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President's Welcome to the Second Meeting of the North American Society for Comparative Endocrinology (NASCE 2013)

It is my sincere pleasure to welcome you to our second meeting. There was a strong positive reaction at the inaugural meeting of NASCE 2011. It was a great success, and the collective feeling was that NASCE should indeed continue. This is largely because of the dedication and enthusiasm of our entire membership. NASCE 2013 promises to be equally exciting, yet most certainly different from Ann Arbor. We have many new participants and a varied scientific program. The Chair of the Local Organizing Committee, Dr. Carlos Aramburo and his team, have put together an excellent cultural and culinary program that will compliment the scientific program. Please also take the time to make new colleagues and friends, be it while enjoying the posters or while walking in Juriquilla and Querétaro. These are beautiful places and thus provide the perfect setting to see old friends and to develop new collaborations.

During the meeting, we must also address important issues about the future of NASCE. This will include our bylaws, future NASCE officers and elections, and also the 3rd meeting of NASCE and beyond. We are targeting the first week of June 2015, and I will be your host in Ottawa, Canada.

Vance L. Trudeau

President NASCE 2011-2013.

LOC's Welcome

On behalf of the Local Organizing Committee (LOC) I extend you the warmest welcome to the Second Meeting of the North American Society for Comparative Endocrinology (NASCE 2013) in Mexico. In this occasion the hosting institution is the Universidad Nacional Autónoma de México (UNAM), with the support of the Institute of Neurobiology at the Juriquilla Campus in Queretaro. As you may know, UNAM is the oldest (1551), biggest (around 330,000 students) and strongest research University in our country, and one of the best in Latin America.

You will find a very hospitable environment that invites to enjoy the richness and tradition of a very well conserved colonial city (founded in 1531), whose downtown has been considered as a World Heritage Site by UNESCO. We are sure that walking in its streets, visiting its many beautiful buildings and lively squares, as well as its baroque temples, will be an unforgettable experience. We have also prepared a nice cultural and social program and hope you will find it gratifying.

In terms of our meeting, we will have a thorough scientific program with over 155 presentations, from colleagues representing 13 countries. Also, around one third of the presentations will be in charge of students, who will have a good chance to increase their experience. This will be an excellent opportunity to exchange ideas, build new friendships, and promote future interactions, to strengthen ties between the comparative endocrinology community.

So, as we say in Mexico: **Bienvenidos y mucho éxito, amigos!!**

Carlos Arámburo

Chair of the Local Organizing Committee, NASCE 2013.

NASCE Purpose and Mission

The North American Society for Comparative Endocrinology (NASCE) is an interdisciplinary scientific organization dedicated to the study of comparative, evolutionary, ecological and model systems endocrinology. The NASCE fosters the study of diverse species, and of non-traditional animal model systems (i.e., invertebrate and vertebrate) to elucidate basic mechanism of hormone actions, the evolution of animal endocrine systems, and factors affecting the health of human and wildlife populations. Biennial meetings of the NASCE, and its official journal, *General and Comparative Endocrinology*, provide essential forums for the communication and exchange of discoveries and ideas in the field of comparative endocrinology.

Comparative endocrinologists work across disciplinary boundaries, from molecular to ecological. Members of the NASCE conduct basic research in diverse areas of the life sciences. Investigations by comparative endocrinologists lead to the development of alternative animal model systems for discovery of novel hormones and hormone-signalling pathways; the discovery of new pharmaceuticals to treat human disease; the design of hormonally-based strategies for pest control; the development of sensitive, representative and high-throughput endocrine-screening assays for EDCs; the analysis of the impact of global climatic change on animal populations; the elucidation of pathways and mechanisms of evolution through the study of endocrine genes and structures; and the development of more efficient means for the production of animal protein to feed the world's growing human population.

NASCE 2013 Committees

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

Vance L. Trudeau, University of Ottawa, Canada (President)
Carlos Arámburo, Universidad Nacional Autónoma de México, México (Vice-President/President Elect)
Robert Denver, University of Michigan, Ann Arbor, USA (Secretary/Treasurer)

NASCE Council

Brian Barnes, University of Alaska, Fairbanks, USA
John Chang, University of Alberta, Edmonton, Canada
Carmen Clapp, Universidad Nacional Autonoma de Mexico, México
Robert Dores, University of Denver, USA
Cunming Duan, University of Michigan, Ann Arbor, USA
Steve Harvey, University of Alberta, Edmonton, Canada
Penny Hopkins, University of Oklahoma, USA
Angela Lange, University of Toronto, Mississauga, Ontario, Canada
David Lovejoy, University of Toronto, Ontario, Canada
Lynn Riddiford, Janelia Farm, Howard Hughes Medical Institute, USA
Nancy Sherwood, University of Victoria, Canada
Yun-Bo Shi, National Institute of Child Health and Human Development, NIH, USA
Stacia Sower, University of New Hampshire, USA
Peter Thomas, University of Texas, Austin, USA
Carlos Valverde Rodríguez, Universidad Nacional Autonoma de Mexico, México
Glen Van Der Kraak, University of Guelph, Ontario, Canada

NASCE 2013 Program Committee (PC)

Carlos Arámburo, Universidad Nacional Autonoma de Mexico, México
Carmen Clapp, Universidad Nacional Autonoma de Mexico, México
Robert Denver, University of Michigan, Ann Arbor, USA
Angela Lange, University of Toronto, Canada
Vance L Trudeau, University of Ottawa, Canada
Pei-San Tsai, University of Colorado, Boulder, USA

NASCE 2013 Scientific Advisory Committe (SAC)

Bill Bendena, Canada
Mark Brown, USA
Fabián Canosa, Argentina
Veerle Darras, Belgium
Penny Hopkins, USA
Gonzalo Martínez de la Escalera, México
Chris Martyniuk, Canada
Marta C. Romano, México
Stacia Sower, USA
Suraj Unniappan, Canada

NASCE 2013 Awards Committee

John P Chang, Canada (Chair of the Committee)
Steve Tobe, Canada
Gonzalo Martínez de la Escalera, México
Marta C. Romano, México
Penny Hopkins, USA
James Carr, USA

NASCE 2013 Local Organizing Committee (LOC)

Carlos Arámburo (Chair)
Martha Armenta
José Ávila
Martha Carranza
Carmen Clapp
Bertha Esquivel
Flor de María Herrera
Maricela Luna
Gonzalo Martínez de la Escalera
Angel Mayrén
Alejandro Mondragón
Aurea Orozco
Raúl Paredes
Enrique Pedernera
Felipe Pedroza
Juan Riesgo
Marta C. Romano
Rogelio Rocha
Oscar Ruiz
Carlos Valverde-R
Juan Villagrán

Sponsors

Universidad Nacional Autónoma de México (UNAM)

Coordinación de la Investigación Científica (CIC)

Instituto de Neurobiología (INB)

Laboratorio de Investigación en Procesos Avanzados de Tratamiento
de Aguas del Instituto de Ingeniería (LIPATA-II)

Coordinación de Servicios Administrativos del Campus Juriquilla

Fundación UNAM Capítulo Querétaro

Consejo Nacional de Ciencia y Tecnología (CONACYT)

Instituto de Ciencia y Tecnología del Distrito Federal (ICyTDF)
Secretaría de Ciencia, Tecnología e Innovación del Distrito Federal (SECITI)

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

Secretaria de Turismo del Estado de Querétaro

Instituto Queretano de la Cultura

Meeting Highlights

Plenary Lectures

PL1. Gustavo M. Somoza, IIB-INTECH (CONICET-UNSAM), Chascomús, Argentina
Cortisol and sex differentiation: the 11β-hydroxysteroid dehydrogenase as a key enzyme
PL2. Ian Orchard, Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada
The Kiss of Death: Hormonal control of diuresis in the kissing bug Rhodnius prolixus, the vector of Chagas disease
PL3 Liliane Schoofs, Animal Physiology and Neurobiology, KU Leuven, Leuven, Belgium
Neuropeptide signalling and behavior
PL4. John P. Chang, Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada
Multifactorial neuroendocrine control and signalling of somatotrope functions – insights from the goldfish study model
PL5. Hugh Drummond, Depto Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de Mexico, Mexico
Sibling conflict, hormones and long-term developmental impacts in the blue-footed booby
PL6. Emilie Rissman, Dept Biochemistry & Molecular Genetics, & Grad. Progr. Neuroscience, University of Virginia,VA, USA
Plastics: immediate and trans-generational actions on brain and behavior
PL7. Penny Swanson, Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA, USA
Seasonal variation in the effects of nutritional plane on age of puberty and the pituitary-gonad axis in salmon

Gorbman-Bern Memorial Award Lecture

Dores, Robert M., University of Denver, Department of Biological Sciences, Denver, Colorado, U.S.A
Analyzing the interaction between the melanocortin-2 receptor and the accessory protein mrap: an example of constructive neutral evolution

Gorbman-Bern New Independent Investigator Award Lecture

Martyniuk, Chris, Canadian Rivers Institute & Dept. Biology, University of New Brunswick, Saint John, New Brunswick, Canada.
The yin and the yang of androgen receptor signaling in teleost fish

Symposia

PS. Sexual development in vertebrates
S1. Multiple actions of thyroid hormones
S2. Comparative endocrinology and application to the management of captive/endangered species
S3. Blood vessels as relevant hormonal targets
S4. Hormones and behavior
S5. Hormonal regulation of metamorphosis across phyla
S6. Development and plasticity of neuroendocrine systems
S7. Comparative endocrinology of leptins and other adipokines
S8. RFamides-Evolution and function
S9. Reproductive endocrinology-From gene to population
S10. Stress and energy metabolism
S11. Endocrine and osmotic modulation of epithelial cells
S12. New hormones and novel functions for old friends
S13. Neuroendocrine disruption
S14. Endocrinology- Advances through genomics, peptidomics and related technologies
S15. Hormone receptors critical in development and reproduction
S16. Diabetic models across species-Lessons from a common pathway

General Information

Conference Information and Contacts

Conference Venue: NASCE 2013 will be held at the Juriquilla Campus of the Universidad Nacional Autónoma de México, in Querétaro. All plenary sessions will be in the **Cultural and Academic Center (CAC)** "Dr. Flavio M. Mena Jara" Auditorium. Symposia and oral presentations will be at the CAC, the Instituto de Neurobiología (INB) Seminar Room, or the Instituto de Ingeniería (II) Auditorium. The poster sessions will be at the Instituto de Neurobiología **Terrace**.

Conference Contacts:
Dr. Carlos Arámburo and/or Dr. Maricela Luna
Depto. Neurobiología Celular y Molecular
Instituto de Neurobiología
Campus Juriquilla
Universidad Nacional Autónoma de México
Blvd. Juriquilla 3001
76230 Querétaro, Qro.
MEXICO

+52 (442)238 1066 phone
Email: aramburo@unam.mx
lunam@unam.mx

Conference e-mail: nasce2013mx@gmail.com

Please direct questions or problems with registration to:
Wendy Arellano
B.P. SERVIMED, SA de CV
Barranca del Muerto 520
Col. Alpes
01010 México, D.F.
MEXICO
+52(55)9171-9570 phone
+52(55)5660-1903 phone
Email: nasce2013@servimed.com.mx

Registration: The meeting registration desk will be located,on Tuesday May 21st at the lobby of the Hotel Mision Juriquilla from 16:00 -21:00 hrs. From May 22 – May 25. The registration desk will be located in the lobby of the Cultural and Academic Center (CAC) at the UNAM's Juriquilla Campus, starting at 8:00 am.

Official Language: All events at NASCE 2013 will use English.

Conference Social Events

NASCE 2013 Welcome Reception, Wednesday May 22, 19:30-21:30 hrs. with the participation of UNAM Choreographic Group, Cultural and Academic Center (CAC), (open to all registrants)

Excursion to the Sta. Rosa de Viterbo Barroque Convent, cultural activity and reception. May 23, 16:00-19:30 hrs. (open to all registrants)

Free-time in downtown Queretaro, May 23, 19:30 – 23:00 hrs. Buses will depart to the venue hotel at 23:00 hrs.

NASCE 2013 Banquet. May 24, 20:00-23:00 hrs at "El Pueblito" in Hotel Mision Juriquilla. (open to ticket holders).

Presenter Information

Posters:: Please prepare your presentation in a horizontal format. Poster boards for NASCE 2013 are **170 cm WIDE x 120 cm HIGH (5.57w x 3.9h ft).** Posters must fit within this space. Pushpins will be provided. You do not need to include the poster number on your poster. All posters should be set up and remain on display on May 22 through May 24. The poster exhibit site will be open for setup at 11am on Wednesday, May 22; please set up your poster 11am-1pm on this day. **Odd numbered posters** will be presented during **Poster Session 1 on Wednesday May 22, 17:15-19:30 hrs**. **Even numbered posters** will be presented during **Poster Session 2 on Friday May 24, 17:15-19:30 hrs**. Posters should be taken down by 20 hrs on May 24.

Plenary, Symposium and Oral Presentations- Plenary amd GB-Award Lectures will be 50 minutes. Symposium presentations will be 25 minutes (20 min with 5 min for discussion and questions). Oral presentations will be 15 min (10 min with 5 min for discussion and questions). Please prepare your presentation in Powerpoint and bring it to the meeting on a flash drive or CD-ROM. 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.

Speaker Ready Room- During the meeting there will be a Speaker Ready Room (Blue class-room on the third floor of CAC) for you to preview your presentation and upload it to the meeting computers. Assistants will be on hand to help you.Please visit the Speaker Ready Room (will be open from 7:30 am every day) and upload your presentation on the morning of the day of your session, but no later than one hour before the session begins.

Coffee Breaks

Coffee breaks service will be provided each day of the meeting. Morning and afternoon coffee breaks will be held at the lobby of CAC.

Final Program

The Local Organizing Committee does not assume responsibilities for any inconsistencies or errors in the abstracts for contributed paper and poster presentations. We regret any possible omissions, changes and/or additions not reflected in this final program book.

Paper Submission Information SPECIAL ISSUE IN *GENERAL AND COMPARATIVE ENDOCRINOLOGY* FOR THE SECOND NASCE MEETING

Selected presentations will be published in a special issue of *General and Comparative Endocrinology* dedicated to NASCE 2013. Plenary speakers are invited to submit either a "major"- or a "mini"-review. Invited symposium speakers (oral presentations) will be invited to submit either a review or an original research manuscript. A number of other speakers or poster presented will be specifically invited at a later date. All manuscripts will be peer-reviewed through the usual GCE process. The Handling Editor will be Vance L. Trudeau (Canada) and the Honorary Editors will be the NASCE 2013 host Carlos Aramburo (Mexico) and Bob Denver (USA). For this Special Issue, we will consider review articles, mini-reviews, and original research manuscripts. Extended abstracts will not be considered. Any submission that appears to be too preliminary, a shorter version of a more complete study, a study that is too short, or resembling previously published work will be returned without external review. We are seeking innovative contributions in comparative endocrinology.

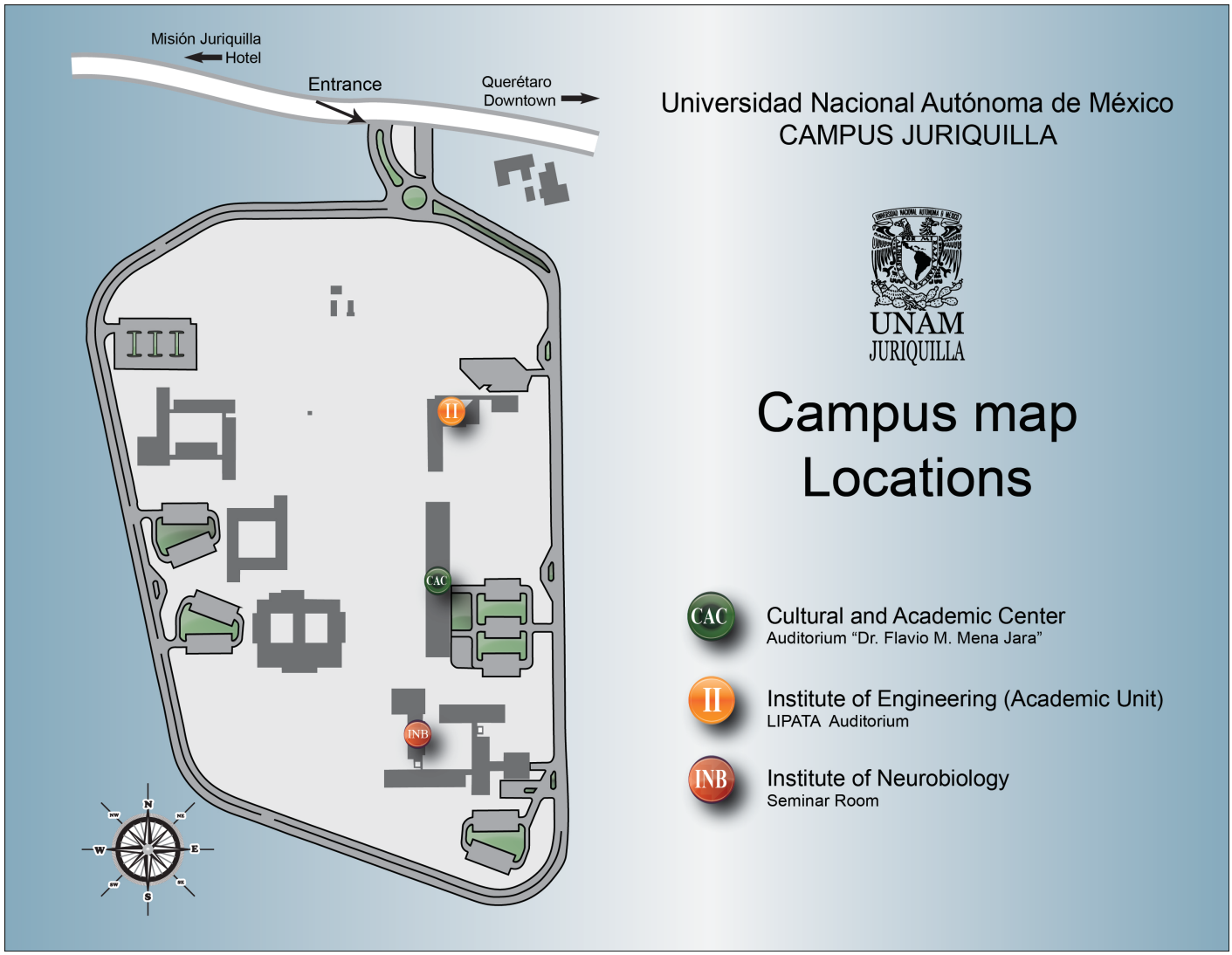
DEADLINES

Target deadline for submission online to GCE: July 1, 2013. There will be a special link identifying the NASCE 2013 special issue. Target publication date in GCE (printed copy): January 2014

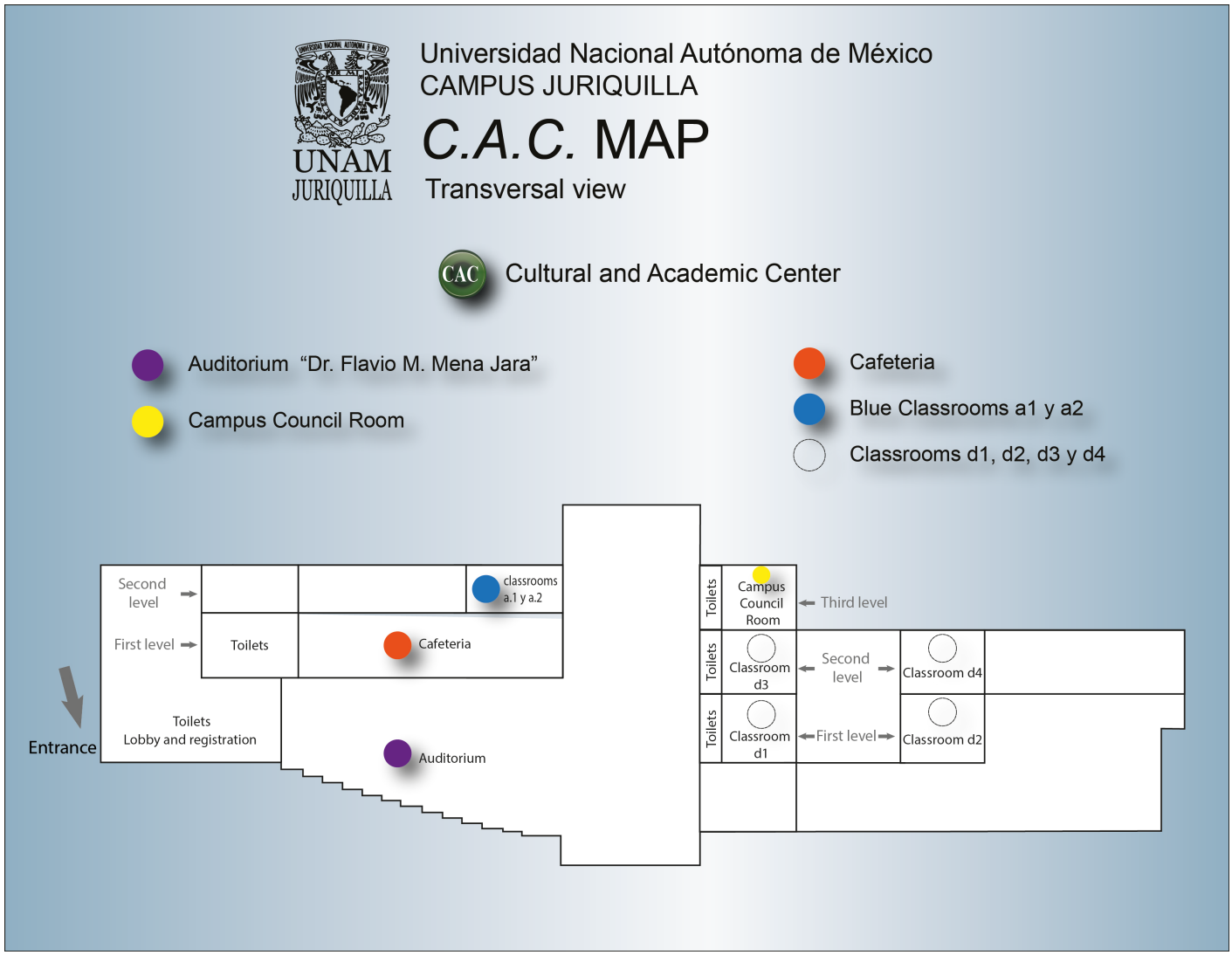
GUIDELINES

Note about all submissions of any type: Because this is a special issue, authors are allowed to publish 4 figures in color at no charge. However, there is a charge for the 5th and additional color figures. Major reviews: 15 to 30 pages doubled spaced, no limit on the number of figures. Mini-reviews: up to 12 pages doubled space; limit of up to four figures.

Maps of the UNAM-Juriquilla Campus and Conference Venue



Cultural and Academic Center (CAC) Map



Maps of Juriquilla and nearby restaurants

PLAZAS

A Plaza Juriquilla

Subway
Sandwiches (Subs)
Suite 8
234 0954
Average cost per person: $70

Cabeson Taurino
Tacos
Suite 37
234 2990
Average cost per person: $120

Emilio's
Italian
Suite 203
234 0338
Average cost per person: $130

El Criollo
Spanish
Suite 220
234 2182
Average cost per person: $130

Los de Colores
Mexican
Suite 14
234 2515
Average cost per person: $70

Toast's
Roasted chicken
Suite 15
239 1517
Average cost per person: $80

Amadeus
International and pastries
Suite 5
312 9380
Average cost per person: $140

El Encuentro
Argentinian
Suite 10
312 8703
Average cost per person: $120

PLAZAS

A Plaza Juriquilla

Mizha Crêpe
Crepes, paninis and salads
Suite 31
234 0434
Average cost per person: $110

Cosi Fan Tutti
Italian
Suite 220
265 2071
Average cost per person: $150

B Plaza Amarras

Porta Via
Italian
Suite 10
234 1168
Average cost per person: $90

Los de Pescado
Fish and shrimp tacos
Suite 13
234 3141
Average cost per person: $70

C Plaza Shopping Center

Burro-T
Mexican subs
Suite 51
234 3152
Average cost per person: $70

UniverSalads
Suite 52
Cel 442 305 5114
Average cost per person: $60

D Plaza Misiones

La Bota
Spanish
Suite 1
234 1000
Average cost per person: $150

PLAZAS

E Plaza Palmas II

El Tizoncito
Tacos
Suite 9, 294-5143
Average cost per person: $90

F Plaza OVNI

Carl's Jr.
Hamburgers
Suite A, 294 5427
Average cost per person: $100

Ihop
International
Suite 200 A 294 5173
Average cost per person: $130

G Plaza Lands

Sushi I
Japanese
Suite 14, 234 2126
Average cost per person: $130

H Plaza Nautica

Porto Buzios
Seafood
Suite 5, 234 00 73
Average cost per person: $270

Koku
Asian
Suite 12, 234 1418
Average cost per person: $300

Rattle Bar
International
Suite 7, 234 1202
Average cost per person: $250

Tinto & Bife
Steak specialty
Suite 3, 234 1418
Average cost per person: $450

RESTAURANTES

1 HOTEL MISIÓN JURIQUILLA

Aranjuez
International and Mexican
Inside Hotel, 234 0000
Average cost per person: $230

Mavericks
Steak (Angus beef)
Inside Hotel, 234 0000
Average cost per person: $250

2 El Camaroncito Express
Seafood, 234 2479
Clemencia Borja Taboada 534
Average cost per person: $100

3 Saint Ross
International buffet, 234 0712
San Felipe de Jesús 510
Average cost per person: $180

Ueik
International , 234 3033
San Felipe de Jesús 510
Average cost per person: $300

4 Dimitris Gyros
Greek
Suite 1, 234 2378
Average cost per person: $180

5 Domino's Pizza
Pizza, 234 3160
Blvd. Universitario 514
Average cost per person: $60

6 Tacos El Pata
Tacos, 3849246
Blvd. Universitario 516
Average cost per person: $100

7 La Terraza
International
Club de Golf Juriquilla, 234 0845
Average cost per person: $170

8 Chilaking
Mexican 455 7015
Clemencia Borja Taboada 552
Average cost per person: $70

NASCE 2013

Program Grid

Tuesday 21			
REGISTRATION 16:00- 21:00 Mision Juriquilla Hotel			
Wednesday 22	**Thursday 23**	**Friday 24**	**Saturday 25**
Shuttles from the hotel to the UNAM Campus will run starting at 7:45 am, every 15 min, until 08:30 am. Then every hour			
Registration (starts at 8:00 am) (CAC Hall)	Registration (starts at 8:00 am) (CAC Hall)	Registration (starts at 8:00 am) (CAC Hall)	Registration (starts at 8:00 am) (CAC Hall)
Opening Ceremony 8:30-8:45(CA	Plenary (3) 08:30-09:20 (CAC) **L. SCHOOFS (BEL)**	Plenary (4) 8:30-09:20 (CAC) **J.P. CHANG (CAN)**	
Plenary (1) 08:45-09:35 (CAC) **G. SOMOZA (ARG)**	Coffee break (09:20-9:45)	Coffee break (09:20-9:45)	Plenary (7) 09:00-09:50 (CAC) **P. SWANSON (USA)**
Coffee break (9:35-09:50)	Morning Symposia (S3-S5) 09:45-11:35 (CAC, INB & II) **S-III: Clapp & Torres-Vázquez** **S-IV: Hoke & Duarte-Guterman** **S-V: Heyland & Schreiber**	Morning Symposia (S9-S11) 09:45-11:35 (CAC, INB & II) **S-IX: Langlois & González-M** **S-X: Carr & Bernier** **S-XI: Seale & Park**	Coffee break (09:50-10:15)
President's Symposium 09:50-11:40 (CAC) **Co-Chairs: Trudeau & Somoza**			Morning Symposia (S14-S16) 10:15-12:05 (CAC, INB & II) **S-XIV: García-Reyero & Hu** **S-XV: Zhu & Vafopoulo** **S-XVI: Riesgo & Klip**
Gorbman-Bern Mem. Lecture 11:45-12:35 (CAC) **R. DORES (USA)**	Gorbman-Bern New Ind. Inv. A. Lec 11:45-12:35 (CAC) **C. MARTYNIUK (CAN)**	Plenary (5) 11:45-12:35 (CAC) **H. DRUMMOND (MEX)**	NASCE Closing Best presentation awards 12:15-13:15
Lunch time 12:35- 14:15 **NASCE Executive Board Meeting** (Campus Council Room)	Lunch time 12:35- 14:15	Lunch time 12:35- 14:15 **NASCE Council Meeting** (Campus Council Room)	**Optional tours (to define)**
Afternoon Symposia (S1-S2) 14:15-16:00 (CAC & II) **S-I: Darras & Orozco** **S-II: Comizzoli & Milnes**	Afternoon Symposia (S6-S8) 14:15-16:00 (CAC, INB & II) **S-VI: Lovejoy & Godwin** **S-VII: Crespi & Londraville** **S-VIII: Bentley & Habibi**	Afternoon Symposia (S12-S13) 14:15-16:00 (CAC, INB & II) **S-XII: Sherwood & Chung** **S-XIII: Martyniuk & Orlando**	
Coffee break (16:00-16:20)		Coffee break (16:00-16:20)	
Plenary (2) 16:20-17:10 (CAC) **I. ORCHARD (CAN)**		Plenary (6) 16:20-17:10 (CAC) **E. RISSMAN (USA)**	
Poster Session 1 (Odd #s) 17:15-19:30 (INB patio)	Trip to Queretaro and Cultural activity & reception Sta Rosa de Viterbo 16:00-19:30:00	Poster Session 1 (Even #s) 17:15-19:30 (INB patio)	
NASCE welcome RECEPTION 19:45-21:30 (CAC)	Free Time in Downtown Queretaro 19:30- 23:00	NASCE party 20:00-23:00 (Mision Juriquilla Hotel)	

CAC: Auditorium of the Cultural and Academic Center (≈380 seats)
INB: Institute of Neurobiology's Seminar room (≈100 seats)
II: Institute of Engineering's auditorium (≈90 seats)

NASCE 2013 Schedule

Wednesday, May 22th:

8:30 – 8:45

NASCE 2013 Opening Ceremony

CAC

8:45 – 9:35

Plenary Session 1

Somoza, Gustavo M.

"CORTISOL AND SEX DIFFERENTIATION: THE 11B-HYDROXYSTEROID DEHYDROGENASE AS A KEY ENZYME"

CAC

9:35 – 9:50

Coffee Break

9:50 – 11:40

President's Symposium

SEXUAL DEVELOPMENT IN VERTEBRATES

Co-Chairs: Vance Trudeau & Gustavo Somoza

CAC

Symposium Speakers		
PS-1	Merchant-Larios, Horacio	*Exogenous Estrogen Prevents Growth Of Sox9+ Medullary Cords And Fails To Mimic The Pattern Of Ovarian Formation Induced By Temperature In The Sea Turtle Lepidochelys Olivacea*
PS-2	Nóbrega, Rafael Henrique	*Diversity Of Spermatogonial Stem Cell Niche Among Teleosts And The Regulation Of Spermatogonial Fate Using Zebrafish As Model*
PS-3	Langlois, Valerie	*Regulation Of Androgen Biosynthesis By Internal And Anthropogenic Compounds In Frogs*
Oral Presentations		
P-OR-1	Flood, Diana E.K.	*Crosstalk Between The Thyroid Hormone And Androgen Axes During Reproductive Development In Male Silurana Tropicalis*
P-OR-2	Luong, Xuan G.	*Variation In Estrogen Sensitivity Across Populations Of African Clawed Frogs*

11:45 – 12:35

Gorbman-Bern Memorial Lecture

Dores, Robert M.

"ANALYZING THE INTERACTION BETWEEN THE MELANOCORTIN-2 RECEPTOR AND THE ACCESSORY PROTEIN MRAP: AN EXAMPLE OF CONSTRUCTIVE NEUTRAL EVOLUTION"

CAC

12:35 – 14:15

Lunch time

NASCE Executive Board Meeting

Campus Council Room

14:15 – 16:00

Afternoon Symposia (S1 – S2)

	Symposium 1 **Multiple Actions of Thyroid Hormones** ***CAC*** Co-Chairs: Veerle Darras & Aurea Orozco		Symposium 2 **Comparative Endocrinology and Application to the Management and Propagation of Captive/Endangered Species** ***II (LIPATA)*** Co-Chairs: Pierre Comizzoli & Matthew R. Milnes
Symposium Speakers			
S1-1	**Orozco, Aurea** *Role and action mechanisms of 3,5-diiodothyronine in teleosts*	**S2-1**	**Kersey, David C.** *Minimally invasive hormone monitoring in endangered species*
S1-2	**Duarte-Guterman, Paula** *The surprising role of thyroid hormones in sexual development*	**S2-2**	**Dehnhard, Martin** *Application of endocrinology to assisted reproduction and/or captive breeding*
S1-3	**Darras, Veerle** *The role of thyroid hormones in neuronal development and regeneration*	**S2-3**	**Tubbs, Christopher** *Potential role of dietary phytoestrogens in the reproductive failure of captive southern white rhinoceros*
Oral Presentations			
OR1-1	**Olvera, Aurora** *Deiodinase selectivity for ord or ird. Involvement of the variable region (TM, H and L domains)*	**OR2-1**	**Aguilar, Fernando** *Seasonal and age dependent differences in testosterone levels in wild mexican Tylvilagus cunicularius*
OR1-2	**Espinoza-Ayala, Carmen** *Maternal separation persistently alters the expression of key elements of the HPT axis and its response to cold exposure in adult male rats*	**OR2-2**	**Ávila-Mendoza, José** *Effect of environmental temperature on the expression of several somatotropic factors (GHRH, SS, PACAP, TRH, GH, IGF-1), in the green iguana*

16:00 – 16:20

Coffee Break

16:20 – 17:10

Plenary Session 2

Orchard, Ian

"THE KISS OF DEATH: HORMONAL CONTROL OF DIURESIS IN THE KISSING BUG RHODNIUS PROLIXUS, THE VECTOR OF CHAGAS DISEASE"

CAC

17:15 – 19:30

NASCE Poster Session 1 – Odd numbered Posters

INB - Patio

19:45 – 21:30

NASCE WELCOME RECEPTION

CAC

Thursday, May 23th:

8:30 – 9:20

Plenary Session 3

Schoofs, Liliane

"NEUROPEPTIDE SIGNALLING AND BEHAVIOR"

CAC

9:20 – 9:45

Coffee Break

9:45 – 11:35

Morning Symposia (S3 – S5)

Symposium 3 **Blood Vessels as Relevant Hormonal Targets** ***CAC*** Co-Chairs: Carmen Clapp & Jesús Torres-Vázquez		**Symposium 4** **Hormones and Behavior** ***INB Seminar Room*** Co-Chairs: Kim Hoke & Paula Duarte-Guterman		**Symposium 5** **Hormonal Regulation of Metamorphosis Across Phyla** ***II (LIPATA)*** Co-Chairs: Andreas Heyland & Alex M. Schreiber	
Symposium Speakers					
S3-1	**Torres-Vázquez, Jesús** *The value of the zebrafish for understanding the vertebrate vasculature*	**S4-1**	**Wilczynski, Walter** *Social and hormonal interactions in the modulation of neural systems underlying reproductive behavior*	**S5-1**	**Buchholz, Daniel** *Beyond synergy: corticosterone and thyroid hormone have numerous interaction effects on gene regulation in Xenopus tropicalis tadpoles*
S3-2	**Clapp, Carmen** *Hormonal regulation of blood vessels: The prolactin-vasoinhibin connection*	**S4-2**	**Lauren O' Connell** *Convergent neuroendocrine mechanisms underlying paternal care*	**S5-2**	**Heyland, Andreas** *Histamine is a regulator of sea urchin metamorphosis*
S3-3	**Albrecht, Eugene D** *Estrogenic regulation of uterine blood vessels in nonhuman primates*	**S4-3**	**Hoke, Kim** *Predator exposure alters stress physiology and behavior in guppies*	**S5-3**	**Schreiber, Alex M.** *How do thyroid hormones induce lateralized swimming behaviors in flatfishes?*
Oral Presentations					
OR3-1	**Moreno-Carranza, Bibiana** *Prolactin stimulates liver regeneration by promoting the proliferation of hepatocytes and endothelial cells*	**OR4-1**	**Siller Pérez, Cristina** *Corticosterone infused into the striatum promotes the use and persistence of a spatial strategy in the tolman maze*	**OR5-1**	**Techa, Sirinart** *The functional importante and significance of ecdysone receptor in the regulation of molt cycle of the blue crab, Callinectes sapidus*
OR3-2	**Moreno-Vega, Aura** *The generation and bioactivity of vasoinhibins are reduced in the vitreous of patients with diabetic retinopathy*	**OR4-2**	**Alward, Beau** *Testosterone modulation of song behavior and consummatory sexual behavior in canaries (Serinus canaria) via action in the medial preoptic nucleus*	**OR5-2**	**Hammond, S. Austin** *Environmental temperature differentially impacts tissue-specific induction of thyroid hormone-responsive genes during precocious metamorphosis of Rana catesbeiana*

11:45 – 12:35

Gorbman-Bern New Independent Investigator Award Lecture

Martyniuk, Chris

"THE YIN AND THE YANG OF ANDROGEN RECEPTOR SIGNALING IN TELEOST FISH"

CAC

12:35 – 14:15
Lunch time

14:15 – 16:00
Afternoon Symposia (S6 – S8)

Symposium 6 **Development and Plasticity of Neuroendocrine Systems** ***CAC*** Co-Chairs: David Lovejoy & John Godwin		**Symposium 7** **Comparative Endocrinology of Leptins and other Adipokines** ***INB Seminar Room*** Co-Chairs: Erica Crespi & Richard Londraville		**Symposium 8** **Rfamides- Evolution and Function *II (LIPATA)*** Co-Chairs: George Bentley & Hamid R. Habibi	
Symposium Speakers					
S6-1	**O´Connell, Lauren** *Evolution of gene modules underlying social systems*	**S7-1**	**Crespi, Erica** *A role for leptin in limb development and regeneration in Xenopus laevis*	**S8-1**	**Habibi, Hamid R.** *Gonadotropin-inhibitory hormone control of gonadotropin hormone production in goldfish*
S6-2	**Adkins-Regan, Elizabeth** *Plasticity and development in zebra finches and other birds*	**S7-2**	**Londraville, Richard** *Exploring leptin function in fish and whales*	**S8-2**	**Calisi-Rodríguez, Rebecca M.** *Sex, stress, and parenting: GNIH response to social environment in birds and rodents*
S6-3	**Denver, Robert J.** *Thyroid hormone regulates DNA methylation and demethylation in the developing brain*	**S7-3**	**Macotela, Yazmín** *Adipocyte precursor cells: implications for obesity and diabetes*	**S8-3**	**Kriegsfeld, Lance J.** *Circadian control of kisspeptin and GNIH in female reproductive function*
Oral Presentations					
OR6-1	**Godwin, John** *Neuroendocrinology of socially controlled sex change in the bluehead wrasse*	**OR7-1**	**Luna-Moreno, Dalia** *Diurnal variations in grelin/leptin signaling in obese females, Neotomodon alstoni*	**OR8-1**	**Ernst, Darcy Fay Kato** *Does chronic corticosterone influence reproductive neuropeptides in female zebra finches?*
OR6-2	**Xing, L** *Dopaminergic regulation of brain aromatase and estrogen receptors and neurotrophic factors in goldfish*	**OR7-2**	**Bernier, Nick** *Appetite suppression, energy mobilization and alterations in the functional role of the endocrine growth axis contribute to the growth-suppressing effects of cortisol in rainbow trout*	**OR8-2**	**Kangas, Kristina A** *Awaking a sleeping dogma: diurnal changes in hypothalamic melatonin synthesis de novo in passerines*

16:00 – 19:30
Trip to Queretaro
and
Cultural Activity & Reception
Sta Rosa de Viterbo

19:30 – 23:00
Free Time in Downtown Queretaro

Friday, May 24th:

8:30 – 9:20

Plenary Session 4

Chang, John P.

"MULTIFACTORIAL NEUROENDOCRINE CONTROL AND SIGNALLING OF SOMATOTROPE FUNCTIONS – INSIGHTS FROM THE GOLDFISH STUDY MODEL"

CAC

9:20 – 9:45

Coffee Break

9:45 – 11:35

Morning Symposia (S9 – S11)

Symposium 9 **Reproductive Endocrinology-From Gene to Population** ***CAC*** Co-Chairs: Valérie Langlois & Gabriela González-Mariscal		**Symposium 10** **Stress and Energy Metabolism** ***INB Seminar Room*** Co-Chairs: James A. Carr & Nicholas Bernier		**Symposium 11** **Endocrine and Osmotic Modulation of Epithelial Cells** ***II (LIPATA)*** Co-Chairs: Andre P. Seale & Yoongseong Park	
Symposium Speakers					
S9-1	**Marlatt, Vicki** *Exploring estrogen action in early life stage salmonids*	**S10-1**	**Deviche, Pierre** *The acute androgen, glucocorticoid, and metabolic stress response of free-ranging birds*	**S11-1**	**Adams, Michael E.** *Endocrine regulation of respiratory epithelia mediating tracheal air filling during the ecdysis behavioral sequence of Drosophila*
S9-2	**González-Mariscal, Gabriela** *Contribution of hormones and exteroceptive signals to the expression of maternal behavior in rabbits*	**S10-2**	**Kozicz, Tamaz** *Midbrain urocortin 1 and energy metabolism*	**S11-2**	**Breves, Jason** *New insights into the molecular and cellular actions of prolactin on teleost osmoregulation*
S9-3	**Gutiérrez, Carlos** *Testosterone involvement in follicular development and ovulation in hens*	**S10-3**	**Lovejoy, David A** *The cryptic evolution of the teneurin C-terminal associated peptides (TCAP): Their role on cellular energy production and inhibition of the stress-response.*	**S11-3**	**Piermarini, Peter** *Cellular and molecular mechanisms of diuresis in mosquitoes*
Oral Presentations					
OR9-1	**Perfito, Nicole** *Physical access to a mate regulates final follicle maturation: endocrine and molecular correlates*	**OR 10-1**	**do Rego, Jean Luc** *Regulation of neurosteroid biosynthesis by corticotropin-releasing hormone: Possible role in the control of stress response*	**OR 11-1**	**Inokuchi, Mayu** *Effects of ambient osmolality and prolactin on branchial osmoregulatory function in cultured gill filaments from freshwater-acclimated mozambique tilapia*
OR9-2	**Stevenson, Tyle** *Epigenetic modifications and photoperiodic time measurement*	**OR 10-2**	**Pham, Vi** *Is nesfatin-1 a modulator of stress response in fish?*	**OR 11-2**	**Moorman, BP** *The effects of steady-state and tidally changing rearing salinities on osmoregulation in the mozambique tilapia*

11:45 – 12:35

Plenary Session 5

Drummond, Hugh

"SIBLING CONFLICT, HORMONES AND LONG-TERM DEVELOPMENTAL IMPACTS IN THE BLUE-FOOTED BOOBY"

CAC

12:35 – 14:15

Lunch time

NASCE Council Meeting

Campus Council Room

14:15 – 16:00

Afternoon Symposia (S12 – S13)

Symposium 12 **New Hormones and Novel Functions for Old Friends** ***CAC*** Co-Chairs: Nancy Sherwood & J. Sook Chung		**Symposium 13** **Neuroendocrine Disruption** ***II (LIPATA)*** Co-Chairs: Chris Martyniuk & Edward F. Orlando	
Symposium Speakers			
S12-1	**Hauser, Frank** *Orthologues of mammalian neuropeptides and receptors in potostomes: the evolutionary orign of GNRH, vasopressin and oxytocin*	**S13-1**	**Trudeau, Vance L.** *Neuroendocrine disruption: upsetting systems control*
S12-2	**Chung, J. Sook** *A gender specific hormone determines the development of adult female features*	**S13-2**	**Rosenfeld, Cheryl** *Effects of bisphenol A on sexually selected cognitive traits in monogamous and polygynous Peromyscus species*
S12-3	**Sherwood, Nancy** *Glycoprotein hormones and their receptors evolved at the origin of metazoans*	**S13-3**	**Orlando, Edward F.** *Environmental gestagens and their effects on progesterone receptor activation in the fathead minnow (pimephales promelas)*
Oral Presentations			
OR 12-1	**Paluzzi, Jean-Paul** *Identification, expression analysis and functional characterization of the myoinhibiting peptide receptor in the Chagas disease vector, Rhodnius prolixus*	**S13-4**	**Richter, Catherine A/Chris Martyniuk** *Characterizing gene regulatory networks in the brain of largemouth bass inhabiting rivers containing high levels of methyl-mercury*
OR 12-2	**Arámburo, Carlos** *Paracrine/autocrine actions of extrapituitary growth hormone in the chicken reproductive system*	**OR 13-2**	**León-Olea, Martha** *Disruption of hypothalamic vasopressin, nitric oxide and PACAP by in utero exposure to PCBS and PBDES in hyperosmotic stimulated rats.*

16:00 – 16:20

Coffee Break

16:20 – 17:10

Plenary Session 6

Rissman, Emilie

"PLASTICS: IMMEDIATE AND TRANS-GENERATIONAL ACTIONS ON BRAIN AND BEHAVIOR"

CAC

17:15 – 19:30

NASCE Poster Session 2 – Even numbered Posters

INB - Patio

20:00 – 23:00

NASCE Party

Mision Juriquilla Hotel

Saturday, May 25th:

9:00 – 9:50

Plenary Session 7

Swanson, Penny

"SEASONAL VARIATION IN THE EFFECTS OF NUTRITIONAL PLANE ON AGE OF PUBERTY AND THE PITUITARY-GONAD AXIS IN SALMON"

CAC

10:15 – 12:05

Morning Symposia (S14 – S16)

Symposium 14 **Endocrinology- Advances through Genomics, Peptidomics and Related Technologies** ***INB Seminar Room*** Co-Chairs: Natália García-Reyero Vinas & Wei Hu		**Symposium 15** **Hormone Receptors Critical in Development and Reproduction** ***II (LIPATA)*** Co-Chairs: Yong Zhu & Xanthe Vafopoulou		**Symposium 16** **Diabetic Models across Species- Lessons from a Common Pathway** ***CAC*** Co-Chairs: Juan Riesgo-Escovar & Amira Klip	
Symposium Speakers					
S14-1	**García-Reyero Vinas, Natalia** *A systems toxicology approach to decipher hormetic effects in Daphnia magna*	**S15-1**	**Evans, Peter D.** *GPCR-mediated rapid, non-genomic actions of steroids in Drosophila*	**S16-1**	**Klip, Amira** *Insulin signals in skeletal muscle regulating glucose transporter Glut4*
S14-2	**Hu, Wei** *Growth hormone transgenesis affects neuroendocrine regulation and reproduction in the common carp Cyprinus carpio l.*	**S15-2**	**Vafopoulou, Xanthe** *Mitochondrial involvement in the non-genomic actions of insect steroid hormones*	**S16-2**	**Corley-Lavine, Laura Sue** *The insulin/insulin like growth factor pathway is a Mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons*
Oral Presentations		**S15-3**	**Zhu, Yong** *Nongenomic progestin receptors and their signaling in zebrafish model*	**S16-3**	**Riesgo-Escovar, Juan** *The insulin pathway in Drosophila as a model for diabetes*
OR 14-1	**Velázquez, Pedro Nicolás** *Estrogenic ovarian cell subpopulations stimulate cell proliferation of germ cells and androgenic cells of 18-day-old chick embryo.*				
OR 14-2	**Moussavi, Mina** *Role of GnIH in paracrine control of gonadal function in goldfish*	**Oral Presentations**			
		OR 15-1	**Harvey, Steve** *Growth hormone (GH) and retinal ganglion cell function: QNR/D cells as an experimental model*	**OR 16-1**	**Lemini, María** *Analysis of pituitary prolactin secretion in two rat diabetes models: role of tumor necrosis factor alpha and transforming growth factor beta*
OR 14-3	**Lee, Dohee** *The roles of crustacean cardioactive peptide in the vector of chagas' disease, Rhodnius prolixus*	**OR 15-2**	**Pérez-Ibave, Diana** *GH locus expression in the eye of higher primates*	**OR 16-2**	**Luna-Acosta, José Luis** *Antiapoptotic effects of growth hormone are mediated by PI3k/akt pathway in the chicken bursa of fabricius*

12:15 – 13:15

NASCE Closing Ceremony
Best Presentation Awards

CAC

List of Abstracts

Plenary Lectures Abstracts		
PL1	Somoza, Gustavo M. IIB-INTECH (CONICET-UNSAM), Chascomús, Argentina	*Cortisol and sex differentiation: the 11β-hydroxysteroid dehydrogenase as a key enzyme*
PL2	Orchard, Ian Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada	*The Kiss of Death: Hormonal control of diuresis in the kissing bug Rhodnius prolixus, the vector of Chagas disease*
PL3	Schoofs, Liliane Animal Physiology and Neurobiology, KU Leuven, Leuven, Belgium	*Neuropeptide signalling and behavior*
PL4	Chang, John P. Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada	*Multifactorial neuroendocrine control and signalling of somatotrope functions – insights from the goldfish study model*
PL5	Drummond, Hugh Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico	*Sibling conflict, hormones and long-term developmental impacts in the blue-footed booby*
PL6	Rissman, Emilie Department of Biochemistry and Molecular Genetics, and Graduate program in Neuroscience, University of Virginia, Charlottesville VA 22908 USA	*Plastics: immediate and trans-generational actions on brain and behavior*
PL7	Swanson, Penny Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA 98112, USA	*Seasonal variation in the effects of nutritional plane on age of puberty and the pituitary-gonad axis in salmon*

Gorbman-Bern Award Lectures		
GBMAL	Dores, Robert M. University of Denver, Department of Biological Sciences, Denver, Colorado, U.S.A	*Analyzing the interaction between the melanocortin-2 receptor and the accessory protein mrap: an example of constructive neutral evolution*
GBNIIAL	Martyniuk, Chris Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, New Brunswick, E2L 4L5, Canada.	*The yin and the yang of androgen receptor signaling in teleost fish*

Invited Symposium Speaker Abstracts		
PS-1	Merchant-Larios, Horacio Departamento de Embriología, Facultad de Medicina. Universidad Nacional Autónoma de México. Mexico City	*Exogenous estrogen prevents growth of Sox9+ medullary cords and fails to mimic the pattern of ovarian formation induced by temperature in the sea turtle Lepidochelys olivacea*
PS-2	Nóbrega, Rafael Henrique Department of Morphology, São Paulo State University – Botucatu, São Paulo, Brazil;	*Diversity of spermatogonial stem cell niche among teleosts and the regulation of spermatogonial fate using zebrafish as model*
PS-3	Langlois, Valerie Department of Chemistry and Chemical Engineering, Royal Military College of Canada, P.O. Box 17 000, Station Forces, Kingston, ON, K7K 7B4, CANADA	*Regulation of androgen biosynthesis by internal and anthropogenic compounds in frogs*
S1-1	Orozco, Aurea Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM). Querétaro, México	*Role and action mechanisms of 3,5-diiodothyronine in teleosts*
S1-2	Duarte-Guterman, Paula Department of Biology, University of Ottawa, Ottawa, Canada	*The surprising role of thyroid hormones in sexual development*
S1-3	Darras, Veerle Department of Biology, Section Animal Physiology and Neurobiology, KU Leuven, Leuven, Belgium.	*The role of thyroid hormones in neuronal development and regeneration*
S2-1	Kersey, David C. College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA USA	*Minimally invasive hormone monitoring in endangered species*

List of Abstracts

S2-2	Dehnhard, Martin Leibniz Institute for Zoo and Wildlife Research, Department of Reproductive Biology, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany	*Application of endocrinology to assisted reproduction and/or captive breeding*
S2-3	Tubbs, Christopher San Diego Zoo Institute for Conservation Research, Escondido, CA USA	*Potential role of dietary phytoestrogens in the reproductive failure of captive southern white rhinoceros*
S3-1	Torres-Vázquez, Jesús Department of Cell Biology, Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine. New York University Langone Medical Center. New York, NY USA.	*The value of the zebrafish for understanding the vertebrate vasculature*
S3-2	Clapp, Carmen Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México	*Hormonal regulation of blood vessels: the prolactin-vasoinhibin connection*
S3-3	Albrecht, Eugene D Departments of Obstetrics, Gynecology, Reproductive Sciences and Physiology, Center for Studies in Reproduction, University of Maryland School of Medicine, Baltimore, MD USA	*Estrogenic regulation of uterine blood vessels in nonhuman primates*
S4-1	Wilczynski, Walter Neuroscience Institute, Georgia State University, Atlanta, GA, USA	*Social and hormonal interactions in the modulation of neural systems underlying reproductive behavior*
S4-2	O´Connell, Lauren Center for Systems Biology, Harvard University, Cambridge, MA USA	*Convergent neuroendocrine mechanisms underlying paternal care*
S4-3	Hoke, Kim Department of Biology, Colorado State University, Ft. Collins, CO USA	*Predator exposure alters stress physiology and behavior in guppies*
S5-1	Buchholz, Daniel Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221, USA	*Beyond synergy: corticosterone and thyroid hormone have numerous interaction effects on gene regulation in Xenopus tropicalis tadpoles*
S5-2	Heyland, Andreas University of Guelph, Integrative Biology Guelph, ON Canada	*Histamine is a regulator of sea urchin metamorphosis*
S5-3	Schreiber, Alex M. Biology Department, St. Lawrence University, Canton, NY USA	*How do thyroid hormones induce lateralized swimming behaviors in flatfishes?*
S6-1	O´Connell, Lauren Center for Systems Biology, Harvard University, Cambridge, MA USA	*Evolution of gene modules underlying social systems*
S6-2	Adkins-Regan, Elizabeth Department of Psychology and Department of Neurobiology and Behavior, Cornell University, Ithaca, NY USA	*Plasticity and development in zebra finches and other birds*
S6-3	Denver, Robert J. Department of Molecular, Cellular and Developmental Biology, The University of Michigan, Ann Arbor, MI USA	*Thyroid hormone regulates DNA methylation and demethylation in the developing brain*
S7-1	Crespi, Erica School of Biological Sciences, Washington State University, Pullman, WA, USA	*A role for leptin in limb development and regeneration in Xenopus laevis*
S7-2	Londraville, Richard Department of Cell and Systems Biology, University of Toronto, Toronto ON, Canada.	*The cryptic evolution of the teneurin C-terminal associated peptides (TCAP): Their role on cellular energy production and inhibition of the stress-response*
S7-3	Macotela, Yazmín Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México	*Adipocyte precursor cells: implications for obesity and diabetes*
S8-1	Habibi, Hamid R. Department of Biological Sciences, University of Calgary, Calgary	*Gonadotropin-inhibitory hormone control of gonadotropin hormone production in goldfish*
S8-2	Calisi-Rodríguez, Rebecca M. Department of Neurobiology, Physiology, and Behavior, University of California, Davis, CA	*Sex, stress, and parenting: GnIH response to social environment in birds and rodents*

List of Abstracts

S8-3	Kriegsfeld, Lance J. Department of Psychology, University of California, Berkeley CA 94720 USA	*Circadian control of kisspeptin and GnIH in female reproductive function*
S9-1	Marlatt, Vicki Nautilus Environmental, Burnaby, BC, Canada and Department of Biology, University of the Fraser Valley, Abbotsford, BC, Canada	*Exploring estrogen action in early life stage salmonids*
S9-2	González-Mariscal, Gabriela Centro de Investigación en Reproducción Animal, CINVESTAV-Universidad Autónoma de Tlaxcala, México	*Contribution of hormones and exteroceptive signals to the expression of maternal behavior in rabbits*
S9-3	Gutiérrez, Carlos Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, México, D.F	*Testosterone involvement in follicular development and ovulation in hens*
S10-1	Deviche, Pierre School of Life Sciences, Arizona State University, AZ USA	*The acute androgen, glucocorticoid, and metabolic stress response of free-ranging birds*
S10-2	Kozicz, Tamaz Department of Anatomy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands	*Midbrain urocortin 1 and energy metabolism*
S10-3	Lovejoy, David A Department of Cell and Systems Biology, University of Toronto, Toronto ON, Canada.	*The cryptic evolution of the teneurin C-terminal associated peptides (tcap): their role on cellular energy production and inhibition of the stress-response.*
S11-1	Adams, Michael E. Departments of Entomology and Cell Biology & Neuroscience, University of California, Riverside, CA, U.S.A	*Endocrine regulation of respiratory epithelia mediating tracheal air filling during the ecdysis behavioral sequence of Drosophila*
S11-2	Breves, Jason Department of Biology & Center for Neuroendocrine Studies, University of Massachusetts, Amherst, MA USA	*New insights into the molecular and cellular actions of prolactin on teleost osmoregulation*
S11-3	Piermarini, Peter Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH USA	*Cellular and molecular mechanisms of diuresis in mosquitoes*
S12-1	Hauser, Frank University of Copenhagen, Denmark	*Orthologues of mammalian neuropeptides and receptors in protostomes: the evolutionary origin of gnrh, vasopressin and oxytocin*
S12-2	Chung, J. Sook Institute of Marine Environmental Technology, University of Maryland Center for Environmental Science	*A gender specific hormone determines the development of adult female features*
S12-3	Sherwood, Nancy Department of Biology, University of Victoria, Victoria, BC Canada	*Glycoprotein hormones and their receptors evolved at the origin of Metazoan*
S13-1	Trudeau, Vance L. Department of Biology, University of Ottawa, Canada	*Neuroendocrine disruption: upsetting systems control*
S13-2	Rosenfeld, Cheryl Department of Biomedical Sciences, University of Missouri, Columbia, MO USA	*Effects of bisphenol a on sexually selected cognitive traits in monogamous and polygynous* Peromyscus *species*
S13-3	Orlando, Edward F. University of Maryland, Department of Animal and Avian Sciences, College Park, MD	*Environmental gestagens and their effects on progesterone receptor activation in the fathead minnow (Pimephales promelas)*
S13-4	Richter, Catherine A U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, USA	*Characterizing gene regulatory networks in the brain of largemouth bass inhabiting rivers containing high levels of methyl-mercury*
S14-1	García-Reyero Vinas, Natalia Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, MS	*A systems toxicology approach to decipher hormetic effects in Daphnia magna*
S14-2	Hu, Wei State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China	*Growth hormone transgenesis affects neuroendocrine regulation and reproduction in the common carp Cyprinus carpio l.*

List of Abstracts

S15-1	Evans, Peter D. The Inositide Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge, UK	*Gpcr-mediated rapid, non-genomic actions of steroids in Drosophila*
S15-2	Vafopoulou, Xanthe York University, Canada	*Mitochondrial involvement in the non-genomic actions of insect steroid hormones*
S15-3	Zhu, Yong Department of Biology, East Carolina University, 1000 E. 5th St. Greenville, NC 27858, USA	*Nongenomic progestin receptors and their signaling in zebrafish model*
S16-1	Klip, Amira Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada	*Insulin signals in skeletal muscle regulating glucose transporter glut4*
S16-2	Corley-Lavine, Laura Sue Department of Entomology, Washington State University, Pullman, WA 99164–6382, USA.	*The insulin/insulin like growth factor pathway is a mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons*
S16-3	Riesgo-Escovar, Juan Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, Querétaro, México	*The insulin pathway in Drosophila as a model for diabetes*

Oral Presentations Abstracts

POR-1	Flood, Diana E.K. Department of Chemistry and Chemical Engineering, Royal Military College of Canada, ON, CA	*Crosstalk between the thyroid hormone and androgen axes during reproductive development in male silurana tropicalis*
POR-2	Luong, Xuan G. Department of Integrative Biology, University of California, Berkeley, CA, USA	*Variation in estrogen sensitivity across populations of african clawed frogs*
OR1-1	Olvera, Aurora Departamento de Neurobiología Celular y Molecular. Instituto de Neurobiología. UNAM Campus Juriquilla, Querétaro, México	*Deiodinase selectivity for ORD or IRD. Involvement of the variable region (TM, H and L domains)*
OR1-2	Espinoza-Ayala, Carmen Department of Physiology and Genetics, Instituto de Biotecnología de la Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Mor., Méx	*Maternal separation persistently alters the expression of key elements of the hpt axis and its response to cold exposure in adult male rats*
OR2-1	Aguilar, Fernando Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, México	*Seasonal and age-dependent differences in testosterone levels in wild mexican cottontails Sylvilagus cunicularius*
OR2-2	Ávila-Mendoza, José Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México.	*Effect of environmental temperature on the expression of several somatotropic factors (GHRH, SS, PACAP, TRH, GH, IGF-I), in the green iguana*
OR3-1	Moreno-Carranza, Bibiana Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México	*Prolactin stimulates liver regeneration by promoting the proliferation of hepatocytes and endothelial cells*
OR3-2	Moreno-Vega, Aura Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM)	*The generation and bioactivity of vasoinhibins are reduced in the vitreous of patients with diabetic retinopathy*
OR4-1	Siller Pérez, Cristina Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, UNAM Campus Juriquilla, Querétaro, Qro. 76230 México	*Corticosterone infused into the striatum promotes the use and persistence of a spatial strategy in the tolman maze*
OR4-2	Alward, Beau Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA	*Testosterone modulation of song behavior and consummatory sexual behavior in canaries (Serinus canaria) via action in the medial preoptic nucleus*
OR5-1	Techa, Sirinart Institute of Marine Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD, USA	*The functional importance and significance of ecdysone receptor in the regulation of molt cycle of the blue crab, Callinectes sapidus*

List of Abstracts

OR5-2	Hammond, S. Austin Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC Canada	*Environmental temperature differentially impacts tissue-specific induction of thyroid hormone-responsive genes during precocious metamorphosis of Rana catesbeiana*
OR6-1	Godwin, John Centre for Reproduction and Genomics, Department of Anatomy, University of Otago, PO Box 913, Dunedin 9054, New Zealand	*Neuroendocrinology of socially controlled sex change in the bluehead wrasse*
OR6-2	Xing, L Department of Biology, University of Ottawa, Ottawa, ON Canada	*Dopaminergic regulation of brain aromatase and estrogen receptors and neurotrophic factors in goldfish*
OR7-1	Luna-Moreno, Dalia Unidad Multidisciplinaria de Docencia e Investigación, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, México	*Diurnal variations in ghrelin/leptin signaling in obese females, Neotomodon alstoni*
OR7-2	Bernier, Nick Department of Integrative Biology, University of Guelph, ON, Canada.	*Appetite suppression, energy mobilization and alterations in the functional role of the endocrine growth axis contribute to the growth-suppressing effects of cortisol in rainbow trout*
OR8-1	Ernst, Darcy Fay Kato Department of Integrative Biology, University of California, Berkeley, CA USA	*Does chronic corticosterone influence reproductive neuropeptides in female zebra finches?*
OR8-2	Kangas, Kristina A Department of Integrative Biology, University of California, Berkeley, CA USA	*Awaking a sleeping dogma: diurnal changes in hypothalamic melatonin synthesis de novo in passerines*
OR9-1	Perfito, Nicole Department of Integrative Biology, Univ. of Calif. Berkeley, CA USA	*Physical access to a mate regulates final follicle maturation: endocrine and molecular correlates*
OR9-2	Stevenson, Tyler Institute for Mind and Biology, The University of Chicago, Chicago, Il., USA	*Epigenetic modifications and photoperiodic time measurement*
OR 10-1	do Rego, Jean Luc *Institute of Research and Innovation in Biomedicine (IRIB), University of Rouen, Rouen, France;*	*Regulation of neurosteroid biosynthesis by corticotropin-releasing hormone: possible role in the control of stress response*
OR 10-2	Pham, Vi Department of Veterinary Biomedical Sciences, Laboratory of Integrative Neuroendocrinology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan, Canada	*Is nesfatin-1 a modulator of stress response in fish?*
OR 11-1	Inokuchi, Mayu Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI, USA	*Effects of ambient osmolality and prolactin on branchial osmoregulatory function in cultured gill filaments from freshwater-acclimated mozambique tilapia*
OR 11-2	Moorman, Benjamin P. Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI, USA	*The effects of steady-state and tidally changing rearing salinities on osmoregulation in the Mozambique tilapia*
OR 12-1	Paluzzi, Jean-Paul Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada	*Identification, expression analysis and functional characterization of the myoinhibiting peptide receptor in the Chagas disease vector, Rhodnius prolixus*
OR 12-2	Arámburo, Carlos Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México	*Paracrine/autocrine actions of extrapituitary growth hormone in the chicken reproductive system*
OR 13-2	León-Olea, Martha Departamento de Neuromorfología Funcional, Instituto Nacional de Psiquiatría Ramón de la Fuente M., México.	*Disruption of hypothalamic vasopressin, nitric oxide and PACAP by in utero exposure to PCBS and PBDES in hyperosmotic stimulated rats*
OR 14-1	Velázquez, Pedro Nicolás Departmento de Biología Celular y Tisular, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F. 04510	*Estrogenic ovarian cell subpopulations stimulate cell proliferation of germ cells and androgenic cells of 18-day-old chick embryo.*
OR 14-2	Moussavi, Mina Department of Biological Sciences, University of Calgary	*Role of GnIH in paracrine control of gonadal function in goldfish*

OR 14-3	Lee, Dohee Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada	*The roles of crustacean cardioactive peptide in the vector of Chagas' disease, Rhodnius prolixus*
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OR 15-2	Pérez-Ibave, Diana Department of Biochemistry and Molecular Medicine, Autonomous University of Nuevo León, Monterrey 64460, México	*GH locus expression in the eye of higher primates*
OR 16-1	Lemini, María Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México	*Analysis of pituitary prolactin secretion in two rat diabetes models: role of tumor necrosis factor alpha and transforming growth factor beta*
OR 16-2	Luna-Acosta, José Luis Departamento de Neurobiología Celular y Molecular. Instituto de Neurobiología. Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro, 76230, México	*Antiapoptotic effects of growth hormone are mediated by PI3k/Akt pathway in the chicken bursa of Fabricius*

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P7	Baroffio, Angelina	Assessment of a full-scale wastewater treatment plant disinfection upgrade– effects on occurrence of disinfection byproduct in effluents and endocrine disruption in fish.
P8	Bourdon, Lisa	Using a combination of lab and field sampling techniques to characterize the estrogenicity gradient of a portion of the south platte river
P9	Cosme, Madelyne	Disruption of ovarian prostaglandin and steroid biosynthetic pathways and reduced spawning in zebrafish exposed in vivo to quinacrine
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Wednesday, May 22th
08:45 – 09:35

PL-1
CORTISOL AND SEX DIFFERENTIATION: THE 11B-HYDROXYSTEROID DEHYDROGENASE AS A KEY ENZYME
Gustavo M. Somoza (1), Juan I. Fernandino (1), Ricardo S. Hattori (2) and Carlos A. Strüssmann (2)
(1) IIB-INTECH (CONICET-UNSAM), Chascomús, Argentina. (2) Tokyo University of Marine Science and Technology, Tokyo, Japan.

In recent years, fish have been extensively studied because they present a variety of mechanisms of sex determination/differentiation. In the present work we review the sex determining mechanisms and the testicular differentiation process in Odontesthes bonariensis, commonly known as pejerrey, in relation to temperature-induced masculinization. We have recently shown that cortisol is involved in the gonadal masculinization process of pejerrey during early development. Larvae exposed to a male-producing temperature showed increased whole-body cortisol and developed as males. Moreover, they also have high 11-KT levels, suggesting a relation between cortisol and 11-oxygenated androgens during the masculinization process. 11β-hydroxysteroid dehydrogenase (11β-HSD) is one of enzymes shared by the glucocorticoid and androgen pathways. This enzyme converts cortisol to cortisone and also participates in the finals steps of the synthesis of the 11-oxigenated androgens. In pejerrey, during the critical period of sex determination, gene expression of the 11β-HSD gene hsd11b2, glucocorticoid receptors gr1, and androgen receptors (ar1 and ar2) were shown to be increased and expressed in the gonads during masculinization. These data suggest that the enzymatic machinery necessary for the local production of 11-oxygenated steroids and the inactivation of cortisol is active in the undifferentiated gonads during sex determination. Also, gonadal explants incubated in the presence of cortisol showed an increase in the synthesis of 11-KT. Based on these data, and also from data taken from the literature we here propose that the masculinization induced by thermal stress can be considered as a consequence of cortisol inactivation and the concomitant synthesis of 11-KT and discuss this as a possible mechanism of masculinization induced by different types of environmentalstressors. Supported by CONICET and ANPCyT (Argentina) and the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Wednesday, May 22th
16:20-17:10

PL-2
THE KISS OF DEATH: HORMONAL CONTROL OF DIURESIS IN THE KISSING BUG *Rhodnius prolixus*, THE VECTOR OF CHAGAS DISEASE
Ian Orchard
Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

Rhodnius prolixus is an obligatory blood-feeding insect found in Central and South America. It is one of 12 Triatominae species acting as a vector for the parasite Trypanosoma cruzi and thereby transmits Chagas disease to humans. Gorging on a blood meal triggers endocrinological events associated with growth, development, metamorphosis and reproduction. Short-term physiological events are also initiated in response to an enormous blood meal. In particular, R. prolixus rapidly excretes a hypo-osmotic fluid of high NaCl content in order to lower its mass, but also concentrate the blood nutrients. In so doing, the volume, ionic, and osmotic balance of the haemolymph are preserved. Pioneering work of Simon Maddrell revealed that this diuresis is under the neurohormonal control of serotonin and neuropeptides. Making use of the R. prolixus genome, these neuropeptides (e.g. Rhopr-CRF/DH, Rhopr-CT/DH) and their GPCRs have been data mined, cloned and sequenced. These diuretic hormones (DHs) act via cAMP on the anterior midgut and Malpighian tubules to stimulate salt and water transport. Combinations of these DHs can be synergistic, and increase fluid secretion 1000 fold. Receptor distribution and physiological assays reveal that these DHs have effects on other tissues. An antidiuretic hormone (ADH) is also present in R. prolixus, and is involved in the cessation of diuresis - an essential phenomenon that prevents excessive loss of water and salts. This ADH (Rhopr-CAPA-2) acts via its GPCR to inhibit the synergism between the DHs, thereby rapidly stopping diuresis. Physiological events associated with gorging on a blood meal are complex, and involve DHs and ADHs that broadcast messages and bias tissues towards a new physiological state. The parasite causing Chagas disease is transmitted from the hindgut in the urine/feces during this post-feeding diuresis. Neuropeptides and serotonin therefore effectively control the transmission of this disease. Funded by NSERC

Thursday, May 23th
08:30 – 09:20

PL-3
NEUROPEPTIDE SIGNALLING AND BEHAVIOR
Liliane Schoofs, Liesbet Temmerman, Tom Janssen, Ellen Meelkop and Isabel Beets
Animal Physiology and Neurobiology, KU Leuven, Leuven, Belgium

Neuropeptidergic signaling is widely adopted by animals for the regulation of physiology and behavior in a rapidly changing environment. The vasopressin/oxytocin (VP/OT) neuropeptide family originates from an ancestral peptide precursor in the antecedent of protostomian and deuterostomian animals. In vertebrates, VP and OT have both hormonal effects on peripheral target tissues, such as in the regulation of reproduction and water balance, and neuromodulatory actions in the central nervous system, controlling social behavior and cognition. Earlier studies on invertebrates have focused on conserved peripheral actions of this neuropeptide family. We recently identified a VP/OT-related signaling system in Caenorhabditis elegans, called nematocin. Neuromodulation by nematocin occurs in at least two functional systems: in reconfiguring the neuronal circuit for salt chemotaxis in light of previous experience1; and in functionally coordinating local sensory-motor circuits for male mating into a coherent reproductive behavior2. As VP/OT-related receptors are expressed in the nervous system of many invertebrates, neuromodulation by the vasopressin/oxytocin family most likely occurs in all these species. In this context, it is reasonable to hypothesize that especially from the moment animals started exploring new environments - where they encountered a variety of novel cues related to e.g. mating partners or food availability - the emergence of a neuropeptidergic system that could accordingly direct their behavior and decisions was of great benefit to their survival. Molecular interactions are comparable to those underlying VP/OT-mediated effects in the mammalian brain. Understanding how the VP/OT family fine-tunes neuronal circuits for social behavior, learning and memory poses a major challenge. Functional conservation in C. elegans enables the development of future models to help answering this question.

Friday, May 24th
08:30 – 09:20

PL-4
MULTIFACTORIAL NEUROENDOCRINE CONTROL AND SIGNALLING OF SOMATOTROPE FUNCTIONS – INSIGHTS FROM THE GOLDFISH STUDY MODEL
Chang, JP, Pemberton, JG, Orr, ME, Grey, CL.
Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada

In goldfish, individual somatotropes possess receptors to multiple neuroendocrine factors. Stimulators of somatotropin (GH) release include two gonadototropin-releasing hormones (sGnRH and cGnRH-II), dopamine (DA), pituitary adenylate cyclase-activating polypeptide (PACAP), ghrelin (GRLN) and GH-releasing hormone (GHRH). Inhibitors include three somatostatins (SS14, [Pro2]SS14, and gbSS28), norepinephrine (NE) and serotonin (5HT). Protein kinase A (PKA) and C (PKC) are two major, largely additive, pathways leading to stimulated GH release and both pathways are linked to increases in intracellular Ca2+ levels. sGnRH, cGnRH-II and GRLN utilize PKC and voltage-sensitive Ca2+ channels (VSCCs). DA, PACAP and GHRH activate PKA, and DA also utilizes VSCCs. Surprisingly, GH responses to GRLN and DA are not additive, suggesting their actions also converge at another major point(s) along their receptor signalling cascades. sGnRH, cGnRH-II, DA and PACAP actions also differ in the use of arachidonic acid (AA), nitric oxide (NO), intracellular Ca2+ stores and Ca2+ buffering systems. Phosphoinositide 3-kinase and extracellular signal-regulated kinase kinase (MEK) mediate sGnRH- and cGnRH-II-induced GH release in a time- and/or ligand-specific manner. Although MEK affects basal GH protein levels and mediates GnRH-induced increases in GH mRNA, neither sGnRH nor cGnRH-II elevates total GH availability indicating that transcription and protein production are regulated differently. The differential abilities of three SSs, NE and 5HT to reduce stimulated GH release, as well as those of the three SSs to affect basal and GnRH effects on GH mRNA levels, may be related to their selective effects on VSCCs, K+ currents, PKC, PKA, Ca2+, AA and/or NO signalling. Overall, these results indicate that interactions at the levels of the cell and intracellular signalling provide the basis of ligand- and function-specific regulation of GH release and synthesis. (Supported by NSERC.)

Friday, May 24th
11:45 – 12:35

PL-5
SIBLING CONFLICT, HORMONES AND LONG-TERM DEVELOPMENTAL IMPACTS IN THE BLUE-FOOTED BOOBY
Drummond, H.
Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de Mexico, Mexico City, Mexico.

In the blue-footed booby's brood of two, the junior (younger) chick is subordinate to its sibling and faces violent aggression, psychological subordination, elevated circulating corticosterone and reduced feeding and growth. Experimental studies have shown that such "poor starts" in life can lead to developmental programming and phenotypic deficits in diverse adult traits including cognition, competitiveness, maturation, attractiveness, reproduction and longevity. Surprisingly, our long-term descriptive study of a natural population of boobies has largely failed to uncover appreciable deficits in adult traits of junior boobies, including body condition, survival, recruitment into the breeding population, natal dispersal, immune response and aggressive nest defence. This does not permit us to conclude that there is no deficit in adulthood because trade-offs between traits are expected and the deficit could always be located in some other trait or expressed later in the lifespan. But scrutiny of the bottom line also failed to reveal inferiority of juniors: at all ages over the lifespan (up to 16 years) they produced as many fledglings as seniors. However, a deficit showed up in the next generation: fledglings produced by former juniors in their first three years of life were less likely to become breeders than those produced by former seniors. Although we know that development of wild vertebrates is sometimes prejudiced by unfavorable weather, they may be less vulnerable to developmental challenges than we have been thinking. Experimental challenges are often posed in environments that limit scope for evasion, and they frequently involve treatments whose ecological validity is unknown. Junior boobies face natural, predictable, socially-imposed stresses, and their developmental impacts are minimal and largely passed to the next generation. How wild vertebrates deal with natural environmental challenges that threaten their development remains largely to be explored. Acknowledgments. Collaborators on the reported research include Cristina Carmona, Sin-Yeon Kim, Alejandra Nuñez, Dani Oro, José Luis Osorno, Cristina Rodríguez, Oscar Sanchez and John Wingfield; funding was provided by CONACYT, the UNAM and the National Geographic Society; permits were granted by SEMARNAT; and logistical support was provided by the fishermen of Nayarit, the Mexican Navy and SEMARNAT.

Friday, May 24th
14:20 – 17:10

PL-6
PLASTICS: IMMEDIATE AND TRANS-GENERATIONAL ACTIONS ON BRAIN AND BEHAVIOR
Emilie Rissman, Jennifer Wolstenholme and Kayla Quinnies
Department of Biochemistry and Molecular Genetics, and Graduate program in Neuroscience, University of Virginia, Charlottesville VA 22908 USA

Anyone old enough to remember the movie "The Graduate" (1967) will recall the friendly career advice that recent graduate, Dustin Hoffman, received from his neighbor. The future he said is "plastics". In this presentation I will prove him correct. I will review the literature on the effects of various plastic components on behavioral endpoints, with an emphasis on humans and rodents. I will also present work from my laboratory where we have been systematically examining the effects of first generation and trans-generational exposure to Bisphenol A (BPA), a man made compound found in hard plastic. We have also started similar work with Di-(2-ethylhexyl) phthalate (DEHP), found in soft plastics. In our studies pregnant mice are exposed to human-relevant doses of the compound either mixed into their diet (BPA) or presented daily in a drop of corn oil (DEHP). These routes of administration mimic the main avenue of exposure for humans and intake is not stressful. At birth exposure is ended and pups are moved to and reared by foster dams to control for actions of theses compounds on maternal behavior. Tests for ultrasonic vocalizations are conducted in pups; tests for social behaviors are conducted in juveniles (days 22-27). To date we have found several behavioral effects of both compounds in first generation exposure animals. Trans-generational actions of BPA are apparent as well. Gene transcription for several candidate genes has been tracked over the generations and I will also present our data and ideas on models for epigenetic mechanisms for these effects. In sum both compounds have long term actions on behavior and gene expression in brain. This work was supported by R01 MH086711 (ER) and F32 ES019404 (JW).

Saturday, May 25th
09:00 – 09:50

PL-7
SEASONAL VARIATION IN THE EFFECTS OF NUTRITIONAL PLANE ON AGE OF PUBERTY AND THE PITUITARY-GONAD AXIS IN SALMON

Swanson, P(1)(2), Luckenbach, JA(1)(2), Yamamoto, Y(1)(3), Dickey, JT (4), Middleton, M (4), and Young G (2)(4). (1) Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA 98112, USA (2) Center for Reproductive Biology, Washington State University, Pullman, WA 98164, USA (3) Department of Marine Biosciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan (4) School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195, USA

Age of puberty is a key component of the species-specific life history in fish, and in some species is highly plastic. But, the underlying mechanisms involved are not understood. Development of reproductive competence relies on the integration of a wide variety of internal and external cues. These signals provide critical information on when an animal should reproduce: whether it is of sufficient size or energy status to reproduce (metabolic cues), whether conditions are optimal for reproductive success (environmental cues), and whether an appropriate mate is present (social cues). Although the mechanisms involved in integrating this information are not clear, the onset and completion of puberty involves increases in gonadotropin-releasing hormone (GnRH) signaling and subsequent effects on pituitary gonadotropins (GTHs) and gonadal physiology. In Pacific salmon, we found that body size/growth rate during critical seasonal periods influences age of puberty onset in both sexes. Further, the rate of previtellogenic oocyte growth is directly related to the rate of body growth. Reductions in body growth during the fall-early winter, when ovarian follicles normally make the transition from the early to late cortical alveolus stage, delayed age of puberty, while in the spring (after vitellogenesis was initiated) fecundity was reduced but not the number of females maturing. Severe nutritional stress (fasting) during previtellogenic stages, reduced pituitary FSH and plasma IGF 1 and E2 levels, retarded follicle development and induced atresia. Fasting also altered ovarian mRNAs for genes associated with FSH signaling, steroidogenesis, apoptosis, autophagy, and follicle development. These results indicate that the effects of environmental factors that alter growth/energy status in salmon depend on reproductive stage and/or season during which growth is altered. IGF1 is one of several factors that may mediate the effects of growth on reproduction in salmonids. Funding from Washington Sea Grant (RB49), NOAA Fisheries, and the Bonneville Power Administration Project 1993-056 are gratefully acknowledged.

Wednesday, May 22th
11:45-12:35

NASCE 2013, Gorbman-Bern Memorial Award Lecture

GBMAL
ANALYZING THE INTERACTION BETWEEN THE MELANOCORTIN-2 RECEPTOR AND THE ACCESSORY PROTEIN MRAP: AN EXAMPLE OF CONSTRUCTIVE NEUTRAL EVOLUTION.
Robert M. Dores, Liang Liang, Perry Davis, and Joseph K. Angleson
University of Denver, Department of Biological Sciences, Denver, Colorado, U.S.A.

The melanocortin receptors are a gene family in the rhodopsin class of G-Protein Coupled receptors. Based on the analysis of several metazoan genome databases it appears that the melanocortin receptors are only found in chordates. The presence of five genes in the family (i.e., mc1r, mc2r, mc3r, mc4r, mc5r) in representatives of the tetrapods indicates that the gene family has undergone two genome duplication events and one local gene duplication event during the evolution of the chordates. The melanocortin receptors are activated by melanocortin ligands (i.e., ACTH, α-MSH, β-MSH, γ-MSH, δ-MSH) which are all derived from the polypeptide hormone/neuropeptide precursor, POMC, and as a result the functional evolution of the melanocortin receptors is intimately associated with the co-evolution of POMC endocrine and neuronal circuits. This presentation will consider the origin of the melanocortin receptors, and discuss the evolutionary relationship between MC2R, MC5R, and MC4R, and the functional co-evolution of the mc2r gene and the mrap (melanocortin-2 receptor accessory protein) gene family. The MC2R/MRAP interaction appears to be an example of constructive neutral evolution.

Thursday, May 23th
11:45-12:35

NASCE 2013, Gorbman-Bern New Independent Investigator Award Lecture

GBNIIAL
THE YIN AND THE YANG OF ANDROGEN RECEPTOR SIGNALING IN TELEOST FISH
Christopher J Martyniuk (1), Sophie Alvarez (2), Anna Ornostay (1)1, Nancy D. Denslow (3), Vicki L. Marlatt (4)
(1) Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, New Brunswick, E2L 4L5, Canada. (2) Donald Danforth Plant Science Center, St Louis, MO, USA (3) Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, 32611 USA(4) Nautilus Environmental, Burnaby, BC, Canada. Current: Department of Biology, University of the Fraser Valley, 33844 King Road, Abbotsford, BC Canada V2S 7M8

Sex steroid hormones (i.e. androgens and estrogens) regulate multiple physiological processes in vertebrates such as growth, development, and reproduction. Androgen receptors (AR) act as transcription factors and regulate gene expression in multiple tissues. In fish, AR signaling plays a role in both males and females; however, less is known about the role of AR pathways in the reproductive axis of females. Recently, omics approaches have provided new insight into molecular pathways regulated by ARs. In the teleost liver, quantitative proteomics studies have shown that the androgens 17β-trenbolone and dihydrotestosterone (DHT) affect a wide array of protein targets, including ribosomal proteins and proteins involved in apoptosis and stress. In addition, proteins related to interferon and epidermal growth factor signaling are altered in response to androgen treatments and these responses are associated with depressed plasma vitellogenin, the egg yolk precursor protein. In the ovary, the progression of oocyte maturation is associated with increased plasma androgens and transcriptomics changes within the ovary. Studies also suggest that the molecular and physiological responses to androgens are not easily elucidated or predicted; for example incubations of ovary with the potent androgen DHT results in increased estrogen production, suggesting that DHT may be converted into estrogenic metabolites. The enzymes responsible for the conversion of T into DHT are the reductases and these isoforms also show tissue and sex-specific differences in expression in both male and female fish gonad and liver. Molecular approaches have improved our understanding of how sex steroids regulate cell pathways and there are common molecular themes associated with androgens (regulation of the immune system, lipid metabolism, and muscular development); however they also highlight the complexity of the AR signaling pathways involved in transducing signals into physiological responses. Acknowledgements: We thank numerous collaborators, both international and national, that have worked on various aspects of this research.

Wednesday, May 22th
9:50-11:40
CAC

NASCE 2013 President Symposium:
Sexual Development in Vertebrates

Chairpersons:
Vance Trudeau, Univ of Ottawa, CAN
Gustavo Somoza, IIB-Intech (CONICET-UNSAM), ARG

PS-1
EXOGENOUS ESTROGEN PREVENTS GROWTH OF SOX9+ MEDULLARY CORDS AND FAILS TO MIMIC THE PATTERN OF OVARIAN FORMATION INDUCED BY TEMPERATURE IN THE SEA TURTLE Lepidochelys olivacea
Díaz-Hernández, V (1), Marmolejo-Valencia, A (2), Merchant-Larios, H (2).
(1) Departamento de Embriología, Facultad de Medicina. Universidad Nacional Autónoma de México. Mexico City. (2) Departamento de Biología Celular y Fisiología, Instituto de Investigaciones Biomédicas. Universidad Nacional Autónoma de México. Mexico City.

Several reptiles have temperature sex determination (TSD). Embryos incubated at female-producing temperature (FPT) or male-producing temperature (MPT) treated with estrogens or estrogen-inhibitors, respectively, counteract the effect of temperature and exhibit partial or total gonadal sex reversal. There is, however, considerable difference to exogenous treatments among species. It is unclear if estrogen levels act by preventing testicular formation, inducing ovarian development, or both. Undifferentiated bipotential gonads of *L. olivacea*, express SOX9 in medullary cords at both, MPT and FPT. Thereafter, SOX9 remains expressed at MPT whereas is down-regulated at FPT. Thus, SOX9 is a reliable indicator of gonadal differentiation in species with TSD. In the current study, embryos of *L. olivacea* were treated with 17-β estradiol (E2) or the non-steroid inhibitor aromatase fadrozole (AI), and their effects on SOX9 expressing cells were studied. Estrogen receptor alpha (ERα) and aromatase were measured by final point PCR. Samples were analyzed at early (stage 26) and later (stage 28 and hatchlings) differentiated gonads. Cytokeratin expression was used as a marker of $SOX9^{+}$ and SOX^{-} epithelial cells in medullary cords and surface epithelium respectively. Current results showed that expression of aromatase is higher at FPT than at MPT, while the ERα is similarly expressed in gonads at both temperatures. Although gonads in hatchlings have an ovarian-like cortex, cells of hypoplastic medullary cords maintain S0X9 expression in contrast to normal ovaries developed at FPT. In contrast, aromatase inhibition fails to counteract the effect of FPT maintaining SOX9 off in medullary cords and form normal-looking ovaries. Conclusion: Exogenous estrogens provoke male to female sex reversal at MPT by reducing the number of SOX9+ cells in medullary cords. This context allows cortex development and a delayed ovary form after hatching. (The authors would like to thank to Martha Harfush, Manuel Rodríguez Gómez, Cuauhtemoc Peñaflores, Erika Peralta, Teresa Luna and Ancelmo Cervantes of the Centro Mexicano de la Tortuga for assistance in collecting nests with permission from SEMARNAT 09109/11. This work was supported by Research Grant PAPIIT-UNAM IN205213 and CONACYT 166012).

PS-2
DIVERSITY OF SPERMATOGONIAL STEM CELL NICHE AMONG TELEOSTS AND THE REGULATION OF SPERMATOGONIAL FATE USING ZEBRAFISH AS MODEL
Nóbrega RH (1,2), Daltro RDVS (2), Male R (3), França LR (4), Bogerd J (2), Schulz RW (2).
(1) Department of Morphology, São Paulo State University – Botucatu, São Paulo, Brazil; (2) Developmental Biology Division, Utrecht University – Utrecht, the Netherlands; (3) Department of Molecular Biology, University of Bergen – Bergen, Norway; (4) Department of Morphology, Federal University of Minas Gerais – Belo Horizonte, Minas Gerais, Brazil.

Fish exhibit a great diversity in testis structure ranging from lobules to anastomosing tubules, which can be subdivided into restricted (e.g, medaka) or unrestricted (e.g., tilapia and zebrafish) according to the distribution of type A spermatogonia in the germinal epithelium. In relation to these testicular types, the spermatogonial stem cell (SSC) niche also differs among teleosts. In medaka and tilapia, SSCs are located exclusively (medaka) or preferentially (tilapia) in close proximity to the testicular capsule. In zebrafish, SSCS are located near to the interstitial area, close to Leydig cells and blood vessels. Therefore, different signals may play a role in the regulation of the SSC fate in different fish species. Using a BrdU pulse-chase, we have found two populations of label (BrdU) retaining cells; one "active" with rapid BrdU dilution among the progenitors; and one "reserve or quiescent" where BrdU was retained for a long time. The "*stemness*" of undifferentiated type A spermatogonia was evaluated also by germ cell transplantation techniques; however, our approach could not differentiate between "active" and "reserve" type stem cells. Donor-derived spermatogenesis was seen in the recipient testes after 2 weeks of transplantation, demonstrating the "*stemness*" potential of the transplanted cells. Activating SSC proliferation towards differentiation by elevated temperature or exposure to a cytostatic drug, resulted in an increase of androgen release, down-regulation of *amh* (anti-müllerian hormone) and up-regulation of *igf3* (*igf1b*) (insulin-like growth factor 3 or 1b) gene expression. These two growth factors may be involved in the regulation "*should I stay or should I go*" of the SSC; suppressing *amh* and stimulating *igf3* may allow spermatogonia "to go" towards meiosis.
(Funding Support: Utrecht University, European Union Project LIFECYCLE, Norwegian Research Council, FAPESP and CNPq).

PS-3
REGULATION OF ANDROGEN BIOSYNTHESIS BY INTERNAL AND ANTHROPOGENIC COMPOUNDS IN FROGS
Valérie S. Langlois (1)*, Diana E. K. Flood (1), Juan I. Fernandino (2), Justine Mathieu-Denoncourt (1) and Sonja Bissegger (1)
(1) Department of Chemistry and Chemical Engineering, Royal Military College of Canada, P.O. Box 17 000, Station Forces, Kingston, ON, K7K 7B4, CANADA
(2) Laboratorio de Biología del Desarrollo, Instituto de Investigaciones Biotecnológicas, Instituto Tecnológico de Chascomús (IIB-INTECH), Dirección: Int. Marino Km. 8.200, Chascomús CP: B7130IWA, Pcia. Buenos Aires. Argentina.

Over the past years, our research group have been investigating the regulation, function and disruption of testosterone reduction pathways in amphibians. We are particularly interested in the steroid 5α-reductases (srd5α) involved in converting testosterone to the potent androgen 5α-dihydrotestosterone (5α-DHT). Failure of these enzymes to synthesize 5α-DHT has resulted in adverse reproductive effects (e.g., steroid-related transcriptional disruption and gonadal feminization) at metamorphosis. Furthermore, changes in thyroid hormone (THs) levels have also shown to alter transcription of *srd5α.* Novel
promoter analysis demonstrated the potential for a direct and vertebrate-wide crosstalk at the transcriptional level in mouse (*Mus musculus*), Western clawed frog (*Silurana tropicalis*), and medaka (*Oryzias latipes*) between TH and androgen axes. A comprehensive literature review revealed that THs would also have considerable influence in the sexual ontogeny of male vertebrates, through direct interactions with select sex-determining-genes and regulation of gonadotropin

production in the hypothalamus-pituitary-gonad axis. Cumulative evidence from previous studies, coupled with novel promoter analysis suggests mechanisms for a more direct crosstalk between TH and male reproductive axis across vertebrate species. This presentation will highlight the importance of srd5α for normal male amphibian development and will focus on the putative regulation of srd5α by THs. (The authors would like to thank to Martin Somoza for helping with promoter analysis. This work was supported NSERC Discovery grant to VSL, Consejo Nacional de Investigaciones Científicas y Técnicas grant D731 and Agencia Nacional de Promoción Científica y Tecnológica grant 2010 No 1980 to JIF, and R. Samuel McLaughlin Fellowship to DEKF).

P-OR-1

CROSSTALK BETWEEN THE THYROID HORMONE AND ANDROGEN AXES DURING REPRODUCTIVE DEVELOPMENT IN MALE SILURANA TROPICALIS

Diana E. K. Flood(1,2) and Valérie S. Langlois(1,2)
(1)Department of Chemistry and Chemical Engineering, Royal Military College of Canada, ON, CA (2)Biology Department, Queen's University, Kingston, ON, CA

Thyroid hormones (THs) have been shown to regulate many developmental processes, including reproductive development. Studies have identified cross-regulation between the TH and androgen systems in numerous vertebrate species, and have demonstrated that males with hypothyroidism exhibited both testis and sperm dysfunctions. It is therefore necessary to clarify the molecular controls behind this hormonal interaction. We examined TH-related and androgen-related mRNA levels in *Silurana tropicalis* (Western clawed frog) larvae chronically exposed to low levels of the goitrogenic chemical potassium perchlorate ($KClO_4$; 0, 25, 50 and 100 ppb) for 3months. Real-time RT-PCR analysis was performed on livers and gonad mesonephros complexes (GMCs); both important tissues for androgen metabolism and synthesis. $KClO_4$ exposure resulted in significant decreases in TH-related gene expression in hepatic tissues, whereas androgen-related mRNA levels increased. In addition, we observed significant transcriptional differences between sexes in GMCs ($p < 0.05$). In order to confirm that the observed gene responses were the result of impeded TH signaling, we exposed larvae to triiodothyronine (T3; 50 nM; 48 h). T3 exposure induced an increase in androgen- and TH-related mRNAs in liver and GMC tissue. These results (i) support the hypothesis that THs are involved in male reproductive development in frogs, (ii) reveal potential molecular mechanisms behind androgen and TH crosstalk; and (iii) highlight a potential regulatory role for the liver in androgen metabolism.

P-OR-2

VARIATION IN ESTROGEN SENSITIVITY ACROSS POPULATIONS OF AFRICAN CLAWED FROGS

Xuan G. Luong (1), Tyrone B. Hayes (1)
Department of Integrative Biology, University of California, Berkeley, CA, USA

Although African clawed frogs (Xenopus laevis) display genetic sex determination, exogenous estradiol (E2) can induce ovarian differentiation in genetic males during larval development. Furthermore, populations can differ 10,000 fold in the minimum E2 concentration required to induce ovaries in genetic males. We examined the response of livers to E2 in juveniles to investigate mechanisms that underlie variation in E2 sensitivity. The livers of juvenile X. laevis contain E2 receptors (ERα1) and respond to E2 with robust increases in vitellogenin (VTGα2) production. Because of this direct interaction and the abundance of tissue available, the liver was an effective model to test E2 sensitivity. Liver E2 sensitivity was defined by the quantity of VTGα2 transcripts resulting from E2 exposure—the higher the expression, the greater the sensitivity. Male frogs from sensitive and insensitive populations were treated with 0, 0.03, 0.3, 3.0, or 30.0 ng/ml E2 for 12 hours. Liver mRNA was extracted and quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure VTGα2 and ERα1 expression. In both populations, VTGα2 and ERα1 expression increased in a concentration-dependent manner from 0.03 to 3.0 ng/ml, but expression declined at the highest concentration, suggesting a non-monotonic concentration response. Males from the sensitive population showed greater increases in VTGα2 expression in response to E2 than their insensitive counterparts. However, there was no correlation between ERα1 and VTGα2 expression. In summary, population differences in E2 sensitivity are observed both in *X. laevis* gonads and livers, but *ERα1* expression may not be the basis of these differences in the liver. (Supported by the National Science Foundation (Grant No. 1209511), National Institute of General Medical Sciences of the National Institutes of Health, the University of California's Chancellor's Fellowship, and the Rose Hill Foundation).

Wednesday, May 22th
14:15 -16:00
CAC

NASCE 2013 Symposium I:
Multiple Actions of Thyroid Hormones

Co-chairs:
Veerle Darras, Katholic University of Leuven, BEL
Aurea Orozco, INB-UNAM, MEX

S1-1
ROLE AND ACTION MECHANISMS OF 3,5-DIIODOTHYRONINE IN TELEOSTS
Aurea Orozco, Arturo Mendoza C, Pamela Navarrete-Ramírez, Gabriela Hernández-Puga, Patricia Villalobos and Carlos Valverde-R.
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología,
Universidad Nacional Autónoma de México (UNAM). Querétaro, México.

Several liganded nuclear receptors have alternative ligands acting in a tissue-specific fashion and playing important biological roles. This is the case of 3,5-T2 (T2), a naturally occurring metabolite of T3 outer-ring deiodination, which is an alternative ligand for TRβ1, at least in teleosts. In this context, binding and transactivation studies showed that T2 is as bioactive as T3 and its effects are mediated by a TRβ1 isoform that contains a 9-amino-acid insert in its ligand-binding domain (long TRβ1), whereas T3 binds to and activates preferentially the short TRβ1 isoform that lacks this insert. Structure-function studies revealed that other regions of the receptor aside from the 9-amino acid insert are implicated in TRβ1 activation by T2. Specifically, the N-terminus is essential for T2-mediated transactivation, but not for that by T3 in the long TRβ1, suggesting a functional interaction between the N-terminus and the insertion in the ligand-binding domain. To establish the functional relevance of T2-mediated TRβ1 binding and activation, mRNA expression and its regulation by T2 and T3 was evaluated for both isoforms *in vivo*. Acute (1-5 days) T3 or T2 treatment differentially regulated the expression of the two TRβ1 isoforms. Furthermore, long-term (1 month) T2 and T3 treatment induced a four-fold increment in tilapia body growth and up-regulated the expression of hepatic GH and IGF-1, while expression of the long and short TRβ1 isoforms increased only in the T2- and T3-treated fish, respectively. Taken together, our results prompted a reevaluation of the role and mechanism of action of thyroid hormone metabolites previously believed to be inactive. Specifically, we propose that T2 acts as an alternative ligand playing a role in the tissue-specific action of receptors.

S1-2

THE SURPRISING ROLE OF THYROID HORMONES IN SEXUAL DEVELOPMENT
Paula Duarte-Guterman(1)(2), Michael J. Ryan(3), Natacha S. Hogan(4), Vance L. Trudeau(1)
(1) Department of Biology, University of Ottawa, Ottawa, Canada (2) Department of Psychology, University of British Columbia, Vancouver, Canada
(3) Section of Integrative Biology, University of Texas, Austin, USA (4) Department of Animal and Poultry Science, University of Saskatoon, Saskatoon, Canada.

In vertebrates, sex steroids (estrogens and androgens) regulate gonadal differentiation and function, while thyroid hormones (THs) regulate metabolism and, in amphibians and some fish, metamorphosis. There is emerging evidence that THs also regulate aspects of the reproductive axis, suggesting that these two endocrine axes interact: disruption of thyroid condition (e.g., inhibiting TH synthesis, exposure to exogenous THs) alters gonadal differentiation and function in different groups of vertebrates. Previous research suggests THs may be involved in male development but the mechanisms that account for these interactions are not understood, let alone how such mechanisms may differ between species. To develop and test new hypotheses on the roles of THs, we chose three species of frogs during tadpole development, a time when the two endocrine axes are active: THs for metamorphosis and sex steroids for gonadal differentiation. We examined transcriptional profiles (mRNA) of enzymes and receptors related to sex steroids and THs during metamorphosis in the brain and gonad and how these targets may be modulated by an acute exposure to TH in the different species. During metamorphosis, most of the transcripts were detected in the brain and gonad, and for each gene, the profile was similar between the three species. In contrast, exposure to TH during development resulted in different patterns of mRNA levels in each species: in one species, androgen-related genes increased, while in the other two species, an estrogen-related gene decreased. Our work in frogs indicates that THs modulate the expression of sex steroid-related genes and play a role in sexual development. The results will be discussed in the context of a potential mechanism for the known masculinising effects of TH in frogs and other vertebrates. Finally, we explore the evolution of the apparently complex molecular mechanisms linking the TH and reproductive endocrine axes. Supported by NSERC-PGS (to PDG), NSERC-DG (to VLT), and NSF IBN 0078150 (to MJR).

S1-3
THE ROLE OF THYROID HORMONES IN NEURONAL DEVELOPMENT AND REGENERATION
Van Herck, SL, Bhumika, S, Delbaere, J, Moons, L, Darras, VM
Department of Biology, Section Animal Physiology and Neurobiology, KU Leuven, Leuven, Belgium

Thyroid hormones (THs) are essential for the developing vertebrate central nervous system (CNS) by regulating cell proliferation, migration and differentiation. To obtain a fully functional brain the different neuronal cell types have to go through these processes in a precise sequence. Therefore the level of active TH needs to be regulated in a cell-specific way by the combined action of TH transporters, deiodinases and receptors. Mutations in one of them can lead to severe neurological defects, as observed in patients with the Allan-Herndon-Dudley syndrome due to deficiency in the TH transporter MCT8. Using the chicken embryo as a model we showed that each of the TH transporters, deiodinases and receptors is expressed in a unique spatiotemporal pattern ensuring that TH-dependent gene transcription occurs at the right time in the maturation sequence. We have developed miRNA-based silencing constructs and use them to knock down TH transporters or deiodinases and elucidate the contribution of each of these regulators in the formation of a mature brain. THs also play a role following neuronal damage where they were shown to stimulate regeneration of peripheral nerves. However, their impact on regeneration in the CNS is still unclear. Since lower vertebrates have a relatively high capacity for CNS regeneration, we here use adult zebrafish as a model. We study the effect of THs on regrowth of retinal ganglion cell axons following optic nerve crush (ONC), in the presence or absence of iopanoic acid (IOP) that blocks peripheral TH activation or the TH receptor blocker methylsulfonylnitrobenzoate. At 4 and 7 days post injury expression of retinal regeneration markers is similar in all groups. However, anterograde tracing at 7 days post injury shows that antithyroid treatment results in a more intense axon regrowth at the level of the optic tectum. Therefore the impact of THs on the different

steps in the regeneration of retinal ganglion cells seems to be different.(This work was supported by grants from the KU Leuven (OT/07/036 and OT/11/041) and the Fund for Scientific Research-Flanders (G.0455.08 and G.0307.12).

OR1-1

DEIODINASE SELECTIVITY FOR ORD OR IRD. INVOLVEMENT OF THE VARIABLE REGION (TM, H AND L DOMAINS)

Olvera A, Orozco A, Villalobos P, Valverde-R C.
Departamento de Neurobiología Celular y Molecular. Instituto de Neurobiología. UNAM Campus Juriquilla, Querétaro, México.

By sequentially and stereo-specifically removing iodine atoms from thyroid hormones (TH), the three distinct isotypes of iodothyronine deiodinases (Ds): D1, D2 and D3, are crucial in regulating the bioavailability of (TH). Thus, T_4 outer-ring deiodination (ORD) generates bioactive products, while its inner-ring deiodination (IRD) produces inactive metabolites. While D2 and D3 exclusively catalyze the ORD and IRD pathways, respectively, D1 catalyzes both; however, the region of the protein that confers this catalytic selectivity is to date unknown. *In silico* studies suggest that the molecular arrangement of the three paralogous includes four functional domains: TM (transmembranal), H (hinge), L (linker), and G (globular). Previously we reported that when the protein sequences of Ds (n=67) are aligned, the G domain that includes the catalytic region, is very similar between the 3 paralogous (60% identity), while the TM, H, and L domains stand as the most variable (20%), but interestingly as relatively conserved domains among orthologous (D1 50%; D2 55% and D3 60%). This information allowed the division of Ds sequences into 2 distinct major regions: "variable region" (VR) and "conserved region" (CR), and led us to propose that the selectivity of Ds depends, to some extent, on the VR arrangement. To test this hypothesis we constructed a chimera combining the VR and CR from shark D3 and D2, respectively. When characterized and despite containing the catalytic site of D2 (CR), the chimera was devoid of ORD activity and only exhibited IRD activity. These results show that due to its key role in removing iodine atoms, CR has been under positive selective pressure thus being highly conserved among chordates. On the contrary, the variable arrangement of RV suggests a more relaxed selective pressure with the consequent gain of change and physiologic novelty, thus conferring the ring selectivity for iodine removal. (**S**upported by: CONACYT 166357 and PAPIIT IN208511).

OR1-2

MATERNAL SEPARATION PERSISTENTLY ALTERS THE EXPRESSION OF KEY ELEMENTS OF THE HPT AXIS AND ITS RESPONSE TO COLD EXPOSURE IN ADULT MALE RATS

Carmen Espinoza-Ayala(1), Jaimes-Hoy Lorraine(1), Charli Jean-Louis(1), Joseph-Bravo Patricia(1).
(1)Department of Physiology and Genetics, Instituto de Biotecnología de la Universidad Nacional Autónoma de México (UNAM), Cuernavaca, Mor., Méx.

Maternal care in rodents is associated with long-term programming of individual differences in behavioral and hypothalamic-pituitary-adrenal responses to stress in the offspring by altering expression levels of key genes through epigenetic marking. As the activity of the HPT axis is susceptible to various forms of stress and is blunted by increased levels of glucocorticoids, we hypothesized that postnatal maternal separation (MS) alters the HPT axis function and its response to a metabolic cue in the adult rat. We studied the effect of MS in adult rats exposed to cold, which stimulates the activity of the HPT axis. Wistar dams were divided into naïve (N) and a maternal separation (MS) group; pups from the latter were separated 3h/day from their mothers from PD 2-21 and fed ad libitum from weaning until adulthood. Male rats were exposed to 4°C for 0.5-4h. Coronal brain sections were cut through the rostrocaudal extent of the paraventricular hypothalamic nucleus (PVN) and mediobasal hypothalamus (MBH) to study levels of levels of proTRH mRNA by quantitative *in situ* hybridization (ISH). TSH and thyroid hormones were analyzed in serum by radioinmmunoanalysis. We show that the expression of proTRH mRNA was higher in the PVN of MS that in N female rats, but this difference was not significant in males. The response to cold exposure was as expected in naïve animals: increased proTRH expression in the PVN, and in serum concentration of TSH and T4. In contrast, cold-exposed MS rats showed no increase in circulating levels of TSH or T4, although the increase in PVN-proTRH mRNA levels prevailed. These results support a dissociation in the dynamics of the hypothalamic response with that of the pituitary or thyroid. In conclusion, maternal separation can alter the expression of genes that control TRH levels in the neuroendocrine axis, and may additionally interfere with peripheral aspects of thyroid hormones turnover. (Supported by CONACYT grant 180009; CEA, CONACYT sholarship; LJH, DGAPA posdoc fellow).

Wednesday, May 22th
14:15 -16:00
II (LIPATA)

NASCE 2013 Symposium II:
Comparative Endocrinology and Application to the Management and Propagation of Captive/Endangered Species

Co-chairs:
Pierre Comizzoli, Smithsonian Conservation Biology Institute, USA
Matthew R. Milnes, University of California, USA

S2-1
MINIMALLY INVASIVE HORMONE MONITORING IN ENDANGERED SPECIES
David C. Kersey
College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA USA

Monitoring hormonal activity without perturbation of study subjects is particularly attractive to studying the physiology, behavior, and general biology of endangered species due to restrictions of access to and handling of animals. In the broadest sense, endocrine function is under influence and production of hormones that are primarily transported in the blood to target tissues to influence cellular function. To control bioactivity and facilitate excretion, hormones undergo conformational changes at peripheral tissues and the excretory organs (e.g. liver, kidneys) that include degradation, metabolization and conjugation. Further biochemical changes occur to hormones between organ elimination and final voidance. As a result, the hormones found in the excreta are a complex of derivates from the parent hormone, but still provide a retrospective proportional relationship to circulating hormone concentrations. In addition to voided hormones, endocrine function can be assessed with minimal invasion via traditional routes (e.g. blood) with animal cooperation, or via novel techniques such as hair and saliva collection. Although study and species-specific circumstances will dictate the type of hormone to be assessed and the sample substrate by which it will be measured, all evaluations must consider the approach to quantifying the hormone. Included in this approach is handling of the sample to limit non-physiological hormone degradation, and liberation of the hormone from the sample matrix so that it may be measured. Measurement running the gamut of qualitative snap tests to highly specific mass spectrometry analyses, all of which require dutiful validation. The final product will yield an objective measure of biological changes that provides new knowledge of the species without interfering with or influencing their biology.

S2-2
APPLICATION OF ENDOCRINOLOGY TO ASSISTED REPRODUCTION AND/OR CAPTIVE BREEDING
Martin Dehnhard and Katarina Jewgenow
Leibniz Institute for Zoo and Wildlife Research, Department of Reproductive Biology, Alfred-Kowalke-Str. 17, 10315 Berlin, Germany

Because of extinction risks there is a growing need for zoos to sustain genetically healthy populations. Many species reproduce poorly. Therefore, efficient methods to analyze ovarian function and pregnancy are demanded. Measurement of blood hormones are widely used to monitor endocrine functions. In wildlife species, however, difficulties associated with blood collection and potential negative impact of stressful procedures on behaviour and physiology hinder this approach or even make it impossible. During the last decades, techniques for urinary and fecal steroid analysis were developed and used for reproduction and health monitoring in mammalian and bird species. Fecal and urinary gestagens are useful to monitor cyclic ovarian and pregnancies. For instance in elephants, gestagen analyses are used to predict parturition. In felid species, measurements of gestagen (P4) metabolites are insufficient to monitor cyclicity. Then, fecal estrogens are helpful to detect estrus. Although pregnancy diagnosis using fecal gestagens became a routine procedure, a differentiation from pseudo-pregnancies is only possible by prolonged sampling. Gestagen elevations during pseudo-pregnancies are shorter than in pregnant cycles. In lynx species, however, fecal P4 metabolites completely failed to demonstrate luteal activities and hence pregnancies. As an alternative, the prostaglandinF2a metabolite (PGFM) allowed a differentiation between pregnant and pseudo-pregnant stages. In pregnant lynxes, a constant PGFM increase over the last trimester of gestation with peak concentrations around parturition was shown in contrast to baseline profiles during pseudo-pregnancy. We described increased PGFM levels during pregnancies in all felid species analyzed (N = 20). To support pregnancy diagnosis in captive settings, we developed a quick PGFM test (less than 4 h). Despite this, the availability of analytical tools to confirm pregnancy at earlier stages might improve breeding of felids.

S2-3
POTENTIAL ROLE OF DIETARY PHYTOESTROGENS IN THE REPRODUCTIVE FAILURE OF CAPTIVE SOUTHERN WHITE RHINOCEROS
Christopher Tubbs1 and Matthew Milnes2
(1) San Diego Zoo Institute for Conservation Research, Escondido, CA USA 92027 (2) Department of Natural Sciences, Mars Hill College, Mars Hill, NC USA 28754

The captive southern white rhinoceros (SWR) population serves as an important genetic reservoir critical to the conservation of this vulnerable species. Unfortunately, this population has been declining for decades due to poor reproductive success of captive-born females. Captive female SWR exhibit reproductive abnormalities suggested to result of continual ovarian activity prolonged exposure to elevated levels of endogenous estrogen. Here we consider the potential for exogenous estrogenic substances in the form of dietary phytoestrogens to contribute to the reproductive failure of this species. We did this by characterizing *in vitro* phytoestrogen activation of recombinant SWR estrogen receptors (ESRs) and comparing it to phytoestrogen activation of greater one-horned rhinoceros (GOHR) ESRs; a species that receives nearly identical diets yet reproduces relatively well in captivity. Using this approach we were able to detect species-specific differences in ESR activation by phytoestrogens. Specifically, coumestrol stimulated greater maximal activation of SWR ESR1 than GOHR ESR1 and SWR ESR2s were significantly more sensitive to both coumestrol and daidzein compared to GOHR ESR2s. The concentrations at which significant differences in ESR activation occur are consistent with circulating concentrations measured in other vertebrate species. Current investigations now focus on comparing phytoestrogen content of diets consumed by SWR in captivity versus the wild, where the population is growing. In contrast to native diets, the majority of captive diet extracts tested are capable of activating SWR ESRs. Taken together, these findings suggest phytoestrogens potentially threaten the reproductive health of captive SWR. However, additional studies examining the bioavailability of phytoestrogens following consumption are needed to clarify the physiological role of dietary phytoestrogens in the reduced fertility of this species.

OR2-1

SEASONAL AND AGE-DEPENDENT DIFFERENCES IN TESTOSTERONE LEVELS IN WILD MEXICAN COTTONTAILS *Sylvilagus cunicularius*

Aguilar F (1), Rödel HG(2), Nicolas L(1), Rodriguez L(1), Bautista A(1), Martínez-Gómez M(1)(3)
(1) Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, México. (2) Université Paris 13, Sorbonne Paris Cité, Laboratoire d'Ethologie Expérimentale et Comparée E.A. 4443 (LEEC), F-93430 Villetaneuse, France. (3)Departamento de Biología Celular y Fisiología. Instituto de Investigaciones Biomédicas. UNAM, México.

We studied serum testosterone concentrations in the endemic Mexican cottontail, *Sylvilagus cunicularius*, which have been reported to show seasonal breeding. Animals were trapped in the wild and in a field enclosure in the National Park *La Malinche* in central Mexico over a period of five years. Testosterone (T) serum concentrations were quantified by ELISA from blood samples. T levels of adult males were lowest around 4 months after the onset of the annual reproductive season and were already high prior to the onset of breeding. In adult females there were no differences in T concentration with respect to female reproductive state (pregnant, lactating or non reproductive), and as expected, the T levels of adult females were consistently lower than in males. In juveniles and subadults, there were no detectable sex-specific differences in T levels, and there was a marked increase in T levels between young and adult males but not in females. Overall, our study clearly reflects and confirms the seasonal breeding strategy of this species, showing high similarities to the much better studied European rabbit. (To the Research Station "La Malinche" and posgraduates programs in Biological Sciences from UAT and UNAM).

OR2-2

EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE EXPRESSION OF SEVERAL SOMATOTROPIC FACTORS (GHRH, SS, PACAP, TRH, GH, IGF-I), IN THE GREEN IGUANA.

Ávila-Mendoza, J(1), Carranza, M(1), Luna Muñoz, M(1), Arámburo, C(1).
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México.

The somatotropic axis regulates growth and development of vertebrates and has critical effects on their metabolism. In ectotherms, environmental temperature is a key metabolic regulating factor, however little is known about its effects on the somatotropic axis in reptiles, which depend on the external temperature to carry on their biological functions. The aim of this study was to investigate how the exposure of green iguanas to suboptimal temperatures (18 and 25°C) influenced the activity of the somatotropic axis, both under acute (A, 48 h) or chronic (Ch, 2 weeks) treatments, in comparison to the control (C, 35°C). Results: GH serum levels increased, under A, only at 18°C (86.24 ng/ml), but not at 25°C (44.2 ng/ml), in comparison to C (46.1 ng/ml). In contrast, GH levels increased to 117.8 and 95 ng/ml, at 25°C and 18°C, respectively, under Ch. These alterations were consistent with changes in the expression of pituitary GH mRNA, which increased 6.6-fold at 18°C under A, and 14-fold at 25°C under Ch, respectively. In addition, changes in the expression of GH-regulating hypothalamic factors were observed. Thus, under A, at 25°C, GHRH mRNA decreased 1.3-fold, while PACAP, TRH and SS mRNAs increased 1.4-, 1.8-, and 1.9-fold, respectively; whereas at 18°C, there was a decrease in GHRH and PACAP mRNA expression (1.4- and 2.7-fold, respectively), but a 2-fold increase in TRH mRNA; compared to C. On the other hand, under Ch, at 25°C, the expression of PACAP and TRH mRNAs increased 1.4- and 1.5-fold, respectively, whilst that of SS was reduced 1.2 times; meanwhile at 18°C, SS mRNA augmented 1.8-fold whereas TRH mRNA decreased 1.6 times. In all treatments, however, the hepatic expression of IGF-1 mRNA was up-regulated between 1.1- and 21-fold. These results indicate that the reptilian somatotropic axis is strongly influenced by external temperature and that GH is involved in metabolic actions, either directly or mediated through IGF-1, for the survival of the green iguana.(Supported by PAPIIT-DGAPA-UNAM IN206813; CONACYT 178335. JAM received a PhD scholarship from CONACYT (267642) and support from the graduate program in Biochemical Sciences, UNAM).

Thursday, May 23th
9:45 -11:35
CAC

NASCE 2013 Symposium III:
Blood Vessels as Relevant Hormonal Targets

Co-chairs:
Carmen Clappp, INB-UNAM, MEX
Jesús Torres-Vázquez, New York University, USA

S3-1
THE VALUE OF THE ZEBRAFISH FOR UNDERSTANDING THE VERTEBRATE VASCULATURE
Torres-Vázquez, J (1).
(1) Department of Cell Biology, Helen L. and Martin S. Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine. New York University Langone Medical Center. New York, NY USA.

In this presentation I will highlight the powerful advantages of the transparent zebrafish embryo for studying the role of genetic and physiological factors of the formation of the vasculature. I will also highlight recent examples of how the zebrafish is being used to elucidate the vascular roles of hormones.(I want to thank all members of my lab, in particular Dr. Florian Ulrich)

S3-2
HORMONAL REGULATION OF BLOOD VESSELS: THE PROLACTIN-VASOINHIBIN CONNECTION
Carmen Clapp
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México

The hormone prolactin (PRL), fundamental for lactation in mammals, exerts a wide diversity of actions in the various vertebrate groups. Blood vessels are surfacing as important PRL targets, contributing to these hormonal functions. PRL promotes the growth of new blood vessels (angiogenesis) and is proteolytically cleaved to vasoinhibins, a family of antiangiogenic peptides with inhibitory effects on vasodilation, vasopermeability, and blood vessel survival. These opposing effects point to the regulation of the proteases responsible for PRL cleavage as an efficient way to balance blood vessel growth, function, and involution. Attention is turned to the roles of PRL and vasoinhibins in blood vessels of the ovary, retina, liver, and cartilage under physiological conditions and diseased states that are characterized by altered angiogenesis and vascular homeostasis, such as diabetic retinopathy, liver regeneration, and rheumatoid arthritis. (Studies supported by the National Council of Science and Technology of Mexico (CONACYT) grants S0008-161594 and 127496, and by UNAM grant PAPIIT200312-3).

S3-3
ESTROGENIC REGULATION OF UTERINE BLOOD VESSELS IN NONHUMAN PRIMATES
Albrecht, ED (1) and Pepe, GJ (2)
(1) Departments of Obstetrics, Gynecology, Reproductive Sciences and Physiology, Center for Studies in Reproduction, University of Maryland School of Medicine, Baltimore, MD USA and (2) Department of Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA USA

During early human and nonhuman primate pregnancy, placental extravillous cytotrophoblasts (EVT) migrate to, invade, and remodel the uterine spiral arteries changing them into low-resistance high-capacity vessels to promote blood flow to the placenta and development of the fetus. Using the baboon as a nonhuman primate model, we show that advancing the physiological surge in estrogen that occurs with normal gestation from the second to the first trimester, by maternal administration of estradiol daily on days 25-59 of gestation (term is 184 days), suppressed placental EVT expression of vascular endothelial growth factor (VEGF) and uterine spiral artery invasion/remodeling. The estrogen-induced suppression of uterine artery remodeling resulted in a marked decrease in uterine artery volume blood flow and fetal heart rate near term, indicative of hypoxia. Preliminary studies suggest that expression of vascular endothelial nitric oxide synthase (eNOS) in the umbilical cord near term was decreased in baboons in which uterine artery remodeling was suppressed by early estrogen treatment, presumably as a result of disruption of the programming of fetal vascular development. We propose that the proper level of uterine artery transformation and fetal vascular development/function are regulated by estrogen and that: (a) the low level of ovarian estrogen in the first trimester ensures a rapid rate of EVT spiral artery invasion; (b) the increase in placental estrogen thereafter has a physiologically important role in controlling the extent to which the uterine arteries are remodeled; and (c) VEGF mediates this estrogen-induced process. The link of estrogen to vessel remodeling is a significant conceptual advance which brings new perspective to the role which estrogen plays during primate pregnancy. (Supported by NIH R01 HD13294).

OR3-1
PROLACTIN STIMULATES LIVER REGENERATION BY PROMOTING THE PROLIFERATION OF HEPATOCYTES AND ENDOTHELIAL CELLS
Moreno-Carranza, B (1), Vega, C (1), López-Barrera, F (1), Nava, G (1), Quintanar-Stephano, A (2), Martínez de la Escalera, G (1), and Clapp, C
(1).Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México.

Liver growth after resection depends on the coordinated proliferation of hepatocytes and blood vessels. After partial hepatectomy (PH), the capillary blood vessels of the liver proliferate allowing nutrients and growth factors to reach the newly replicating hepatocytes. Prolactin is a potent liver mitogen and a proangiogenic factor in different organs. Here, we studied the effects of PRL on liver growth and angiogenesis after 70% PH. Male Wistar rats were implanted with two anterior pituitary glands (AP) under the kidney capsule to increase circulating PRL levels, and after fifteen days they were subjected to 70% PH. AP-implanted rats showed a 10-fold increase in serum PRL levels compared to the non-grafted controls. Injection of grafted rats with dopamine D2 receptor agonist CB-154, an inhibitor of anterior pituitary PRL release, blocked the increase of serum PRL. On day two after PH, hyperprolactinemia in AP-implanted rats correlated with a significant increase in the ratio of liver to body weight and this increase was prevented by CB-154. AP-implanted rats showed an increase in the number of proliferating hepatocytes and in the expression of the pro-angiogenic factor, vascular endothelial growth factor (VEGF) as compared to sham and 70% PH controls. Moreover, liver endothelial cells proliferated at a higher rate in the hyperprolactinemic rats vs. normoprolactinemic animals on day four after PH. In agreement with the supporting role of endogenous PRL in liver growth and regeneration, PRL receptor-deficient (PRLR-/-) mice showed smaller livers and reduced liver growth after

PH compared to PRLR+/+ counterparts. In conclusion, PRL promotes liver growth and regeneration, and these actions involve VEGF-induced angiogenesis. These findings support the potential of PRL as an effective addition to therapies improving hepatic function after liver resection and transplantation. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant 127496. We thank D. Mondragón, A. Prado, and M. García their technical assistance).

OR3-2

THE GENERATION AND BIOACTIVITY OF VASOINHIBINS ARE REDUCED IN THE VITREOUS OF PATIENTS WITH DIABETIC RETINOPATHY

Moreno-Vega, A (1), Triebel, J (1), Vázquez-Membrillo, M (2), Jeziorsky, MC (1), García-Franco, R (2), Macotela, Y (1), López-Star, E (2), Martínez de la Escalera, G (1), Clapp, C (1).
(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) Instituto Mexicano Oftalmología, Querétaro, México

Disruption of the quiescent state of blood vessels in the retina leads to aberrant vasopermeability and angiogenesis, the major causes of vision loss in diabetic retinopathy. Vasoinhibins derived from prolactin by proteolytic cleavage, are a family of antiangiogenic peptides that inhibit retinal vasopermeability and angiogenesis in experimental diabetic retinopathy. Given that vasoinhibins are found in the retina, we hypothesized that their down-regulation in diabetic retinopathy favors vascular alterations. Here, we investigated the presence and generation of vasoinhibins in the vitreous from non-diabetic patients and from patients with proliferative diabetic retinopathy (PDR). We detected vasoinhibins by Western blotting and demonstrated that the vitreous from non-diabetic patients (n=11), but not from patients with PDR (n=26), inhibits basic fibroblast growth factor-induced proliferation of endothelial cells in culture. Immunodepletion of vasoinhibins eliminated the anti-proliferative effect, indicating that vasoinhibins contribute substantially to the anti-angiogenic action of the vitreous from non-diabetic patients. Cathepsin D is known to generate vasoinhibins from prolactin, and we found that the incubation of prolactin with the vitreous from non-diabetic patients, but not from patients with PDR, cleaved PRL to vasoinhibins and that this cleavage was abolished by the cathepsin D inhibitor, pepstatin A. We conclude that vasoinhibins and cathepsin D are present in human vitreous and suggest that a reduction in the bioactivity and generation of these peptides favors the progression of diabetic retinopathy. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant S0008-161594. We thank F. López-Barrera, G. Nava, D. Mondragón, and A. Prado their technical assistance).

Thursday, May 23th
9:45 -11:35
INB

NASCE 2013 Symposium IV:
Hormones and Behavior

Co-chairs:
Kim Hoke, Colorado State University, USA
Paula Duarte Guterman, University of British Columbia, CAN

S4-1
SOCIAL AND HORMONAL INTERACTIONS IN THE MODULATION OF NEURAL SYSTEMS UNDERLYING REPRODUCTIVE BEHAVIOR
Wilczynski, W
Neuroscience Institute, Georgia State University, Atlanta, GA, USA

Social communication is an integral part of reproductive behavior in anuran amphibians, where males produce an advertisement call that both attracts females and serves as a male-male agonistic signal. In temperate zone anurans the behavior is seasonal, present in the summer and absent in the winter. This behavior pattern coincides with seasonal changes in gonadal steroids, which are high during the breeding season and decline in the winter, and the pineal hormone melatonin, which shows the opposite pattern. The influence of melatonin on two brain neuromodulator systems may in part regulate the seasonal pattern of male calling. Both the neuropeptide arginine vasotocin (AVT) and the catecholamine neurotransmitter dopamine stimulate calling. Implanting male green treefrogs (*Hyla cinerea*) with melatonin decreases the number of immunoreactive AVT cells in the nucleus accumbens and suprachiasmatic nucleus and decreases immunoreactive tyrosine hydroxylase cells (marking dopamine neurons) in the posterior tuberculum. Androgen facilitates male calling, and in several species has been shown to facilitate AVT action. Melatonin treatment does not affect androgen levels, and therefore is likely not the cause of the seasonal decline in circulating androgens. The seasonal androgen decline thus appears to independently reduce social communication just as the seasonal rise in melatonin may do the same via its negative effects on AVT and dopamine. The seasonal decrease in androgen levels could be slowed by engaging in social behavior, as hearing conspecific calls increases testosterone levels. Social interaction could thus potentially extend an individual's effective tenure in a breeding chorus by extending the period of high androgens. There is no evidence that social activity influences melatonin levels. If it does not, melatonin-mediated decreases in AVT and dopamine could lead to a cessation of seasonal reproductive behavior regardless of any stimulatory effect of androgens.

S4-2
CONVERGENT NEUROENDOCRINE MECHANISMS UNDERLYING PATERNAL CARE
O´Connell, Lauren
Center for Systems Biology, Harvard University, Cambridge, MA USA

S4-3
PREDATOR EXPOSURE ALTERS STRESS PHYSIOLOGY AND BEHAVIOR IN GUPPIES
Fischer, EK (1), Harris, RM (2), Hofmann, HA (2), Hoke, KL (1).
(1) Department of Biology, Colorado State University, Ft. Collins, CO USA (2) Section of Integrative Biology, Institute for Cellular & Molecular Biology, The University of Texas at Austin, Austin, TX USA

In vertebrates, glucocorticoids mediate physiological, morphological, reproductive, immunological, and behavioral responses to stressors and are implicated in adaptive evolution following changes in predation pressure. We used the Trinidadian guppy (*Poecilia reticulata*) to disentangle genetic and environmental effects of predation on cortisol levels. Guppies from high-predation environments have repeatedly and independently colonized and adapted to low-predation environments, resulting in parallel changes in life history traits, morphology, and behavior. To distinguish genetic and environmental influences, we compared cortisol levels in guppies from different source populations reared with and without exposure to predator chemical cues. Evolutionary history with predators and lifetime exposure to predator cues were both associated with lower cortisol levels. The concordant evolutionary and developmental influences on basal cortisol levels depended on distinct physiological mechanisms that suggest active modulation of cortisol release. We propose that the coupling of genetic and environmental effects at a phenotypic, but not a mechanistic, level increases the flexibility of this system, and we assess the consequences of cortisol manipulation on anti-predator and social behaviors. (We acknowledge financial support from Sigma Xi - The Scientific Research Society (to EKF), Sigma Delta Epsilon - Graduate Women in Science (to EKF), NSF (DEB-0846175 to CK Ghalambor), and the Alfred P. Sloan Foundation (to HAH).

OR4-1
CORTICOSTERONE INFUSED INTO THE STRIATUM PROMOTES THE USE AND PERSISTENCE OF A SPATIAL STRATEGY IN THE TOLMAN MAZE
Siller Pérez C (1), Serafín N (1), Espinoza-González V (1), Prado-Alcalá RA (1), Roozendaal B (2) and Quirarte GL (1).
(1)Departamento de Neurobiología Conductual y Cognitiva, Instituto de Neurobiología, UNAM Campus Juriquilla, Querétaro, Qro. 76230 México (2) Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, The Netherlands.

It is well established that stressful experiences induce the release of hormones such as corticosterone (CORT), which can modulate cognitive processes. Strong evidence demonstrates that CORT enhances the memory consolidation. The multimodal information from the at CORT enhances memory consolidation. The multimodal information from the subject's environment is collected, stored and retrieved by different neural substrates. It has been proposed that in tasks which involve allocentric and egocentric navigation is first acquired through spatial learning, but as training continues, behavior is guided by procedural learning. There is ample research indicating that the hippocampus is a core structure intimately engaged in spatial learning and memory. The dorsomedial striatum is also a region that participates in this kind of memory, although literature supporting this view is scarce. Importantly the dorsolateral region of the striatum is involved with procedural memory. The aim of this investigation was to evaluate the potential facilitating effects of CORT on spatial learning in the Tolman maze task. To address this issue, male *Wistar* rats were implanted with bilateral cannulae into the anterodorsal, dorsomedial or dorsolateral striatum. After recovery, rats were restricted to an 85% of their *ad libitum* feeding weights and this deprivation was maintained throughout the experiment. They were trained for 12 sessions in a Tolman maze apparatus with extra maze cues. Vehicle solution or one of three doses of CORT were infused into the anterodorsal (10, 30, or 60/µL/side), dorsomedial or,

dorsolateral region (10, 30 or 60ng/0.5µL/side) of the striatum immediately after sessions 2, 3 and 4. Performance was tested after the 5th and 12th sessions. The results showed that CORT administration into the anterodorsal striatum interferes with the spatial strategy after limited (5 sessions) and with procedural strategy after extensive (12 sessions) training. On the other hand, CORT administration into the dorsomedial striatum facilitates the spatial strategy also after limited and extensive training. These effects are conservative regardless the striatum region, given that CORT administration into the dorsolateral part showed that the spatial strategy is enhanced after both limited and extensive training. Despite it has been proposed that the striatum is anatomically and functionally heterogeneous with regard to the types of memory that it mediates, our results show that corticosterone can modulate the processes and drive memory to use and prevalence of spatial strategy. We thank the excellent technical assistance from Martín García, Ángel Méndez, Cristina Medina, Omar González and Leonor Casanova. (This work was supported by PAPIIT-UNAM (IN214111) and CONACYT (Grant 130524 and Scholarship 371741 to C.S.P).

OR4-2

TESTOSTERONE MODULATION OF SONG BEHAVIOR AND CONSUMMATORY SEXUAL BEHAVIOR IN CANARIES (SERINUS CANARIA) VIA ACTION IN THE MEDIAL PREOPTIC NUCLEUS

Alward, B.A., Ball, G.F.
Department of Psychological and Brain Sciences, Johns Hopkins University, Baltimore, MD, USA 21218

Studies utilizing a variety of mammalian and avian species indicate that the medial preoptic nucleus (POM) is a critical site for the regulation of male sexual behavior. For example, POM lesions block the expression of male-typical sexual behaviors and the action of testosterone in the POM is sufficient to activate male sexual motivation and performance in birds and mammals. There is some evidence that the POM is essential for the expression of sexually motivated song in songbirds. Testosterone has multiple effects on song regulating both the motivation to sing as well as its quality. However, the brain region(s) where testosterone acts to regulate the motivation to sing has not been definitively characterized. We hypothesized that song, at least in part, is regulated by testosterone action in the POM. We tested this by implanting testosterone into the POM of castrated male songbirds, (canaries; *Serinus canaria*) and assessing song output and acoustic features as well as the degree of stereotypy, a measure of quality. We also presented males with a female to assess sexual behaviors in her presence. We demonstrate here that T in the POM is sufficient to increase song output and improve acoustic measures (loudness, pitch, and complexity) to levels of males with high concentrations of circulating T; however, T in the POM is not sufficient to induce song stereotypy to the levels of males with globally circulating T. We also show that T is sufficient to activate copulation attempts in canaries but not the number of calls produced in response to the presence of a female, suggesting that the function of T in the POM is specific to regulating sexual behaviors. These results suggest while T in the POM is sufficient for activating song output, T in brain regions such as HVC of the song control system is required to induce high song stereotypy. Moreover, these results support an evolutionarily conserved role of T action in the POM in regulating male sexual behavior.

Thursday, May 23th
9:45 -11:35
II (LIPATA)

NASCE 2013 Symposium V:
Hormonal Regulation of Metamorphosis Across Phyla

Co-chairs:
Andreas Heyland, University of Guelph, CAN
Alex M. Schreiber, St. Lawrence University, USA

S5-1
BEYOND SYNERGY: CORTICOSTERONE AND THYROID HORMONE HAVE NUMEROUS INTERACTION EFFECTS ON GENE REGULATION IN XENOPUS TROPICALIS TADPOLES
Saurabh S. Kulkarni and Daniel R. Buchholz
Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221, USA

Thyroid hormone (TH) and glucocorticoids (CORT) synergize to accelerate metamorphic events in tadpoles. Synergy at the level of gene expression is known for three genes in frogs, but the nature and extent of TH and CORT cross-talk in gene regulation is otherwise unknown. Therefore, to examine TH and CORT interactions, we performed microarray analysis on tails from *X. tropicalis* tadpoles treated with CORT (100 nM), TH (50 nM), CORT+TH, or vehicle for 18 hours. We found over 5000 genes significantly altered in response to either or both hormones. We used Venn diagrams and cluster analysis to identify 16 main patterns of gene regulation, due to up-or down-regulation by TH and/or CORT. Many genes were affected by only one of the hormones, and a large proportion of regulated genes (22%) required both hormones. Surprisingly, a total of 928 genes (17%) were regulated by novel interactions between the two hormones, described as inhibitory, subtractive, and annihilatory interactions. These data greatly expand our understanding of the hormonal cross-talk underlying the gene regulation cascade directing tail resorption. Synergy at the morphological level is underlain by a variety of hormonal interactions and suggests the possibility that CORT affects not only the timing but also the nature of TH-dependent tissue transformation.

S5-2
HISTAMINE IS A REGULATOR OF SEA URCHIN METAMORPHOSIS
Heyland Andreas,
University of Guelph, Integrative Biology Guelph, ON Canada

Many marine invertebrate taxa evolved a complex life history that involves a drastic morphological and habitat transition from the planktonic to the benthic environment. Mechanisms regulating the habitat transition (settlement) and morphological and physiological transition (metamorphosis) remain poorly understood. Recent work on metamorphosis in echinoids (sea urchins and sand dollars) depicts a complex regulatory hormonal network responsible for the successful timing and completion of this transition within the life history of the organism. New data now suggest that histamine (HA) interacts with this regulatory network by modulating metamorphic competence in sea urchin larvae. Specific HAnergic neurons create a complex network within the larval and juvenile nervous system and interact with nitric oxide (NO), a metamorphic inhibitor in sea urchins. Furthermore, HA functions specifically through caspase mediated apoptosis in sea urchin metamorphosis. (This work was supported by NSERC Discovery and RTI funds to AH as well as CFI funds).

S5-3
HOW DO THYROID HORMONES INDUCE LATERALIZED SWIMMING BEHAVIORS IN FLATFISHES?
Schreiber, A.M. (1), Francis, A. (2), Bergstrom, C. (3), Seikai, T. (4)
(1) Biology Department, St. Lawrence University, Canton, NY USA (2) Biology Department, Armstrong Atlantic State University, Savannah, GA USA (3) Biology Department, University of Alaska Southeast, Juneau, AK USA (4) Department of Marine Bioscience, Fukui Prefectural University, Obama, Fukui, Japan.

Flatfish begin life as bilaterally symmetrical larvae that swim up-right, then abruptly metamorphose into asymmetrically shaped juveniles with lateralized swimming postures. Flatfish metamorphosis is mediated entirely by thyroid hormone (TH). Changes in flatfish swim posture are thought to be regulated via vestibular remodeling, although the influence of TH on teleost inner ear development still remains unclear. We have previously shown that TH induces abrupt growth and mineralization of one component of the vestibular system, the otoliths, during early larval development and metamorphosis. In typical upright swimming fish, the shapes and sizes of otoliths from the left and right sides are known to be bilaterally symmetrical. Here we show the existence of directional left-right asymmetry in the sizes and shapes of otoliths from the adults of several flatfish species: 'left-sided' species (both eyes always located on the animal's left side), 'right-sided' species, and 'ambidextrous' species (both eyes are located either on the right or left sides). The directionality of otolith shape asymmetry from the left-sided species was reversed compared with that of the right-sided species. Furthermore, the directionality of otolith shape asymmetry of left- and right-sided siblings from ambidextrous species was also reversed. These findings suggest that bilateral asymmetry of flatfish otoliths corresponds with the lateralized swim postures adpoted during metamorphosis. Though we know TH mediates flatfish otolith growth, it is not yet known if otolith asymmetry develops during metamorphosis.

OR5-1
THE FUNCTIONAL IMPORTANCE AND SIGNIFICANCE OF ECDYSONE RECEPTOR IN THE REGULATION OF MOLT CYCLE OF THE BLUE CRAB, Callinectes sapidus
Sirinart Techa and J. Sook Chung
Institute of Marine Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD, USA

Successful molting in arthropods requires a coordinated multiple hormonal interactions. In crustaceans, crustacean hyperglycemic hormone (CHH) family suppresses the activities of molting glands, Y-organs where ecdysteroids are synthesized. It has been proposed that circulating ecdysteroids may have a feedback effect on the synthesis of molt-inhibiting hormone (MIH) in the eyestalk. However, this hypothesis has not yet been fully examined. Ecdysteroids exert their signals through a nuclear heterodimer complex: ecdysone receptor (EcR) and retinoid-X receptor (RXR). The response to ecdysteroids can be inferred by the levels of EcR-RXR. First, *MIH* and *CasEcR/CasRXR* expressions are established during embryonic and juvenile molt cycle using qPCR assays. And, we aimed to understand the direct role of ecdysteroids and their nuclear receptors in the molt regulation of the blue crab, *Callinectes sapidus* using *in vitro* incubation study.

The eyestalks were incubated with ecdysteroids at two different concentrations which represent two molt stages: early and mid premolt, and examined for the changes in the levels of *MIH*/*CHH*, *EcR* and *RXR* isoforms. We found that *MIH* expressions are sensitive to the presence of ecdysteroids, and the levels are significantly elevated in eyestalk cultured in premolt-conditioned media. However, no changes in the levels of CasEcR/CasRXR expression are noted. Our data indicate that circulating ecdysteroids may directly influence *MIH* expression in the eyestalk, possibly *via* a feedback loop.(Supported by Grant MB-8714-08 from the United States-Israel Binational Agricultural Research and Development (BARD) to JSC and Thai government scholarship to ST).

OR5-2

ENVIRONMENTAL TEMPERATURE DIFFERENTIALLY IMPACTS TISSUE-SPECIFIC INDUCTION OF THYROID HORMONE-RESPONSIVE GENES DURING PRECOCIOUS METAMORPHOSIS OF RANA CATESBEIANA

Hammond, SA, Veldhoen, N, Helbing, CC.
Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC Canada

Amphibian metamorphosis is a complex process initiated solely by the thyroid hormones (THs) thyroxine (T_4) and triiodothyronine (T_3). In cold climates, the American bullfrog (*Rana catesbeiana*) typically does not metamorphose until its second year of life, and endures low winter temperatures in its functionally-athyroid larval form. Injection of premetamorphic tadpoles with T_3 at the permissive temperature of 24°C induces precocious metamorphosis with apparent physical changes and increased levels of thyroid hormone receptor beta (*thrb*) mRNA within hours. Tadpoles acclimated to the non-permissive temperature of 5°C then exposed to T_3 do not show these changes even after three weeks, although a slight increase in liver *thrb* expression is detectable at this time. Animals injected at 5°C then shifted to 24°C after one week undergo accelerated development and display increased abundance of liver *thrb* mRNA. In this study, we further investigated the degree of responsiveness of classic TH-responsive gene transcripts in tadpole liver, brain, tail fin, and back skin at permissive and non-permissive temperatures to determine how widespread the response patterns are to temperature changes. In the absence of T_3, animals acclimated to 5°C showed decreased abundance of *thrb* mRNA relative to animals held at 24°C in all tissue types examined. At the non-permissive temperature, a T_3 challenge resulted in no increase in liver or brain *thrb* mRNA whereas tail fin and back skin tissues showed a classic induction pattern. Shifting animals from 5°C to 24°C resulted in restoration of non-induced baseline and T_3-induced *thrb* levels similar to those observed at the permissive temperature. The present work illustrates the dynamic response to TH action between permissive and non-permissive environmental conditions and identifies differential temperature-associated effects on tissue-specific developmental programs.(We thank Mitchel Stevenson for expert technical assistance. This work was supported by a NSERC discovery grant to CCH).

Thursday, May 23th
14:15 -16:00
CAC

NASCE 2013 Symposium VI:
Development and Plasticity of Neuroendocrine Systems

Co-chairs:
David Lovejoy, University of Toronto, CAN
John Godwin, North Carolina State University, USA

S6-1
EVOLUTION OF GENE MODULES UNDERLYING SOCIAL SYSTEMS
O'Connell, LA
Center for Systems Biology, Harvard University, Cambridge, MA USA

Context appropriate behavioral decisions are made by all animals daily and can ultimately influence individual fitness. Little is known, however, about how neuroendocrine systems have evolved to contribute to these behavioral decisions. I will discuss my work comparing the specification and spatial distribution of neural phenotypes across vertebrates that has suggested some major brain evolution trends. I will then discuss comparative experiments examining how the repeated and independent evolution of monogamy relies on similar gene modules across vertebrates and even invertebrates. This work investigating the evolution of brain form and function holds great promise for facilitating a mechanistic understanding of behavior and how variation in brain morphology, neural phenotypes, and neural networks influences behavioral diversity across organisms.(LAO is supported by the NIGMS National Centers for Systems Biology grant 5P50GM068763).

S6-2
PLASTICITY AND DEVELOPMENT IN ZEBRA FINCHES AND OTHER BIRDS
Adkins-Regan, E.
Department of Psychology and Department of Neurobiology and Behavior, Cornell University, Ithaca, NY USA

Recent research has revealed marked individual variation in the behavior and endocrinology of birds that is likely to have fitness consequences. Non-genetic processes, including several kinds of maternal effects, are thought to contribute to the developmental origins of this variability. As one of the few songbirds that breeds sufficiently well in the laboratory for developmental experiments, the zebra finch has considerable potential for elucidating such processes. A set of studies will be presented as examples. One asks whether sex steroid levels of juveniles underlie individual differences in plumage and beak color maturation. Another shows that birds raised by fathers alone (adult females removed from the breeding aviaries) exhibit a long-term alteration in the dynamics of the corticosterone response to a social and environmental stressor, revealing developmental programming of the response through a maternal effect. Finally, several experiments show a surprisingly high degree of plasticity in sexual (pairing) partner preference mediated by early estradiol action or social environment. Such plasticity has implications for the functions of the continuous permanent pair relationships seen in many birds. (Research supported by the US National Science Foundation).

S6-3
THYROID HORMONE REGULATES DNA METHYLATION AND DEMETHYLATION IN THE DEVELOPING BRAIN
Kyono, Y (1) and Denver, RJ (1)(2),
Neuroscience Graduate Program (1) and the Department of Molecular, Cellular and Developmental Biology (2), The University of Michigan, Ann Arbor, MI USA.

Thyroid hormone (TH) has pleiotropic actions in the developing brain, influencing neural cell proliferation, migration, differentiation, morphology and function. The TH receptors (TRs) are ligand-activated transcription factors that function as 'epigenetic switches'; they regulate gene transcription by recruiting cofactors that modify histones, thereby altering chromatin structure. In addition to histone modifications, methylation of cytosine residues in DNA is an important epigenetic mechanism for gene regulation. Using *Xenopus* tadpole metamorphosis as a model system we discovered that TH regulates expression of genes coding for proteins that regulate DNA methylation and demethylation. Genes involved with DNA demethylation (*tet3, gadd45c* and *TDG*) were activated in tadpole brain during early prometamorphosis, and this corresponded with a dramatic decrease in 5-methyl cytosine levels. We investigated DNA methylation at two genomic loci (*Klf9* and *dnmt3a*) and found that both underwent progressive demethylation during metamorphosis, which corresponded with increased expression of the associated genes. Furthermore, treatment of premetamorphic tadpoles with TH caused induction of *tet3, gadd45c* and *TDG* mRNAs, which was followed by demethylation of *Klf9* and *dnmt3a* genes. During prometamorphosis expression of the *de novo* DNA methyltransferase DNMT3a increased as cell proliferation decreased; *in situ* hybridization histochemistry showed that *dnmt3a* mRNA was expressed outside of the neurogenic zone in tadpole brain. DNMT3a may function to promote cell differentiation and to maintain cell cycle exit during later postembryonic brain development. Our findings show for the first time that TH can regulate genes that control DNA methylation and demethylation in the developing brain. Parallel studies in mouse brain suggest that these actions are ancient and evolutionarily conserved. (Supported by NSF grants IOS 0922583 to R.J.D.; Y.K. was supported by a Ruth L. Kirschtein NRSA from the NIH).

S6-4
NEUROENDOCRINOLOGY OF SOCIALLY CONTROLLED SEX CHANGE IN THE BLUEHEAD WRASSE
Melissa A. Slane (1), Hui Liu (2), Neil J. Gemmell (2), and John Godwin (2),
(1) Department of Biology and W.M. Keck Center for Behavioral Biology, Box 7617, North Carolina State University, Raleigh, NC, USA (2) Centre for Reproduction and Genomics, Department of Anatomy, University of Otago, PO Box 913, Dunedin 9054, New Zealand

Coral reef fishes display an extraordinary diversity of sexual patterns including socially-controlled, functional, sex change. Despite decades of study, the transduction of social cues into reproductive responses by sex-changing fishes remains poorly understood. The Caribbean bluehead wrasse (Thalassoma bifasciatum) exhibits female-to-male functional sex change and discrete alternate male mating phenotypes. We are exploring possible roles for kisspeptin and gonadotropin-releasing hormone (GnRH) signaling in the brain and gonads in regulating sex change. We are also exploring potential influences of estrogenic and arginine vasotocin signaling on kisspeptin systems. Consistent with a possible role for GnRH in regulation of sex change, we find that large terminal phase (TP) males have greater forebrain GnRH1 mRNA abundances than females. Large males also have higher forebrain kisspeptin receptor mRNA abundances than females. In situ hybridization shows that bluehead wrasses express kisspeptin 2 (kiss2) mRNA in the ventral tuberal hypothalamus in a pattern that broadly overlaps

sites of vasotocin receptor expression. Interestingly, kiss2 and kiss receptor mRNAs are also expressed in the gonads where initial findings suggest lower expression of kiss2 mRNA in testes than ovaries, but higher expression of kiss receptor mRNA. We also find lower abundances of mRNA for the forkhead box transcription factor FoxL2 in testes than ovaries. These findings are consistent with a role for changes in the gonadotropic axis and potentially kisspeptin signaling in the gonads mediating socially-controlled sex change.

OR6-1

DOPAMINERGIC REGULATION OF BRAIN AROMATASE AND ESTROGEN RECEPTORS AND NEUROTROPHIC FACTORS IN GOLDFISH

Xing L, Hamilton CK, Navarro-Martín L, Trudeau VL.
Department of Biology, University of Ottawa, Ottawa, ON Canada

Aromatase cytochrome P450 is the only enzyme that performs the conversion of androgens into estrogens. Aromatase B is expressed exclusively in radial glial cells in teleost fish brains. While it is clear that brain aromatization plays important roles in neuroendocrine functions, neural plasticity, sexual behavior, many questions about the regulation of brain aromatase still exist. Previous microarray screens of goldfish hypothalamus suggest that dopamine (DA) is able to regulate radial glial cell related gene expression including aromatase B. The purpose of this study is to determine if dopaminergic compounds can regulate aromatase B, estrogen receptors (ER), glial cell derived neurotrophic factor (GDNF), brain derived neruotrophic factor (BDNF) and glial fibrillary acidic protein (GFAP) expression in female goldfish brain. A time course study (3h, 6h, 12h, 24h) was performed to detect expression function mRNA levels in telencephalon and hypothalamus using real-time RT-PCR after injecting a D1 agonist (SKF 38393; 40μg/g body weight) and antagonist (SCH 23390; 40μg/g body weight) separately. The data indicate that expression of ERα and ERβ, GDNF, but not ERγ and BDNF, are increased by DA D1 agonist and antagonist. Both SCH and SKF decrease GFAP and aromatase B expression in the telencephalon and hypothalamus. These paradoxical effects of SCH to affect gene expression similarly to SKF suggest that it is not acting as a true antagonist in vivo. Nevertheless, it is clear that DAergic compounds modulate radial glial cell function. It will be important to determine the specificity of this regulation, and the compliment of functional DA receptors expressed in radial glial cells. (Funding from NSERC-DG. Ontario Trillium Scholarship Program and U. Ottawa are acknowledged).

Thursday, May 23th
14:15 -16:00
INB

NASCE 2013 Symposium VII:
Comparative Endocrinology of Leptins and Other Adipokines

Co-chairs:
Erica Crespi, Washington State University, USA
Richard Londraville, University of Akron, USA

S7-1
A ROLE FOR LEPTIN IN LIMB DEVELOPMENT AND REGENERATION IN XENOPUS LAEVIS
Erica J. Crespi
School of Biological Sciences, Washington State University, Pullman, WA USA

The study of leptin in amphibians has led to a greater understanding of its role as a modulator of early developmental processes. The initial characterization of leptin function in amphibians revealed that treatment with recombinant *Xenopus* leptin protein accelerated limb growth and development in early prometamorphic tadpoles, suggesting a role as a growth factor during tadpole stages. Subsequent studies in *Xenopus laevis* support this hypothesis, as leptin treatment accelerates limb development and growth in part by increasing cell proliferation. Leptin receptor (long form) mRNA is distributed throughout the limb during early stages of development, and leptin treatment activates receptors in the limb, as indicated by an increase in phosphorylated STAT-3 staining. Leptin also is involved in limb regeneration, as a single injection of *Xenopus* leptin at the time of limb amputation (Nieuwkoop-Faber stages 52-53) increases cell proliferation and accelerates re-growth. Leptin injection also allowed for continued development in the unamputated limb, whereas development either ceased or regressed in saline-injected control animals. These are the first described effects of leptin on morphogenesis in vertebrates, and given that leptin expression is positively correlated with nutritional state at this stage of tadpole development, it may serve as a link between nutrition and early developmental processes. (Supported by NIH grant R15 HD057604-01 to EJC).

S7-2
EXPLORING LEPTIN FUNCTION IN FISH and WHALES
Richard L. Londraville
Department of Biology and Program in Integrated Bioscience, University of Akron, Akron, OH, USA

Even though leptin soon will enter its second decade of study, most of the research effort toward understanding its function is still focused on model mammals. Our group asserts that studying leptin expression pattern and function in a comparative context can inform both how leptin influences the phenotype of the study organism and how leptin functions in humans. Using morpholino knockdown technology, we have manipulated leptin expression in developing zebrafish embryos. Leptin A-deficient zebrafish have dramatically altered development, with aberrant sensory systems (non-functional eyes and inner ear), reduced yolk absorption, metabolic rate, and cardiac output. Recombinant zebrafish leptin rescues these effects. We also are investigating leptin expression in one of the world's most lipid-rich mammals. Bowhead whales (*Balaena mysticetus*) have the thickest blubber layer of any whale (0.5m), and can approach 50% body fat. In humans, this level of adiposity would produce leptin resistance, yet our data suggest that Bowhead leptin responds to feeding state (higher in Fall whales), sex (higher in females), and age (higher in adults). Additionally, leptin transcript copy number follows a gradient within the blubber column, and can be as much as 50x higher than in a mouse (per 50 ng total RNA). These investigations (with others in the comparative leptin community) allow unique hypotheses to be tested about leptin's evolutionary history. (This work was supported by NIH R15 DK079282-01/02 to Richard Londraville. This work was done in collaboration with Qin Liu, Robert J. Duff, Brian Bagatto, Mark Dalman, Hope Ball (University of Akron) and Johannes G.M. Thewissen (Northeast Ohio Medical University). Tissue samples for bowhead whales were acquired by J.G.M. Thewissen (permit: NOAA-NMFS 814–1899). The collection permit follows the provisions of the Marine Mammal Protection Act of 1972 as amended (MMPA: 16 U.S.C 1361 et seq) as well as the Endangered Species Act of 1973 as amended (ESA 16 U.S.C 1531 et seq).

S7-3
ADIPOCYTE PRECURSOR CELLS: IMPLICATIONS FOR OBESITY AND DIABETES.
Yazmín Macotela
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México.

Obesity constitutes a worldwide epidemic and is one of the main risk factors for the development of type 2 diabetes and cardiovascular disease. Understanding how increased fat accumulation underlies metabolic disease is crucial to develop effective therapies against obesity and its comorbidities. Excessive accumulation of adipose tissue differs in metabolic risk depending on its localization. In contrast to subcutaneous fat, accumulation of visceral or intra-abdominal fat conveys a high risk for the development of metabolic disease. In recent years, we have been interested in elucidating the differences between visceral and subcutaneous fat depots, particularly at the level of adipocytes and adipocyte precursor cells. We have characterized how different environmental and genetic factors affect adipocyte precursor cell number and also analyzed their genetic signatures and differentiation capacities. We are also searching for factors that may rescue the adipogenic potential of adipose tissue, which is altered during obesity and is believed to contribute to metabolic dysfunction. One such factor may be the hormone prolactin, which we have found to reduce insulin resistance in obese animals due at least in part to reduced adipocyte hypertrophy and increased adipocyte number. These findings represent a first step in identifying targets able to influence the metabolic phenotype of visceral fat and possibly modify it into a healthier, subcutaneous-like phenotype.(Supported by the National Council of Science and Technology of Mexico (CONACYT) grants 164423 and 174984, and by UNAM PAPIIT IA200113-2)

OR7-1

DIURNAL VARIATIONS IN GHRELIN/LEPTIN SIGNALING IN OBESE FEMALES, Neotomodon alstoni

Dalia Luna-Moreno and Miranda-Anaya Manuel
Unidad Multidisciplinaria de Docencia e Investigación, Universidad Nacional Autónoma de México, Juriquilla, Querétaro, México

Obesity is currently a major health problem that is increasing worldwide. The Mexican volcano mouse *Neotomodon alstoni* is a useful model to understand the causes and consequences of this condition because when is kept in captivity, circa 50% of individuals develop obesity. Ghrelin and leptin are hormones that act as crucial signals to indicate nutritional status as well as to modulate feeding behavior through a variety of distinct pathways. They target overlapping CNS regions in order to mediate their opposing effects on energy balance. In this sense, it is interesting to know how the food intake is modulated and for this crucial to study endocrine signals that are integrated in the hypothalamus. Females N alstoni develop obesity with hyperleptinemia more often than males. In this study we used female mice 25±5 WO in diestrous state and compare lean and obese condition. Obese mice were condsidered above 65 g body weight. Room temperature was between 18 and 23°C, and light–dark cycles were set at 12:12 hours (photophase: 06:00–18:00, 200–250 lx). Tissue and blood samples were collected at 10:00, 15:00, 19:00, 24:00 and 05:00 h. We quantified the circulating levels of total and active ghrelin by means of ELISA as well as ghrelin and leptin receptors in hypothalamus by Western Blotting. Our results indicate that in obese animals there is a significant decrease in total ghrelin at 24:00 h during the dark phase regarding the observed in lean mice, however not differences were noted in active ghrelin. In addition, there are not differences in leptin and ghrelin receptors between groups Also a further evaluation of STAT3 and STAT3-p, nuclear receptors that are activated during leptin action in hypothalamus, surprisingly we did not show differences either. These results suggest that although LRb–STAT3 signals contribute to leptin action on both energy homeostasis and glucose homeostasis, there are STAT3-independent signals. To know hypothalamic mechanisms that contribute to differences in obesity of *Neotomodon alstoni*, we look forward to study leptin-regulated hypothalamic neuropeptides such as NPY. (Supported by DGAPA-PAPIIT IN225311, Dr. Mauricio Díaz-Muñoz and Elvira Arellanes and Dr. Stephanie Thebault for helpfull assistance).

OR7-2

APPETITE SUPPRESSION, ENERGY MOBILIZATION AND ALTERATIONS IN THE FUNCTIONAL ROLE OF THE ENDOCRINE GROWTH AXIS CONTRIBUTE TO THE GROWTH-SUPPRESSING EFFECTS OF CORTISOL IN RAINBOW TROUT

Madison, BN, Tavakoli, S, Bernier, NJ.
Department of Integrative Biology, University of Guelph, ON, Canada.

To gain a better understanding of the mechanisms by which cortisol suppresses growth during chronic stress in fish, we characterized the effects of chronically elevated plasma cortisol on food intake, growth, the expression of key appetite- and growth-regulating factors, and the status of energy stores in rainbow trout. Fish given osmotic pumps that maintained plasma cortisol levels at ~70 and 120 ng/ml for 34d were sampled at 14, 28 and 42d post-implantation. Relative to controls, chronically elevated cortisol resulted in sustained 40-60% reductions in food intake that were associated with dose-dependent increases in the gene expression of liver leptin and of brain preoptic area corticotropin-releasing factor and neuropeptide Y. The cortisol treatments also elicited 40-80% dose-dependent reductions in growth rate that were sustained throughout the 42d experiment. During the hypercorticoid period, cortisol increased the mRNA levels of pituitary growth hormone (GH) as well as liver GH receptor (GHR), insulin-like growth factor-I (IGF-I) and IGF binding protein (IGFBP)-1 and -2, but had no consistent effect on plasma GH and IGF-I levels. During recovery, plasma GH levels and the expression of pituitary GH, liver GHR and IGF-I did not differ between treatments but the cortisol-treated fish had lower plasma IGF-I and elevated liver IGFBP-1 and -2 mRNA levels. Finally, the cortisol-treated fish had elevated plasma glucose levels, significantly reduced liver glycogen and lipid reserves, and only modest reduction in both muscle lipid and tissue protein content. Our findings suggest that the growth-suppressing effects of cortisol in rainbow trout result from a chronic reduction in food intake, a mobilization of energy reserves to sustain metabolic processes other then biosynthetic pathways, and changes in the GH/IGF-I/IGFBP axis that promote the catabolic effects of GH while disabling the growth-promoting actions of IGF-I.(Supported by NSERC to NJB).

Thursday, May 23th
14:15 -16:00
II (LIPATA)

NASCE 2013 Symposium VIII:
Rfamides-Evolution and Function

Co-chairs:
George Bentley, University of California, Berkeley, USA
Hamid R. Habibi, University of Calgary, CAN

S8-1
GONADOTROPIN-INHIBITORY HORMONE CONTROL OF GONADOTROPIN HORMONE PRODUCTION IN GOLDFISH
Moussavi, M (1), Wlasichuk, M (1)(2), Chang, JP (1)(2), Habibi, HR (1).
(1) Department of Biological Sciences, University of Calgary, Calgary and (2) Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Control of reproduction is mediated by a number of stimulatory and inhibitory neurohormones. Gonadotropin-releasing hormone (GnRH) is a key stimulator of gonadotropin hormone (LH and FSH) production, and goldfish brain contains two GnRH variants [salmon GnRH (sGnRH) and chicken GnRH-II (cGnRH-II)]. In the past decade, a number of RF-amide homologs (LPXRF-amide) were shown to be present in the brain of vertebrates, named as gonadotropin inhibitory hormone (GnIH) because of their observed inhibitory effects on pituitary LH and FSH production in birds and mammals. While GnIH may inhibit LH production in some species, its physiological significance is not fully understood. The presence of GnIH homologs has since been demonstrated in the brain of fish, frogs, birds, rodents, ovines, monkeys, and humans. Studies in fish revealed that GnIH action is complex and not always inhibitory as described in mammals and birds. In goldfish, GnIH action is both stimulatory and inhibitory, depending on mode of treatment (*in vivo* vs. *in vitro*), season, and stage of gonadal maturity. GnIH was found to differentially regulate expression of LH-ß and FSH-ß subunits, as well as modulating circulating level of LH in goldfish. In contrast, neither acute nor prolonged GnIH exposure *in vitro* was found to alter basal LH release but selectively modulated the cGnRH-II-induced LH secretion. Injection with GnIH was found to reduce basal and sGnRH/cGnRH-II induced serum LH levels in goldfish in early and mid gonadal recrudescence. The results indicate that in goldfish, GnIH differentially affects sGnRH and cGnRH-II regulation of LH secretion and expression directly at the pituitary level, as well as indirectly by affecting production of other neurohormones, depending on stage of gonadal development. The findings provide a framework for better understanding of the role of GnIH in the control of reproduction in fish and other vertebrates. (Funded by NSERC grants to HRH and JPC).

S8-2
SEX, STRESS, AND PARENTING: GNIH RESPONSE TO SOCIAL ENVIRONMENT IN BIRDS AND RODENTS
Calisi, RM (1)(2), Geraghty, A (2), Wingfield, JC (1), Kaufer, D (2)(3), Bentley, GE (2)(3)
(1) Department of Neurobiology, Physiology, and Behavior, University of California, Davis, CA USA (2) Department of Integrative Biology, University of California, Berkeley, CA USA (3) Helen Wills Neuroscience Institute, University of California, Berkeley, CA USA

Discoveries of how social environment can influence the plasticity of gonadotropin-inhibitory hormone (GnIH) are providing new insights into the neural mechanisms controlling reproduction and behavior. Since GnIH was first reported in 2000, its inhibitory effects on various levels of the vertebrate hypothalamo-pituitary-gonadal (reproductive) axis have been documented; and yet, very little is known of how its actions are affected by the external environment. We review what is known about how social environment affects GnIH expression in the brain, specifically how it is similarly impacted by stress in birds and rodents, and how it is affected by social competition in birds. We report new data characterizing GnIH expression over the course of avian and rodent parental care. We examined hypothalamic GnIH in male and female European starlings (*Sturnus vulgaris*) and female Sprague Dawley rats over the course of parental care. In birds, GnIH-ir peptide expression increases with the first day of incubation and first day of chick care. In rats, GnIH-ir does not significantly change. However, we observed a trend for the decrease of GnIH-ir on the day after pups were born and are further exploring this relationship. Additionally, we conducted egg/pup removal experiments to examine how unpredictable events (i.e. nest predation) can affect this relationship. GnIH-ir expression changes in response to egg loss in birds but not to pup loss in rats. Thus, changes in avian GnIH-ir expression during specific points in parental care may implicate it in the mediation of such behaviors, and we are currently conducting manipulations to better understand its role. However, lack of significant changes in rat GnIH-ir expression suggests the role of GnIH in parental care may be species specific and/or specific to housing environment (wild birds housed in semi-natural field conditions vs. captive, laboratory rodents).(We thank Nicole Perfito, Jesse Krause, Lance Kriegsfeld, Eileen Lacey, Alessandro Avila, Shanna Tucker, and Sandra Muroy for their help and/or discussion of this work. This research was supported by NSF DBI 1003112 (to RMC), NIMH BRAINS R01MH087495 (to DK), and NSF IOS 1122044 (to GEB)

S8-3
CIRCADIAN CONTROL OF KISSPEPTIN AND GNIH IN FEMALE REPRODUCTIVE FUNCTION
Lance J Kriegsfeld,
Department of Psychology, University of California, Berkeley CA 94720 USA

In females, precision in the circadian (daily) timing of reproductive hormone secretion is required for normal sexual motivation and fecundity. The preovulatory luteinizing hormone (LH) surge that triggers ovulation, for example, is generated by the timed activation of the gonadotropin-releasing hormone (GnRH) system by the suprachiasmatic nucleus (SCN), the main circadian pacemaker in mammals. During most of the ovulatory cycle, LH is maintained at low concentrations through estradiol negative feedback. However, at the time of the LH surge, negative feedback is suppressed and the SCN integrates with estradiol signaling to stimulate the LH surge (i.e., estradiol positive feedback). The neural pathways and cellular targets underlying the integration and coordination of circadian and estrogenic signaling required for ovulation are not fully understood. Our work explores the roles of kisspeptin and GnIH neurons as part of the essential, circadian-controlled network responsible for ovulation and female sexual motivation. Through these studies, we have identified a novel neurochemical pathway by which the SCN communicates with estradiol-responsive kisspeptin neurons to initiate the LH surge, along with a parallel pathway by which the SCN concomitantly removes the negative influence of estradiol via projections to the gonadotropin-inhibitory hormone (GnIH) system. We have also uncovered an endogenous timing mechanism in GnRH cells that gates daily changes in responsiveness to upstream, stimulatory neurochemicals, including kisspeptin, pointing to a hierarchy of oscillators in ovulatory control. This presentation will provide an overview of our work and others underscoring a role for kisspeptin and GnIH in the circadian control of ovulation and the interaction of these neurochemical mediators with other, well-established mechanisms of reproductive control. Supported by NIH grant HD50470

OR8-1

DOES CHRONIC CORTICOSTERONE INFLUENCE REPRODUCTIVE NEUROPEPTIDES IN FEMALE ZEBRA FINCHES?

Ernst, DFK (1), Lopes, PSC (1)(2), Bentley, GE (1)(3).
(1) Department of Integrative Biology, University of California, Berkeley, CA USA (2) Programa Graduado em Áreas da Biologia Básica e Aplicada, University of Porto, Porto, Portugal (3) Helen Wills Neuroscience Institute, University of California, Berkeley, CA USA

Chronic stress in birds has the potential to influence all levels of the hypothalamo-pituitary-gonadal (HPG) axis. In addition to direct actions via glucocorticoid receptor, acute and chronic stress impact the HPG axis via the actions of gonadotropin-inhibitory hormone (GnIH) in birds and rats. We hypothesized that chronic corticosterone (CORT) treatment would increase hypothalamic synthesis and content of GnIH, and thereby decrease gonadotropin-releasing hormone-I (GnRH-I) and gonadotropin-releasing hormone-II (GnRH-II). Adult female zebra finches (*Taeniopygia guttata)* were implanted with blank or CORT-filled silastic implants for two weeks, after which blood and brain tissue were collected. CORT treatment elevated plasma CORT to physiological levels. Immunocytochemistry revealed no significant differences in numbers of GnRH-I and GnRH-II immunoreactive neurons between groups and no significant difference in GnIH projections to GnRH-I and GnRH-II cells. Quantitative RT-PCR showed no significant differences between CORT implant and control birds in expression of mRNA for GnRH-I or GnIH. These data indicate that two weeks of chronic CORT exposure via implants does not measurably influence levels of GnRH-I or GnIH in the hypothalamus, nor affect levels of available GnRH-I, GnIH, and GnRH-II peptide in the midbrain, suggesting that this treatment either may not have adverse effects on reproductive physiology in female zebra finches, or that chronically high levels of CORT provided by implants are not a good mimic of chronic stress.
Acknowledgements: The authors would like to thank their funding sources, the National Science Foundation [1122044 to G.E.B] and the Ministério para Ciência, Tecnologia e Ensino Superior (MCTES-Lisbon, Portugal) for financial support through doctoral grant [SFRH/BD/33251/2007 to P.C.L.].

OR8-2

AWAKING A SLEEPING DOGMA: DIURNAL CHANGES IN HYPOTHALAMIC MELATONIN SYNTHESIS DE NOVO IN PASSERINES

Kangas, KA(1), Rosenblum, MK(1), Rodriguez, G(1), Guerrero, V(1), Bentley, GE(1)(2).
(1) Department of Integrative Biology, University of California, Berkeley, CA USA (2) Helen Wills Neuroscience Institute, University of California, Berkeley, CA USA

Annual fluctuations in photoperiod, associated with seasonal availability of resources, mediate physiological and behavioral changes across vertebrate taxa. Variations in the duration of nocturnal melatonin secretion in temperate zones provide information about the time of day and year. Changes in photoperiod regulate gonadal growth and regression in seasonal breeders, and administration of melatonin has profound effects on mammalian gonadal status. Unlike in mammals, there is a long-standing dogma that pineal melatonin is not involved in avian seasonal reproduction, based on just a handful of experiments. Recent evidence suggests hypothalamic melatonin synthesis could well be involved in the timing of reproduction in birds. Our preliminary data indicate that passerine birds *Sturnus vulgaris* are likely to produce hypothalamic melatonin with diurnal fluctuations, causing us to reconsider the role of melatonin in avian seasonal reproduction. This project investigates if melatonin can be synthesized de novo in the avian hypothalamus by confirming the expression of all four enzymes of the melatonin biosynthesis pathway: tryptophan-5-hydroxylase (TPH), 5-hydroxytryptophan decarboxylase (DDC), aralkylamine N-acetyltransferase (AANAT), and hydroxyindole-O-methyltransferase (HIOMT). This is the first time the expression of these four enzymes has been identified in the songbird hypothalamus. By comparing the diurnal and nocturnal expression levels of these enzymes in the hypothalamus relative to other tissues known to synthesize melatonin de novo (i.e. the pineal and the retina), we demonstrate the potential for daily fluctuations of encephalic melatonin to influence avian photoperiodic responses. This will better our understanding of the crucial role of melatonin in the evolution of photoperiodic responses across vertebrates.(The project described was supported by award number R25GM090110 from the National Institute of General Medical Sciences to KAK and NSF IOS 0956338 to GEB. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or the National Institutes of Health).

Friday, May 24th

9:45 -11:35
CAC

NASCE 2013 Symposium IX:
Reproductive Endocrinology-From Gene to Population

Co-chairs:
Valérie Langlois, Royal Military College, CAN
Gabriela González-Mariscal, CIRA-CINVESTAV, MEX

S9-1
EXPLORING ESTROGEN ACTION IN EARLY LIFE STAGE SALMONIDS
Vicki L. Marlatt (1)(2), Jinying Sun(3), Ryan Sherrard(3) , Cat Curran(2), Chris J. Kennedy(4), Howard Bailey(1), James R. Elphick(1), Christopher J. Martyniuk(3)
(1) Nautilus Environmental, Burnaby, BC, Canada (2) Department of Biology, University of the Fraser Valley, Abbotsford, BC, Canada Canadian Rivers Institute and Department of Biology, University of New Brunswick, Saint John, NB, Canada (3) Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

These studies examined the several molecular biomarkers for estrogen exposure in early life stage salmonids (swim-up fry), and whether these molecular level effects corresponded with higher level physiological effects. The experimental design was based on an existing in situ early life stage salmonid bioassay used in environmental monitoring programs whereby hatch boxes containing salmonids (cutthroat or rainbow trout) at the eyed embryo stage are deployed into a stream bed, and are monitored until the swim-up fry stage is reached. The validated endpoints include higher level physiological effects (survival, deformities, body morphometrics), and the experimental design includes concurrent laboratory reared animals as a control treatment. To explore the effects of 17β-estradiol (E2) on early life stage salmonids, laboratory exposure experiments of 1 µg/L of E2 initiated pre-hatching as eyed-embryos or post-hatching upon entering alevin stage were conducted. High mortality (~90%) was observed when E2 exposures were initiated at the eyed embryo stage compared to the alevin stage (<44% mortality), demonstrating the dynamic sensitivity and significant role of E2 *in vivo* during very early critical developmental stages. Gene expression analyses revealed that vitellogenin (*vtg*) was detectable in the liver of swim-up fry, and was highly inducible by 1 µg/L E2 (> 100 fold higher levels compared to control animals). Several other genes relevant to the reproductive endocrine axis (e.g. estrogen receptors and androgen receptors) exhibited decreased expression levels compared to control animals. Laboratory experiments also confirmed the induction of *vtg* protein levels in head/tail preparations of swim-up fry from E2 treatments. Although further studies are needed (i.e. dose-response) to fully elucidate the sensitivity of these early life stage salmonids to estrogens, combining molecular biomarkers and higher level physiological measures in this *in situ* bioassay design holds considerable promise for further defining estrogen action and detecting endocrine disruptors in the environment.

S9-2
CONTRIBUTION OF HORMONES AND EXTEROCEPTIVE SIGNALS TO THE EXPRESSION OF MATERNAL BEHAVIOR IN RABBITS
Gabriela González-Mariscal
Centro de Investigación en Reproducción Animal, CINVESTAV-Universidad Autónoma de Tlaxcala, México

Rabbit maternal behavior consists of building an underground nest (prepartum) and nursing the litter once a day for ca. 3 min with circadian periodicity. Nest building involves: digging a burrow, carrying straw into it (or into a nest box), and plucking body hair to line the straw nest. The onset and offset of these activities are regulated by the changing levels of estradiol (E2), progesterone (P), testosterone, and prolactin (PRL) across pregnancy. E2 acts on the medial preoptic area to stimulate nest building in ovariectomized P-injected rabbits but the brain sites where P acts are unknown. Throughout lactation, the precise timing of nursing relies critically on a threshold of nipple stimulation: does suckling less than 4 pups *do not* show a circadian periodicity of nursing, enter multiple times into the nest box, and stay inside it for more than 5 min. PRL and oxytocin (OT) may participate in the timing of nursing as: a) bromocryptine, which blocks PRL release, does not prevent maternal responsiveness but induces longer times inside the nest box; b) OT-immunoreactive neurons increase in size and number from late pregnancy into early lactation; c) there is a correlation between the number of suckling pups and the amount of OT released. The brain sites where PRL and OT act to regulate the timing of nursing are under investigation. (Supported by CONACYT and CINVESTAV).

S9-3
TESTOSTERONE INVOLVEMENT IN FOLLICULAR DEVELOPMENT AND OVULATION IN HENS
Rangel, PL and Gutierrez, CG
Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, México, D.F.

Follicular development and the ovulatory process in avian species involve an array of coordinated endocrine systems of which steroids are a major player. Progesterone (P) is responsible for ovulation through the induction of the ovulatory peak of LH; estradiol (E) induces the formation of P receptors in the hypothalamus, and in the liver stimulates yolk production. In contrast, the role of testosterone (T) remains controversial; nonetheless a significant amount of information sustains an essential role for T in the ovulatory process. As for P and E, a peak of T occurs prior to the LH surge that induces ovulation, and a positive correlation exists among these hormones. In addition, in the absence of a T peak the rise of P and LH will not occur in 90% of the cases. Moreover, the inhibition of T action either through passive or active immunization or by the use of a specific antagonist abolishes the occurrence of the preovulatory peaks of P and LH, impeding ovulation. Testosterone also has a paracrine action within the ovary by stimulating mRNA expression for the steroidogenic enzymes STAR and P450scc, and for the LH receptor in the preovulatory follicle granulosa cells. This synergistic action, both at the hypothalamic and ovarian levels, appears to be necessary to ensure the positive feedback between P and LH needed for ovulation. In birds, follicular development is characterized by a group of dominant follicles arranged in a hierarchy so that the order of ovulation is determined by their size. The steroid production of these dominant follicles changes as they mature and progress within the hierarchy. Removal of the smaller dominant follicles (F4 to F6) results in the abolition of the T preovulatory peak, and consequently of P and LH surges, as well as ovulation. The latter suggest that T is mainly secreted by smaller dominant follicles and that an endocrine complementation among follicles in the hierarchy occurs, ensuring an adequate milieu for continuous ovulation. (Funding for these studies were provided by PFAMU and PAPIIT, UNAM; CONACYT, Mexico; and the International Found for Science).

OR9-1

Physical access to a mate regulates final follicle maturation: endocrine and molecular correlates

Nicole Perfito(1), Matthew D. MacManes(2), Daisy Guardado(1) and George E. Bentley(1).
(1) Department of Integrative Biology, Univ. of Calif. Berkeley, CA USA (2) California Institute for Quantitative Biosciences, Univ. of Calif. Berkeley, CA USA

Producing fertile eggs requires that mates produce functional gametes at the same time. Prior to fertilization, female birds' ovarian follicles must have formed a pre-ovulatory hierarchy and accumulated sufficient amounts of yolk. Birds use behavioral displays and sexual behavior to synchronize internal physiology between pair members, and these interactions are particularly important for fertilization. We manipulated female follicle development by restricting physical access to males (restricted), but not visual or auditory access. Restricted females did not begin final follicle maturation, whereas females with mates (controls) had begun to lay eggs during the same time period. A subsequent period of seven days with *de novo* access to males was sufficient to induce final follicle maturation and pre-ovulatory hierarchy formation in the previously restricted females. To identify differences in gene expression in females with delayed follicle development (restricted group), versus normal follicle development (controls) and accelerated follicle development (females given males for 7 days), we used Illumina RNA sequencing to generate a transcriptome in the hypothalamus and in ovarian follicles. These data will provide a resource for the discovery of genes related to final follicle maturation, both in the ovary and in the hypothalamus. (Supported by NSF IOS 1122044 (to GEB).

OR9-2

EPIGENETIC MODIFICATIONS AND PHOTOPERIODIC TIME MEASUREMENT

Tyler J Stevenson and Brian J Prendergast
Institute for Mind and Biology, The University of Chicago, Chicago, Il., USA.

Local synthesis of triiodothyronine (T_3) in the hypothalamus is an evolutionarily-conserved mechanism for photoperiodic time measurement in seasonally breeding animals. Exposing Siberian hamsters to short winter photoperiods (SD) increase hypothalamic iodothyronine deiodinase Type III (DIO3) expression; this creates a hypothyroid local neuroendocrine environment in the mediobasal hypothalamus, which quenches T_3 signaling, and inhibits neuroendocrine-to-periphery gonadotrophin signaling. It is currently unknown whether epigenetic mechanisms participate in *dio3* mRNA responses to photoperiod. We tested the hypothesis that DNA methylation in the *dio3* promoter region affords photoperiodic control of *dio3* mRNA. DNA sequence analyses indicated that the hamster *dio3* proximal promoter region is a "CpG island" suggesting high susceptibility to methylation. Increased *dio3* mRNA expression in hamsters following transfer from long photoperiods (LD) to SD was associated with a marked decrease in DNA methyltransferase (DNMT) expression and demethylation of the *dio3* proximal promoter region. Prolonged (~40 weeks) exposure to SD photoperiods led to neuroendocrine refractoriness to SD, gonadal recrudescence and was associated with a complete reversal in DNMT expression and *dio3* promoter methylation patterns-- leading to *dio3* mRNA and promoter methylation indistinguishable from that of LD hamsters. Finally, hypermethylation attenuated gonadal involution in response to SD. Together, these data illustrate that SDs decrease DNMT activity in the hypothalamus and demethylate the *dio3* promoter; demethylation increases *dio3* mRNA expression and inhibits the HPG axis. These data suggest that DNA methylation is dynamic and provides evidence for reversible epigenetic mechanisms in the adult mammalian brain. Seasonal regulation of DNA methylation may be an evolutionarily-ancient, conserved timing mechanism.

Friday, May 24th
9:45 -11:35
INB

NASCE 2013 Symposium X:
Stress and Energy Metabolism

Co-chairs:
James A. Carr, Texas Tech University, USA
Nicholas Bernier, University of Guelph, CAN

S10-1
THE ACUTE ANDROGEN, GLUCOCORTICOID, AND METABOLIC STRESS RESPONSE OF FREE-RANGING BIRDS
Deviche, P. (1), Beouche-Helias, B (2), Davies, S (1), Gao, S (1), Lane, S (1), Valle, S (1).
(1) School of Life Sciences, Arizona State University, AZ USA (2) University of Poitiers, France

Plasma testosterone (T), the main testicular androgen in terrestrial vertebrates, can rapidly decrease during acute stress. The objectives of the present work were to (1) characterize this decrease, (2) determine its relationships to the stress hormone, corticosterone (CORT), and (3) investigate its metabolic correlates. In free-ranging male Rufous-winged Sparrows, *Peucaea carpalis*, plasma T decreases within 10 min of capture and handling stress, and the magnitude of this decrease increases linearly with the stress duration. Acute stress also rapidly increases plasma CORT, but little evidence exists that this increase is responsible for the stress-mediated suppression of plasma T. The rapid effects of acute stress on plasma T and CORT correlate with metabolic changes consisting in decreased plasma levels of glucose and the antioxidant uric acid. These changes may reflect enhanced uptake of these metabolites by tissues, in turn increasing energy substrate availability while protecting metabolically active tissues from the potentially damaging effects of oxidative stress resulting from glucose oxidation. The respective roles of T and CORT in the control of acute stress-mediated metabolic changes remain, however, unknown. The endocrine effects of acute stress persist long after birds are no longer exposed to the experimental stressor. Indeed, plasma T and CORT levels in sparrows released on their breeding territory 30 min after capture and handling and then recaptured one day later, are similar to levels at the time of release. At this time plasma uric acid levels do, however, not differ from those at capture, indicating full metabolic recovery. The results emphasize the importance of considering rapid changes in plasma androgen and glucocorticoid levels in the interpretation of the behavioral and physiological effects of these hormones. They reveal a higher level of plasticity of plasma T regulatory mechanisms than demonstrated to date in free-ranging vertebrates.
(Support: National Science Foundation Award 1026620 to P.D).

S10-2
MIDBRAIN UROCORTIN 1 AND ENERGY METABOLISM
Tamas Kozicz MD, PhD
Department of Anatomy, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Urocortin 1 is a member of the corticotropin releasing factor neuropeptide superfamily. Besides its proposed role in controlling the animal's neuroendocrine and behavioral stress adaptation response, urocortin 1 is also a potent inhibitor of food intake. Both of these functions of urocortin 1 are mediated by the brain corticotropin releasing factor receptors. One of the largest populations of brain urocortin 1 neurons can be found in the centrally projecting Edinger-Westphal nucleus (EWcp) in the midbran. Our group have provided various lines of evidence on the significance of EWcp urocortin 1 neurons in the stress adaptation response. Our recent data point to another potential role of EWcp urocortin1 neurons. Specifically, we have found that urocortin 1 neurons in the EWcp express receptors sensitive to peripheral fuel signals such as ghrelin and leptin, and represent a subset of presympathetic-premotor neurons controlling the white adipose tissue and the stomach. We have also found that the activity of EWcp urocortin 1 neurons is controlled by the metabolic state of the animal (fasting, high fat diet, etc.) mediated by peripheral fuel signals. In line with this notion, we have shown that leptin is a potent inhibitor of EWcp urocortin 1 neuronal activity, whereas ghrelin activates EWcp urocortin 1 neurons. In my talk, I will give a comprehensive summary on EWcp urocortin 1's role in regulating energy metabolism and draft a neuronal circuitry whereby EWcp urocortin 1 neurons might contribute to the coordination of the energy-dependent stress adaptation response.

S10-3
THE CRYPTIC EVOLUTION OF THE TENEURIN C-TERMINAL ASSOCIATED PEPTIDES (TCAP): THEIR ROLE ON CELLULAR ENERGY PRODUCTION AND INHIBITION OF THE STRESS-RESPONSE.
Lovejoy, DA, De Lannoy, L, Chen, Y, Song, L, Crosier, R, Otchengco A,
Department of Cell and Systems Biology, University of Toronto, Toronto ON, Canada.

The teneurin transmembrane proteins are ubiquitous in the Metazoa. In vertebrates, there are four paralogous genes that are highly expressed in the brain and in most metabolically active tissues. As type II proteins, the carboxy-terminus is displayed on the extracellular face of the cell. At the tip of the carboxy-terminus, lies a 40- or 41-residue sequence that can be cleaved to release a bioactive peptide termed teneurin C-terminal associated peptide (TCAP). However, TCAP-1 can be transcribed as a separate mRNA, also. The TCAP/teneurin gene system evolved initially as a polymorphic proteinaceous toxin in prokaryotes, then introduced into a choanoflagellate by horizontal gene transfer. During the subsequent radiation of the Metazoa, the proto-teneurin system evolved into a multifunctional protein system associated with adhesion, interaction with the cytoskeleton and increased energy production by stimulation of glucose transport into the cell. TCAP evolved into a soluble signaling peptide that binds β-dystroglycan to elicit a MEK-ERK1/2-mediated signal transduction system to phosphorylate key cytoskeletal-regulating proteins. In neurons and skeletal muscle, TCAP-1 stimulates an insulin-independent glucose transport into the cells to ATP production. In vitro, TCAP-1 stimulates neurite growth and protects cells against stressful conditions. In rodents, picomolar concentrations of TCAP-1 administration inhibits the corticotropin-releasing factor (CRF)-induced increase in cocaine-seeking, acoustic startle and forced swim tests. Thus, the TCAP family of peptides represent an ancient peptide signaling system associated with the regulation of cellular energy.

OR10-1

Regulation of neurosteroid biosynthesis by corticotropin-releasing hormone: Possible role in the control of stress response

do Rego, JL (1)(2)(3), Haraguchi, S (4), Moon, MJ (5), do Rego, JC (1)(6), Hasunuma, I (4), Luu-The, V (7), Pelletier, G (7), Seong, JY (5), Tsutsui, K (4), Vaudry, H (1)(2)(3)

(1) Institute of Research and Innovation in Biomedicine (IRIB), University of Rouen, Rouen, France (2) Regional Platform for Cell Imaging (PRIMACEN), University of Rouen, Rouen, France (3) National Institute of Health and Medical Research (INSERM) (4) Laboratory of Integrative Brain Sciences, Waseda University, Tokyo, Japan (5) Laboratory of GPCR, Korea University College of Medicine, Seoul, Korea (6) Platform of Behavioral Analysis (SCAC), Faculty of Medicine and Pharmacy, University of Rouen, Rouen, France (7) Research Center in Molecular Endocrinology, Oncology and Genetics, Laval University Hospital Center, Quebec, Canada

There is now clear evidence that the brain, in very much the same as the adrenal gland, gonads and placenta, is a steroidogenic organ. However, the neuronal mechanisms controlling the activity of steroid-producing cells in the brain have received little attention. In this study, we have investigated the effect of CRH, a neuropeptide that plays a crucial role in the control of stress, on neurosteroid biosynthesis, using the brain of the frog *Rana esculenta* as an experimental model. Double labeling of brain sections, showed that steroid-synthesizing neurons are often innervated by CRH-containing fibers. The diencephalic regions where most steroidogenic cell bodies are located are also enriched with CRH receptor (CRH-R)-like immunoreactivity. Exposure of frog hypothalamic explants to graded concentrations of CRH produced a dose-dependent increase in the formation of various neurosteroids. The stimulatory effect of CRH on neurosteroid biosynthesis was mimicked by stressin-1, urocortin, sauvagin and urotensin-I. CRH-induced neurosteroid production was markedly attenuated by CRH-R1 and CRH-R2 receptor antagonists, suggesting that the effect of CRH on neurosteroidogenesis is mediated through both receptors. To determine the relationships between CRH, stress and neurosteroid biosynthesis we exposed frogs to shaking stress before incubation of hypothalamic explants with tritiated pregnenolone. HPLC analysis revealed that stress enhances the production of several neurosteroids. The stress-induced stimulation of neurosteroid formation was significantly reduced by icv pretreatment with CRH-R1 or CRH-R2 receptor antagonists. These observations indicate that CRH exerts a direct stimulatory effect on the synthesis of neurosteroids. Since several neurosteroids can modulate the activity of the $GABA_A$ receptor and thus exert sedative effects, our data indicate that the stimulatory effect of CRH on neurosteroid production may attenuate the stress response induced by this neuropeptide. (Supported by European Regional Development Fund (FEDER), INSERM, PRIMACEN, a France-Japan exchange program (INSERM-JSPS), a France-Korea exchange program (STAR), a France-Québec exchange program (INSERM-FRSQ) and the Région Haute-Normandie).

OR10-2

IS NESFATIN-1 A MODULATOR OF STRESS RESPONSE IN FISH?

Vi Pham (1), Brent Kerbel (1), Ron Gonzalez (1), Joshua Pemberton (2), Nicholas J. Bernier (3), John P. Chang (2), and Suraj Unniappan (1).

(1) Department of Veterinary Biomedical Sciences, Laboratory of Integrative Neuroendocrinology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatchewan, Canada (2) Department of Biological Sciences, University of Alberta, Alberta, Canada (3) Department of Integrative Biology, University of Guelph, Ontario, Canada

Stress in fish and mammals is mediated by the hypothalamo-pituitary-adrenal (stress) axis, which produces the corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH) and corticosteroids, respectively. Nesfatin-1 is a novel metabolic protein encoded in the precursor, nucleobindin-2. Nesfatin-1 is emerging as a protein that influences the stress axis in mammals. Nesfatin-1 co-express CRF in the rat brain and stimulates both ACTH and cortisol secretion in rats. Moreover, central co-injection of astressin$_2$-B, a CRF2 receptor antagonist, attenuates the anorectic effects of nesfatin-1 central injection. While the mechanisms by which nesfatin-1 acts remain unclear, the GPCR12 has been proposed as a nesfatin-1 receptor. The main aim of this research is to understand the relationship between the stress axis and nesfatin-1 in goldfish, a well-characterized representative non-mammalian model. Immunohistochemical studies found abundance of nesfatin-1, nesfatin-1 colocalizing CRF receptor 1 in the goldfish hypothamalus and in the pituitary corticotropes. GPCR12 immunoreactivity was also found in the goldfish hypothalamus and pituitary. Acute netting and restraint stress upregulate nucleobindin-2 mRNA expression by 50% in the hypothalamus and by 150% in the forebrain. Intracerebroventricular injection of 25ng/g bodyweight nesfatin-1 into the third ventricle causes a 4-fold increase in CRF mRNA expression in the hypothalamus at 30 minutes post-injection. Intraperitoneal injection of 50 ng/g bodyweight nesfatin-1 resulted in a 2 fold increase in serum cortisol levels at 15 minutes post-administration. However, nesfatin-1 did not directly affect cortisol secretion from perifused interrenal tissues *in vitro*. Our preliminary studies found 0.01 nM nesfatin-1 stimulating ACTH from a mixed culture of dispersed pituitary cells from goldfish. Overall, nesfatin-1 is a new endocrine factor that modulates the stress axis in fish. Character Count: 1953 (Funding for these studies was provided by NSERC Discovery and Discovery Accelerator Awards, Ontario Ministry of Economic Development and Innovation, CFI-LOF funds, NSERC RTI grant, University of Saskatchewan and York University. We gratefully acknowledge Dr. Scott Kelly (Biology, York University) and Susan Cook (Veterinary Biomedical Sciences, University of Saskatchewan) for their guidance and support of our research; and Dr. Rolando Ceddia (Kinesiology, York University) for allowing us to use his equipment).

Friday, May 24th
9:45 -11:35
II (LIPATA)

NASCE 2013 Symposium XI:
Endocrine and Osmotic Modulation of Epithelial Cells

Co-chairs:
Andre P. Seale, University of Hawaii, USA
Yoongseong Park, Kansas State University, USA

S11-1
ENDOCRINE REGULATION OF RESPIRATORY EPITHELIA MEDIATING TRACHEAL AIR FILLING DURING THE ECDYSIS BEHAVIORAL SEQUENCE OF *DROSOPHILA*
Do-Hyoung Kim (1), Young-Joon Kim (2), and Michael E. Adams (1)
(1) Departments of Entomology and Cell Biology & Neuroscience, University of California, Riverside, CA, U.S.A (2) Department of Life Science, Gwangju Institute of Science and Technology, Gwangju, South Korea

In terrestrial organisms, respiratory epithelia are unique in regulating transport of molecules between fluid- and air-filled compartments. Efficient gas exchange across this barrier depends upon maintenance of an optimally thin fluid layer at the apical membrane of the cell. The insect tracheal system allows diffusion of gaseous oxygen to within microns of every cell in the organism, an evolutionary adaptation of this highly aerobic group. Nevertheless during the molting process, the tracheal system becomes fluid-filled until onset of ecdysis, when gas filling occurs following release of ecdysis triggering hormones (ETH). ETH release triggers a peptide signaling cascade in the CNS that mediates tracheal air filling and ecdysis behaviors. In response to ETH, central neurons release the neuropeptide drosokinin. Disruption of drosokinin signaling interferes with tracheal air filling during larval stages. Tracheal air filling defects are observed following drosokinin cell ablation, expression of inward rectifier potassium channels (K_{ir}), or RNA silencing of kinin or ETH receptors in drosokinin neurons. A hypomorphic drosokinin receptor mutant fly line (*Lkr*^{f02594}) also exhibits tracheal air filling defects, as does RNA silencing of Lkr using *pickpocket* and *breathless* drivers. Direct exposure of tracheal epidermal cells to drosokinin (10 nM) results in calcium mobilization. Our findings provide new evidence for a tracheal air filling mechanism involving peptidergic regulation of calcium in respiratory epithelial cells. (Supported by NIH grant R01-GM067310.

S11-2
NEW INSIGHTS INTO THE MOLECULAR AND CELLULAR ACTIONS OF PROLACTIN ON TELEOST OSMOREGULATION
Breves, JP (1), Serizier, SB (1), Goffin, V (2), McCormick, SD (1)(3), Karlstrom, RO (1).
(1) Department of Biology & Center for Neuroendocrine Studies, University of Massachusetts, Amherst, MA USA (2) Inserm, Research Center Growth and Signaling, Prolactin/GH Pathophysiology Laboratory, University Paris Descartes, Paris, France (3) USGS, Conte Anadromous Fish Research Center, Turners Falls, MA USA

Prolactin (PRL) is recognized as a key regulator of ion and water transport within osmoregulatory tissues across vertebrate species, but the cellular mechanisms underlying these actions remain poorly understood. Research in teleosts has established PRL as a direct regulator of ion uptake in response to hyposmotic conditions. We recently turned to the experimentally accessible zebrafish (*Danio rerio*) as a model to investigate how PRL directs the function of ionocytes in the gill. We show that acclimation to ion-poor water leads to the concurrent activation of genes encoding a Na^+/Cl^- cotransporter (*ncc*), Na^+/H^+ exchanger (*nhe3b*), epithelial Ca^{2+} channel (*ecac*), and two PRL receptors (*prlra* and *prlrb*). Injecting oPRL into adults increased expression of *ncc* and *prlra*, but not *nhe3b* or *ecac*. *In vitro* incubation of gill filaments with oPRL stimulated *ncc* in a concentration-dependent manner, an effect blocked by a PRL receptor antagonist, Δ1-9-G129R-hPRL. Considered with our previous findings in euryhaline tilapia, our data suggest that *ncc* represents a conserved transcriptional target of PRL in fishes that employ NCC-dependent Cl^- uptake pathways. We are now using zebrafish to precisely map PRL receptor expression in relation to differentiated ionocytes and ionocyte precursors in osmoregulatory tissues of both embryos and adults. We have developed tools for the up-and down- regulation of PRL signaling that allow us to investigate for the first time how PRL regulates gene transcription and/or cell differentiation events *in vivo*. Our work in a series of models is providing mechanistic insight into PRL-mediated ionoregulatory action in teleost epithelia. Given the high level of conservation in this endocrine system across vertebrate species, this work promises to shed light on endocrine control of osmoregulation in humans that may lead to unique treatments for kidney-related diseases. (Supported by NIH T32-MH020051, F32-DK095575 and RO1-NS039994 and NSF HRD 0450339).

S11-3
CELLULAR AND MOLECULAR MECHANISMS OF DIURESIS IN MOSQUITOES
Piermarini, PM.
Department of Entomology, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH USA.

Mosquitoes face distinct challenges to salt and water balance at different stages of their life cycle. Larvae require a perpetual diuresis to excrete the constant osmotic uptake and ingestion of water from their aquatic habitat. In contrast, adults require a perpetual anti-diuresis to limit the excretory loss of water to their terrestrial environment. Adult female mosquitoes are an exception when they feed on vertebrate blood as part of their reproductive cycle; they require a rapid post-prandial diuresis to excrete the excess salt and water that are ingested. The diuresis in both larval and adult mosquitoes is mediated by the Malpighian tubules. This renal epithelium consists of two cell types (principal and stellate cells) and generates urine via active transepithelial fluid secretion. The goal of the present paper is to highlight recent efforts by our group to dissect the cellular and molecular mechanisms of transepithelial ion transport in Malpighian tubules of adult female mosquitoes (*Aedes aegypti*). We show and discuss evidence for: 1) a Cl/HCO_3 anion exchanger in stellate cells that contributes to the metabolic regulation of fluid secretion; 2) a K,Cl cotransporter in principal cells that plays a central role in fluid secretion; and 3) a putative intercellular pathway between principal and stellate cells mediated by gap junctions (innexins) that allows for cross-talk between the epithelial cells. The potential roles of diuretic neuropeptides (e.g., kinin and calcitonin-like peptides) in regulating the above mechanisms are also discussed.(Supported by NIH grants K01DK080194 and R03DK090186).

OR11-1

EFFECTS OF AMBIENT OSMOLALITY AND PROLACTIN ON BRANCHIAL OSMOREGULATORY FUNCTION IN CULTURED GILL FILAMENTS FROM FRESHWATER-ACCLIMATED MOZAMBIQUE TILAPIA

Inokuchi, M (1)(2), Lerner, DT (1)(3), Grau, EG (1), Watanabe, S (2), Kaneko, T (2), Seale, AP (1).
(1)Hawaii Institute of Marine Biology, University of Hawaii, Kaneohe, HI, USA (2)Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Tokyo, Japan (3)Sea Grant College Program, University of Hawaii, Honolulu, HI, USA

Euryhaline teleosts alter their branchial osmoregulatory function to cope with changes in environmental salinity. Although hormones and extracellular osmolality are known to modulate gill function, identifying and characterizing the unique effects of the endocrine system and environment remain a challenge. We aim to characterize the effects of osmolality and prolactin (PRL) on gill function by using an *in vitro* experimental model. To examine the effect of ambient osmolality, gill filaments were dissected from freshwater (FW)-acclimated tilapia and incubated in media of various osmolalities. These gill filaments were cut sagittally to allow culture medium to access mitochondria-rich cells. The mRNA expression levels of Na^+/K^+-ATPase a1a (NKAa1a) and Na^+/Cl^- cotransporter (NCC) in the gills were increased with reducing medium osmolality, while NKAa1b and $Na^+/K^+/2Cl^-$ cotransporter 1 (NKCC1) expression was upregulated by increasing osmolality. The expression of PRL receptor (PRLR) 1 and 2 revealed negative and positive correlations with medium osmolality, respectively. To investigate the effect of PRL, we incubated gill filaments in media with various concentrations of PRL and measured mRNA levels of ion transporters and PRLRs. Na^+/H^+ exchanger 3, NCC, NKAa1a and PRLR1, which are involved in ion uptake in FW, were positively correlated with an increase in PRL concentration. Our findings indicate that osmolality and PRL independently and directly modulate gene expression of PRLRs and ion transporters in the gill. (This study was supported by grants from the National Science Foundation, the Edwin W. Pauley Summer Program, United States Department of Agriculture and Japan Society for the Promotion of Science).

OR11-2

THE EFFECTS OF STEADY-STATE AND TIDALLY CHANGING REARING SALINITIES ON OSMOREGULATION IN THE MOZAMBIQUE TILAPIA

Moorman, BP (1) (2), Inokuchi, M (1), Lerner, DT (1)(3), Grau EG (1), Seale, AP (1).
(1) Hawai'i Institute of Marine Biology, University of Hawaii, Kaneohe, HI, USA. (2) Department of Molecular Biosciences and Bioengineering, University of Hawaii, Manoa, HI, USA. (3) Sea Grant College Program, University of Hawai'i, Honolulu, HI, USA.

Mozambique tilapia, Oreochromis mossambicus, evolved in estuaries and rivers where the salinity varies between fresh water (FW) and seawater (SW) with tidal frequency. Adaptation to changes in environmental salinity is largely mediated by the neuroendocrine system and involves the activation of ion uptake and ion extrusion mechanisms in branchial ionocytes. Exposure to a fall in extracellular osmolality stimulates the release of prolactin (PRL) which acts to promote ion uptake in osmoregulatory tissues, including the gill. We compared plasma osmolalities, plasma PRL levels, pituitary PRL mRNA levels, and branchial ion pump and transporter mRNA levels in tilapia reared in FW, SW, and under a tidal regimen (TR), characterized by changes in salinity between FW (TF) and SW (TS) every six hours. We also investigated the ability of tilapia reared under these conditions to adapt to osmotic challenges. Plasma PRL levels were higher in tilapia reared in FW than those reared in SW, TF, and TS. Unlike tilapia reared in steady-state FW or SW, TR tilapia did not show any correlation between plasma osmolality and PRL levels. Branchial Na+/Cl- cotransporter (NCC) mRNA levels were highest in fish reared in FW, followed by those reared in TF, TS and SW. Na+/K+/Cl- cotransporter (NKCC) levels were lower in FW than in SW, TF, and TS. Immunocytochemistry revealed that TF and TS ionocyte morphology closely resemble that of SW fish. FW tilapia were unable to survive direct transfer to SW, while TF tilapia showed no increase in mortality when transferred directly to SW. These data indicate that the ionocyte physiology of TR tilapia is geared towards ion extrusion. This study also suggests that tilapia reared in a tidal environment are better able to adapt to osmotic challenges than their steady-state counterparts.

Friday, May 24th

14:15 -16:00
CAC

NASCE 2013 Symposium XII:
New Hormones and Novel Functions for Old Friends

Co-chairs:
Nancy Sherwood, University of Victoria, CAN
J. Sook Chung, University of Maryland, USA

S12-1
ORTHOLOGUES OF MAMMALIAN NEUROPEPTIDES AND RECEPTORS IN PROTOSTOMES: THE EVOLUTIONARY ORIGIN OF GnRH, VASOPRESSIN AND OXYTOCIN
Frank Hauser and Cornelis C. J. P. Grimmelikhuijzen
University of Copenhagen, Denmark

Gonadotropin-Releasing Hormone (GnRH) is a neuropeptide regulating reproduction in mammals. 15 years ago, we identified a first invertebrate orthologue of mammalian GnRH receptors in *D. melanogaster*. Surprisingly, the ligand for this receptor turned out to be adipokinetic hormone (AKH), a metabolic neuropeptide regulating sugar and lipid homeostasis. Structurally, AKH is closely related to two other insect neuropeptides, corazonin and AKH/corazonin-related peptide (ACP). Corazonin has cardioacceleratory activity in the cockroach, but steers male sexual behavior in *Drosophila*. AKH, corazonin and ACP each activate a specific G protein-coupled receptor (GPCR). These three independent hormonal systems regulate and link different aspects of insect metabolism and reproduction and have common evolutionary origins with the vertebrate GnRH system. Closely related to this GnRH receptor superfamily is another superfamily, consisting of vasopressin/oxytocin-like receptors and crustacean cardioactive peptide (CCAP) receptors. Vasopressin and oxytocin are cyclic neuropeptides that are involved in various aspects of reproduction. Also many insects contain a vasopressin/oxytocin-like peptide (inotocin) and an inotocin receptor. Our work and that of others suggest that already before the split of deuterostomes and protostomes (about 700 million years ago), there existed two related GPCR superfamilies: the GnRH and oxytocin/vasopressin GPCR superfamilies. The two families were conserved in the deuterostome branch (mammals), while the GnRH receptor family split into three branches (ACP, AKH, and corazonin-like) and the oxytocin/vasopressin family into two branches inotocin- and CCAP-like) in protostomes.

S12-2
A GENDER SPECIFIC HORMONE DETERMINES THE DEVELOPMENT OF ADULT FEMALE FEATURES.
J. Sook Chung(1) and Nilli Zmora(2)
(1)University of Maryland Center for Environmental Science (2) University of Maryland Baltimore County, Baltimore, MD, USA

The current paradigm in sex differentiation of malacostracan crustaceans is based solely on the androgenic gland, a male specific endocrine organ, and its hormonal product, the androgenic gland hormone (AGH). Accordingly, female differentiation and secondary traits essential for reproduction are determined by default, in the absence of the masculinizing action of AGH. Adult female crustaceans display a wide range of reproductive strategies but the endocrine systems supporting them have not yet been found. We identified a hetherto unknown crustacean female sex hormone (CFSH), predominantly expressed in distinct neurons in the eyestalk ganglia of female blue crab *Callinectes sapidus*. The full-length cDNA encodes a precursor of a novel protein consisting of a signal peptide, a precursor-related peptide and a mature protein of 167 amino acids. *CFSH* knock-down by double-stranded RNA interference reduced the levels of *CFSH* expression and CFSH protein in the eyestalks of pubertal females, resulting in abnormal development of structures essential for successful mating and brooding, such as a pair of gonopores and a brooding egg attachment system comprised of enlarged semi-circular abdomen and ovigerous setae. The ovigerous setae in *CFSH* knocked-down females displayed smaller and less complex structure and the gonopores were small, misplaced or absent. These data provide the first evidence for the presence of a female specific hormone in decapod crustaceans and its functional role in the development of adult female-secondary morphology. CFSH is probably needed to support the specific reproductive strategy of crustacean species with internal fertilization and brooding behavior.(Supported by NSF IOS 1146774 to JSC).

S12-3
GLYCOPROTEIN HORMONES AND THEIR RECEPTORS EVOLVED AT THE ORIGIN OF METAZOANS
Graeme J. Roch and Nancy M. Sherwood
Department of Biology, University of Victoria, Victoria, BC Canada

Pituitary hormones such as LH, FSH and TSH in vertebrates are glycoprotein hormones (GPHs) that have a number of roles including reproduction and metabolism. The evolution of these hormones is thought to have occurred in early vertebrates due to the duplication of an ancient GPH, thyrostimulin. Both the ancient and more recent GPHs are active as heterodimers that bind to receptors known as leucine-rich G protein-coupled receptors (LGRs). To date these hormones have been identified in animals from nematodes to humans; the receptors have been traced even further back from sea anemones to humans. In this study we have used bioinformatic techniques to trace the evolution of these hormones and receptors to the earliest metazoans.
From the genome and transcriptome databases of multiple sponge and cnidarian species we have identified homologs for both glycoprotein hormones and LGRs. The hormones share characteristic features with their bilaterian homologs, including the conserved cystine-knot motif. Additional motifs help clarify the relationship of these ancient hormones within a larger group of related cystine-knot proteins, including the insect glycoprotein hormone bursicon, DAN-domain proteins, norrin-disease protein, and sclerostin. Peptide and receptor phylogenetics confirm the homology of the sponge, sea anemone and coral sequences, and allow us to construct an evolutionary history for this group of ancient endocrine and mitogenic factors. In addition, the origin of the relaxin family is considered as relaxin-related LGRs can be found in cnidarians with corresponding insulin/relaxin homologs that may represent a pre-bilaterian origin for this endocrine system as well. (Funded by the Natural Sciences and Engineering Research Council (NSERC) of Canada).

OR12-1

IDENTIFICATION, EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF THE MYOINHIBITING PEPTIDE RECEPTOR IN THE CHAGAS DISEASE VECTOR, RHODNIUS PROLIXUS

Paluzzi, J.P.(1) and Lange, A.B. (1)
(1) Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

Myoinhibiting peptides (MIPs) are a family of insect neuropeptides whose primary structure is characterized by an amidated carboxyl-terminal motif consisting of a conserved pair of tryptophan residues separated by six non-conserved amino acids ($WX_6W\text{-}NH_2$). Interestingly, the MIPs appear to be the ancestral ligands of the sex peptide receptor in the fruit fly *Drosophila melanogaster*, which plays an important role in courtship and reproduction. Recently, several endogenous MIPs were discovered in the Chagas disease vector, *Rhodnius prolixus*, having both conserved ($WX_6W\text{-}NH_2$) and atypical ($WX_7W\text{-}NH_2$) carboxyl-terminal motifs. Physiological functions of MIPs are plentiful and include inhibition of visceral muscle activity; a role that has been illustrated on hindgut in *R. prolixus*. In order to establish novel physiological targets and elucidate actions for the MIPs in *R. prolixus*, we have isolated and functionally-characterized the endogenous MIP receptor and examined the binding affinity of the MIPs with both the typical $WX_6W\text{-}NH_2$ and atypical $WX_7W\text{-}NH_2$ carboxyl-terminal motifs. Interestingly, alternative splicing of the RhoprMIP receptor gene yields two receptor isoforms, namely RhoprMIPr1 and RhoprMIPr2. Notably, only the RhoprMIPr1 isoform, which has a seven transmembrane predicted topology in common with other G protein-coupled receptors, is dose-dependently activated by the endogenous MIPs with EC_{50} values in the nanomolar range. Finally, we utilized RNAi-mediated knockdown of transcripts encoding the MIP prepropeptide as well as the MIP receptor in order to infer novel physiological roles for the MIP neuropeptides in *R. prolixus*. (This research was supported through an NSERC Discovery Grant to A.B.L).

OR12-2

PARACRINE/AUTOCRINE ACTIONS OF EXTRAPITUITARY Growth Hormone in the chicken reproductive system.

Arámburo, C (1), Martínez-Moreno C.G (1), Ahumada-Solórzano, M (1), Harvey, S (2), Carranza, M (1), and Luna, M (1).
(1)Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México. (2) Department of Physiology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada.

It is known that GH plays a role in the control of both female and male reproductive tract development. Acting as an endocrine, paracrine and/or autocrine regulator, GH influences proliferation, differentiation and function of reproductive tissues. We studied GH mRNA and GH protein local expression in both the chicken testis and in ovary, and found it is distributed mainly in germinal and Leydig cells, and in follicular granulosa cells (GC), respectively. GH immunoreactivity was located in the cytoplasm and within the nucleus. We also found co-localization of GH receptor (GHR) in the same locations. Locally expressed GH was found to be heterogeneous, with a 17 kDa variant being predominant. Both testicular and follicular cells in primary cultures were able to synthesize and release GH to the culture medium. Addition of GH (0.1, 1.0, 10 nM) in the media stimulated progesterone production in cultured GCs in a dose-dependent manner (1.5, 2.9, 5.4 times, respectively). This effect was mediated by regulating the expression of cytochrome P450cc mRNA. GH (10 and 1 nM) also stimulated cell proliferation in both testicular cell (70%) and GC cultures (78%) as determined by ^{3}H-thymidine incorporation, respectively. GH immune-neutralization inhibited these effects. Data suggest local GH may have important autocrine/paracrine effects.(Supported by PAPIIT-DGAPA, UNAM (IN208812) and CONACYT, México (118353, 60296, 178335).

Friday, May 24th
14:15 -16:00
II (LIPATA)

NASCE 2013 Symposium XIII:
Neuroendocrine Disruption

Co-chairs:
Chris Martyniuk, University of New Brunswick, CAN
Edward F. Orlando, University of Maryland, USA

S13-1
NEUROENDOCRINE DISRUPTION: UPSETTING SYSTEMS CONTROL
Trudeau, VL, Waye A.
Department of Biology, University of Ottawa, Canada.

A vast array of industrial pollutants, agricultural pesticides, pharmaceuticals and other chemicals are now ubiquitously present in the environment. This includes contamination at all levels, from water to soil to air to animal tissues, including those in humans. Research across vertebrate and invertebrate taxa is revealing significant effects of pollutants on the nervous system. More specifically, it is now clear that many pollutants can affect neuroendocrine neurons and the physiological processes they control. We have previously proposed (Waye & Trudeau. J. Tox Env. Health B, 14:270, 2011) that "neuroendocrine disruption extends the concept of endocrine disruption to include the full breadth of integrative physiology—that is, neuroendocrine disruption is more than just hormones. It is possible that pollutants disrupt numerous other neurochemical pathways, upsetting diverse physiological and behavioral processes". Such upsets in 'systems control' can affect an animal's ability to maintain homeostasis, undergo reproduction, develop normally or cope with classical stress and pathologies. Our definition of neuroendocrine disruption encompasses all physiological targets and subsequently their direct and indirect downstream effects that may impact proper neuroendocrine function. We will explore the impacts of these neuroendocrine effects from the level of an organism to the ecosystem, using several examples (fish, frogs, mammals) to illustrate this emerging field. Our intention is to generate discussion and refinement of the concept of neuroendocrine disruption. (Funding from NSERC-DG, Environment Canada, CWN and uOttawa-URC programs is acknowledged).

S13-2
EFFECTS OF BISPHENOL A ON SEXUALLY SELECTED COGNITIVE TRAITS IN MONOGAMOUS AND POLYGYNOUS PEROMYSCUS SPECIES
Rosenfeld, CS (1)(2)(3)
(1) Department of Biomedical Sciences, University of Missouri, Columbia, MO USA (2) Bond Life Sciences Center Investigator, University of Missouri, Columbia, MO USA (3) Genetics Area Program, University of Missouri, Columbia, MO USA

Sexually selected traits include those regulating intrasexual competition and intersexual choice and many of these traits are programmed by early exposure to steroid hormones. In polygynous deer mice (*Peromyscus maniculatus*), selection for home range expansion during the breeding season has resulted in males exhibiting enhanced spatial navigational abilities than females. We hypothesized that this male behavior would be susceptible to developmental exposure to BPA. Indeed, BPA-exposed male deer mice showed compromised spatial abilities and exploratory behavior. Females engaged in less contact with BPA-exposed males than controls. Another *Peromyscus* species, California mice (*P. californicus*) displays both social and genetic monogamy. No selection for sex differences in spatial learning and memory are apparent in California mice. Rather, male mate guarding and territorial defense are sexually selected traits in these males. Male California mice developmentally exposed to BPA, however, demonstrate reduced territorial marking relative to unexposed males. BPA exposure had no effect on spatial navigational skills in male or female California mice. In California mice, both parents provide essential parental investment. Yet, females, whose offspring was fathered by BPA-exposed males, nursed his pups less than those fathered by control males. BPA-exposed males and their control female partners spent less time in the nest and grooming the pups compared to control pairs. In sum, developmental exposure to BPA can compromise later adult behaviors in a species and sex-dependent manner. By using a socially monogamous animal model, we have the first evidence that developmental exposure to BPA compromises both paternal and maternal behaviors. Comparative animal models that display contrasting reproductive behaviors may allow for sex-specific predictions regarding which traits are sensitive to the effects of BPA, and thereby provide a framework for human risk assessment studies. (This work was supported by a National Institutes of Health Challenge Grant RC1 ES018195 (to CSR) and a University of Missouri College of Veterinary Medicine Faculty Grant Award (to CSR).

S13-3
ENVIRONMENTAL GESTAGENS AND THEIR EFFECTS ON PROGESTERONE RECEPTOR ACTIVATION IN THE FATHEAD MINNOW (*PIMEPHALES PROMELAS*)
Laura E. Ellestad[1], Kyle Stevens[2], Daniel L. Villeneuve[2], Emily F. Goodell[1], Ian G. Chambers[1], Jennifer L. Farmer[1], and Edward F. Orlando
University of Maryland, Department of Animal and Avian Sciences, College Park, MD, [2]USEPA, Mid-Continent Ecology Division, Duluth, MN

Native progestagens are important regulators of reproductive physiology and behavior of teleost fishes. Synthetic progestins are constituents of human contraceptives and hormone replacement therapy, and studies show they can alter fish reproduction. Gestagen is the collective term for native and synthetic progesterone receptor (PR) agonists. At least four PRs are known in teleosts, including the nuclear PR (nPR) and membrane PRs (mPR). Broadly distributed in US freshwater ecosystems, the fathead minnow (FHM) is an important ecotoxicology model and has economic relevance as an aquacultured baitfish. Here, we have cloned and begun to characterize nPR, mPRα, mPRβ, and the splice variants mPRγ-1 and mPRγ-2 in FHM, including tissue specific expression of the PRs in adults as evaluated by RT- and Q-PCR. In both sexes, nPR was detected in the brain, gonad, pituitary, and spleen. Surprisingly, nPR was detected in the male, but not the female, kidney. Of the mPRs, mPRα was most widely expressed and was detected in all tissues of both sexes. In both male and female FHM, mPRβ was detected only in the brain, gonad, and gill. Expression of mPRγ was detected in male and female gill and kidney, and in female intestine. We also examined the functional activity of FHM nPR as measured by activation of an MMTV promoter-driven firefly luciferase reporter construct. Cos-7 cells were transiently transfected with FHM nPR, in combination with the reporter construct, and treated with progesterone (P4), or the fish progestagens: 17,20β-dihydroxy-4-pregnen-3-one (DHP) or 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S). FHM nPR responded to each of the steroids, with DHP > 20β-S > P4. In addition to P4, levonorgestrel activates nPR. P4 has been measured in US surface waters, and levonorgestrel is the most commonly used contraceptive progestin. This is the first evidence that environmental gestagens bind a FHM PR and suggests that PR-mediated reproduction physiology and behavior could be affected.
(We thank J. Cavallin, M. Severson, and K. Jensen for their assistance and the Morris Animal Foundation for a grant to EFO and LEE (D12ZO-046).

S13-4

CHARACTERIZING GENE REGULATORY NETWORKS IN THE BRAIN OF LARGEMOUTH BASS INHABITING RIVERS CONTAINING HIGH LEVELS OF METHYL-MERCURY

Catherine A. Richter(1), Christopher J. Martyniuk(2), Nancy D. Denslow(3) and Donald E. Tillitt(1)
(1) U.S. Geological Survey, Columbia Environmental Research Center, Columbia, MO, USA (2) Department of Biology and Canadian Rivers Institute, University of New Brunswick, Saint John, New Brunswick, Canada (3) Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, USA

Methylmercury (MeHg) is a potent neuroendocrine disruptor. In fish, exposure to MeHg represses reproduction in both sexes, and depresses sex steroid hormone levels, gonad development, and gametogenesis. The molecular targets mediating the neuroendocrine effects of MeHg have not been fully elucidated. The objectives of this study were to investigate the impacts of MeHg exposure in the female largemouth bass (LMB) central nervous system transcriptome. We conducted a laboratory injection experiment with 2.5 µg MeHg /g body weight and a field survey of LMB from three river systems with varying levels of MeHg (St. Mary's, Big Wekiva and Santa Fe Rivers, Florida, USA). Mercury in the blood of LMB collected from St Mary's River was significantly elevated approximately 3-fold (0.1 ng Hg/ml) compared to the other two sites (0.03 ng Hg/ml). A LMB 8 x 15 K microarray was used for hypothalamic (laboratory) and whole brain (field) transcriptomics analysis. Functional enrichment revealed that genes involved in protein folding and targeting, regulation of protein metabolic process, and protein degradation were over-represented. Interestingly, gene set enrichment analysis identified common expression targets between the laboratory experiment and field collected LMB, including homeobox transcription factors PROP paired-like homeobox 1, LIM homeobox 3 and paired-like homeodomain 1 and 2. Individual genes up-regulated in response to MeHg exposure in both the laboratory and field experiments included the cell adhesion proteins cadherin 1 and integrin beta 1, and a myelin proteolipid protein homolog. Lastly, the gene network analysis suggested that MeHg regulated the expression targets of neuropeptide receptor signaling, steroid signaling, and structural components, such as beta-actin, integrins, and stress fibres. This study provides novel data on the molecular effects of MeHg in the brain and generates hypotheses regarding the mechanisms underlying neuroendocrine disruption. (This research was funded by a National Institutes of Health Pathway to Independence Award granted to CJM (K99 ES016767-01A1).

OR13-2

DISRUPTION OF HYPOTHALAMIC VASOPRESSIN, NITRIC OXIDE AND PACAP BY IN UTERO EXPOSURE TO PCBS AND PBDES IN HYPEROSMOTIC STIMULATED RATS.

Leon-Olea Martha (1), Sánchez-Jaramillo Edith (2), Mucio-Ramirez Samuel (1), Sánchez-Islas Eduardo (1), Curras-Collazo Margarita (3), Romero Arteaga Fidelia (4) y Gómez González Berenice (4).
(1) Departamento de Neuromorfología Funcional y (2) Lab. de Neuroendocrinología. Dirección de Investigaciones en Neurociencias. Instituto Nacional de Psiquiatría Ramón de la Fuente M., México. (3) Laboratorio de Neurobiología celular y molecular. Instituto de Biotecnología UNAM. Cuernavaca, Morelos. México. (4) Dept. Cell Biology & Neuroscience, Univ. of California, Riverside, Riverside, CA

Vasopresin (VP) is released from hypothalamo-neurohypophysial system and acts as a hormone to regulate body fluid and cardiovascular functions. Magnocellular neuroendocrine cells within the hypothalamic supraoptic (SON) and paraventricular nucleus (PVN), release VP, in response to hyperosmotic stimulation, from axon terminals located in the neurohypophysis and from central soma/dendrites. Central release of VP serves an autoregulatory role over hormonal VP output. PACAP and nitric oxide (NO) contributes to VP regulation. It was demonstrated that polychlorinated biphenyls (PCBs) markedly inhibit central VP release from SON punches while exaggerating VP hormonal output during hyperosmotic activation. Since this effect may be due, in part, to alterations in synthesis and/or turnover. We examined toxicant effects of PCBs and PBDEs on VP, PACAP and NO immunoreactivity and mRNA expression in the hypothalamic SON and PVN. Pregnant dams were exposed with Aroclor 1254 via diet (30 mg/kg/d; GD 10-19) or DE-71 (30 mg/kg/d; GD 6 to Pn 20; controls received corn oil. At 3 months old, male rats were salt loaded (drinking tap water or tap water with 2% NaCl, X 5 days). Anesthetized rats were fixed, brains processed for VP-neurophysin (VP-NP), PACAP and NOS immunoreactivity (IR). For RT-PCR fresh brains were obtained and freezed (-80ºC). Mean integrated optical density (IOD) values for VP-NP, NOS and PACAP IR was significantly lower in hyperosmotic PCB and PBDE-treated than PCB and PBDE-naïve rats for PVN and SON. The Aroclor-1254 exposure showed significant changes in the mRNA expression levels of VP and PACAP in the PVN and SON. mRNA from Hyperosmotic PCB treated rats showed signal depletion for PACAP and VP in the PVN and SON. In combination, these data suggest that developmental exposure to Aroclor 1254 or DE-71 permanently disrupts physiologically activated neuroendocrine responses, perhaps via effects on NO and PACAP signaling. (Support: INPRFM (MLO) and UCMEXUS (MCC & ML-O).

Saturday, May 25th
10:15 -12:05
INB

NASCE 2013 Symposium XIV:
Endocrinology-Advances Through Genomics, Peptidomics and Related Technologies

Co-chairs:
Natalia García-Reyero Vinas, Mississipi State University, USA
Wei Hu, Institute of Hidrobiology, China

S14-1
A systems toxicology approach to decipher hormetic effects in Daphnia magna
Natalia Garcia-Reyero (1), Jacob Stanley (2), Tanwir Habib (3), Lynn Escalon (2,) Mitch Wilbanks (2), Jerre Simms (2), Pornsawan Chappell (3), Ed Perkins (2)
(1) Institute for Genomics, Biocomputing and Biotechnology, Mississippi State University, Starkville, MS. (2) U.S. Army Engineer Research and Development Center, Environmental Laboratory. 3909 Halls Ferry Road, Vicksburg, MS 39180. (3) Badger Technical Services, 12500 San Pedro Avenue, Suite 450, San Antonio, TX 78216

A hormetic response is characterized by an opposite effect at small and large doses of toxicant exposure, often having a beneficial effect at low concentrations. Here, we used an integrated, systems toxicology approach to decipher hormetic mechanisms in *Daphnia magna*. *Daphnia magna* were exposed to the energetic compound trinitrotoluene (TNT) for 21 days and several endpoints such as number of neonates, length, growth and survival were measured. TNT elicited hormetic responses in reproduction and growth at very low concentrations. In order to elucidate the mechanisms leading to hormesis, microarray analysis was performed at 0.004 (hormetic), 0.12 (sometimes hormetic), and 1.85 (toxic) mg/L TNT. Functional and transcriptional analyses identified concentration dependent differentially expressed genes that suggested the involvement of lipid metabolism in hormetic responses. Lipidomic analysis of the TNT exposed *Daphnia* supported the hypothesis that TNT exposure affected lipid metabolism, This analysis leads us to conclude that TNT hormetic effects are related to increases in some polyunsaturated fatty acids and ecosanoids known to be involved in *Daphnia* growth and reproduction. This conclusion is consistent with observations that high levels of TNT and other nitroaromatics change fatty acid metabolism in fathead minnow, Bobwhite quail, and rats.(This work was funded by the US Army Environmental Quality Research Program (including BAA 11-4838).

S14-2
GROWTH HORMONE TRANSGENESIS AFFECTS NEUROENDOCRINE REGULATION AND REPRODUCTION IN THE COMMON CARP CYPRINUS CARPIO L.
Mengxi Cao, Ji Chen, Wei Peng,, Zuoyan Zhu, Wei Hu
State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

The interaction between growth and reproduction has been focus of research in the field of fish neuroendocrinology. GH transgenic carp showed delayed gonadal development compared with non-transgenic carp. We used GH-transgenic carp to study the relationship between GH and the HPG axis. We found that the kiss and gnrh-III expression was upregulated while the gthα, fshβ, and lhβ expression was downregulated during early gonadal development in transgenic carp. Throughout the experiment, LH secretion was inhibited in transgenic carp. Pituitary containment of LHβ decreased and the distribution of LHβ cells reduced in transgenic carp. An in vitro incubation experiment was performed, and exogenous GH was found to induce kiss and gnrh-III transcription while inhibiting gthα, fshβ, lhβ transcription. The study suggested GH interacted with the HPG axis at the hypothalamus and the pituitary level to regulate fish reproduction. LH deficiency is the main cause of delayed reproductive development in GH-transgenic carp. (This work was supported financially by National Natural Science Foundation (Grant No.30930069), the Key Research Program of the Chinese Academy of Sciences (Grant No. KSCX2-EW-N-004), the"863" High Technology Project (Grant No. 2011AA100404) and the Development Plan of the State Key Fundamental Research of China (Grant No. 2010CB126302).

OR14-1
ESTROGENIC OVARIAN CELL SUBPOPULATIONS STIMULATE CELL PROLIFERATION OF GERM CELLS AND ANDROGENIC CELLS OF 18-DAY-OLD CHICK EMBRYO.
Velázquez P.N., Peralta D.I., and Hernández GE.
Departmento de Biología Celular y Tisular, Facultad de Medicina, Universidad Nacional Autónoma de México, México D.F. 04510.

In recent years, exciting progress has been made towards unraveling the complex intraovarian control mechanisms that, in concert with systemic signals, coordinate early gonadal development and differentiation as well as synthesis of steroid hormones and posnatally, the recruitment, selection and growth of follicles from the primordial stage through to ovulation and corpus luteum formation. The aim of the present study was to evaluate the proliferative effect of rhFSH on four different cell subpopulations (S_1,S_2,S_3 and S_4), isolated from the ovary of 18-day-old chick embryo by means of subsequent metrizamide gradients (0-15%). Dissociated cells from the whole ovary and cell subpopulations obtained by metrizamide gradients were cultured for 60h on polycarbonate membranes, which was floating on 2 ml of DMEM-BSA plus 0.1 µCi of ^{3}H-thimidine, rhFSH (1 IU/ml) was added to the medium at the beginning of the culture without any subsequent change of medium or replenishment. At the end of the culture period, polycarbonate membranes with the cellular aggregates were processed for ^{3}H-thimidine measurement as an indicator of cell proliferation. The results showed that rhFSH had no proliferative effect in the typical androgenic cells isolated in S_1 (d= 1.012 g/ml). Primary oocytes present in S_2 (d= 1.037 g/ml) did not show any effect induced by hormonal treatment. S_3 (d= 1.055 g/ml) and S_4 (d= 1.071) which contained a mixture of prefollicular and poorly differentiated epithelial cells were the cellular subpopulations that showed an increment in cell division induced by rhFSH. On the other hand when S_1 or S_2 were mixed with S_4, there was an increase in cell proliferation in non stimulated or treated group with rhFSH. According to the results obtained we can suggest that estrogenic cells could synthesize some proteins or growth factors that could modulate the cellular division of germ cells and androgenic cells in early gonadal development of the birds.

OR14-2
ROLE OF GNIH IN PARACRINE CONTROL OF GONADAL FUNCTION IN GOLDFISH

M. Moussavi[1], M. Wlasichuk[1,2], J. P. Chang[1,2], H. R. Habibi[1a]
[1] Department of Biological Sciences, University of Calgary; [2] Department of Biological Sciences, University of Alberta

Gonadotropin-inhibitory hormone (GnIH) was named because of its ability to inhibit gonadotropin (GTH) production in avian species and mammals. Subsequently, variants of GnIH peptides have been discovered in different vertebrate species and demonstrated to both stimulate and inhibit the release of gonadotropins from fish pituitary. In our studies, we observed the expression of GnIH and GnIH receptors in the goldfish ovary and testis, indicating a possible role in the regulation of gonadal function. To investigate this possibility, we tested direct actions of native goldfish GnIH (gGnIH) on cultured ovarian follicles and testis, *in vitro*. We tested the hypothesis that GnIH plays a role in paracrine control of gonadal function in goldfish. The effects of GnIH on basal and hCG induced changes in gonadal gene expression was tested in goldfish at early and mid stages of gonadal recrudescence. Treatment with gGnIH alone resulted in sexually dimorphic and seasonally dependent changes in gene expression pattern. Furthermore, we used ^{1}H-NMR metabolomics to detect the effects of GnIH on metabolic patterns of goldfish testis and ovary. Our results provide evidence for the role of GnIH in the control of gene expression and demonstrated sex specific changes in the expression of GnIH, LH, FSH, estrogen, androgen receptors as well as steroidogenic acute regulatory protein, aromatase, activin βA, and follistatin mRNA levels. The results of gene expression and metabolomics results collectively provide a framework for better understanding of the physiological significance of GnIH as a paracrine regulator of gonadal function in goldfish and other vertebrates. Supported by NSERC of Canada.

OR14-3
THE ROLES OF CRUSTACEAN CARDIOACTIVE PEPTIDE IN THE VECTOR OF CHAGAS' DISEASE, *RHODNIUS PROLIXUS*

Lee, DH (1) and Lange, AB (1).
(1)Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

In insects, ecdysis is an important feature of growth and development and is tightly controlled by a variety of peptide hormones such as ecdysis triggering hormone, eclosion hormone, crustacean cardioactive peptide (CCAP) and bursicon. CCAP, a cyclic nonapeptide (PFCNAFTGCamide), is highly conserved in insects and other arthropods and is one of the key neuropeptides that executes ecdysis by turning on motor neurons involved in ecdysis behaviour. In our research, we have cloned CCAP and its receptor genes from *Rhodnius prolixus* central nervous system (CNS). We have described the expression patterns of RhoprCCAP gene and the distribution of neurons and their processes containing CCAP-like immunoreactivity using *in situ* hybridization and immunohistochemistry respectively. Recently, we expressed the RhoprCCAP receptor (RhoprCCAPR) in Chinese hamster ovary (CHO) W11 cells and showed that RhoprCCAPR is activated by RhoprCCAP with an EC_{50} value of 15.03nM. By using real time PCR, we found that expression of RhoprCCAP and its receptor genes increased prior to ecdysis in the CNS and peripheral tissues of 4th and 5th instar *R. prolixus*. Also, we found that RhoprCCAP increases heartbeat frequency in a reversible, dose-dependent manner, with threshold close to 10^{-11}M and maximum response at 10^{-10}M CCAP. *In vivo* heartbeat frequency in dsCCAPR treated bugs (28.03 ± 2.70 beats/min, n =10) was lower than in the control group (40.68 ± 1.77 beats/min, n =10). Moreover, we have found that RhoprCCAP plays crucial roles in *R. prolixus* ecdysis since 80% of *R. prolixus* with knocked-down RhoprCCAP and its receptor transcripts fail to undergo ecdysis. (We acknowledge gratefully funding by NSERC and OGS).

Saturday, May 25th
10:15 -12:05
II (LIPATA)

NASCE 2013 Symposium XV:
Hormone Receptors Critical in Development and Reproduction

Co-chairs:
Yong Zhu, East Carolina University, USA
Xanthe Vafopoulou, York University, CAN

S15-1
GPCR-MEDIATED RAPID, NON-GENOMIC ACTIONS OF STEROIDS IN DROSOPHILA
Peter D. Evans (1), Asha Bayliss (1) and Vincenzina Reale
(1),The Inositide Laboratory, The Babraham Institute, Babraham Research Campus, Cambridge, UK.

Steroid hormones classically mediate their actions by binding to intracellular receptor proteins that migrate to the nucleus and act as transcription factors to change gene expression. However, evidence is now accumulating for rapid non-genomic effects of steroids. There is considerable controversy over the mechanisms underlying such effects. Evidence has been presented for direct actions of steroids on cell surface ion channels and on classical steroid activated transcription factors localized at, or close to, the plasma membrane. Further, in a number of cases evidence has been presented for the direct activation of G-protein coupled receptors (GPCRs) by steroids either at the plasma membrane or at intracellular locations. Here, we will focus on the non-genomic actions of ecdysteroids on a Drosophila GPCR, DopEcR (CG18314), which can be activated by both ecdysone and the catecholamine, dopamine. We will also point out parallels between this system and the activation of the vertebrate GPCR, GPR30 (GPER), which is thought to be activated by 17β-estradiol. DopEcR, heterologously expressed in either Chinese hamster ovary cells or insect Sf9 cells, can be activated by dopamine to produce increases in intracellular cyclic AMP levels and the activation of the PI3Kinase pathway, effects which can be blocked by ecdysteroids. In addition, the receptor can be induced by ecdysteroids to produce activation of the MAPKinase pathway. Evidence from a number of laboratories is now uncovering modulatory effects for DopEcR on a range of Drosophila behaviours. The role of GPR30 in vertebrates is also controversial, as is its cellular expression pattern and its apparent pharmacology. We have investigated its pharmacological activation when heterologously expressed in Xenopus oocytes. We propose that the cellular localization and signalling properties of both DopEcR and GPR30 may be cell specific and depend upon their interactions with both accessory molecules and signalling pathways. (Supported by the BBSRC via the Babraham Institute).

S15-2
MITOCHONDRIAL INVOLVEMENT IN THE NON-GENOMIC ACTIONS OF INSECT STEROID HORMONES
Vafopoulou, Xanthe.
York University, Canada

Insect steroid hormones, ecdysteroids, exert both genomic and non-genomic actions. We provide evidence here that both actions are mediated by the ecdysteroid receptor, EcR. Using *Rhodnius prolixus* (Hemiptera), we showed that genomic actions involve circadian cycling of EcR between cytoplasm and nucleus using microtubules as tracks for nucleocytoplasmic shuttling. Here, we report localization of EcR in mitochondria in the perinuclear and sub-plasmalemmal cytoplasm of several cell types and axons of brain neurons, using both Western blots of subcellular fractions and confocal immunohistochemistry using mitochondrial markers. Disruption of the microtubules with colchicine causes dispersal of the mitochondria aggregates, showing that mitochondria are intimately connected to microtubules. However, EcR remains associated with these dispersed mitochondria, confirming their close relationship. Ecdysteroid treatment of brains *in vitro* induced appearance of EcR in mitochondria, concomitantly with an increase in COX I protein. We infer a role of EcR in regulation of mitochondrial gene expression. These findings align EcR with various vertebrate steroid receptors, which are known to mediate non-genomic actions of steroids on mitochondria.

S15-3
NONGENOMIC PROGESTIN RECEPTORS AND THEIR SIGNALING IN ZEBRAFISH MODEL
Yong Zhu
Department of Biology, East Carolina University, 1000 E. 5th St. Greenville, NC 27858, USA

The receptor for deciphering extracellular progestin signaling and initiating meiosis resumption in vertebrate oocytes has been hotly contested as accumulated evidence suggested GPCR-like membrane progestin receptors (mPRs) and the nuclear progestin receptor (nPR or Pgr) are likely candidates. The nPR transcript and protein were expressed abundantly in follicular cells that were surrounding stage IV oocytes in zebrafish. The most significant daily changes of nPR transcript were observed in stage IV follicular cells, with the highest level observed just prior to ovulation. In contrast, the expressions of mPRα and mPRβ transcripts and proteins were abundant and increased significantly in late stage denuded oocytes prior to oocyte maturation. Moreover, over-expression of mPRα in follicle-enclosed oocytes significantly increased the activity of MAPK, the production of cyclin B protein, and the number of oocytes that underwent oocyte maturation without exogenous progestin, while over-expression of mPRβ or nPR alone had no such effect. Intriguingly, significant acceleration of oocyte maturation was observed when follicle-enclosed oocytes were incubated with the maturation inducing steroid, 4-pregnen-17,20β-diol-3-one (DHP) following over-expression of nPR or mPRα. Interestingly, this acceleration in oocyte maturation was observed approximately 1h later in oocytes over-expressing nPR compared to those over-expressing mPRα. Importantly, the acceleration of maturation in the nPR injected group was blocked by treatment with the transcription inhibitor actinomycin D, implying requirement of the genomic signaling pathway, while the same treatment did not affect the accelerated rate of maturation in mPRα injected oocytes. We are conducting additional experiments including generating and characterizing receptor knockouts and transgenic lines in order to establish precise roles of nPR and mPRs in nongenomic progestin signaling in the zebrafish model.

OR15-1

GROWTH HORMONE (GH) AND RETINAL GANGLION CELL FUNCTION: QNR/D CELLS AS AN EXPERIMENTAL MODEL

Martínez-Moreno CG, Andres A, Giterman D, Harvey S.
Department of Physiology, University of Alberta, Edmonton T6G 2H7 Canada

Retinal ganglion cells (RGCs) have been shown to sites of growth hormone (GH) production and GH action in the embryonic (embryo day 7, ED7) chick neural retina. Primary RGC cell cultures were previously used to determine autocrine or paracrine actions of GH in the retina, but the antibody used in their immunopanning (anti-Thy-1) is no longer available commercial. We have therefore characterized an immortalized neural retina (QNR/D) cell line derived from ED7 embryonic quail as a replacement experimental model. These cells express the GH gene and have GH receptor (GHR)-immunoreactivity. They are also immunoreactive for RGC markers (islet-1, calretinin, R4A) and neural fibers (neurofilament, GAP 43, vimentin) and they express the genes for Thy-1, neurotrophin 3 (NTF3), neuritin 1 (NRN1) and brn3 (POU4F). These cells are also electrically active and therefore resemble the RGCs in the neural retina. They are also similarly responsive to exogenous GH, which induces overexpression of the neurotrophin 3 and insulin-like growth factor (IGF) 1 genes and stimulates cell survival, as in the chick embryo neural retina. QNR/D cells are therefore a useful experimental model to assess the actions of GH in retinal function. (Supported by NSERC).

OR15-2

GH LOCUS EXPRESSION IN THE EYE OF HIGHER PRIMATES

Pérez-Ibave DC (1) , Rodríguez-Sánchez IP (2), Garza-Rodríguez ML (1), Mohamed-Hamsho J (3), Cavazos-Adame H (3), Mohamed-Noriega J (3), Arámburo C (4), Bautista de Lucío VM (5), Matsui R (5), Barrera-Saldaña HA (1)(6), Luna M(4).
(1)Department of Biochemistry and Molecular Medicine, (2) Department of Genetics, (3) Department of Ophthalmology, University Hospital "Dr. José Eleuterio González " Medicine School, Autonomous University of Nuevo León, Monterrey 64460 , México,.(4), Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Querétaro 76230, México. (5) Institute of Ophthalmology "Fundación de Asistencia Privada Conde de Valenciana IAP" Chimalpopoca 14, Colonia Obrera, México D.F. 06800, (6)Vitaxentrum. Blvd. Puerta del Sol 1005, Colinas de San Jerónimo, Monterrey, Nuevo León 64460, México.

In humans the growth hormone (GH) locus is pentagenic located in chromosome 17, harboring five genes: two of GH type (GH-N and GH-V) and three of chorionic somatommamotropin (CSH) type (CSH-L, CSH-1 and CSH-2). GH-N gene is expressed specially in the somatotrophs of the anterior pituitary gland, while the remaining four genes are expressed in the syncytiotrophoblast of the placenta. GH expression in extra-pituitary tissues has recently been reported, being one of them the eye. We are investigating the molecular biology of this *locus* in humans and baboons eyes. We quantified transcripts derived from the locus (from GH-N, GH-V, CSH-1, CSH-2) and from the GH receptor (GHR) gene, using TaqMan probes in different ocular tissues (sclera, cornea, retina, trabecular meshwork, iris, lens, conjuctiva, optic nerve, choroid and muscle eye). For immunohistochemistry assays, tissues were fixed in 4% paraformaldehyde and incubated with anti-GH antibodies and anti-human GHR, which were observed by fluorescence microscopy. We collected 152 tissues (we analyzed at least three different samples for each type of ocular tissue). Six fresh complete eyes from adult baboon and two complete eyes of baboon embedded in paraffin (one of fetus and one of an adult). In human eyes we found the GH-N mRNA in: choroid, retina and trabecular and GHR mRNA in: conjunctiva, cornea, choroid, iris, retina and tenon. Baboon GH-N gene expression was documented by RT-PCR in retina of fetal and adult eyes, showing that the baboon retina GH mRNA sequence is identical to its pituitary counterpart. As in humans, we did not find the expression of other gene members of the GH locus (CSHs and GH-v genes) in this ocular tissue. The above findings are unprecedented in both human and baboon. We conclude that this hormone may be acting in retina in an autocrine and paracrine manner. (We appreciate the donation of tissues by Dr. Peter W. Nathanielsz, from the Center for Pregnancy and Newborn Research, University of Texas Health Science Center Medical School, Department of Obstetrics and Gynecology, San Antonio, TX, USA. IPRS enjoyed an SNI assistantship and student fellowship to visit the SFBR. This work was supported by grants from the Mexican Council of Science and Technology, CONACyT (CB-2011-01/167697), UANL's PAICyT (SA972-04).

Saturday, May 25th
10:15 -12:05
CAC

NASCE 2013 Symposium XVI:
Diabetic Models Across Species-Lessons From a Common Pathway

Co-chairs:
Juan Riesgo, INB-UNAM, MEX
Amira Klip, The Hospital for Sick Children, CAN

S16-1
INSULIN SIGNALS IN SKELETAL MUSCLE REGULATING GLUCOSE TRANSPORTER GLUT4
Klip, A.
Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada

Skeletal muscle is the major site for glucose disposal following a meal. Glucose enters skeletal muscle through the GLUT4 glucose transporter. Insulin causes a net gain in surface GLUT4 units in muscle. This is brought about through a redistribution of GLUT4 from intracellular compartments to the plasma membrane, and this process fails in insulin resistance accompanying –and often causing- type 2 diabetes. These signals trigger the mobilization of GLUT40containing vesicles, all the way to vesicle docking and fusion with the plasma membrane. Insulin binding to its receptor at the cell surfaces leads to the phosphorylation of the Insulin Receptor Substrate 1, which binds and activates phosphatidyl inositol 3-kinase. –Downstream of this enzyme, signalling bifurcates to activate Akt on the one hand, and to activate Rac-1 that causes actin remodelling on the other. Both signalling arms are required for GLUT4 translocation. Akt phosphorylates the Rab-GAP AS160, allowing its target Rabs on GLUT4 vesicles to become active and interact with their effectors. In muscle cells, the insulin-stimulated Rabs that lie downstream of AS160 are Rab8A and Rab13. Active Rab8A-GTP interacts with its effector myosin Va, to allow GLUT4 vesicle interaction with actin filaments. Active Rab13-GTP interacts with the linker MICAL-L2, which in turn links to actinin-4, close to the plasma membrane. The concomitant insulin-induced actin remodelling, triggered by activated Rac-1, provides the cortical actin filaments that bind actinin-4. This allows the vesicles to concentrate in the cortical region. Here, a final event involves the tethering of vesicles through myosin 1c to actin. Collectively, this series of steps culminates in the interaction of VAMP2 on the GLUT4 vesicles with syntaxin-4 and SNAP-23 on the membrane, for the final insertion of GLUT4 into the membrane. Identifying which steps fail in insulin resistance should provide potential therapeutic targets to prevent type 2 diabetes. (This work was supported by the Canadian Institutes of Health Research).

S16-2
The Insulin/insulin like growth factor Pathway is a Mechanism of Extreme Growth and Reliable Signaling in Sexually Selected Ornaments and Weapons
Douglas J. Emlen (1) Ian A. Warren (2) Annika Johns (1) Ian Dworkin (3) Laura Corley Lavine (2)
(1) Division of Biological Sciences, The University of Montana, 104 Health Science Building, Missoula, MT 59812, USA. (2) Department of Entomology, Washington State University, Pullman, WA 99164–6382, USA. (3) Department of Zoology, Michigan State University, East Lansing, MI 48824, USA.

Many male animals wield ornaments or weapons of exaggerated proportions. We propose that increased cellular sensitivity to signaling through the insulin/insulin-like growth factor (IGF) pathway may be responsible for the extreme growth of these structures. We document how rhinoceros beetle horns, a sexually selected weapon, are more sensitive to nutrition and more responsive to perturbation of the insulin/IGF pathway than other body structures. We then illustrate how enhanced sensitivity to insulin/IGF signaling in a growing ornament or weapon would cause heightened condition sensitivity and increased variability in expression among individuals—critical properties of reliable signals of male quality. The possibility that reliable signaling arises as a by-product of the growth mechanism may explain why trait exaggeration has evolved so many different times in the context of sexual selection.

S16-3
THE INSULIN PATHWAY IN DROSOPHILA AS A MODEL FOR DIABETES
Riesgo-Escovar, J. R. (1), Peña-Rangel, M.T. (1), Reyes De la Torre, A. (1), Maldonado-Murillo, J. M. (1).
(1) Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UNAM Juriquilla, Querétaro, Querétaro, México.

The insulin pathway is an evolutionarily conserved intercellular signaling pathway amongst metazoans. Despite differences in lifestyle, adult size, and metabolism, the insulin pathway has proven to be a common key regulator of growth at the cell, organ, and organismal level. The main genes of the pathway are highly conserved, and constitute a core set of molecular machinery, perhaps best exemplified by *chico*, the *Drosophila* homolog of vertebrate IRS genes. Besides this, the pathway also controls metabolism and other bodily functions. We show, using viable mutant alleles and heteroallelic mutant combinations of different key genes of the pathway, that total lipid and carbohydrate content are affected, and that these affections can be traced throughout adult life and larval stages. In these mutants, nervous system function is affected as well: We quantitated electroretinograms in response to flashes of light, and found that they were reduced, as were also the activity of cholinesterase enzymes from fly heads. Since acetylcholine is the main excitatory neurotransmitter in the fly brain, we take this to evidence general nervous system phenotypes. We will discuss benefits and pitfalls of this genetic model for diabetes.(We acknowledge support from CONACYT, PAPIIT and UNAM to JRR-E.)

OR16-1
ANALYSIS OF PITUITARY PROLACTIN SECRETION IN TWO RAT DIABETES MODELS: ROLE OF TUMOR NECROSIS FACTOR ALPHA AND TRANSFORMING GROWTH FACTOR BETA.
Lemini, M, Vega, M, Ruíz-Herrera, X, Martínez de la Escalera, G, Macotela, Y, Clapp C.
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México.

Prolactin (PRL) may play a role in diabetes and its complications. It acts on pancreatic β cells to stimulate their proliferation, survival, and insulin production, and it inhibits retinal vascular alterations associated with diabetic retinopathy (DR). Serum PRL decreases in diabetic patients with severe DR, suggesting a role of factors able to inhibit anterior pituitary (AP)-PRL secretion. Here, we show that the levels of AP mRNA and circulating PRL are reduced in the type-2 diabetes (T2D) model of male rats fed a high-fat diet, which are hyperglycemic and insulin resistant. Moreover, the AP PRL mRNA is also reduced in streptozotocin-induced type-1 diabetic (T1D) male rats with confirmed hyperglycemia. Given that tumor necrosis factor-α (TNF-α) stimulates and transforming growth factor-β (TGF-β)

inhibits AP-PRL expression, we hypothesized that changes in TNF-α and TGF-β expression in the AP could influence PRL secretion in diabetes. The AP mRNA levels of TGF-β were up-regulated in both diabetes models, although those of TNF-α were lower in T2D and higher in T1D. Moreover, these factors antagonized each other´s effect on PRL synthesis and release by the GH4C1 lactotroph cell line. TNF-α stimulated and TGF-β inhibited PRL mRNA and protein levels in cell lysates and conditioned medium. The effects of all doses of TNF-α and TGF-β were blocked by co-incubation with a single concentration of TGF-β (10 ng/ml) and TNF-α (50 ng/ml), respectively. Although NFκB is a key transcription factor mediating AP-PRL induction by TNF-α, TGF-β did not modify TNFα-induced mRNA up-regulation of the NFκB inhibitor IκB-α or its protein degradation, suggesting that TGF-β counteracts TNF-α stimulation by mechanisms independent of IκB-α. In conclusion, we suggest that up-regulation of TGF-β in the AP reduces PRL secretion in diabetes by counteracting the stimulatory effect of TNF-α. Experiments are underway to characterize the molecular basis of TGF-β and TNF-α interaction. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant S0008-161594 and by PAPIIT-UNAM grant IA200113. We thank F. López-Barrera, G. Nava, D. Mondragón, and A. Prado for their technical assistance).

OR16-2

ANTIAPOPTOTIC EFFECTS OF GROWTH HORMONE ARE MEDIATED BY PI3K/AKT PATHWAY IN THE CHICKEN BURSA OF FABRICIUS

Luna-Acosta, JL, Alba-Betancourt, C, Martínez-Moreno, C, Ramírez, C, Luna, M, Carranza, M, and Arámburo C.
Departamento de Neurobiología Celular y Molecular. Instituto de Neurobiología. Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México.

Growth hormone (GH) has several effects on the immune system. In birds, it has been shown that GH is involved in B cell differentiation and maturation in the bursa of Fabricius (BF). This unique avian lymphoid tissue is able to express and produce GH,, which suggests a paracrine and/or autocrine modulator role for the hormone. We have shown GH mRNA expression and a cell specific GH distribution pattern (mainly on B cells and epithelial cells) during BF development that indicates a functional role in this organ. Thus we studied the antiapoptotic effects of GH in BF and the intracellular pathways that may be involved. Bursal cell cultures were prepared to study the effect of GH on cell viability and apoptosis. Results showed that treatment with 10 nM GH significantly increased B cell viability (16.7±0.67 %) when compared with the untreated control. On the other hand the presence of apoptotic bodies (TUNEL) dramatically decreased after GH treatment. Likewise, caspase-3 activity was significantly inhibited (by 40±2.0%) in these cultures. The addition of 10μM wortmaninn (a PI3K/Akt inhibitor) blocked the GH protective effects. These data indicate that GH antiapoptotic activity might be mediated by the PI3K/Akt pathway. (Supported by CONACyT (F1-60296, 118353, 178335) and PAPIIT-DGAPA-UNAM (IN-208812, 206813). We acknowledge the technical support of Gerardo Courtois).

NASCE 2013 POSTER PRESENTATIONS ABSTRACTS

TOPIC: BIOLOGICAL RHYTMS

P-1.

FUNCTIONAL ADAPTATIONS OF THE GHRELIN, GH-IGF-1 AXIS DURING DAYTIME FOOD SYNCHRONIZATION IN RATS

Arellanes-Licea, E (1), Báez-Ruiz, A (1), Carranza, M (1), Ávila-Mendoza, J (1), Arámburo, C (1), Luna, M (1), and Díaz-Muñoz, M (1).

(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México.

Feeding time is the dominant zeitgeber for peripheral oscillators. In mammals, a circadian food entrainable oscillator (FEO) emerges ensuing daytime restricted feeding, driving behavioral and physiological adaptations. The mechanisms underlining the physiology of the FEO are not entirely elucidated. The interrelation of signals involving fasting, feeding and the nutritional status, could be implicated in the FEO onset. We explored if the activity of the axis ghrelin, growth hormone (GH)-insulin-like growth factor-1 (IGF-1) could be playing a role in the FEO expression. In rats fed Ad libitum (AL) and with restricted feeding schedule (RF, food access from 12:00 to 14:00h) we determined nutrient-handling peripheral organs somatometry, serum levels of ghrelin isoforms, GH, IGF-1, as well as the content of these hormones in stomach, hypophysis and liver. Also, we studied the quantitative mRNA expression of pituitary GH and by Western blot clock gene BMAL1 protein level in the tissues. We observed a hyperphagia, stomach distention and delayed gastric emptying during RF. The weight of the pituitary gland did not change. The liver size showed a phase shift towards the dark phase and epididymal white adipose tissue (WAT) was reduced ~32% in RF. Total and unacylated ghrelin (UAG) significantly increased with a modest reduction of stored total ghrelin during RF. The ratio UAG to acylated ghrelin was 66% higher in RF. GH secretion in RF decreased 32% and showed a rhythmic pattern, along with 30% reduction of IGF-1 release. Diminished pituitary GH levels were observed in RF without changes in hepatic IGF-1. RF promoted a phase shift of BMAL1 daily levels. In the condition of daytime RF, these hormones could be acting as integrators of the metabolic rheostatic adjustments during the FEO expression, covering slow stomach emptying, catabolism of lipids from WAT, changes in hepatic triacylglycerols and a decline of anabolic functions towards the efficacious handling of nutrients. (Work supported by grants: PAPIIT IN201209, IN210209, IN208812; FONCICYT 91984 and CONACYT 49047, 178335).

P-2.

REPRODUCTION AND FERTILITY IN THE ARRHYTHMIC SIBERIAN HAMSTER (*PHODOPUS SUNGORUS*)

Erin J. Cable(1), Xiaoli Peng (2), Sandra J. Legan(2) Brian J. Prendergast(1,3)

(1) Department of Psychology, Integrative Neuroscience Program, University of Chicago, Chicago, IL 60637, U.S.A.

(2) Department of Physiology, University of Kentucky, Lexington, KY, 40536, U.S.A.

(3) Committee on Neurobiology, University of Chicago, Chicago, IL 60637, U.S.A.

The circadian timing of behavior and physiology has implications for reproduction and survival. A circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) gates the preovulatory LH surge and the onset of behavioral estrus to occur shortly before the active phase. In several rodent species, the LH surge or normal estrous cycles require a functional SCN, which dictates a circadian gate during which LH surges may occur. To circumvent limitations inherent in lesion and transgenic models, we used a hamster model of SCN arrhythmia generated noninvasively to investigate consequences of complete circadian desynchrony on multiple aspects of female reproduction. In Siberian hamsters (*Phodopus sungorus*), brief nocturnal light treatments were used to eliminate circadian locomotor activity rhythms and to determine if behaviorally arrhythmia led to deficits in reproductive neuroendocrine function. Spontaneous 4 day cycles in serum LH were indistinguishable between normally-entrained (ENTR) and circadian arrhythmic (ARR) females. In ovariectomized, steroid-clamped females, LH surges (when evident) were comparable in amplitude between ENTR and ARR hamsters but, in contrast to those of ENTR hamsters, occurred at random times of day in ARR females. ARR females also exhibited higher thresholds for steroid induction of LH surges. Fertility was assessed in multiple paradigms (5 and 90 cohabitation), and the latency to mate, number of pups, survival to weaning, pup weight were all comparable in ARR and ENTR females. Persistent reproductive function and the presence of LH surges in hamsters lacking a functional circadian system suggest a circadian-independent mechanisms can sustain reproductive neuroendocrine function in this species.

P-3.

IS THE PINEAL GLAND OF THE NEOTROPICAL CATFISH "SIERRA NEGRA" (*OXYDORAS SIFONTESI*) A PHOTORECEPTIVE STRUCTURE? EVIDENCE FROM EX VIVO PINEAL GLAND CULTURES.

Hilda Y. Guerrero(1), Rubén Carvajal(1), Anabel Espinoza-Luna(1), Elizabeth Cardillo(1), Germán Poleo(2), Daissy Marcano(1)

(1) Laboratorio de Neuroendocrinología Comparada, Escuela de Medicina "José María Vargas" e Instituto de Medicina Experimental, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. (2)Decanato de Agronomía, Estación de Piscicultura, Universidad Centroccidental, "Lisandro Alvarado", Barquisimeto, Venezuela

In some teleosts, the pineal organ performs three functions: photoreception, generation of circadian rhythms and production of melatonin (MEL). In neotropical teleosts, however, the physiology of this structure has been barely studied. In the present work we examined this aspect in the Venezuelan catfish Oxydoras sifontesi. O. sifontesi shows a well developed pineal window with the pineal vesicle (PV) located immediately below it. Histological study of PV reveals a multilamellar system resembling the outer segment of photoreceptive pinealocytes. In vivo studies, performed under controlled photoperiod conditions (CPC), show that plasma melatonin rises during the subjective night, suggesting the presence of endogenous melatonin circadian rhythm. The aim of the present study was to determine whether MEL synthesis is controlled by photoreceptive pinealocytes in this teleost species. Experimental procedure included PV static cultures during five continuous days with 3 alternated CPC (LD: 12h Light/12h Dark; DD: 12h Dark/12h Dark; LL: 12h Light/12h Light). Every 3 hours, culture medium was renewed and MEL concentration was quantified by HPLC coupled to fluorescence detection. A rhythmic MEL secretion was found in cultivated PV from O. sifontesi, with lower MEL secretion values when compared to those reported for other teleost. The highest concentrations of MEL were found during the scotophase in LD cycles, with a periodicity about 24h. However, results for DD and LL cycles were variable. Although preliminary, these results suggest that MEL synthesis in the pineal organ of O. sifontesi presents a cyclic pattern of secretion associated to illumination conditions. However, due to variable data observed in constant conditions (DD and LL), we could not assign to PV a major role in the generation of circadian cycle of plasma melatonin in this species.

TOPIC: BRAIN AND BEHAVIOR

P-4.

EFFECTS OF *HYPERICUM CAPRIFOLIATUM* CHAM & SCHLTDT (GUTTIFERAE) EXTRACT AND OF TWO ESTABLISHED ANTIDEPRESSANTS ON BASAL AND STRESS-INDUCED INCREASE IN SERUM AND BRAIN CORTICOSTERONE LEVELS.

do Rego, JC (1)(2)(3), Viana, FA (4), Costentin, J (1)(2), Rates, SMK (4).

(1) Institute of Research and Innovation in Biomedicine (IRIB), University of Rouen, Rouen, France (2) Platform of Behavioral Analysis (SCAC), Faculty of Medicine and Pharmacy, University of Rouen, Rouen, France (3) National Center of Scientific Research CNRS, France (4) Programa de Pós-Graduação em Ciências Farmacêuticas, Faculty of Pharmacy, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Since depressive patients present alterations in the hypothalamo-pituitary-adrenal (HPA) axis that are normalized by antidepressants, this HPA axis has been considered as a target of their actions. We have investigated the mechanism of action of a cyclohexane extract of *Hypericum caprifoliatum* (HCP), which displays antidepressant-like activity, by studying, in mice, the influence of HCP and of two established antidepressant drugs**,** imipramine and bupropion, administered either acutely or semi-chronically (once a day, three consecutive days), on serum and brain cortex corticosterone levels, either in basal conditions or shortly after a forced swimming test (FST). Administered acutely, imipramine (20 mg/kg, per os (p.o.)), bupropion (30 mg/kg, p.o.) and HCP (360 mg/kg, p.o.) reduced significantly the immobility time and had no effects on FST-induced increase of serum and cortical corticosterone levels. Conversely, three days repeated treatment with imipramine or bupropion resulted in a significant reduction of immobility time and FST-induced increase of serum and cortical corticosterone levels. In a different way, repeated treatment with HCP significantly reduced the immobility time and only cortical corticosterone levels in stressed mice. These results indicate that short-term treatments with antidepressants are sufficient to induce modifications in the HPA axis reactivity to stress; and that apparently HCP has an influence on corticosterone levels by a mechanism diverse from the other tested antidepressants. (Supported by European Regional Development Fund (FEDER), SCAC, CNRS, CAPES-COFECUB (France-Brazil exchange program) and the Région Haute Normandie).

P-60

BRAIN ESTROGEN SYNTHESIS INCREASES WITH PARENTAL EXPERIENCE AND BEHAVIOUR IN MALE MICE

Paula Duarte-Guterman(1)(2), Ariane Merkl(1), Diana B. Geissler(1), Günter Ehret(1)

(1) Department of Neurobiology, University of Ulm, Ulm, Germany (2) Department of Psychology, University of British Columbia, Vancouver, Canada

Parental care optimises offspring fitness and survival, and while maternal care is universal and its neural bases are well-studied in mammals, paternal care is much rarer and poorly understood. In house mice, paternal care is gained with experience. Naïve males ignore and often kill pups and only display parental behaviour after several days' exposure to pups. This delay coincides with altered neuroendocrinology; injection of estrogen helps naïve males become parental and experience with pups increases estrogen receptor content in limbic brain areas. Here we asked, whether locally produced estrogen (from testosterone via aromatase) may contribute to becoming paternal. We used immunocytochemistry to quantify aromatase positive cells in limbic brain areas in three male groups also assessed for parental care by retrieval of lost pups back into the nest: naïve males (M0, no previous sexual experience or contact with pups), fathers co-caring their first litter of pups with female partner for 5 days (M5, 5 days of pup exposure), and males co-caring with female partner their first litter of pups until weaning and their second litter of pups for 5 days males (M27, 27 days of pup exposure). Only males with previous pup experience retrieved pups (0% in M0, 83% in M5 and 92% in M27). The number of aromatase positive cells increased in M5 and M27 relative to M0 in the lateral septum, amygdala, piriform cortex, and ventromedial hypothalamus. In contrast, there were no significant changes in nuclei associated with maternal care, i.e., the medial preoptic area, bed nucleus of the stria terminalis, and nucleus accumbens. Our results show increased paternal performance with increased aromatase activity in brain areas regulating emotional/motivational and olfactory signal processing and learning. this suggests local estrogen is involved in a paternal brain network that is different from the network regulating the maternal instinct in females.

TOPIC: DEVELOPMENTAL ENDOCRINOLOGY

P-5.

RETINAL GROWTH HORMONE: REGULATON OF SYNTHESIS AND RELEASE

Martínez-Moreno CG, Giterman D, Henderson D, Gazala F, Harvey S.

Department of Physiology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada

Retinal ganglion cells (RGCs) in the chick embryonic neural retina are extrapituitary sites of growth hormone (GH) synthesis and release. The regulation of GH secretion by these cells is largely unknown, although we recently discovered several of the hypothalamic releasing factors involved in pituitary GH regulation (including GH-releasing hormone (GHRH) and thyrotropin releasing hormone, TRH) to be present in the cytoplasm of immortalized quail RGCs (QNR/D cells). The GH gene is also expressed in these cells, in which GH immunoreactivity is colocalized with SNAP-25 and chromogranin A. GH is thus likely released from these RGCs in secretory granules, as in pituitary somatotrophs. QNR/D cells may therefore provide an experimental model for studies GH regulation in the chick neural retina. The possibility that GHRH and TRH might stimulate GH synthesis and release in QNR/D cells was therefore investigated. Both peptides depleted the GH content of the QNR/D cells, as demonstrated by immunohistochemistry, Western blotting and ELISA and increased GH synthesis, determined by real-time PCR. These results demonstrate secretagogue actions of GHRH and TRH on GH secretion in QNR/D cells, comparable to that observed in pituitary somatotrophs. This is the first demonstration of GH regulation in any avian extrapituitary tissue and the first evidence for autocrine or paracrine pathways in the actions of GHRH and TRH in GH regulation. (Supported by NSERC)

P-6.

PRODUCTION OF FOLLICLE STIMULATING HORMONE IN THE ADENOHYPOPHYSIS OF THE CHICKEN EMBRYO

Enrique Pedernera, Guadalupe Sánchez-Bringas, Maria José Gómora, and Maria del Carmen Méndez-Herrera

Departamento de Embriología, Facultad de Medicina, Universidad Nacional Autónoma de México, México DF.

The involvement of pituitary gonadotropins in the development of gonads has been demonstrated by hypophysectomy in the chicken embryo. The aim of the present study was to evaluate changes in the production of follicle stimulating hormone (FSH) in the adenohypophysis of 8-17 day-old chicken embryo. A bioassay for FSH was developed to measure the in vitro secretion of pituitary cells. The expression of FSH mRNA was analyzed by qPCR. The secretion of FSH was demonstrated in the adenohypophysis from 9 days of incubation onwards, the maximum levels were observed in 13-14 day-old embryos; at 8 days of incubation

FSH secretion was not detected. In male embryos FSH levels were higher than in female pituitary cells at 9-10, 12-16 days of development. Levels of mRNA for beta-chain of chicken FSH were also higher at 9-17 days of incubation in adenohypophysis of males compared to females. These results support the involvement of FSH at early stages of gonad development, that it has been proposed in the chicken embryo ovary. Moreover, days 13-14 of incubation seems to be important in pituitary-gonad axis development. The importance of FSH highest levels observed in male adenohypophysis deserves further study.

TOPIC: ENDOCRINE DISRUPTION

P-7.

ASSESSMENT OF A FULL-SCALE WASTEWATER TREATMENT PLANT DISINFECTION UPGRADE– EFFECTS ON OCCURRENCE OF DISINFECTION BYPRODUCT IN EFFLUENTS AND ENDOCRINE DISRUPTION IN FISH

Angelina Baroffio and Alan Vajda
Department of Integrative Biology, University of Colorado Denver, CB 171, Denver, Colorado 80217

An important aspect of infrastructure development is understanding the impacts of operational scale engineering upgrades on non-target objectives. For example, in 2012, the City of Boulder (COB) wastewater treatment plant (WWTP), switched disinfection methods from chlorination with chlorine gas followed by dechlorination with bisulfite, to low pressure ultraviolet (UV) light oxidation. This switch from chlorine gas to UV disinfection is driven primarily from public safety concerns associated with handling a hazardous gas. One of the obvious non-targeted effects of the switch in disinfection process will be a shift in the nature of the chemical disinfection byproducts (DBPs) that are formed from primarily halogenated to primarily oxygenated compounds. One of the questions that needs to be asked about the switch from chlorination to UV is the effect of the DBPs on fish and other organisms living tin Boulder Creek. Because one of the major chemical structural features required to interact with the estrogen receptor (ER), which is an extremely important step in initiating a complex cascade of biochemical and physiological events leading to reproductive impairment, is the presence of a phenolic moiety, it is important to assess whether the UV oxidation increases the potential endocrine disruption qualities of the effluent. Beginning in mid September 2012, the first fish exposure experiment was conducted using Boulder Creek Water upstream from the WWTP outfall, or final effluent after chlorination and dechlorination. The endocrine disruption biomarker include secondary sexual characteristics, vitellogenin mRNA and plasma concentrations, NMR metabolomics, and gonad histology.

P-8.

USING A COMBINATION OF LAB AND FIELD SAMPLING TECHNIQUES TO CHARACTERIZE THE ESTROGENICITY GRADIENT OF A PORTION OF THE SOUTH PLATTE RIVER

Lisa Bourdon and Alan Vajda
Department of Integrative Biology, University of Colorado Denver, CB 171, Denver, Colorado 80217

Endocrine disrupting compounds (EDCs) can be found in many natural waterways that are utilized for drinking water and other anthropogenic uses. The South Platte River represents a significant source of water for the state of Colorado, yet little or no data exists concerning EDCs. The aim of this study is to evaluate the upstream and downstream concentrations of EDCs in relation to two major metropolitan wastewater treatment plants, and determine if one wastewater treatment plant's contribution is still present downstream of the next wastewater treatment plant, where a major drinking water intake is present. This study will characterize the estrogenicity gradient of the South Platte River in the Denver Metro area by combining data from a 5 day in-stream exposure as well as two 48 hour in-lab exposures. Exposed fish were dissected and their livers used to obtain NMR metabolomic, and vitellogenin mRNA data, while gonads will be used to obtain ex vivo steroidogenesis data. Finally, water samples collected from the start and end times of the exposures will be used to obtain chemical data. By combining both lab and field data, the goal is to obtain a comprehensive dataset that will incorporate the best aspects of both lab and field studies.

P-9.

DISRUPTION OF OVARIAN PROSTAGLANDIN AND STEROID BIOSYNTHETIC PATHWAYS AND REDUCED SPAWNING IN ZEBRAFISH EXPOSED IN VIVO TO QUINACRINE

Madelyne Cosme (1), Andrea Lister (2), Glen Van Der Kraak (1)
(1) Department of Integrative Biology, University of Guelph, Guelph, ON Canada
(2) Biology Department, Wilfrid Laurier University, Waterloo, ON, Canada

The zebrafish is a useful model species for studies examining the endocrine changes associated with oocyte maturation and ovulation. Knowledge of these pathways may be useful in defining the adverse outcome pathways that describe the impacts of endocrine disrupting compounds (EDCs) during the periovulatory period. While the effects of EDCs at the molecular, cellular and organ levels have been well documented, the linkages between these responses to impacts at the whole organismal level (e.g., egg production) are not well understood. In the current study, we examined the effects of the phospholipase A2 (PLA2) inhibitor quinacrine on spawning success in the zebrafish. Exposure to 100 ug/L of quinacrine resulted in a complete inhibition of spawning. On the fifth day of exposure, fish were sampled at midnight and 6 AM. Levels of prostaglandin $F_2\alpha$ and the maturation inducing steroid 17α, 20β-dihydroxy-4-pregene-3-one in ovarian tissue were the highest at 6 AM in both the control and the exposed group. The group exposed to quinacrine exhibited lower levels of the hormones compared to the control group at both timepoints. Expression of cytosolic phospholipase A2 (*cpla2)* and cycloxygenase-2 (*ptgs2*) was also measured. In the control and the exposed group the highest levels of *cpla2* and *ptgs2* was observed at 12 AM. The group exposed to quinacrine had lower levels of cpla2 and ptgs2, compared to the control group, at both timepoints. These results suggest that a PLA2 inhibitor acts at multiple sites controlling steroid and prostaglandin synthesis and imply that these pathways play an important role in spawning.

P-10

TESTICIULAR APOPTOSIS AND CELLULAR PROLIFERATION IN FATHEAD MINNOW EXPOSED TO WASTEWATER TREATMENT PLANT EFFLUENT

Andrea Hallagin and Alan Vajda
Department of Integrative Biology, University of Colorado Denver, CB 171, Denver, Colorado 80217

Environmental toxicants and their effects on both wildlife and human populations have been a worldwide concern as human populations are growing larger, water sources are becoming scarce, and anthropogenic waste is becoming more prevalent. Municipal wastewater treatment plants (WWTPs) are principally designed to effectively remove nutrients and pathogens from urban wastewater and release the effluent into local streams, however, many compounds such as steroids, pharmaceuticals, and other inorganic and organic compounds are rereleased into the environment, and have been found to have deleterious effects even in minute quantities on biological life. Because these compounds occur as complex mixtures, their additive effects as well as their individual effects must be considered in how physiological systems are affected at multiple sites and through multiple mechanisms. This complex problem has been evaluated using a multifaceted approach in Boulder Creek, CO, USA, which involved longitudinal studies examining water sources, anthropogenic pollutants, purification efficiency of the WWTP, effluent compounds, and effects of these compounds both individually and in complex mixtures on fathead minnow. In this study, a double labeling approach using both proliferating cell nuclear antigen (pCNA) and terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) was used to examine end points of physiological disruption in fathead minnow exposed to Boulder Creek effluent waters in 2005, 2006, 2008, and 2011.

P-11

INFRASTRUCTURE INVESTMENT IMPROVES ECOSYSTEM HEALTH

Alan Vajda
Department of Integrative Biology, University of Colorado Denver, CB 171, Denver, Colorado 80217

Stream ecosystems are strongly influenced by urban development, chemical loading, and other landscape-scale processes. The urban water cycle modifies natural stream hydrology, and domestic and commercial activities increase the burden of anthropogenic chemicals, including steroidal hormones and nonylphenol, that can disrupt endocrine system function in diverse aquatic organisms. Municipal wastewater treatment plant (WWTP) infrastructure changes can impact stream hydrology, water chemistry, and ecology. An important aspect of infrastructure development is understanding the impacts of operational scale engineering upgrades on non-target objectives. This study summarizes results from a series of integrated chemical and biological investigations into the occurrence, fate, and effects of endocrine disrupting chemicals in the City of Boulder, Colorado's WWTP and Boulder Creek, the receiving stream. Results are presented for the effects of modifying the WWTP from a trickling filter/solids contact to an activated sludge process on the concentrations of endocrine disrupting chemicals through each of the major treatment units. Corresponding effects of pre- and post-upgrade effluent chemistry on fish reproductive endpoints were evaluated in free-living white suckers (Catostomus commersoni) above and below the WWTP effluent. We have previously observed gonadal intersex, female-biased sex ratios, and other markers of endocrine disruption in fish downstream from the WWTP effluent. The upgrade of the WWTP resulted in improved removal efficiency for most endocrine disrupting chemicals and fish exposed to the post-upgrade effluent indicated reduction in endocrine disruption relative to pre-upgrade conditions, including balanced sex ratios and the absence of gonadal intersex. The improved effluent quality following the WWTP modification likely will result in improved long-term stream ecosystem health.

TOPIC: ENDOCRINE-IMMUNE SYSTEM INTERACTION

P-12

PROLACTIN INHIBITS CHONDROCYTE APOPTOSIS INDUCED BY PRO-INFLAMMATORY CYTOKINES AND ARTHRITIS IN RATS

Adán, N (1), Guzmán-Morales, J (1), Ledesma-Colunga, MG (1), López-Barrera, F (1), Quintanar-Stephano, A (2), Martínez de la Escalera, G (1), Thébault, S (1), Clapp, C (1).
(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México.

Chondrocytes are the only cells in cartilage and their death by apoptosis contributes to cartilage loss in rheumatoid arthritis (RA). A potential therapeutic intervention for RA is the inhibition of apoptosis-mediated cartilage degradation. Prolactin (PRL) stimulates the survival of chondrocytes induced by serum deprivation and increases in the circulation of patients with RA. Here, we demonstrate that PRL inhibits the apoptosis of chondrocytes in culture in response to proinflammatory cytokines (Cyt: TNF-α, IL-1β, and IFN-γ) by preventing the induction of p53 and decreasing the bax/bcl-2 ratio through a JAK2-STAT3-dependent, NO-independent pathway. Also, local treatment with PRL or inducing hyperprolactinemia by placing two anterior pituitary glands under the kidney capsule prevented the apoptosis of chondrocytes evoked by injecting Cyt into the knee-joints of rats. Given that Cyt cause apoptosis-mediated cartilage loss in RA, we evaluated the survival effect of PRL in the adjuvant-induced RA model in rats. Osmotic minipumps delivering PRL or subcutaneous tablets releasing haloperidol (Hal), a dopamine D2 receptor antagonist that causes hyperprolactinemia, implanted before or after the injection of complete Freund's adjuvant (CFA), resulted in elevated serum PRL levels, lowered CFA-induced expression of proapoptotic mediators (caspase-3, bax, and p53) in ankle joints, and reduced chondrocyte apoptosis in knee cartilage as revealed by TUNEL and active caspase-3 immunostaining. These findings reveal the protective effect of PRL against inflammation-induced cartilage loss and the therapeutic potential of hyperprolactinemia to reduce permanent joint damage in RA. (This study was supported by the Universidad Nacional Autónoma de México, grant PAPIIT-UNAM 200312-3. We thank G. Nava, D. Mondragón, A. Prado, and M. García for their technical assistance).

P-13.

PROLACTIN DOWN-REGULATES INTERLEUKIN-6 DURING THE PRIMING PHASE OF LIVER REGENERATION, POSSIBLY THROUGH SUPRESSOR OF CYTOKINE SIGNALING-3 INDUCTION

Goya-Arce, M (1), López-Barrera, F (1), Triebel, J (1), Binart, N (2) Martínez de la Escalera, G (1), Moreno-Carranza, B (1), and Clapp, C (1).
(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) INSERM U693, Faculté de Médecine Paris-Sud, Le Kremlin-Bicetre, France.

Interleukin-6 (IL-6) is an important mediator of hepatocyte proliferation, body homeostasis, and survival after partial hepatectomy (PH). Although IL-6 is necessary to induce liver regeneration, sustained elevated levels can delay and inhibit hepatocyte proliferation and decrease animal survival. PRL is a liver mitogen that increases in the circulation after PH and promotes liver regeneration. Given that PRL inhibits LPS-induced expression of IL-6 in hepatic Kupffer cells and stimulates the synthesis of the suppressor of cytokine signaling-3 (SOCS-3) in various cells, and that SOCS-3 can blunt IL-6 production and action in the liver, we hypothesized that PRL promotes liver regeneration through SOCS-3-mediated modulation of the detrimental effects of IL-6. Here, we investigated the action of PRL on hepatic IL-6 and SOCS-3 production during liver regeneration in PRL receptor-deficient mice ($PRLR^{-/-}$). $PRLR^{-/-}$ mice subjected to 60% PH displayed reduced survival between 24 and 48 hours after PH relative to wild-type animals. Reduced survival could be linked to the up-regulation of IL-6. $PRLR^{-/-}$ mice showed enhanced hepatic IL-6 mRNA levels that were associated with higher concentrations of serum IL-6 at 3, 6, and 24 h post-PH, compared to $PRLR^{+/+}$

animals. Moreover, the absence of the PRL receptor resulted in reduced expression of hepatic SOCS-3 at 3 and 6 h after PH. Consistent with these results, we found that in rats, a 70% PH increases PRL circulating levels and that lowering these levels with CB-154, a dopamine receptor agonist that inhibits anterior pituitary PRL release, increases hepatic IL-6 expression and reduces SOCS-3 synthesis in the liver. In conclusion, we suggest that PRL promotes survival after PH via SOCS-3 modulation of IL-6 and that this hormone has potential clinical utility for ensuring survival following liver injury. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant 127496. We thank G. Nava, D. Mondragón, A. Prado, and M. García their technical assistance).

P-14.

PROLACTIN REDUCES JOINT INFLAMMATION IN ADJUVANT-INDUCED ARTRITHIS IN RATS

Ledesma-Colunga, MG (1), Adán, N (1), Méndez, I (1), Quintanar-Stephano, A (2), Martínez de la Escalera, G (1), Clapp, C (1).
(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, México.

Prolactin (PRL) has immunomodulatory properties and may play a protective role against joint inflammation in rheumatoid arthritis (RA). It is elevated in the circulation of patients with RA and of women who are breast-feeding, a condition associated with reduced risk of RA in non-arthritic individuals. Here, we investigated the effect of PRL on joint inflammation in the adjuvant-induced model of RA in rats. Osmotic minipumps delivering PRL or subcutaneous tablets releasing haloperidol (Hal), a dopamine D2 receptor antagonist that causes hyperprolactinemia, were implanted 3 days before the injection of complete Freund´s adjuvant (CFA) and resulted in elevated serum PRL levels throughout the experiment. PRL or Hal infusion delayed the onset and ameliorated the severity of joint inflammation, as indicated by reduced swelling, pain, and expression of pro-inflammatory mediators (iNOS, TNFα, IL-1β, IFNγ, IL-6) in ankle joints. Notably, starting the osmotic minipump delivery of PRL after inflammatory onset (15 days post-CFA injection) also mitigated ankle swelling, pain, and expression of the pro-inflammatory factors. These findings show the anti-inflammatory action of PRL in adjuvant-induced arthritis and suggest the clinical utility of this hormone for the prevention and reduction of joint inflammation in RA. (This study was supported by the Universidad Nacional Autónoma de México grant PAPIIT-UNAM 200312-3. We thank F. López-Barrera, G. Nava, D. Mondragón, A. Prado and M. García for their technical assistance).

P-15.

SEXUAL HORMONES ARE INVOLVED IN MALARIA PATHOLOGY

Legorreta-Herrera M and Mosqueda-Romo NA.
Molecular Immunology Laboratory. FES Zaragoza, National University of Mexico, Mexico.

Malaria incidence between both sexes is similar. However, male develop higher parasitaemias and more severe pathologies than female mice. On the other hand, both reactive oxygen and nitrogen species play an important role on malaria parasite elimination. We studied whether differences in pathology and parasitaemias could be associated with sexual hormones, as estradiol is a natural antioxidant and testosterone is associated with immunosuppression. In addition, the expression of some immune response genes is modulated by hormones. Furthermore, we studied the effect of gonadectomy on the activity of catalase in CBA/Ca mice infected with *P. berghei* ANKA. Groups of male and female mice were gonadectomised and after 28 days were infected with *P. berghei* ANKA. Additional groups of mice, were opened and closed (sham). Two additional groups of mice were left intact as controls. All groups of mice were infected with *P. berghei* ANKA, parasitaemia, body weight and haemoglobin concentration was daily analysed. Nine days post infection mice were sacrificed. Gonadectomy in female mice, increased both parasitaemia and body weight. In contrast, in male mice was detected a decreased parasitaemia and loss of body weight. Interestingly, gonadectomy increased the level of haemoglobin in both female and male mice, indicating that sexual hormones are involved in anaemia. The specific activity of catalase was substantially decreased in gonadectomised female mice, while in male mice we did not detected significant changes.Our results suggest that sexual hormones are involved in pathology (anaemia), regulation of body weight (cachexia) and oxidative stress in *P. berghei* ANKA infected mice. (This work was supported by PAPIIT IN217412, UNAM).

P-16.

ESTRADIOL INDUCES THYMUS GLAND REGRESSION VIA BOTH ESTROGEN AND GLUCOCORTICOID RECEPTOR PATHWAYS IN XENOPUS LAEVIS TADPOLES

Schreiber, A.M., McCaffrey, A., Garcia, J., Priyamvada, L., Heckman, K.,
Biology Department, St. Lawrence University, Canton, New York USA

High levels of endogenously-produced or exogenously-administered estrogen are known to cause the thymus to atrophy in mammals, similar to the suppressive effects of glucocorticoids on the immune system. However, the influence of estrogen and estrogenic compounds on thymus gland development in tadpoles and other aquatic vertebrates remains unknown. Here we show that treatment of young tadpoles (7 days-post fertilization; Nieuwkoop and Faber stage 50) for 5 days with estradiol (10 uM), or dexamethasone (DEX, 2 uM; a glucocorticoid receptor agonist) significantly reduces the thymus gland size by 35%, and 67%, respectively. Treatment of tadpoles with estradiol induces maximum active caspase-3 expression (a mediator of programmed cell death) in thymocytes within 48 hours, after which levels of thymus cell apoptosis decrease. Concurrent treatment of tadpoles with fulvestrant (25 uM; an estrogen receptor-specific antagonist) rescued both the estradiol-induced reduction in thymus size and apoptosis. Compared with tadpoles treated with DEX alone, those treated with DEX + RU-486 (200 nM; a glucocorticoid receptor antagonist) completely rescued tadpoles from reduction in thymus size. Interestingly, treatment of tadpoles with estradiol + RU-486 also produced a partial rescue of the estradiol-induced reduction in thymus size. These findings suggest that some of the effects of estradiol and of estrogenic compounds on thymus gland regression may be mediated through concurrent activation of both estrogen receptors and glucocorticoid stress-response pathways.

TOPIC: ENVIRONMENTAL ENDOCRINOLOGY

P-17.

COLLECTING DUCTS AQUAPORIN-2 IMMUNOREACTIVE EXPRESSION AND MORPHOMETRY IN NECTAR-FEEDING BIRDS INHABITING HUMID AND ARIDS HABITATS.

Mata, A (1), Guerrero, HY (2), Salazar, V (3). (1) Laboratorio de Biología de Organismos, Centro de Ecología, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela. (2) Laboratorio de Neuroendocrinología Comparada, Facultad de Medicina, Universidad Central de Venezuela, Caracas, Venezuela. (3) Servicio de Microscopía de Luz, Centro de Biofísica y Bioquímica, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

In arid environments, birds should avoid water loss while in humid environments excess of water should be eliminated. Both events are performed through complex physiological mechanisms. We investigated some morphological and molecular parameters of collecting ducts (CD) in the avian kidney. Aquaporins (AQP) are transmembrane proteins involved in water reabsorption from CD. The avian kidney expresses Aquaporin subtypes 1, 2, and 4. We detected the presence of AQP-2 homologs by immunohistochemistry and measured its expression by semi quantitative image analysis using the integrated optical densities (IOD) in 3 species of nectar-feeding birds: hummingbirds *Amazilia tobaci* (*At*) that inhabits montane forests, *Leucipus fallax* (*Lf*) from arid zones, and the passerine *Coereba flaveola* (*Cf*), common to both environments. Also, we determined the following morphometric variables of CD: density, total diameter, and lumen diameter. The highest IOD was observed in *At*, followed by *Lf* and *Cf*. Density of CD was higher in *Lf*, followed by *At*, and *Cf*. CD`s total diameter was greater in *At*, however, the lumen diameter was lower in *Lf* and *Cf*. Both the IOD and the luminal diameter of the CD were correlated in *Lf* and *Cf*, but not in *At*. *Lf*, a species facing restrictions on water availability, had the highest CD density and medium level of IOD. *Cf*, living in both habitats, shows a positive correlation between the lumen diameter and IOD, but has a low density of CD. *At*, seems to have a different way of regulation of water loss, modulating CD density and AQP-2ir expression. Although *At* showed more IOD that *Lf*, CD density was lower. Differences in the density, size of the CD, and AQP-2ir expression reveal the existence of complex regulatory mechanisms of water loss among similar nectar-feeding birds occupying different habitats.

TOPIC: GENERAL NEUROENDOCRINOLOGY

P-18.

NEURO-PROTECTIVE EFFECT OF GROWTH HORMONE (GH) IN CHICKEN CEREBELLAR CELL CULTURES. A POSSIBLE ANTI-APOPTOTIC ROLE OF GH DURING THE HYPOXIA INJURY.

Alba-Betancourt, C (1), Luna-Acosta, JL (1) , Ramírez-Martínez, CE (1), Carranza, M (1), Martínez-Coria, H (2), Arámburo, C (1) and Luna, M (1).
(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, Qro., 76230, México, (2) Department of Neurobiology and Behaviour, University of California, Irvine, CA 92697-4545, USA

It is known that GH is involved in cell survival and may inhibit apoptosis in several cell types, including those of the CNS. Recent reports indicate that GH and its receptor are expressed in the CNS of several species, where it may act as a neuroprotective factor in addition to its capacity to stimulate growth and development in the brain. A common insult that can cause severe damage on the CNS is ischemia, which can be induced by hypoxia and low glucose condition (HLG). In this work we studied the possible neuroprotective role of GH in a model of ischemic neuronal injury (HLG) using primary cerebellar neuron cultures. The viability of cerebellar neurons exposed to HLG decreased to 38.4±7.2% compared to the control (97.8%), and it increased to 66±12.8% after GH (1 nM) treatment. Likewise, the addition of GH (1 nM) decreased the number of apoptotic cells (15.3±2.2%) when compared with those exposed only to HLG (68.1±12.1%) and reduced 1.5-fold the activity of caspase-3 in cerebellar neurons exposed to HLG. Furthermore, addition of GH induced the activation of the PI3K/Akt pathway during the HLG insult, and this activation was blocked by Wortmannin (a PI3K/Akt inhibitor) suggesting that GH exerts its effects through this signaling pathway. Also, the antiapoptotic Bcl-2 protein increased following GH treatment in HLG exposed cells. On the other hand, the addition of 10 nM 15kDa GH variant also increased cell viability (63.2±11.9%) compared to HLG exposed cultures (28.4±6.2%), and was able to reduce caspase-3 activity in comparison to the control (5.1±1.2 vs 7.5±1.3 units, respectively). These results indicate that locally expressed GH may act as an autocrine/paracrine survival factor that preserves cellular viability and inhibits apoptotic cell death in response to ischemia. *(Supported by PAPIIT-DGAPA,* IN210209, IN208812, IN206813, and CONACYT 118353, 178335. Thanks to G. Courtois for excellent technical support*)*.

P-19.

PROLACTIN-DERIVED VASOINHIBINS ANTAGONIZE THE KALLIKREIN-KININ SYSTEM IN THE CONTEXT OF DIABETIC RETINOPATHY

David Arredondo Zamarripa (1), Olivia Baldivieso-Hurtado (2), Daniel Ochoa (2), Carmen Clapp (1), and Stephanie Thebault (1).
(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México(2) Hospital "Dr. Luis Sanchez Bulnes", Asociación Para Evitar la Ceguera en México, I.A.P., Distrito Federal, México

Excessive retinal vasopermeability (RVP) contributes to diabetic retinopathy, the leading cause of visual impairment in working-age adults. The kallikrein-kinin system, able to mediate vasopermeability in pathological conditions, is over-activated in retinas of patients with proliferative diabetic retinopathy (PDR) and in experimental models of the disease. Here, we investigated whether vasoinhibins derived from the proteolytic cleavage of the hormone prolactin target key components of the kallikrein-kinin system, i.e. both B1 and B2 bradykinin receptors, to attenuate the increase in RVP associated with diabetes. We observed that vasoinhibins prevented the increase in RVP induced by intravitreal injection of bradykinin in a similar manner as the B2 receptor antagonist Hoe-140. In addition, levels of B1 receptor mRNA were increased by four- and two-fold in retinas from rats injected with vitreous from PDR patients and diabetic rats compared to rats injected with vitreous from non-diabetic patients and healthy controls, respectively. Notably, vasoinhibins prevented the induction of B1 receptor expression in both models. Together, these data support that vasoinhibins regulate the kallikrein-kinin system at the level of the two bradykinin receptors in order to prevent the increase in RVP associated with diabetes.(This study was supported by the Universidad Nacional Autónoma de México UNAM and the National Council of Science and Technology of Mexico grant CONACYT S0008-161594. We thank F. López-Barrera, G. Nava, D. Mondragón, A. Prado, and M. García for their technical assistance).

P-20.

CHARACTERIZATION OF PACAP IN TWO LATIN AMERICAN SILVERSIDE FISH (ATHERINOPSIDAE)

Mauricio N. Kraemer(1), Mónica Chavez-Maldonado (2), Rodolfo Cárdenas-Reygadas (2), Luis Fabián Canosa (1).
(1) Laboratorio de Neuroendocrinología Comparada. IIB-INTECH, CONICET-UNSAM, Argentina. (2). Laboratorio de Endocrinología de Peces, Unidad de Morfología y Función, Facultad de Estudios superiores Iztacala, Universidad Autónoma de México, México.

Silverside fish in both Mexico and Argentina represent an important economical resources but their aquaculture fails to reach industrial levels and economical sustainability. One of the factors for this phenomenon is found in the slow growth rate of this fish. Therefore, it is important to study the endocrine regulation of

growth in this species. In the present work pituitary adenyl cyclase activating polypeptide (PACAP) was cloned and the brain distribution was investigated in two members of Atherinopsidae family, *Chirostoma humboldtianum* and *Odontesthes bonariensis*. The isolated cDNAs encode for a signal peptide (1-25), a cryptic peptide (26-83) a PACAP related peptide (PRP; 84-128) and PACAP itself (131-168). In both species, two cDNA variants were found representing an alternative splicing of the same gene as it was found for other teleost. In the short form, exon 4 is spliced out and the transcript expresses PACAP but not PRP. Phylogenetic analysis shows that the transcripts from these species fall into the catfish-like clade. Distribution of PACAP expressing-cells in the brain was studied by immunohistochemistry (IHC) in *C. humboldtianum* and *in situ* hybridization (ISH) in *O. bonariensis*. PACAP mRNA was detected by ISH wide spread in the brain of *O. bonariensis*. PACAP expressing-cells were found in the telencephalon, the preoptic area, the ventral hypothalamus, thalamus and the hind brain. Similarly, IHC reveals PACAP expressing-cells in the brain of *C. humboldtianum* in the preoptic area and several hindbrain nuclei but not in the telencephalon or ventral hypothalamus. IHC also reveals axons across the telencephalon, the diencephalon, the optic tectum, cranial nerves and the pituitary. These results suggest that PACAP functions as a neuroendocrine peptide at the pituitary level as well as a neuromodulator in several brain areas.(This work was partially supported by ANPCyT-Argentina (PICT 2006-074, PICT 2010-1493) and CONICET-Argentina (PIP 2011-0271) to LFC.)

P-21.

ISOLATION OF GONADOTROPIN-RELEASING HORMONE RECEPTORS FROM THE BRAIN OF CHIROSTOMA HUMBOLDTIANUM.

Marin-Ruiz MA (1), Chavez M (1), Cardenas R (1)
1.- Laboratorio de Endocrinología de peces. Unidad de Morfología y Función. FES Iztacala UNAM.Tlalnepantla; Edo.de Mexico, Mexico.

The gonadotropin-releasing hormone and their receptors play an important role in the reproductive axis in vertebrates. The gonadotropin-releasing hormone receptors (GnRH-Rs) are receptors coupled to G proteins. In this study, males and females of *Chirostoma humboldtianum* fish were collected from Zacapu laggon, (Michoacan, Mexico). The brains were removed and kept in dry ice. Total RNAs were extracted with Trizol reagent. cDNAs were synthesized using commercial kit. For PCR amplification, the primers used were designed according the consensus sequences for GnRH-Rs reported in Gen Bank. Partial sequences of two types of GnRH-R were isolated from the brain of *Chirostoma*. One sequence was 213 bp corresponding from intracellular loop 3 to TM 7 and identify like GnRH-R1 type and the second sequence was 425 bp corresponding from TM 4 until TM 7, this sequence was identified like GnRH-R2 type. The sequence of GnRH-R1 type of *Chirostoma humboldtianum* keeps an identity of 90%, 88% and 86% with *Odontesthes bonariensis*, *Astatotilapia burtoni* and *Dicentrarchus labrax*, respectively. For the GnRH-R2 sequence of *Ch. humboldtianum* keeps an identity of 92% with *O. bonariensis*, 88% *Morone saxatilis* and 86% *Acanthopagrus schlegelii* and *D. labrax*. Also, the sequences of the both receptor types were found in the gonads of *Chirostoma*.(To UNAM, DGAPA, PAPIIT IN 216910 to R.C.)

P-22.

GROWTH HORMONE (GH) CHARACTERIZATION IN GREEN IGUANA (IGUANA IGUANA) RETINA

Carranza, M(1), Avila-Mendoza, J (1), Ahumada, M(1), Luna, M (1), and Arámburo, C(1). (1)Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro 76230, México.

The expression of growth hormone (GH), a protein mainly synthesized in the pituitary of all vertebrates, has recently been described in several tissues such as in the nervous, immune and reproductive systems, among others. However, in reptiles there is no information regarding the expression or the distribution of extrapituitary GH. In this study we characterized the presence of local GH in the retina of green iguana (Iguana iguana). GH-immunoreactivity (IR) (105.8 ± 12.7 ng/mg of protein) was determined employing a heterologous ELISA. It was associated, by SDS-PAGE and western blot, with several size variants (55, 41, 26 y 15 kDa). By far, the 15 kDa isoform predominated (65 ± 0.71%). GH mRNA expression was analyzed by in situ hybridization, and the distribution of GH-IR was studied by immunohistochemistry. In both cases, a strong signal was found in retinal rods and cone cells, as well as in ganglionar cells. These data suggest that GH is locally expressed in the retina, where it may act as a local autocrine/paracrine factor. (We thank Lorena López (Microscopy Unit) and Gerardo Courtois (Lab assistant). This work was supported by grants from CONACyT (178335 and 267642) and PAPIIT-DGAPA-UNAM (IN206813 and 20881223).

P-23.

EXPRESSION OF GH AND IGF-I IN RESPONSE TO HYPOXIA IN THE CHICKEN EMBRYO CEREBELLUM.

Granados, E.(1), Cruz, C. .(1), Aparicio, M. (1), Alba-Betancourt, C.(2), Carranza, M .(1), Avila J .(1), Arámburo, C.(1), and Luna, M. (1) .
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro 76230, México

Growth hormone (GH), GH RNAm and it receptor (GHR); have been found not only in the pituitary gland, but also in the Central Nervous System (CNS). This data suggests that, besides the endocrine role, this hormone could have autocrine/paracrine effect in extrapituitary tissues, such as neuro-protection in the CNS after a severe damage, for instance hypoxia. In this work, we evaluated the expression of cerebellar GH and apoptosis in an *in vivo* model: chicken embryos at 15 days of embryonic development, after treatment under 24h of hypoxia and 24 h of re-oxygenation. The results showed that GH (determined by ELISA) increased 1.74 times under hypoxia compared to the control. Cerebellar GH showed a molecular heterogeneity pattern (15, 26, 29, 35, 52 kDa) by SDS-PAGE and western blot, no changes in the size variants pattern was observed, however the relative proportion of 35 and 52 kDa variants increased 1.58- and 1.54-fold, respectively, when compared with the control. The expression of GH RNAm in the cerebellum (645-bp, similar to the one found in the pituitary) dramatically increased (99 fold) after the hypoxia insult. On the other hand, the expression of IGF-1 was also increased (2.4-fold) in response to the treatment when compare with the control. Also, caspase-3 activity increased 1.6 times after exposure to hypoxia. Data suggest that the expression of cerebellar GH and IGF-1 may be involved in autocrine/paracrine mechanisms related to neuroprotection in response to an ischemic insult. (Technical support from: Courtois G, Proteogenomic Unit. Supported by grants: PAPIIT IN210209, IN208812 and CONACYT 118353, 178335)

P-24.

EXPRESSION OF 5-HT5A RECEPTOR IN THE HIPPOCAMPUS DURING THE ESTROUS CYCLE OF THE RAT

Rodríguez-Cruz A, González-Torres A, García-Alcocer G, Escobar-Cabrera J, Berumen LC.
Faculty of Chemistry, Universidad Autónoma de Querétaro, Querétaro, México.

The 5-hydrxotryptamine (5-HT, serotonin) is an important monoamine neurotransmitter with a wide range of functions, including body temperature regulation, appetite, sleep and mood. In mammals it is mainly synthesized by the enterochromaffin cells in the intestine and by raphe nuclei in central nervous system. Serotonin exerts its function when it binds to specific receptors; there are more than 14 different serotonin receptors, which have been structural, operational and pharmacologically classified in seven families (5-HT_{1-7}), of which the 5-HT_{5A} is one of the least understood. The 5-HT_{5A} in the brain has been reported, finding a

large concentration in hippocampus. The hippocampus is related with important roles in spatial navigation, memory and mood, which has also been associated with serotonin and steroid hormones. Furthermore, it is known that the density of 5-HT receptors is regulated by steroid hormones that fluctuate during the reproductive cycle. The objective of this study was to evaluate the expression of 5-HT_{5A} in the hippocampus during the estrous cycle, by western blot. The results indicate a relationship between steroid hormone levels and 5-HT_{5A} receptor in the rat hippocampus, where the expression levels of the receptor showed the highest levels in the early stages of the cycle with a higher concentration of gonadal hormones. Differences between exogenous administration of steroid hormones and gonadal hormones in estrous cycle need to be further investigated. The results correspond with 5-HT_{5A} expression in hippocampus associated with mood, where high levels of receptor expression were found in the different phases of the cycle with lower incidence of depression and lower levels of anxiety. (This work was supported by ProMeP for Berumen and UAQ).

P-25.

THE HORMONE PROLACTIN IS PROTECTIVE AGAINST RETINAL DEGENERATION

Stéphanie Thebault (1), Edith Arnold (1), Andrés Quintanar-Stéphano (2), Miguel Condés-Lara (3), Gerardo Rojas-Piloni (3), Nadine Binart (4), Gonzalo Martínez de la Escalera (1), and Carmen Clapp (1)
(1) Departmento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México (2) Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Avenida Universidad 940, 20100 Aguascalientes, México (3) Departmento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Campus UNAM-Juriquilla, Querétaro, México(4) INSERM, Unit 845, University Paris-Descartes, Faculty of Medicine, Paris, France

Retinal degeneration is characterized by the progressive destruction of retinal cells, causing deterioration and eventual loss of vision. We explored whether the hormone prolactin (PRL) provides trophic support to retinal cells, thus protecting the retina from degeneration. Retinas from PRL receptor-deficient mice exhibited photoresponsive dysfunction and gliosis that correlate with the down-regulation of neurotrophins (bFGF, GNDF, and BDNF) and antioxidant glutathione S-transferase μ-type 2. Most of these effects were exacerbated in aged (9 month-old) compared with young (3 month-old) mice. Furthermore, PRL receptors were up-regulated in retinal glia of rats exposed to bright continuous light. In this model of photoreceptor degeneration, inducing hyperprolactinemia limited photoreceptor apoptosis, prevented gliosis and changes in neurotrophin expression, and maintained photoresponse. Thus, this study unveils PRL as a trophic factor regulating glial-neuronal cell interactions in the retina, and a potential therapeutic molecule against retinal degeneration.(This study was supported by the Universidad Nacional Autónoma de México UNAM and the National Council of Science and Technology of Mexico grant CONACYT 176393. We thank F. López-Barrera, D. Mondragón, A. Prado, G. Nava, and M. García for their technical assistance).

P-61

AGGRESSIVE RESPONSES TO FOOD INSECURITY: A NOVEL STEROID-NEUROPEPTIDE INTERACTION

Fokidis, HB (1)(2) , Prior, NH (1), Ma, CC (1), Granados-Samayoa, J (1), Taves, MD (1), Adomat, HA (2), Guns, ES (2), Saldanha CJ (3), Soma, KK (1).
(1) Department of Psychology, University of British Columbia, Vancouver, BC, Canada (2) The Prostate Centre, Vancouver General Hospital, Vancouver, British Columbia, Canada (3) Department of Biology, American University, Washington D.C, USA

Aggression enables individuals to access limited resources. Most research has focused on the regulation of male breeding aggression where circulating testosterone (T) is converted to estradiol (E_2) by aromatase (AROM) within the brain to act on receptors in behavioral circuits. Food insecurity is common in nature and can promote aggression, but is percieved as a stressor, stimulating corticosterone (CORT) secretion in birds. Higher CORT lowers circulating T, thus suggesting aggression during food insecurity is mediated by another, as yet unknown, mechanism. Using novel behavioral tests we can explore how food insecurity impacts aggression and the endocrine system in both gregarious and territorial birds. In zebra finches, fasting increased aggressive interactions between individuals competing for a food source. Fasting also decreased T and elevated both CORT and the levels of the prohormone, dehydroepiandrosterone (DHEA). Circulating DHEA can be metabolized to E_2 within the brain to maintain aggression when T levels are low (e.g., non-breeding season). Although fasting increased CORT content in many organs, only adrenals and liver had elevated DHEA levels suggesting them as sites of synthesis. In microdissected brain tissue, fasting increased E_2 content within the vertebrate social behavior network in brain regions with roles in both food intake and social interaction thus serving to link these disparate functions. In territorial song sparrows, fasting increased aggression in a resident-intruder test and immunoreactivity for neuropeptide-Y (NPY), an orexigenic regulator of food intake. To establish neuroanatomical connection between NPY and E_2, we assessed cellular colocalization of AROM and the NPY Y1 receptor and how fasting may alter this association. In summary fasting may promote adrenal and hepatic DHEA secretion and its conversion to E_2 where it may interact with NPY to promote aggression during food insecurity.

TOPIC: GONADAL DEVELOPMENT AND GAMETE MATURATION

P-26.

GROWTH HORMONE (GH) EFFECTS ON PROLIFERATION OF OVARIAN GRANULOSA CELLS IN THE HEN.

Ahumada-Solórzano, M(1), Carranza, M(1), Luna Muñoz, M(1), and Arámburo, C(1).
(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México.

Preovulatory follicular development is mainly regulated by gonadotropins (FSH, LH) and other intraovarian hormones and factors (steroids, GH, IGF-I). It is now known that GH participates in the differentiation, proliferation, steroidogenesis, and cell survival in the ovary. The ovarian expression of GH has been described in human, rat, bovine and poultry but little is known about its local activity in this organ. Recently, we showed that ovarian GH is involved in the regulation of steroidogenesis in the hen's granulosa cells (GC). Here we analyzed the effect of GH on the proliferation of GC. We used follicles at the beginning of hierarchical stage, F4 (1-2 cm of diameter) of adult (25-35 weeks old) hens. GH treatment (0.01, 0.1, 1, 10 nM) increased the proliferation of cultured GC, as determined by the 3H-thymidine incorporation assay (1.6-, 2.5-, 4-fold) or the MTT assay (1.5-, 2-, 3-, 3.5-fold), respectively. This GH-dependent proliferative effect was substantially decreased when a specific αGH antibody (1:100 dilution) was employed. The addition of conditioned media (CM), containing the GH produced locally by cultured GC, was capable to stimulate the proliferation of freshly cultured GCs (0.8-, 1.33-, 2.27-fold at 0.01, 0.1, 1nM GH, respectively), and this effect was suppressed by co-incubation with the αGH antibody. Also, CM increased (1.5-fold) the expression of PCNA in cultured GC, and this decreased in a dose-dependent manner with the addition of αGH antibody. Several GH molecular variants were found in the CM by western blot, with the 17 kDa isoform being the most abundant one. These data suggest that locally produced GH may be involved in follicular development through paracrine and/or autocrine mechanisms. (Supported by PAPIIT-DGAPA-UNAM IN210209, IN206813; CONACYT 178335 and 161791. MAS received a PhD scholarship from CONACYT and support from the PhD program in Biomedical Sciences, UNAM. Technical support of G. Courtois is acknowledged).

P-27.

DEPOLARIZATION WITH HIGH-K INDUCES INTRACELLULAR CA2+ CONCENTRATION ([CA2+]I) RISES IN THE THECA CELL LAYER OF INTACT PREANTRAL FOLLICLES RECORDED IN ACUTE OVARIAN SLICES IN VITRO.

Daniel Bahena-Alvarez (1), Diana Millán-Aldaco(1), Tatiana Fiordelisio(2) and Arturo Hernández-Cruz(1).
Instituto de Fisiología Celular1 y Facultad de Ciencias2. Universidad Nacional Autónoma de México, Ciudad Universitaria, México City DF, CP 04510, México.

Spontaneous [Ca2+]i fluctuations occur in mouse oocytes isolated from preantral follicles and in cultured granulose and theca cells. Fluctuations increase in size and frequency in adult oocytes, suggesting a role in follicle maturation. In mouse ovarian slices we recently recorded spontaneous [Ca2+]i fluctuations involving the entire theca cell layer of intact preantral follicles. Here we investigated if these fluctuations could result from the opening of voltage-gated Ca2+ channels (VGCC). Female Balb/c mice 8-10 weeks old were sacrificed at different stages of the estrous cycle. Ovaries were removed and 130 µm thick slices were cut, incubated with fluo-4 AM (22 µM), mounted on the stage of an upright Nikon microscope and perfused with normal, oxygenated saline. Imaging (488 nm ex; 510 nm em) was performed with an Andor camera and a spinning-disk confocal microscope (10 ms exposure; 200 ms interval, 5 min-long movies). The cortical region, containing follicles at different stages was imaged with a 10X water-immersion objective, while 1 sec-long puffs of high [K+] (120 mM) were applied through a glass micropipette near the slice surface. Ovarian histological sections were reacted with antibodies raised against alpha 1 subunits of VGCC and stained with secondary fluorescent antibodies. Ca2+ signals recorded in ovarian follicles upon exposure to high K+ are similar in organization (involving the entire theca layer) and duration (~ 1-3 sec) to those observed both spontaneously and occasionally after the application of luteinizing hormone. K+ -induced Ca2+ signals were frequently observed in preantral follicles at diestrus and metaestrous and less frequently at estrus. Anti-alpha1B antibodies bind specifically to theca cells, Anti-alpha1C and D reacted more weakly. These results suggest that K+ -induced Ca2+ signals and possibly spontaneous Ca2+ fluctuations result from the opening of VGCC present in the theca cell membrane. These findings should be confirmed by appropriate electrophysiological and pharmacological experiments.

P-28.

USING A MODIFIED BIOASSAY TO DETERMINE THE ROLE OF EICOSANOIDS IN ZEBRAFISH OVULATION AND SPAWNING

Olivia M. Knight(1) and Glen Van Der Kraak(1)
(1) Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

The physiological processes underlying ovulation and spawning in fishes are poorly understood. While eicosanoids such as prostaglandins (PGs), leukotrienes, and hydroxyeicosatetraenoic acids are known to play an essential role in these events, their specific function and relative contribution to each have yet to be established. The goal of this research was to investigate these factors by profiling gene expression of enzymes in the eicosanoid biosynthesis pathway (EEBPs) and measuring eicosanoid levels during ovulation and spawning in a model teleost, the zebrafish. To isolate changes specific to ovulation from those specific to spawning, this study modified a recently developed bioassay demonstrating that ovulation can be induced in female zebrafish *in vivo* following waterborne exposure to the oocyte maturation-inducing hormone 17-α, 20-β-dihydroxy-4-pregnen-3-one (17,20βP). Solitary female zebrafish exposed to 10 nM waterborne 17,20βP ovulated within 4 hours but did not spawn their eggs, regardless of experimental duration, whereas male-female pairs spawned within the 4 hour period. This modified design enabled comparison of ovarian physiology of periovulatory fish destined for spawning to those not destined to spawn. Real-time PCR revealed a significant increase in the mRNA expression of cytosolic phospholipase A2 and cyclooxygenase 2, enzymes that liberate the eicosanoid precursor arachidonic acid and synthesize PGs respectively, in periovulatory ovaries of solitary females. Results from mass spectrometry screening for 25 different eicosanoids showed no change in eicosanoid levels at this stage. Ongoing studies will reveal if mRNA expression of EEBPs and eicosanoid levels are elevated in post-ovulatory ovaries of non-spawning fish and how these findings compare to fish destined to spawn. This work will provide the foundation from which to ultimately clarify the role of eicosanoids in the culminating events of female zebrafish reproduction.

TOPIC: GROWTH AND AGING

P-29.

LIPID AND CARBOHYDRATE ANALYSIS ON A DROSOPHILA MELANOGASTER TYPE 2 DIABETES MODEL

Reyes De la Torre, Alejandro (1); Riesgo-Escovar, Juan R (1). (1) Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus Juriquilla, Querétaro, México.

Type 2 diabetes (T2D) shows insulin resistance, high glucose, and elevated free fatty acids. The insulin pathway is evolutionarily conserved. Drosophila insulin pathway mutants have elevated lipid and carbohydrate levels, yet chronic aspects of this T2D model have not been investigated. We measured total lipid and carbohydrate levels from larvae and adult female flies of 1, 7, 14, and 28 days in wild type, homozygous chico1/chico1, heteroallelic mutant InRE19/InR3T5, Dp110A/Dp1105W3, PKB1/PKB3, and S6KL-1/S6KP1713 and heterozygous controls. InR is the fly insulin receptor, Chico the IRS homologue, DP110 the PI3 kinase catalytic subunit, and S6K S6 kinase. We also measured total cell area occupied by Nile Red stained lipid droplets in isolated abdominal adipocytes in one-day-old adult flies, mutants, and controls. Results show that carbohydrate levels do not change with time in wild type adults, but increase in insulin pathway mutants, being always higher in InR, chico, and PKB mutants ($P<0.001$). On the other hand, lipid levels do increase in wild type adults with time ($P<0.001$), but this increase is not seen in insulin pathway mutants. Insulin pathway mutants do show, however, significantly higher lipids levels throughout time ($P<0.001$). Adipocyte lipid volume does not change between mutants and controls. We conclude that carbohydrate and lipid homeostasis is altered in mutant flies, it being a chronic effect. Since there are no significant differences in lipid volume in abdominal adipocytes between mutants and controls, lipid differences may be due to different numbers of abdominal adipocytes or lipid accumulation in other tissues.(We thank Dr. Peña-Rangel, Ana Pinedo, and Claudia González. Funding: UNAM, CONACYT).

TOPIC: INTRACELLULAR SIGNALLING

P-30.

SPHINGOSINE 1-PHOSPHATE AS A REGULATOR OF BOVINE DOMINANT FOLLICLE FATE

Hernández-Coronado CG (1), Guzmán A (1), Romano-Pardo MC (2), Verde-Calvo JR (3), Rosales-Torres AM (1).

1Laboratorio de Bioquímica de la Reproducción UAM-X México Distrito Federal. 2Departamento de Fisiología CINVESTAV México Distrito Federal, 3Departamento de Biotecnología UAM-I México Distrito Federal.

The balance among survival and death cell signals determine follicular fate throughout follicular development and atresia. In several cells types, sphingosine-1-phospate (S1P) promotes cellular proliferation and survival, whereas ceramida (CER) triggers cell death; thus ratio S1P/CER decides cellular destiny. The aim of present experiments was to quantify S1P and CER concentrations and its ratio in bovine healthy and atretic dominant follicles (DF). Follicles with 8 to 17 mm of diameter were dissected from cow ovaries collected slaughtered in a local abattoir. Follicular sac (FS), granulose cells (GC) and follicular fluid (FF) were separated; 17β-estradiol (E2) and progesterone (P4) concentrations were measured by RIA in FF and based in their E2/P4 ratio follicles were classified as healthy (3.2 ± 0.44) and atretic (0.3 ± 0.05). In GC and FS, sphingolipids were extracted and S1P and CER concentrations were quantified by HPLC (X Terra RP-18; 5µm, 3.0x150mm column). Results showed that in both GC and FS, S1P concentrations were higher in healthy DF than in atretic DF ($P<0.05$) without changes in CER concentrations. Ratio S1P/CER in both follicular compartments was also higher in healthy DF. Interesting, in healthy DF there was 45 fold more S1P that CER in the GC ($P<0.05$) whereas in FS SIP was just 17 folds greater than CER. Results suggest that SIP have a role in ovarian DF health, probably increasing cell proliferation and survival. In contrast, reduction of DF SIP could be triggering cellular death and atresia.

P-31.

REGULATION OF OVARIAN INSULIN-LIKE GROWTH FACTOR EXPRESSION IN THE ZEBRAFISH OVARY

Glen Van Der Kraak (1), David Irwin (1) and Hannah Anderson (1)
(1) Department of Integrative Biology, University of Guelph, Guelph, ON, Canada

Our earlier studies have shown that insulin-like growth factors (IGFs) regulate cell survival, oocyte maturation and prostaglandin synthesis during the periovulatory period in the zebrafish. This work established that igf3, igf2a, and igf2b are expressed in full grown immature (FG) and mid-vitellogenic (MV) ovarian follicles. The current studies investigate the hormonal and intracellular signaling cascades involved in regulating the expression of ovarian-derived IGFs in zebrafish . Using quantitative real-time PCR we showed that the gonadotropin analogue human chorionic gonadotropin (hCG) and the adenylate cyclase activator forskolin increased igf3 expression in FG and MV follicles, but had no effect on igf2a or igf2b expression. The effects of hCG on igf3 expression were blocked by the addition of the protein kinase A (PKA) inhibitor H-89. Pituitary adenylate cyclase activating peptide (PACAP) also stimulated a small increase in igf3 expression in FG follicles, while growth hormone and salmon gonadotropin releasing hormone had no effect on igf3, igf2a, or igf2b expression. We also showed that showed that the protein kinase C activators, PMA and A23187, strongly down-regulate igf3, igf2a and igf2b expression in FG follicles,. Given that these compounds also stimulate prostaglandin synthesis in zebrafish ovarian follicles it was perhaps not surprising that the addition of prostaglandin E2 and prostaglandin F2α also attenuated igf3 expression in full grown follicles suggesting that that production of prostaglandins by PKC may be the driving force behind the down-regulation of the IGFs. Collectively these results demonstrate that IGF expression in the ovary is stimulated by PKA activators and inhibited by PKC activators and imply that there is a dynamic regulation of ovarian IGFs during the periovulatory period.

TOPIC: ION AND WATER BALANCE

P-32.

GROWTH HORME, PROLACTIN AND CORTISOL REGULATE AQUAPORIN EXPRESSION IN THE GASTROINTESTINAL TRACT OF SALMONIDS.

Steffen S. Madsen, (1,2) and Christian K. Tipsmark (2)
(1) Institute of Biology, University of Southern Denmark, Odense, Denmark (2) Department of Biological Sciences, University of Arkansas, Fayetteville, AR USA

Aquaporins (Aqp's) are integral membrane proteins that are permeable to water and in some cases small noncharged solutes such as glycerol and urea. In fish, tandem and genomic duplication events have led to at least 18 paralogs with widespread tissue distribution. Aqp's are suspected to have a major role in the absorption of imbibed water in the gastrointestinal system of seawater (SW)-acclimated euryhaline fish, such as salmonids; however, the redundant expression of at least 6 paralogs here makes the situation quite complex. Based on our previous work, 3 paralogs may be of particular importance for transcellular water absorption: Aqp1a, -1b and -8ab. All three are permeable to water and Aqp8ab is also permeable to glycerol and urea. They are all localised in the apical brush border of pyloric, middle and posterior gut segments, and the further presence of Aqp8ab in basolateral membranes opens a transcellular pathway for water movement. Transfer of fish from freshwater (FW) to SW stimulates the mRNA and protein expression of all three paralogs, in particular Aqp8ab. We report here the effect of GH (200 ng/g), PRL (200 ng/g) and cortisol (4 µg/g) on *aqp8ab* mRNA levels when administered *in vivo* (2 injections) and during 24 h *in vitro* tissue culture. GH (trout or carp) stimulated whereas cortisol inhibited *aqp8ab* expression in pyloric and middle intestine when injected into FW fish. The two hormones had an additive (antagonistic) effect when administered together. Neither hormone (0.2 and 1 µg/mL, respectively) had an effect when applied to intestinal biopsies *in vitro*. Both PRL (salmon) and cortisol inhibited *aqp8ab* expression when injected into SW fish. PRL (200 ng/mL) furthermore inhibited *aqp8ab* expression when applied to intestinal biopsies *in vitro*. The study supports and elaborates the role of GH and PRL as being SW- and FW-hormones, respectively, by having profound effects on the functional development of the intestine into a water absorbing organ.(Supported by the Danish Research Council and the Carlsberg Foundation).

P-33.

IDENTIFICATION, EXPRESSION ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF A SEROTONIN RECEPTOR ESSENTIAL FOR INITIATING THE RAPID DIURESIS THAT FOLLOWS BLOOD GORGING IN RHODNIUS PROLIXUS

Paluzzi, J.P.(1) Bhatt, G.(1) and Orchard, I. (1)
(1) Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada

In the Chagas disease vector, Rhodnius prolixus, two diuretic hormones have been identified and are known to work synergistically to increase Malpighian (renal) tubule (MT) fluid secretion rates by over 1000-fold during the rapid diuresis that follows engorgement of vertebrate blood. One of these diuretic hormones is the biogenic amine, serotonin (5-hydroxytryptamine, 5-HT), which plays a variety of additional roles in R. prolixus including plasticization of the cuticle, stimulation of salivary gland secretion, stimulation of absorption by the anterior midgut, increasing dorsal vessel contractions (i.e. cardioactive), and myotropic activities on various visceral tissues (e.g. salivary glands, anterior midgut, hindgut). In order to better understand the signaling mechanisms linked to the various physiological roles of 5-HT, we have isolated a 5-HT receptor in R. prolixus, Rhopr5HTR, and functionally characterized its activation characteristics using various receptor agonists, antagonists, and other insect biogenic amines. The Rhopr5HTR receptor, which shares high sequence similarity to the mammalian 5HT2A receptor subtypes, was dose dependently activated by 5HT with an EC50 in the nanomolar range. Rhopr5HTR expression analysis by quantitative PCR reveals a variety of

tissues that may be sensitive to this neurohormone, including those previously established (MTs, anterior midgut, salivary glands, hindgut), as well as novel targets which will be investigated in future studies. We utilized RNAi-mediated knockdown of Rhopr5HTR in order to examine the importance of the 5HT neuroendocrine system in processes related to the rapid diuresis that ensues after engorgement of vertebrate blood. These findings confirm that 5HT signaling plays an essential role in the rapid diuresis that follows gorging, and in addition, 5HT signaling is vital for coordinating an array of other feeding-related activities, which are required for normal development. This research was supported through an NSERC Discovery Grant to I.O.

P-34.

CONTROL OF OSMOREGULATORY ORGAN SALIVARY GLANDS IN HEMATOPHAGOUS TICKS

Yoonseong Park and Ladislav Simo,
Department of Entomology, Kansas State University, Manhattan, KS, USA

Ticks are obligatory ectoparasites causing global animal and human health problems. Tick salivary secretions are required for initiation and continuation of the feeding by injections of bioactive salivary components into the host and by osmoregulations upon ingestion of large quantity of blood. Nervous control of the salivary glands has been previously speculated. Dopamine has been described as a potent activator of salivary secretion. Our recent finding indicates presence of more complex mechanisms for control of the salivary glands. We identified at least two neuropeptides, myoinhibitory peptide and SIFamide, innervating the salivary glands in blacklegged ticks (Ixodes scapularis Say) which transmits the most important tick-born disease Lyme disease. We hypothesize that these neuropeptides control dopaminergic cells located on the base of acini in the salivary glands. Additionally, we find at least two different dopamine receptors with putative different functions in the salivary secretion. The fundamental knowledge obtained from this research is eventually expected to lead to the design of compounds and vaccines to prevent disease transmissions by ticks.

P-35.

REGULATION OF SEAWATER AND FRESHWATER INDUCED GILL TIGHT JUNCTION PROTEINS IN TILAPIA BY CORTISOL AND OSMOLALITY CHANGES IN VITRO

Tipsmark CK (1,3), Breves JP (2,3), Rabeneck DB (1), Trubitt RT (1), Lerner DT (3) and Grau EG (3)
(1) Department of Biological Sciences, University of Arkansas, Fayetteville, AR USA (2) Department of Biology & Center for Neuroendocrine Studies, Univ. of Massachusetts, Amherst, MA, USA (3) Hawaii Institute of Marine Biology, Univ. of Hawaii, Kaneohe, HI, USA

This study examined expression of tight junction proteins in the gill of Mozambique tilapia in response to salinity changes and in vitro treatment with cortisol, hyperosmotic and hypoosmotic media. Claudins and occludin identified in branchial expressed sequence tag libraries were used to pinpoint likely relevant targets for the study (claudin-10c, claudin-10e, claudin-28a, claudin-30 and occludin). Transcript levels of claudin-10c and claudin-10e increased in seawater (SW), while claudin-28a and occludin were found to be higher in freshwater (FW). To evaluate if effects of cortisol or changed osmolality in situ could be involved in the observed changes, a series of experiments were performed with gill filaments incubated in vitro. The experiments included isosmotic media as control. Some applied hypoosmotic media combined with cortisol treatment others hyperosmotic media combined with cortisol. The data suggest a dissimilar expression pattern of FW and SW induced tight junction proteins in response to the treatments.

P-36.

OSMOREGULATORY HORMONES MODULATE TIGHT JUNCTION PROTEIN EXPRESSION AND TRANSEPITHELIAL RESISTANCE IN AN EPITHELIAL GILL CELL LINE FROM RAINBOW TROUT.

Trubitt RT (1), Bujak JK (1), Madsen SS (2), Rabeneck DB (1) AND Tipsmark CK (1)
(1) Department of Biological Sciences, University of Arkansas, Fayetteville, AR USA (2) Institute of Biology, University of Southern Denmark, Odense, Denmark

Much in vitro research of gill function in euryhaline fishes relies on primary cell cultures. However, the use of a novel model for branchial epithelia, the immortal trout gill cell line RTgill-W1, could make this research more efficient and easier to replicate. So far the cell line has been applied in studies of fish pathology and toxicology. We examined the trout cell line grown to confluent epithelial cultures when applied in studies of osmoregulatory physiology. Immunostaining for tight junction proteins showed the presence of tight junctions. Transepithelial resistance (TER), measured in seeded trans-wells, develops to a constant level within 1-2 days and was stimulated by the addition of cortisol. The epithelial sheet formed by RTgill-W1 cells has a relative low TER that compares to what can be observed in opercular membranes of seawater killifish and proximal kidney tubules. TER is elevated significantly by tetrabromocinnamic acid, an agent well known to reduce paracellular cation flux. We then examined responses of the cell line to treatment with growth hormone (GH) alone or in combination with cortisol. Expression of tight junction proteins zonula occludens 1 (ZO-1), claudin-10e and claudin-30 were studied. While ZO-1 was unresponsive to the hormonal treatment, claudin-10e was up-regulated by GH and claudin-30 stimulated by cortisol. A parallel combination experiment with cortisol and GH measuring TER showed that GH very significantly antagonizes cortisol induced TER increases. The stimulatory effect of GH on claudin-10e and in silico analysis suggesting that this claudin are likely to form cation pores in tight junctions suggests that claudin-10e induction by GH may be functionally coupled to the hormone antagonistic effect to the "tightening" effect of cortisol. Given the analogy to current in vivo data on salmonid claudins this in vitro model appears to have potential in studies of endocrine control of ion transport in euryhaline fishes.

P-37.

FGLAMIDE-RELATED ALLATOSTATINS IN RHODNIUS PROLIXUS

Meet Zandawala (1) and Ian Orchard (1)
(1) Department of Biology, University of Toronto Mississauga, Mississauga, ON, Canada.

Allatostatins (ASTs) are insect neuropeptides that were first identified as inhibitors of juvenile hormone biosynthesis by the corpora allata. There are three families of ASTs in insects, defined by their C-terminus conserved regions, one of which is FGLamide. Previously, we cloned and characterized the complete cDNA sequence encoding the Rhodnius prolixus FGLa/ASTs (Rhopr-FGLa/ASTs). Fluorescent in situ hybridization showed transcript expression in neurons in each ganglion of the CNS, but notably in five dorsal unpaired median (DUM) neurons in the mesothoracic ganglionic mass, which produce neurohaemal sites over the abdominal nerves. Rhopr-FGLa/ASTs inhibit spontaneous contractions of the anterior midgut and leucokinin-1-induced hindgut contractions; suggestive of a role in physiological events post-feeding. To investigate this possibility, we have examined the potential release of Rhopr-FGLa/ASTs into the haemolymph following feeding. Immunohistochemical analyses indicate that FGLa/AST-like immunoreactivity (FLI) is reduced in DUM neurons and their neurohemal sites at 48-50 hours post-feeding. Moreover, FLI is also absent in the open-type endocrine cells of the anterior midgut from 48-50 hours post-fed insects. However, no apparent

change in immunoreactivity was observed in posterior midgut and hindgut up to 48-50 hours post-feeding. Rhopr-FGLa/ASTs inhibit hindgut contractions induced by an endogenous kinin, Rhopr-kinin-2, but do not inhibit serotonin-stimulated anterior midgut absorption and Malpighian tubule secretion suggesting that they may only indirectly affect post-feeding diuresis in R. prolixus. Lastly, we have cloned and characterized a putative receptor for FGLa/ASTs in R. prolixus. Spatial expression analysis identifies novel target tissues that have not previously been examined in this species. (Supported by the Natural Sciences and Engineering Research Council of Canada).

TOPIC: METABOLISM AND FEEDING.

P-38.

IDENTIFICATION OF DEPOT-SPECIFIC GENE CHANGES AS A RESPONSE TO A HIGH-FAT DIET DURING ADIPOCYTE PRECURSOR CELL DIFFERENTIATION

Lucía Martínez de la Escalera, Xarubet Ruíz-Herrera, Carmen Clapp, Yazmín Macotela
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México

The metabolic syndrome is associated with an inability of adipocyte precursor cells (APCs) to differentiate into new adipocytes capable of adequately managing the excess fat, resulting in hypertrophic adipocytes that develop inflammation and secrete excess fatty acids. All adipose tissue depots are intrinsically different. Visceral APCs have higher requirements for differentiation in vitro than those from subcutaneous depots, and excess visceral but not subcutaneous fat is strongly associated with metabolic disease. Understanding their intrinsic characteristics and how these are affected by obesity may help explain why each poses a different metabolic risk. Thus, we compared ten preferentially expressed genes between visceral and subcutaneous APCs from mice fed a high-fat (HFD) or control-chow diet (CD) before and after induction of in vitro differentiation. APCs were isolated from male mice after 8 weeks on either diet using MACS separation technology. Two days before 100% confluence (day -2), cells were incubated with APC commitment factor bone morphogenetic protein 4 (BMP4); on day 0 they were treated with differentiation cocktail (IBMX, dexamethasone, insulin and rosiglitazone) and on day 3 with insulin alone. Cells were collected before BMP4 was added (day -2) and after the differentiation protocol was completed (day 9). Gene expression was analyzed using qRT-PCR. Of the ten genes analyzed, five [haptoglobin (hp), angiotensinogen (agt), cadherin-9 (cdh-9), vascular endothelial growth factor-c (vegfc) and matrix metalloproteinase-3 (mmp-3)] showed a marked rise in expression that was differentially affected by the HFD in association with their depot origin. In subcutaneous APCs, the HFD attenuated the rise of hp and agt observed with a CD but up-regulated vegfc and mmp-3. The HFD caused no apparent change in expression of agt in APCs from the visceral depot, but it down-regulated mmp-3 and attenuated the rise of hp and vegfc. Finally, in APCs from both depots, the HFD enhanced the expression of cdh-9 on day -2, blunting its up-regulation at the end of the differentiation protocol. The marked depot-specific changes in expression of these genes as a response to HFD and adipogenic stimuli may indicate their participation in the metabolic alterations of adipose tissue biology that arise from obesity. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grants 164423 and FOMIX 174984. We thank D. Mondragón, and M. García their technical assistance).

P-39.

DAYTIME RESTRICTED FEEDING MODIFIED THE DIURNAL PROFILE OF CORTICOSTERONE, INSULIN AND GLUCAGON: ASSOCIATION WITH LIVER GLUCONEOGENESIS AND GLYCOGEN PRODUCTION

Pérez-Mendoza M. (1), Díaz-Muñoz M. (1)
(1) Departament of Cellular and Biology Molecular, Instituto de Neurobiología, UNAM-Juriquilla. Querétaro, Qro. MÉXICO.

Limited food access during daytime promotes circadian adaptations that are advantageous for the restricted processing of nutrients. The liver is the main organ that responds to this protocol by shifting the phase of diverse anabolic and catabolic pathways, such as gluconeogenesis (production of new glucose) and glycogenesis (production of glycogen). These 2 activities are under endocrine regulation by corticosterone, insulin and glucagon. We measured a 24 h-profile of these 3 hormones as well as the liver content of glycogen and the presence of total and phosphorylated glycogen synthase in rats with food access only for 2 h in the light period. We found that all these parameters changed in function of the time of the day and the feeding condition. The restricted feeding promoted high levels of glucagon, low levels of insulin and changes in the rhythmicity of corticosterone and glycogen-handling by the liver.(We are thankful to CONACyT (project U-49047) and CONCyTEQ for financial support. It is acknowledged the technical assistance of Lic. Nut. Fernando López-Barrera, Lorena López and Dr. Olivia Vázquez–Martínez).

P-40.

HYPOTHALAMIC DIFFERENTIAL GENE EXPRESSION IN BABOONS WITH AND WITHOUT OBESITY.

Rodríguez-Sánchez IP (1), Treviño-Alvarado VM (2), Tejero-Barrera ME (3), Cole SA (4), Comuzzie AG (4), Martínez de Villarreal LE (1) Barrera-Saldaña HA (5)(6) and Pérez-Ibave DC (5)

(1) Department of Genetics, School of Medicine of the Autonomous University of Nuevo León, Monterrey 64460 , México. (2) Department of Computation Science, Mechatronics Division and Information Technology, ITESM, Campus Monterrey, Monterrey Nuevo León 6484, México. (3) Department of Nutrigenetics and Nutrigenomics of the INMEGEN, Tlalpan Delegation, México D.F. 14610. (4) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio Tx 78227, USA. (5) Department of Biochemistry and Molecular Medicine, School of Medicine of the Autonomous University of Nuevo León, Monterrey 64460, México. (6) Vitaxentrum. Blvd. Puerta del Sol 1005, Colinas de San Jerónimo, Monterrey, Nuevo León 64460, México.

Obesity is defined as the presence of an excessive amount of fat that varies depending on age and gender, a consequence being considered is the positive calorie balance, either by high energy intake and/or reduced energy expenditure . Obesity is considered as a pandemic and is recognized as a complex and multifactorial disease. The causes of obesity are many, but the evidence points to an important role of heredity, specifically changes in the nervous system, affecting the endocrine, immune and metabolic functions. The baboon (Papio hamadryas) is used as an experimental model for multiple diseases, including obesity because, like in humans, the condition develops spontaneously. METHODS: The study groups were composed by 22 adult baboons which were divided into two groups: lean and obese, matched for age and sex. From these hypothalamic tissue biopsies were obtained an total RNA was extracted and hybridized to microarrays gene expression determined by more than 13000 transcripts. The differentially expressed transcripts were amplified, cloned, sequenced and compared. RESULTS: This study allowed identifying differentially expressed genes between obese and lean baboons both males and females in hypothalamic tissue.. Of these genes differentiated, 3 (TMEM107, POLR3A and MPPE1) had significant differences between obese and lean male baboons and 9 (RPL12, PSAP, MED30, KIF1A, TMBIM1, PKD1, SLC7A14, NELF and MRPS33) had significant differences between obese and lean female baboons. From the genes identified, 38% participates directly or indirectly in the endocrine system, 22% in the regulation of immunity and inflammation, 8% in lipid metabolism, 4% in glycolysis and glycogenesis and

the remaining 28% has not yet been linked to obesity. CONCLUSION: These results describe for the first time differentially expressed genes in obesity by gender. The baboon was found to be a excellent model to know the role of hypothalamic tissue in obesity.

P-41.

GHRELIN O-ACYL TRANSFERASE IN FISH

Roman Yufa (1) and Suraj Unniappan (2)

(1) Department of Biology, York University, Toronto, Ontario, Canada; (2) Department of Veterinary Biomedical Sciences, Laboratory of Integrative Neuroendocrinology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Ghrelin is an evolutionarily conserved multifunctional hormone in vertebrates. It has significant stimulatory effects on growth hormone secretion, feeding and anabolism in fish and mammals. Ghrelin O-acyl transferase (GOAT) is the enzyme responsible for the acylation of ghrelin, a multifunctional metabolic hormone. The addition of an acyl group to the third amino acid in the n-terminal region is essential for the biological activity of ghrelin. GOAT, due to its indirect role in regulating bioactive ghrelin, is currently being explored as an anti-obesity therapy. The protein sequences of GOAT appear to be conserved from humans to zebrafish. However, GOAT in non-mammalian vertebrates remain poorly understood. We studied GOAT in zebrafish and goldfish, two well characterized and widely used model organisms in comparative endocrinology. We hypothesized that GOAT is highly conserved in fish, and is expressed in a meal and energy status dependent manner in the brain and gut of fish. GOAT mRNA is expressed in the brain and gut of zebrafish. We found abundance of GOAT-like immunoreactivity (ir) in the gut of zebrafish and goldfish. GOAT-like-ir colocalizes ghrelin-like-ir in the enteroendocrine cells of both zebrafish and goldfish. GOAT-like-ir is visible in the gut of both fed and fasted zebrafish and goldfish. Preliminary studies did not find any significant difference in GOAT mRNA expression in the brain or gut during a 7 day fast. While an increase during the regular feeding time was observed, no clear peri-prandial profile in gut GOAT mRNA expression was visible. Further studies are required to elucidate whether nutrient status affects GOAT mRNA expression in fish. Our results indicate the presence of a very highly conserved GOAT in fish. The co-expression of GOAT in the gut ghrelin cells suggest acylation occurs at sites proximal to the enteroendocrine cells, the most abundant source of ghrelin in fish.(This research is generously supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant, and a Discovery Accelerator Supplement (DAS) Award to SU. SU is a Canadian Institutes of Health Research New Investigator).

P-42.

THE GALANINERGIC SYSTEM REGULATES FEEDING AND GUT CONTRACTIONS IN GOLDFISH

Elsie Tachie Mensah (1), Andrew Donini (1), Suraj Unniappan (2)

(1) Department of Biology, York University, Toronto, Ontario, Canada; (2) Department of Veterinary Biomedical Sciences, Laboratory of Integrative Neuroendocrinology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

The regulation of energy balance is achieved by the coordinated actions of multiple hormones. The galaninergic system, comprised of galanin, galanin-like peptide (GALP), and its receptors (GALR1, GALR2 and GALR3), is a major player in the regulation of feeding and metabolism. Galanin stimulates feeding in goldfish. We previously characterized the galanin genes in goldfish. The identity of GALP and galanin receptors remain unknown in fish. The aim of this study was to further elucidate the role of the galaninergic system in regulating energy balance in fish. In this study, we identified partial mRNA sequences encoding GALR1 and GALR2 in goldfish. Hydropathy analysis found that goldfish GALR1 and GALR2 are seven transmembrane domain containing G-protein coupled receptors with high similarity to their chicken and human counterparts. Messenger RNAs encoding both receptors are expressed in the brain and gut of goldfish. Both GALR1, GALR2 and GALP-like immunoreactivity were also detected in the goldfish hypothalamus, a brain region important in coordinating feeding and metabolism in fish. Similarly, GALR1- and GALR2-like immunoreactivity was also detected in the goldfish gut. Galanin (10-7 M) caused a significant decrease in the amplitude of spontaneous contractile activity of goldfish gut in vitro. Meanwhile, galanin at 10-6 M increased the amplitude of acetylcholine-induced contractions of goldfish gut in vitro. A single intracerebroventricular injection of 1 ng/g body weight human GALP caused a significant increase in food intake at one hour post-injection in goldfish. These results show that the non-galanin members of the galaninergic system are also present in the brain and gut, and influences feeding and gut motility in fish. These new results highlight the importance of galanin and related peptides in the maintenance of energy homeostasis in goldfish. (This research is generously supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada Discovery Grant, and a Discovery Accelerator Supplement (DAS) Award to SU, and a NSERC Discovery Grant to AD. SU is a Canadian Institutes of Health Research New Investigator).

P-43.

DIETARY INTAKE OF INULIN AND PROMOTER GROWTH FLAVOMICYN AND ITS IMPACT ON MONO- AND POLY-UNSATURATED FATTY ACIDS CONTENT IN RABBIT MEAT.

Salas R (1), Juárez ME (1)

(1) Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México D.F.

Rabbit meat is a product whose characteristics are beneficial for human consumption, since meat is rich in protein, easily digestible, low cholesterol. The fat content of rabbit meat depends on food, sex or channel portion analyzed. It is an excellent source of polyunsaturated acid. Within the field of animal production have been several studies on the effect of the inclusion of inulin as prebiotic diets and as an alternative to the use of antibiotic growth promoters (AGP), flavomycin. The aim of this study was to evaluate the effect of inulin and AGP on the content of polyunsaturated fatty acids and monounsaturated. We used 40 New Zealand rabbits aged 40 days, divided into 4 groups of 10 animals. The control received a diet free of AGP and Inulin, the second was supplemented with 0.25% inulin. The third, treated with AGP (0.01%) and the fourth group, inulin/AGP, was 50:50. In the rabbit loin, the inulin group had higher levels of oleic and heptadecanoic than AGP and control groups ($P \leq 0.04$). Also, α-linolenic acid concentration was higher than the control and inulin/AGP groups ($P \leq 0.02$). In arm-back, the supplemented with inulin had heptadecanoic acid concentrations greater than the control and Inulin/AGP groups ($P \leq 0.05$) and higher levels of α-linolenic acid that AGP groups and Inulin/AGP ($P \leq 0.04$). Regarding the leg-thigh meat the supplemented with inulin had higher heptadecanoic acid concentration than control and AGP groups ($P \leq 0.05$); also with Inulin/AGP had higher levels of arachidonic acid ($P \leq 0.03$). With linoleic, Inulin and AGP treatments showed higher concentration than the control and Inulin/AGP groups ($P \leq 0.05$) and finally inulin group had significantly higher values α linolenic than the other groups ($P \leq 0.01$). We conclude that the dietary intake of inulin, improved rabbit meat quality, respect to mono- and polyunsaturated fatty acids, mainly by increasing the concentration of linoleic acid.

P-44.

SENSORY EVALUATION OF MEAT CHICKEN FED WITH DIFFERENT CONCENTRATIONS OF INULIN

Juárez ME (1), Salas R (1), Pérez-Gil F (1.)
(1) Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán, México D.F.

The need for healthy foods of animal origin for human consumption, leading the way for research and development of alternatives to ensure safe, achieving an improvement in the quality of chicken meat that requires increasingly demanding market. The use of prebiotics such as inulin in the diet is a healthy choice for consumers by offering chicken richer in protein, low in fat, and has a characteristic flavor and color. The aim of this study was to evaluate the effect of added inulin in the diet on the quality of meat in different cuts, assessing the preference for untrained judges. The treatments were: T1 without inulin, inulin 0.1% T2, T3 0.2% inulin, inulin 0.4% T4 and T5 with a common food. Samples were taken randomly (leg, thigh and breast) at slaughter (46 days). Sensory analysis of chicken meat under the same cooking method was evaluated on a hedonic scale of 4 grade levels, measuring sensory attributes such as appearance, color, smell and taste of each treatment and each piece. Statistical analysis was used Kruskal-Wallis test with $P < 0.05$. The acceptance level for breast appearance in descending order was: T1, T4, T2, T3 and T5, Leg T3, T5, T2, T4 and T1, in thigh no difference. Regarding breast color was first T1, T4, T2, T3 and T5, for the most accepted leg T5, T3, T2, T4 and T1, in thigh was not different. For odor in any parts difference was found. The flavor of the leg from highest to lowest preference was T1, T4, T3, T2 and T5. For preference thigh was T3, T4, T1, T2 and T5, for breast no difference. It can be concluded that with the exception of the leg in color, consumers preferred chickens containing no growth promoters and pigments.

P-45.
HYPERPROLACTINEMIA PREVENTS VISCERAL ADIPOSE TISSUE HYPERTROPHY AND REDUCES INSULIN RESISTANCE IN OBESE RATS.

Ruiz-Herrera, X, Lemini, M, López-Barrera, F, Martínez de la Escalera, G, Clapp, C, Macotela, Y.
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México

Obesity is the main risk factor for the development of type 2 diabetes and cardiovascular disease. However, not all fat depots posses the same risk; increased visceral adipose tissue (VAT) mass is associated with metabolic disease, whereas excess subcutaneous fat (SAT) is not. Prolactin (PRL) may regulate adipose tissue function and metabolic homeostasis. Human adipose tissue secretes PRL, and preadipocytes and adipocytes from mice VAT express higher levels of the PRL receptor (PRLR) compared to their SAT counterparts. Also, PRLR knockout mice have reduced adipose tissue mass compared to wild type animals and are protected against the deleterious effects of high fat diet-induced obesity. We analyzed whether hyperprolactinemia (hyperPRL) influences adipose tissue expansion in a depot specific manner and insulin resistance in obese rats. Male Wistar rats were maintained with either a control (CD) or a high fat diet (HFD) and 4 weeks later, divided to form groups: CD, CD+hyperPRL, HFD and HFD+hyperPRL. HyperPRL was induced by subcutaneous osmotic pumps releasing PRL for 28 days, and PRL circulating levels were evaluated by the Nb2 bioassay. An insulin tolerance test was performed after 3 weeks of hyperPRL induction, and adipose tissues and serum were collected at the end of the following week. HyperPRL significantly reduced HFD-induced insulin resistance and resulted in increased VAT and SAT mass in the HFD-fed animals. However, this was the result of increased hyperplasia in both fat tissues but reduced hypertrophy specifically in the visceral. Increased levels of adiponectin were found in HFD-HyperPRL rats, which may also account for their increased insulin sensitivity. HyperPRL had no effects on insulin sensitivity and adipose tissue expansion in CD-fed rats, or on food intake or body weight in rats under either diet. In conclusion, hyperPRL could exert beneficial metabolic effects by modulating adipose tissue expansion in obesity. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grants 164423 and Fomix 174984. We thank D. Mondragón, and A. Prado for their technical assistance).

TOPIC: MOLECULAR EVOLUTION

P-46.
EXPRESSION OF RECOMBINANT GREEN IGUANA GROWTH HORMONE IN PICHIA PASTORIS

Flores-Peña, BE (1), Ávila-Mendoza, J(1), Luna Muñoz, M(1) and Arámburo, C(1).
(1) Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Campus Juriquilla, Universidad Nacional Autónoma de México, Querétaro, Qro., 76230, México

In all vertebrates, growth hormone is involved in important functions in development, growth and metabolism. Little is known, however, about the role and regulation of GH in reptiles. It is therefore necessary to develop tools to address the study of GH function in these organisms. In this work we aimed to produce functional recombinant green iguana Growth Hormone (rgiGH) in the Pichia pastoris system, in order for the recombinant protein to keep the main structural features (folding and post-translational modifications) as the native protein. The giGH cDNA was amplified by PCR from pituitary RNA extracts and ligated to pPIC9 vector, which contained the alcohol oxidase (AOX1) promoter. Yeast cells were electroporated with the expression cassette linearized by Sal I and the integration of the construction was confirmed by PCR. The positive colonies were identified as 1 Kb product corresponding to giGH cDNA and a AOX1 gene fragment. This PCR product was sequenced and analyzed to confirm the absence of mutations. With the positive strains, fermentation was performed in two phases, one to stimulate growth (with glycerol in the culture medium) and the other one to induce the rgiGH expression (with methanol). The induction phase was extended for 8 days and in each day a sample was taken from culture medium to analyze the presence of the recombinant protein by Western blot, using a heterologous antibody directed against chicken GH, that cross-reacts with giGH. The luminograms obtained showed the presence of a 26 kDa band, under reducing conditions, corresponding to the rgiGH. The major recombinant hormone production was observed on the fifth day of induction. Results indicated that the Pichia pastori system is a good alternative to produce sufficient rgiGH amounts, which will boost future GH research in this reptilian model. (Supported by PAPIIT-DGAPA-UNAM IN206813, IN208812; CONACYT 178335).

TOPIC: NEURAL DEVELOPMENT AND PLASTICITY

P-47.

AN EXPERIMENTAL PLATFORM FOR IDENTIFYING KRÜPPEL-LIKE FACTOR TARGET GENES AND PROTEIN INTERACTING PARTNERS IN HIPPOCAMPAL NEURONS

Knoedler, J (1) and Denver, RJ (1, 2), Neuroscience Graduate Program (1) and the Department of Molecular, Cellular and Developmental Biology (2), The University of Michigan, Ann Arbor, MI USA.

Krüppel-like factors (KLFs) are zinc finger transcription factors that have diverse roles in physiology and development. There are 17 different KLFs in mammals, but little is known about their genomic targets and the cellular pathways that they influence. We have established a platform using the mouse hippocampus-derived neuronal cell line HT22 to investigate genomic targets and protein interacting partners for KLFs. Our initial focus is on KLF9 which has functions in neural development and nuclear hormone receptor signaling. We engineered stable tetracycline-inducible HT22 cell lines using the T-Rex system to allow for temporal control of Klf9 expression. A time course experiment showed maximal Klf9 mRNA at 2 h which was maintained through 24 h. We engineered a luciferase reporter vector with three basic transcription element sequences in tandem (pGL4.23-3xBTE) to assay for KLF9 protein. Inducing Klf9 led to repression of this reporter, demonstrating expression of functional protein. Our preliminary results show that expression of Klf9 in HT22 cells reduced cell proliferation (MTT assay) and altered morphology. We are applying deep sequencing of RNA from this cell line to identify early KLF9 response genes. We have also developed HT22 cells that stably express the biotin ligase BirA and a peptide-tagged KLF9 that can be biotinylated in vivo. We confirmed by Western blotting that the stable lines express BirA protein and FLAG-tagged KLF9, and biotinylated KLF9 by blotting with streptavidin-HRP. We are now using these cells to identify KLF9 genomic targets by chromatin precipitation with streptavidin beads combined with deep sequencing. We will also use these cells to identify KLF9 protein interacting partners by mass spectrometry. This platform will help define the KLF9 cistrome and protein interaction networks in hippocampal neurons, and establish a platform for investigations of other KLFs to understand the diversity of KLF actions in development and physiology. (We thank Dr. Anthony Hollenberg (Beth Israel Deaconess Medical Center) and Dr. Jianlong Wang (Boston Children's Hospital) for BirA and biotin fusion protein vectors and Dr. David Schubert (Salk Institute, La Jolla, California) for HT22 cells. This work was supported by NIH grant 1 R01 NS046690 and NSF grant IOS 0922583 to RJD. JK was supported by a NIH training grant (T-32-NS076401).

TOPIC: OTHER

P-48.

ANALYSIS OF RETINAL VASOINHIBINS AND THEIR OCULAR GENERATION IN DIABETIC AND NON-DIABETIC RATS.

Adán E, Díaz-Lezama N, Ramírez M, Triebel J, Moreno-Vega A, Martínez de la Escalera G, Macotela Y, Carmen Clapp.
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México.

Vasoinhibins are a family of antiangiogenic peptides derived from prolactin (PRL) by proteolytic cleavage. They prevent retinal angiogenesis in the healthy eye and inhibit retinal angiogenesis in experimental diabetic retinopathy. Given that vasoinhibins are present in the retina and retinal angiogenesis determines diabetic retinopathy, we hypothesized that their down-regulation in diabetic retinopathy favors the progression of this disease. Here, we investigated the levels of retinal vasoinhibins and the activity of PRL cleaving enzymes in the eye of diabetic and non-diabetic rats. Diabetes was induced with streptozotocin and retinal vasoinhibins were evaluated by Western blotting using monoclonal antibodies directed against the N- and the C-terminus of PRL since vasoinhibins correspond to the N-terminal region of the molecule. Contrary to expected, higher levels of vasoinhibins were detected in the retina of diabetic vs. non-diabetic rats. PRL injected intravitreally was partially converted to vasoinhibins at 2 and up to 24 hours following injection. Both PRL and the resulting vasoinhibins disappeared over time and such disappearance was higher in eyes from diabetic rats. These findings suggest that the diabetic condition upregulates ocular proteases able to generate but also degrade vasoinhibins. The balance between these proteolytic actions may promote the stability of retinal vasoinhibins to help counteract pathological angiogenesis in diabetic retinopathy and warrant further investigation. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant S0008-161594. We thank F. López-Barrera, G. Nava, D. Mondragón, A. Prado and M. García their technical assistance).

P-49.

AAV2-MEDIATED TRANSDUCTION IS ENHANCED IN THE RETINA OF DIABETIC RATS

Díaz-Lezama, N (1), Wu, Z (2), Ramírez, M (1), Moreno-Carranza, B (1), Martínez de la Escalera, G (1), Colosi, P(2), Carmen Clapp (1).
(1) Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México (2) Ocular Gene Therapy Laboratory, National Eye Institute, National Institutes of Health, Bethesda, MD USA.

Ocular gene therapy based on the adeno-associated virus (AAV) vector-mediated delivery of antiangiogenic molecules offers considerable promise for the treatment of diabetic retinopathy. AAV type-2 (AAV2) uses cell surface heparan sulphate proteoglycans (HSPG) as the primary receptors for cell entry. HSPGs are present on retinal ganglion cells, and likely mediate vector transduction. Here, we compared the transduction of retinal ganglion cells in diabetic and normal rats following intravitreal delivery of AAV2 vectors, and investigated whether such transduction correlates with the level of HSPG expression in the respective retinas.
An AAV2 CMV EGFP reporter vector was delivered by intravitreal injection (2.8e10 vg/eye) to adult non-diabetic rats (controls) and diabetic rats treated two weeks prior with a single injection of streptozotocin. One month after vector administration, retinal flatmounts were examined for EGFP expression, by confocal microscopy, and expression of the HSPGs syndecan, glypican, and perlecan, by quantitative PCR. In normal rat retinas, transduction was limited to occasional cells in the ganglion cell layer. In the diabetic rats, the transduction was enhanced more than 4-fold in ganglion cell somas and processes. The expression of mRNAs for syndecan and glypican was elevated 2- and 1.5-fold in the diabetic rat retina, respectively, whereas that of perlecan was not modified. Retinal transduction by AAV2 vectors is enhanced in diabetes and may be mediated by elevated expression of HSPGs on ganglion cells. The diabetes-induced factors responsible for these changes warrant further study. AAV2 vectors may be desirable for gene therapeutics targeting diabetic retinopathy. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant S0008-161594 (C.C.) and the National Eye Institute, NIH, USA (P.C.). We thank F. López-Barrera, G. Nava, D. Mondragón, A. Prado, M. García and Nydia Hernández-Rios for their technical assistance).

P-50.

STRUCTURE-FUNCTION RELATIONSHIP OF RECOMBINANT VASOINHIBINS

Robles, JP, Cruces-Solís, H, Jeziorski, MC, Martínez de la Escalera, G, Moreno-Carranza, B, Clapp, C.
Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM), Querétaro, México

Vasoinhibins are a family of peptides derived from the proteolytic cleavage of prolactin that act on endothelial cells as negative regulators of angiogenesis. Biochemical and recombinant strategies have generated vasoinhibins that vary in yield and biological activity due to conformational changes during purification or residual endotoxin contamination when produced in bacterial systems. Moreover, cysteine at position 58 (Cys58) is regularly replaced by serine (Ser58) to prevent the formation of anomalous disulfide bonds, but the effect of this mutation is not well documented. We compared the secretion and biological activity of two commonly used forms of recombinant vasoinhibins (123 or 139 aa) containing either Cys58 or Ser58. Vasoinhibin cDNAs were engineered from human prolactin, cloned into the pcDNA3 vector, and transfected into HEK293T/17 cells. Cell lysates and conditioned medium collected 48 h after transfection were analyzed for vasoinhibins by Western blot, and the antiangiogenic activity of the secreted vasoinhibins was assessed in bovine umbilical vein endothelial cells (BUVEC) using a [3H]-thymidine incorporation assay. For both forms studied, replacement of Cys58 with Ser58 increased secretion and apparent molecular weight of the vasoinhibins. In silico analysis revealed that the presence of Ser58 creates an additional glycosylation site (Asn56) that was confirmed by PNGase F deglycosylation experiments. However, greater glycosylation was correlated with diminished inhibitory potency upon BUVEC proliferation. In conclusion, we have found that substitution of Ser58 for the native Cys58 increases secretion and glycosylation of vasoinhibins, but that glycosylation interferes with the antiantiogenic properties of these molecules. This study has unveiled important aspects of the production of recombinant vasoinhibins relevant to their use in clinical studies. (This study was supported by the National Council of Science and Technology of Mexico (CONACYT) grant 127496. We thank A. Prado, F. Lopez-Barrera, G. Nava, and D. Mondragon for their technical assistance).

P-51.

FOOT COLOR IS RELATED TO TESTOSTERONE IN MALES AND FEMALES OF MASKED BOOBIES

López-Rull, I (1), Nicolás, L (1), Torres, R (2) and Martínez-Gómez, M (3,1).
(1) Centro Tlaxcala Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, México. (2) Instituto de Ecología, Departamento de Ecología Evolutiva, Universidad Nacional Autónoma de México, México. (3) Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México.

Testosterone-dependent ornaments should signal male quality since only superior males can express extravagant ornamentation without compromising their immune defenses. This idea could also explain the honesty of conspicuous signals in females, yet the role of testosterone (T) in mediating such traits is not as well known as in males. Sulids have colorful feet that display during courtship and in Sula nebouxii seem to be favored by sexual selection through mutual mate preferences. The masked booby (Sula dactyltra) exhibit variation in foot color ranging from olive to orange. We investigated whether such variation is related to T-levels in both sexes. The study was performed in Isla Muertos, Yucatán in the breeding season of 2011. Forty breeding pairs were captured during courtship period. Within 5 minutes of capture blood samples were extracted to determine plasma T-levels. Ulna length and body mass were measured to estimate body condition. Foot color was measured with a spectrophotometer with CIELAB parameters, where L* indicates brightness, and a* and b*indicate the chromaticity co-ordinates. We found sexual dichromatism in foot color with males showing more orange chromaticity and females more olive chromaticity. Males had higher T-levels than females. In both sexes olive coloration was positively related to body condition and T-levels. Finally, assortative mating (i.e. the tendency to mate with an individual with similar traits) by foot color was found. The association of T and body condition to the expression of ornamental traits in males and females suggests that foot coloration in the masked booby may be a condition-dependent honest trait that might be used by males and females in mate choice. (We thank N. Neri and V. Argáez for their invaluable help during fieldwork. L. García kindly helped in the laboratory. This project was supported by CONACYT and the UNAM. Permission to carry out the research was granted by SEMARNAT and SEGOB. Logistic support was provided by E. Avila, CONANP, and SEMAR).

P-52.

CLONING, CHARACTERIZATION AND ACTIVITY OF TAENIA SOLIUM 17BETA-HYDROXYSTEROID DEHYDROGENASE

Aceves A, Valdez RA, de la Torre P (1), García-Villegas R, Laclette JP (1), Bobes RJ (1), Romano MC.
Dpto. de Fisiología, Biofísica y NC, CINVESTAV del I.P.N., México D.F. México. (1) Dpto. de Inmunología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, México D.F., México.

17β-hydroxisteroid dehydrogenases are key enzymes responsible for the formation (reductive) and relative inactivation (oxidative) of sex steroids. Several types of 17β-HSDs have been found in vertebrate, fishes and some invertebrates like Caenorhabditis elegans, Ciona intestinalis and Haliotis diversicolor supertexta. To date not much information is available in parasites. Previous work from our lab showed that Taenia solium cysticerci in vitro synthesizes sex steroid hormones when steroid precursors were provided. The aim of this study was to clone, characterize, and then investigate the expression of Taenia solium 17β-HSD in HEK293T transfected cells. Total RNA was obtained from Taenia solium cysticerci. RT-PCR was performed with SuperScriptTM One-Step RT-PCR and Platinum Taq using gene specific primers designed from an EST sequence identified in the larval cDNA library of the Taenia solium Genome Project. The full length cDNA contains 957 bp, including the open reading frame (ORF) of 319 aa. The PCR product was cloned into the pcDNA3.1 (+) vector and its identity confirmed by sequencing. Homology of the T. solium 17b-HSD with the proteins of other species in example Glossina morsitans morsitans and Clonorchis sinensis, respectively was 30 and 44%. HEK293T cells were seeded in 6–well culture plates and transiently transfected with the construct pcDNA3.1 (+)-Tsol17β-HSD or the empty vector using lipofectamine, and cultured for different periods. Tritiated steroid precursors were added to the culture media. After the incubation period culture media was ether extracted and transformation was assayed by thin layer chromatography. We found that transfected cells catalyzed the transformation of 4-androstenedione into testosterone. In conclusion, Taenia solium cysticerci express a 17β-HSD that catalyzes the androgen transformation. The enzyme belongs to the short chain dehydrogenase/reductase family.

P-53.

TAENIA SOLIUM TAPEWORMS SYNTHESIZE GLUCOCORTICOIDS IN VITRO.

Valdez RA, Aguilar Vega L(1), Willms K(1), Romano MC.
Dpto. de Fisiología, Biofísica y Neurociencias, Cinvestav, Apdo. Postal 14-740, 07000, México D.F.; 1 Depto. de Microbiología y Parasitología, Facultad de Medicina, UNAM, México 04510, D.F.

Cysticerci, the larval stage of Taenia solium (T.s) and Taenia crassiceps WFU tapeworms have the capacity to synthesize sex steroid hormones in vitro. Furthermore, T. crassiceps WFU cysticerci have the ability to synthesize corticosteroids, mainly deoxycorticosterone. Taenia solium tapeworms live for months in the host gut without causing an important inflammatory response or rejection of the taenia. Many factors could be responsible for this situation, among them the secretion of molecules by the worm. To date there is no information on the capacity of T.s. tapeworms to synthesize corticosteroids, therefore the aim of this work was to investigate the ability of T.s tapeworms to synthesize these hormones. Tapeworms were obtained from the intestine of golden hamsters previously infected by oral administration of cysticerci dissected from pig meat. The tapeworms were thoroughly washed and pre-incubated for 24 h in DMEM plus antibiotics/antimycotics solution (n=12). After this period tritiated progesterone (3H-P4) was added to the culture media of each tapeworm. Blanks containing the culture media plus 3H-P4 were simultaneously incubated. After 48h culture media were ether extracted and analyzed by thin layer chromatography (TLC) in

bencene:acetone 50:50 v/v and in toluene:acetone:methanol 78:20:2 v/v. Data were expressed as percent transformation of the tritiated precursor. Results indicated that after 48h in culture the tapeworms had the ability to transform 3H-P4 to tritiated metabolites, mainly 17OH-progesterone, 11-deoxycorticosterone and 11-deoxycortisol (13, 23, and 16.5 % of transformation respectively). Data indicated that 11-deoxy corticosterone and 11-deoxy cortisol were the main corticosteroids synthesized by Taenia solium tapeworms. These results show for the first time that tapeworms synthesize corticosteroids.

P-54.

SEROTONERGIC SYSTEM EXERTS NEUROENDOCRINE MODULATION OF TESTICULAR CYCLE IN THE BAT MYOTIS VELIFER.

Martínez-Méndez L. A. (1), Tapia-Rodríguez M. (2), León-Galván M. A.(3), Mendoza-Rodríguez C. A. (1), López-Wilchis R. (3), Vela-Hinojosa (3), Cerbón M. (1), Jiménez-Trejo F. (1)*.

(1) Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, México, Distrito Federal, México. (2) Unidad de Microscopía, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, México, D. F., México. (3) Laboratorio de Biología y Ecología de Mamíferos, Departamento de Biología, Universidad Autónoma Metropolitana-Iztapalapa. 09340. México D.F., México.

The reproduction is a complex process that involves factors such as sexual behavior and environmental conditions in the habitat of species, which are essential to ensure the perpetuation of species. In some mammals, males show an important temporal asynchrony in the development and function of reproductive organs along the year. Bats are the only flying mammals and some of them show seasonal reproduction; Myotis velifer species are present in Mexico and show this feature. These bat species present a testicular annual cycle that goes from recrudescence to regression (spermatogenesis to inactivity period, respectively), and these extend for some months during the year. After recrudescence, the spermatozoa arrive at epididymis and wait to be expelled at the time of ejaculation during the mating period, which occurs some months later. Because serotonin (5-HT) has gained reproductive importance in the last years, the aim of the present study was to analyze the expression of this indolamine and both tryptophan hydroxylase and monoamine oxidase isoform A -enzymes involved in its metabolism- in Myotis velifer testes, a seasonal reproductive bat species that shows temporal asynchrony in its sexual cycle, across the principal periods of their reproductive cycle. By using both Falck-Hillarp histochemistry and immunofluorescence techniques, we found serotonin in vesicles of Leydig cells and probably Sertoli cells too; interestingly, both intracellular localization and concentration was variable across the different stages of the reproductive cycle, being lower during spermatogenesis phase and increasing during the mating phase. These results suggest that 5-HT is present in bat testes and is possible that 5-HT could be an ancient mechanism of modulation in the reproductive functions, but more work must to be done to confirm this hypothesis.

P-55.

NOVEL RNA INTERFERENCE PLATFORM FOR GENE SILENCING IN XENOPUS

Nae, AM (1), Knoedler, J (1,2) and Denver, RJ (1,2), The Department of Molecular, Cellular and Developmental Biology (1) and the Neuroscience Graduate Program (2), University of Michigan, Ann Arbor, MI USA

RNA interference (RNAi) is a powerful tool for knocking down gene expression in eukaryotes. However, RNAi has not been widely applied in amphibian models due to poorly defined technical limitations. We developed a new RNAi method to knock down gene expression in Xenopus tissue culture cells and tadpole brain in vivo. The method uses RNA polymerase II expression vectors (SIBR vectors) for expressing small hairpin RNAs (shRNAs) based upon the non-coding RNA BIC. Within BIC there is a micro RNA (miRNA) precursor that forms a hairpin loop that is efficiently processed by the RNA-induced silencing complex (RISC). To create the RNAi vector the endogenous miRNA sequence is exchanged for a shRNA targeting the gene of interest. The vectors are constructed such that they can be engineered to contain tandem copies of a shRNA against a single gene or multiple shRNA cassettes targeting different genes. We constructed a frog version of the mammalian SIBR vector by isolating the BIC gene from X. tropicalis genomic DNA and subcloning it into UI4-GFP-SIBR (to give UI4-GFP-xtSIBR). We first engineered UI4-GFP-xtSIBR to target firefly luciferase (UI4-GFP-xtSIBR-luc) to validate the method. We introduced the RNAi vector into the fibroblast cell line XTC-2 by lipid-based transfection, or the tadpole brain by electroporation-mediated gene transfer. We cotransfected a pGL3 luciferase reporter vector, UI4-GFP-xtSIBR-luc (or UI4-GFP-xtSIBR empty vector as control), with or without a vector to express human Argonaut 2 (Ago2). Published findings suggest that Ago2 is rate-limiting for RNAi in amphibians, as it is necessary for proper functioning of RISC. The RNAi vector reproducibly reduced luciferase expression ranging from 40-64% in XTC-2 cells and 50-67% in vivo. Generation of a functional shRNA required co-expression of Ago2. We are now targeting endogenous genes with this method. Vector-based RNAi can provide a versatile and efficient method for gene silencing experiments in Xenopus.(We are grateful to Dr. David Turner for providing the SIBR plasmid which we used as a backbone for creating a Xenopus version of the vector, and for providing advice on vector design. Yuliang Ma isolated the X. tropicalis BIC gene and created the frog SIBR vector backbone. This work was supported by NSF grants IOS 0922583 and IOS 0641587 to RJD; AMN was supported by a summer fellowship from the Women in Science and Engineering Residential Program (WISE RP) at the University of Michigan.(We thank James Dahlberg for providing the hAgo2 expression vector).

P-56.

DIETARY INDOL-3-CARBINOL AFFECTS ENZYME ACTIVITY AND GENE EXPRESSION OF CYTOCHROME P450 1A1 AND CELL DIFFERENTIATION IN THE NEONATAL RAT OSTEOBLASTS MODEL.

Enríquez, J (1), Velázquez-Cruz, R (2), Larrea, F (1).

(1) Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMSZ), Mexico City, Mexico (2) Genomics of Bone Metabolism Laboratory, Instituto Nacional de Medicina Genómica, Mexico City, Mexico

Indole-3-carbinol (I3C) is a natural antiestrogen present at high levels in cruciferous vegetables and possess the capability to induce gene expression of cytochromes P450 belonging to 1A family, which is responsible of 2-hidroxylation of steroids and constitutes the main route for estrogen inactivation. In the bone, estradiol (E2) plays an essential role in the bone remodeling. Estrogen metabolism in bone has been poorly studied; however, the metabolic fate of estrogens might be determinant for bone mass density. Studies in rat and estrogen-receptor-positive cell lines have showed that the estrogens 2-hydroxylated appear to have no estrogen agonistic activity. Other study has demonstrated an inverse correlation between estrogens 2-hydroxylated formed with the bone mass density and osteoporosis. These data suggest that estrogen metabolism into 2-hydroxy derivatives could be of a negative impact on cellular processes of bone formation. Based on these observations, the aim of this study was to investigate in the neonatal rat osteoblasts model, the effects of I3C upon the enzymatic activity and gene expression of Cyp1A1, as well as alkaline phosphatase activity (AP) and osteocalcin content (OC), two biomarkers of osteoblasts differentiation. The results demonstrated in the rat osteoblasts model, the ability of I3C to increase, in a dose- and time-dependent manner, the bioconversion of 3H-E1 to 2-hydroxy derivatives. On the other hand, I3C induced a significant and early (2h) increase on the level of expression of Cyp1A1. Incubations of osteoblasts in the presence of increasing concentrations of I3C (1-250 µM) or OC content while E2 was an efficient inducer. Co-incubation of I3C (1 or 250 µM) with E2 (50 nM) abolished AP activity and OC content increase induced by E2. In conclusion, the dietary I3C exhibited antiestrogenic effects upon osteoblasts differentiation, which were

mediated through the induction of Cyp1A1 expression, increasing the 2-hydroxylation of estrogens. (This study was supported by grants from the Department of Biology Reproduction, INCMSZ, Mexico City, Mexico).

TOPIC: THYROID

P-57.

3,5-T2 BINDS TO A SPECIFIC THYROID HORMONE RECEPTOR ISOFORM IN FISH.

Hernández-Puga G., Villalobos P., Orozco A.
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, UNAM Campus Juriquilla, Querétaro, México.

The bioactive thyroid hormone (TH) triiodothyronine or T3 has pleiotropic effects in early and adult life in vertebrates by binding to the thyroid hormone receptors (TR) in the cell nucleus and promoting or blocking gene transcription. We have shown that another TH, 3,5-diiodothyronine (3,5-T2 or T2), induces the transcription of genes in the same way as T3 in fish and activates a specific isoform of the TRβ1 different from that activated by T3. These isoforms differ in the presence (long or L-TRβ1) or absence (short or S-TRβ1) of a 9-amino acid insert in the ligand binding domain of the receptor. To investigate if T2 binds to a specific TR, we conducted binding assays with the long, short and human TRβ1. The isoforms were cloned and expressed in X. laevis oocytes and saturation and competition assays were realized. For the saturation assays, increasing concentrations of 125-I-T3 (0.01-1 nM) with a constant amount (~20 fmoles) of TRβ1 were used. The ligand bound fraction was separated (Sephadex G-25 columns) and quantified on a γ-counter. Competition assays were conducted with a constant 125I-T3 concentration and increasing concentrations of unlabeled T2 or T3 (0.01-500 nM). The data were analyzed to calculate dissociation and inhibition constants (kd, ki). Our results show that T3 is a high affinity ligand for the human, short and long TRβ1 with kd values of 0.2 nM for all receptors. However, T2 shows to be a more selective ligand, with a ki value of 0.1 nM for L- TRβ1, 8 nM for the human receptor and 17 nM for S-TRβ1. These results show that T2 binds to L-TRβ1 with high affinity and suggest that it may exert its effects through this receptor in fish. The lower affinity of T2 for the human TRβ1 correlates with the effects reported in mammals, suggesting that it has pharmacological potencial as a selective agonist. Current studies are aimed to identify coregulators that interact with the T2-long TRβ1 complex. (Supported by CONACYT 166357 and PAPIIT 208511).

P-58.

TISSUE-SPECIFIC EXPRESSION OF THE LONG AND SHORT ISOFORMS OF TRB1 IN TILAPIA BRAIN, HEART AND SKELETAL MUSCLE.

Hurtado E., Navarrete-Ramírez P., Villalobos P., Valverde-R C. and Orozco A.
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología campus Juriquilla, Querétaro, Universidad Nacional Autónoma de México.

Thyroid hormones (TH) play a fundamental role during development and have metabolic effects throughout life in all vertebrates. These effects are mediated by their binding to TH nuclear receptors (TR), which function as ligand-dependent transcription factors to regulate gene expression. At least in teleosts, two TH are bioactive: triiodothyronine (T3) and 3, 5- diiodothyronine (T2). Interestingly, their effects seem to be differentially mediated by two isoforms of the TRβ1 which differ in the presence (long TRβ1) or not (short TRβ1) of a 9 amino acid insert in the ligand binding domain. Thus, T3 and T2 bind to and activate the short and long TRβ1, respectively. Until now, these isoforms have only been described in liver. In the present study we investigated if the expression of these receptors are more widely expressed and if their corresponding ligands regulate this expression. To this end, we treated juvenile tilapia (~2 g) with T2 or T3 (10, 25, 50, 100 and 150 nM in rearing water) during 24 hours (n=20/experimental group). mRNA was extracted from brain, heart and muscle, and qRT-PCR was performed to quantify the expression of short- and long TRβ1. Our initial results indicate that: 1) the three tissues express both short and long TRβ1; 2) in all tissues, the expression of long TRβ1 is at least 2-fold higher than the short isoform; 3) both isoforms respond to TH treatment in all tissues examined; and 4) there is tissue-specific regulation of the two isoforms, being down-regulated in the brain and up-regulated in skeletal and cardiac muscle. These results suggest that the pleiotropic effects of TH can in part be explained by tissue-specific control of gene expression mediated by the different TR isoforms and their ligands. (Supported by CONACYT 166357 and PAPIIT 208511).

P-59.

3,5-T2 ACTION MECHANISM IN TILAPIA GROWTH

Navarrete-Ramírez Pamela, Patricia Villalobos, Maricela Luna, Carlos Valverde-R and Aurea Orozco.
Departamento de Neurobiología Celular y Molecular, Instituto de Neurobiología, Universidad Nacional Autónoma de México (UNAM). Querétaro, México.

We have recently shown that as triiodothyronine (T3), 3,5-T2 (T2) induces tilapia growth with the same potency. However, the effects of T2 are mediated by a different TRβ1 isoform that contains a 9 amino acid insert in its ligand binding domain, and which we have denominated long TRβ1 (L-TRβ1). To further evaluate the effect of T2 in growth, we partially blocked TH synthesis with methimazole (MMI) and simultaneously supplemented with 1 nM of T3 or T2. The experimental groups were treated for 8 hours, three times a week for one month. We recorded growth gain and quantified intrahepatic T3 concentrations and mRNA expression of TH-responsive genes involved either in growth (GH, IGF-1), or in its signaling pathway [S-TRβ1 (short TRβ1) and L-TRβ1]. Our results showed that MMI treatment significantly decreased T3 in all groups, however, T3 supplement (MMI+T3) stimulated growth, while that with T2 (MMI+T2) maintained growth similar to untreated fish. GH and IGF-1 mRNA expression remained euthyroidal in all treated tilapia, except in the group treated with MMI+T2 which exhibited a significant increase in the expression of GH. Interestingly, L-TRβ1 and S-TRβ1 expression was up-regulated in fish treated with MMI+T2 and MMI+T3, respectively. Altogether our results support the notion that T3 and T2 participate in the growth process, however, their effects are differently mediated by specific TRβ1 isoforms. In order to further understand T2 action mechanism, organotypic tilapia liver cultures were standardized. Hepatic slices were treated with different concentrations (0.1-100 nM) of T3 and T2 and the expression of the two isoforms of TRβ1 was assessed. Under these experimental conditions, L-TRβ1 was down-regulated in a dose-dependent manner by T2, while S-TRβ1 expression decreased only with T3 treatment. Current studies are aimed to further study T2 signaling pathways using ex-vivo systems. (Supported by PAPIIT 208511 and CONACyT 166357).

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