypoxic-ischemic (hi) injury*

OR7-2	María Magdalena Zamora Corona Instituto de Neurobiología, UNAM, México	*Vasoinhibin acts directly on cancer cells to inhibit thrombin-induced proliferation and invasion*
OR8-1	Alexander Baugh Swarthmore College, University of Pennsylvania,USA	*Sex is stressy. Is stress sexy? Glucocorticoids and mate choosiness in Cope's gray treefrogs*
OR8-2	Ya-Xiong Tao Auburn University, USA	*Unique pharmacology of fish neural melanocortin receptors and origin of melanocortin receptors*
OR9-1	Laura Cristina Berumen Universidad Autónoma de Querétaro, México	*Ace-2 differential expression in A549 type II alveolar cell line*
OR9-2	Jinghan Tan York University, Canada	*Characterization and insight into the physiological role of the CCHamides in the yellow fever mosquito, Aedes aegypti*
OR10-1	Yudong Jia Yellow Sea Fisheries Research Institute, China	*Characterization of IGF3 in turbot and its expression patterns during ovarian and embryonic development*
OR10-2	Aurelien Chuard Boston College, USA	*Functional characterization of viral Insulin/IGF-1 like peptides in host-pathogen interactions*
OR11-1	Erica Crespi Washington State University, USA	*Salinity increases differentiation of mucus secreting cells and secretion of mucus in amphibian embryos: a potential role for leptin?*
OR11-2	Daniel R. Buchholz University of Cincinnati	*Role of mineralocorticoid receptor in tadpoles of Xenopus tropicalis*
OR12-1	Verónica Angélica Alves Institut National de La Recherche Scientifique (INRS), Canada	*Gonadal development in the frog Silurana tropicalis: does thyroid hormone signaling play a role?*
OR12-2	Gaganpreet Sidhu York University, Canada	*NDR3 in zebrafish sex differentiation and fertility regulation*
OR13-1	Michael McKay Simon Fraser University, Canada	*Copper impacts on endocrine processes in the liver of developing rainbow trout (Oncorhynchus mykiss)*
OR13-2	Paisley Thomson Institut National de la Recherche Scientifique, Canada	*Emerging evidence that the synthetic progestin, melengestrol acetate, disregulates three of the major endocrine pathways in silurana tropicalis*
OR14-1	Chunyu Lu University of Ottawa, Canada	*Simultaneous extraction and detection of neuropeptides, steroids, and proteins in small tissue samples*
OR14-2	Robin Warne Southern Illinois University, USA	*Microbiome mediation of animal life histories via metabolites and insulin-like signaling.*
OR15-1	Enezi Khalid University of Alberta, Canada	*Small GTPase involvement in basal and GNRH-dependent luteinizing hormone and growth hormone secretion from dispersed goldfish pituitary cells*
OR15-2	Melissa Pamela Lozano Staines Universidad Autónoma de Ciudad Juárez, México	*Adiponectin, resistin and chemerin as obesity asociated biomarkers in a murine model supplemented with probiotics and prebiotics*
OR16-1	David Epardo Instituto de Neurobiología, UNAM, México	*Neuroprotective effects of growth hormone in an optic nerve crush model as an experimental model for glaucoma*

OR16-2	Ana Luisa Ocampo-Ruíz Instituto de Neurobiología, UNAM, México	*Prolactin receptor deficiency promotes hypomyelination during central nervous system maturation of suckling and prepubertal mice*
OR17-1	Tracy Larson University of Virginia, USA	*Seasonal plasticity in songbirds as novel model for uncovering mechanisms that limit neural degeneration and regeneration*
OR17-2	Robert M. Dores University of Denver, USA	*Re-evaluation the evolution of the POMC gene: a study on melanocortin peptides and melanocortin receptors of the hagfish, Eptatretus stoutii*
OR17-3	Jessica Paloma Alvarez-Rendón Instituto de Neurobiología, UNAM, México	*Characterization of behavioral changes in insulin-signaling impaired males and females in Drosophila Melanogaster*
OR17-4	Andre Barany United States Geological Survey, USA	*In vitro and in vivo studies on the function and osmoregulatory action of neurohypophysial hormones and receptors in the sea lamprey (Petromyzon marinus*
OR17-5	Sajid Alvi York University, Canada	*The role of NDR1 and NDR2 in regulating zebrafish ovarian function*
OR17-6	Liezhen Fu NICHD, National Institutes of Health, USA	*Disruption of T3-induced epigenetic regulator mbd3 leads to growth inhibition and development retardation in Xenopus*
OR18-1	Glen Van der Kraak University of Guelph, Canada	*Ammonia inhibits oocyte maturation, ovulation and spawning in zebrafish*
OR18-2	Yuta Tanizaki NICHD, National Institutes of Health, USA	*Thyroid hormone regulates iron transport via ferroportin to stimulate erythropoiesis during postembryonic development*
OR18-3	Tharindu Malintha Gardi University of Hawaii, USA	*Hewage Salinity-dependent endocrine pathways in tilapia gill and kidney*
OR18-4	Rosario Baltazar-Lara Instituto de Neurobiología, UNAM, México	*Effect of growth hormone (GH) on neuroinflammation in the postnatal cerebellum of rats subjected to hypoxia*
OR18-5	Yorgui Santiago Andres Facultad de Ciencias, UNAM, México	*The contribution of the communication systems in the hypothalamus-pituitary unit is plastic among vertebrates*
OR18-6	Brianna Raven University of Ottawa, Canada	*Effects of kisspeptins on gene expression in the hypothalamo-pituitary-gonadal axis of the female western clawed frog, Silurana tropicalis*

Poster Presentation Abstract		
P1	Ryan Chang	Changes in cortisol, glucose and corticosteroid receptors during dynamic salinity challenges in Mozambique tilapia
P2	Yiming Yue	Disruption of bone morphogenetic protein type II receptors results in reproductive and non-reproductive dysfunction in zebrafish
P3	Alma Lorena Pérez Gómez	Effect of the vasoinhibin analogue crivi45-51 on melanoma leukocyte infiltration and metastasis in mice
P4	Oscar Fernando Martínez Díaz	Vasoinhibin analogs as a new therapeutic strategy for the treatment of arthritis
P5	Nancy Denslow	Lipid profiles in different regions of the brain of fathead minnows are altered upon exposure to steroid hormones
P6	Edgar Rodrigo Juvera	Molecular iodine decreases the invasive potential of neuroblastoma xenografts in zebrafish.
P7	Ruijing Geng	NMN supplementation reverses female reproductive aging and rescues Nampta and Namptb deficiency-induced gonadal ageing in female and male zebrafish respectively
P8	A.F. Boan	Knockout of somatostatin receptor 5 has no effect on medaka growth
P9	Juan Manuel Murillo-Maldonado	A comparative study of lifestyles and metabolism of *Drosophila lutzii*, a floridosa group of species, and sympatric *D. simulans*, a generalist species
P10	José Luis Dena-Beltrán	Milk prolactin participates on enterocyte maturation in lactating mice
P11	Elizabeth Brammer-Robbins	Plasma progesterone and lipids as bioindicators of pregnancy in the Florida manatee (Trichechus manatus latirostris)
P12	Isui Abril Garcia-Montoya	Transcriptional and histological response of liver in an obese mice model due to the effect of a symbiotic supplementation.
P13	David Salvador Diaz Ortegon	Thyrotropin-Releasing Hormone (TRH) decreases hyperglicemia in adult zebrafish (danio rerio)
P14	Jose Fernando Garcia Rodrigo	Mechanisms mediating dual actions of prolactin in joint inflammation
P15	Erandi Arvizu-Hernández	A Laurencia johnstonii extract reverses the effect of 17β-estradiol in early lesions in the k14e7hpv16 murine model of cervical carcinogenesis
P16	Denisse Guadalupe Rivera Bautista	Epigenetic changes associated with pair bond formation in the prairie vole female
P17	Francisco Freinet Nuñez Ramirez	Renin generates vasoinhibin in the retina of newborn mice
P18	Elva Adán-Castro	The vasoinhibin analogue crivi45-51 inhibits excessive vasopermeability in the retina of rats
P19	Juan Pablo Robles	Identification of the antiangiogenic determinant of vasoinhibin and design of a therapeutic orally active oligopeptide
P20	Miriam Ulloa	Prolactin regulates the antioxidant response of astrocytes through the activation of STAT3 signaling pathway
P21	Lourdes Montserrat Siqueiros Márquez	Sulpiride-induced hyperprolactinemia protects retinal function in streptozotocin-induced diabetes
P22	Jerusa Elienai Balderas-Márquez	Effect of growth hormone (GH) in the retinal microglia during inflammation
P23	Pamela Reyes Ortega	Clinical relevance of the contribution of the retina to the spontaneous oscillations detected by electroretinogram (ERG) in mice and humans
P24	Fernando Macias	Prolactin protects hippocampal neurons *in vitro* from H_2O_2-induced oxidative stress and reduces NOX4 activation via NF-kB inhibition
P25	Erin Legacki	The pre and post ovulatory steroid hormone profile of North American Atlantic salmon (*Salmo salar*) as measured by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)
P26	Maya Zanardini	Role of arginine vasotocin in the regulation of zebrafish spermatogenesis (ORAL PRESENTATION: S12.3)

P27	Alma Guadalupe Petry Ticante Carrizales	Growth hormone effects on axon regeneration in *Klf13*-deficient retinal ganglion cells
P28	Karen Delgado Rueda	Depletion of Krüppel-like factor 13 (KLF13) enhances GH-dependent JAK/STAT activity in hippocampal neurons
P29	David González Aretia	Sphingosine-1-phosphate (S1P) synthesis in granulosa cells and its effects on cell survival and steroidogenesis
P30	Yudong Jia	Melatonin improves turbot oocyte meiotic maturation and antioxidant capacity, inhibits apoptosis-related genes mRNAs *in vitro*
P31	Zaire Belen Medina Moctezuma	Importance of sphingosine 1-phosphate (S1P) synthesis stimulated by Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) on viability of bovine theca and granulosa cells
P32	Lydia Marín López	Does Sphingosine 1-Phosphate (S1P) promote the viability and steroidogenesis of bovine theca cells in culture?
P33	Veronica Viñuela-Berni	The neuroendocrine interactions during chronic kidney disease: the role of prolactin
P34	Cynthia Alejandra Rodriguez Arzate	Sex impact on the alterations of spontaneous activity of the retina induced by obesity: comparison between humans and mice
P35	Victoria Giovanna Spadacini	Glutamatergic activation of *Erk* expression in neurons and pituitary cells of the female zebrafish
P36	Laura Nayeli Del Pilar Martinez	Olfactory alterations in a model of chronic kidney disease in female mice
P37	Jazmin Wynter	Identifying the implications of hypoxia and DMOG on reproductive processes in adult female zebrafish (*Danio rerio*)
P38	Jesus Angel Aguirre	Mineralocorticoids regulate the expression of Na+/K+ ATPase in *T. crassiceps* WFU cysticerci
P39	Samuel Palacios Pérez	Alterations in the myelination patterns in a hyper- or hypothyroidism state in early zebrafish development
P40	Fernanda Maldonado-Lira	Thyroid hormone status modifies retinal morphology and color preference during zebrafish development
P41	Robert M. Dores	Trends in the evolution of elasmobranch melanocortin-2 receptors (MC2Rs): insights from an analysis of the pacific dogfish MC2R

Sunday, May 28th, 2023
16:30-17:30
Cultural Academic Center "Flavio Mena Jara"
Campus UNAM Juriquilla

PL-1. IMPACTS OF ENDOCRINE DISRUPTORS ON THE DEVELOPING BRAIN: FROM MOLECULAR INSIGHTS TO NOVEL TEST METHODS

Joëlle Rüegg
Uppsala University, Sweden.

Evidence is mounting that exposure to endocrine disrupting chemicals (EDCs) can affect neurodevelopmental processes, leading to cognitive impairments and changed behaviours. Yet, the pathways through which EDCs affect brain development are understudied and mechanisms underlying long term changes in cognition and behaviour far from understood. As a result, chemical testing and hazard assessment hardly covers endocrine disruption (ED-)induced developmental neurotoxicity (DNT). This presentation will describe how integration of molecular and cellular analyses in in vitro models and physiological outcomes in in vivo studies and human data leads to the identification of novel pathways for ED-induced DNT and the development of assays and biomarkers for chemical testing and assessment. A large part of this work was conducted in the context of the ENDpoiNTs project, a European project including 16 participants in Europe, USA and Australia. Based on human data, a number of EDCs were identified that are associated with cognitive and behavioural outcomes in children. Using in silico predictions, putative hormonal pathways were identified that could be affected by these chemicals. Subsequently, in mammalian in vitro models, links between disruption of these pathways and cellular key events crucial for neurodevelopmental processes, such as neural progenitor proliferation, differentiation, myelination and network formation, were established. Using the same exposures in rat and zebrafish models, the molecular and cellular effects were linked to behavioural changes. Using this integrative approach, pathways beyond the frequently studied ones in the context of EDCs (estrogen-, androgen-, and thyroid hormone), were identified as important to mediate ED-induced DNT. Based on these insights, novel assays for chemical testing are currently under development. Furthermore, by analyzing epigenomic changes induced by EDCs in experimental settings and linking them to human data, epigenetic markers could be identified that can predict cognitive impairments induced by prenatal EDC exposures. While this work has filled some of the knowledge and regulatory gaps concerning neurodevelopmental effects of EDCs, further integrative studies are needed to understand, and regulatory account for, ED-induced DNT.

(EU grant No 825759)

Monday, May 29th, 2023
9:00 – 10:00
Salón Juárez

PL-2. THE LIVES OF GROWTH HORMONE: NEWS FROM THE PAST, STORIES WITH FUTURE.

Hugo A. Barrera-Saldaña
Autonomous Universtity of Nuevo Leon,México.

STUDYING OUR GENOME. The human growth hormone locus harbors the pituitary gene (hGH-N) and four placental genes. Two of this code the same chorionicsomatomammotropin hormone (hCSH-A and hCSH-B), one is considered a pseudogene (hCSH-L), and the product of the other (hGH-V) replaces the pituitary one during pregnancy.

NEWS FROM THE PAST. In prosimians, this locus is unigenic; New World Monkeys harbor multiple GH genes and pseudogenes; in Old World Monkeys, the CSH and GH-V genes emerged. In hominids, the variety of encoded CSHs is reduced, with three hCSH genes contributing a single CSH in man.

STORIES WITH FUTURE. cDNA analyses prepared from human ocular tissues revealed the expression of the hGH-N gene and the gene for this hormone´s receptor in the retina. The former is in the choroid and trabecular network, and the latter is in the choroid, crystalline, and conjunctiva. The potential neuroprotective role of HGH could lead to new ocular therapies.

FROM GENOMICS TO NEW-WAVE DIAGNOSTICS. Over a decade before the HGP was officially launched, we sequenced the hGH locus. With the genes´ restriction maps, the polymerase chain reaction, and restriction enzymes cutting each gene differently, we invented a test for tracking these genes. With it, in 1996 we explained a case of rHGH replacement failure by evidencing the absence of the hGH-N gene. Therefore our test, published two years before the supposedly first companion diagnostic (CDx) was announced (HercepTest?, Dako), was the first CDx.

FROM GENOMICS TO BIOTECH. Only a minority in Mexico of children with growth retardation could access said therapy. Thus, we used the cDNA of HGH to reprogram yeast (Pichia pastoris) to produce rHGH. Since it turned out to be identical to that synthesized by the pituitary and abundantly secreted into the fermented yeast media in its biologically active form, we patented and licensed it to a Mexican biotech firm.

(Many students and colleagues made these discoveries and inventions possible)

Monday, May 29th, 2023
12:30 – 13:30
Salón Juárez

PL-3. THYROID HORMONE DEPENDENCE OF ADULT PHENOTYPE IN ZEBRAFISH

David Parichy
Department of Biology, University of Virginia, Charlottesville, Virginia, USA

Thyroid hormone is required for the abrupt metamorphosis of anuran amphibians and some teleost fishes. To understand roles for TH in post-embryonic development more generally our group has focused on the larva-to-adult transformation of zebrafish, *Danio rerio*. Here I will show how TH and TH receptors regulate the numbers and behaviors of pigment cells and dermal cells to correctly pattern adults stripes and scales, respectively. Our work suggests critical functions for TH in coordinating adult trait development in zebrafish and points to modulation of TH activity as a plausible mechanism contributing to evolutionary changes in these and other phenotypes of teleost fishes.

Tuesday, May 30th, 2023
9:00 – 10:00
Salón Juárez

PL-4. COMPARATIVE ENDOCRINOLOGY OF BLOODY VECTORS TOWARDS USEFUL OUTCOMES: PROBING GPCRs FROM MULTIFUNCTIONAL NEUROPEPTIDES IN TICKS AND MOSQUITOES

Patricia Pietrantonio
Texas A&M University, USA

Our G protein-coupled receptor (GPCR) research focuses on two arthropod vectors of deadly diseases for which control with pesticides is routinely used to prevent disease transmission. Novel targets to control these vectors are needed due to globally widespread pesticide resistance in their populations: these are the cattle fever tick, Rhipicephalus microplus, the vector of Babesia bovis and B. bigemina, causative agents of bovine babesiosis, and the yellow fever mosquito, Aedes aegypti, which is also the vector of dengue, chikungunya and Zika viruses. The validation of the octopamine/tyramine receptor (GPCR) as acaricide target prompted us to explore invertebrate-specific GPCRs as novel control targets. Under the working hypothesis that disrupting diuresis and osmoregulation in blood feeders could be lethal, we investigated the kinin neuropeptide signaling system, as kinins are neurohormones and neurotransmitters. Insect kinins are myotropic, and diuretic in mosquitoes, but functions in ticks are uncertain. For both species we performed: 1) receptor functional analyses in CHO-K1 recombinant clonal cell lines to investigate the potency of endogenous ligands and peptide mimetics, 2) receptor silencing by RNAi 3) immunohistochemistry 4) small molecule pharmacology. Further, we identified the endogenous tick kinins, previously unknown, and cDNA analyses demonstrated expansion of kinins in hard ticks. All kinins tested activated their cognate recombinant receptor. A potent kinin analog, ID 1728 (designed by R. Nachman; USDA), was antifeedant when offered to mosquito females in sugar solution and elicited aversive behavior. Electrophysiology from a labellar sensilla revealed 1728 shut down the sucrose neuron in ms. This proved for the first time a GPCR modulates taste in an insect. HTS yielded kinin receptor antagonists that decreased mosquito hindgut contractions.

(This project was supported by AFRI Competitive Grant no. 2022-67015-36336 from the USDA NIFA.)

Wednesday, May 31st, 2023
9:00 – 10:00
Salón Juárez

PL-5. UNCOVERING ROLES FOR ALLATOSTATIN-LIKE RECEPTORS IN CAENORHABITIS ELEGANS

William Bendena
Queen's University, Canada

Metamorphosis in insects is initiated and regulated by two hormonal systems, namely the sesquiterpenoid and the ecdysteroid hormone systems. The corpus allatum in insects produces the sesquiterpenoid juvenile hormone (JH) that postpones the initiation of metamorphosis, prolonging the juvenile characteristics of the larvae. Allatostatins (ASTs) are neuropeptides that serve as inhibitors of the JH biosynthetic pathway. Different insects use one of three identified AST peptides. The C-terminal -T/YXaaFGL-NH2 family of ASTs (AST-As) function as inhibitors of JH in cockroaches, the WXaa6W-NH2 (AST-Bs) in crickets and the -PISCF family (AST-Cs) in Lepidoptera and Diptera. All three types of peptide sequence exit in all Arthropods but only one family serves as regulators of JH biosynthesis in any given insect. The others have adopted other roles which include acting as regulators of muscle contraction, feeding decisions/foraging, circadian rhythm, sleep activity and growth.

The nematode model organism Caenorhabditis elegans has orthologs of AST-A and AST-C peptides as well as receptors that have similarity to Drosophila AST receptors DAR1/2 (termed NPR-9/NPR-14) and Drostar1/2 (termed NPR-16/NPR-24) which bind AST-As and AST-Cs, respectively. C. elegans does not make JH! We therefore hypothesized that some of the alternate functions for ASTs found in insects might be an ancestral function in worms.

Through receptor mutational analysis NPR-9 was shown to have altered foraging behaviour, altered ability to go into a daur state as well as increased lipid accumulation. Mutations in NPR-14 induced spontaneous bouts of quiescence/sleep and works with other neuropeptides and receptors known to be in the stress-induced sleep pathway. Mutations NPR-16 and 24 display enhanced longevity, daur entry defects and enhanced lipid accumulation.

(This work was supported by an NSERC Discovery grant RGPIN 36481-08)

Thursday, June 1st, 2023
9:00 – 10:00
Salón Juárez

PL-6. MOLECULAR MECHANISM ANALYSIS OF SEX DETERMINATION AND DIFFERENTIATION OF TILAPIA BASED ON GENOME EDITING

Deshou Wang, Minghui Li
Key Laboratory of Freshwater Fish Reproduction and Development (Ministry of Education), Key Laboratory of Aquatic Science of Chongqing, School of Life Sciences, Southwest University,

El measurement. Its sexual maturation time (about 5 months) is shorter than that of most cultured fish. Tilapia can be artificially propagated in the laboratory all year round to obtain genetically all XX female and all XY male fry. Its genome sequence has been opened, and a perfect gene editing platform has been established in our laboratory. In recent years, we have established dozens of single, double and triple gene mutation lines to study sex determination and differentiation, and identified amhy, dmrt1, gsdf as key male pathway genes and foxl2, foxl3, cyp19a1a as key female pathway genes in tilapia.Loss of function study of genes challenges the traditional views: 1) whether estrogen is required for ovarian differentiation; 2) whether androgen is needed for testicular development and spermatogenesis; 3) whether germ cell fate is determined by its somatic environment. By gene editing, we demonstrated that steroidogenic enzymes can be classified into two categories based on their expression, enzyme activities and function in tilapia. Mutation of star2, cyp17a1 and cyp19a1a, which are dominantly expressed in the gonads and involved in estrogen production, results in up-regulation of male pathway genes and female to male sex reversal in XX fish. In contrast, mutation of star1, cyp11a1, cyp17a2 and cyp19a1b, as well as cyp11c1, which are expressed both in gonads and extra-gonadal tissues, alters steroids (androgen, DHP and cortisol) production and spermatogenesis, fertility, secondary sexual characteristics and sexual behavior, but does not affect sex differentiation in XY fish. Consistently, mutation of estrogen receptors results in female to male sex reversal in XX fish, while mutation of androgen receptors results in no sex reversal in XY fish. Amhy, which is the master sex determining gene of Nile tilapia, determine male sex by inhibiting the transcription of cyp19a1a and estrogen production through Amhr2/Smads signaling. In addition, the differentiated ovary could be transdifferentiated into functional testis by down-regulation of estrogen level, and the differentiated testis could be

transdifferentiated into ovary by simultaneous administration of androgen synthetase inhibitor and exogenous estrogen, demonstrating the maintenance of sexual plasticity even after sex differentiation in gonochoristic fish. Interestingly, germ cells lost sexual plasticity in dmrt1 XY and foxl3 XX single mutants, as aromatase inhibitor (AI) and estrogen treatment failed to rescue the respective phenotypes. However, recovery of germ cell sexual plasticity was observed in dmrt1/foxl3 double mutants. Antagonism between Dmrt1 and Foxl3 determines the fate of germ cells, while antagonism between Dmrt1 and Foxl2 determines the fate of somatic cells. Further, we found that once dmrt1 is mutated, the gonad cannot be rescued to a functional testis by mutation of any female pathway gene. In contrast, if dmrt1 is present, double or triple mutation of male and female pathway genes resulted in functional testes. The sex reversal caused by mutation of male pathway genes other than dmrt1, including sex determining gene amhy, can be rescued by mutation of the female pathway gene. The fundamental reason why dmrt1 is indispensable to males is that it is expressed in both germ cells and somatic cells, unlike the other male pathway genes. These results demonstrate that dmrt1 is the only gene indispensable for male sex determination and testicular development. This also explains why dmrt1 is conservatively used as the key male pathway gene in all vertebrates and even in some invertebrates. Overall, these studies demonstrate that tilapia is a good animal model for studying comparative endocrinology, sex determination and differentiation.

(This work was supported by grants from National Natural Science Foundation of China (NSFC key project 31630082, NSFC-NSF 31861123001).

NASCE 2023 Gorbman-Bern Award Lecture

Tuesday, May 30th, 2023
12:30 -13:30
Salón Juárez
Gorbman- Bern Memorial Award Lecture
GBMAL. NEUROENDOCRINE CONTROL OF REPRODUCTION: IS IT LOVE OR IS IT JUST OVULATION?
Vance Trudeau
Dept. of Biology, University of Ottawa, CANADA

In the spirit of comparative endocrinology and to honour Professors Gorbman and Bern, I will contrast mammalian and teleost models to reveal several concepts and controversies in reproductive neuroendocrinology. Surge release of luteinizing hormone (LH) from the pituitary is essential for reproduction as it triggers ovulation. Competent expression of sexual behaviours by both sexes ensures optimal fertilization rates. The central dogma is that specific hypothalamic kisspeptin (KISS) neurons stimulate gonadotropin-releasing hormone (GNRH) that in turn, stimulates LH and induces ovulation in mammals. Data in emerging models such as zebrafish and medaka now challenge this concept of essentiality of GNRH and KISS. Alternatively, oxytocin (OXT) controls sexual behaviour but not ovulation. Yet, mutation or deletion of genes encoding these, and other well-known peptides, hardly impact breeding in fish, contradicting pharmacological evidence for their roles. This has accelerated research to uncover other critical neurohormones. One such candidate is secretogranin 2 (SCG2). There are 2 teleost genes, the phylogenetically ancient SCG2a and the duplicated SCG2b. Prohormone convertase-mediated precursor processing leads to the production of the secretoneurin (SNa/SNb) peptides. Injection of SNa robustly stimulates LH release in vivo in goldfish. Similarly, in vitro studies established that SNa could directly stimulate LH production and release from dispersed goldfish pituitary cells and mouse L?T2 tumour cells in the absence of GNRH. Subfertile zebrafish SCG2a/b mutants resemble other mutant lines lacking LH in which female infertility is linked to disrupted ovulation. Concurrent mass spectrometry measurements of multiple neuropeptides and sex steroids in zebrafish brain, pituitary and ovaries reveal timely increases in SNa, GNRH and OXT in relation to the LH surge and oviposition. Injection of SNa stimulates ovulation in otherwise anovulatory zebrafish females. Complete gene knockout of SCG2 genes quantitatively decreases sexual interactions, delays oviposition, and significantly reduces spawning success. Fully developed ovaries in SCG2 knockouts suggests that failed reproduction may be a result of failed ovulation rather than defects in ovarian development. Sexual activity and ovulation in zebrafish appear dependent on the normal functioning of the SCG2 genes. Together these data place the evolutionarily conserved SN peptides amongst other critical neuroendocrine regulators of ovulation.
(The financial support of the University of Ottawa Research Chair in Neuroendocrinology and the NSERC Discovery program is acknowledged. The contributions of numerous insightful and dedicated graduate and undergraduate students, along with international collaborators has been fundamental to the success of the outlined studies. The author recognizes that he works and lives on the unceded Algonquin territory of the Three Fire Confederacy, Anishinaabewaki. He respects the traditional guardians of these and other regions of the world he is privileged to visit.)

Monday, May 29th, 2023
10:30 – 12:00
Salón Juárez

NASCE 2023 Symposium 1:
State-of-the-art in invertebrate comparative endocrinology
Co-chairs:
Jean-Paul Paluzzi and Meet Zandawala

S1-1

RECONSTRUCTING THE EVOLUTION OF NEUROPEPTIDE SIGNALLING IN NON-BILATERIAN METAZOA

Luis Alfonso Yanez Guerra(1), Daniel Thiel(2), Adriano Senatore(3), Gaspar Jekely(4)
(1)(2)University of Exeter, UK (3)University of Toronto Mississauga (4)Centre for Organismal Studies (COS), University of Heidelberg

Neuropeptides regulate important functions in animals, including feeding, reproduction and behaviour. Because of the short sequences and the high divergence of the precursors encoding them, it is difficult to identify how neuropeptides across animal phyla are related. Advances in comparative genomics have reshaped our understanding of the evolution of peptidergic signalling. Recently, it has been shown that some of the neuropeptide precursors can be traced back to pre-metazoan origins. Despite these advances, we still know little about the origin of neuropeptide signalling. Here, using sequence-similarity and machine learning-based searches, we identified 29 neuropeptide precursors and several mature peptides in the placozoan model Trichoplax adhaerens. Then, using hidden-Markov models, cluster-based analyses and large-scale combinatorial deorphanisation methods, we were able to experimentally characterise more than 20 neuropeptide GPCRs in this species, demonstrating that neuropeptide signalling is present in this neuronless animal. Finally, the phylogenetic analysis of bilaterian and non-bilaterian receptors revealed that most non-bilaterian neuropeptide GPCRs are many-to-many orthologues of bilaterian neuropeptide receptors. This suggests independent strong diversification in both clades from only a few ancestrally shared peptidergic systems. Our discoveries provide experimental evidence of metabotropic neuropeptide GPCRs and their activation by endogenous ligands in placozoans. These resources will be helpful for the further characterisation of neuropeptides and their signalling pathways in other non-bilaterian species, such as ctenophores, and provide the first insights to understand the origin of neuropeptide signalling in animals.
(The work was funded by a Leverhulme Trust Research Project Grant RPG-2018-392 to G.J. and the BBSRC discovery fellowship (BB/W010305/1) awarded to L.A.Y.G.)

S1-2

AN ANTI-DIURETIC SIGNALING SYSTEM IN THE FEMALE MOSQUITO, AEDES AEGYPTI

Farwa Sajadi(1), Lulia Snan(2), María Fernanda Vergara-Martínez(3), Chiara Di Scipio(4), Jean-Paul Paluzzi(5)
(1)(2)(4)(5)York University, Canada (3)Universidad Nacional Autónoma de México

Haematophagus insects, such as the female yellow fever mosquito, Aedes aegypti, ingest bloodmeals equivalent to twice their body volume that introduces considerable amounts of salts and water, threatening the osmotic and ionic balance of their haemolymph. Aedes mosquitoes have evolved mechanisms to achieve strict regulation of their hydromineral balance through the neuroendocrine control of their specialized excretory system, consisting of the Malpighian ?renal? tubules (MTs), which are responsible for primary urine formation, and the hindgut, which functions primarily as a reabsorptive organ. While extensive studies have examined this process of hydromineral balance in A. aegypti focusing on diuretic regulation, the roles of anti-diuretic hormones remain elusive. Herein, we investigate the role and signaling system of the CAPA neuropeptide in the female A. aegypti mosquito. In adult MTs, CAPA peptides elicit a selective anti-diuretic role, inhibiting DH31- and 5HT-stimulated secretion through the NOS/cGMP/PKG pathway. Bafilomycin, a V-type H+ ATPase (VA) inhibitor, significantly inhibits DH31-and 5HT-stimulated secretion by MTs, while having no effect on other diuretic hormones. CAPA and bafilomycin treatment cause alkalization of the secreted fluid suggesting inhibition of the VA, which may lead to constrained entry of cations across the apical membrane. Additionally, adult female MTs treated with DH31 resulted in an increased VA activity, while tubules incubated with both DH31 and CAPA had a lower VA activity comparable to unstimulated controls. To determine whether CAPA promotes VA disassembly, cytosolic and membrane protein fractions were isolated from DH31- and CAPA-incubated MTs. V1 protein expression was found higher in the membrane fractions of DH31-incubated MTs while higher levels were seen in the cytosolic fractions of CAPA-treated tubules. Lastly, functional characterization using a heterologous receptor assay confirmed the release of the natriuretic hormone (DH31) immediately post-bloodmeal, with levels remaining elevated for 15 minutes and peaking at 5 minutes, corresponding with the peak-phase of post-prandial diuresis. These results provide evidence of a coordinated action of both diuretic and anti-diuretic hormones on the MTs to maintain haemolymph homeostasis by fine-tuning primary urine secretion.
(Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an NSERC Discovery Grant (JPP) as well as NSERC CGS, Vernon Stong, and Carswell Scholarships in Science (FS))

S1-3

VISUALIZING CONTEXT-DEPENDENT MODULATION IN-VIVO USING TANGO-MAP MKII NEUROPEPTIDE ACTIVITY SENSORS

Meet Zandawala(1), Maria Steigmeier(2), Altar Sorkac(3), Jayati Gera(4), Selina Hilpert(5), Gilad Barnea(6)
(1)(2)(4)(5)Julius-Maximilians-University of Würzburg, Germany (3)(6)Brown University

Neuropeptides and peptide hormones (hereafter referred to as NPHs) are the largest class of neuronal signaling molecules that allow animals to flexibly adapt their behavior and physiology to varying environmental conditions and internal states. Functional studies on NPH signaling have revealed that most NPHs are pleiotropic. Furthermore, multiple NPHs can influence a given behavior. But how multiple NPH signaling pathways interact to orchestrate organismal behavior and physiology under a given context are poorly understood. Moreover, resolving the spatial and temporal dynamics of NPH signaling (what are the targets of NPHs, when are they modulated and for how long?) has also been challenging due to a lack of tools to observe neuromodulatory activity in-vivo. To address this knowledge-gap, we have developed a powerful genetic tool, TANGO-Map MkII, to analyze paracrine or hormonal signaling in Drosophila in-vivo. We showcase the power of this technique by utilizing TANGO-Map MkII sensors to visualize modulation of neural circuits and peripheral tissues under diverse contexts (mated vs. virgin, fed vs starved and daytime vs nighttime). For instance, we uncover time-of-day dependent modulation of specific circuits by the clock neuropeptide, pigment-dispersing factor (PDF). PDF also signals to peripheral tissues under different contexts to regulate feeding-associated physiology. Using TANGO-Map MkII sensors for additional NPHs we provide unprecedented insights on the temporal as well as spatial dynamics of context-dependent neuromodulation. Taken together, TANGO-Map MkII provides a strong foundation to examine signaling dynamics of other neuromodulators that regulate state-dependent behavioral plasticity. Ultimately, this will allow us to discover fundamental principles of neuromodulatory signaling which will be an important step towards decoding the functioning of the brain.
(Research supported by NIH and DFG.)

OR1-1

GLYCOPROTEIN HORMONE (GPA2/GPB5) AND CORTICOTROPIN-RELEASING FACTOR (CRF) SIGNALING ARE INVOLVED IN REPRODUCTION IN ADULT FEMALE RHODNIUS PROLIXUS

Areej N. Al-Dailami, Ian Orchard, Angela B. Lange
University of Toronto Mississauga, Canada

Glycoprotein hormones are formed by the heterodimerization of alpha and beta subunits. In vertebrates, there are five glycoprotein hormones, four of which have a common alpha subunit (GPA1) bound to a specific beta subunit (GPB1, GPB2, GPB3, or GPB4). The fifth, thyrostimulin, is also found in invertebrates, and is formed by the dimerization of GPA2 and GPB5 subunits. These hormones mediate physiological events such as development, metabolism, and reproduction. The mammalian corticotropin-releasing factor (CRF) is a neurohormone involved in stress responses, and in insects can act as a diuretic hormone. Previous reports on invertebrates suggest that GPA2/GPB5 and CRF play critical roles in feeding, diuresis, and reproduction. Immunohistochemistry reveals that GPB5 and CRF are co-localized in neurosecretory cells in the brain and mesothoracic ganglionic mass and in their neurohemal organs in adult female Rhodnius prolixus, a blood-gorging insect and a vector for human Chagas disease. qPCR reveals that transcripts for the GPA2/GPB5 receptor (LGR1) and the CRF receptor (CRFR) are expressed in reproductive tissues and fat body in adult female R. prolixus, and their expression increases post-blood meal, a stimulus that triggers diuresis and reproduction. To examine the involvement of the GPA2/GPB5 and CRF signaling pathways in reproduction, transcript expression of the receptors, LGR1 and CRFR, were knocked down using RNA interference, and reproductive success monitored by examining the production of the major yolk protein, vitellogenin (Vg), the number and quality of eggs laid and their hatching ratio. The results will be discussed in this presentation.
(This work is supported by NSERC discovery grants to A.B.L and I.O.)

OR1-2

THYROID HORMONES SIGNALING MECHANISMS IN SEA URCHINS AND OTHER ECHINODERMS

Elias Taylor, Andreas Heyland
University of Guelph, Canada

Thyroid hormones (THs) are crucial regulators of morphogenesis, metamorphosis, and metabolism in chordates. Emerging evidence suggests a role for THs in regulating non-chordate development. We have found that THs act via a membrane integrin receptor non-genomically, and via a nuclear hormone receptor genomically. A fluorescently-labelled TH analogue reveals TH binding locations in the membrane and nucleus of echinoderms, including sea urchins, a sea star, and a brittle star. These binding locations coincide with TH actions, including acceleration of skeletal development and metamorphosis. We show that THs bind to sea urchin membrane proteins, and may be displaced by ligands of RGD-binding integrins. Inhibitors of TH signaling via an integrin membrane receptor are sufficient to disrupt TH regulation of skeletogenesis in sea urchin embryos. We also find additional evidence for genomic signaling via the canonical nuclear hormone receptor: analysis of thyroid hormone response elements in the sea urchin genome demonstrates enrichment near sites of TH-induced transcriptional regulation. TH signaling via both genomic and non-genomic mechanisms may regulate sea urchin development. In the context of previous work showing that THs are synthesized by sea urchin embryos and larvae and contribute to metamorphic development, our data suggest that TH signaling mechanisms may be a shared feature of bilaterians.
(We would like to acknowledge the contributions of Drs. V. Lewis, S. Jonusaite, M. Coppolino, T. Gillis, S. George, J. Allen, and T. Van Raay. We would also like to thank H. Belanco, N. Schuh, H. Wynen, and the Hagen Aqualab, Friday Harbour Laboratories, and the UoG Advanced Analysis Centre.)

Monday, May 29th, 2023
10:30 – 12:00
Salón Claustro II

NASCE 2023 Symposium 2:
Development of sex in animals
Co-chairs:
Chun Peng and Yong Zhu

S2-1
SEX STEROID REGULATION OF SEXUALLY DIMORPHIC BEHAVIORAL RESPONSES TO CONSPECIFICS IN MEDAKA
Kataaki Okubo
The University of Tokyo, Japan

Across species, adult males and females exhibit very different behavioral responses when encountering same- and opposite-sex conspecifics. Typically, adult males court and attempt to mate with females while attacking other males, whereas adult females are receptive to male courtship and do not mate or attack other females. The neural and hormonal mechanisms underlying these sexually dimorphic behavioral responses have long remained elusive, but recent studies in mice have revealed that activation of the estrogen receptor (ESR) subtype ESR1 in the ventromedial hypothalamus and medial preoptic area is central to these mechanisms. However, the specific role of estrogen/ESR1 signaling in shaping sexual dimorphism in behavioral responses is unclear. Furthermore, and importantly, it is unlikely that the findings in mice apply to many other vertebrates, including primates and teleost fish, in which androgen/androgen receptor (AR) signaling, rather than estrogen/ESR signaling, has been implicated in the display of male-typical behavioral responses. In the present study, we genetically dissected the roles of estrogen/ESR and androgen/AR signaling in the display of male- and female-typical behavioral responses toward conspecifics, using the teleost fish medaka (Oryzias latipes) as a model species. Our findings revealed that in medaka, sex steroid signaling ensures appropriate behavioral responses of females and males in a very different manner than in mice.
(This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan, and the Japan Society for the Promotion of Science (JSPS) (MEXT/JSPS grant numbers 17H06429, 19H03044, and 21J20634)

S2-2
SINGLE-CELL ANALYSIS OF ZEBRAFISH GERM CELL LINEAGE AND SEX DEVELOPMENT.
Bon-chu Chung
Academia Sinica, Taiwan

Zebrafish represents an important model organism, but its mode of sex determination has been unclear. The types and differentiation of zebrafish germ cells also remain uncharacterized. Here, we performed single cell RNAseq on single germ cells isolated by fluorescence-activated sorting from piwi1:GFP larval gonads. We applied gene-expression similarity mapping to identify 14 clusters of cells that form a pattern matching the progression from germline stem cells (nanos2+), through early progenitors (selenow2a+), committed progenitors (foxl2l+), to pre-meiotic (rec8a+) and meiotic germ cells (zp2.3+). We report the expression of a transcription factor Foxl2l solely in the committed progenitors and ovaries. CRISPR-Cas9-mediated mutation of foxl2l resulted in production of 100% fertile male fish, indicating a fate change in females. Single-cell profiling of foxl2l mutant germ cells revealed cell clusters matching only with the wildtype germline stem cells and early progenitors, so progression of early progenitors into committed progenitors had been blocked. In addition to aberrant elevation of male transcripts such as dmrt1, we demonstrate that expression of a transcription repressor id1 was aberrantly elevated, together with concomitant elevation of stem cell marker nanos2, indicating that these foxl2l mutant germ cells had reverted to the stem cell stage. Overall, we have delineated all germ cell types in zebrafish and reveal that Foxl2l drives zebrafish germ cells out of stemness towards committing to female progenitors.
(This work was funded by grants from Academia Sinica, AS-101-TP-B05, NHRI- EX107-10506SI, MOST 107-2321-B-001-034, MOST 108-2311-B-001 -038 -MY3.)

S2-3
Genetic analysis for roles of gonadotropins in zebrafish sex differentiation, gonadal development, and function
Wei Ge
Department of Biomedical Sciences and Centre of Reproduction, Development and Aging (CRDA), Faculty of Health Sciences, University of Macau, Taipa, Macao Special Administrative Region, China

Vertebrate reproduction is controlled by two gonadotropins (FSH and LH) from the pituitary. Despite numerous studies on FSH and LH in fish species, their functions in reproduction remain poorly defined. Using zebrafish as the model, we have created mutant lines for both FSH (fshb) and LH (lhb) as well their receptors (fshr and lhcgr) and carried out systematic genetic analysis for functional roles of FSH and LH in controlling gonadal differentiation, puberty onset, gametogenesis, final maturation, and fertility. FSH-deficient zebrafish (fshb-/-) were surprisingly fertile in both sexes; however, the development of both the ovary and testis or puberty onset was significantly delayed. In contrast, LH-deficient zebrafish (lhb-/-) showed normal gonadal growth, but the females failed to spawn and were therefore infertile. Neither fshb-/- nor lhb-/- alone seemed to affect gonadal differentiation; however, the double mutant (fshb-/-;lhb-/-) turned out to be all males with significant delay in spermatogenesis. We went on to analyze the functions of FSH and LH receptors (fshr and lhcgr). In contrast to the deficiency of its cognate ligand FSH, the fshr-deficient females showed a complete failure of follicle activation with all ovarian follicles arrested at the primary growth-previtellogenic transition, which is the marker for puberty onset in females. These mutant females all reversed to males, and all these males were fertile with normal spermatogenesis. In contrast to fshr, the deletion of the lhcgr gene alone caused no obvious phenotypes in both males and females; however, double mutation of fshr and lhcgr resulted in infertile males. In summary, our results in the present study showed that the signalling via Fshr was indispensable to folliculogenesis and the disruption of the fshr gene resulted in a complete failure of follicle activation followed by masculinization

into males. In contrast, lhcgr does not seem to be essential to zebrafish reproduction in both males and females. Neither Fshr nor Lhcgr deficiency could phenocopy the deficiency of their cognate ligands FSH and LH, which is likely due to the fact that Fshr can be activated by both FSH and LH in the zebrafish.

OR2-1

GONADAL DEVELOPMENT IN ZEBRAFISH WITH CHROMOSOMAL SEX DETERMINATION

John Postlethwait, Catherine Wilson, Yi-lin Yan
University of Oregon, USA

Sex-determination genetics varies across taxa, sometimes even within a species. For zebrafish (Danio rerio), the laboratory strains AB and TU lack a strong genetic sex determinant but strains more recently derived from nature (e.g., NA) possess a sex-determining locus on chromosome-4 with a ZZ male/ZW female system sensitive to the environment. AB fish form oocytes that survive in fish that become females but die in fish that become males. Oocyte survival is likely mediated by oocyte signals that maintain estrogen production by support cells. To learn if ZZ fish make a juvenile ovary, we studied histology, bulk RNA-seq, and single cell RNA-seq in ZZ and ZW fish. ZW fish developed oocytes by 22 days post-fertilization (dpf) but ZZ fish avoided the juvenile ovary phase and formed testes directly, presumably without estrogen production. Some ZW fish, however, developed oocytes that died as the gonad became a testis, like AB fish. Single-cell RNA-seq of 19dpf gonads showed similar cell types in ZZ and ZW fish, including precursors of gonadal support cells and germline stem cells, consistent with a bipotential juvenile gonad. In contrast, scRNA-seq of 30dpf gonads revealed cells with transcriptomes characteristic of testicular Sertoli, Leydig, and germ cells in ZZ fish and ovarian theca and granulosa cells and developing oocytes in ZW fish. These results show that juvenile NA zebrafish initially develop a bipotential gonad; that a factor on the NA W chromosome is essential to initiate oocyte development and maintain cyp19a1a expression and hence estrogen production; and that fish lacking the feminizing W-chromosome factor directly develop testes. Gonad development in the laboratory stocks AB and TU mimics NA ZW and WW zebrafish, suggesting Z chromosome loss during domestication, and hence, maintenance of estrogen in about half the population, raising the question about the mechanisms that dampen oocyte persistence in the other half that become males.
(Supported by NIGMS.)

OR2-2

ADAMTS9 MODULATES GONAD SEX IN JUVENILE ZEBRAFISH

Yong Zhu
East Carolina University, USA

ADAMTS9 is a conserved extracellular matrix metalloprotease that is critical for the development. Knockouts of ADAMTS9 in mice and invertebrate ortholog in Drosophila are embryonic lethal. Loss of Adamts9 causes male-biased sex development, underdeveloped ovaries, female infertility, and intersex phenotype. In current study, we observed Adamts9 expression in zebrafish ovarian stroma, primary follicles, stage I follicles, and preovulatory follicles in juveniles and adults. Surprisingly, adamts9-/- had no effect on primary sex determination, even had slow germ cell proliferation and development of primary follicles. In contrast to continued development through later stages of follicles (Stage II, III) in wildtype, whereas most follicles in Adamts9 KO sibling remain arrested as primary follicles and do not progress further in mid- or late-juveniles. In newly sexual matured adults, the sex ratio of Adamts9 KO shifted to heavily male biased (~90%). We found drastically overfeeding of juvenile zebrafish was sufficient to rescue growth deficiency and female development in Adamts9 KO fish. These rescued Adamts9 KO females, however still possessed dramatically underdeveloped ovaries, with most follicles (~95%) remaining arrested as primary follicles. Additionally, we also found morphological evidence of active sex reversal in Adamts9 KO including degenerating follicles or coexistence of ovarian follicles and spermatic cyst with mature sperm in the same gonad. By knocking out tp53, a major apoptosis pathway, in Adamts9 KO, we were able to partially rescue the underdeveloped ovaries. Finally, RNAseq analysis on rescued Adamts9 ovaries found significant disruption of chemokine and cytokine signaling in KO ovary. Taken together, we demonstrated that adamts9 expression is critical for the development of primary ovarian follicles and sex determination in juvenile zebrafish.
(Supported by NIH GM100461 and HD109785 to YZ.)

Monday, May 29th, 2023
10:30 – 12:00
Salón Claustro III

NASCE 2023 Symposium 3:
Nothing to stress about: Endocrine and oxidative responses to diet
Co-chairs:
Pierre Deviche and Karen Sweazea

S3-1

HOW BIRDS DURING MIGRATION MAINTAIN (OXIDATIVE) BALANCE

Clara Cooper-Mullin(1), Wales Carter(2), Kristen DeMoranville(3), Abigail Frawley(4), Barbara Pierce(5), Megan Skrip(6), Scott McWilliams(7)
(1)(2)(3)(4)(7)University of Rhode Island, USA (5)Sacred Heart University (6)North Carolina State University.

Animals dynamically adjust their physiology and behavior to survive in changing environments, and seasonal migration is one life stage that demonstrates these dynamic adjustments. As birds migrate between breeding and wintering areas, they incur physiological demands that challenge their antioxidant system. Migrating birds presumably respond to these oxidative challenges by up-regulating protective endogenous systems or accumulating dietary antioxidants at stopover sites, although our understanding of the pre-migration preparations and mid-migration responses of birds to such oxidative challenges is as yet incomplete. Here we review evidence from field and captive-bird studies that address the following questions: (1) Do migratory birds build antioxidant capacity as they build fat stores in preparation for long flights? (2) Is oxidative damage an inevitable consequence of oxidative challenges such as flight, and, if so, how is the extent of damage affected by factors such as the response

of the antioxidant system, the level of energetic challenge, and the availability of dietary antioxidants? (3) Do migratory birds recover from the oxidative damage accrued during long-duration flights?, and, if so, does the pace of this rebalancing of oxidative status depend on the quality of the stopover site? The answer to all these questions is a qualified ?yes? although ecological factors (e.g., diet and habitat quality, geographic barriers to migration, and weather) affect how the antioxidant system responds. Furthermore, the pace of this dynamic physiological response remains an open question, despite its potential importance for shaping outcomes on timescales ranging from single flights to migratory journeys. In sum, the antioxidant system of birds during migration is impressively dynamic and responsive to environmental conditions, and thus provides ample opportunities to study how the physiology of migratory birds responds to a changing and challenging world.
(The authors were supported by a grant from the National Science Foundation to SM and BP (IOS-1354187), and by The University of Rhode Island, College of the Environment and Life Sciences.)

S3-2
PROLONGED FASTING STIMULATES ENDOGENOUS ANTIOXIDANT PRODUCTION IN ELEPHANT SEALS
Jose Pablo Vazquez-Medina
University of California, Berkeley, USA

Elephant seals experience spontaneous long-term absolute food deprivation (fasting) while breeding, molting, and undergoing postnatal development. Prolonged fasting in elephant seals promotes insulin resistance, induces a pro-inflammatory phenotype and increases circulating cortisol without causing deleterious consequences. Our previous work shows that prolonged fasting stimulates endogenous antioxidant production in elephant seals. This response is likely coupled to hormonal regulation involving the renin-angiotensin system and glucocorticoid receptor signaling. Here, I will present data my group has collected over the years studying wild elephant seals during their natural prolonged fasting period. Using a combination of in vivo work with exogenous hormone infusions, receptor blocker studies, and in vitro work with primary cells derived from elephant seals, we have found that elephant seals are a prime example of physiological adaptation to extreme conditions, including prolonged fasting.
(Julia Maria Torres-Velarde, David C Ensminger and Diana Daniela Moreno Santillan contributed with experimental analyses. Research funded by UC Berkeley.)

S3-3
ACUTE METFORMIN INDUCES HYPERGLYCEMIA IN HEALTHY ADULT MOURNING DOVES, ZENAIDA MACROURA
Karen Sweazea, Anthony Basile, Avin Kreisler, Ryan Hassen, Kavita Singh, Maggie Symes, Gabrielle Larson
Arizona State University, USA

Birds naturally have high blood glucose concentrations compared to other vertebrates. Several mechanisms have been proposed to explain their relative hyperglycemia including the lack of an insulin-responsive glucose transport protein, high glucagon concentrations, and reliance on lipid oxidation to fuel the high metabolic demands of flight. We suspected the latter may result in the production of the gluconeogenic precursor, glycerol. Therefore, we examined the hypothesis that glycerol gluconeogenesis contributes to the naturally high glucose concentrations in birds. We captured adult mourning doves, Zenaida macroura, and acclimated the birds to captivity for two weeks. In this crossover design study, birds received one of the following treatments administered once per week, until they received each treatment: gluconeogenesis antagonist (150 or 300 mg/kg metformin), glycogenolysis inhibitor (2.5 mg/kg DAB), or water (50 ul). Blood glucose concentrations were measured using a glucose meter at baseline, 30, 60, and 120 minutes following the oral dose. In contrast to mammals and chickens, 300 mg/kg metformin did not alter blood glucose (p>0.05) and 150 mg/kg metformin significantly increased blood glucose concentrations (p=0.043) compared to the oral bolus of water. To examine whether the low dose of metformin stimulated glycogenolysis, thus causing the hyperglycemic effect, we administered the low dose of metformin along with an inhibitor of glycogenolysis, 2.5 mg/kg 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), which prevented the hyperglycemic response. In a separate set of experiments, we examined the early effects of 150 mg/kg metformin and observed significant increases in blood glucose within 5 minutes. Administration of DAB did not prevent the early glucose response to metformin. In addition, DAB alone had no effect on blood glucose concentrations. In conclusion, and in contrast to the hypothesis, metformin increased endogenous glucose production via glycogenolysis in healthy mourning doves. These findings are similar to what has been reported for healthy humans.
(This study was supported by Barrett, The Honors College (AK, RH) and the Graduate and Professional Student Association (AJB) at Arizona State University.)

OR3-1
MODERATE ACTIVATION OF NRF2 IN INSULIN-SIGNALING IMPAIRED DROSOPHILA MELANOGASTER IMPROVES RESISTANCE TO OXIDATIVE STRESS
Juan Rafael Riesgo-Escovar, Jéssica Paloma Álvarez-Rendón
Instituto de Neurobiologia, UNAM. México

Disequilibria between production and exposure to reactive oxygen species, coupled with inadequate responses from the antioxidant machinery, may lead to the accumulation of damage in cell components such as lipids, proteins, and DNA. This phenomenon, known as oxidative stress, contributes to the deterioration of the organism in a vast array of diseases, including diabetes. In Drosophila, organismal deficiency in insulin signaling leads to hyperglycemia and dyslipidemia. The evolutionarily conserved Nrf2 signaling pathway functions to counteract oxidative stress. Here we sought to understand whether moderate Nrf2 pathway activation could enhance resistance to pro-oxidant conditions (20 mM paraquat or 3% hydrogen peroxide), in adult insulin-compromised and control fruit flies. We used one-week-old male and female flies with 1) heteroallelic mutations for InR (the fly homologue of the insulin receptor) or dS6K (the fly homologue of ribosomal protein S6 kinase beta-1, under the control of TORC1), and 2) with or without a Keap1 mutation in heterozygosity (Keap1 being a negative regulator of CncC, the Nrf2 homolog in Drosophila), as well as wild-type controls with the same genetic background. We found that wild-type and insulin signaling-affected flies often showed similar resistance to both pro-oxidants, whereas males with the heterozygous Keap1 mutation in addition to the insulin pathway mutations have statistically significant enhanced survival to paraquat and hydrogen peroxide por-oxidant conditions. Our results point to an altered oxidative state

in insulin-signaling impaired flies, a state of affairs where moderate activation of the Nrf2 pathway provides enhanced resistance to exogenous pro-oxidant agents, particularly in male flies.
(Supported by the Instituto de Neurobiología, Universidad Nacional Autónoma de México, PAPIIT #IG200216 to JRR-E and CONACYT scholarship #612316 to JPAR.)

OR3-2
DO HEALTHY MANATEES FROM A POOR-QUALITY HABITAT EXHIBIT EVIDENCE PHYSIOLOGICAL OF STRESS?
Elizabeth Brammer-Robbins(1), Mohammad-Zaman Nouri(2), Emily Griffin(3), Juan Aristizabal-Henao(4), Nancy Denslow(5), John Bowden(6), Iske Larkin(7), C.J Martyniuk(8)
(1)University of Florida, USA (2)Center for Environmental and Human Toxicology, University of Florida, (3)Department of Physiological Sciences, College of Veterinary Medicine, Center for Environmental and Human Toxicology, University of Florida (4)BPGbio Inc., 500 Old Connecticut Path, Framingham, MA 01701, the United States of America, (5)Department of Physiological Sciences, College of Veterinary Medicine,Center for Environmental and Human Toxicology,Genetics Institute, University of Florida (6)Department of Physiological Sciences, Center for Environmental and Human Toxicology, Department of Chemistry,University of Florida (7)Aquatic Animal Health Program, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida Gainesville, FL, the United States of America., (8)Department of Physiological Sciences, College of Veterinary Medicine,Center for Environmental and Human Toxicology,University of Florida.

Florida manatees (Trichechus manatus latirostris), a federally protected species, are classified as threatened due to anthropogenic stressors. Manatees inhabit sites that are impacted by human activities that can negatively affect stress physiology and metabolism. Samples collected from healthy manatees (pregnant females, non-pregnant females, and males) at Crystal River and Indian River Lagoon in Florida, were assessed for adrenal hormones, proteins, glucose, and lipid content in plasma. The objective was to determine if healthy manatees sampled between 2010-2014 from the Indian River Lagoon exhibited evidence of stress compared to healthy manatees sampled between 2012-2019 from Crystal River. Plasma cortisol concentrations were not different in male and non-pregnant female manatees between sites but were elevated in pregnant manatees. Plasma aldosterone concentrations were elevated in Indian River Lagoon manatees relative to those at Crystal River, possibly due to differences in salinity and available freshwater between the two environments. Site differences were noted for plasma protein and glucose concentrations in manatees; additionally, differences between the sexes were also observed in glucose concentrations. Fifteen lipid subclasses, including oxidized lysophosphatidylcholines, oxidized phosphatidylcholines, and oxidized triacylglycerols, were elevated in manatees from the Indian River Lagoon relative to manatees from Crystal River. Evidence of a stress response in healthy Indian River Lagoon manatees was lacking compared to Crystal River manatees. Differences in metabolites related to energy (glucose, protein, and lipids) may be related to site-specific variables, such as salinity and food availability/quality. This study generates novel data on plasma lipid profiles and provides cortisol, aldosterone, glucose, and protein values from healthy Florida manatees in two disparate sites that can be referenced in future studies. These data contribute to an improved understanding of manatee physiology to better inform population management.
(We would like to acknowledge Dr. Margaret Hunter and Dr. Jason Ferrante with the Sirenia Project at the U.S. Geological Survey Wetland and Aquatic Research Center, for providing samples. We would also like to thank the Aquatic Animal Health Program at the University of Florida in cooperation with the Florida Fish and Wildlife Conservation Commission for providing samples. We acknowledge the U.S. National Institutes of Health (NIH) Shared Instrumentation (Grant 1S10OD018141 to ND). Finally, we acknowledge that the material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. (2019285699 to EB-R).)

Monday, May 29th, 2023
14:30 – 16:00
Salón Juárez

NASCE 2023 Symposium 4:
Endocrinological changes induced by feeding
Co-Chairs:
Ian Orchard and Angela Lange

S4-1
INTEGRATION OF FEEDING WITH THE CONTROL OF GROWTH AND METABOLISM: INSIGHTS FROM STUDIES IN FISH
Mark Sheridan
Texas Tech University, USA

Fish, which occur in a diverse range of habitat types and have evolved often elaborate life history patterns, have provided important insight about how feeding integrates with the regulation of growth and metabolism. Appetite and food intake are controlled by many hormones within the feeding centers of the hypothalamus as well as by hormones produced in the periphery. Many of these hormones serve to coordinate feeding with growth and with the metabolic state of the animal. For example, ghrelin (GRLN) from the hypothalamus and from the gut (produced by the presence of nutrients) stimulates feeding and the release of growth hormone (GH) and insulin (INS). GH, in turns, stimulates growth via the GH-insulin-like growth factor (IGF) system. GRLN and INS upregulate elements of the GH-IGF system to further promote growth. GH, GRLN, and INS also stimulate anabolic actions to support growth and energy storage (e.g., lipids). As feeding progresses, a number of feedbacks are initiated. IGF downregulates the GH-IGF system, and INS and Leptin (LEP; produced in peripheral sites, e.g., liver) inhibit feeding. LEP downregulates elements of the GH-IGF system and blocks GH-stimulated effects on IGF production. LEP and GH during periods of fasting promote mobilization of stored energy. These actions, in concert with many others, demonstrate how feeding is coordinated with growth and metabolism and help to explain how GH has both anabolic and catabolic actions depending on nutritional status.
(Supported by the National Science Foundation (NSF), USA, grant IOS 1558037.)

S4-2
INSULIN LIKE PEPTIDES AS MULTITASKERS FOR SUCCESSFUL REPRODUCTION IN RHODNIUS PROLIXUS, A VECTOR OF CHAGAS DISEASE
Jimena Leyria
University of Toronto Mississauga, Canada

The blood-sucking hemipteran Rhodnius prolixus is one of the main vectors of Chagas disease, a neglected tropical disease that affects several million people worldwide. Consuming a blood meal is an event with a high epidemiological impact since after each feed, mated females can lay eggs that result in hundreds of offspring. Insulin-like peptides (ILPs) are peptide hormones that mediate metabolism, growth, lifespan and reproduction in vertebrates and invertebrates. We have used a combination of hormone treatments, gene expression analyses, hormone measurements, RNA interference (RNAi) and ex vivo experiments to investigate how insulin signaling is particularly important in defining the overall hormonal environment for egg production in R. prolixus. The results show that after a blood meal, insulin signaling interprets and responds to nutrient levels to coordinate egg production. The insulin tyrosine kinase pathway is activated to modulate the synthesis of yolk protein precursors (YPPs) in the fat body (the principal organ of intermediate metabolism in insects). In addition, the insulin signaling pathway modulates juvenile hormone (JH) synthesis and release from the corpus allatum, which stimulates egg growth, in part via YPPs and ecdysteroids, synthesised and released from the ovaries, and involved in egg laying as well. Recently, a new ILP named gonadulin was found to be highly expressed in the reproductive system of R. prolixus, particularly in the calyx, through which eggs move into the lateral oviducts at ovulation. Interestingly, we found that gonadulin works through a signaling pathway mediated by LGR3, a leucine-rich repeat-containing G protein-coupled receptors (LGRs) subfamily of G protein-coupled receptors, modulating movements of mature eggs through the calyx; thus, promoting ovulation. This study highlights the ability of ILPs to use different signaling pathways to promote a successful reproductive cycle, coordinating oogenesis, ovulation and oviposition. Understanding the physiological processes involved in reproduction in R. prolixus sheds light on potential targets for effective production of biopesticides by translational research, thereby controlling insect populations and transmission of the disease.
(Supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) through Discovery Grants to Angela B. Lange and Ian Orchard)

S4-3
REGULATION OF BLOOD DIGESTION IN LYME DISEASE VECTOR TICK, IXODES SCAPULARIS
Monika Gulia-Nuss, Jeremiah Reyes, Arvind Sharma, Michael Pham
University of Nevada, Reno, USA

Ixodes scapularis is the principal vector of Lyme disease in the Eastern United States. Each active life stage (larva, nymph, and adult) takes a blood meal either for developing and molting to the next stage (larvae and nymphs) or for oviposition (adult females). This protein-rich blood meal is the only food Ixodes ticks take; therefore, efficient blood digestion is critical for survival. Studies in partially engorged ticks have shown that cathepsin proteases carry out the initial stages of digestion within acidic digestive cells. However, understanding the regulation of blood digestion beyond six days of feeding on the vertebrate host is still lacking. Here, we investigated the changes in gene expression during and after blood feeding and the potential role of serine proteases in blood digestion in ticks. RNAseq and TMT proteomics techniques were used for gene expression, RNA interference was used for functional gene analysis, and a trypsin-benzoyl-D, L-arginine 4-nitoanilide assay was used to measure active trypsin levels. Our data suggest that blood digestion shifts from early feeding (while still attached to the host) to replete females. Serine proteases were among the most differentially expressed genes in our dataset. Knockdown of serine proteases negatively impacted blood feeding, survival, fecundity, and levels of active trypsin in the midgut, resulting in lower hemoglobin degradation. Incubation of midgut extract with a trypsin inhibitor resulted in 65% lower hemoglobin degradation. We provide evidence of the serine proteases as digestive enzymes in fully engorged, replete females. Understanding the digestive profile of trypsin during blood meal digestion in I. scapularis improves our understanding of the basic biology of ticks.
(This work was funded by the Plymouth Hill Foundation, NY, and the UNR Genomics Core.)

OR4-1
EFFECTS OF FASTING UPON THE SOMATOTROPIC AXIS AND METABOLISM IN THE GREEN IGUANA
Valeria Urban, José Ávila-Mendoza, Martha Carranza, Maricela Luna, Carlos Guillermo Martínez-Moreno, Carlos Arámburo
Instituto de Neurobiología, Universidad Nacional Autónoma de México, México

The somatotropic axis plays an important role in modulating metabolism. In many species, it has been shown that fasting induces an increase in GH levels to favor lipolysis and obtain energy from fat. However, little is known about the neuroendocrine factors regulating this increase in serum GH. In vertebrates, most of the studies focused on elucidating the mechanisms involved in the regulation of the somatotropic axis components and its relationship with metabolism have been carried out in mammals, and to a lesser extent in birds and fish; while work on amphibians and reptiles is still very scarce. In this study, we evaluated the effect of acute (2 days) and chronic (10 days) fasting upon several metabolic and somatotropic axis parameters in the green iguana. Results showed that stomach weight decreased in both experimental conditions, while caecum weight decreased only under chronic fasting. Serum glucose decreased significantly only after 10 days of fasting, while the concentration of free fatty acids increased in both conditions. In turn, serum GH concentration increased significantly after 2 days of fasting, but after ten days its levels were similar to the control group. On the other hand, pituitary GH mRNA expression significantly increased in both fasting periods. The rise in serum GH was not reflected in IGF-1 concentrations, which decreased both in the liver and in circulation. Additionally, hepatic IGF-1 mRNA expression decreased considerably in comparison with the control. Our results also showed that, regarding the mRNA expression of several GH hypothalamic secretagogues, only PACAP mRNA was significantly increased after 2 days of fasting. These data indicate that, in reptiles, the adaptive response to fasting involves a complex regulation of the somatotropic axis, that deserves further investigation.
(We thank Gerardo Courtois for animal care and Adriana González Gallardo for technical support at the Proteogenomic Unit, INB, UNAM. This work was supported by PAPIIT-DGAPA (IN227020, IN209621, IN215522, IA200622,); CONACYT (CF214971), PILGRIM´S México and a fellowship from CONACYT to VAUS 787975/612881)

OR4-2

SULPIRIDE REDUCES HYPERGLYCEMIA AND INSULIN RESISTANCE IN DIET-INDUCED OBESE FEMALE AND MALE MICE

Dina Iathzil Vázquez Carrillo(1), Ana Luisa Ocampo Ruiz(2), Arelí Báez Meza(3), Ericka Gabriela Ramírez Hernández(4), Elva Hortencia Adán Castro(5), Ericka Alejandra de los Ríos Arellano(6), Jose Fernando García Ródrigo(7), José Luis Dena Beltrán(8), Magdalena Karina Sánchez Martínez(9), María Georgina Ortiz Arballo(10), Gonzalo Martínez de la Escalera Lorenzo(11), María del Carmen Clapp Jiménez(12), Yazmín Macotela Guzmán(13)
(1)UNAM, México (2)(5)(6)(7)(8)(9)(11)(12)(13)Instituto de Neurobiología, UNAM, Campus Juriquilla, (3)Biología Molecular Diagnostica SA de CV (4)Joslin Diabetes Center/ Harvard Medical School. Boston, MA, (10) Instituto de Neurobiología, UNAM; Anáhuac Querétaro.

Low PRL levels correlate with higher risk/prevalence of metabolic diseases1. In obese male rats with low PRL levels, treatment with PRL improves metabolic parameters2. Therefore, our hypothesis is that drugs that increase PRL, such as sulpiride, an antagonist of dopamine D2 receptors, could have beneficial effects against obesity-derived metabolic alterations. Previous studies have shown that sulpiride exerts sexually dimorphic effects: producing hyperglycemia and hyperinsulinemia in male rats3, and increasing body weight while reducing insulin levels without altering glucose levels in females4. To test whether we could recapitulate the beneficial effects of PRL in obese rats, we evaluated whether sulpiride would improve obesity-derived metabolic alterations in obese male and female mice. For this, C57BL/6 8-week-old mice fed a high-fat diet (HFD) for 8 weeks to induce obesity, were administered daily with 30 mg/kg of sulpiride during the last 4 weeks of the diet. Sulpiride induced a larger increase in PRL levels in females than in male mice: around 70 ng/mL in control and obese male mice (OMM), and 113 ng/mL in control and 147 ng/mL in obese female mice (OFM). Regarding metabolic parameters, in OMM, sulpiride decreased hyperglycemia, insulin resistance, triglyceride levels, and energy expenditure, without affecting body weight or caloric intake; and in OFM, sulpiride treatment decreased hyperglycemia, without alterations in insulin, triglyceride levels, body weight or caloric intake. Sulpiride reduced hypertrophy in visceral adipose tissue (AT) of OMM and normalized the expression of hypoxia inducible factor 1a (a marker of hypoxia), and of the Prlr, the insulin receptor and the glucose transporter 4 (markers of insulin sensitivity), all altered in obesity. Meanwhile, in OFM, sulpiride increased subcutaneous AT weight and the number of adipocytes. In conclusion, in both obese male and female mice, sulpiride reduces hyperglycemia and improves metabolic parameters, however, it exerts sexually dimorphic actions.
(We thank Xarubet Ruiz-Herrera, Fernando López-Barrera, Daniel Mondragon, Antonio Prado, Deisy Gasca-Martínez, Juan Ortiz, Martín García-Servín, Alejandra Castilla and María Antonieta Carbajo for their technical assistance. Project supported by CONACYT grants: Fondo Sectorial de Investigación para la Educación 284771 to YM. Student PhD CONACYT fellowship 531683.)

Monday, May 29th, 2023
14:30 – 16:00
Salón Claustro II

NASCE 2023 Symposium 5:
Thyroid hormone and vertebrate development.
Co-Chairs:
Yun-bo Shi and Aurea Orozco Rivas

S5-1

THYROID HORMONE RECEPTOR ALPHA 1 IN UTERUS FUNCTIONS

Sheue-yann Cheng (1), Elijah Edmondson(2), Minjun Kim(3), Jack Zhu(4)
(1) National Cancer Institute, NIH, USA (2)Molecular Histopathology Laboratory, Leidos Biomedical Research, Inc. Frederick National Laboratory, (3)(4)Laboratory of Cancer Biology, Center for Cancer Research, National Cancer Institute.

Thyroid hormone receptors (TRs) mediate the genomic actions of the thyroid hormone (T3). Due to the critical functions of TR, mutations of THRA gene cause a human disease known as resistance to thyroid hormone (RTHalpha). We created a mouse model expressing a dominant negative mutated TRalpha1 (ThraPV/+ mice) that exhibits severely retarded growth, bone abnormalities, constipation, and anemia as shown in RTHalpha patients. In addition, we also found that the uterus of mutant mice atrophied with 50-60% reduction in weight as compared with wild-type (WT) mice. The mutant uterus showed reduced proliferation, increased apoptosis, and loss of ~90% of uterine glands. Moreover, squamous metaplasia of the uterus was observed in >70% of the uterine epithelium of all ThraPV/+ mice examined. Bioinformatic analysis of the RNA-Seq data from laser captured micro-dissected endometrium identified 592 differentially expressed genes (DEG) (>2-fold changes; p<0.1) in which 489 genes were up-regulated and 102 genes were down-regulated in the mutant endometrium. Remarkably, the top 10 canonical pathways identified by Ingenuity Pathway Analysis (IPA) of DEG were mainly related to altered signaling in lymphoid immune cells and related functions. Consistently, histological analysis showed that cytotoxic T-cells (CD3+/CD8+) were significantly increased in the mutant endometrium, which could account for the uterus atrophy and squamous metaplasia of the mucosa. Among five differentially expressed cytokines identified by RNA-Seq, interleukin-33 (IL-33) was validated to be highly elevated in mutant endometrium by qRT-PCR and western blot analysis. IL33 regulates immune responses and is elevated in tissue inflammation and injuries. These findings show that the ThraPV/+ mouse is a valuable model for understanding the critical role of aberrant immune responses in infertility frequently associated with female patients with thyroid dysfunctions.
(Supported by the Intramural Research Program of NIH.)

S5-2

GENETIC ANALYSIS OF THYROID HORMONE REGULATION OF INTESTINAL REMODELING IN XENOPUS

Yuki Shibata(1), Kenta Fujimoto(2), Ken-ichi T. Suzuki(3), Takashi Hasebe(4)
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Xenopus species, anuran amphibians, are one of the most widely used vertebrate model organisms in the history of physiological, cell, and developmental biology research. Of particular interest is intestinal remodeling, including the removal of larval epithelial cells via apoptosis and de novo formation of adult intestinal stem cells (ISCs), as seen in mammals. Importantly, ISCs are formed through dedifferentiation of some larval epithelial cells during frog metamorphosis that is controlled by thyroid hormone (TH) via gene regulation by its receptor (TR). We have reported that knockout of two TR genes, TR-alpha and TR-beta, resulted in suppression of apoptosis of larval epithelial cells and complete disruption of ISC formation, development, and proliferation, indicating the essential role of TR-mediated TH signaling in intestinal remodeling. We have also shown that TH regulates several signaling pathways during intestinal remodeling, including Sonic Hedgehog (Shh)/ Bmp4, Wnt, Notch, and Hippo pathways, and that these pathways are involved in the stem cell niche formation. Recently, we have identified for the first time a novel safe harbor site, transforming growth factor beta receptor 2-like (tgfbr2l) locus in the X. laevis genome and established the non-homologous end joining (NHEJ) based strategy to generate transgenic X. laevis by using CRISPR/Cas9 (NEXTrans). In this method, the donor plasmid containing a tissue-specific promoter driving a reporter gene can be integrated into the tgfbr2l locus with relatively higher efficiency than previous methods. For future investigation, we are now preparing to utilize the NEXTrans method to identify the precursor of ISCs (pre-ISCs), which dedifferentiate into ISCs in response to TH, by the spatiotemporal expression patterns of the receptor tyrosine-like orphan receptor 2 (Ror2) gene, a marker of pre-ISC, during intestinal development. We expect that this will open new avenues to elucidate the molecular mechanisms of how and when the origin of ISCs is determined.
(We are grateful to Dr. Yun-Bo Shi for inviting us to present our research at this symposium.)

S5-3

THYROID HORMONE AND GLUCOCORTICOID EFFECTS ON TRANSCRIPTOME AND METHYLOME IN XENOPUS TROPICALIS TADPOLES

Laurent M. Sachs(1), Nicolas Buisine(2), Vincent Jonchere(3), Alexis Grimaldi(4), Muriel Rigolet(5), Daniel Buchholz(6), Evelyne Duvernois-Berthet(7), Corinne Blugeon(8)
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Thyroid hormones (TH) have been shown to govern post-embryonic development in vertebrates inducing during Anuran amphibian metamorphosis spectacular morphological and biochemical modifications. However, TH do not act alone. In particular, corticosterone (CS) play important roles during this process including the proper timing of metamorphosis and the main mediators of the stress response. Thus, the physiological processes of metamorphosis and stress coping are very much intertwined. In order to probe the effects of TH, CS, and the crosstalk between these two hormones on the transcriptome of three Xenopus tropicalis organs with contrasted fate (either proliferation for the hind-limbs no morphological changes for the liver or cell death for the tail fin epidermis), we performed systematic sequencing of RNA (RNA-Seq). We found that the presence of the two hormones does not have the effect of adding the effects of these hormones alone. The biological functions affected by crosstalk are mostly related to cell proliferation, extracellular matrix remodeling and the immune system in both tissues, the set of genes involved is very tissue specific. In limb, we further showed that the crosstalk affects expression of a restricted number of genes, mostly involved in skeletal development and homeostasis and causing the appearance of phenotype. TH was also shown to regulate the expression of genes coding for DNA methylation modifying enzymes. Acting on DNA methylation is an important means of regulating the expression of gene clusters and a central mechanism for coordinating developmental transitions. However, DNA methylation is dynamic and also integrate signals from the constantly changing environment. We have then initiated the study to locate TH and CS effects on DNA methylation profiles to characterize potential determinants for stress and thus adaptation to a variation of the environment. Following treatment with TH and CS independently or in combination, we have used a method for capturing methylated DNA (MethylCap) combined with high-throughput sequencing (MethylCap-Seq). Again,the presence of the two hormones does not have the effect of adding the effects of these hormones alone. A strong tendency for DNA methylation markers to be removed following treatment with TH and GC is observed. The majority of differential methylated regions (DMR) are tissue specific. The DMRs are generally not localize near genes regulated or not by the two hormones.
(This work has been carried out in the context of ?IDEAL?, a large European Integrated Project funding from FP7 (contract n°259679), ?TRIGGER?, an ANR JCJC funding and « MethylDev », a PICS CNRS funding program.)

OR5-1

DISRUPTION OF THYROID HORMONE RECEPTOR ALPHA A (THRAA) PROMOTES HEART REGENERATION IN ZEBRAFISH

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(1)(2)(3)The Chinese University of Hong Kong, Hong Kong (4)Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health.

Myocardial infarction (MI) is an ischemic event that commonly occurs due to decreased or blocked coronary circulation and results in massive cardiomyocyte (CM) death and damage to the cardiac muscle. Induction of post-MI cardiac regeneration is a topic of intensive research in regenerative medicine. A recent study suggested that reducing thyroid hormone (TH) can promote heart regeneration in mice. Some vertebrate animals, such as zebrafish, show remarkable cardiac regenerative capacity after ventricular apex resection. We employed the zebrafish mutant of thraa, the paralogue of human Thyroid Hormone Receptor alpha (THRA), to assess the influence of TH on heart regeneration. Using CRISPR/Cas9 genome editing technology, a mutant zebrafish line at the thraa gene locus was generated, expressing a C-terminal truncated mutation, as found in patients resistant to THs. A time-course experiment conducted with RNA-sequencing techniques revealed that attenuation of TH signaling shows beneficial effects on heart regeneration in zebrafish. The thraa mutant exhibited a stronger inflammatory response upon injury and has an extended period observed with proliferating cardiomyocytes. Lower metabolic activity at the regenerative stage altered the process switching between the proliferation and differentiation of cells, and more proliferating cardiomyocytes could be advantageous to the reconstruction of cardiac muscle structure. Moreover, the thraa mutant showed a significant reduction in the fibrotic area. Our data suggested that attenuation of TH signaling can enhance the process of regeneration through its regulation of inflammation and metabolism.
(Supported by the Research Grants Council of Hong Kong (14112618 and 14119120) to HZ. Additional support was provided by the Hong Kong Branch of CAS Center for Excellence in Animal Evolution and Genetics to HZ (8601012))

OR5-2

SPECIFIC TEMPORAL WINDOWS OF T3 ACTION IN OLIGODENDROGENESIS. LESSONS FROM THE ZEBRAFISH.

Aurora Olvera Vidal(1), Isela García-Martínez(2), Ximena Meza(3), Santiago Pech-Pool(4), Iván Lazcano(5), Aurea Orozco(6)
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), México

During oligodendrogenesis, neural precursor cells (NPC) differentiate into oligodendrocyte (OL) precursor cells (OPC), which give rise to mature myelinating OL, these lasts are the specialized cells that synthesize myelin in the CNS. In vertebrates, the bioactive thyroid hormone T3 is a critical key element that regulates this process. Zebrafish (Danio rerio) is an excellent model to study oligodendrogenesis since in this species a major generation of OPC takes place as early as 30 hours post-fertilization (hpf) and mature OL are identified after 72 hpf. During this period, the maternal T3 load is the only source of this hormone, and its importance during early development was highlighted when we blocked its bioavailability with iopanoic acid (IOP) from 0 to 72 hpf and observed a significant decrease in OPC marked with anti-NG2 antibodies. Furthermore, reduced myelination in the brain accompanied by a decrease of transcripts involved in OL differentiation (olig2, mpz and plp1b) were also observed. IOP + T3 (5 nM) co-administration rescued these impairments. Given that T3 acts on specific critical windows during CNS development, we were interested in analyzing early T3 effects in oligodendrogenesis. To this aim, we treated zebrafish embryos at 0-1, 1-2 and 2-3 days post-fertilization (dpf), when NPC, OPC, and OL start to differentiate, respectively, and found a reduction of myelin only in larvae treated during 0-1 dpf as revealed by myelination patterns and OPC staining, suggesting that the input of T3 at early developmental stages affects oligodendrogenesis maybe due to the OPC pool proliferation disruption. We treated larvae with the demyelinating drug cuprizone for 5 h at 3 dpf when CNS myelin is evident and observed a decrease in its content at 7 dpf. This alteration was not perceptible in fish co-treated with 0.1 nM T3. These results suggest that exogenous T3 could rescue demyelination by promoting OPC proliferation or by promoting the maturation of OL from the preexisting OPC pools. Thus, T3 seems to act during different critical windows during oligodendrogenesis, very early (0-1 dpf) during OPC differentiation and later (3 dpf) in OL maturation, demonstrating its role in cell fate and cell lineage differentiation.
(Supported by UNAM PAPIIT IN210823 and IA201122; CONACyT Ciencia de Frontera, Paradigmas y Controversias de la Ciencia 2022 399880.)

Monday, May 29th, 2023
14:30 – 16:00
Salón Claustro III

NASCE 2023 Symposium 6:
Comparative endocrinology of osmoregulation.
Co-Chairs:
Jason P. Breves and Stephen D. McCormick

S6-1

CENTRAL AND PERIPHERAL CONTRIBUTIONS OF THE CORTICOTROPIN-RELEASING FACTOR (CRF) SYSTEM TO SMOLTIFICATION AND SEAWATER ACCLIMATION IN ATLANTIC SALMON

Nicholas J. Bernier(1), Brett M. Culbert(2), Stephen D. McCormick(3)
(1)(2)University of Guelph / Department of Integrative Biology, Canada (3)U.S. Geological Survey, Leetown Science Center, Conte Anadromous Fish Research Laboratory, Turners F

While CRF-related peptides can stimulate pituitary ACTH secretion in vitro, the physiological conditions under which these factors contribute to the regulation of the corticotropic axis remain unclear. In fishes, the presence of CRF-related peptides in regions of the central nervous system responsive to osmotic challenges, and the expression of CRF system components in the gills and intestine, suggest an involvement in osmoregulatory control. In this study, we took advantage of the known increase in the activity of the corticotropic axis associated with smolt development and seawater transfer in Atlantic salmon to address these questions. Specifically, we evaluated the changes between migratory smolts and pre-migratory parr in circulating levels of cortisol and ACTH, as well as transcript abundance of major components of the CRF system in hypophysiotropic regions of the brain, in the CNSS, and in key osmoregulatory tissues. Smolts had higher plasma cortisol levels than parr throughout the spring and plasma ACTH peaked in May for smolts. While transcript abundance of preoptic area crfb1 was upregulated in smolts compared to parr throughout the spring, the transcriptional changes in other CRF-related peptides were generally unrelated to changes in circulating ACTH and/or cortisol levels. In the hypothalamus, smolts had much higher urotensin 1a (UTS1a) expression compared to parr in May through July. Smolts also consistently had higher CNSS crfb1 and uts1a mRNA levels than parr throughout the spring. Seawater transfer in May increased the expression of central and peripheral components of the CRF system in both smolts and parr. Most notably, the osmotic stressor elicited increases in transcript levels of hypothalamic uts1a, gill crfa2 and CRF receptor 1b, and posterior intestine urocortin 2a and CRF receptor 2b. Together, our results implicate central and peripheral CRFb1 and UTS1a in the neuroendocrine regulation of the corticotropic axis during smoltification, and suggest that specific components of the CRF system are involved in seawater acclimation via direct osmoregulatory functions in the gills and intestine of Atlantic salmon.
(Supported by an NSERC Discovery grant provided to NJB. BMC was supported by a NSERC Doctoral Canadian Graduate Scholarship and an Ontario Graduate Scholarship.)

S6-2

STRATEGIES OF ANADROMOUS SEA LAMPREY FOR COPING WITH SALINITY CHALLENGES BY REGULATING HORMONE SIGNALING SYSTEMS AND ION-TRANSPORTERS IN THE GILL

Ningping Gong(1), Jessica Norstog(2), Stephen McCormick(3), Mark Sheridan(4)
(1)Texas Tech University, USA (2)University of Massachusetts, (3)US Geological Survey (4)Texas Tech University.

Anadromous sea lamprey are native to the Atlantic Ocean and have a complex lifecycle involving fresh water (FW) and seawater (SW) phases. Larval sea lamprey live exclusively in FW and acquire SW tolerance during metamorphosis before their migration to the ocean. We investigated sea lamprey, a representative of one of the oldest extant lineages of vertebrates, to understand their strategies for coping with salinity changes and to provide new insight into the evolution of hormonal control of osmoregulation. Growth hormone (GH) and prolactin (PRL) play important roles in the osmoregulation of teleosts by promoting adaptation to SW and to FW, respectively. GH and PRL-like and their signaling systems have been identified in the sea lamprey, and we found that sea lamprey PRL-like facilitates the transition from FW to SW through downregulation of some key ion-transporters that are crucial to FW adaption and salt uptake. Correspondingly, the distribution of PRL-like protein was mapped in the gill filaments of juvenile sea lamprey; PRL-like immunoreactive staining was significantly weaker in filaments of juveniles acclimated to SW compared to filaments in juveniles acclimated to FW and to ion-poor water (IPW). Furthermore, the hormonal effects on the ion-transporters as well as their signaling systems were studied in juvenile sea lamprey injected with recombinant sea lamprey GH or PRL-like following transfer from SW to FW, or without salinity change. The receptors for GH and PRL-like (GHR and PRLR, respectively) were upregulated in the gill of the control group, 2 days after the transition from SW to FW, concomitant with upregulation of several genes of FW-adapting ion-transporters; however, hormone treatments did not affect the expression of ion-transporters following FW transfer nor under conditions of constant salinity. These studies indicate that juvenile sea lamprey adapt to various salinity changes by regulating the expression of branchial ion transporters. Although hormonal effects are profound in the regulation of ion transporters during adaption to high salinity (from FW to SW), their role in the adaption to lower salinity (from SW to FW) is unclear and merits further investigation.
(Supported by National Science Foundation (USA) grant 1558037 to MAS and SDM)

S6-3

THE PROLACTIN CELL AS A NEXUS FOR THE INTEGRATION OF OSMOTIC AND THERMAL SENSORY MODALITIES

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University of Hawai, USA

With over 300 described biological functions in vertebrates, the pleiotropic hormone prolactin (Prl) is well known for its role in osmoregulation in euryhaline fish. In the Mozambique tilapia, Oreochromis mossambicus, Prl cells, which comprise over 95% of the rostral pars distalis (RPD) of the pituitary gland, have been employed as a model for studying osmoreception, the first step in osmoregulation, and recently described to be thermosensitive. The native distribution of Mozambique tilapia is characterized by estuarine areas subject to salinity variations between fresh water (FW) and seawater (SW). Acclimation to changes in environmental salinity is largely mediated by Prl, which restores hydromineral balance by acting through specific receptors, Prlr1 and Prlr2. Consistent with multiple biological functions, the release of Prl177 and Prl188, the two isoforms of this hormone found in tilapia, is directly stimulated by a fall in extracellular osmolality, rise in temperature, and modulated by various autocrine, hypothalamic, and extra-hypothalamic factors. Multiple transcription factors (TFs) predicted to bind promoter regions of prl177 and prl188 genes, including the TF modules OCT1_PIT1 01, CEBP_CEBP 01 and BRNF_RXRF 01, were activated in FW- acclimated tilapia RPDs, while SORY_PAX3 02 and SP1F_SP1F 06, SP1F_SP1F 09 were activated in SW fish. Moreover, prl177, prl188, prlr1, prlr2 and TF transcripts, including pou1f1, pou2f1b, creb3l1, cebpb, stat3, stat1a and nfat1c, were sensitive to both thermal and osmotic stimuli, in vitro. Combined, these findings set a path for resolving how adaptive patterns of Prl secretion are linked through the integration of thermosensitive and osmoreceptive processes.
(HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01), and NSF (IOS-1755016) to A.P.S.)

OR6-1

THE INFLUENCE OF DIURETIC AND ANTI-DIURETIC FACTORS ON AQUAPORIN EXPRESSION IN FEMALE A. AEGYPTI

Britney Picinic, Farwa Sajadi, Andrew Donini, Jean-Paul Paluzzi.
York University, Canada

Adult female Aedes aegypti mosquitoes acquire a blood meal from vertebrate hosts for egg maturation which creates an osmoregulatory challenge requiring a diuretic response, where neuroendocrine factors are released to initiate an increase in fluid secretion, to maintain homeostatic fluid levels. A dilute urine is formed in the Malpighian tubules (MTs) of blood-fed mosquitoes, followed by selective reabsorption in the hindgut before excretion. Diuresis in A. aegypti is tightly controlled by the release of diuretic factors such as diuretic hormone 31 (DH31), corticotropin releasing factor (CRF)-like peptide, and serotonin (5-HT), allowing females to eliminate excess fluid and ion waste post blood meal. However, the diuretic process must be finely controlled, to avoid excess loss of water and this regulation can involve anti-diuretic factors, including CAPA. The movement of ions across the MT epithelium has been extensively studied while the movement of water post blood meal in response to diuretic and anti-diuretic factors remains understudied. Water can be transported through the tubule epithelium by aquaporin (AQP) channels, which are transmembrane domain proteins that allow for the single file movement of water through the cell membrane. In A. aegypti, six AQP genes have been identified, including both water-specific AQPs and entomoglyceroporins that can transport water along with other small, uncharged solutes. This study looks at the influence of three diuretic hormones found in A. aegypti (DH31, CRF, and 5-HT) and the anti-diuretic CAPA on AQP1 and 4 abundances in female mosquitoes, 0.5hr and 24hr post-blood meal. The objective is to determine if individual neuropeptides or a combination of them influences AQP abundance or localization in the MTs. Through western blotting, we have shown that diuretic CRF in combination with anti-diuretic CAPA induces a significant increase in AQP4 protein in 24hr post blood meal MTs. Immunohistochemistry further confirms this with increased staining intensity for AQP4 24hr post blood meal. Immunohistochemistry also provides evidence that DH31 and 5-HT influence AQP abundance in the MTs of blood fed females. Ongoing studies with AQP knockdown are examining the influence of both diuretic and anti-diuretic factors on AQP1 expression in adult female A. aegypti.
(Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an NSERC Discovery Grant (JPP).)

OR6-2

REGULATION OF THE HYPOTHALAMUS-PITUITARY-INTERRENAL (HPI) AXIS IN ATLANTIC STURGEON (ACIPENSER OXYRINCHUS) DURING SALINITY ACCLIMATION AND ACUTE STRESS

Ciaran Shaughnessy(1), Valorie Myhre(2), Liam Doherty(3), Amy Regish(4), Daniel Hall(5), Stephen McCormick(6), Robert Dores(7)
(1)(2)(3)(7) University of Denver, USA (4)(5)(6)USGS / University of Massachusetts

In bony fishes, the hypothalamic-pituitary-interrenal (HPI) axis is a highly conserved neuroendocrine axis that regulates corticosteroid production in interrenal tissue via a signaling network including corticotropin releasing hormone (CRH) released from the hypothalamus causing adrenocorticotropic hormone (ACTH) release from the anterior pituitary. In actinopterygians, including sturgeons, cortisol is the terminal corticosteroid produced in the interrenal tissue and acts as both a mineralocorticoid and glucocorticoid. In the present study, we examined the tissue distribution of genes involved in HPI axis signaling and cortisol biosynthesis in the Atlantic sturgeon (Acipenser oxyrinchus). We examined whether and which components of HPI axis signaling are regulated during salinity acclimation and recovery from acute stress. In the first experiment, juvenile Atlantic sturgeon were acutely exposed to dilute seawater (SW) then sampled at 1, 3, and 10 days during SW acclimation. In the second experiment, juvenile Atlantic sturgeon were exposed to an acute handling stress then sampled at 1, 6, and 24 h during post-stress recovery. In both experiments, circulating levels of cortisol and glucose in the blood plasma transiently increased above control values. The transcriptional regulation of hypothalamus crh, pituitary crhr and pomc, and interrenal mc2r, mrap1, star, and cyp11a1 were examined for both experiments. We discuss the role in sturgeons of an HPI axis in mediating the production of cortisol in the contexts of responding to osmoregulatory and non-osmoregulatory stressors.
(This research was supported by the Long Research Endowment at the University of Denver to R.M.D., a National Science Foundation Postdoctoral Fellowship (DBI-2109626) to C.A.S., a National Science Foundation grant (IOS-1558037) to S.D.M., and a University of Denver Undergraduate Research Center Summer Fellowship to V.D.M.)

Tuesday, May 30th, 2023
10:30 – 12:00
Salón Juárez

NASCE 2023 Symposium 7:
Growth hormones: from physiological roles to clinical science.
Co-Chairs:
Farwa Sajadi and Santiago Pech-Pool

S7-1

CURRENT INSIGHTS INTO THE SPONTANEOUS ACTIVITY OF THE RETINA: FROM BASICS TO CLINICS

Stephanie Thebault(1), Rodríguez-Arzate(2) CA, Noguez-Imm R(3), Pérez-Félix M(4), Reyes Ortega P(5), Rodríguez Ortiz LR(6), García Peña MF(7), Hughes-Cano JA(8), Rojas-Piloni G(9), Ordaz B (10), Peña-Ortega F(11), Epardo D(12), Martínez CG(13), Arámburo de la Hoz C(14), Quiroz-Mercado H(15), Hernández-Zimbrón LF(16), García-Franco R(17), Rubio Mijangos JF(18), López-Star E(19), García-Roa M(20), Lansingh VC(21), Muñoz-Benitez JC(22), Medina D(23), Amaro-Cantoral E(24), Silva-Barrera B(25), Barcenas E(26) Molero-Castillo G(27)

(1)(2)(3)(4)(5)(8) Laboratorio de Investigación Traslacional en Salud Visual, México (6)(7)Laboratorio de Neurobiología Molecular y Celular, (9)Laboratorio de Integración Sensoriomotora, (10)(11)Laboratorio de Circuitos Neuronales (12)(13)(14)Laboratorio de Bioquímica de las Hormonas; Instituto de Neurobiología; Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, 76230 Querétaro, Mexico, (15)Research Department, Asociación Para Evitar la Ceguera, Mexico City, Mexico (16)Research Department, Asociación Para Evitar la Ceguera, Mexico city, Mexico/ Clínica de Salud Visual, Escuela Nacional de Estudios Superiores, Unidad León, Universidad Nacional Autonóma de México (UNAM), León, Guanajuato, Mexico. (17)Instituto de la Retina del Bajío (INDEREB), Prolongación Constituyentes 302 (Consultorios 410 y 411, torre 3, Hospital San José), El jacal, 76187, Santiago de Querétaro, Querétaro, Mexico, (18)(19)(20)(21)Instituto Mexicano de Oftalmología (IMO), I.A.P., Circuito Exterior Estadio Corregidora Sn, Centro Sur, 76090 Santiago de Querétaro, Querétaro, Mexico, (22)Instituto Nacional de Astrofísica, Óptica y Electrónica, San Andrés Cholula, Puebla, Mexico, (23)(24)(25)(26)(27) Facultad de Ingeniería; Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Ciudad de México, Mexico

The eyes, considered as the windows of the soul, can also tell about how healthy is an organism. In particular, examining the retina helps predict cardiovascular risk, multiple sclerosis, and obesity. This is usually done using retinal images that capture the structure of the tissue and partially its function, or via the analysis of the retinal response to light using an electroretinogram (ERG). Our group recently introduced a new, quick, and easy ERG modality to assess the intrinsic activity of the retina, characterized by slow, spontaneous oscillations. Current evidence shows that this activity is modified in rodent models of diverse diseases, like obesity, type 1 diabetes, hereditary retinopathy, and glaucoma. In humans, variables extracted from the mock ERG allow the predictive diagnosis of risk factors for type 2 diabetes, including overweight, obesity and metabolic syndrome. Our ongoing research focuses on gaining insights into the basics of this non-evoked ERG signal and its relevance in clinics.
Using pharmacological approaches *in vivo* and *ex vivo*, as well as functional connectivity analyses, we are approximating the origin of this activity and we are studying the predictive power of the retina-specific signal for the diagnosis of metabolic conditions using supervised algorithms. We also provide preliminary data showing that the slow spontaneous oscillations of the retina are responsive to neuroprotective treatment using growth hormone in the optic nerve crush model of glaucoma in rats. Finally, we are introducing the zebrafish as a model where spontaneous retinal oscillations are also present and discuss its potential to study the physiopathology of obesity.
(Acknowledgements: Supported by the Instituto de Neurobiología-UNAM, the UNAM DGAPA-PAPIIT grants IN205420 and IN212823, and CONACYT grant CF-2019-1759 and INFRA-299625.)

S7-2

NEURAL GROWTH HORMONE (GH): FROM BASIC TO APPLIED RESEARCH

Carlos G. Martinez-Moreno, Jerusa E. Balderas-Márquez, David Epardo, Martha Carranza, José Ávila-Mendoza, Maricela Luna
Instituto de Neurobiología UNAM, México

In addition to its well-known roles in tissue development and growth, growth hormone (GH) has potent neurotrophic actions that could lead to the discovery of novel neuroprotective and neuroregenerative treatments. It has been known for over two decades that GH expression is not limited to the pituitary gland, and many of its classical target tissues also express GH in extrapituitary sites. In the nervous system, it is possible to identify neurons that express both GH and its receptor (GHR), suggesting complementary autocrine and paracrine actions. Using the chicken retina as a model to study the functions of locally expressed GH, it was demonstrated that GH has anti-apoptotic actions during early retinal development and can induce axonal growth in retinal ganglion cells (RGCs). The pro-survival actions of GH include its ability to be internalized after GHR activation and to prevent/reverse excitotoxic damage induced by glutamate and kainate in a quail-derived neuroretinal cell line (QNR/D) and in the green iguana (in vivo). Additionally, administration of chicken GH can exert anti-inflammatory actions when applied after a lipopolysaccharide (LPS) insult in vitro and in vivo. Given the observed neurotrophic and anti-inflammatory actions of GH in avian retinal cells under different types of distress, it was investigated whether these effects are conserved in mammals. Using a mechanical compression-induced optic nerve damage model (ONC) to induce cell death, neuroinflammation, and axonal degeneration, it was shown that GH can suppress damage-induced expression several genes of glial activity (eg. Iba1, CD86, CD206, vimentin, GFAP) and inflammatory markers (eg. TNF, IL6, IL1B, iNOS) in the rat retina. Furthermore, in an ONC mammal model, GH was found to promote survival in RGCs and regenerate axons in the injured optic nerve. In conclusion, the neurotrophic and antinflammatory actions of GH are conserved in reptiles, birds, and mammals.
(Supported by grants IN227020, IN209621, IN215522, IA200622, PAPIIT-DGAPA-UNAM; UNAM grant 1130-202-002 to C.A.; grant CF-214971, CONACYT, Mexico. We thank Adriana González-Gallardo at the INB-UNAM Proteogenomic Unit; Ramón Martínez-Olvera at the INB-UNAM Computing Unit: as well as Dr. José Martín García-Servín and Dr. Alejandra Castilla-León at the INB-UNAM vivarium, for technical support.)

S7-3

PI3K AND ERK SIGNALLING IN GOLDFISH ACUTE BASAL AND AGONIST-STIMULATED GH SECRETION, AND LONG-TERM BASAL HORMONE RELEASE AND AVAILABILITY IN VITRO.

John P Chang, Federico Sacchi, George Kinley, Enezi Khalid.
University of Alberta, Canada

Phosphatidylinositol-3-kinase (PI3K, Class I) and extracellular signal regulated kinase (ERK) signalling systems are known to mediate acute growth hormone (GH) release responses to neuroendocrine regulators such as GnRH in goldfish pituitary. Recent results with a pan PI3K inhibitor and selective inhibitors of Class I and III PI3Ks indicate that Class I, but not Class III, PI3Ks also mediate acute ghrelin-elicited GH secretion, and reveal that PI3K classes also differentially modulate acute basal GH release from perifused goldfish pituitary cells. How PI3K-dependent/sensitive downstream signalling mechanisms regulate longer-term basal hormone release and availability are further investigated. Results with inhibitors of PI3K and ERK kinase (MEK)/ERK signalling elements in 2-hr static incubation of primary cultures of dispersed goldfish pituitary cells suggest that Class I PI3Ks exert a negative influence on basal GH release, but not GH availability. Relative to Class I, Class II and/or III PI3Ks may also have different effects on hormone availability. Common PI3K downstream elements phosphoinositol-dependent kinase 1, protein kinase B/Akt, target of rapamycin complex 1 (TORC1) and TORC2 have negative influences on basal GH release; however, these components play dissimilar roles in GH availability. Conversely, ERK signalling negatively and positively regulates basal GH secretion and availability, respectively. Measurements of phospho-Akt and phospho-ERK levels following inhibitor treatments also reveal cross-talks between PI3K- and MEK/ERK-dependent signalling elements in unstimulated goldfish pituitary cells. These results indicate complex interactions between classical signal transduction modules may play important roles in the differential regulation of stimulated and basal activities in goldfish somatotrophs.
(Funded by NSERC Canada.)

OR7-1

NEUROPROTECTIVE AND REGENERATIVE EFFECTS OF GROWTH HORMONE (GH) IN THE EMBRYONIC CHICKEN CEREBRAL PALLIUM EXPOSED TO HYPOXIC-ISCHEMIC (HI) INJURY

Juan Olivares, Martha Carranza, Jerusa Balderas Márquez, David Epardo, Carlos Martínez, José Ávila Mendoza, Maricela Luna, Carlos Arámburo de la Hoz, María del Rosario Baltazar Lara
Instituto de Neurobiología, Universidad Nacional Autónoma de México, México

Prenatal hypoxic-ischemic (HI) injury inflicts a severe damage on the developing brain provoked by a pathophysiological response that leads to neural structural lesions, synaptic loss, and neuronal death. It has been proposed that growth hormone (GH) may act as a neurotrophic factor inducing neuroprotection and neuroregeneration after HI injury. We used the chicken embryo to develop both in vitro and in vivo models of prenatal HI injury in the cerebral pallium (the equivalent of brain cortex in mammals), to examine if GH exerts neuroprotective and regenerative effects in this tissue. For in vitro experiments, pallial cell cultures obtained from chick embryos were incubated under HI conditions (<5% O2, 1 g/L glucose) for 24 h and treated with 10 nM GH, and then collected for analysis. For in vivo experiments, embryos (ED14) were injected in ovo with GH (2.25 µg), exposed to hypoxia (12% O2) for 6 h, and then the pallial tissue was obtained to perform the studies. GH exerted an anti-apoptotic effect, promoted cell survival and proliferation of HI-injured pallial neurons, in both models. Neuroprotective actions of GH involved the activation of Bcl-2 and ERK1/2 signaling pathways. Remarkably, GH protected mature neurons, which were particularly harmed by HI injury, but also stimulated neural precursors. In addition, GH increased the number and length of neurite outgrowth and branching in HI-injured pallial neurons, and these effects were blocked by a specific GH antibody. Furthermore, it was found that the local expression of several synaptogenic markers (NRXN1, NRXN3, GAP-43, NLG1) and neurotrophic factors (GH, BDNF, NT-3, IGF-1, and BMP4) were increased after GH treatment during HI damage. GH exerts protective and restorative effects in brain pallium during prenatal HI injury, and these actions could be the result of a joint effect between GH and endogenous neurotrophic factors. Also, they encourage further research on the potential role of GH as a therapeutic complement in HI encephalopathy treatments.

(Acknowledgements: Supported by PAPIIT-DGAPA (IN227020, IN209621, IN215522, IA200622) and CONACYT (285004). Postdoctoral fellowships to J.D.O.-H were provided by CONACYT (# 261533, 2020-2021) and Fondo Alejandro Bayón (2021-2022).

OR7-2

VASOINHIBIN ACTS DIRECTLY ON CANCER CELLS TO INHIBIT THROMBIN-INDUCED PROLIFERATION AND INVASION

Maria Magdalena Zamora Corona, Juan Pablo Robles, Alma Lorena Perez, Jessica Martinez, Mauricio Chaveste, Gonzalo Martinez de la Escalera, Carmen Clapp
Instituto de Neurobiología, Universidad Nacional Autónoma de México, México

Thrombin is a multifaceted protease promoting blood coagulation, tissue repair, and tumor growth and invasion. We recently showed that thrombin cleaves prolactin into anti-angiogenic, profibrinolytic vasoinhibin during the clotting process suggesting vasoinhibin as a thrombin-activated mechanism for the regulation of hemostasis and angiogenesis in response to tissue injury. However, the functional interaction between thrombin and vasoinhibin extends beyond tissue repair. Here, we show that vasoinhibin acts on cultured cancer cells (melanoma, prostate, and breast) to inhibit thrombin-induced proliferation and invasion. Furthermore, vasoinhibin treatment of melanoma cells stimulated with thrombin reduced the number of metastases in an in vivo murine model. Although vasoinhibin is known to inhibit tumor growth and metastasis via its anti-angiogenic properties, this is the first report of a direct action of vasoinhibin on cancer cells. The fact that vasoinhibin has both vascular and non-vascular-dependent inhibitory effects on cancer encourages the potential use of newly discovered vasoinhibin analogs as therapeutic agents in the clinic.

(Supported by CONACYT grant A1-S-9620B. We thank Xarubet Ruíz Herrera, Fernando López Barrera, Alejandra Castilla León, José Martín García Servín, and María A. Carbajo for their excellent technical assistance.)

Tuesday, May 30th, 2023
10:30 – 12:00
Salón Claustro II

NASCE 2023 Symposium 8:
Role of the endocrine system in ecological tradeoffs.
Co-Chairs:
James Carr and Breanna Harris

S8-1

VISUAL SYSTEM NEUROPEPTIDES MODULATE PREDATOR/PREY TRADEOFFS

James Carr, Breanna N. Harris, Peter A. Keyel, Christine M. Prater, Caoyuanhui Wang
Texas Tech University, USA

Subcortical visual pathways play an important role in controlling prey capture and predator avoidance behaviors. Identifying the precise role of subcortical visual pathways in defense and avoidance tradeoffs in mammals is complicated by the presence of a visual cortex, which also responds to visual threats. Amphibians are an excellent model for studying the contribution of subcortical pathways to these behavioral decisions as they lack a visual cortex but otherwise engage similar defensive mechanisms (visual thalamus, amygdala, HPA axis, etc) in response to a visual threat. Work from our laboratories, and from others, indicates that the optic tectum of anuran amphibians has an intrinsic CRF signaling system and that CRF, acting on CRFR1 receptors, can modulate food intake as well as certain aspects of prey capture and antipredator behavior. Tectal CRF neurons also appear to be active in response to non-specific stressors and tectal CRFR1 receptors modulate non-specific stressor-induced changes in food intake. We also have identified a potential role for NPY and Y2R receptors in suppressing the approach to food when a predator is present. We are currently using physiological and imaging methods to identify which classical neurotransmitter systems are involved in mediating the modulatory effects of CRF and NPY on appetitive and avoidance behaviors. Collectively, our data suggest a role for neuropeptides in modulating discrete aspects of innate approach and avoidance behaviors and suggest the possibility that visual system neuropeptides play a role in responding to novel threats.

(This work was supported by the National Science Foundation (IOS, #1656734).)

S8-2

STRESS AND ECOLOGICAL TRADEOFFS: PREDICTIONS, PERSEVERANCE, AND THE PACING OF PANDEMIC PRODUCTIVITY

Breanna N. Harris
Texas Tech University, Lubbock, TX, USA

All organisms, including humans, make behavioral decisions that impact reproduction, survival, and ecological interactions. These decisions are made by integrating complex information about the environmental landscape and ideally result in behaviors that maximize survival and fitness. Endocrine systems related to stress, feeding, reproduction, and growth are hypothesized to respond to ecological and environmental changes and thus influence behavioral decisions, often resulting in tradeoffs. This talk has two parts, both relating to stressors and ecological tradeoffs. First, I will categorize 131 hypothesized relationships among stress, the endocrine stress responses, and tradeoffs as well as life-history transitions in vertebrates. Of those 131, the majority made predictions about reproduction (n =43), the transition from health to disease (n = 38), development (n = 23), and stress coping (n = 18). Additional hypotheses were classified as stage-spanning or models (n=37). I will conclude with recommendations for testing stress hypotheses. Next, I will reverse the lens and apply the framework of ecological tradeoffs to explore how the pandemic-induced chronic stress impacted productivity of academics. COVID19 upended our personal and professional lives, but certain groups were disproportionately affected, particularly when it comes to the currency of academic: publications. I will describe the role that differential identities played on the pause vs. persevere tradeoff during COIVD19 and then show submission data from General and Comparative

Endocrinology. Several journals saw submissions by women fall precipitously during the pandemic. I compiled submission data for 1375 articles submitted to GCE from May, 2019 through Dec, 2021 and used Google/Research Gate/ORCID/LOOP photos and pronoun use to code the gender (woman, man, unknown) of the first and corresponding authors over time (pre-pandemic; year 1; year 2). Gender was recorded on 85% of corresponding authors and 77% of first authors. Overall submission number was lowest in year 2; additional analyses underway. Lastly, I will connect parts 1 and 2 with broad hypotheses and study questions and highlight the importance of not only interrogating the ecological context of our study organisms, but also of ourselves to assess the ecological context of stressors in academia and how they shape (career) survival and persistence.
(The National Science Foundation (IOS, #1656734), which partially funded my time. Dr. James Carr for inviting me to co-edit the first special issue of General and Comparative Endocrinology and to co-chair a symposium at this year's meeting, and for co-editing the GCE SI about COVID with me.)

S8-3
A COMPARATIVE APPROACH TO THE STUDY OF MAMMALIAN MATERNAL BEHAVIOR: NEST-BUILDING IN RABBITS, SOWS, AND RATS
Gabriela González Mariscal
Centro de Investigación en Reproducción Animal, CINVESTAV- Universidad Autónoma de Tlaxcala, México.

Mammalian maternal behavior has been explored from the perspectives of ethology,neuroscience, endocrinology, and pharmacology. Studies have revealed common neuroendocrine factors regulating the expression of activities directly related to caring for the young (e.g., nest-building and nursing) and others indirectly associated with maternal behavior (e.g., social isolation and foraging). Comparisons among specific mammals regarding the ways by which ?essential? maternal activities are expressed and controlled can illuminate the evolution of maternal care and the mechanisms underlying hormone actions on the brain. Rabbits, sows, and rats build a nest where they will give birth and nurse the young. The time when this occurs varies among these species: doe rabbits start building a nest already in early pregnancy, while sows and rat dams perform this activity close to parturition. Nest-building in does is a three-stage process involving: digging, straw-carrying, and hair-plucking. Sows also dig a shallow burrow that they line with hay, sawdust or tree branches. Dams use almost any available soft material and shape it into a sphere in the place selected for delivery. In these three species nest-building is displayed only by pregnant animals and its onset/offset is regulated by a combination of hormones and environmental stimuli. Estradiol and progesterone ?whose levels in blood change across gestation- are key players in stimulating nest-building in rabbits, sows, and rats, despite differences in the particular activities displayed by each species. Additionally, prolactin and testosterone promote straw-carrying and hair-plucking in does while in sows prolactin and prostaglandin F2-alpha stimulate nest-building before farrowing. External factors contribute to the cessation of nest-building in does and sows, specifically the visual or tactile perception of a ?ready-built nest?. The lack of materials suitable for nest-building in the environment leads to deficits in maternal care at delivery and nursing postpartum in does, sows, and dams. This has led to changes in the ways pregnant and lactating animals are raised on the farm. Hopefully, maternal behavioral neuroendocrinology will continue to evolve as a field and interact more fruitfully with animal science to provide us with new models for the study of topics relevant to neuroscience and physiology.

OR8-1
SEX IS STRESSY. IS STRESS SEXY? GLUCOCORTICOIDS AND MATE CHOOSINESS IN COPE'S GRAY TREEFROGS.
Alexander Baugh
Swarthmore College, USA

Glucocorticoids (GCs) are rarely studied in the context of female mate choice, despite the expression of receptors for these products in sexual, sensory and decision-making brain areas. Here I investigated the effects of GC concentrations on three aspects of female sexual behavior in breeding Cope's gray treefrogs (Hyla chrysoscelis): proceptivity? a measure of sexual motivation, intraspecific mate preferences, and mate choosiness. To my knowledge this is the first experimental study on the endocrine basis of mate choosiness. I predicted that mate choosiness pursuing the most attractive mate under costly (locomotor) conditions would be particularly impacted by elevated GCs with moderate GC levels associated with greater choosiness. I found support for this predicted inverted-U relationship. Females in the control group (no injection) showed no change in choosiness across timepoints. In contrast, females in the vehicle, Low (20 ng/g) and High (180 ng/g) corticosterone groups exhibited a nominal decline in choosiness after injection, suggesting that the experience of injection has little or perhaps slightly suppressive effects on female choosiness. Females in the moderate dose group (60 ng/g), however, exhibited a significant increase (>100%) in choosiness. Further, I found no effect of elevated GCs on sexual proceptivity or the species-typical preference for longer calls. These findings may reflect a buffering of primary sensory areas in the brain against elevated GCs. The recruitment of other cognitive processes during active decision-making, however, may facilitate GC modulation of mate choosiness, thereby promoting tactical plasticity at this critical life history juncture.
(I am grateful to members of the Baugh lab including N. Ambiel, S.Gray-Gaillard, N. LaScala, J. Lavigne and N. Lee for assistance with collecting frogs, behavioral testing and record keeping. I thank Mark Bee and members of his lab and S. Gupta in particular, for assistance in collecting frogs.)

OR8-2
Unique pharmacology of fish neural melanocortin receptors and origin of melanocortin receptors
Ya-Xiong Tao, Ren-Lei Ji
Auburn University, USA

Neural melanocortin receptors (MCRs), melanocortin-3 and -4 receptors (MC3R/MC4R), are critical non-redundant regulators of energy homeostasis. The MC3R does not regulate food intake but affect feed efficiency and fat storage, whereas MC4R activation results in decreased food intake and increased energy expenditure, resulting in negative energy balance. During the past few years, we have systematically studied neural MCRs in several fishes. We showed that fish neural MCRs have significantly higher constitutive (basal) activities when compared with corresponding human receptor, suggesting that inverse agonist for the MC4R might be used for increasing feed intake and decreasing energy expenditure in fish. Furthermore, small molecule orthosteric ligands at human MC4R are allosteric ligands. These small molecule ligands also serve

as biased ligands, with distinct effect on different signaling pathways. For example, ligands that are antagonists or non-functional in the Gs-cAMP pathway can serve as agonists at the ERK1/2 pathway. To investigate the origins of MCRs, we studied experimentally four putative, sequence based predicted MC4R-like receptors and one putative MC1R-like receptor from urochordata and cephalochordate, including Styela clava (sc), Ciona intestinalis (ci), Branchiostoma floridae (fl), and Branchiostoma belcheri (bb). No genes coding for the endogenous MCR ligands were observed in these species. We showed that four receptors (so far named flMC4R, bbMC4R-1, bbMC4R-2, and ciMC1R) had high cell surface expression in HEK293T cells, whereas scMC4R had low cell surface expression. Four receptors (except scMC4R) showed high basal cAMP signaling, suggesting that these receptors are likely coupled to the stimulatory Gs protein. However, no specific binding to alpha-MSH were observed at these five receptors, and in accordance they had no alpha-MSH-induced cAMP signaling coupled to Gs stimulation. All five receptors had low basal and no alpha-MSH-stimulated ERK1/2 signaling. Four human MC4R inverse agonists had no effect on cAMP and ERK1/2 signaling of the four predicted MC4R-like receptors. In summary, our results suggested that the four predicted MCR-like receptors (except scMC4R) in ancient chordates are indeed functional receptors, with high constitutive activity, but they are not MCRs. These receptors might be ancient G protein-coupled receptors with unidentified ligands. MCRs first appeared in lampreys.
(None)

Tuesday, May 30th, 2023
10:30 – 12:00
Salón Claustro III

NASCE 2023 Symposium 9:
Trends in the evolution of hormone receptors.
Chair:
Robert Dores

S9-1
APPLYING ANCESTRAL SEQUENCE RECONSTRUCTION TO RESOLVE THE FUNCTIONAL EVOLUTION OF MELANOCORTIN 2 RECEPTOR (MC2R)
Ciaran Shaughnessy, Robert Dores
University of Denver, USA

The melanocortin 2 receptor (Mc2r) is a critical component of the hypothalamus-pituitary-adrenal/interrenal (HPI/HPA) axis in vertebrates, receiving neuroendocrine signaling from the anterior pituitary in the form of Acth and initiating glucocorticoid synthesis. Agnathans only possess two Mcr genes (Mca and Mcb), which later diversified to the family of 5 receptors (Mc[1-5]r) present in all gnathostomes. In bony fishes, the functionality of Mc2r is exclusively selective for ACTH and dependent on chaperoning by its accessory protein, Mrap1. In elasmobranchs, Mc2r can be activated by either ACTH or MSH-sized melanocortin ligands and does not necessarily require Mrap1 chaperoning. In agnathans, it appears that Mca (the primitive orthologue to Mc1r/Mc2r) is a promiscuous receptor with no dependance on interaction with Mrap1. Taken together, functional studies on the Mc2rs of extant vertebrates illustrate that this receptor has evolved from a promiscuous and independent receptor to an Acth-exclusive and Mrap1-dependent receptor. Here, we briefly present recent studies in our laboratory have identified and/or functionally characterized Mc2rs in species at critical phylogenetic positions, including the basal actinopterygian (Senegal bichir, Polypterus senegalus), the basal sarcopterygian (West African lungfish, Protopterus annectens), the basal cartilaginous fishes (Elephant shark, Callorhinchus milii), and the agnathans (inshore hagfish, Eptatretus burgeri; sea lamprey, Petromyzon marinus). Then, we present state-of-the-art methods of ancestral sequence reconstruction using phylogenetic analyses by maximum likelihood to infer the ancestral sequences of Mc2rs at key nodes in vertebrate evolution, the ancestral gnathosome Mc2r and the ancestral osteichthyan Mc2r. Finally, we present functional studies on these resurrected ancestral Mc2r sequences. We synthesize our studies on extant and phylogenetically inferred ancestral Mcrs and propose hypotheses for the evolution of Mc2r ligand selectivity and accessory protein interaction throughout the course of the diversification of the Mcr gene family and vertebrate evolution.
(This work was supported by the Long Endowment (R.M.D.) and an N.S.F. Postdoctoral Fellowship (DBI-2109626; C.A.S.).)

S9-2
FUNCTIONAL EVOLUTION OF THE CORTICOSTEROID RECEPTORS IN A PISCINE MODEL
Mathilakath Vijayan, Femila Antomagesh, Erin Faught
The University of Calgary, Canada

Corticosteroids are stress-hormones, and they are produced in every vertebrate phylogenetic clade. However, corticosteroid receptors (CRs), which confer the function of these hormones, gained additional roles and ligand specificity throughout evolution. The ancestral form is a single CR present in the Agnathans, and duplicated into distinct paralogues, namely the type I CR or mineralocorticoid receptor (MR) and the type II CR or glucocorticoid receptor (GR), in the Elasmobranchs. These two receptors, as well as additional paralogues can be found in most other vertebrates, retaining much of their structural homology. However, the ligands for CRs are diverse, including 11-deoxycortisol (agnathan), 1a-hydroxycorticosterone (elasmobranchs) and cortisol (ray-finned fish and humans), all functioning as principal corticosteroids. Additionally, the increased demands of fluid and ion balance has led to the emergence of aldosterone, an MR-specific ligand in the tetrapods. Teleosts lack aldosterone, and studies suggest that cortisol is the primary ligand for both MR and GR, while the mineralocorticoid role is mediated by GR and not MR. This raises the question why ancestrally one hormone would need two receptors for stress coping? MR has a 10-fold higher affinity compared to GR, suggesting that these CRs may be selectively activated depending on the circulating cortisol levels. The recent developments in gene editing and the generation of GR and MR knockouts in zebrafish (Danio rerio) have advanced our knowledge about the distinct and overlapping roles of GR and MR in teleosts, while also informing their conserved roles. The emerging evidence points to a key role for GR and MR, either singly or in combination, in shaping the tissue-specific metabolic changes to cortisol stimulation. Our results suggest that MR activation is essential for the metabolic homeostasis necessary for growth and development, while elevated cortisol levels due to stress or circadian changes

and the associated GR-mediated responses may lead to allostasis. We propose the activation of MR during stress is essential in restricting the allostatic load and for efficient stress coping. Overall, the evolution of these receptors may have a key functional role in maintaining energy homeostasis, which allowed animals to grow and reproduce in a challenging environment.
(This work was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV.)

S9-3
BOTH GR AND MR ARE IMPORTANT FOR THE INCREASED METABOLIC FLUX OF GLUCOSE DURING STRESS IN THE ZEBRAFISH BRAIN
Femilarani Antomagesh, Mathilakath Vijayan
University of Calgary, Canada

Coping with stress is an energy demanding process, and the corticosteroids play an important role in facilitating the fuel mobilization and utilization. In teleosts, as in mammals, cortisol is the principal glucocorticoid secreted in response to activation of the hypothalamus-pituitary-adrenal (interrenal tissue in fishes) axis. Cortisol stimulates tissue-specific energy substrate reallocation, and that is essential for meeting the increased energy demand during stress. However, the mechanism by which cortisol facilitates metabolic adjustments are far from clear. A primary target for energy utilization during stress is the brain and recent studies clearly point to a role for the glucocorticoid receptor (GR) signalling in altering brain metabolism. Here we tested the hypothesis that activation of both the GR and the mineralocorticoid receptor (MR) by cortisol mediate the brain energy substrate partitioning during stress. To test this, we assessed the brain metabolic flux from U-13C glucose after treating zebrafish (Danio rerio) with cortisol to mimic chronic stress. A ubiquitous GR or MR knockout (KO) zebrafish was utilized to assess the role of these receptors in brain metabolic adjustments. The 13C enrichment of metabolic intermediates from U-13C glucose were measured using the liquid chromatography-mass spectrometry. Cortisol did not affect brain glucose uptake in the wildtype; however, lack of GR but not MR enhanced brain glucose uptake compared to the wildtype. Metabolic profiling using U-13C glucose showed a high 13C enrichment of pyruvate, lactate, and alanine in the GRKO brain, highlighting an enhanced glycolytic activity associated with the increased glucose uptake. Our results suggest that the enriched pyruvate was not utilized in the TCA cycle in the GRKO fish, given the higher enrichment of 2-13C labeled lactate and malate, supporting a partial pyruvate recycling. Also, other 2-13C labeled TCA intermediates, including citrate, succinate, and fumarate were enriched in the GRKO and MRKO fish. However, only the cortisol-treated wildtype zebrafish brain showed higher enrichment of 4-13C labeled glutamate, suggesting anaplerosis from two rounds of the TCA cycle. Overall, our results indicate a key role for GR and MR in coordinating the glucose metabolic flux to enhance the brain energy metabolism during stress in zebrafish.
(Acknowledgement: This work was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to MMV.

OR9-1
ACE-2 DIFFERENTIAL EXPRESSION IN A549 TYPE II ALVEOLAR CELL LINE
Laura Cristina Berumen, Miriam Aguilar Ugalde, Jazmín Soto Hernández, Irasema Mendieta Trejo, Guadalupe García Alcocer, Jesica Escobar Cabrera, Laura Cristina Berumen
Universidad Autónoma de Querétaro, México

Angiotensin converting enzyme (ACE) and ACE-2 are key enzymes involved in the endocrine control of blood pressure, with the angiotensin processing from the protein precursor (with renin activity) to the active oligopeptide signal molecule that will be detected by the appropriate receptors, involving the participation of the lungs, with their low-pressure vascular system, to balance the response between tissue injury or tissue protection with vasoconstriction (AT1Rs) and vasodilation (MasR), for example. The expression of this transmembrane protein ACE-2 in type II alveolar cells (AT2) is related to lung homeostasis and immune response, but in A549 cells it was reported to be absent. In this work we found ACE-2 expression in A549 cells by rt-PCR, with differences between variants detected, as expected for neuroendocrine phenotype of transdifferentiated A549 cells (with cAMP increasing agents). One of the ACE-2 variants (GenBank: MT505392.1; ENST00000677282.1) has been reported as an interferon-stimulated gene, which lacks N-terminal region and is non-functional (UNIPROT ID Q9BYF1-3). We found differences in expression for transcripts recognized in region X:15581270-15581289, which is lacking for the short variant. Some other models of cell lines for study of ACE-2 have been proposed (e.g. knockout, knockin, conditional KO and humanized ACE-2 mouse models), and the correspondent differential expression of variants, that are responsive to specific cytokines should be investigated. Differential expression is important between variants of ACE-2, which might be related to efficiency in actual therapeutic strategies or susceptibility to viral infections.
(Acknowledgments: research supported by CONACYT A1-S-25275.)

OR9-2
CHARACTERIZATION AND INSIGHT INTO THE PHYSIOLOGICAL ROLE OF THE CCHAMIDES IN THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI
Jinghan Tan, Jean-Paul Paluzzi
York University, Canada

As a widely distributed anthropophilic mosquito species and vector of various arboviruses, Aedes aegypti poses a significant threat to human health on a global scale. Investigating mosquito neuropeptides allows us to better understand their physiology. The neuropeptides CCHamide1 (CCHa1) and CCHamide2 (CCHa2) and their associated G protein-coupled receptors (CCHa1R and CCHa2R) were recently identified and studied across insects. However, expression profiles and physiological roles of CCHamides and their receptors in many other insects, including A. aegypti, remains unclear. This research aimed to quantify and localize expression of CCHamides along with their receptors and to elucidate their physiological function in the yellow fever mosquito.
RT-qPCR analysis revealed transcript abundance of CCHamides and receptors changes over development. Differential expression was also observed in tissues/organs of adult mosquito indicating CCHa1 and CCHa2 transcripts are most highly enriched in the female head and midgut, while receptors are expressed across various tissues. Further, CCHamides were immunolocalized in neurons in the ventral nerve cord along with enteroendocrine cells in the posterior midgut adjacent to the midgut-hindgut junction, corroborating their transcript expression profiles. A heterologous functional assay was used to confirm the specificity and sensitivity of the two CCHamide receptors by assessing their activity in

response to diverse peptidergic ligands, which revealed CCHa1 and CCHa2 exhibited the strongest response. Interestingly, using a capillary feeder (CAFÉ) bioassay, our results suggest that CCHa2 modulates feeding behaviour in female mosquitoes.
(This research was supported by an NSERC Discovery Grant to J.P.P.)

Tuesday, May 30th, 2023
14:30 – 16:00
Salón Júarez

NASCE 2023 Symposium 10:
Insulin/Insulin-like growth factor peptides
Chair:
Cunming Duan

S10-1
INSULIN RESISTANCE AND METABOLIC ADAPTATION IN CAVEFISH
Nicolas Rohner
Stowers Institute for Medical Research, USA

Adapting to extreme environments requires drastic changes to an animal?s metabolism. Adaptation to the total darkness and food limitation of caves can be particular challenging. The cavefish Astyanax mexicanus is a promising research organism to unravel the genetic basis of starvation resilience. Extant surface and cave morphs of the same species remain interfertile and can be bred outside their natural environments. We have previously shown that cavefish evolved impressive adaptations such as increased appetite, starvation resistance, and altered feeding due to mutations in mc4r. In addition, we found that cavefish display elevated blood sugar levels and insulin resistance caused by a mutation in the insulin receptor. In contrast to human patients, carrying the exact same mutation, cavefish do not display common markers of diabetes and live long and healthy lives. Furthermore, cavefish develop hypertrophic visceral adipocytes without obvious signs of inflammation due to reduced amounts of pro-inflammatory cytokines. Taken together, our work suggests that cavefish develop these phenotypes as part of their starvation resistance and have evolved resilience phenotypes that allow them to tolerate stark deviations from what would be considered normal physiology in other vertebrates, including humans. This positions cavefish as a promising model to gain mechanistic insights into disease phenotypes from an evolutionary and adaptive perspective.
(This work is supported by institutional funding from the Stowers Institute and by the NIH New Innovator Award 1DP2AG071466-01)

S10-2
INSULIN-MEDIATED NUTRIENT PARTITIONING IN A NON-MODEL INSECT: THE WESTERN TARNISHED PLANT BUG, LYGUS HESPERUS
Andrew Nuss(1), Devin Mazolewski(2), Joe Hull(3), Colin Brent(4)
(1)(2) University of Nevada, Reno, USA (3)(4)USDA-ARS Arid Land Agricultural Center

Our understanding of insect physiology was pioneered through the use of model insects selected for their ease of use in the lab, body size, or importance to human activities. However, the continued improvement and reduced cost of high throughput genetic sequencing has enabled opportunities for the physiological study of less explored insect species. Lygus hesperus, the western tarnished plant bug, is an agricultural pest of several crops and has a recently improved transcriptome. We took advantage of this newly available resource to explore the dynamics and functions of insulin-like peptides (ILPs) in L. hesperus. It is as yet unknown if all lessons learned from other insects apply to this relatively unexamined species. This talk will cover our work on one of the fundamental hallmarks of insulin signaling: as a signal for the cellular uptake of hemolymph carbohydrates. These explorations have set the foundation for examination of other ILP functions in this system. Overall, the information gained from these studies will contribute to a long-term goal of our lab to target neuropeptide receptors for new mode of action pesticides, gravely needed in response to developing insecticide resistance.

S10-3
REGULATION OF CELL PLASTICITY BY IGF SIGNALING: LESSONS FROM FISH IONOCYTES
Cunming Duan
University of Michigan, USA

During development, cells gradually adapt to different phenotypic states or cell type. This directional cell fate specification process was conceptualized by Conrad Waddington in his classic landscape model in the 1950s. Recent development of single-cell technologies has revealed the tremendous diversity of cell states and phenotypic plasticity. Many differentiated cells are endowed with the ability to change cell type or cell state in normal tissue homeostasis and regeneration. Interestingly, cancer cells often gain increased plasticity and this plasticity has emerged as a major hallmark of cancer. Despite the importance, the underlying molecular mechanisms are still poorly understood. To address this gap, we have developed a zebrafish model. In this whole organism model, a population of Ca2+-transporting epithelial cells or ionocytes are genetically labeled by GFP expression. When transferred to an induction medium, these differentiated cells are reactivated and reenter the cell cycle. Using this model, chemical biology screens, transcriptomics, and genetic studies have elucidated a number of cell autonomous and non-autonomous factors regulating this form of cell plasticity. These all converge on the nutrient sensitive insulin/insulin-like growth factor (IIS)-PI3 kinase-Akt-Tor signaling pathway. Our new and unpublished findings suggest that IIS-Tor signaling acts by modulating mitochondria activity and ROS signaling. These findings will be presented and discussed.
(This work was supported by NSF IOS-1755268.)

OR10-1
CHARACTERIZATION OF IGF3 IN TURBOT AND ITS EXPRESSION PATTERNS DURING OVARIAN AND EMBRYONIC DEVELOPMENT
Yudong Jia(1), Feixia Li(2), Jiarong Zhang(3)
(1)Yellow Sea Fisheries Research Institute, China (2)(3)Shanghai Ocean University

Accumulating evidence suggests insulin-like growth factor 3 (IGF3) as teleost-specific endocrinological factor plays key roles in gonadal sexual differentiation. However, the roles of IGF3 during economic marine fish ovarian and embryonic development is poorly understand. In this study, full-length sequences coding for IGF3 were isolated from turbot (Scophthalmus maximus) ovary by homology cloning and a strategy based on RACE-PCR. Results showed that the full-length IGF3 cDNA was 1255 bp long and contained a 780 bp open reading frame that encoded a mature protein of 259 amino acids (aa) and a signal peptide of 33 aa. The nucleotide and amino acid sequences of turbot IGF3 showed high homologies with the corresponding sequences of other fish species and significant homology with that of Hippoglossus hippoglossus. Interestingly, igf3 mRNA was found to be abundant in the brain of female and male puberty turbot, but deficient in eyes, intestine, gonad, muscle, gill, spleen, stomach, heart, and kidney. Furthermore, igf3 was gradually increased from pre-vitellogenesis to migratory nucleus stages, with the highest values observed at the late vitellogenesis stage throughout reproductive cycle. However, igf3 was dramatically decreased at the atresia stage. Meanwhile, IGF3 predominantly expressed in the follicle cells surrounding the oocytes. There was a sharp increase of igf3 expression from blastula to gastrulae stage, but significantly decreased at hatching stage. These results indicate turbot IGF3 is involved in the regulation of oocyte maturation and germ layer formation, give novel insights into the understanding of its new function.
(Supported by the National Natural Science Foundation of China (31972811) and Central Public-interest Scientific Institution Basal Research Fund (NO. 2020TD51).)

OR10-2
FUNCTIONAL CHARACTERIZATION OF VIRAL INSULIN/IGF-1 LIKE PEPTIDES IN HOST-PATHOGEN INTERACTIONS
Aurelien Chuard(1), Kaitlin Reiners(2), Khadija Danazumi(3), Kalaimagal Nesarajah(4), Martina Chrudinova(5), Fa Zhang(6), Richard Dimarchi(7)
(1)(2)(3)(4)Boston College, USA (5)The Czech Academy of Sciences (6)(7)Indiana Univeristy

Viruses, encode proteins that are similar to host proteins to manipulate the host. Until our recent discovery of viral hormones, examples of viral mimicry were limited to immunomodulatory proteins and growth factors. We recently showed for the first time that six viruses in Iridoviridae family encode genes mimicking human insulin and IGF-1. Chemically synthesizing these viral insulin/IGF-1 like peptides (VILPs), showed that VILPs can bind to human insulin and IGF-1 receptor and further stimulate post-receptor signaling. VILPs also stimulate glucose uptake and proliferation in mammalian cells and lower the blood glucose in mice. Although the main functions of fish insulin (regulating metabolism, development and feeding) and fish IGFs (proliferation, survival, differentiation and growth) are similar to that in mammals, fish show some important differences. Originally, VILP-carrying viruses were isolated from fish; however, the role of VILPs in viral pathogenesis is not studied in depth. Therefore, we examined the effects of Grouper Iridovirus (GIV, one of the VILP carrying viruses) and its VILPs on fish insulin/IGF signaling system. To this end, we first chemically synthesized GIV-VILP in its single chain (sc, IGF-1 like) and double chain (dc, insulin-like) forms. Stimulation of grouper kidney (GK) and AB9 zebrafish cells with these peptides, insulin and IGF-1, showed that IGF-1 was the most potent ligand and both forms of the VILPs were as potent as insulin in its activity on receptor phosphorylation. Examining the viral kinetics, we showed that GIV32 gene (encoding VILP) is an early gene in both cell types. Mass spectrometry analysis revealed that VILPs are not a part of the viral particle. Using supernatants of infected cells, we showed that , VILPs are secreted during the viral cycle and stimulate receptor phosphorylation and activates downstream metabolism cell signaling.
Taken together, GIV VILP is an active member of insulin/IGF superfamily. Characterizing the function of VILPs in host-pathogen interactions will enable us to define a novel viral pathogenesis mechanism in which viruses mimic host hormones to manipulate the host?s endocrine system.
(Cunming Duan for the invitation to the NASCE, DiMarchi Richard for the synthesis of the VILPs McMenamin lab for the Zebrafish facility)

Tuesday, May 30th, 2023
14:30 – 16:00
Salón Claustro II

NASCE 2023 Symposium 11:
Regulation of salinity tolerance in amphibians
Co-Chairs:
Daniel Buchholz and Erica Crespi

S11-1
SALT-REGULATION IN ANURANS: WHAT DO WE KNOW?
Molly Albecker(1), Molly Womack(2)
(1)University of Houston, USA (2)Utah State University

The increased salinization of freshwater habitats is a global consequence of climate change and urbanization. At least 80 percent of the anurans (frogs and toads) rely on aquatic ecosystems for at least one life stage. Anuran populations are expected to be severely affected by increased salinity. Although studies investigating anuran salt tolerance have accumulated in recent years, we lack a current synthesis of the effects of salt across different species and life stages. Using a meta-analysis, we synthesized the literature to provide a quantitative foundation for anuran salt tolerance, evaluate evidence for hypotheses on the physiology of salt regulation, and identify gaps in knowledge. These results provide a useful foundation for additional studies on anuran salt regulation, particularly in response to salinity changes associated with global climate change. We hope that this study will motivate additional work that links physiological consequences of salinization within and across anuran life stages.

(We thank members of the WoLab for thoughtful discussion in the development of this project. This work was funded by Utah Agricultural Experimental Station Project (UTA01574) awarded to MCW.)

S11-2

DETERMINING WHETHER ADRENDAL STEROIDS MEDIATE PHENOTYPIC AND PHYSIOLOGIC EFFECTS OF ELEVEATED SALINITY ON LARVAL AMPHIBIANS

Brian Tornabene(1), Creagh Breuner(2), Blake Hossack(3), Erica Crespi(4)
(1)(2)University Of Montana, USA (3)U.S. Geological Survey (4)Washington State University

Salinity (sodium chloride, NaCl) from anthropogenic sources is a persistent contaminant that negatively affects freshwater taxa, but some species are innately or adaptively tolerant. Physiological mechanisms mediating tolerance to salinity are still unclear, but changes in adrenal steroids (corticosterone [CORT] and aldosterone [ALDO]) are prime candidates. We exposed larval salamanders and frogs to environmentally relevant NaCl treatments (<32?4000 mg/L) for 24 days to test effects on growth, survival, and waterborne hormone responses. Using a glucocorticoid antagonist (RU486), we also experimentally suppressed CORT signaling of some larvae to determine if CORT mediates effects of salinity. Phenotypic and physiologic responses to increased salinity differed between frogs and salamanders. For frogs, mortality was higher than for salamanders and there was a growth-survival tradeoff mediated by CORT. Suppressing CORT signaling reduced survival further but also attenuated negative effects of salinity on growth, development, and water content of larval frogs. For salamanders, survival did not differ among salinity treatments, but salinity reduced dry mass, snout?vent length, and body condition while increasing water content of larvae. High survival and sublethal effects provided evidence that salamanders were physiologically challenged but were tolerant of experimental concentrations whereas salinity dysregulated CORT physiology for frogs. CORT of control larvae increased or was stable with growth and development (frogs) or body condition (salamanders) but decreased for those exposed to salinity. CORT attenuated negative effects of salinity for frogs but not for salamanders. We also quantified waterborne ALDO from a subset of larvae and found it was correlated with CORT, suggesting it may be difficult to decouple effects of these adrenal steroids. In frogs, ALDO increased when larvae were exposed to RU486, suggesting RU486 may also suppress mineralocorticoid receptors or that negative feedback of ALDO is mediated through glucocorticoid receptors. To our knowledge, this is the first study to concomitantly measure tradeoffs between growth and survival and experimentally link these changes to CORT physiology. Our findings expand our understanding of the roles of adrenal steroids in mediating effects of a prominent stressor, describes variation among species and populations, and provides new hypotheses regarding the co-regulation of ALDO and CORT.
(We thank the U.S. Geological Survey and University of Montana for funding and the U.S. Fish and Wildlife Service in Crosby, North Dakota for assistance with sampling and housing.)

S11-3

INTEGRATING THE GUT MICROBIOTA INTO OUR UNDERSTANDING OF AMPHIBIAN RESPONSES TO SALINITY AND PATHOGEN STRESS

Myra Hughey(1), Robin Warne(2)
(1)Vassar College, USA (2) Southern Illinois University

Host-associated microbial communities are increasingly recognized as an important factor influencing stress-mediated disease outcomes. In particular, gut microbiota have the potential to affect susceptibility to infectious disease through direct interactions with pathogens, by increasing resources available for host immune function, and by regulating the neuroendocrine and immune systems? responses to infection. Assembly of the gut microbiota, however, depends upon environmental conditions, and its function may therefore vary, for instance, based on host exposure to pollutants or diet. We investigated how increasing salinization of freshwaters and associated increases in growth of nutritional algae influenced gut bacterial assembly, host physiology, and responses to ranavirus exposure in larval wood frogs (Rana sylvatica). Ranaviruses are ubiquitous in breeding ponds in the northeastern US and contribute to die-offs of wood frog larvae. These die-offs are more likely to occur in ponds impacted by the application of road de-icing salts, as elevated salinity exacerbates a potentially maladaptive stress response to infection. However, we found that supplementing a basic larval diet with algae reversed the effects of elevated salinity on the stress response; that is, larvae that were fed algae did not exhibit elevated kidney corticosterone levels, accelerated development, or weight loss post- infection, whereas larvae fed a basic diet did. Salt pollution and algae supplementation had unique and interactive effects on the gut microbiota that are consistent with the way in which hosts responded to infection across treatments. Elevated salinity led to the proliferation of Proteobacteria that have been associated with disrupted metabolic function and disease. By contrast, algal supplementation selected for bacteria (e.g., Firmicutes) that may contribute to the diminished stress response to infection via the production of bacterially-produced short chain fatty acids that influence host metabolism and endocrine function. The effect of algal supplementation on the gut microbiota was observed even under high salt conditions. This study highlights how early-life exposure to pollutants and resource availability can influence the gut microbiota, which are integrators of both host and pathogen responses to infection on multiple fronts.
(Supported by NSF DEB 1754474, Washington State University College of Arts and Sciences, and Vassar College.)

OR11-1

SALINITY INCREASES DIFFERENTIATION OF MUCUS SECRETING CELLS AND SECRETION OF MUCUS IN AMPHIBIAN EMBRYOS: A POTENTIAL ROLE FOR LEPTIN?

Erica Crespi, Kourtnie Whitfield
Washington State University, USA

Increased salinity in freshwater environments due to anthropogenic factors is an emerging stressor in natural populations of amphibians, and embryonic stages are the most vulnerable to mortality with increasing salinity. To better understand cellular mechanisms underlying salt tolerance, we challenged embryos of Xenopus laevis, a salt-tolerant species along a gradient of salinities. We hypothesized that salt-tolerant embryos will be better able to adjust their epidermal landscape to reduce salt permeability either by reducing the number of ionocytes and increasing the number of mucus-secreting cells. To test this hypothesis, we chronically exposed X. laevis and R. sylvatica embryos to one of four salinities (150-200, 560, 1500, 3000 ?Si/cm) from fertilization through Nieuwkoop and Faber stage 42. Scanning electron microscopy revealed that X. laevis embryonic

epidermis exhibited a proportional increase in the number of mucus-secreting goblet cells and an increase in the percentage of small secretory cell secreting mucus. We also show, using fluorescent immunohistochemistry, that leptin is highly expressed in mucus-secreting cells (co-localized with PNA) in the X. laevis epidermis at this stage and CRISPR/CAS9 knock-down of leptin reduces the number of mucin-secreting cells in the epidermis. These findings support the idea that embryos can alter cellular differentiation and mucus secretion in response to saline conditions, and leptin may be involved in either mucus production or mucus secretion, similar to associations found between leptin and intestinal mucosal epithelia in mammals. Future experiments are planned to determine specific roles of leptin in epidermis differentiation and mucus production or secretion capacities, and future studies planned to compare salt tolerance abilities of embryos across phylogenetic groups to better understand the ecology and evolution of salt tolerance in amphibians.
(This research is supported by graduate research grants from the American Microscopical Society, Sigma Xi, and Society for Integrative and Comparative Biology to KW, and a National Science Foundation (#1754474)to EJC. We also thank the Franceschi Microsophy and Imaging Center (Dan Mullendore and Valerie Lynch-Holm).)

OR11-2
ROLE OF MINERALOCORTICOID RECEPTOR IN TADPOLES OF XENOPUS TROPICALIS
Daniel R. Buchholz, Bidisha Paul, Zachary R Sterner, Leena H. Shewade, Rejenae Dockery
Department of Biological Sciences, University of Cincinnati, USA

Corticosteroid signaling is vital for proper organ maturation and survival during development in terrestrial vertebrates. The contributions of corticosterone and/or aldosterone via the glucocorticoid and/or mineralocorticoid receptors are not well resolved. To better characterize corticosteroid signaling during development, we used RNA-seq to identify mineralocorticoid receptor (MR) response genes in tadpoles of the model frog Xenopus tropicalis. We made MR mutant Xenopus lines with CRISPR to examine the role of MR during development. In contrast to the death that occurs in tadpoles with impaired glucocorticoid signaling, MR knockout tadpoles survived through metamorphosis to adulthood and exhibited no differences in growth or development throughout the larval period in both normal and low-salt rearing conditions. RNA-seq analysis on tadpole tails treated in culture with aldosterone and/or thyroid hormone produced very few candidate MR response genes, even though plasma levels of aldosterone peak and MR is induced in tail by thyroid hormone during metamorphosis. These and previous results suggest that vital corticosteroid signaling is mediated principally if not exclusively by the glucocorticoid receptor in Xenopus tadpoles.

Tuesday, May 30th, 2023
14:30 – 16:00
Salón Claustro III

NASCE 2023 Symposium 12:
Hormonal control of gonadal development: from biological sex to reproduction
Co-Chairs:
Juan I Fernandino and Diana Castañeda-Cortés

S12-1
THYROID AXIS PARTICIPATES IN HEAT TEMPERATURE-INDUCED MALE SEX REVERSAL THROUGH ITS ACTIVATION BY THE STRESS RESPONSE
Juan Ignacio Fernandino(1), D.C. Castañeda-Cortés(2), I.F. Rosa(3), A.F. Boan(4), N. Pagliaro(5), V.S. Langlois(6), R.H. Nobrega
(1)INTECH (CONICET-UNSAM), Argentina (2)(4)(5) Instituto Tecnológico de Chascomús, INTECH (CONICET-UNSAM), Chascomús, Argentina, Escuela de Bio, (3)(7) Reproductive and Molecular Biology Group, Department of Structural and Functional Biology, (6)Institut national de la recherche scientifique (INRS) - Centre Eau Terre Environnement, Québec, Canada

Environmental changes alter the sex fate in about 15% of vertebrate orders, mainly in ectotherms such as fish and reptiles. However, the effects of temperature changes on the endocrine and molecular processes controlling gonadal sex determination are not fully understood. Here, we provide evidence that thyroid hormones (THs) act as co-players in heat-induced masculinization through interactions with the stress axis to promote testicular development. We first demonstrated that the thyroid axis (through thyroid-related genes and T3 levels) is highly active in males during the gonadal development in medaka (Oryzias latipes). Similarly, T3 treatments promoted female-to-male sex reversal in XX embryos. Subsequently, embryonic exposure to temperature-induced stress up-regulated the genes related to the thyroid and stress axes with a final increase in T3 levels. In this context, we show that blocking the stress axis response by the loss of function of the corticotropin-releasing hormone receptors suppresses thyroid-stimulating hormone expression, therefore, heat-induced activation of the thyroid axis. Thus, our data showed that early activation of the stress axis and, in consequence, the TH axis, too, leaves us with that both are the leading endocrine players in inducing female-to-male reversal, which can help predict possible upcoming physiological impacts of global warming on fish populations.
(Supported by the Agencia Nacional de Promoción Científica y Tecnológica Grants 2501/15, 1875/18 and 3231/20 (to J.I.F.). VSL holds a Canada Research Chair (950-232235). RHN and IFR were supported by São Paulo Research Foundation (FAPESP), Brazil (grant numbers 14/07620-7; 18/10265-5; 20/15237-0; 21/06742-5).)

S12-2
ROLE OF STEROID-5α-REDUCTASE TYPE 2 ON DEVELOPMENT AND REPRODUCTION IN AMPHIBIANS
Diana Carolina Castañeda-Cortés, Alison Mombert, Valerie S Langlois
Institut national de la recherche scientifique (INRS), Canada.

The enzyme steroid-5α-reductase type 2 (Srd5α2) produces one of the most potent androgens in frogs, 5 α -Dihydrotestosterone (5 α -DHT). With the increased presence of environmental contaminants acting as endocrine disruptors to animals, including humans, it is critical to assess how androgens are regulated and produced to understand the health consequences of altering androgen biosynthesis. Frog mutants

(srd5 α2+1 α4) to mimic androgen disruption were created using one of the best aquatic models for endocrine disruptor testing, the Western clawed frog (Silurana tropicalis), and the CRISPR gene-editing technology. In addition to their metamorphosing ability, frogs represent a unique animal model featuring large externally developing embryos to study all aspects of developmental physiology (compared to any mammalian models). The research aims to characterize how SRD5 α2 regulates gonadal development, differentiation, and other reproduction-related factors like maintaining reproductive capacity and secondary sex characteristics. Our preliminary results reveal that homozygotes mutants did not exhibit external morphological differences compared to the wild-type in both sexes. However, the nuptial pads, androgen-dependent secondary sex characteristics in male frogs, are absent in homozygous male mutants. Besides, the reproductive capacity seems to be decreased in homozygous and heterozygotes couples; inducing reproduction using the Human chorionic gonadotrophin (hCG) does not successfully breed observed; nonetheless, sexual behavior assays and gonadal histology characterization are necessary to elucidate this observation. Since mutants cannot reproduce naturally using in vitro fertilization, homozygous embryos were generated. Surprisingly the laking of SRD5 α 2 has several implications in the early development of S.tropicalis; several spinal cord malformations were observed in the mutants individuals in the first 24 hours after fertilization compere with the wild-type embryos. These results highlight that the SRD5 α 2 may have crucial functions for embryonic development and not only in the reproduction of amphibians. On a more practical scale, this research may have applications targeting environmental contamination that would act through the activation or deactivation of androgen production in aquatic and semi-terrestrial species.
(Marko Horb (National Xenopus Resource (NXR), Marine Biological Laboratory) and Émie Cantin.)

S12-3

ROLE OF ARGININE VASOTOCIN IN THE REGULATION OF ZEBRAFISH SPERMATOGENESIS

Maya Zanardini(1), Nicolas Parker(2), Weimin Zhang(3), Hamid Habibi(4)
(1)(2)(4)University of Calgary, Canada, (3)Sun Yat-sen University

The nonapeptide hormone arginine vasotocin is known for its critical role in the regulation of osmotic balance and social behavior in teleost. Although vasotocin is primarily expressed in the brain, recent studies have indicated its expression in peripheral tissues, including the male and female gonads. This study investigated the direct effect of vasotocin on spermatogenesis using zebrafish as a model organism. Results demonstrate that vasotocin receptors (avpr1aa, avpr2aa, avpr1ab, avpr2ab and avpr2l) are expressed in the zebrafish testes, indicating that vasotocin may play a role in the regulation of testicular function. Using ex vivo culture of zebrafish testis, we investigated the direct action of three concentrations of arginine vasotocin (1nM, 10nM, 100nM) on spermatogenesis. Morphological and stereological evaluation of the testis demonstrated an effect of vasotocin on spermatogonia stem cell renewal, mitotic and meiotic germ cell development over a period of seven days. The results showed that vasotocin directly influences the number of spermatozoa and early mitotic cell stages. In addition to changing basal spermatogonial self-renewal, the presence of vasotocin altered gonadotropin-induced response in both the early and late stages of spermatogenesis. The results support the hypothesis that vasotocin is involved in the regulation of synchronized testicular development and gametogenesis. Overall, our findings provide insights into the physiological significance of vasotocin in vertebrates as a factor regulating male reproductive function.
(This work was supported by funding from the Natural Sciences and Engineering Research Council (NSERC) of Canada.)

OR12-1

GONADAL DEVELOPMENT IN THE FROG SILURANA TROPICALIS: DOES THYROID HORMONE SIGNALING PLAY A ROLE?

Verónica Angélica Alves(1), Diana C. Castañeda Cortés(2), Michael G. Wade(3), Valérie S. Langlois.
(1)(2)(4) Centre Eau Terre Environnement, Institut National de La Recherche Scientifique (INRS), Canada (3)Environmental Health Science & Research Bureau, Health Canada

Thyroid hormones (THs) are essential in all vertebrates to regulate growth, development, and homeostasis. THs are also known to interact with other hormonal axes, such as the hypothalamus-pituitary-gonadal (HPG) axis. For example, alterations in TH signalling affect the sex ratios, reproductive function, and sex steroid hormone levels in several species. Many aspects of this complex interplay between THs and the reproductive system remain to be elucidated, notably in non-mammalian species, such as amphibians. For example, whether THs are required for normal gonadal differentiation in tadpoles remains an open discussion. Some studies suggest that THs are important to maintain balanced sex ratios in tadpoles, while others argue that gonad development is independent of TH signalling. The current study examines the role of TH signalling on gonadal development in frogs. We monitored gonadal development in Silurana tropicalis lacking either or both functional genes coding for TH receptors (TRs) (tralpha and trbeta). TRs function as ligand-modulated transcription factors and can repress or activate the expression of a myriad of genes sensitive to THs. S. tropicalis tadpoles lacking TRalpha, TRbeta, both TRs and wild-type were sampled from the beginning of gonadal development and differentiation until the completion of their metamorphosis (Nieuwkoop-Faber (NF) development stages 50, 52, 54, 58, 62 and 66). Initial results of gonadal morphology in NF stages 62 and 66 reveal abnormal gross morphology in ovaries from double knockout (TRalpha TRbeta KO) and TRalpha KO tadpoles. The presence of structures that resemble mononuclear cells infiltrations (MCI) is observed in the ovaries of TRalpha TRbeta KO, TRalpha KO, and TRbeta KO tadpoles. This preliminary data suggest that TRs influence normal ovary development in S. tropicalis tadpoles, although further work is necessary to confirm the nature and the physiological implications of these observed effects. Interestingly, the presence of MCI was observed in frogs? ovaries exposed to the fungicide pyrimethanil, an endocrine disruptor that is related to TH function disruption.
(We thank Yun-Bo Shi Lab (from the National Institutes of Health, Bethesda, United States) for generously giving us a couple of TRalpha heterozygous and TRbeta heterozygous Silurana tropicalis frogs.)

OR12-2

NDR3 IN ZEBRAFISH SEX DIFFERENTIATION AND FERTILITY REGULATION

Gaganpreet Sidhu(1), Sajid Alvi(2), Yara Zayed(3), Yong Zhu(4), Raymond Kwong(5), Chun Peng(6).
(1)(2)(3)(5)(6) York University, Canada (4)East Carolina University.

Nodal is a member of the transforming growth factor-ß (TGF-ß) superfamily known to play important roles during embryonic development. Zebrafish have three Nodal homologs, nodal related (ndr) 1, 2, and 3. Recent studies in our lab suggest that ndr1 and ndr2 play a role regulating ovarian functions. However, the role of ndr3 in reproduction is unknown.
Zebrafish sex differentiation occurs between 15- and 45-days post fertilization (dpf). All zebrafish first develop juvenile ovaries, where oocytes develop from a primordial germ cell (PGC) population. These oocytes either continue developing in females or undergo apoptosis in approximately half the fish around 20-25 days post fertilization (dpf) and testes begin to develop. Sex determination in lab strains is polygenic and influenced by environmental factors, and the abundance of PGCs in the bipotential larval gonad is also associated with sex determination. Since it has been reported that Nodal reduces germ cell number in mouse and zebrafish embryos with lower germ cell numbers predominantly develop into males, we hypothesize that ndr3 may play a role in sex differentiation, testis development and male fertility. To investigate the potential functions of ndr3, we used CRISPR/Cas9 to generate ndr3 knockout zebrafish. We identified a line with a 7-bp deletion that results in a premature stop codon close to the N-terminus region of the mature peptide and found that ndr3-/- embryos were viable. Interestingly, we observed that knockout of ndr3 resulted in a female-biased sex ratio in a dose-dependent manner, with heterozygous and homozygous knockouts showing increasingly higher female sex ratios. To determine if ndr3 affects male fertility, adult male ndr3-/- or ndr3+/+ fish were paired with wild type females and spawning frequency was recorded over a 4-week period. Compared with ndr3+/+ control, ndr3-/- male had a significantly lower spawning frequency. These findings suggest that ndr3 plays a role in promoting testis differentiation and functions. The mechanisms underlying ndr3 actions in the testis are currently under investigation.
(Acknowledgment: This work was supported by a Discovery Grant from Natural Science and Engineering Research Council of Canada to CP.)

Wednesday, May 31st, 2023
10:30 – 12:00
Salón Juárez

NASCE 2023 Symposium 13:
Estrogenic, androgenic, thyroidal, and steroid biosynthetic (EATS) and non-EATS research advancements and testing
Co-Chairs:
Vicki Marlatt and Valerie Langlois

S13-1

IDENTIFICATION OF MOLECULAR MARKERS ON ZEBRAFISH EMBRYO FOR THYROID DISRUPTION BY TRANSCRIPTOMIC ANALYSIS

Celia Marti(1), Nicolas Buisine(2), Laurent Sachs(3), Noemie De Croze(4), Marc Leonard(5)
(1)(2)(3)UMR 7221 – CNRS, France (4)(5)Ramp;D L'OREAL

Environmental pollution is a rising concern for both human and environmental health. Among others, the evaluation of endocrine disruptor chemicals is a challenge for both regulators and industry. Only a few methods exist to identify Thyroid Disruptor Chemicals (TDC). Those methods are low throughput and expensive or do not often comply with the animal testing ban of cosmetic regulation. Therefore, it is becoming urgent to develop novel strategies and screening methods to identify TDCs. As thyroid signaling is highly conserved between teleost and mammals, zebrafish embryo is an alternative model for studying both physiological regulations and disruption. We design a transcriptomic analysis where embryos are exposed to reference compounds alone or in combination with thyroid hormones (T3 and T4) following the FET test (Fish Embryo Test - OECD guideline, No.236). We selected four reference compounds: Iopanoic acid (IOP), Sodium Perchlorate (PCL), Tetrabromobisphenol A (TBBPA) and Propylthiouracil (PTU) based on their different modes of action on the thyroid signaling pathway. We choose to expose the embryos at dose corresponding to FET EC10 and analyzed their transcriptomes by RNA sequencing. After quality control, reads were mapped to Danio rerio genome. Lists of differentially regulated genes (DEGs) are classified in several clusters, each corresponding to a type of biological response. Despite a great diversity of biological responses, DEGs are classified into a few: chemical dependent, TH (Thyroid Hormone) dependent and crosstalk responses. There are specific effects due to the action of selected compounds (chemical or TH) alone or in co-treatment with thyroid hormones (crosstalk responses). The crosstalk responses represented the most observed pattern in all conditions. We also found that there is a huge amount of different crosstalk responses in all treatments which is consistent to the endocrine disruptor properties of our selected reference compounds. However, there is a variable distribution of genes in response patterns. In the case of PCL, IOP and TBBPA the highest quantity of genes is grouped in chemical effects. In contrast regarding PTU exposure, most genes were in crosstalk responses. All procedures and results are described in the poster.
(This work has been carried out in the context of ERGO Consortium (No.825753).)

S13-2
KNOCK(IN) KNOCK(OUT): WHO'S THERE? GENE EDITING APPROACHES FOR DEVELOPING? NON-EATS? ZEBRAFISH SCREENS.
Christopher Martyniuk, Chris Souders, Christine Larrea, Hunter Davis, David Kim, Francisco Paneque, Andrea Guzman, J.T Schmidt, J Zubcevic, Jr Bisesi J
University of Florida, USA

The Organization for Economic Co-operation and Development has validated several in vitro and in vivo bioassays to detect estrogen, androgen, thyroid, and steroidogenesis (EATs) modalities of chemicals. However, chemicals that exert their effects through non-EATS modalities are less established. The CRISPR/Cas system has proven an efficient method for advancing zebrafish (Danio rerio) models to study endocrine disruptors. Here we present "knock-in" and "knock-out" strategies to develop zebrafish screens for two non-EATS modalities: peroxisome proliferator activated receptor (PPAR) signaling and the adrenergic system. Lipid metabolism is regulated by PPARs and chemicals can alter PPAR signaling. Such chemicals are labeled obesogens, and modulate fat deposition in individuals. We present our strategy for developing a humanized PPARα zebrafish model to reduce time and costs associated with screening of chemicals for obesogenic potential. As a second example, chemical screens for the adrenergic system will be discussed. Adrenergic hormones like epinephrine regulate heart rate and blood pressure, as part of the fight-or-flight response. Exposure to environmental chemicals can modulate this system. Zebrafish have one α1AR gene and two α2AR genes (α2aAR and α2bAR) that mediate the effects of epinephrine. We describe a CRISPR/Cas9 knockout model for adrenergic receptor α2a to study compounds that disrupt the sympathetic nervous system. High resolution melt curve analysis was employed to genotype the F2 zebrafish for gene disruption. To validate our model, behavioral responses in larval zebrafish were assessed for locomotor responses using norepinephrine and the selective α2 adrenoreceptor agonist procaterol. Heterozygote pairs were bred, and individual embryos were reared in 96 well plates and exposed to each agonist. Heart rate was assessed at 3 days post fertilization (dpf) to determine resting differences between ADRB2+/+, ADRB2+/-, and ADRB2-/- individuals. Fish exposed to norepinephrine showed elevated activity compared to untreated fish, however, no difference in activity was detected between the three genotypes for norepinephrine (non-selective for receptor isoforms). Interestingly, procaterol reduced activity in homozygote fish. Zebrafish models show potential for high-throughput chemical screens for obesogens and sympathetic disruption. Translational models are necessary for understanding how chemical exposures affect diseases like obesity and cardiovascular disease, two significant health-related crises.
(Acknowledgements. Funding for the project was supported by a UF Seed Opportunity Fund Grant (AWD08478). We also thank InVivo Biosystems (Project JBIS01).

S13-3
RAPID SCREENING TOOL FOR DISRUPTION OF ENDOCRINE STRESS RESPONSES LINKED TO THE DEVELOPMENTAL ORIGINS OF DISEASE
Emmanuelle Monniez(1), Vance Trudeau(2), Carole Yauk(3), Errol Thomson(4).
(1)(2)(3)University of Ottawa, Canada (4)Health Canada.

Selective serotonin reuptake inhibitors (SSRIs) are widely used to treat depressive disorders, anxiety, and affective disorders. They are also the first line of pharmacological treatment for perinatal depression in expectant mothers. These compounds cross the placenta and bioaccumulate in milk, so fetuses and young children are exposed during vulnerable development stages. SSRIs also enter the aquatic environment due to insufficient wastewater treatment, exposing aquatic vertebrates. Our recent studies demonstrated that male zebrafish (Danio rerio) exposed to Fluoxetine (FLX; Prozac) during early development exhibit reduced exploratory behaviour and hypocortisolism as adults, as did their descendants over three generations. Developmental exposure to FLX also results in life-long dysregulation of pathways involved in nervous system development, stress response, and lipid metabolism. We are characterizing the effects of four antidepressants and their metabolites on the stress response and will conduct a comprehensive gene expression analysis with high-throughput transcriptomics. To accomplish this, the stress-responsive SR4G transgenic zebrafish reporter line expressing a short half-life green fluorescent protein (eGFP) will be employed in a rapid testing protocol we developed for zebrafish larvae. After exposure to drugs for the first 6 days post-fertilization, larvae undergo a standardized stress protocol to assess the effects of SSRIs on whole body cortisol and eGFP mRNA levels. This study is essential to ensure that eGFP mRNA is an amenable and reliable biomarker of cortisol responses to stress. The results obtained will allow us to improve and validate this new rapid screening assay to quantify impaired stress responses after exposure to SSRIs and other potential endocrine disruptors for human and environmental health risk assessment.
(Support from the NSERC Discovery Program and Health Canada.)

OR13-1
Copper impacts on endocrine processes in the liver of developing rainbow trout (Oncorhynchus mykiss)
Michael McKay(1), Laura Baseler(2), Jason Rogalski(3), Renata Moravcova(4), Jordan Below(5), Mark Cleveland(6), Vicki Marlatt(7)
(1)(7)Simon Fraser University, Canada (3)(4)UBC, (5)(6)Gitanyow Fisheries Authority

Metal pollution is a global issue that is mainly caused by anthropogenic activities, most notably associated with urbanization and industry, especially metal mining. Despite being essential for biological processes in trace amounts, copper (Cu) is a common pollutant of particular concern because it exerts toxic effects on aquatic wildlife at low, environmentally relevant concentrations. The molecular mechanisms underlying the plethora of whole organism adverse effects are not well characterized for Cu, yet frequently observed adverse effects on growth, development, and reproduction suggest complicated impacts on the endocrine system during multiple life stages. Therefore, the objective of the present study was to examine the impacts of Cu on endocrine processes during early growth and development of rainbow trout. To achieve this, rainbow trout (Oncorhynchus mykiss) waterborne Cu (31, 47, 70, and 104 μg/L) exposure experiments initiated at the eyed embryo stage through to the onset of the swim-up fry life stage were conducted. In addition to examining gill and liver pathologies, growth, development, and survival, label-free LC-MS/MS was employed to assess differences in protein expression (n = 8 individual liver samples per treatment) and to identify pathways associated with sub-chronic hepatic Cu toxicity. A total of 36 proteins were differentially expressed in the livers of Cu-exposed rainbow trout relative to unexposed controls (10% false discovery rate), including vitellogenin type III. Proteomics data mined for endocrine-related functional associations (Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) terms) using the stringApp plugin within the Cytoscape

platform revealed the potential involvement of several biological pathways/processes (i.e., peroxisome proliferator-activated receptor (PPAR), steroid hormone biosynthesis, aromatase activity, cholesterol metabolism, glycolysis and gluconeogenesis, and fatty acid degradation). These observations at the protein level were generally consistent with organ level pathologies, growth, and development adverse effects. Not only do these data suggest a possible interaction of Cu upon several endocrine pathways, but this study also demonstrates the value of label-free proteomics in identifying biochemical modes of action and biomarkers in ecotoxicology
Despite being essential for biological processes in trace amounts, copper (Cu) is a common pollutant of particular concern because it exerts toxic effects on aquatic wildlife at low, environmentally relevant concentrations. The molecular mechanisms underlying the plethora of whole organism adverse effects are not well characterized for Cu, yet frequently observed adverse effects on growth, development, and reproduction suggest complicated impacts on the endocrine system during multiple life stages. Therefore, the objective of the present study was to examine the impacts of Cu on endocrine processes during early growth and development of rainbow trout. To achieve this, rainbow trout (Oncorhynchus mykiss) waterborne Cu (31, 47, 70, and 104 μg/L) exposure experiments initiated at the eyed embryo stage through to the onset of the swim-up fry life stage were conducted. In addition to examining gill and liver pathologies, growth, development, and survival, label-free LC-MS/MS was employed to assess differences in protein expression (n = 8 individual liver samples per treatment) and to identify pathways associated with sub-chronic hepatic Cu toxicity. A total of 36 proteins were differentially expressed in the livers of Cu-exposed rainbow trout relative to unexposed controls (10% false discovery rate), including vitellogenin type III. Proteomics data mined for endocrine-related functional associations (Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) terms) using the stringApp plugin within the Cytoscape platform revealed the potential involvement of several biological pathways/processes (i.e., peroxisome proliferator-activated receptor (PPAR), steroid hormone biosynthesis, aromatase activity, cholesterol metabolism, glycolysis and gluconeogenesis, and fatty acid degradation). These observations at the protein level were generally consistent with organ level pathologies, growth, and development adverse effects. Not only do these data suggest a possible interaction of Cu upon several endocrine pathways, but this study also demonstrates the value of label-free proteomics in identifying biochemical modes of action and biomarkers in ecotoxicology research.

OR13-2
EMERGING EVIDENCE THAT THE SYNTHETIC PROGESTIN, MELENGESTROL ACETATE, DISREGULATES THREE OF THE MAJOR ENDOCRINE PATHWAYS IN SILURANA TROPICALIS
Paisley Thomson(1), Diana Castañeda-Cortés(2), Stacey Robinson(3), Valerie Langlois(4).
(1)(2)(4) Institut National de la Recherche Scientifique, Canada (3)Department of Biology, Carleton University, ON, Canada; Environment and Climate Change Canada (ECCC)

Melengestrol acetate (MGA) is a synthetic steroid hormone used in beef cattle agriculture to promote growth and synchronize estrus by mimicking progesterone (P4). In Silurana tropicalis tadpoles, MGA has been previously shown to induce differential effects than P4 on morphological, metamorphic, and transcriptional endpoints. This study aimed to investigate the non- progestogenic mechanisms of action of MGA in larval amphibians. As tadpole growth is dependent on hypothalamic-pituitary-prolactin (HPP) and metamorphosis is mediated by both the HP-thyroid (HPT) and HP-interrenal (HPI) axes, we hypothesized that MGA alters the development through interference with these signalling pathways. Chronic exposure (22 d) to 1.7 μg/L MGA induced abnormal and asynchronous development, including smaller size, delayed metamorphic timing, and inhibited forelimb emergence by unsynchronized development of skin. In contrast, exposure to a mixture of 1.7 μg/L MGA and 43 μg/L mifepristone (RU486) did not alter these morphological endpoints. RU486 is an anti-progestogen and anti-glucocorticoid, suggesting that RU486 may block the action of MGA through antagonism of glucocorticoid signalling. Gene expression was assessed in the tadpole (NF stage 56-59) brain. Expression of HPI-related transcripts (gcr, crh, mr) was not significantly altered at this developmental stage when the transcriptional response had likely ended. MGA and mixture treatments induced upregulation of prl and downregulation of tr? in the brain. However, dio2 was upregulated by MGA but not the mixture treatment, suggesting that metabolism of THs may explain the normal morphology observed in the mixture group. This provides further evidence that MGA affects metamorphosis and acts through different pathways than in mammals. These mechanisms are likely complex and involve neuroendocrine disruption of HPI, HPT and HPP axes.
(Rachel Cheong, Kim Ménard, Émie Cantin. Supported by Environment and Climate Change Canada, an NSERC Discovery Grant, and Canadian Research Chair.)

Wednesday, May 31st, 2023
10:30 – 12:00
Salón Claustro II

NASCE 2023 Symposium 14:
Evolution of neuropeptide and hormonal signaling systems
Chair:
Andreas Heyland

S14-1
FUNCTION AND EVOLUTION OF THYROID HORMONE SIGNALING IN SEA URCHIN EMBRYONIC AND POST-EMBRYONIC DEVELOPMENT
Andreas Heyland
University of Guelph, Canada

Thyroid hormones (THs) are small amino acid-derived signaling molecules with critical functions in animal development, metabolism and disease. Their synthesis requires iodine, an essential, environmentally derived trace element ubiquitous in marine environments and limited in many terrestrial environments. In contrast to mammals and other vertebrates, which use a specialized thyroid gland to synthesize and secrete THs, the large majority of animals living in marine environments can readily concentrate iodine and synthesize THs from it, or concentrate it from food sources, equally enriched in iodine and TH metabolites. While information on TH function in these animals remains scarce, our new data show that T4 and to a lesser degree T3 regulate skeletogenesis and larval development in several echinoderm groups. Furthermore, THs can signal via

both genomic and non-genomic pathways in sea urchin embryos and larvae. Specifically, skeletogenesis is regulated, in part, via membrane receptors, while apoptosis later in development is regulated via nuclear hormone receptor action. We propose a new model of TH signaling for larval echinoderms, and propose that this system provides a unique opportunity to study the integration of non-genomic and genomic TH signaling, shedding light on the evolution of TH signaling in animals.
(NSERC DG to AH 400230 Elias Taylor, Hannah Wynen, Katherine Leaper)

S14-2
NEUROPEPTIDE REGULATION OF SHELL BIOMINERALIZATION IN BIVALVES
Joao CR Cardoso(1), Joao CR Cardoso(2), Zhi Li(3), Maoxiao Peng(4), Deborah M Power(5)
(1)Centre of Marine Sciences (CCMAR), Portugal (2)(3)(4)(5)Comparative Endocrinology and Integrative Biology, Centre of Marine Sciences, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal, International Research Center for Marine Biosciences

The shell is a natural hard-biomineralized structure in bivalves and is produced by the mantle that secretes shell matrix proteins that form a scaffold in which the calcium carbonate crystals of the shell are deposited. Explaining bivalve shell diversity and the likely consequences of ocean acidification has prompted many studies of shell formation and a biomineralization ?toolbox? has been assembled from mantle transcriptomes and shell proteomes. Surprisingly little attention has been paid to the regulation of shell growth, which is changed by season and a range of other environmental factors. Our recent studies on the symmetrical marine bivalve, the Mediterranean mussel (Mytilus galloprovinciallis), revealed that the mantle transcriptome is enriched with neuropeptide/peptide hormone precursors and GPCRs and we hypothesise that neuropeptide-GPCRs may play a key role in controlling mantle function including regulation of shell growth. In the Mediterranean mussel posterior mantle edge transcriptomes, the most active shell building region, we found at least 40 different partial and full-length neuropeptide precursors that shared a similar organization and sequence with those described in neural ganglia transcriptomes of other bivalves. At least 200 transcripts for GPCRs were found and representatives of the main GPCR superfamilies were identified and putative neuropeptide-GPCR ligand-receptor pairs were assigned. Furthermore, preliminary analysis of the mussel nervous system revealed a rich network of fibres and cell bodies projecting from the cerebral and visceral ganglia to the mantle and that damage and regrowth of the shell was associated with significant changes in expression of some neuropeptide gene transcripts in the ganglia and mantle. The evolution and role of the Mediterranean mussel mantle neuropeptidome in shell growth and repair will be discussed with particular focus on the homologues of the calcitonin-GPCR system, that plays a key role in the regulation of calcium homeostasis and bone formation in vertebrates.
(This study received Portuguese national funds from FCT - Foundation for Science and Technology through projects UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020 and from the FCT-AGAKHAN/541666287/2019 HealthyBi4Namibe project. ZL was supported by a PhD scholarship from the China Scholarship Council.)

S14-3
OXYTOCIN/VASOPRESSIN-RELATED NEUROPEPTIDE DISTRIBUTION IN OVARIES OF POGONOMYRMEX BARBATUS ANT
María Fernanda Vergara Martínez, Carlos Rafael Zavaleta Zamora, Ingrid Fetter Pruneda
(1)Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México.

The neuropeptides oxytocin and vasopressin and their receptors are one of the best-studied signaling systems in vertebrates where they are known to play major roles in regulating social and reproductive behaviors as well as memory and learning. However, their role in invertebrates is poorly understood because they are absent in the common insect model organisms fruitflies and honeybees. On the other hand, ants possess an oxytocin/vasopressin-related neuropeptide orthologue called inotocin, which provides a great opportunity to study its potential role in modulating social and reproductive behavior.
In ants, it has been demonstrated that the expression of inotocin and its receptor in the brain changes according to age and correlates with the propensity to perform certain tasks associated with the age and caste. Moreover, it is known that in ants, ovarian activity changes according to the caste. Since inotocin receptor expression has been found in the ovaries of clonal raider ants, we are interested in knowing if this peptide has any role in the modulation of ovarian activity in ants of different ages and castes.
In the present work, we performed immunofluorescence to identify the presence of inotocin as well as its spatial distribution in the ovaries of the red harvester ant Pogonomyrmex barbatus. We compared the localization of inotocin in ovaries of young and old adult workers as well as ant queens using confocal microscopy. We identified inotocin in the ant ovary and we analyzed the signal shown between the different groups described above.
(Acknowledgements: Supported by Global Consortium for Reproductive Longevity and Equality (GCRLE) of the Buck Institute, Universidad Nacional Autónoma de México (UNAM), Instituto de Investigaciones Biomédicas UNAM, Posgrado de Ciencias Biológicas UNAM.)

OR14-1
SIMULTANEOUS EXTRACTION AND DETECTION OF NEUROPEPTIDES, STEROIDS, AND PROTEINS IN SMALL TISSUE SAMPLES
Chunyu Lu(1), C Lu(2), D Peng(3), WKC Erandani(4), C.J. Martyniuk(5), V.L. Trudeau(6)
(1)(2)(3)(4)(6)University of Ottawa, Canada (5)University of Florida

Accurate detection and quantification of hormones is critical for assessing the reproductive and stress status of experimental models, as well as diagnosing diseases in both human and veterinary clinics. However, traditional methods using antibodies with radioactive or non-radioactive tracers suffer from sensitivity, specificity, and inter-laboratory repeatability issues. To overcome these challenges, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as a promising solution. Nonetheless, extracting both lipophilic steroidal compounds and hydrophilic peptide hormones from the same tissue sample can introduce variation. To address this, we developed a novel approach to sensitively detect peptide, steroid, and protein hormones in small tissue samples, including those as small as a zebrafish pituitary. Our approach demonstrates over 85% extraction efficiency for both peptide and steroid analytes, with standard deviation for extraction and LC-MS/MS analysis of each compound varying between 5-10%. The method enables the quantification of peptide and steroid hormones in the low to medium fmol/µL range and is compatible with both targeted and untargeted peptidomics and proteomics analysis. Using this method, we discovered novel versions of an

emerging reproductive hormone, secretoneurin. Time-dependent variations in SN-related peptides with well-known reproductive hormones like gonadotropin hormone-releasing hormone and oxytocin were evident. Our results demonstrate the potential of this approach for improving hormone discovery, detection characterization, and specifically to assess covariations and interactions of multiple hormone classes under numerous physiological or pathological conditions.
(This research is funded by NSERC and the University of Ottawa Research Chair in Neuroendocrinology.)

OR14-2
MICROBIOME MEDIATION OF ANIMAL LIFE HISTORIES VIA METABOLITES AND INSULIN-LIKE SIGNALING
Robin Warne, Jason Dallas
Southern Illinois University, USA

The regulatory pathways by which gut microbiota potentially shape host life histories remain largely untested, however, a constellation of research suggests that gut bacteria likely have significant effects on their hosts via metabolites. In this article we review known and hypothesized pathways by which gut microbiota influence host life histories through interfacing with the neuroendocrine system, with a focus on the IGF signaling pathway. Bacterially derived metabolites including SCFAs, polyamines, and peptides likely impact host life histories as metabolic substrates, essential nutrients, and via molecular signaling with well-studied neuroendocrine pathways. The hypothalamus-pituitary axis and ILS signaling pathways are central regulatory networks for development, growth, reproductive maturity, reproduction, and senescence and are likely targets for tests of how gut bacterial metabolites shape host life histories. SCFAs in particular, as metabolites derived from bacterial fermentation, are implicated as significant microbiome signaling molecules shown to interface with the ILS pathway, as well as bind receptors on neuroendocrine and peripheral nervous tissues. For example, experimental increases of SCFA production have been shown to affect IGF-1 levels in circulation and are associated with robust development, growth, reproduction, and delayed senescence. Finally, emerging -omics approaches are providing integrative ways to test and detail the potential diverse ways in which gut microbiota interact with their hosts and the likely important roles they play in shaping host life history responses to varied environmental conditions.
(The authors have no conflicts of interest or funding support to declare.)

Wednesday, May 31st, 2023
10:30 – 12:00
Salón Claustro III

NASCE 2023 Symposium 15:
Hormones, behavior and reproduction
Co-Chairs:
Rebeca Corona and Maricela Luna

S15-1
ADULT NEUROGENESIS INDUCED BY SOCIO-SEXUAL BEHAVIOR IN THE PRAIRIE VOLE, A SOCIAL MONOGAMOUS MAMMAL
Wendy Portillo Martínez(1), Daniela Ávila González(2), Analía E Castro(3), Raymundo Domínguez Ordoñez(4), Italo Romero Morales(5), Lizette Caro(6), Alejandro Martinez Juárez(7), Francisco J Camacho Barrios(8), Omar Martínez Alarcón(9), Larry J Young(10), Raúl G Paredes(11), Néstor F Díaz(12)
(1)Instituto de Neurobiología, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, México, (2)Instituto de Neurobiología, Universidad Nacional Autónoma de México ,Instituto Nacional de Perinatología Isidro Espinosa, (3)Instituto de Neurobiología, Universidad Nacional Autónoma de México,Silvio O. Conte Center for Oxytocin and Social Cognition, Center for Translational Social Neuroscience, Emory National Primate Resea (4)Instituto de Neurobiología, Universidad Nacional Autónoma de México, (5)(6)(7)(9)(12)Instituto Nacional de Perinatología Isidro Espinosa de los Reyes, (8)Instituto de Neurobiología, Universidad Nacional Autónoma de México, (10)Silvio O. Conte Center for Oxytocin and Social Cognition, Center for Translational Social Neuroscience, Emory National Primate Research Center, Emory University, (11)Instituto de Neurobiología, Universidad Nacional Autónoma de México,Escuela Nacional de Estudios Superiores Juriquilla.

The prairie vole (Microtus ochrogaster) is a socially monogamous rodent that establishes an enduring pair bond after cohabitation with or without mating. The plastic mechanisms involved in this pair bonding need to be better understood. Our research group demonstrates in vivo that social exposure to the opposite sex and social cohabitation with mating increase cell proliferation and differentiation into the neuronal fate in neurogenic niches in male voles. Additionally, in female voles, this socio-sexual stimulation increases cell survival and differentiation into mature neurons in the olfactory bulb. In contrast, these new cells decreased in males that were exposed to receptive females or mates. We also evaluated the differentiation potential in neural progenitor cells isolated from the subventricular zone from both female and male adult voles in vitro as a function of socio-sexual experience. We found that exposure to the opposite sex and social cohabitation with mating in male and female voles increases the proliferation of neurosphere derived Nestin+ cells, as well as an increase in mature neurons and a decrease in glial cells. Brain-derived neurotrophic factor, estradiol, prolactin, oxytocin and progesterone modulate the proliferation and differentiation of the neural progenitor cells. Our studies demonstrate that adult neurogenesis is a neuronal plastic mechanism in pair bond formation.
(This research was supported by grants CONACYT (252756), UNAM-DGAPA-PAPIIT (IN208221 and IN203518), INPER (2018-1 163and 2022-1-13), and NIH (P51OD11132).)

S15-2

REPRODUCTIVE ISOLATION BARRIERS IN ARGIA DAMSELFLIES

Angela Nava-Bolaños(1), Rosa Ana Sanchez-Guillén(2), Roberto Munguía-Steyer(3), Gabriela Castaño-Meneses(4), Alex Córdoba-Aguilar(5)
(1)UMDI-Juriquilla, Facultad de Ciencias, UNAM, México (2)Instituto de Ecología A. C., (3)Facultad de Estudios Superiores Iztacala, UNAM. (4)UMDI-Juriquilla, Facultad de Ciencias, UNAM., (5)Instituto de Ecología, UNAM.

A central question in evolutionary biology concerns the accumulation of reproductive barriers during speciation. Actually, one long-standing information gap concerns the order in which the reproductive barriers accumulate at the initial steps of diversification, and the intensity with which these barriers prevent genetic exchange. In this work, we studied how reproductive behavior works at different times in the mating process as reproductive isolation barriers in damselflies. We investigated the strength of isolation barriers between four pairs of closely related species of the non-territorial Argia damselflies (A. anceps, A extranea, A. oenea and A. tezpi). First, to establish a basis for isolation barriers, we estimated the strength of sexual isolation in terms of visual, mechanical and tactile isolation. For this, alive female models were presented repeatedly to males of the four species, to record male responses categorized as: (1) no sexual response; (2) attempt of tandem, which was when the male approached the female and attempted the tandem by curving his abdomen; (3) tandem, which was when the male grasped the female with his anal appendages; and (4) mating, which was when female does genital contact with the male. Second, we linked the strength of such isolation with the genetic divergence among all four species. Third, we studied the importance of the degree of sympatry between the four studied species in their complete distribution, using techniques of ecological niche modelling. We detected a strong reproductive isolation between all pairs of species by the joint action of the three studied barriers [visual (90.6%), mechanical (8.7%), and tactile (0.7%)]. Sexual (visual) isolation was the most important barrier, perhaps driven by learning of mating preferences. In addition, we detected an insignificant ecological niche differentiation between the studied species (70% shared distribution). Our results suggest that sexual (visual) isolation may be an important force driving speciation in non-territorial Argia species. These reproductive barriers can play an important role in the context of climate change, in contact zones, maintaining the isolation between species.
(ANB (CVU294446) is grateful to the Consejo Nacional de Ciencia y Tecnología for the postdoctoral current fellow.)

S15-3

PROLACTIN MODULATES THE OLFACTORY SYSTEM RESPONSE TO REPRODUCTIVE CHEMOSIGNALS

Rebeca Corona, Viridiana Cerbantez-Bueno, Verónica Viñuela-Berni, Daniel Muñoz-Mayorga, Teresa Morales, Rebeca Corona
Instituto de Neurobiología UNAM, México

Olfactory communication is essential for reproduction. Processing of pheromones profoundly influences neuroendocrine responses that triggers social interactions. Pheromones are detected mainly by the vomeronasal olfactory system that includes the accessory olfactory bulb (AOB); however, the volatile compounds of pheromones can be detected by the olfactory epithelium (OE) and processed by the main olfactory bulb (MOB). We previously observed that during female sexual maturation, MOB and AOB circuits are sensitive to prolactin (PRL) hormone altering their response towards chemosignals in adulthood. Additionally, PRL receptors (PRLR) are extensively expressed within OE and the mitral cells (MC) of the olfactory bulb (OB), suggesting an important role of PRL on olfaction. To understand the effects of PRL in the female olfactory processing of opposite sex cues, we explore the PRLR expression within OB during sexual maturation and the direct responses of PRL by the time of pheromonal exposure. C57BL6/J female mice were evaluated for the expression of PRLR within the OB during the onset of puberty (PB), the first estrous indicating sexual maturity (SM) and during adulthood in estrous stage (A). Additionally, we assessed the behavioral response of an A female exposed to male soiled bedding after PRL administration, and the participation of the olfactory systems by the quantification of cFos within MOB, AOB, piriform cortex (Pir) and medial amygdala (MeA) as the first central relays coming from the OB. At last, we looked for the intracellular pathway that could be activated by PRL within OB. Our results indicate that PRLR expression within MOB remains constant during all maturational stages, however in AOB this expression decreases in A. Behaviorally, females that received PRL explored actively the male stimuli, along with an increased activation of the MOB-MC towards the male stimuli. AOB-MC activation showed by control females was impaired after PRL. Interstingly, ERK pathway was enhanced after odor exposure only in MOB. Centrally, MeA showed an augmented response to the male stimuli after PRL. Overall, our results suggest that PRL participates in the processing of chemosignals and behavioral response by activating the main olfactory system mainly and switching the classical vomeronasal response to pheromones.
(Supported by UNAM-DGAPA-PAPIIT IN214822, IA202218, IA200820 e IN204718, IN205423 y CONACYT A1S8948.)

OR15-1

SMALL GTPASE INVOLVEMENT IN BASAL AND GNRH-DEPENDENT LUTEINIZING HORMONE AND GROWTH HORMONE SECRETION FROM DISPERSED GOLDFISH PITUITARY CELLS.

Enezi Khalid, John Chang
University of Alberta, Canada

The pituitary hormones luteinizing hormone (LH) and growth hormone (GH) control processes of sexual maturation and metabolism, respectively. Pituitary cells, such as LH-producing gonadotrophs and GH-producing somatotrophs, are in turn regulated by multiple hypothalamic factors, which, although generally activating unique receptors, ultimately propagate signals through conserved intracellular regulatory elements to influence hormone secretion. One major family of intracellular regulators is the monomeric small GTPases, a subset of which (Arf1/6, Rac, RhoA, and Ras) has highly conserved functions across vertebrates, including the control of secretory vesicle exocytosis in several cell types. However, the participation of these effectors in neuroendocrine systems, particularly in basal vertebrate models, has been under studied. Here, we utilized the well-characterized goldfish (Carassius auratus) neuroendocrine model to investigate the roles of these proteins in both unstimulated and agonist-induced hormone secretion from dispersed goldfish pituitary cells in column perifusion experiments. Pharmacological inhibition of these small GTPases elevated basal LH and GH secretion, except for Ras inhibition which only increased LH release. Furthermore, distinct effects were observed in acute LH and GH secretion responses to the two goldfish native gonadotropin-releasing hormone isoforms, GnRH2 and GnRH3. Results indicate that Arf1/6 GTPases participate in GnRH-dependent LH, but not GH release. In contrast, Rac and RhoA GTPases selectively exert negative modulatory actions on acute GnRH3- and GnRH2-dependent GH release, respectively, whereas Ras negatively affects GnRH3-evoked LH secretion.

Together, our results demonstrate novel divergent cell-type- and ligand-selective roles for small GTPases in the control of goldfish pituitary hormone exocytosis during both unstimulated and GnRH-evoked release.
(Supported by grants from NSERC and Faculty of Science, University of Alberta.)

OR15-2
ADIPONECTIN, RESISTIN AND CHEMERIN AS OBESITY ASOCIATED BIOMARKERS IN A MURINE MODEL SUPPLEMENTED WITH PROBIOTICS AND PREBIOTICS
Melissa Pamela Lozano Staines, Florinda Jiménez Vega, Isui Abril García Montoya, Jose Alberto López Díaz, Ana Lidia Arellano Ortiz, Yolanda Loya Méndez, Alejandra Rodríguez Tadeo
Universidad Autónoma de Ciudad Juárez, México

Introduction: In obesity there is an increase in adipose tissue, which has an endocrine role since it secretes peptides called adipokines, involved in inflammatory and metabolic processes. In this project, the following were studied: resistin, associated with proinflammatory effects in obesity, in this same context chemerin, capable of modulating adipogenesis and finally adiponectin as a
biomarker involved in anti-inflammatory processes.
Hypothesis: The supplementation with probiotics and prebiotics could modify the genic expression of adipokines associated to the low-grade inflammation of obesity.
Main Methods: A murine model (n=50) was used, 5 mice constituted the control group and the remaining 45 were treated with an obesogenic diet for 8 weeks. Afterwards, the control group and 5 obese mice were sacrificed; the rest divided into 4 groups with 4 different diets: 1.- Normocaloric (NC), 2.- NC + probiotic (Lactobacillus acidophillus), 3.- NC + prebiotic (inulin), 4.- NC + symbiotic (L. acidophillus and inulin). A sacrifice is made at 8 weeks of treatment, dissecting the epididymal adipose tissue from which the RNA was extracted for cDNA synthesis. Finally, semiquantitative RT-PCR was performed to evaluate, by means of a densitometric analysis, the relative expression index (REI) of the genes of interest, using the gen 18sRNA as constitutive.
Results: Supplementation for 8 weeks triggers repression of adipokine genes related to obesity and inflammation. The better results were obtained when the animal model was supplemented with the NC + synbiotic diet, this repression was specifically observed in chemerin: it starts with a REI of 0.722 (±0.951) in the model with obesity and is repressed to an REI of 0.488 (± 0.713), also in resistin, REI where the value was modified from 1.980 (±1.976) to 1.064 (± 1.128). On the other hand, in adiponectin an increase in expression was observed, obtaining an REI of 0.134 (±0.100) to an IER of 0.912 (±0.891).
Conclusion: A synbiotic composed of L. acidophillus and inulin, can be postulated as a viable additive for modifying the expression of genes that promote a negative regulation of the development inflammation associated with obesity, thus we can consider synbiotics an complementary anti-obesity therapy.
(Our gratitude to the support given from CONACYT, with the grant 1080026)

Thursday, June 1st, 2023
10:30 – 12:00
Salón Juárez

NASCE 2023 Symposium 16:
Hormonal control of regeneration in vertebrates.
Chair:
José Ávila-Mendoza

S16-1
NEURONAL REGENERATION IN SPINAL CORD INJURY BY GONADOTROPIN-RELEASING HORMONE.
J. Luis Quintanar, Denisse Calderon-Vallejo, Carmen Díaz-Galindo, Irma Hernández-Jasso
Universidad Autónoma de Aguascalientes, México

Introduction: The neurotrophic factors such as neurotrophin-3 (NT-3), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), have been used in different models of spinal cord injury ant they have demonstrated to be effective in neural regeneration. It has been demonstrated that the hypothalamic decapeptide gonadotropin-releasing hormone (GnRH) has neurotrophic effects in vitro.
Hypothesis: The administration of GnRH in preclinical and clinical studies of spinal cord injury has neurotrophic effects that favor recovery.
Methods: Animals with spinal cord injury (SCI) treated with GnRH were evaluated for motor, sensory, and micturition behavior, as well as biochemical and histological markers of injury recovery. Likewise, patients with spinal cord injury treated with a GnRH agonist were evaluated with the ASIA and SCIM scales to identify the degree of injury and the level of independence.
Results: In the rat model of SCI, recovery of motor, sensory, and micturition function was found, as well as less damage to spinal cord tissue and an increase in axonal regeneration marker proteins. On the other hand, in the group of injured patients, an increase in sensitivity and a greater degree of movement were found, as well as an improvement in the capacity for independence in daily life.
Conclusions: Treatment with GnRH or its agonists may act as neurotrophic factors particularly in recovery of SCI in animal models and in patients.
(CONACyT CF 214971)

S16-2
PLEIOTROPHIC ROLES OF LEPTIN SIGNALING IN XENOPUS TAIL TIP REGENERATION
Robyn Reeve, Grace Curtis, Erica Crespi
Washington State University, USA

Although hormones influence many cellular processes that occur during regeneration, endocrine control of regeneration is not well understood. Leptin, a hormone known for modulating appetite and metabolism, increases immune function, angiogenesis, and wound closure rate in adult mammals. Previous work in our lab has shown that leptin increases regeneration rate in Xenopus laevis larval limbs and that leptin increases in vitro wound closure speed in adult X. laevis. We now show that leptin similarly increases regeneration rate in the larval tail tip, both when leptin was administered via intraperitoneal injection and via implantation of a leptin-secreting bead at the amputation plane. Food restriction reduces expression of leptin protein in the tail, and both leptin injection and bead application rescue regenerative ability. We are currently using RNAseq to determine how leptin administration changes gene expression during X. laevis tail regeneration at 6, 24, and 48 hours post amputation (hpa). Preliminary results show that leptin modulates genes associated with cellular metabolism, apoptosis, and immune response. At 6 hpa, leptin upregulates transcription of pro-inflammatory factors including complement factor C3b and cytokine receptor common subunit beta as well as oxidative stress mediators such as glutathione peroxidase 1. Leptin treatment downregulates transcription of genes associated with apoptosis and suppressing proliferation. Using lurpGFP+ transgenic tadpoles (expressing GFP in neutrophil-like cells), we show that leptin is a chemokine for neutrophil-like cells in X. laevis supporting our hypothesis that leptin is increasing both innate and cellular inflammatory responses immediately after amputation. Because leptin signaling has been shown to stimulate angiogenesis in cancer and adipogenesis, we tested whether leptin would increase angiogenesis during tail regeneration. We found that leptin injection promotes angiogenesis in the uninjured tadpole tail fin, and upon injury, accelerates blood vessel growth into the blastema and regenerated ventral tail fin. Leptin protein is present in blood vessel endothelial cells, and leptin injection induced phosphorylation of STAT3 in the blood vessels of the developing ventral tail fin, indicating leptin receptor activation of JAK/STAT signaling. Together, this work shows that leptin signaling has pleiotropic roles throughout tail tip regeneration, and leptin may be a nutritional modulator of regeneration.
(This research was supported by an American Microscopical Society Student Research Fellowship to R. Reeve and G. Curtis, Grants in Aid of Research from Sigma Xi and the Society for Integrative and Comparative Biology awarded to G. Curtis, and NSF-DEB 1754474 awarded to E. Crespi.)

S16-3
COMPARATIVE ANALYSIS OF KLF TRANSCRIPTION FACTORS IN AXONAL REGENERATION: INSIGHTS FROM FISH AND MAMMALIAN STUDIES
José Ávila-Mendoza, Iván Lazcano, Valeria Alejandra Urban-Sosa, Aurea Orozco, Carlos Guillermo Martínez-Moreno, Maricela Luna, Carlos Arámburo
Instituto de Neurobiología, UNAM, México

Mammals and fish exhibit significant differences in their ability to regenerate axons in the central nervous system (CNS). Fish have a remarkable ability to regenerate axons, which enables them to recover from injuries in the optic nerve, spinal cord and other nervous system lesions. In contrast, mammals, including humans, have limited capacity for axon regeneration in the CNS, often resulting in permanent disabilities following nervous system injury. This difference in regenerative capacity is influenced by a combination of both stimulatory and inhibitory extrinsic and intrinsic factors. Krüppel-like factors have emerged as important intrinsic factors involved in the control of axonal growth. They constitute a family of eighteen transcription factors characterized by three C-terminal C2H2 zinc finger motifs that recognize GC/GT rich sequences in DNA. Here, we investigated whether KLFs play a role in the differential ability of axon regeneration between fish and mammals. We compared gene expression patterns in response to optic nerve crush (ONC) and spinal cord injury in both zebrafish and rodents by analyzing previously published RNA sequencing databases. The analysis revealed that some KLFs (KLF6 and KLF7) exhibit similar expression patterns between fish and mammals, while others (KLF9 and KLF13) show differential expression between the two vertebrates. These in silico findings were validated experimentally using the ONC injury model and RT-qPCR in both in fish and mice. Our results showed that KLF6 expression increased in both species after damage, whereas KLF7 expression decreased. However, the expression of KLF9 decreased and that of KLF13 increased in zebrafish, while remaining unchanged in mice. KLF9 and KLF13 are thyroid hormone-inducible factors that play essential roles in promoting and maintaining neuronal differentiation. Therefore, we hypothesize that changes in their expression patterns in zebrafish could be associated with the establishment of a pro-regenerative genetic program, which does not occur in mammals. We are currently conducting research to test this hypothesis.
(Acknowledgments. Supported by Grants PAPIIT-DGAPA IA200622, IN215522, IN209621, IN227020; and CONACYT CF-319938, CF-214971. We thank M. en C. Adriana González, Gallardo, M.V.Z. José Martín García Servín, Dra. Alejandra Castilla León, for technical support.)

OR16-1
NEUROPROTECTIVE EFFECTS OF GROWTH HORMONE IN AN OPTIC NERVE CRUSH MODEL AS AN EXPERIMENTAL MODEL FOR GLAUCOMA
David Epardo, Jerusa Elienai Balderas-Márquez, Martha Carranza, Maricela Luna, José Ávila-Mendoza, Carlos Arámburo, Carlos Guillermo Martínez-Moreno
Departamento de Neurobiología Molecular y Celular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, México

Glaucoma is a neurodegenerative disease that leads to the death of retinal ganglion cells (RGCs) and eventually blindness. We have previously demonstrated that growth hormone (GH) has neuroprotective effects by inducing multi-activation of intracellular signaling pathways, synaptogenesis, axogenesis, and cell survival in different retinal injury models in birds and reptiles. In this study, we evaluated the neuroprotective effects of GH in an optic nerve crush (ONC) model in a mammal (6-week-old male Wistar rats). The lesion was induced by compressing the optic nerve for 10 seconds. Subcutaneous injections of GH (0.5 micrograms per gram every 12 hours) were administered immediately after the injury for either 24 hours or 14 days. The effects of GH in the retina were evaluated using qPCR and immunohistochemistry for several markers. We also assessed optic nerve integrity using CTB Alexa 488 tracer injection.
Results showed that GH treatment improved RGC survival 14 days after ONC. The ONC+GH group had significantly more positive RGCs compared to the ONC group, as shown by Brn3a immunohistochemistry. The RGC layer also had more immunoreactivity to the anti-apoptotic protein Bcl-XL

at that time compared to the ONC group. CTB axonal labeling revealed some regeneration in the optic nerve since a few labeled axons posterior to the lesion were observed in the ONC+GH group, in comparison to the ONC group where all the axons were lost in that area. Furthermore, 24 hours after the damage GH treatment partially restored the mRNA expression levels of CNTF, Gap43, SNAP25, NRXN1, NLGN1, and IL-6. Finally, 14 days after the lesion, GH downregulated the mRNA levels of GFAP, IL-6, and NGF while upregulated Gap43, as compared to the ONC group.
In conclusion, GH treatment showed neuroprotective effects in the ONC model in rats. It upregulated the expression of neurotrophic factors and prevented the downregulation of synapse and neuronal activity-related genes. GH also reduced the loss of RGCs and promoted the maintenance of retrograde axonal transport. These findings suggest that GH neuroprotective actions in the retina and many intermediate molecules are conserved across vertebrates. Therefore, GH could potentially be a therapeutic option for neurodegenerative diseases.
(Supported by PAPIIT-DGAPA-UNAM (IN227020, IN209621, IN215522, IA200622) and CONACYT (CF-214971). DE received a PhD fellowship (1083209) from CONACYT.)

OR16-2

PROLACTIN RECEPTOR DEFICIENCY PROMOTES HYPOMYELINATION DURING CENTRAL NERVOUS SYSTEM MATURATION OF SUCKLING AND PREPUBERTAL MICE

Ana Luisa Ocampo-Ruiz, Ana Luisa Ocampo-Ruiz, Dina Iathzil Vazquez-Carrillo, José Luis Dena-Beltrán, Ana Gabriela Cárdenas, Rogelio Arellano, Abraham Cisneros-Mejorado, MA Dimas-Rufino, X Castillo, E Garay, G Martínez de la Escalera, C Clapp, Y Macotela
National Autonomous University of Mexico (Neurobiology institute), México.

Prolactin (PRL) plays an important role in different biological processes such as sexual behavior, parental care, development, metabolism, angiogenesis, immunomodulation, and osmoregulation. Recently, its role in myelination and remyelination has been reported in adult female mice. Myelination in the Central Nervous System (CNS) is performed by oligodendrocytes (OLs), which extend their cell membranes to surround neuronal axons forming myelin sheaths. This phenomenon accelerates action potential conduction, participates in neuronal plasticity, connectivity, and modulates maturation, survival, and axonal regeneration. In pregnant mice lacking an allele of the PRL receptor (Prlr), there is a lower rate of OLs precursor cell proliferation and hypomyelination. Moreover, systemic PRL treatment promotes myelin repair in a model of spinal cord demyelination. Here, we explored the effect of Prlr deficiency on myelination during early postnatal development. We evaluated myelination in suckling mice (Postnatal day (P) 12), age at which they are exposed to high concentrations of PRL via maternal milk, and in prepubertal stage (P28). Using Prlr null mice (Prlr-KO) and their wild-type (Prlr-WT) counterparts, we analyzed the levels of myelination by Black Gold II (BGII) staining, expression levels of myelin proteins by rt-qPCR and immunofluorescence (IF) and assessed behavior using open field (OF) test. In cingulum, corpus callosum (cc) and dorsal fornix of Prlr-KO suckling mice, BGII revealed a hypomyelinating phenotype; IF showed decreased myelin basic protein (MBP) expression and less OLs numbers by the OL marker OLIG2 compared to Prlr-WT mice. mRNA assessment in cc of Prlr-KO showed a significant reduction in Olig2 expression level compared to Prlr-WT. This phenotype was maintained until prepubertal stage as shown by less BGII staining in the three structures evaluated, less expression of MBP by IF, and significant reduction in myelination transcription factors Olig2 and Sox10 mRNA expression levels in Prlr-KO compared to Prlr-WT. In addition, OF test revealed behavioral alterations as distance traveled and velocity of movement was lower in Prlr-KO prepubertal mice. Taken together, these data indicate that lack of Prlr activity leads to hypomyelination in white matter structures in early development and has adverse consequences in prepubertal age.
(Work supported by PAPIIT IN207321 to YM, IN205822 and 319740 to ROA and CONACYT 1771 to AC-M. We thank Ruiz-Herrera X, García-Servín M, Castilla A, Carbajo M, Hernandez N, Palma Ma, Gasca D, Ortiz-Retana J, González L, and López-Barrera for their technical assistance.)

Thursday, June 1st, 2023
10:30 – 12:00
Salón Claustro II

NASCE 2023 Symposium 17:
Emerging topics in comparative endocrinology I.
Chair:
Christopher Martyniuk

OR17-1

Seasonal plasticity in songbirds as novel model for uncovering mechanisms that limit neural degeneration and regeneration

Tracy Larson, William Tucker
University of Virginia, USA

The birth and incorporation of new cells into neural circuits in the adult vertebrate brain is a fundamental process of neural plasticity. Given the relationship between neural plasticity and the maintenance of behavior, it is crucial to understand the functional relationships within and across cell types of the adult brain. In the songbird Gambel?s white-crowned sparrow (Zonotrichia leucophrys gambelli), extreme seasonal plasticity in neuronal number in a region of the brain that controls seasonal singing behavior, called HVC, coincides with changes in singing behavior quality and quantity. In white-crowned sparrows, as testosterone levels rise during entry into breeding season, over 60,000 new neurons are added into a pre-existing pool of 100,000 neurons in HVC. As testosterone levels drop during transition out of breeding season, an equal number of neurons die. Both addition and death of neurons occurs within days of changes in testosterone levels and coincides with performance and cessation of singing behavior, respectively. To uncover how the extent of seasonal growth and degeneration of HVC is limited, we have begun exploring the role of astrocytes in supporting both the addition and death of HVC neurons. We find that astrocytes themselves are quite plastic in number with large turnover events occurring just after both the addition and death of neurons. We are now exploring whether or not astrocyte participate in the clearance of dead neurons and then die to allow newly born astrocytes to repopulate HVC. The unique and extensive turnover of astrocytes that occurs in the avian brain during both addition and death of neurons suggests that astrocyte turnover might serve to limit the extent of

degeneration and regeneration by promoting rapid return to homeostasis. Our discovery raises the possibility that astrocyte turnover occurs following natural neuronal loss in the mammalian brain and that diseases associated with alteration in neuronal number might be triggered or manifested through alterations in astrocyte number and behavior.
(Work is supported by start-up funds from the University of Virginia.)

OR17-2
RE-EVALUATION THE EVOLUTION OF THE POMC GENE: A STUDY ON MELANOCORTIN PEPTIDES AND MELANOCORTIN RECEPTORS OF THE HAGFISH, EPTATRETUS STOUTII
Robert M. Dores(1), Ciaran Shaughnessy(2), Ian Bouyoucos(3), Robert M. Dores(4),
(1)(2)(4)University of Denver, USA (3)University of Manitoba

Opioid and orphanin (nociceptin) peptides are derived from large precursor proteins that belong to the Opioid/Orphanin gene family. For the gnathostomes there are four genes in this family, proenkephalin, prodynorphin, proorphanin, and pomc that originated as a result of the two genome duplication events that have punctuated the evolution of the chordates. In gnathostome genomes (R2) each precursor gene codes for at least one opioid-like peptide (i.e., YGGF/FGGF), but only the pomc gene codes for melanocortin-related peptides. The origin of the melanocortin peptides in pomc is the subject of this study. We tested the hypothesis that the genome of an R1 agnathan vertebrate may contain a gene that only codes for melanocortin-related end products. To this end, an analysis of a transcriptome of the hagfish Eptatretus stouti revealed a cDNA that codes for a 168 amino acid long protein that has a signal sequence, and the sequence of a 34 amino acid ACTH-like peptide that is flanked by a monobasic cleavage N-terminal to the peptide sequence and a tetrabasic cleavage site C-terminal to the peptide sequence. The ACTH-like peptide has the HFRW ?message? motif and the KKRR ?address? motif that is characteristic of ACTH-related ligands. This precursor sequence was also detected in the genome of the hagfish Eptatretus burger. A cAMP reporter gene assay was done to show that the melanocortin-a receptor (Mca) of E. stouti expressed in CHO cells could be activated by the hagfish ?ACTH? with an EC50 value of 2.3x10-08M+7.8x10-09. The efficacy of this ligand on E. stouti Mcb, and the implication of this ACTH precursor protein with respect to the origins of POMC will be discussed.
(Acknowledgements: Support for this research was provided by the Long Endowment (University of Denver; 143246; R.M.D.), a National Science Foundation Postdoctoral Fellowship (DBI-2109626; C.A.S), and a Company of Biologists Travelling Fellowship (JEBTF2210863; I.A.B.).)

OR17-3
CHARACTERIZATION OF BEHAVIORAL CHANGES IN INSULIN-SIGNALING IMPAIRED MALES AND FEMALES IN DROSOPHILA MELANOGASTER.
Jessica Paloma Alvarez-Rendón, Juan Rafael Riesgo-Escovar
INB UNAM, México

In vertebrates, insulin signaling regulates metabolic control whereas insulin-like growth factors regulate development and growth. In insects like Drosophila melanogaster, insulin-like peptides (ILPs) participate in both functions, coupling nutritional cues with development. Homozygous null mutations in insulin pathway genes are lethal in flies, but milder reductions in insuling signaling, while viable, delay development, final organ and adult size, and lead to metabolic dysregulations like hyperglicemia and dyslipdemia. We sought to characterize Drosophila behavioral defects consequence of reduced insulin signaling. We quantified fertility, feeding behavior, and locomotor activity levels of InR (the sole insulin receptor fly homologue) heteroallelic mutant flies, as well as wildtype controls with the same genetic background. We found no significant differences in adult progeny from crosses between InR mutant or wildtype females with wildtype males, or between wildtype females and mutant males, but crosses between mutant males and females produced no progeny. InR mutant females exhibited higher food consumption and starvation resistance compared to their wildtype controls. Males showed no significant differences in total consumption. Lastly, InR mutant males and virgin females are hyperactive. Mated females exhibited a reduction in activity levels. These results highlight the all-encompassing impact insulin-signaling impairment can have in Drosophila melanogaster, which extends to behavioral changes, and the influence of sex and mating status on these changes.
(Supported by the Instituto de Neurobiología, Universidad Nacional Autónoma de México, PAPIIT #IG200216 to JRR-E and CONACYT scholarship #612316 to JPAR)

OR17-4
IN VITRO AND IN VIVO STUDIES ON THE FUNCTION AND OSMOREGULATORY ACTION OF NEUROHYPOPHYSIAL HORMONES AND RECEPTORS IN THE SEA LAMPREY (PETROMYZON MARINUS)
Andre Barany(1), Ciaran Alvar Shaughnessy(2), Amy Regish(3), Juan Miguel Mancera(4), Stephen Daniel McCormick(5), Robert M. Dores(6)
(1)(4) Universidad de Cádiz, Spain (2)(6)University of Denver, (3)U.S. Geological Survey, Conte Anadromous Fish Research Laboratory, (5)University of Massachusetts

Sea lamprey (Petromyzon marinus) are extant basal vertebrates that undergo a larvae-to-juvenile metamorphosis that includes the development of seawater (SW) tolerance. Although the basic osmoregulatory mechanisms in the lamprey appear to be similar to those of teleosts, relatively little is known about the role(s) of neurohypophysial hormones in controlling osmoregulation in freshwater (FW) or SW. A single pre-pro-peptide gene existed before the origin of vertebrates, arginine vasotocin (AVT), and many gene orthologs (AVP, OXT, IT, and MT) now exist across vertebrate taxa. The present study examined physiological and pharmacological function of neurohypophysial hormones and receptors in the juvenile sea lamprey through a series of in vitro and in vivo approaches aimed at understanding thier role in supporting osmoregulation. The transcriptional expresion of neurohypophysial hormone receptors (v1r1, v1r2, v1r3, v2r1, v2r2) was examined in tissues from FW- and SW-acclimated sea lamprey, and throughout metamorphosis and salinity acclimation. The effect of AVT on intestinal water absorption ex vivo and systemic osmoregulation in vivo were examined in FW-and SW-acclimated sea lamprey. Stimulation of lamprey V1- and V2-type receptors using AVT, OXT, and IT were examined in vitro. This work is the first to provide an integrative analysis of the role(s) AVT role at various levels (molecular function to physiological action) in the sea lamprey.

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OR17-5

THE ROLE OF NDR1 AND NDR2 IN REGULATING ZEBRAFISH OVARIAN FUNCTION

Sajid Alvi(1), Zayed Y (2), Malik R (3), Kwong RWM (4), Zhu Y(5), Peng C(6)
(1)(2)(3)(4)(6) Department of Biology, York University, Canada (5) Department of Biology, East Carolina University, Greenville, NC, USA

Nodal-related (ndr) 1 and 2 are homologs of Nodal, a member of the transforming growth factor-? (TGF-?) superfamily. Zebrafish ndr1 and ndr2 are essential regulators of germ layer cell organization, left-right asymmetry, and midline signaling during embryogenesis. Complete silencing of ndr1 or ndr2 is lethal and results in characteristic morphological defects in larvae. However, their roles in adult physiology are unclear. We have demonstrated that ndr1 and ndr2 are highly expressed in adult zebrafish ovaries and that Nodal inhibits follicular cell proliferation while inducing oocyte maturation, in vitro. Additionally, siRNA-mediated knockdown of ndr1 and ndr2 decreases levels of the key steroidogenic enzymes in follicular cells. These results suggest that ndr1/2 play key roles in zebrafish ovarian function, particularly in follicle development and oocyte maturation.
To study the role of Nodal in ovarian function, in vivo, we used CRISPR/Cas9 technology to generate ndr1 and ndr2 knockout zebrafish lines with 7 and 4 bp deletions, respectively. The homozygotes displayed morphological defects characteristic of ndr1/2 silencing and embryonic lethality. Therefore, heterozygotes were used for analysis. In ndr2+/- females, we found that spawning frequency and embryo production was significantly reduced, indicating a reduction in fertility. We also found that levels of the maturation inducing hormone precursor 17?-hydroxyprogesterone were significantly reduced in ndr2+/- as compared to wildtype. In ndr1+/- females, ovarian mRNA expression of several genes involved in steroidogenesis were altered. These results support the notion that ndr1/2 play key roles in regulating follicle development and oocyte maturation, and hormone regulation. We are currently establishing inducible ndr1 and ndr2 knockout lines to further investigate the role of these genes in regulating gonadal functions.
(Supported by a discovery grant from Natural Science and Engineering Research Council of Canada to CP.)

OR17-6

DISRUPTION OF T3-INDUCED EPIGENETIC REGULATOR MBD3 LEADS TO GROWTH INHIBITION AND DEVELOPMENT RETARDATION IN XENOPUS

Liezhen Fu, Shouhong Wang, Yun-Bo Shi
NICHD/National Institutes of Health, USA

Adult organ-specific stem cells are critical for organ homeostasis as well as tissue repair and regeneration. However, it has been difficult to study the formation of adult stem cells during vertebrate development. The development of adult intestine during thyroid hormone (T3)-dependent frog metamorphosis, which involves apoptotic degeneration of the larval epithelium and de novo formation of adult stem cells, offers a unique opportunity to study adult stem cell development. T3 controls frog metamorphosis through T3 receptor (TR)-mediated regulation of T3 response genes. We previously carried out a ChIP-on-chip analysis with anti-TR antibody on the tadpole intestine and identified many putative TR target genes. Among them is the methyl-CpG binding domain protein 3 (MBD3) gene, which has been implicated to play important roles in epigenetic regulation of cellular processes as a subunit of the Mi-2/NuRD (Nucleosome Remodeling Deacetylase) complex. We showed that MBD3 is upregulated in the intestine by T3 and its expression peaks at stage 62, the climax of metamorphosis. We further showed that a putative TRE within the first intron of the MBD3 gene binds to TR/RXR in vitro and in vivo, and mediates T3 regulation of the MBD3 promoter in vivo. To investigate how MBD3 functions to regulate cell fate during intestinal metamorphosis, we generated, through CRISPR/Cas9-mediated genome editing, a germline mutant of 8nt-deletion within the Xenopus tropicalis MBD3 coding sequence to knockout MBD3 (MBD3-KO). Preliminary data suggested that homozygous MBD3-KO significantly impeded tadpole growth and development, and delayed the onset of metamorphic climax. Further studies is under way to investigate the impact of complete MBD3-KO on the entire metamorphic process and tissue-specific metamorphic changes, including intestinal remodeling and adult stem cell formation, and the underline molecular mechanisms.
(This work was supported by the Intramural Research Program of NICHD, NIH.)

Thursday, June 1st, 2023
10:30 – 12:00
Salón Claustro III

NASCE 2023 Symposium 18:
Emerging topics in comparative endocrinology II.
Co-Chairs:
Aurora Olvera Vidal and Ivan Lazcano

OR18-1

AMMONIA INHIBITS OOCYTE MATURATION, OVULATION AND SPAWNING IN ZEBRAFISH

Glen Van Der Kraak, Cory Schilling, Jacquie Matsumoto
University of Guelph, Canada

There is considerable interest in understanding the potential for municipal wastewater effluents to affect reproduction in fish. A common constituent of municipal wastewater effluents is ammonia which arises from amino acid catabolism and the runoff of agricultural fertilizers. Surprisingly little is known of its effects on reproduction in fish or its possible interactive effects with endocrine disrupting chemicals present in sewage treatment plant effluents. The purpose of this study was to investigate the effects of ammonia on reproductive processes in adult female

zebrafish. Exposure of zebrafish to unionized ammonia at 0.15 mg/L or higher for 5 to 10 days led to a significant reduction in the numbers of eggs spawned through reductions in both the number of spawning events and the number of eggs produced per spawn. Ammonia exposure had no effects on ovarian testosterone or 17?-estradiol content or on the expression of genes involved in steroidogenesis (lhcgr, star, cyp19a1a), prostaglandin synthesis (pgr, pla2g4aa) or oocyte meiotic maturation (mpr?). Separate studies showed that the birth control estrogen ethinylestradiol also inhibited spawning success in the zebrafish but did so through mechanisms that was distinct from the actions of ammonia. In other studies, 96 hr exposure to 0.3 mg/L unionized ammonia exposure completely blocked spawning in response to an intraperitoneal injection of human chorionic gonadotropin (hCG). Interestingly, a high proportion (40%) of the ammonia exposed fish ovulated in response to the hCG injection. Additionally, exposure to ammonia for 24 led reduced the effectiveness of the progestin 17?, 20? dihdroxy-4-pregnen-3-one on the in vitro induction of oocyte maturation in zebrafish ovarian follicles. Collectively, the current studies demonstrate that ammonia is a reproductive toxicant in the zebrafish and this provides the impetus to examine the responses of fish inhabiting regions of high ammonia content such as areas immediately downstream of wastewater treatment plants.
(Supported by NSERC, the Canadian Water Network and the Ontario Ministry of the Environment, Conservation and Parks)

OR18-2

THYROID HORMONE REGULATES IRON TRANSPORT VIA FERROPORTIN TO STIMULATE ERYTHROPOIESIS DURING POSTEMBRYONIC DEVELOPMENT

Yuta Tanizaki, Yun-Bo Shi
NICHD, National Institutes of Health, USA

Aberrant production of erythrocytes and anemia have been observed in patients with thyroid diseases for more than a century, but the molecular mechanisms underlying thyroid hormone (T3) regulation of erythropoiesis are unknown. We have generated T3 receptor (TR)? and TR? double knockout (TRdKO) Xenopus tropicalis tadpoles to study T3 function during postembryonic development and found that TRdKO tadpoles exhibited iron deficiency anemia. Surprisingly, TRdKO tadpoles had large amounts of iron stored in the hematopoietic organs, the liver, during metamorphosis when fetal erythrocytes are removed and adult erythrocytes are formed, suggesting a failure in iron metabolism in TRdKO tadpoles. Our molecular analysis demonstrated that the expression of the iron exporter ferroportin in liver macrophages, which captures fetal erythrocytes for destruction and iron recycling, is directly regulated by T3. Our study suggests that liganded TR is required for iron recycling to facilitate adult erythropoiesis during metamorphosis by activating ferroportin expression.
(This work was supported by the intramural Research Program of NICHD, NIH. Y. Tanizaki was supported in part by Japan Society for the Promotion of Science (NIH) Fellowship (No. 29-71715) and a FY22 NICHD Early Career Award.

OR18-3

SALINITY-DEPENDENT ENDOCRINE PATHWAYS IN TILAPIA GILL AND KIDNEY

Tharindu Malintha Gardi Hewage(1), Fritzie Celino-Brady(2), Alan Hudson(3), Andre Seale(4), Jason Breves(5), Sandaruwan Rathnayake(6)
(1)(2)(3)(4)University of Hawaii at Manoa, USA (5)Skidmore College, Saratoga Springs, New York, USA (6)Danforth Plant Science Center, MO, USA

Euryhaline fishes, such as Mozambique tilapia (Oreochromis mossambicus), are capable of maintaining hydromineral balance in conditions ranging from fresh water (FW) to seawater (SW) through the endocrine control of osmoregulatory organs such as the gill and kidney. In FW, for example, prolactin (Prl) promotes ion uptake and retention. Two Prl isoforms in tilapia, denoted Prl177 and Prl188, bind Prl receptors (Prlr1 and Prlr2) in target tissues to activate downstream signaling pathways. We compared the branchial and renal transcriptomes of tilapia acclimated to FW and SW. Differentially expressed genes (DEGs) were clustered through co-expression analysis. Major expression profile clusters of tissue-specific DEGs, 16 in the gill and 8 in the kidney, were identified in FW- and SW-acclimated tilapia. Focusing on the activation of endocrine pathways in FW versus SW, we further analyzed the cluster of DEGs containing prlr1 in the gill and kidney. Branchial transcription factor (TF) transcripts associated with Prl signaling, including stat5b, mknk2b, junbb, and socs2, were co-expressed with prlr1 in FW-acclimated fish. In contrast, impa, an enzyme involved in the biosynthesis of myo-inositol (a compatible osmolyte) was the highest expressed branchial transcript in SW-acclimated fish while the transcription repressors bcl6ab and pax1b were co-expressed. The kidney cluster in FW-acclimated fish containing prlr1 co-expressed jakmip1, jak3, and stat5b, which are involved in the Prl signaling pathway along with other TF transcripts: crebzf, wt1a, wt1b, crem, pou2af1, pou6f1, and sall4. In the kidney of SW-acclimated tilapia, transcripts of the TF, sall3a, and the hypocalcaemic hormone, stanniocalcin, were highly co-expressed. Our collective findings shed light on the molecular mechanisms associated with salinity-dependent and tissue-specific endocrine pathways in a euryhaline teleost.
(Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), NIH (1R21DK111775-01), and NSF (IOS-1755016) to A.P.S..)

OR18-4

EFFECT OF GROWTH HORMONE (GH) ON NEUROINFLAMMATION IN THE POSTNATAL CEREBELLUM OF RATS SUBJECTED TO HYPOXIA

María del Rosario Baltazar Lara(1), Martha Carranza(2), D Gasca Martínez(3), Carlos Guillermo Martínez Moreno(4), José Ávila Mendoza(5), Carlos Arámburo(6), Maricela Luna(7)
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Perinatal asphyxia is a leading cause of mortality and neurodevelopmental impairment in infants. The cerebellum, which undergoes a prolonged developmental process, is particularly vulnerable to asphyxia-induced damage.Postnatal rats exposed to hypoxia have shown persistent inflammation in the cerebellum, with microglial cells implicated in Purkinje neuron death. Growth hormone (GH) is a pleiotropic protein that increases in the cerebellum in response to hypoxia and can exert neuroprotective, regenerative, and anti-inflammatory effects. This study aimed to investigate whether administering GH systemically to newborn rats after inducing hypoxic injury can mitigate inflammation and enhance cerebellar function. The study used 1-day postnatal Wistar rats exposed to hypoxia damage (8% O 2 for 2 hours), which then received 5 doses of bovine GH (0.1mg/kg/day, sc.). The expression of proinflammatory mRNA markers and activation of NF-kB cell signal, was evaluated using qPCR and Western blot, respectively. Behavioral tests of motor coordination, locomotion, and anxiety were analyzed at 60 days old. The results showed

that hypoxia damage induced a persistent (7-day) inflammatory response in the cerebellum, which was mediated by the increase of IL-1?, TNF-?, IL-6, COX-2, and iNOS mRNAs expression. GH administration reduced cerebellar inflammation by significantly decreasing the expression of these factors and diminished the activation of NF-kB. Hypoxia damage increased deficits in motor coordination and anxiety behavior. In contrast, GH treatment improved motor coordination and reduced anxious-like behavior. In conclusion, these data suggest that GH treatment reduces inflammation and improves cerebellar function.
(We thank the technical assistance of Santiago Pech-Pool, Ericka Ríos Arellano, Elsa Nydia Hernández Ríos, and Gerardo Curtois. Supported by PAPIIT-DGAPA UNAM (IN227020, IN209621, IN215522, IA200622) and CONACYT (285004, 214971). B-L R received a Ph.D. fellowship (696979) from CONACYT.)

OR18-5

THE CONTRIBUTION OF THE COMMUNICATION SYSTEMS IN THE HYPOTHALAMUS-PITUITARY UNIT IS PLASTIC AMONG VERTEBRATES

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Traditionally, the pituitary has been viewed as a randomly organized collection of cells that respond to hypothalamic stimuli by secreting their content. However, recent studies have established that pituitary cells in mammals are organized in tightly wired large-scale networks that communicate with each other in both homo and heterotypic manners, allowing the gland to quickly adapt to changing physiological demands. We describe that the existence of pituitary cell networks exists in all vertebrates as presented in this work by representative species of fish, amphibians, reptiles, and mammals. These networks functionally decode and integrate the hypothalamic and systemic stimuli and serve to optimize the pituitary output into the generation of physiologically meaningful hormone pulses. We also describe that the organization of pituitary cell networks is highly determined by the communication with the hypothalamus. In particular the grade of vascularization in the portal system of vertebrates allows transport of hypothalamic hormones to the pituitary but in species where vascularization is poor neurons pervade the pituitary facilitating hormone devilevy and communication.
(We would like to thank Edgar Jiménez-Díaz and the LANSBIODYT-UNICUA-UNAM (CONACYT) for equipment and the technical support in the acquisition of confocal images. We would like to thank Berta-Levavi Sivan for her permission to use the zebrafish pituitary images. YSA would like to thank the Posgrado en Ciencias Biolo?gicas (UNAM) for the academic formation through its Master?s degree program in Biological Sciences and CONACyT for graduate scholarship (CVU: 686018).)

OR18-6

EFFECTS OF KISSPEPTINS ON GENE EXPRESSION IN THE HYPOTHALAMO-PITUITARY-GONADAL AXIS OF THE FEMALE WESTERN CLAWED FROG, SILURANA TROPICALIS

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The neuropeptide kisspeptin (Kiss) and its receptor Gpr54 are vital for proper mammalian reproduction with variable importance reported in teleost fish. Little research exists on the function of kisspeptinergic systems in amphibians but may have a local intratesticular role to regulate steroidogenesis and spermiation. Genome searches have revealed that S. tropicalis likely produces Kiss-1a, Kiss-1b, and Kiss-2 and therefore we are investigating their role in the hypothalamo-pituitary-gonadal (HPG) axis of this species. Genes for the kisspeptins and Gpr54 (-1a, -1b, -2) are expressed in hypothalamus, pituitary, and ovarian tissues. We successfully synthesized and purified S. tropicalis Kiss-1a and Kiss-2 then studied the effects of lymph sac injections of several doses (1, 10, 100, 1000ng/g) compared to saline-treated adult females. Collection of hypothalamus, pituitary, and ovary was performed 3 hours post-injection and subsequent gene expression analysis was conducted using droplet digital PCR (ddPCR). Injection of Kiss-1a at doses of 1, 100, and 1000ng/g caused significantly increased expression of gpr54-1b, kiss2, and gpr54-2 within hypothalamus and pituitary tissues compared to saline. Surprisingly, there was no significant effect on gnrh-1, gnrh-2, lhb, or fshb gene expression in these tissues with any of the doses. These results indicate that Kiss-1a may be stimulating the other kisspeptins and thus it will be necessary to compare the effects of Kiss-1a to Kiss-1b and Kiss-2 to establish the importance of kisspeptins in the reproduction of this frog.
(The financial support of the Ontario Graduate Scholarship and NSERC Discovery program is acknowledged.)

P1

CHANGES IN CORTISOL, GLUCOSE AND CORTICOSTEROID RECEPTORS DURING DYNAMIC SALINITY CHALLENGES IN MOZAMBIQUE TILAPIA

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In estuarine environments, euryhaline fish maintain a stable internal osmolality despite frequent fluctuations in environmental salinities. The capacity of fish to maintain osmotic homeostasis is facilitated by the endocrine system, including the hypothalamus-pituitary-interrenal (HPI) axis. While most actions associated with the HPI axis investigated models of seawater (SW) acclimation, fewer studies have focused on the effects of hyposmotic and/ or dynamically-changing environments. We characterized plasma glucose and cortisol, and mRNA expression of pituitary pomc, and hepatic and branchial gr1, gr2, and mr in Mozambique tilapia (Oreochromis mossambicus) subjected to salinity challenges employing two experimental paradigms. In one experiment, fish were transferred from freshwater (FW) to SW and from SW to FW; in the second, fish were transitioned from either FW or SW to a tidal regimen (TR), where FW and SW in-flow changed every 6 h to simulate a tidal environment. Following one-time transfers, fish were sampled at 0, 6 h, 1 d, 2 d, and 7 d while in the transfer to TR, fish were sampled at time 0 and 15 d. Pituitary pomc expression was higher in SW and the SW phase of the TR. Upon transfer to SW, pomc expression transiently increased in FW to SW transferred fish. While plasma cortisol did not change with salinity, plasma glucose increased in fish transferred from SW to FW or in the FW phase of the TR. Further, branchial gr1, gr2, and mr expression decreased following transfer from SW to FW and in the FW phase of the TR. Together, these responses suggest that responses of the HPI axis to salinity challenges are largely mediated by changes in branchial corticosteroid receptors. These findings further clarify the role of the HPI axis in euryhaline fish during dynamic salinity challenges.
(Supported by HATCH (#HAW02051-H), NOAA (#NA18OAR4170347), and NSF (IOS-1755016) to A.P.S.)

P2

DISRUPTION OF BONE MORPHOGENETIC PROTEIN TYPE II RECEPTORS RESULTS IN REPRODUCTIVE AND NON-REPRODUCTIVE DYSFUNCTION IN ZEBRAFISH

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Bone morphogenetic protein type II receptor (BMPRII) is one of the most important receptors in TGF? superfamily. It plays vital roles in cell growth and differentiation, embryonic development, and bone formation in both mammals and teleosts. There are two Bmpr2 genes in zebrafish, bmpr2a and bmpr2b. In this study, we generated bmpr2a and bmpr2b mutants in zebrafish using CRISPR/Cas9. Phenotype analysis showed that deficiency of bmpr2a caused gonadal hypertrophy in both males and females. In the ovary, despite normal folliculogenesis in young fish, there was a significant accumulation of primary growth (PG) follicles, while the male mutant had a super large testis with enormous number of spermatogonia. The bmpr2b mutants also caused defective folliculogenesis in the ovary with follicle development arrested at mid-vitellogenic (MV) stage. We further demonstrated that the ovary of double mutant (bmpr2a-/-;bmpr2b-/-) was also hypertrophic with follicles arrested completely at the PG stage, and the testis showed abnormal spermatogenesis. In addition to gonadal abnormalities, the double mutant (bmpr2a-/-;bmpr2b-/-) also showed obstruction of oviduct, indicating a role for BMP signaling in cloaca development. The loss of BMP signaling also impacted non-reproductive functions. The double mutants developed protruding lower jaw and abnormal bone structures. The shape of neural and hemal arches were irregular in the double mutant, which were wider than those in the control fish. The defective skeleton development in double mutant also caused defective locomotion. This study will provide strong evidence for the importance of BMP signaling in fish development and reproduction.
(This study was supported by grants from the University of Macau (MYRG2019-00123-FHS, MYRG2020-00192-FHS and CPG2022-00028-FHS) and The Macau Fund for Development of Science and Technology (FDCT173/2017/A3, FDCT0132/2019/A3 and NSFC-FDCT0086/2022/AFJ).)

P3

EFFECT OF THE VASOINHIBIN ANALOGUE CRIVi45-51 ON MELANOMA LEUKOCYTE INFILTRATION AND METASTASIS IN MICE

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Vasoinhibin is an endogenous protein that reduces tumor growth by inhibiting angiogenesis and promoting leukocyte infiltration. Difficulties in the clinical translation of vasoinhibin were recently overcome by the development of the vasoinhibin analogue CRIVi45-51, a cyclic retro-inverse heptapeptide that is orally active to inhibit the growth and vascularization of melanoma tumors in mice. Here, we further investigated the potential therapeutic benefit of CRIVi45-51 by studying its effect on melanoma leukocyte infiltration and vascular permeability associated with tumor growth and metastasis. We examined the effect of CRIVi45-51 on the RT-PCR expression of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1) in cultured human umbilical vein endothelial cells (HUVEC) and quantified the number of stained peripheral blood leukocytes adhered to HUVEC monolayers. Also, the expression of the leucocyte marker CD45 was determined in primary tumors derived from the subcutaneous inoculation of the mouse melanoma cell line (B16-F10) in mice treated intravenously (IV) with CRIVi45-51. Finally, the IV effect of CRIVi45-51 was tested on the number of macroscopic pulmonary metastatic nodules generated by the IV administration of B16-F10 cells in mice. CRIVi45-51 did not modify the expression of VCAM1 and ICAM1 nor the adhesion of leukocytes to HUVEC. Nonetheless, it did lower the expression of CD45 in melanoma primary tumors suggesting reduced leukocyte infiltration through inhibition of vascular permeability, a known effect of CRIVi45-51 and vasoinhibin. Consistently, vascular permeability is indispensable for cancer metastasis and CRIVi45-51 reduced by 60% the number of macroscopic melanoma nodules in lungs. In conclusion, CRIVi45-51 inhibits leukocyte infiltration in melanoma primary tumors and reduces melanoma lung metastasis. Ongoing studies are investigating whether reduced vascular permeability in tumors and remote organs (lung) mediates both actions of CRIVi45-51.
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P4

VASOINHIBIN ANALOGS AS A NEW THERAPEUTIC STRATEGY FOR THE TREATMENT OF ARTHRITIS

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Rheumatoid arthritis (RA) is a progressive autoimmune inflammatory pathology dependent on angiogenesis. In the treatment of RA, anti-inflamatory drugs are prescribed that trigger severe cardiovascular adverse reactions, dependence, and even reactive RA, so the search for new therapeutic strategies is important [1]. Since angiogenesis is a key event in RA, anti-angiogenic factors represent a promising therapy for its control. Vasoinhibin (Vi) is an anti-angiogenic fragment of prolactin with potential therapeutic effects in arthritis [2]. However, Vi has pro-inflammatory effects on the arthritic joint due its actions on various cell types, including synovial fibroblasts (SF) [3]. It was recently described that the anti-angiogenic functional domain of Vi is found in amino acids H46-G47-R48 and heptapeptides that contain them in their linear and cyclic form that contain them retain the same anti-angiogenic potency [4], but it?s still unknown whether they conserve its pro-inflammatory actions. In the present work, we analyzed the possible pro-inflammatory effect of Vi45-51 and CRIVi45-51 on the joint. SF was isolated from the joint of male C67BL/6 mice and treated with or without recombinant vasoinhibin (positive control), Vi45-51 and CRIVi45-51. Vi but not the analogs induced the expression of Tnfa and nitric oxide (NO) via iNOS. To highlight the effect of CRIVi45-51 on joint inflammation, antigen-induced arthritis (AIA) was induced via intra-articular administration of 20 µg (severe inflammation) or 2.5 µg (mild inflammation) of mBSA (methylated bovine serum albumin) to observe the antiangiogenic and pro-inflammatory effects of vasoinhibin, respectively [3]. As expected, CRIVi45-51 decreased joint inflammation through its antiangiogenic actions on the endothelium in severe AIA but didn?t increase inflammation in mild AIA. We conclude that Vi45-51 and CRIVi45-51 are potential therapeutic agents for the treatment of arthritis because they conserve the beneficial antiangiogenic properties of Vi but are lack of its deleterious proinflammatory effects.
(Acknowledgments: Supported by CONACYT (A1-S-9620B) and UNAM (DGAPA-PAPIIT IN202321).
Reference: (1) Smolen et al., Ann Rheum Dis 2023 Jan;82(1):3-18. (2) Ortiz et al., Lab Invest. 2020 Aug;100(8):1068-79. (3) Ortiz et al., Endocrinology 2022 May;163(5): bqac036. (4) Robles et al., Angiogenesis 2022 Feb;25(1):57-70.)

P5

LIPID PROFILES IN DIFFERENT REGIONS OF THE BRAIN OF FATHEAD MINNOWS ARE ALTERED UPON EXPOSURE TO STEROID HORMONES

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Sex steroid hormones activate both nuclear and membrane receptors in brains of fish. In previous work, we reported alterations in brain transcriptomics and proteomics profiles in fathead minnows treated with 17 alpha ethinylestradiol (EE2) and levonorgestrel (LVN), showing that estrogen and progestins elicit changes in pathways related to neuronal processes, synaptic plasticity, and neurite outgrowth. Lipid composition of brains is proportionately high as myelin sheaths are composed of 85% lipids and 15% proteins while other biological membranes are roughly 50% lipids and 50% proteins. Some lipids are bioactive molecules made on site when they are needed and may target G-coupled proteins, among other bioactivities. We tested the lipidomes of male and female fathead minnows and found the profiles to be distinct from each other overall and distinct in different brain regions. We hypothesized that exposure of fathead minnows to EE2 and LVN would result in altered lipid profiles, especially for bioactive lipids in the brain of fathead minnow. Fathead minnow adult males were treated with 5 ng/L EE2 or 100 ng/L LVN for 24 h and the lipidome was investigated in different regions of the brain using a targeted LC MS/MS approach on an AB Sciex QTRAP 6500 mass spectrometer. The lipidomes of these regions were altered by the treatments, especially for several bioactive lipids including ceramides, sphingomyelins, diacylglycerols and lysophospholipids, suggesting these lipid species may play a role in the dimorphism of the brain. Further experiments with MALDI imaging mass spectrometry of brain slices from the fish showed that the alterations in the lipids were cell specific and were distributed heterogeneously throughout different brain regions. Transverse sections of flash-frozen minnow brains were collected at 10 µm thickness and thaw mounted onto indium tin oxide-coated microscope slides. 2,5-dihydroxyacetophenone (DHA) MALDI matrix was applied using a custom-built sublimation apparatus. MALDI imaging experiments were performed at 100 µm spatial resolution on a Bruker Daltonics QhFTICR hybrid mass spectrometer equipped with an Nd:YAG laser system. These findings open this area as a novel research area to understand the role of lipids in brain function.
(University of Florida, College of Veterinary Medicine Faculty Research Development)

P6

MOLECULAR IODINE DECREASES THE INVASIVE POTENTIAL OF NEUROBLASTOMA XENOGRAFTS IN ZEBRAFISH.

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Neuroblastoma (NB) is one of childhood's most common extra-cranial solid tumors, with 90 % of the cases occurring in those <5 years of age. NB is highly heterogeneous, with variable biological and clinical characteristics; exhibits spontaneous or induced regression processes (low-risk NB) up to highly invasive capacities and drug resistance tumors (high-risk NB). High-risk NB overexpresses the N-MYC gene associated with invasion and chemoresistance. On the other hand, molecular iodine (I2) exerts antiproliferative and differentiation effects on cancer cells. Our previous studies have shown that I2 synergies the neurodifferentiation effect of all-trans-retinoic acid in low-risk (SN-K-AS) and the antiproliferative and apoptotic action of cyclophosphamide in high-risk (SK-N-BE2) neuroblastoma cell lines. The cellular mechanisms include the activation of antioxidant pathways (Nrf2) and the overexpression of the peroxisome proliferator-activated receptor gamma (PPAR?). The present aim is to analyze the effect of I2 on the invasive capacity of SNK-AS and SK-N-BE2 in the zebrafish model. NB cells were cultured in DMEM medium with and without I2 400 uM for 72 hours. 400 to 600 cells labeled with Fast DiI (4 ug/mL) were injected cells into the perivitelline space of zebrafish larvae (2 days post fertilization). The larvae were incubated for 48 hours at 28°C in E3/PTU medium to prevent pigment formation. Cancer cell dissemination to the caudal vein plexus was analyzed with a fluorescence Nikon Eclipse E-600 microscope (10X/0.30 magnification).
Cell line Control I2 400 uM

SNK-BE2	14.78 ± 9.9	15.75 ± 7.5
SNK-AS	13.33 ± 7.9	9.71 ± 5.4

Preliminary data show that only the I2-pretreated low-risk cells exhibited partial impairs in migration capacity. Analysis with higher iodine concentrations in high-risk cells is currently under evaluation.
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P7

NMN SUPPLEMENTATION REVERSES FEMALE REPRODUCTIVE AGING AND RESCUES NAMPTA AND NAMPTB DEFICIENCY-INDUCED GONADAL AGEING IN FEMALE AND MALE ZEBRAFISH RESPECTIVELY

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Female reproductive aging is characterized with a steady decline in both quantity and quality of follicles. Here, we report that the decrease of oocyte quality in aging zebrafish was accompanied by a decline of niacinamide adenine dinucleotide (NAD+), an important enzyme cofactor. A supplementation of NAD+ precursor nicotinamide mononucleotide (NMN) could effectively enhance the quality and quantity of eggs produced by aged female zebrafish with increased levels of NAD+. Supplementation with NMN could not only improve the fertility of aged females, but also increase their fertilization capacity by improving maturation of oocytes in vitro. In addition, the increase in ovarian NAD+ levels in aging zebrafish also led to increase in the number of ovarian follicles and their ovulatory potential. Further analysis showed that NMN supplementation reduced reactive oxygen species in the oocyte while enhanced the activity of antioxidant enzymes. It also elevated the ATP levels and reduced inflammatory cytokine contents to suppress apoptosis in aged oocytes. Mutation of nampta, the enzyme responsible for the conversion of NMN to NAD+ caused premature ovarian failure, which could be rescued by NMN supplementation. Interestingly, mutation of namptb reduced spermatogenesis in males, which could also be ameliorated by NMN supplementation. In general, our data suggest that NMN supplementation is an effective way to slow down or even reverse the process of reproductive aging in zebrafish, and that Nampta and Namptb may be responsible for maintaining NAD+ levels in the ovary and testis respectively.
(This study was supported by grants from the University of Macau (MYRG2019-00123-FHS, MYRG2020-00192-FHS and CPG2022-00028-FHS) and The Macau Fund for Development of Science and Technology (FDCT173/2017/A3, FDCT0132/2019/A3 and NSFC-FDCT0086/2022/AFJ))

P8

KNOCKOUT OF SOMATOSTATIN RECEPTOR 5 HAS NO EFFECT ON MEDAKA GROWTH

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In vertebrates, somatic growth is regulated by the somatotropic axis. It is well known that the primary hormone in the axis is the growth hormone (GH), which is synthesized in the pituitary gland and induces the expression of insulin-like growth factor I (IGF-I) in the liver, which is ultimately responsible for promoting tissue growth and differentiation. In fish, the release of GH is under constant negative regulation, mainly by the hormone somatostatin (SST). This hormone is synthesized in the hypothalamus of all vertebrates and acts as an inhibitor of GH secretion in the pituitary. In turn, the action of SST is carried out through its receptors. In fish, 4 SST receptors (SSTR 1-3 and 5) have been described, with SSTR 2 and 5 showing the highest expression in the pituitary gland. Therefore, we hypothesized that blocking this inhibition produces an increase in GH secretion, with concomitant somatic growth. We tested this by generating an SSTR 5 mutant using the CRISPR/Cas9 system. First, we analyzed the effect of loss of function on medaka growth by performing a growth experiment in which we breed knock-out fish and WT fish from the hatch and measure the standard length every week until week 7. We observe that the knockouts do not grow significantly faster than the WT fish. We then evaluated whether the SSTR 5 is involved in compensatory growth. Comparing WT fish after a short-term fasting exposure with normal-fed WT fish, we observe a differential expression of GH but no difference in the somatostatin or its receptors (SSTR5). When we performed a fasting test with the knockouts, we did not observe a significant variation in body weight between the feeding protocols. Taken together, the results suggest that the SSTR 5 is not the only somatostatin receptor mediating the SST-GH interaction and is not, or at least not acting alone, for the changes in compensatory growth.
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P9

A COMPARATIVE STUDY OF LIFESTYLES AND METABOLISM OF DROSOPHILA LUTZII, A FLORIDOSA GROUP OF SPECIES, AND SYMPATRIC D. SIMULANS, A GENERALIST SPECIES

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The Drosophila genus of the family Drosophilidae comprises around 1600 described species. These species differ in their geographic distribution and ecologies, and consequently, in ecological niches and lifestyles. As they evolved in different environments, species may differentially regulate their metabolism and behavior as they adapt to these local conditions. We hypothesize that Drosophila lutzii, a phytophagous specialist species; and Drosophila simulans, a saprophytic generalist, regulate their metabolism and insulin signaling in a different manner allowing them to adapt to their particular lifestyles. We are conducting a comparative study between both species. We have found D. lutzii eggs, larvae, pupae, and adults living inside Ipomoea sp. flowers, suggesting a restricted diet and mobility, while D. simulans is widely distributed within the same environment (sympatric species). D. simulans feeding is based on rotting plants and fruits. Freshly caught D. lutzii from the wild have higher carbohydrates levels, but similar lipid content, to sympatric freshly caught D. simulans. Consistent with a restricted diet and specialist lifestyle, D. lutzii flies survive less in culture in diets that differ in the amounts of carbohydrates. Body triglycerides and carbohydrates levels were differentially

affected in both species when fed with diets varying in sugar content. Interestingly, D. lutzii flies accumulated higher carbohydrates content when fed diets with high sugar concentrations, compared to D. simulans. D. lutzii flies are also significantly and dramatically less motile, but possess a circadian rhythmicity akin to that of D. simulans. Both species showed a differential feeding behavior when exposed to food with different amounts of sugar. In order to analyze if the species differentially modulate insulin signaling at the genome and gene expression level, we sequenced the transcriptome and plan to sequence the genome of D. lutzii, and compare it with those of D. simulans, fed with different diets. As illustrated in insulin compromised flies in D. melanogaster, we expect to see significant differences in insulin signaling in these species. So far, our results show that specialist species like D. lutzii, with more restricted ecological niches and feeding, are less capable of metabolic adjustments, compared to generalist species like D. simulans.
(This work was funded by grants from UNAM-DGAPA PAPIIT IN209622 and INB UNAM UR-307.)

P10
MILK PROLACTIN PARTICIPATES ON ENTEROCYTE MATURATION IN LACTATING MICE
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After birth, enterocytes experience a gradual maturation process that transforms the intestinal epithelium to switch from maternal milk to solid food feeding. This transition has important implications for neonatal health. Neonatal enterocytes (Neo-Ent) have different functions than adult-type enterocytes (Ad-Ent). Milk components including growth factors and hormones regulate the transition from Neo-Ent to Ad-Ent. Prolactin (PRL) is a pituitary hormone present in milk in high quantities, and it is a bioactive component because it ameliorates metabolic alterations in pups nursed by dams on a high fat diet during lactation. However, the target tissues that mediate milk PRL (mPRL) actions on the offspring are unknown. Here, we hypothesized that mPRL could participate in the transition from Neo-Ent to Ad-Ent. To test this, we evaluated the morphometry of proximal small intestine with hematoxylin-eosin stain at postnatal day (PD) 14, and gene expression by RT-qPCR of neonatal and mature epithelium (PD 7, 14 and 21) of lactating PRL receptor null mice (Prlr-KO) and their wild type (Prlr-WT) pairs. Neo-Ent is characterized by high expression of Lactase (Lct), Argininosuccinate synthetase 1 (Ass1) and neonatal-Fc-receptor (Fcrn), that are decreased on Ad-Ent. Conversely, Ad-Ent have higher expression of Sucrase-isomaltase (Si) and Adenosine deaminase 1 (Ada1) than Neo-Ent. Duodenum morphometry showed more crypts/field in Prlr-KO than in Prlr-WT mice at PD14, these results could be associated with accelerated gut maturation in Prlr-KO mice. In addition, gene expression of duodenum from Prlr-KO showed reduced expression of Neo-Ent markers, such as Lct and Fcrn compared to Prlr-WT. Consistently, the jejunum of Prlr-KO showed lower expression of markers of Neo-Ent: Lct, Ass1 and Fcrn, and higher expression of Ad-Ent markers than Prlr-WT at PD14. In summary, Prlr-KO mice at PD14 show a phenotype of precocious intestinal epithelium maturation, which could lead to adverse health consequences as poor digestion of lactose and reduced passive immunity. In this regard, Fcrn participates in the regulation of passive immunity in Neo-Ent by allowing protection against degradation of milk´s IgG. These results suggest that mPRL modulates Neo-Ent maturation, avoiding precocious intestinal maturation and facilitating absorption of milk components.
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P11
PLASMA PROGESTERONE AND LIPIDS AS BIOINDICATORS OF PREGNANCY IN THE FLORIDA MANATEE (TRICHECHUS MANATUS LATIROSTRIS)
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Identification of pregnancy status is essential to the management and conservation of threatened wildlife populations. However, pregnancy detection is limited to ultrasonography in the threatened species, Florida manatee (Trichechus manatus latirostris). This study aimed to (1) quantify plasma steroid hormones in Florida manatees from Crystal River and Indian River Lagoon field sites at different gestational stages and to (2) determine the relationship between plasma progesterone concentrations and lipid biochemistry to develop a diagnostic lipid panel for pregnant manatees. Ultra-high performance liquid chromatography-tandem mass spectrometric analysis was used to measure plasma steroid hormones and lipid concentrations. Pregnant female manatees were morphometrically distinct from male and non-pregnant female manatees, characterized by larger body weight and maximal girth. Progesterone concentrations in manatees were also elevated during early gestation versus late gestation. Cholesterol, a lipid precursor for reproductive steroids, was not different between groups. We detected 949 lipid species from 39 lipid subclasses in the serum of manatees. Cholesteryl esters, triacylglycerols, and lysophosphatidylcholines were the lipid subclasses with the first, second, and third largest plasma concentrations, respectively. Plasma concentrations of a sphingolipid, ceramide non-hydroxy fatty acid-sphingosine and several glycerophospholipids, including lysophosphatidylcholine, phosphatidylethanolamines, plasmenyl-phosphatidylserines and monomethyl phosphatidylethanolamines, were associated with pregnancy status in the Florida manatee. These data are expected to advance the understanding of manatee reproductive physiology and contribute to pregnancy detection in archived and fresh blood samples and generates a novel dataset of plasma lipids in healthy Florida manatees.
(We would like to acknowledge Dr. Margaret Hunter and Dr. Jason Ferrante with the Sirenia Project at the U.S. Geological Survey Wetland and Aquatic Research Center, for providing samples. We would also like to thank the Aquatic Animal Health Program at the University of Florida in cooperation with the Florida Fish and Wildlife Conservation Commission for providing samples. We acknowledge the U.S. National Institutes of Health (NIH) Shared Instrumentation (Grant 1S10OD018141 to ND). Finally, we acknowledge that the material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. (2019285699 to EB-R).)

P12

TRANSCRIPTIONAL AND HISTOLOGICAL RESPONSE OF LIVER IN AN OBESE MICE MODEL DUE TO THE EFFECT OF A SYNBIOTIC SUPPLEMENTATION.

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Introduction. Obesity is a pathology characterized by excessive fat accumulation that alters the functioning of pathways such as bile acid production, predisposing the liver to ectopic lipid accumulation, a histological visible characteristic of non-alcoholic fatty liver disease (NAFLD). Clinical studies indicate that the consumption of probiotics and prebiotics modulates the intestinal microbiota, promoting weight loss and decreasing fat deposits influencing metabolic pathways.
Hypothesis. The use of Lactobacillus acidophilus + inulin (synbiotic) as a supplement in a normocaloric diet, can modify the liver transcriptome reducing the negative effects of NAFLD
Main methods. The model murine used was C57BLACK6, inducing obesity with a high-fat diet for 8 weeks followed by synbiotic supplements in a normocaloric diet for another 8 weeks. Pool screening analysis (5 samples) was completed using a synthesis of cDNA. The transcriptome was analyzed by DNA microarrays hybridizing on 22,000 mouse genes. Differentially expressed genes (DEGs) were analyzed under 3 hybridization processes with the aid of GenArise software using the z-score value. As a result of transcriptome analysis, fatty acid-binding genes (Cyp7a1 and Acox2) were selected to analyze the liver response, molecular and histologically.
Results. The transcriptome analysis results indicate 1.26% overexpression and a 2.2% average repression in relation to the hybridized genome; DEGs allow us to identify genes associated with fatty acid metabolism. The synbiotic treatment increases the expression of Cyp7a1 and Acox2 significantly (p<0.05) in correlation with a decrease in the histological level of accumulated fat in the tissue.
Conclusion. The synbiotic treatment could be an adjuvant to obesity and NAFLD as it can increase the production of bile acids coming from the classical pathway which promotes the absorption of ectopically accumulated lipids thus reducing the development of NAFLD at histological and molecular level.
(IAGM thanks CONACYT for the postdoctoral grant.)

P13

THYROTROPIN-RELEASING HORMONE (TRH) DECREASES HYPERGLICEMIA IN ADULT ZEBRAFISH (DANIO RERIO)

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The expression of thyrotropin-releasing hormone (TRH) and its receptor (TRH-R) has been demonstrated on pancreatic tissue in murine and human models. In vitro studies have described the potentiating effect of TRH upon insulin synthesis and secretion in the presence of glucose and have suggested that during hyperglycemia, a mechanism of action at the ?-cell level could favor insulin release. However, the fine-tune mechanisms of this insulin balance are not yet well understood. In recent years, the zebrafish (Danio rerio) has been developed as a useful model for biomedical research due, among other characteristics, to its genetic similarity to humans. In this sense, this teleost conserves the pancreatic functionality as occurs in humans, as well as the signaling elements of glucose regulation, including TRH expression in the pancreas. Given the lack of in vivo studies, we here used the zebrafish to test if TRH indeed regulates hyperglycemia. Male adult zebrafish were immersed in a 2 % glucose solution for 3 days. During this period, glucose-immersed zebrafish received a daily ip injected of saline solution or 0.1, 1 and 10 µg of TRH. At the end of the experiment, all the animals were submitted to 24 h of fasting and sacrificed for blood glucose measurement. Our values of serum glucose in non-fasting fish were similar to previous reports (97.1 ± 31 mg/dL). As expected, intact control animals showed a 1-fold glucose reduction (48.4 +/-2.6 mg/dL) due to fasting, whereas glucose-immersed animals displayed increased glucose levels (165.3 +/-19 mg/dL). Ip injection of 0.1, 1 and 10 µg of TRH in glucose-immersed animals reduced glucose levels to similar values of non-fasting animals (89.2 ± 5.7 mg/dL; 71.5 ± 19 mg/dL, and 103.1 ± 19 mg/dL, respectively). Thus, TRH is able to decrease hyperglycemia as previously demonstrated in mammals. Together, our preliminary results show the in vivo effects of TRH administration on regulating glucose homeostasis in adult zebrafish, probably through acting in the pancreas, as well as places the zebrafish as a plausible model to further study TRH-glucose regulation interactions.
(Dra. Erika A. de los Ríos Arellano, Unidad de Microscopía, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM) Campus Juriquilla, Querétaro, Qro., México. This work was supported by Grants: UNAM PAPIIT IN210823 and IA201122; CONACyT Ciencia de Frontera, Paradigmas y Controversias de la Ciencia 2022 399880.)

P14

MECHANISMS MEDIATING DUAL ACTIONS OF PROLACTIN IN JOINT INFLAMMATION

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The close association between rheumatoid arthritis (RA), sex, reproductive state, and stress have long-linked the sexually dimorphic, reproductive, stress-related hormone prolactin (PRL) to disease progression. However, this role is questioned by the fact that PRL has both pro-inflammatory and anti-inflammatory outcomes in RA. Here, we show that PRL modifies in an opposite manner the inflammatory action of IL-1? and TNF-? in mouse synovial fibroblasts (SF) in culture and in joints in vivo. SF treated with IL-1? or TNF-? upregulated the metabolic activity and the expression of proinflammatory genes (Il1b, Inos, and Il6) via the activation of NF-?B. However, IL-1? increased and TNF-? decreased the levels of the long PRL receptor (PRLR) isoform and this differential regulation associated with dual effects of PRL. PRL decreased the proinflammatory action and activation of NF-?B in response to IL-1?, but increased the inflammatory response and NF-?B signaling stimulated by TNF-?. The double-faceted regulatory role of PRL against the two cytokines also manifested in vivo. IL-1? or TNF-? with or without PRL were injected into the intra-articular space of the knee joint of mice and, after 24 h, joint inflammation was monitored through the expression of pro-inflammatory mediators. Both IL-1? and TNF-? upregulated the joint expression of Il1b and Inos, and PRL inhibited the action of IL-1? but not that of TNF-?. We conclude that the outcome of PRL action on joint inflammation is dependent on its interaction with specific proinflammatory cytokines, the level of the PRLR,

and the activation of NF-?B. Opposite effects of PRL may help balance joint inflammation in RA and understanding their mechanisms could provide insights into the pathophysiology of RA and the development of new treatments.
(We thank Fernando López Barrera, Alejandra Castilla, María Antonieta Carbajo Mata and Martín García Servín for their technical assistance.)

P15

A LAURENCIA JOHNSTONII EXTRACT REVERSES THE EFFECT OF 17b-ESTRADIOL IN EARLY LESIONS IN THE K14E7HPV16 MURINE MODEL OF CERVICAL CARCINOGENESIS

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Cervical Cancer (CC) is a major health problem worldwide. Persistent high-risk Human Papillomavirus (HR-HPV) infection can lead to precancerous intraepithelial lesions classified as Cervical Intraepithelial Neoplasia (CIN) that can progress into CC being hormonal environment one of the main cofactors associated. In most cases, timely treatment of women diagnosed with CIN 1 or 2 would have a high probability that the patients would be cured effectively and, therefore, progression to cancer would be prevented; however, it is necessary to continue in the search for novel, non-invasive treatments, complementary to the existing ones. In recent decades, alternatives have been sought for the treatment of cancerous and precancerous lesions using Natural Products. Among these, Laurencia johnstonii Setchell & Gardner (L. johnstonii) algae have been reported to have cytotoxic and/or antitumor properties. With the objective of determining the effect of a crude extract obtained from L. johnstonii (ELJ), we used a murine model of cervical carcinogenesis which expresses E7 oncoprotein and develops cervical cancer after chronic 17b-estradiol (E2) treatment and evaluated histopathological alterations as well as cell proliferation and apoptosis levels by immunohistochemistry. We found that this model develops CIN 1 at three months of age after a single E2 exogenous hormonal stimulus. Our results show that the treatment with ELJ allows the partial recovery of the cervical epithelium, decreasing the levels of cell proliferation and increasing the levels of apoptosis. Based on these results, we propose that the use of L. johnstonii could be an alternative for the treatment of early lesions of the cervical epithelium related to hormonal stimulus.
(This work was supported by Escuela Nacional de Medicina y Homeopatía, Instituto Politecnico Nacional, grant SIP20220540)

P16

EPIGENETIC CHANGES ASSOCIATED WITH PAIR BOND FORMATION IN THE PRAIRIE VOLE FEMALE

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In the prairie vole (monogamous specie), the socio-sexual stimulation generates a pair bond that lasts for the animal's life cycle. Our research group and others have demonstrated that socio-sexual stimulation modulates adult neurogenesis. It has been proposed that epigenetic modifications might change the proliferative and differentiative potential of the newly generated cells in the subventricular zone and subgranular zone of the dentate gyrus. Within these modifications, the trimethylation of the lysine 4 in the histone 3 (H3K4me3) and the trimethylation of the lysine 27 in histone 3 (H3K27me3) have a fundamental role in genetic regulation. In addition, the administration of deacetylase inhibitors facilitates pair bond formation, increasing the acetylation in the H3 in the oxytocin promotor and vasopressin promotor in the nucleus accumbens. Similarly, a dopamine surge in this region is fundamental to this behaviour. Given all the above, in this work, we determine if the pair bond formation in the female prairie vole induces changes in the neural precursors in the H4K4me3 and H3K27me3 marks. Additionally, we evaluated if the pair bond formation changes the catecholaminergic afferents and the epigenetic marks in the nucleus accumbens. Our data suggest there aren't global changes in the methylation patterns of H3K4 and H3K27 associated with the pair bond. Nevertheless, the surge in the subventricular zone of H3K27me3 opens the possibility that methylation might be involved in the proliferation and differentiation of neural precursors. The application of different methodological strategies is required to evaluate the deposition of the mark on specific genes related to the pair bond formation
(Deisy Gasca, Martín García, Alejandra Castilla y Nydia Hernández, for all their technical assistance)

P17

RENIN GENERATES VASOINHIBIN IN THE RETINA OF NEWBORN MICE

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The hormone prolactin (PRL) acquires antiangiogenic properties upon its proteolytic cleavage to vasoinhibin, a family of PRL fragments ranging from 5.6 to 18 kDa that inhibits angiogenesis in the retina. Excessive retinal angiogenesis underlies retinopathy of prematurity, a major cause of blindness in children. Cathepsin D (CD) is an acidic protease that generates vasoinhibin in the retina of neonate mice as revealed by the CD inhibitor, pepstatin A (PA). However, PA also inhibits renin, an acidic and neutral protease activating the angiotensin-aldosterone system. Here we investigate whether renin cleaves PRL to vasoinhibin in the newborn mouse retina. PRL was incubated (24h at 37°C) under acidic pH with retinal extracts from CD null and wild type mice at postnatal day 8 (P8) and with recombinant renin at acidic and neutral pH in the presence and absence of PA or a selective renin inhibitor (VTP27999). PRL proteolytic products were evaluated by Western blot. PRL was partially converted to vasoinhibin by retinal extracts from wild-type mice and such conversion was prevented by heat- inactivation and by PA. The generation of vasoinhibin was not eliminated when retinal extracts from CD null mice were used, to suggest that another acidic protease, inhibited by PA, contributes to the production of vasoinhibin. In support of renin as the putative retinal PRL cleaving protease, VTP27999 inhibited the generation of vasoinhibin by both, wild-type, and CD null mice. Moreover, incubation of PRL with recombinant renin at acidic and neutral pH generated

fragments of ~14 and ~16 kDa. Renin acted upon PRLs of different species where the location of the main renin cleavage site in rodent PRL(Leu124-Leu125) and in human, bovine, and ovine PRLs (Leu126-Leu127) is consistent with the generation of ~14 kDa vasoinhibin. These findings suggest that renin generates vasoinhibin across species. Ongoing research investigates the physiological
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P18

THE VASOINHIBIN ANALOGUE CRIVi45-51 INHIBITS EXCESSIVE VASOPERMEABILITY IN THE RETINA OF RATS

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Excessive retinal vasopermeability causes visual impairment in diabetic macular edema (DME). Vascular endothelial growth factor (VEGF) is a major vasopermeability factor in DME and intravitreal inhibitors of VEGF have become first-line treatment for this disease. However, anti-VEGF administration is invasive, not always effective, and temporary, emphasizing the need for other therapeutic options. Vasoinhibin is a fragment of the hormone prolactin that inhibits diabetes- and VEGF-induced retinal hypervasopermeability. CRIVi45-51, a cyclic retro-inverse heptapeptide, is an orally active vasoinhibin analogue with promising therapeutic potential. Here, we tested whether CRIVi45-51 inhibits the retinal hypervasopermeability induced by diabetes or VEGF in rats. Diabetes was induced with streptozotocin and six weeks later, rats were injected intravitreally with vehicle, CRIVi45?51, or the anti-VEGF monoclonal antibody ranibizumab. In other experiments, healthy rats were treated orally or topically (eye drops) with CRIVi45-51 one hour before the intravitreal injection of vehicle or VEGF. The Evans blue method was performed 24 hours after CRIVi45-51 administration in all experiments to evaluate the retinal vasopermeability. CRIVi45?51 inhibited the excessive retinal vasopermeability induced by diabetes and VEGF. Inhibition was like ranibizumab and effective after intravitreal, oral, or topical administration and did not modify basal retinal vasopermeability. CRIVi45-51 inhibits excessive retinal vasopermeability in diabetes via the blockage of VEGF action. This vasoinhibin analog has significant therapeutic potential as a non-invasive (oral, topical) treatment for preventing and restricting the progression of diabetic macular edema, diabetic retinopathy, and other vasoproliferative retinopathies.
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P19

IDENTIFICATION OF THE ANTIANGIOGENIC DETERMINANT OF VASOINHIBIN AND DESIGN OF A THERAPEUTIC ORALLY ACTIVE OLIGOPEPTIDE

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The hormone prolactin acquires antiangiogenic and antivasopermeability properties after undergoing proteolytic cleavage to vasoinhibin, an endogenous prolactin fragment that inhibits the action of multiple pro-angiogenic factors. Preclinical and clinical evidence supports the therapeutic potential of vasoinhibin against angiogenesis-related diseases, including diabetic retinopathy, peripartum cardiomyopathy, rheumatoid arthritis, and cancer. However, the therapeutic use of vasoinhibin has been limited by difficulties in its recombinant production and by the partial understanding of its structure-function relationship. This barrier was recently removed by finding that a linear motif of just three residues (His46-Gly47-Arg48) (HGR) is the functional determinant of vasoinhibin. The HGR motif is conserved throughout evolution, its mutation leads to vasoinhibin loss of function, and oligopeptides containing this sequence inhibit angiogenesis and vasopermeability with the same potency as whole vasoinhibin. Monoclonal antibodies directed to this region are vasoinhibin-specific and inhibit vasoinhibin function. The HGR motif is obscured in prolactin by salt bridges formed between Arg48 and a conserved motif comprising Glu161 and Glu162 residues, rendering it unable to inhibit angiogenesis. Furthermore, the oral administration of an optimized cyclic retro-inverse vasoinhibin heptapeptide containing HGR inhibits tumor growth and vascularization in mice and exhibits equal or higher antiangiogenic potency than other antiangiogenic molecules currently used as anti-cancer drugs in the clinic. Therefore, we have developed potent vasoinhibin analogs that are easy to produce, stable, and orally active as potential therapeutics against angiogenesis-dependent diseases.
(Supported by CONACYT A1-S-9620-B. We thank Fernando López Barrera, Xarubet Ruíz Herrera, Alejandra Castilla León, José Martín García Servín, and María A. Carbajo Mata for their excellent technical assistance.)

P20

PROLACTIN REGULATE THE ANTIOXIDANT RESPONSE OF ASTROCYTES THROUGH THE ACTIVATION OF STAT3 SIGNALING PATHWAY

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Astrocytes exert a wide variety of functions in health and disease, including regulating defense against oxidative stress. Agents that improve astrocytes antioxidant defenses could be potential therapies for brain pathologies associated with oxidative stress The STAT3 signaling is a key pathway through which astrocytes regulate their antioxidant response. In this regard, prolactin (PRL) displays antioxidant and cytoprotective effects in retinal pigmented epithelial cells and JAK/STATs are an important pathway associated with prolactin receptor (PRLR) signaling. This study sought to determine the protective effect of PRL against hydrogen peroxide (H2O2)-induced cytotoxicity in primary rat cortical astrocytes in culture. PRL treatment led to increased expression of the long PRLR isoform, and this response was associated with upregulation of STAT3 phosphorylation/activation, increased antioxidant capacity, and both the transcriptional upregulation and increased enzymatic activity of the antioxidant enzymes: superoxide dismutases, glutathione peroxidase and peroxiredoxins. H2O2 induced cell death, increased ROS generation, lipid peroxidation and protein oxidation. Preincubation with PRL protected astrocytes against H2O2-induced cell death and increased lipids and proteins oxidative damage. Pharmacological blockade of STAT3 (S31-201) or genetic deletion of the long PRLR isoform (Prlr-/-) suppressed the protective effect of PRL against H2O2-induced cell death and oxidative damage, by decreasing both antioxidant capacity and the activity of

antioxidant enzymes. Our data suggest that PRL might represent a promising strategy for the treatment of brain pathologies associated with oxidative stress.
(We thank Fernando López, Xarubet Ruíz and Alejandra Castilla for their technical assistance. Supported by PAPIIT-IN201621)

P21
SULPIRIDE-INDUCED HYPERPROLACTINEMIA PROTECTS RETINAL FUNCTION IN STREPTOZOTOCIN-INDUCED DIABETES
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Visual dysfunction in diabetes associates with inflammation. The hormone prolactin (PRL) is a neurotrophic factor that downregulates retinal inflammation. Sulpiride is a dopamine D2 receptor blocker, prokinetic drug that elevates circulating PRL levels as a side effect. Here, we investigated whether sulpiride-induced hyperprolactinemia improves visual outcome in diabetic rats. Diabetes was induced with streptozotocin, and four weeks later, rats were treated for two weeks with daily intraperitoneal injections of sulpiride or with osmotic minipumps delivering PRL. Visual function was studied by evaluating visual acuity with the OptoKinetic tracking virtual system and by the electroretinogram (ERG). Expression of inflammatory (IL6, IL1b) and gliosis (GFAP) markers indicated the level of retinal inflammation. Visual acuity and the amplitude of the mesopic ERG B-wave decreased in diabetic vs. non-diabetic rats (p<0.050), and this reduction was prevented by sulpiride or PRL treatment. Likewise, both sulpiride and PRL inhibited the diabetes-induced increase in the expression of IL6, IL1b, and GFAP. In conclusion, sulpiride-induced hyperprolactinemia protects against visual loss observed in diabetes by mechanisms including reduced retinal inflammation. Sulpiride has significant therapeutic potential for the treatment of visual loss in diabetic retinopathy and diabetic macular edema.
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P22
EFFECT OF GROWTH HORMONE (GH) IN THE RETINAL MICROGLIA DURING INFLAMMATION
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It is known that neuroinflammation is associated to various retinal diseases, which may lead to cell death and neurodegeneration. Previous studies have demonstrated that growth hormone (GH) has neuroprotective actions against excitotoxicity-induced damage and LPS-induced inflammation in chicken retina. The current study aims to evaluate the effects of GH following an inflammatory stimulus to determine if its neurotrophic effects target neuroinflammation and prevent cell death and tissue injury in the rat retina, as previously observed in chicken retina. Male Wistar rats underwent optic nerve compression (ONC) to induce injury and neuroinflammation. bGH was administered via subcutaneous injection (0.5 mg/kg every 12 h), and samples were collected 24 h after injury to evaluate the acute effects of GH on cytokine expression and microglial markers. GH significantly reduced the expression of proinflammatory cytokines (IL6, TNFa, iNOS) and microglial activation markers (Iba1, CD86, and CD206). Neuroinflammation has been linked to microglial activation. Therefore, we utilized a mouse microglial cell line (SIM-A9) to evaluate the effects of GH on microglia. Inflammation was induced using LPS (1 μ/mL), and bGH (100 nM) was used for treatment. After 6 h of LPS-induced inflammation, we analyzed the activation of the NFkB pathway and cytokine expression. GH decreased the phosphorylation of the P65 protein, reduced TNFa expression, and increased the expression of anti-inflammatory cytokines such as IL10 and TGFb. These findings suggest that GH targets microglial phenotypes and cytokine expression, thus demonstrating its neuroprotective effects in the retina by preventing neuroinflammation, and this property appears to be conserved in vertebrates.
(Adriana González Gallardo, Proteogenomic Unit. Supported by PAPIIT-DGAPA UNAM (IN227020, IN209621, IN215522, IA200622) and CONACYT (214971).)

P23
CLINICAL RELEVANCE OF THE CONTRIBUTION OF THE RETINA TO THE SPONTANEOUS OSCILLATIONS DETECTED BY ELECTRORETINOGRAM (ERG) IN MICE AND HUMANS
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Background: Type 2 diabetes mellitus (T2DM) could be faced as a modern preventable pandemia. Systems that help predict early tissue-specific damage caused by risk factors of T2DM can be very useful to accompany modifiable lifestyle changes. In this sense, we developed a predictive diagnostic method for the main risk factors of DM2 (overweight and obesity), based on the non-invasive recording of spontaneous electroretinograms (ERG) in the 0.3 to 40 Hz range. However, the exact contribution of the retina to this activity remains nuclear, spontaneous ERG oscillations may be contaminated by other sources than the retina such as the cardiorespiratory system and the brain. Similarly, we also found that spontaneous ERG oscillations in the 0.1 to 10 Hz range help discriminate mice with obesity and insulin resistance from lean ones. Therefore, our goal is to determine the origin of spontaneous ERG oscillations at the tissue level in both mice and humans, and subsequently, assess the predictive power of the retina-specific activity.
Methods. Spontaneous ERGs were measured in C57BL6 mice in conjunction with movements of their rib cage. Coherence and phase analyzes were performed between the simultaneously recorded ERGs and cardiac-respiratory activity to determine the frequency range of the activity coming specifically from the retina. Next, a similar study will be carried out in healthy human volunteers, using simultaneously recorded spontaneous ERGs with electrooculogram, electroencephalogram, and electrocardiogram,
Preliminary results. In mice, the overlap of the spectral components derived from both the ERG and chest sensor signals showed that only the low (0.1?0.8 Hz) and medium-low (1.0?2.5 Hz) frequencies of spontaneous ERG oscillations did not overlap with the spectral components related to breathing. Therefore, the <2.5 Hz spontaneous ERG activity comes from the retina. Humans studies are ongoing.

Conclusion. We found that the slow, spontaneous ERG activity (<2.5 Hz) comes from the mouse retina. It remains to be determined whether the slow waves of the spontaneous ERG in humans also come from the retina.
(Acknowledgements: Supported by the UNAM DGAPA-PAPIIT grant IN212823 and CONACYT grant CF-2019-1759.)

P24

PROLACTIN PROTECTS HIPPOCAMPAL NEURONS IN VITRO FROM H2O2-INDUCED OXIDATIVE STRESS AND REDUCES NOX4 ACTIVATION VIA NF-KB INHIBITION

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Oxidative stress has been linked to neuronal apoptosis and progression of several neurodegenerative diseases. Oxidative damage elevates NADPH oxidases (NOX) activity, one of the main endogenous sources of ROS, which contribute for neuronal cell death. Efforts have been made to find NOX inhibitors as a neuroprotective strategy. Prolactin (PRL) is neuroprotective against glutamate excitotoxicity-induced oxidative damage in mouse hippocampal neurons and hydrogen peroxide (H2O2)-induced apoptosis of human retinal pigment epithelial cells. Furthermore, PRL regulates NOX activation in fish macrophages. Using primary cultures of hippocampal neurons isolated from the brain of E16 mice, we investigated the neuroprotective effect of PRL against H2O2-induced oxidative stress, cell death, and NOX activation. H2O2 treatment induced apoptotic cell death and increased ROS generation, lipoperoxidation and NOX activity in hippocampal neuronal cultures. In contrast, PRL pretreatment prevented H2O2-induced apoptotic cell death, reduced ROS levels, lipid peroxidation and NOX activity. PRL-induced protection against H2O2 was abolished by using a PRL receptor antagonist 1-9 G129R-hPRL. To assess the molecular mechanism involved in these PRL actions, we evaluated the mRNA expression of apoptosis mediators and NOX. PRL downregulated H2O2-induced BAX, BAD, and NADPH oxidase 4 (NOX4) expression. Additionally, we demonstrated the involvement of NF B inhibition in PRL-mediated neuroprotection. PRL prevented NF B nuclear translocation and decreased I B and I B expression in response to H2O2. Furthermore, the pharmacological inhibition of NF B with BAY 11-7082 prevented H2O2-induced reduction in cell viability and increase in NOX4 expression, similarly to PRL. In summary, our results demonstrate that PRL is an antioxidant neuroprotective factor that reduces the expression of NOX4, ROS production and apoptosis in hippocampal neurons, that could serve as a potential therapeutical strategy for neurodegenerative diseases.
(We thank Fernando López-Barrera, Xarubet Ruíz-Herrera, Alejandra Castilla, Nydia Hernández and María Carbajo for their technical assistance. Supported by PAPIIT-IN201621.)

P25

THE PRE AND POST OVULATORY STEROID HORMONE PROFILE OF NORTH AMERICAN ATLANTIC SALMON (SALMO SALAR) AS MEASURED BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Using pharmacological approaches in vivo and ex vivo, as well as functional connectivity analyses, we are approximating the origin of this activity and we are studying the predictive power of the retina-specific signal for the diagnosis of metabolic conditions using supervised algorithms. We also provide preliminary data showing that the slow spontaneous oscillations of the retina are responsive to neuroprotective treatment using growth hormone in the optic nerve crush model of glaucoma in rats. Finally, we are introducing the zebrafish as a model where spontaneous retinal oscillations are also present and discuss its potential to study the physiopathology of obesity.
(NCWMAC staff SC DNR)

P26

ROLE OF ARGININE VASOTOCIN IN THE REGULATION OF ZEBRAFISH SPERMATOGENESIS

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The nonapeptide hormone arginine vasotocin is known for its critical role in the regulation of osmotic balance and social behavior in teleost. Although vasotocin is primarily expressed in the brain, recent studies have indicated its expression in peripheral tissues, including the male and female gonads. This study investigated the direct effect of vasotocin on spermatogenesis using zebrafish as a model organism. Results demonstrate that vasotocin receptors (avpr1aa, avpr2aa, avpr1ab, avpr2ab and avpr2l) are expressed in the zebrafish testes, indicating that vasotocin may play a role in the regulation of testicular function. Using ex vivo culture of zebrafish testis, we investigated the direct action of three concentrations of arginine vasotocin (1nM, 10nM, 100nM) on spermatogenesis. Morphological and stereological evaluation of the testis demonstrated an effect of vasotocin on spermatogonia stem cell renewal, mitotic and meiotic germ cell development over a period of seven days. The results showed that vasotocin directly influences the number of spermatozoa and early mitotic cell stages. In addition to changing basal spermatogonial self-renewal, the presence of vasotocin altered gonadotropin-induced response in both the early and late stages of spermatogenesis. The results support the hypothesis that vasotocin is involved in the regulation of synchronized testicular development and gametogenesis. Overall, our findings provide insights into the physiological significance of vasotocin in vertebrates as a factor regulating male reproductive function.
(This work was supported by funding from the Natural Sciences and Engineering Research Council (NSERC) of Canada.)

P27

GROWTH HORMONE EFFECTS ON AXON REGENERATION IN KLF13-DEFICIENT RETINAL GANGLION CELLS

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During postnatal development, mammalian neurons of the central nervous system (CNS) lose their ability to elongate projections. Extrinsic and intrinsic factors, both stimulatory and inhibitory, regulate this complex process. Krüppel-like factors have emerged as important intrinsic factors involved in the control of axonal growth. Our previous work showed that KLF13 promotes and maintains neuronal differentiation and, consequently, is an intrinsic inhibitor of axonal regeneration. The action mechanisms underlaying this effect include KLF13-dependent inhibition of signaling pathways involved in axonal growth and regeneration, including the JAK/STAT pathway, which is the canonical mediator of growth hormone (GH) signaling. In addition to its primary role as regulator of growth, GH is known for its neurotrophic actions on neurons of the CNS. To test the hypothesis that depletion of an intrinsic inhibitor of axonal growth, KLF13, would potentiate the regenerative effects of a stimulatory extrinsic factor, GH, we established two paradigms to study the neuroprotective and neuroregenerative roles of GH in KLF13-deficient retinal ganglion cells (RGC) of mice: 1) AAV-mediated ectopic expression in RGCs, and 2) peripheral GH injection. Initially, we packaged the mouse Gh coding sequence into AAV viral particles of the PHP.eB seroptype. Also, a vector containing the Enhanced Green Fluorescent Protein (EGFP) was produced as control. The viral vectors were then injected intravitreally into the eyes of adult WT mice and, twenty-one days later, we confirmed by Western Blot that AAV-mGH causes ectopic expression of GH in mouse retinas. Moreover, by retinal flat mounts immunohistochemistry (IHC), it was found that that the viral vector transduced RGCs and other retinal cells. On the other hand, we performed subcutaneous injections of bovine GH (2 mg/kg) and analyzed GH-mediated signaling pathway activation in the retina. Our results showed that JAK/STAT and PI3/AKT signaling pathways were activated by GH treatment, as evidenced by STAT5 and AKT phosphorylation, respectively. Currently, we are analyzing and comparing these experimental strategies to deliver GH to RGCs for survival and axon regeneration in an optic nerve crush injury model, in both WT and Klf13-/- mice.

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DEPLETION OF KRÜPPEL-LIKE FACTOR 13 (KLF13) ENHANCES GH-DEPENDENT JAK/STAT ACTIVITY IN HIPPOCAMPAL NEURONS

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Krüppel-like factor 13 (KLF13) has emerged as an important transcription factor involved in essential processes of the central nervous system (CNS). It predominantly functions as a transcriptional repressor by interacting with GC-rich regions on promoters of its target genes. KLF13 impacts the activity of several signaling pathways with essential roles in the CNS, including the JAK/STAT, which is the canonical mediator of growth hormone (GH) signaling. Cumulative evidence has shown that GH has important actions as a neurotrophic factor. To test the hypothesis that KLF13 is a negative regulator of the JAK/STAT signaling pathway and that its depletion could enhance GH-dependent JAK/STAT activity, we analyzed the effect of KLF13 on the mRNA levels of several genes involved in the JAK/STAT pathway and how the GH-dependent gene expression of JAK/STAT output genes was impacted by KLF13 depletion in hippocampal neurons. We used the hippocampus-derived cell line HT22, which was previously engineered to force the expression of KLF13 with doxycycline, and the CRISPR/Cas9 genome edited Klf13-KO HT22 cell line; as well as the Klf13-/- mice as in vivo model. Our results confirmed that KLF13 directly regulates the expression of several genes involved in the JAK-STAT pathway: Jak1, Jak2, Jak3 and Socs1 were repressed up to 80 % while Stat5a was induced 2-fold by forced expression of Klf13. We also found that in KLF13-deficient HT22 neurons, the expression of Jak1, Stat3, Socs1, Socs 3 and Igf1 was dysregulated with mRNA levels up to 7-fold higher than the control cell line. The experiment of JAK/STAT activity stimulation showed that GH treatment increased the expression of Socs3, Igf1 and Bdnf in control cells between 2 to 10-fold. Interestingly, this stimulation was strongly enhanced (up to 20-fold) in the absence of KLF13. Some of these results were recapitulated in a in vivo model where the basal expression of Jak3 and Socs1 was upregulated (0.5- and 3-fold, respectively), in Klf13-/- mice compared to WT, while subcutaneous injection of GH enhanced the phosphorylation of STAT3 in the liver of Klf13-/- mice compared to WT. These findings support the notion that KLF13 is a regulator of the JAK/STAT activity.

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SPHINGOSINE-1-PHOSPHATE (S1P) SYNTHESIS IN GRANULOSA CELLS AND ITS EFFECTS ON CELL SURVIVAL AND STEROIDOGENESIS.

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The FSH stimulates proliferation, survival and estradiol synthesis of granulosa cells binding to its Gs-protein-coupled receptor and it has been showed that FSH activate SK1 to induce S1P synthesis, to mediate the biological effect. The mechanisms by which FSH promote S1P synthesis as well as the role of this sphingolipid on estradiol synthesis has been no reported. The objectives were to evaluate the importance of FSH-induced S1P synthesis as mediator of the effects of this gonadotrophin on granulosa cell viability, steroidogenesis and determinate if FSH-induced S1P synthesis depends of estradiol, cAMP, PKA or PKC. We used bovine granulosa cell cultures and determined in four experiments the effects of FSH,

SKI-178, estradiol and inhibitors of aromatase, cAMP, PCA and PKC on S1P and estradiol concentration, granulosa cells viability and the mRNA expression of CYP19a1 and StAR. Results showed that FSH (1 ng/mL) to culture media, increase (P<0.05) the number of viable granulosa cells and S1P concentration in culture media. However, SKI-178 (10 ?M) reduce S1P concentration in culture media and block the effect of FSH on cell viability. Inhibition of PKC and PKA but not cAMP reduce (P<0.05) S1P secretion by granulosa cells treated with FSH. The addition of 5 or 10 ng/mL of estradiol increase (P<0.05) S1P secretion to culture media. FSH increase (P<0.05) estradiol concentration in culture media, but this effect is not blocked by the inhibition of S1P synthesis and FSH, SKI-178 or its combination modified the mRNA expression of CYP19a1 and StAR mRNA. In conclusion, S1P synthesis mediated by FSH in granulosa cells depends of PKC and S1P seems to mediate the survival effects of FSH but not the effects on estradiol synthesis. High concentration of estradiol may stimulate S1P production by granulosa cells and estradiol synthesis induced by FSH seems to be not necessary for the FSH-induced S1P synthesis.
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MELATONIN IMPROVES TURBOT OOCYTE MEIOTIC MATURATION AND ANTIOXIDANT CAPACITY, INHIBITS APOPTOSIS-RELATED GENES mRNAs IN VITRO

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High-quality egg is essential for the sustainability of commercial aquaculture production during fish farming. Melatonin is a potent candidate in the regulation of fish oocyte growth and maturation. However, litter information about the role of melatonin on marine fish oocytes in vitro has been reported. The present study was undertaken to establish a oocyte culture system in vitro and investigate the influence of melatonin on meiotic maturation, antioxidant capacity and apoptosis-related genes expression in turbot (Scophthalmus maximus). Results showed that turbot late vitellogenic denuded oocytes of 0.5-0.7 mm in diameter had a low spontaneous maturation rate and exhibited a sensitive response to 17?, 20?-dihydroxyprogesterone (DHP) treatment in vitro. Melatonin significantly increased the oocyte germinal vesicle breakdown (GVBD) rate in a concentration- and time-dependent manner. Melatonin receptor 1 (mtnr1) mRNA were significantly upregulated in oocyte and follicle after treatment with melatonin (10-6 g/L) for 24 h in vitro, whereas mtnr2 and mtnr3 remained unchanged. In addition, melatonin significantly increased the catalase, glutathione peroxidase, superoxide dismutase activities, and glutathione levels, decreased the malondialdehyde and reactive oxygen species (ROS) levels in turbot oocytes and follicles culture in vitro. Meanwhile, the p53, caspase3 and bcl2 mRNAs were significantly down-regulated in oocyte and follicle. bax mRNAs was significantly up-regulated. In conclusion, turbot late-vitellogenesis oocytes (0.5-0.7 mm) were suitable for establish a culture system in vitro. Melatonin could promote oocyte meiotic maturation and antioxidative capacity, inhibited apoptosis by p53-bax-bcl2 and the caspase-dependent pathways, which have important potential for improving oocytes maturation and quality.
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IMPORTANCE OF SPHINGOSINE 1-PHOSPHATE (S1P) SYNTHESIS STIMULATED BY FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) ON VIABILITY OF BOVINE THECA AND GRANULOSA CELLS

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Previous results from our laboratory have shown that sphingosine 1-phosphate (S1P) is produced by granulosa and theca cells in response to follicle stimulating hormone (FSH) and luteinizing hormone (LH), and that this sphingolipid seems to mediate the effects of gonadotropins on follicular cell viability. However, the sphingosine kinase 1 (SphK1) inhibitor (SKI-178) that we used in our previous studies, in addition to inhibiting SphK1, also induces cell cycle arrest. Therefore, the aim of the present experiment was to evaluate if inhibition of S1P synthesis by using a specific SphK1 inhibitor (PF-543) affects viability of bovine ovarian follicular cells. Five granulosa cell cultures treated with FSH (1 ng/mL) and five theca cell cultures treated with LH (0.1 ng/mL), in the presence of two different Sphk1 inhibitors (PF-543 at a concentration of 0.1, 1 and 10 µM and SKI-178 at a concentration of 5 µM) were performed. Our results showed a decrease in S1P concentration in culture media of granulosa and theca cells treated with 0.1 and 1 µM PF543 respectively. However, whereas in theca cells the use of 1 µM as well as the use of 0.1 and 10 µM of PF543 reduced cell viability, in granulosa cells the reduction in S1P secretion by using 0.1 µM PF543 is not reflected in a reduction in cell viability. Therefore, we can suggest that synthesis of S1P induced by gonadotropins is important to regulate theca cells viability, however in granulosa cells the importance of this sphingolipid is no clear.
(Consejo Nacional de Ciencia y Tecnología, proyecto A1-S-21990)

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DOES SPHINGOSINE 1-PHOSPHATE (S1P) PROMOTE THE VIABILITY AND STEROIDOGENESIS OF BOVINE THECA CELLS IN CULTURE?

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Sphingosine 1-phosphate (S1P) is a sphingolipid involved in the regulation of proliferation, survival, and steroidogenesis in various cell types. At ovarian level there is evidence indicating that S1P increased proliferation and survival of bovine granulosa cells in culture, however the evidences of the role of S1P in theca cells are scarce. Thus, the objective of the present experiment was to evaluate in vitro, the effects of S1P on the function of bovine theca cells. Two experiments were carried up to evaluate the effect of 0, 0.1, 1 and 10 µM of S1P on theca cells viability and secretion of sexual steroids. In the first experiment, theca cell cultures were establishment for 48 hours and then the treatment were added. In the second experiment S1P was added to culture media at seeding. In both experiments cultures were finish and 48 ad 96 hours of treatment and theca cells viability and concentration of sexual steroid in culture media were determined. The results indicated that in pre-established bovine theca cell cultures, the addition of S1P to the culture medium did not affect theca cell viability or testosterone and progesterone secretion after 48 or 96 hours of culture. In contrast in culture of theca cells without establishment the addition of 0.1 and 1 µM of S1P to the culture media increased testosterone production and the number of viable theca cells after 96 but not at 48 hours of treatment. To corroborate the steroidogenic effects of S1P, we determine if S1P (0.1 µM) alone or in combination with LH (0.1 ng/mL) modified the mRNA expression of CYP11A, 3BHSD and CYP17A. The results showed that the mRNA expression of CYP11A, 3BHSD was not modified by S1P, whereas the mRNA of CYP17A could not be determine. In conclusion, results of the present experiment suggest that S1P may favors cell viability and testosterone synthesis in theca cells without establishment but not in cells with establishment.
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THE NEUROENDOCRINE INTERACTIONS DURING CHRONIC KIDNEY DISEASE: THE ROLE OF PROLACTIN

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Mexico has a high prevalence of Chronic Kidney Disease (CKD) and the most common causes of this pathology are diabetes and hypertension. CKD is characterized by progressive decline of glomerular filtration rate, which is conditioned by the accumulation of proteins at glomerular and tubule interstitial levels. The gradual loss of kidney functions leads to endocrine alterations affecting the metabolic functions. Prolactin (PRL) is a peptide hormone synthesized and secreted by the anterior pituitary gland and one of its many functions is in osmoregulation. Alterations in PRL circulating levels have been reported with a high prevalence of hyperprolactinemia patients, ranging from 30% in early stages to 65-80% in the advanced stages. The systemic accumulation of PRL have been associated to the inadequate clearance because of a deficient renal filtration, or a disruption of the regulatory loop between hypothalamus and PRL secretion. However, the mechanisms and the progression of hyperprolactinemia in CKD are yet to be unraveled. In the aim to create tools to study this PRL dysregulation in a CKD condition, we develop a mouse model of adenine-induce CKD. C57BL6/J adult female mice received daily 50 mg/kg of adenine by oral gavage for four weeks to induce CKD. At the end of the treatment biochemical and morphological parameters were evaluated to verify our CKD model. As expected, serum creatinine and urea levels were significantly increased in the CKD-treated group. Additionally, hematoxylin-eosin histological kidney sections were analyzed and showed significant differences in tubular dilation that confirm renal damage. To evaluate the PRL level state during the progression of the CKD, the PRL level was determined by ELISA, at one, two, three and four weeks after adenine administration. A suppression in PRL serum levels during CKD induction was observed, from one to four weeks of treatment. We can conclude that adenine effectively induces CKD via altering the biochemical parameters that triggers the progression to kidney failure, however, opposite to expected, peripherical levels of PRL decreased dramatically in adenine-induced CKD at least for the first four weeks on the pathology. Further experiments are needed to unravel the PRL regulation in a CKD mouse model.
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SEX IMPACT ON THE ALTERATIONS OF SPONTANEOUS ACTIVITY OF THE RETINA INDUCED BY OBESITY: COMPARISON BETWEEN HUMANS AND MICE

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Obesity, defined as the excess of adipose tissue in the body, is the main risk factor for developing type 2 Diabetes Mellitus(T2DM) and cardiovascular diseases. Even though obesity affects both men and women, worldwide studies have shown that Body Mass Index(BMI) based obesity is more prevalent in women than men. However, men are more susceptible to develop cardiovascular diseases associated with obesity, because they accumulate more pro-inflammatory visceral fat than women. However, this favorable situation towards women disappears at menopause because of the female sex hormone drop. Obesity has also been shown to associate with early changes in retinal function. We recently showed that spontaneous oscillations can be detected by non-evoked electroretinogram (ERG) and that this activity help predict obesity in both humans and mice. The impact of sex on spontaneous ERG oscillations needs to be investigated to provide insights into the mechanisms underlying sex/gender differences in obesity. In humans, spontaneous retinal activity showed three main oscillations in the 0.3?2.0, 10-20, and 20-40 Hz range (n=357 subjects).In metabolically healthy, norm weight groups, the only significant difference observed between men and women was a slower peak frequency of the 10-20 Hz oscillation in women(12±1 vs. 14±1 Hz, respectively).We found no change in the peak frequency of any of the oscillations in men with overweight, obesity, or metabolic syndrome but no yet T2DM. In contrast, the peak frequency of the 10-20Hz oscillation rose in women with overweight, obesity, or metabolic syndrome but not yet T2DM, compared to women of the metabolically healthy, norm weight group. To gain insights into these observations, we decided to study spontaneous retinal oscillations in cafeteria diet-fed mice used as a model of obesity. After three weeks of obesogenic diet, mice showed overweight, insulin resistance, and elevated levels of blood glucose. Mice fed with a control diet showed spontaneous retinal activity in the 0.1?2.0 Hz range, with a peak frequency lower in photopic conditions than in dark conditions. This difference disappeared in the cafeteria diet-fed group. We are currently studying if sex and more precisely the estrous cycle stage, has an effect on the spontaneous activity of the retina in control diet and cafeteria diet-fed mice. Our data indicate that sex influences spontaneous retinal oscillations in humans and mice under both control and obesity conditions.
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GLUTAMATERGIC ACTIVATION OF ERK EXPRESSION IN NEURONS AND PITUITARY CELLS OF THE FEMALE ZEBRAFISH

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The hypothalamic-pituitary-gonadal (HPG) axis is central to vertebrate reproduction. Hypothalamic neurons release gonadotropin-releasing hormone that acts on gonadotrophs of the anterior pituitary to stimulate luteinizing hormone and follicle stimulating hormone synthesis and release. These gonadotropins, in conjunction with other hormones, neuropeptides, and neurotransmitters, trigger and regulate essential reproductive events including gametogenesis, oocyte maturation, modulation of courtship behaviors, and ovulation. However, many of the key players suspected to also contribute to this ovulatory trigger, their neural origins and activity, remain elusive in teleosts. Phosphorylated extracellular signal-regulated kinase (p-ERK), a downstream target of the MAPK/ERK signaling pathway, is stimulated by calcium influx into neurons upon their activation. It is a robust biomarker for neuronal activation and a tool to potentially track critical circuits triggering pituitary-ovarian function. We have validated a mouse phospho-ERK1/2 (Thr202, Tyr204) monoclonal antibody in adult zebrafish brain and pituitary. Positive p-ERK immunoreactivity was identified throughout the olfactory bulb, dorsal and ventral telencephalic area, hypothalamus, and pituitary of periovulatory female zebrafish, with immunoreactivity being completely blocked by pre-absorption using a phosphorylated peptide derived from ERK. Clear induction of p-ERK expression was observed within 3-minutes of intraperitoneal injection of female zebrafish with the specific glutamate receptor agonist AMPA. The mean number of p-ERK positive cells in the hypothalamus (periventricular and lateral hypothalamic nuclei) significantly increased 1.75-fold from 343 to 601 in saline and AMPA injected fish respectively (n = 3, p < 0.05). Strong p-ERK immunoreactivity was observed in preoptic neurons in close proximity to, but not in secretoneurin (SN)-positive magnocellular neurons. SN is a stimulatory neuropeptide emerging as a key regulator of HPG function, and glutamate may indirectly regulate these cells. Areas also sensitive to glutamate action via AMPA receptors include the olfactory bulbs and the pars distalis of the pituitary. This reinforces these as important areas of neuroendocrine control. Tracing time-dependent p-ERK expression is one strategy to identify key neuroendocrine neurons activated in the periovulatory period.
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OLFACTORY ALERATIONS IN A MODEL OF CHRONIC KIDNEY DISEASE IN FEMALE MICE

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Chronic Kidney Disease (CKD) encompasses alterations in the structure and function of the kidney characterized by its irreversibility and progressive evolution. CKD is established when the glomerular filtration rate is below 60 mL/min/1.73 m2 and/or the presence of renal damage markers remains for at least 3 months. A large percentage of CKD patients report olfactory alterations, which could contribute to metabolic problems of malnutrition, one of the main determinants of mortality in this disease affecting quality of life of patients. However, the onset and evolution of the pathophysiology of these alterations have been little addressed. Currently, there are no laboratory models that replicate the olfactory dysfunction in CKD that allow the study of the underlying processes. In the present work, a CKD model was generated in adult C57BL/6J female mice by intragastric adenine administration (50 mg/kg, n=12) to evaluate olfactory capacity. The CKD was confirmed by metabolic, biochemical, and morphological parameters. To determine possible olfactory alterations, we used two behavioral tests, the Buried Food test and the Habituation/Dishabituation test, that allow us to evaluate olfactory capacity, as well as olfactory memory and discrimination processes, respectively. The morphology of the olfactory epithelium (OE), structure in which olfactory processing begins by odorant detection, was also analyzed. No alteration in the ability to detect buried food was observed under the treatment. Both control and adenine groups, decreased performance in the habituation/dishabituation test was observed over time, possibly due to a habituation component to the test. For the OE, no changes on the olfactory epithelial thickness or cell density were detected. Overall, our results indicate that the adenine-CKD induced model did not alter the olfactory behavioral responses nor the OE morphology, however, further tests should be included as well as increasing the sample size and the time adenine-treatment to confirm the presence or absence of olfactory alterations in this model.
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IDENTIFYING THE IMPLICATIONS OF HYPOXIA AND DMOG ON REPRODUCTIVE PROCESSES IN ADULT FEMALE ZEBRAFISH (DANIO RERIO)

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Over the last several decades, dissolved oxygen (DO) availability in aquatic ecosystems has decreased in both shallow and deep-water habitats. Previous studies have shown that decreased DO affects reproduction in fish by altering in sex steroid production, gonadal development, oocyte maturation and endocrine function. The current study examined the effects of hypoxia on reproduction the zebrafish in vivo and explores the role of hypoxia inducible factor (HIF) within the ovary using an in vitro approach. Many studies have looked at long term hypoxia exposure on reproductive effects, therefore, the following study aimed to determine reproductive effects from short term hypoxia exposure. Female and male zebrafish were exposed to hypoxia for 24 - 96 hours to explore spawning success and steroidogenic gene expression. After 24 hours of hypoxia, the number of eggs spawned were significantly decreased. The expressions of steroid acute regulatory protein (StAR) and 3?-hydroxysteroid dehydrogenase (3?HSD) in the ovary were significantly decreased after 96 hours of hypoxia. To explore the impacts of HIF on the ovary, full grown ovarian follicles were incubated in vitro with dimethyloxalylglycine (DMOG; a HIF stabilizing compound) and Human Chorionic Gonadotropin for 6 hours and analyzed for steroidogenic gene expression. The expressions of StAR and 3BHSD gene expression were significantly reduced in groups exposed to DMOG. These results demonstrate that hypoxia exposure as short as 24 hours has negative effects on reproduction in the zebrafish and suggests that HIF could impact steroidogenic pathways within the ovary.
(Jacquie Matsumoto, Hagen Aqualab)

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MINERALOCORTICOIDS REGULATE THE EXPRESSION OF ?-NA+/K+ ATPASE IN T. CRASSICEPS WFU CYSTICERCI

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In vertebrates, mineralocorticoids regulate extracellular fluid volume and salt balance, in part by controlling the expression and activity of Na+/K+ ATPase, the enzyme that generates and maintains Na+ and K+ gradients across the plasma membrane of animal cells. However, the presence and function of mineralocorticoids are less studied in invertebrates. Taenia crassiceps WFU cysticerci synthesize mineralocorticoids in vitro and express an alpha subunit of Na+/K+ ATPase (?-Na+/K+ ATPase). In this work we studied the role of mineralocorticoids in the regulation of ?-Na+/K+ ATPase expression in T. crassiceps WFU cysticerci in vitro. Cysticerci were obtained from the peritoneal cavity of female mice after 3-5 months of infection, pre-cultured for 24 h, and then cultured in the presence of the mineralocorticoids corticosterone or deoxycorticosterone, or a mineralocorticoid receptor antagonist (MR) spironolactone, at different concentrations and for different periods of time. The expression of ?-Na+/K+ ATPase was evaluated by Western Blot. Mineralocorticoids were found to affect ?-Na+/K+ ATPase expression in a concentration-dependent manner. On the other hand, blockade of the MR significantly decreased the expression of the enzyme. These results suggest a role for corticosteroids in the regulation of Na+ and K+ transport in T. crassiceps. A deep understanding of these mechanisms could also contribute to improve therapeutic strategies for cysticercosis.
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ALTERATIONS IN THE MYELINATION PATTERNS IN A HYPER- OR HYPOTHYROIDISM STATE IN EARLY ZEBRAFISH DEVELOPMENT

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It is well known that triiodothyronine (T3) is responsible for regulating several processes associated with the development and function of the central nervous system (CNS), including the differentiation of the oligodendroglial lineage. T3 acts through its binding to nuclear receptors (thr) to regulate the transcription of target genes, and this action mechanism and the corresponding signaling components are highly conserved in all vertebrates. Using zebrafish (Danio rerio), we confirmed that the differentiation of oligodendrocytes (OLs) depends, at least in part, on an adequate supply of T3 during early developmental stages. However, the action mechanisms involved in this process are still obscure and very little is known about the thr genes that mediate such process. In the present study, native 3 or 5 days post-fertilization (dpf) zebrafish larvae were subjected to a T3 surplus induction or decreased bioavailability by an immersion treatment with 0.025 nM T3, or 5?M iopanoic acid (IOP), respectively. In all cases, the myelination patterns were visualized with the Black Gold II staining technique and a semi-quantification of myelin content was performed using the Fiji software. Myelin content of wild-type larva CNS increased after T3 treatment and decreased when T3 bioavailability was reduced. By using the CRISPR/Cas9 technique, we obtained zebrafish larvae for thraa, thrab and thrb which were grown for 3 or 5 dpf and myelination patterns were visualized (see above). Crispants for all thr genes showed hypomyelination patterns in the central region of the telencephalon and midbrain at 3 dpf. Interestingly, only thrb crispants recovered CNS control myelination patterns after 5 dpf. These results show that T3 has a positive effect upon CNS myelin content and that each of the thr genes (thraa, thrab and thrb) seems to play different roles on OL differentiation and subsequent myelin synthesis in the CNS of the zebrafish.
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THYROID HORMONE STATUS MODIFIES RETINAL MORPHOLOGY AND COLOR PREFERENCE DURING ZEBRAFISH DEVELOPMENT

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Thyroid hormones (THs) regulate several processes of early development including that of the retina. Indeed, experimental evidence has shown that disruption of the thyroidal status affects photoreceptor cell differentiation from fish to mammals, but the intrinsic mechanisms involved in this developmental event are far from being elucidated. In the present study, we investigated the ontogeny of thyroid hormone receptors (TRs) expression in microdissected retinas of zebrafish at 3 and 5 days post-fertilization (dpf) using qRT-PCR. Additionally, we compared the effects of TR?2 mutation and of hypo- and hyperthyroidism on retinal morphology and function at 5 dpf. We created TR?2 larvae mutants using CRISPR/Cas9 technology; thyroid status was modified through treatments with 0.025 µM T3 or 0.5 µM iopanoic acid (IOP) from 0 to 5 dpf. At the end of the treatments, larvae were submitted to a color preference paradigm and/or sacrificed for histological analysis of retinal cell layers. qRT-PCR showed that TR?2 was the predominantly expressed isoform in retina and its expression increased at 5 dpf, coincident with the maturation of photoreceptor cells. Compared with controls, crispants for TR?2 presented a modified color preference at 5 dpf, suggesting that mutations in this TR isoform impaired color preference, as previously reported. In these crispants, histologically observable characteristics in the retina such as layer thickness were not affected. In contrast, hypo- and hyperthyroidism selectively affected the development of retinal cell layers. T3 induced an increase of the ganglion cell layer thickness and a decrease of the outer nuclear layer thickness while IOP treatment slightly decreased the thickness of the outer nuclear layer. Both, T3 and IOP exposure modified color preference, suggesting that TH bioavailability affects retinal development and visual perception in a more dramatic way than TR?2 depletion. Together, these results show that TR?2 is the predominant TR isoform in the retina; its depletion affects color preference; however, TH level imbalance produces more dramatic effects. These results highlight the critical role of the thyroid system during early retinal development.
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TRENDS IN THE EVOLUTION OF ELASMOBRANCH MELANOCORTIN-2 RECEPTORS (MC2RS): INSIGHTS FROM AN ANALYSIS OF THE PACIFIC DOGFISH MC2R

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The melanocortin-2 receptor (Mc2r), one of five GPCR paralogs in the Melanocortin Receptor Gene family, is expressed on adrenal cells of amniote tetrapods, and interrenal cells of anamniote tetrapods, bony fishes, and cartilaginous fishes to promote the release of glucocorticoids to facilitate recovery from chronic stress experiences. While the Mc2r orthologs of osteichthyan vertebrates are exclusively selective for the pituitary hormone, ACTH, and require co-expression with the accessory protein, Mrap1, for trafficking and activation, the Mc2r orthologs of cartilaginous fishes are more promiscuous. Cartilaginous fish Mc2r orthologs can be activated by either ACTH or MSH-sized ligands. In addition, the Mc2r ortholog of the holocephalan, Callorhinchus milii does not require co-expression with Mrap1 for activation or trafficking. However, the Mc2r orthologs for the elasmobranchs, the stingray, Hemitrygon akajei and the whale shark, Rhincodon typus, are dependent on interaction with Mrap1 to facilitate trafficking from the ER to the plasma membrane. This study was done to determine whether a squalomorph elasmobranch also is dependent on interaction with Mrap1. To this end, the Mc2r ortholog of the Pacific spiny dogfish (pd; Squalus suckleyi) was transfected into CHO cells either alone or co-expressed with pdMrap1 or pdMrap2) and stimulated with a cartilaginous fish ACTH(1-24. Activation of pdMc2r was quantified using a cAMP luciferase reporter gene assay. pdMc2r was not activated when expressed alone, but gave a robust response when co-expressed with the two homologous accessory proteins. In addition, pdmc2r/pdmrap1 transfected CHO cells could be activated by either ACTH, ACTH(1-23)NH2, or ?MSH. ACTH was 3 orders of magnitude more potent at activating pdMc2r than the MSH-related ligands. The role of the ?message? motif (i.e., HFRW) and the ?address? motif (i.e., KKRRP) of ACTH with respect to the activation of pdMc2r was also evaluated. The unique features of elasmobranch Mc2r orthologs and the insights they provide on the evolution of gnathostome Mc2r orthologs will be discussed. (Acknowledgements: Support for this research was provided by the Long Endowment (University of Denver; 143246; R.M.D.), a National Science Foundation Postdoctoral Fellowship (DBI-2109626; C.A.S.), a Natural Sciences and Engineering Research Council Discovery Grant (05328; W.G.A.), and a Company of Biologists Travelling Fellowship (JEBTF2210863; I.A.B.))

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