The front- and backsides of this abstract book were designed by Jason Grigoropoulos, Cynthia Lenaerts and Marijke Christiaens; with credits to professional designer Jason Grigoropoulos.

Els Wellens and Marijke Christiaens are kindly acknowledged for their administrative support in making this abstract book.

CECE 2016

21 - 25 August 2016

Leuven, Belgium

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WELCOME

Welcome to Leuven

by Jozef Vanden Broeck, Conference Organizer

Dear colleague,

I am very pleased to welcome you to the 28th Conference of European Comparative Endocrinologists, CECE 2016, held on August 21-25, 2016.

This year's conference takes place in the historic centre of Leuven, a famous city situated close to the Belgian and European capital, Brussels. The University of Leuven (KU Leuven) was founded in 1425, making it the oldest university in the Low Countries. Today, KU Leuven ranks as one of the largest and most renowned universities in Europe.

At CECE 2016 we have the honour of celebrating the 50th anniversary of the European Society for Comparative Endocrinology (ESCE), which endorses the CECE conferences. In this 'omics' era comparative studies are gaining momentum, and ESCE promotes research on endocrinology of all organisms at the molecular, cellular, systemic and evolutionary levels. Furthermore, it strives to integrate the field of comparative endocrinology with many related disciplines including biochemistry, physiology, neurobiology, immunology and ecology. Meetings are organized every other year and special support is provided for young investigators. Interested parties are invited to apply for membership to ESCE at the following website: http://www.escendo.info/.

As for the conference itself, we are pleased to welcome attendees from more than 35 countries and great efforts have been made to ensure both a stimulating scientific programme and an enjoyable stay in Leuven. The scientific programme includes five selected plenary lectures and 23 half-day sessions with 150 oral communications. Two symposia have arisen as joint initiatives with CECE 2016: the *International GPCR Symposium* organized by Marc Parmentier and colleagues, and the *Leopoldina Symposium on Seasonal Rhythms* organized by Horst-Werner Korf. A special symposium has also been organized for young investigators. There are four poster sessions, one of which (on Monday evening) will be accompanied by extra food and drinks. Finally, prizes

for the best oral and poster presentations will be awarded to young investigators at the end of the conference.

Major social events include the Welcome Reception at KU Leuven's historic University Hall (Sunday 21st August), and the Social Banquet in the Faculty Club at the 'Groot Begijnhof' UNESCO World Heritage Site (Wednesday 24th August). A fantastic selection of Excursions is also offered for the Tuesday afternoon (August 23rd). For accompanying persons, an interesting and affordable programme is available to discover more of Leuven and Belgium.

I am very grateful to everyone who has assisted in the organization of this international meeting. In particular, I would like to thank the Conference Office at KU Leuven, our local collaborators, session organizers, scientific committees, suppliers, sponsors, and - last but not least - all attendees. Your personal contribution to a successful CECE 2016 is highly appreciated. I hope you enjoy your stay in Leuven, and wish you an inspiring and scientifically fruitful meeting!

Please do not hesitate to contact us at the registration desk with any questions you might have about the conference programme.

With best wishes,

Jozef Vanden Broeck

CONTENTS

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GENERAL INFORMATION

Conference information and contacts

Venue: The conference sessions will be held at the Faculty of Social Sciences.

Address: Parkstraat 45, 3000 Leuven

- Coordinates: 50°52'25.5"North, 4°42'12.6" East
- Bus stop: *Leuven H. Hart Kliniek* or *Leuven Rustoord Remy*
- Phone: 0032 478 38 71 79
- Security: 0032 16 32 20 00

Contact:

Conference organization - coordinator: Jozef Vanden Broeck
E-mail address: jozef.vandenbroeck@bio.kuleuven.be

Conference Office: Stefanie Verbeeck
Mobile phone: 0032 478 38 71 79
Conference E-mail address: CECE2016@kuleuven.be
Conference website: https://kuleuvencongres.be/CECE2016

Registration

The registrations at the conference site will take place in the entrance hall of the Max Weber Auditorium (AP 00.15).

Certificates of attendance and receipts

Certificates of attendance to the conference and receipts are delivered by the Conference Office (please contact: CECE2016@kuleuven.be).

Language

The official language of the conference is English.

Belgium is a federal country with a complex political system consisting of three different regions and three language communities. Leuven is situated in the Dutch speaking part of Belgium. Many people in the street will also be able to communicate in English, French and German.

T-shirts

KU Leuven t-shirts can be ordered via the e-shop of the university:
https://www.kuleuven.be/shop/en/clothes

Transportation

The meeting will take place at the KU Leuven Faculty of Social Sciences, which is easily accessible by:

- **City bus** (about 20 minutes from the train station): from the train station, you can take bus N°1 *Heverlee Boskant* or N°2 *Campus Arenberg*, and get off at "*H. Hartkliniek*". You can also take bus N°4 *Herent Haasrode*, N°5 *Wakkerzeel Vaalbeek*, or N°6 *Wijgmaal Hoegaarden* and get off at "*Rustoord Remy*".

  From both bus stops, the Faculty of Social Sciences can be reached by foot within a few minutes.

- **By car**: the Leuven city center is accessible by car. Follow the "**P**" signs to access the parking lots. See also: http://www.leuven.be/en/tourism/useful-information/how-to-get-there/parkings-en-busstops.jsp

Weather

Belgium and Leuven have a temperate climate with relatively mild winters, mild summers and possible rainfall throughout the year. In August, the average weather statistics are: maximum temperature 23°C, minimum temperature 13°C; number of days with rainfall 15; hours of sunshine 190.

Social Programme

- **Welcome reception**: Sunday, 21st of August at 19:15 in the main building of the University. The opening reception is included in the conference fee and in the accompanying persons programme.
- **Excursions**: Tuesday, 23rd of August at 12:45. With these excursions we would like to give you some time to discover Belgium.
- **Social Banquet**: Wednesday, 24th of August at 19:00 - The *Faculty Club* is located near the *'Groot Begijnhof'* of Leuven, which is a well preserved and completely restored historical quarter containing dozens

of streets in the south of downtown Leuven. The price for joining the social banquet is **40 euro** for registered persons.

All these activities had to be reserved upon registration. If you would be interested in a social activity but did not yet register for it, please contact the registration desk to check whether there are still places available.

Presenter information

Poster information

Poster presentations will allow presenters and attendees to communicate their new research findings, new ideas, innovations, and scientific advances and engage in extended discussions. These will be scheduled during dedicated poster sessions. Presenters are asked to be at their assigned poster board location for the entire length of the poster session. The poster boards are situated in ALMA2.

Oral presentations

The oral presentations will consist of plenary, state-of-the-art and regular oral communications, to which time is formally scheduled in the timetable of CECE 2016 for both the presentation of the work and the discussion part.

Plenary lectures take 45 minutes. State-of-the-art lectures take 25 minutes, and then there is 5 minutes time for discussion. Oral presentations take 15 minutes plus 5 minutes for discussion.

Lunch and Breaks

Lunch can be taken in the ALMA2 restaurant.

During the sessions, breaks with coffee and other drinks will be provided every day in the morning and during the afternoon.

Awards

There will be two prizes for young researchers: one for the Best Oral Presentation and one for the Best Poster Presentation. The maximum age limit of the candidates is 35y.

Publication Policy

The speakers of the plenary and state-of-the-art lectures, and of the 'International GPCR Symposium' and the 'Leopoldina Symposium' are invited to submit a manuscript (review or research type) for a special issue of *General and Comparative Endocrinology*. For this special issue, the editors can also select from the regular oral and poster abstracts and invite these authors to submit a manuscript. Instructions for authors and peer reviewing process are according to the journal's rules and policy. When submitting your manuscript via the online system of the journal, please indicate the "CECE2016 special issue" option. The ultimate deadline for manuscript submission to this special issue is February 15, 2017.

Accompanying persons programme

During the conference, we also have a programme for those who are accompanying you. We invite them to discover Leuven and Belgium, which have a lot to offer!

The programme for accompanying persons includes:

- a conference bag (including tourist information about Leuven)
- access to the welcome reception on Sunday, August 21st
- a day trip to Bruges on Monday, August 22nd
- a day trip to Antwerp on Wednesday, August 24th
- lunch on Thursday, August 25th

In addition, these accompanying persons can also register for an excursion on Tuesday afternoon and for the social banquet on Wednesday evening.

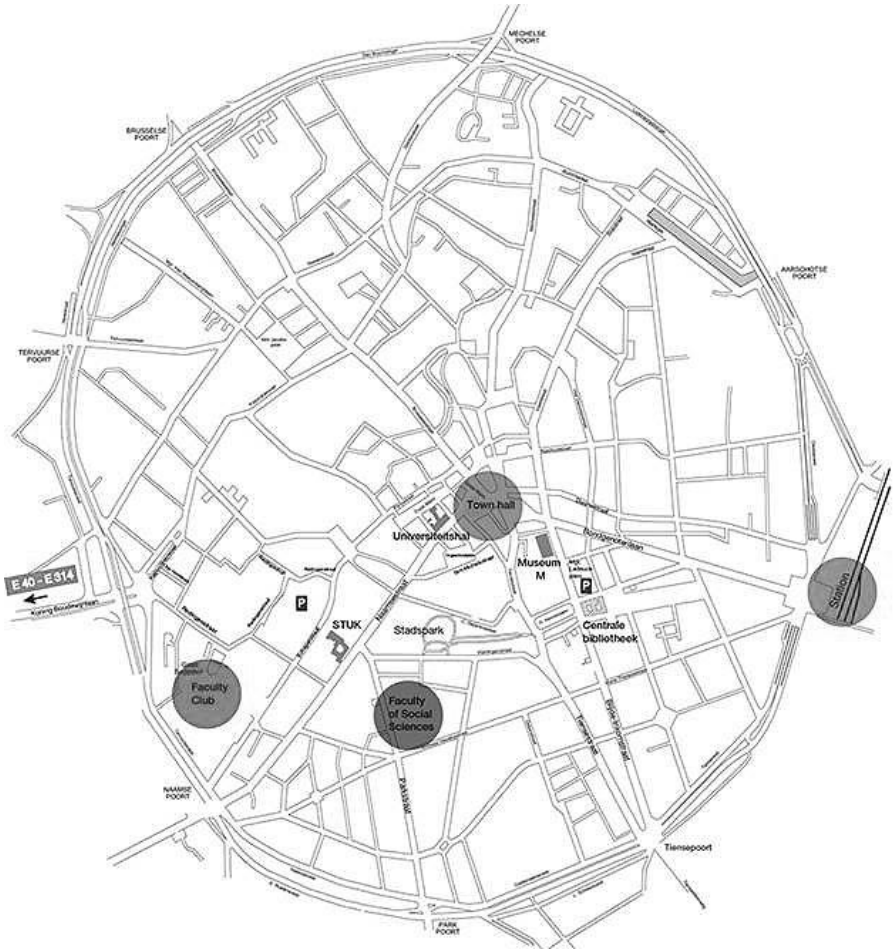
Persons who would like to join this programme but did not yet register for it, can always contact the registration desk to check whether there are still places available.

VENUE

The conference will take place on the campus of the Faculty of Social Sciences, Parkstraat 45, 3000 Leuven.

Below you find 2 maps: the first is a map of Leuven, where you will see the most important places of the conference. Next, you will find a more detailed map of the campus of Social Sciences.

Important places of the conference



Campus of Social Sciences in detail

Registration takes place where the AP-marking is.

CONFERENCE PROGRAMME

General Scheme

	Sunday August 21		**Monday August 22**	**Tuesday August 23**	**Wednesday August 24**		**Thursday August 25**
		08.00	*Registration*	*Registration*	*Registration*	*08.15*	*Registration*
		08.30-09.15	**PL2**	**PL3**	**PL4**	08.30-09.30	SOTA
		09.20-10.20	SOTA	SOTA	SOTA	09.30-11.10	Orals
		10.20-10.50	*Break*	*Break*	*Break*	*11.10-11.40*	*Break*
		10.50-12.30	Orals	Orals	Orals	*11.20-11.40*	*General assembly ESCE*
		12.30-14.30	*Lunch & Posters*	*Lunch (package)*	*Lunch & Posters*	11.40-12.25	**PL5**
		14.30-15.30	SOTA	***Excursions***	SOTA	12.25-13.00	Closing ceremony
		15.30-15.50	Orals		Orals	*13.00*	*Lunch*
15.00-18.00	*Registration*	*15.50-16.10*	*Break*		*Break*	14.00-19.00	Leopoldina Symposium
18.00-18.30	Opening ceremony	16.10-17.30	Orals		Orals		
18.30-19.15	**PL1**	17.30-19.30	Posters				
19.15-21.15	***Reception***	*19.00*			***Social Banquet***		

Symposium Scheme

	Lecture hall A Max Weber	Lecture hall B AV 00.17	Lecture hall C AV 02.17	Lecture hall D AV 01.12
Monday morning	*International GPCR Symposium*	Hormones in Development and Reproduction	Osmoregulation	
Monday afternoon	*International GPCR Symposium*	Hormones in Development and Reproduction	Neurobiology and Behaviour	
Tuesday morning	Molecular Evolution, Structures and Functions of GPCRs-1	Signalling Pathways in Immunity	Endocrine Disruption	Feeding and Metabolism
Tuesday afternoon				
Wednesday morning	Novel Hormones and Receptors in the Animal Kingdom	Omics and Physiology of Insect Neuropeptides	Functions of Steroids, their Receptors and Binding Proteins	Model Organisms in Comparative Endocrinology and Neurobiology
Wednesday afternoon	Comparative Genomics and Evolution	Omics and Physiology of Insect Neuropeptides	Regulation of Thyroid Hormone Action at Multiple Levels	Young Investigator Symposium-1
Thursday morning	Endocrinology and Chronobiology	Molecular Evolution, Structures and Functions of GPCRs-2	Stress and Adaptation	Young Investigator Symposium-2
Thursday afternoon	*Leopoldina Symposium on Seasonal Rhythms*			

Detailed programme of CECE2016

Sunday, August 21*:*

UNIVERSITY HALL - Naamsestraat 22

15.00-18.00: Registration

18.00-18.30: Opening Ceremony (*Promotiezaal - 01.46*)

Welcome in Leuven and opening of CECE2016:

- Prof. Dr. Rik Torfs - Rector KU Leuven
- Prof. Dr. Horst-Werner Korf - ESCE President
- Prof. Dr. Jozef Vanden Broeck - CECE2016 Coordinator

18.30-19.15: Opening Lecture (*Promotiezaal - 01.46*):

Chair: Shireen Davies (Glasgow, UK)

Pierre Leopold (Nice, France) - "*Growth coordination mechanisms during* Drosophila *development*" (PL1)

19.15-21.15: Welcome Reception (*Jubileumzaal - 01.05*)

Monday, August 22*:*

Conference site - Parkstraat 45

8.00: Registration

8.30-9.15: Plenary Lecture (Lecture Hall A - Max Weber):

Chair: Marc Parmentier (Brussels, Belgium)

Marc Caron (Durham, North Carolina - USA) - "*Physiological Consequences of GPCR Functional Selectivity and Therapeutic Approaches*" (PL2)

LECTURE HALL - A - MAX WEBER

International GPCR Symposium: *"G protein-coupled receptors: Structure, Evolution and Function"*

Organizers: *Marc Parmentier (Brussels, Belgium), Paul Proost (Leuven, Belgium), Silvano Sozzani (Brescia, Italy), Jan Steyaert (Brussels, Belgium), Johan Thevelein (Leuven, Belgium), Jozef Vanden Broeck (Leuven, Belgium)*

Morning session

9.15: Chris Tate (Cambridge, UK) - "Structure of the human adenosine A2A receptor bound to an engineered G protein" (G1)

9.45: Torsten Schöneberg (Leipzig, Germany) - "What are they waiting for? - Tethered agonism in GPCRs" (G2)

10.15-10.30: Morning Break

10.30: Céline Gales (Toulouse, France) - "Natural biased agonism at Angiotensin II-type 1 receptor" (G3)

11.00: Jean-Yves Springael (Brussels, Belgium) - "Coexpression of CCR7 and CXCR4 during B cell development controls CXCR4 responsiveness and bone marrow homing" (G4)

11.30: Johan Thevelein (Leuven, Belgium) - "Fructose-1,6-bisphosphate couples glycolytic flux to activation of Ras downstream in the GPCR-controlled glucose sensing network of yeast" (G5)

12.00: Young-Joon Kim (Gwanju, South Korea) - "Two for one, one for two: Myoinhibitory peptide and sex peptide receptor" (G6)

12.30: Heleen Verlinden (Leuven, Belgium) - "The pleiotropic allatoregulatory neuropeptides and their receptors" (G7)

13.00-14.00: Lunch Break (+ Posters) *(ALMA2)*

Afternoon session

14.00: Henrik Dohlman (Chapel Hill, NC-USA) - "Protons as second messenger regulators of GPCR signaling" (G8)

14.30: Francisco Ciruela (Barcelona, Spain) - "Unraveling the function of GPR37 in the brain: more than a Parkinson's disease-associated receptor" (G9)

15.00: Mauro Teixeira (Belo Horizonte, Brazil) - "Developing anti-inflammatory drugs for infectious diseases" (G10)

15.30: Rik Janssens (Leuven, Belgium) - "Natural nitration of CXCL12 reduces its signaling capacity and chemotactic activity *in vitro* and abrogates intra-articular lymphocyte recruitment" (G11)

16.00-16.15: Afternoon Break

16.15: Mette Rosenkilde (Copenhagen, Denmark) - "Chemokine receptor activation - progress towards novel therapeutic principles" (G12)

16.45: Martine J. Smit (Amsterdam, The Netherlands) - "Oncogenic potential of HCMV-encoded chemokine receptors" (G13)

17.15: Marc Parmentier (Brussels, Belgium) - "Chemerin and its receptors in leukocyte trafficking, inflammatory diseases, metabolism and cancer" (G14)

17.45-19.30: Poster session (GPCRs)

This international symposium is jointly organized in the frame of the Belgian Interuniversity Attraction Poles programme (BELSPO - IAP P7/40) "G protein-coupled receptors: from structures to functionally validated targets" coordinated by Marc Parmentier.

LECTURE HALL - B - AV 00.17

Hormones in Development and Reproduction

Organizers: *Charlotte Cornil (Liège, Belgium), Carlo Di Cristo (Benevento, Italy), Angela Lange (Mississauga/Toronto, Canada), Rüdiger Schulz (Utrecht, The Netherlands)*

Morning session

SOTA lectures:

9.20: You Lee Son (Tokyo, Japan) - "The effect of GnIH on the signaling pathways leading to the activation of GnRH neurons triggered by kisspeptin and VIP" (S1)

9.50: Juli Wade (East Lansing, Michigan - USA) - "The Roles of Estradiol and Specific Z-Chromosome Genes in Masculinization of the Zebra Finch Song System" (S2)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Xianyu Lin (Gent, Belgium) - "FoxO directs the timing of larval-pupal metamorphosis through ecdysteroid signaling in the red flour beetle, *Tribolium castaneum*" (O1)

11.10: Joris M. Koene (Amsterdam, The Netherlands) - "The neuroendocrine consequences of being male and female at the same time" (O2)

11.30: Almas Juma (Melbourne, Australia) - "Effect of Plag1 deficiency on gene expression in the hypothalamo-pituitary-gonadal axis in male mice" (O3)

11.50: Vincent Hellier (Liège, Belgium) - "Sexual motivation is driven through kisspeptin neuron activity" (O4)

12.10: Anne Houbrechts (Leuven, Belgium) - "Permanent deiodinase type 2 deficiency alters local thyroid hormone levels, disturbs development and strongly reduces fertility" (O5)

12.30-14.30: Lunch and Poster Session 1 - *(ALMA2)*

(*ESCE Council meeting during Lunch - Raadzaal SW 00.113*)

Afternoon session

SOTA lectures:

14.30: Maurice Elphick (London, UK) - "Neuropeptide signalling in echinoderm reproduction and development" (S3)

15.00: Mark R. Brown (Athens, Georgia - USA) - "Different neuropeptides convergently activate egg maturation in mosquitoes" (S4)

ORAL presentations:

15.30: Scott Cummins (Sippy Downs, Australia) - "Neurohormones that regulate molluscan and echinoderm reproduction" (O6)

15.50-16.10: Afternoon Break

16.10: Cynthia Lenaerts (Leuven, Belgium) - "Functional and pharmacological characterization of the ecdysis triggering hormone receptor in the desert locust, *Schistocerca gregaria*" (O7)

16.30: Masatoshi Mita (Tokyo, Japan) - "Species specificity of relaxin-like gonad-stimulating peptides in starfish" (O8)

16.50: Katleen Crabbé (Leuven, Belgium) - "The role of SGPP-5 in the reproductive cycle of the desert locust, *Schistocerca gregaria*" (O9)

17.10: Carlo Di Cristo (Benevento, Italy) - "Nervous control of reproduction in *Octopus* in the time of Genomics" (O10)

17.30-19.30: Poster Sessions 2&3

LECTURE HALL - C - AV 02.17

Osmoregulation *(morning session)*

Organizers: *Ian Orchard (Mississauga/Toronto, Canada), Patrick Prunet (Rennes, France)*

SOTA lectures:

9.20: Shireen Davies (Glasgow, UK) - "Osmoregulation and stress tolerance in *Drosophila melanogaster*" (S5)

9.50: Pung-Pung Hwang (Taipei, Taiwan) - "Molecular physiology of hormonal actions on body fluid ionic and acid-base homeostasis in zebrafish" (S6)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Tom Ole Nilsen (Bergen, Norway) - "Effects of androgens on osmoregulatory mechanisms in Atlantic salmon (*Salmo salar* L.)" (O11)

11.10: Yung-Che Tseng (Taipei, Taiwan) - "Convergent signaling of neurohypophysial hormones on epithelial ion regulation in cephalopods" (O12)

11.30: Anthony Dornan (Glasgow, UK) - "Mapping water transport in insect renal systems" (O13)

11.50: Meet Zandalawa (Stockholm, Sweden) - "Unraveling of the corazonin circuit identifies novel roles for this signaling system in *Drosophila*" (O14)

12.10: Ian Orchard (Mississauga/Toronto, Canada) - "Neurohormonal control of diuresis: discoveries from the blood-gorging bug, *Rhodnius prolixus*" (O15)

12.30-14.30: Lunch and Poster Session 1 - *(ALMA2)*

(*ESCE Council meeting during Lunch - Raadzaal SW 00.113*)

Neurobiology and Behaviour *(afternoon session)*

Organizers: *Lut Arckens (Leuven, Belgium), Darron Cullen (Leuven, Belgium)*

SOTA lectures:

14.30: Stephen M. Rogers (Tempe, Arizona, USA) - "Mechanisms of locust phase change: parallels and differences across several different lineages" (S7)

15.00: Antón Barreiro-Iglesias (Santiago de Compostela, Spain) - "Serotonin promotes the development and regeneration of spinal cord motor neurons in zebrafish" (S8)

ORAL presentations:

15.30: Dušan Žitnan (Bratislava, Slovakia) - "Expression and functional analysis of neuropeptides in ticks" (O16)

15.50-16.10: Afternoon Break

16.10: Emiel Geeraerts (Leuven, Belgium) - "Unravelling mouse behaviour upon optogenetic activation of the superior colliculus" (O17)

16.30: Jan Watteyne (Leuven, Belgium) - "Behavioral phenotyping of neuropeptidergic mutants in *C. elegans* experience-dependent salt chemotaxis" (O18)

16.50: Shobha Bhargava (Pune, India) - "Orexigenic response of Neuropeptide Y to varying nutritional status in the tadpole brain of frog *Euphlyctis cyanophlyctis*" (O19)

17.10: Carlos Aramburo (Queretaro, Mexico) - "Neuroprotective effects of growth hormone in the green iguana neuroretina" (O20)

17.30: Jean-François Picimbon (Jinan, China) - "RNA trafficking in moth pheromone gland cells" (O21)

17.50-19.30: Poster Sessions 2&3

Tuesday, August 23:

Conference site - Parkstraat 45

8.00: Registration

8.30-9.15: Plenary Lecture (Lecture Hall A - Max Weber):

Chair: Deborah Power (Faro, Portugal)

Charlotte Cornil (Liége, Belgium) - "*The dual action of neuroestrogens on sexual behavior*" (PL3)

LECTURE HALL - A - MAX WEBER

Molecular Evolution, Structures and Functions of GPCRs - 1

Organizers: *Christian Gruber (Vienna, Austria), Heleen Verlinden (Leuven, Belgium)*

SOTA lectures:

9.20: David Gloriam (Copenhagen, Denmark) - "Comparative GPCR structure and sequence analyses in GPCRdb and in ligand design" (S9)

9.50: Christian Gruber (Vienna, Austria) - "Pharmacology of nature-derived neuropeptide ligands" (S10)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Daiane Boff (Leuven, Belgium) - "Role of receptor CXCR2 in the pathogenesis of experimental septic arthritis" (O22)

11.10: Anne Lemaire (Brussels, Belgium) - "Mouse P2Y4 nucleotide receptor is a negative regulator of cardiac adipose-derived stem cell differentiation and cardiac fat formation" (O23)

11.30: Julien Hanson (Liège, Belgium) - "Molecular determinants for constitutive activity of GPR101, an orphan GPCR associated to X-linked acrogigantism syndrome (X-LAG)" (O24)

11.50: Els Lismont (Leuven, Belgium) - "Using BRET biosensors to detect direct G protein activation" (O25)

12.10: Elisabeth Marchal (Leuven, Belgium) - "Ligand specificity of insect neuropeptide G protein-coupled receptors" (O26)

12.30: Lunch (package for persons going on excursion)

12.45: Start of the Excursions

LECTURE HALL - B - AV 00.17

Signalling Pathways in Immunity

Organizers*: Elisabeth Eppler (Basel, Switserland), Paul Proost (Leuven, Belgium)*

SOTA lectures:

9.20: Niels Wynant (Leuven, Belgium) - "RNA interference based antiviral immunity in insects" (S11)

9.50: Mieke Gouwy (Leuven, Belgium - "The cytokine-serum amyloid A-chemokine network" (S12)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Roger Huybrechts (Leuven, Belgium) - "In *Locusta migratoria*, bacterial PAMPs make GBP activating the hemocytes whereas angiotensin converting enzyme regulates pro-inflammatory peptides" (O27)

11.10: Fariba Poosti (Leuven, Belgium) - "Interferon gamma peptidomimetic targeted to interstitial myofibroblasts attenuates renal fibrosis after unilateral ureteral obstruction in mice" (O28)

11.30: Pieter Ruytinx (Leuven, Belgium) - "The role of the CXC chemokine CXCL4 and its variant CXCL4L1 in monocyte differentiation" (O29)

11.50: Elisabeth Eppler (Basel, Switzerland) - "CD40 mediated hepatitis in TNF-receptor 1 gene knockout mice" (O30)

12.10: Vincent Vanheule (Leuven, Belgium) - "The COOH-terminal GAG binding CXCL9(74-103) peptide inhibits CXCL8-induced neutrophil extravasation and monosodium urate crystal-induced gout in mice" (O31)

12.30: Lunch (package for persons going on excursion)

12.45: Start of the Excursions

LECTURE HALL - C - AV 02.17

Endocrine Disruption

Organizers: *Barbara Demeneix (Paris, France), Jean-Baptiste Fini (Paris, France), Dries Knapen (Antwerp, Belgium), Lucia Vergauwen (Antwerp, Belgium)*

SOTA lectures:

9.20: Helmut Segner (Bern, Switzerland) - "The vertebrate immune system as a target of endocrine disrupting compounds" (S13)

9.50: Jean-Baptiste Fini (Paris, France) - "Mixtures of environmental xenobiotics affect endocrine signalling and brain development" (S14)

10.20-10.50: Morning Break

ORAL presentations:

10.50: David Du Pasquier (Evry, France) - "Inter-laboratory OECD validation of the *Xenopus* Embryonic Thyroid Signalling Assay" (O32)

11.10: Salima Aroua (Le Havre, France) - "Long term dietary exposure of adult common sole (*Solea solea*) to the flame retardants PBDEs – impact on thyroid and reproductive status" (O33)

11.30: Ellen Michiels (Antwerp, Belgium) - "Development of a zebrafish embryo test for environmental risk assessment of endocrine disrupting pharmaceuticals using nano-injection" (O34)

11.50: Hamid Habibi (Calgary, Canada) - "Reproductive and developmental impairment by low concentrations of environmental contaminants with hormone-like activity in fish using Omics approach" (O35)

12.30: Lunch (package for persons going on excursion)

12.45: Start of the Excursions

LECTURE HALL - D - AV 01.12

Feeding and Metabolism

Organizers: *Encarnación Capilla (Barcelona, Spain), Dick Nässel (Stockholm, Sweden)*

SOTA lectures:

9.20: Mark Sheridan (Lubbock, Texas - USA) - "Mechanisms that underlie nutrition-associated 'metabolic shifting' of growth hormone" (S15)

9.50: Angela Lange (Mississauga/Toronto, Canada) - "Insulin-like peptides in *Rhodnius prolixus*: the vector of Chagas disease" (S16)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Ayelén M. Blanco (Madrid, Spain) - "Ghrelin Modulates Digestive Enzymes and Glucose Transporters in Goldfish Gut and Hepatopancreas in vitro via the GHS-R1a Receptor, and PLC-PKC and AC-PKA Signaling Pathways" (O36)

11.10: Mark R. Brown (Athens, Georgia - USA) - "Endocrines and microbes get mosquitoes through feast and fast" (O37)

11.30: Martina Gáliková (Göttingen, Germany) - "*Drosophila melanogaster* Adipokinetic hormone regulates food intake and expression of other neuropeptide regulators of fly metabolism" (O38)

11.50: Dick Nässel (Stockholm, Sweden) - "Effects of reproductive dormancy on metabolism and genome-wide transcriptome in *Drosophila melanogaster*" (O39)

12.10: Encarnación Capilla (Barcelona, Spain) - "Use of primary cultured adipocytes to better understand adipogenesis, lipid metabolism and fat accumulation in fish" (O40)

12.30: Lunch (package for persons going on excursion)

12.45: Start of the Excursions

Wednesday, August 24:

Conference site - Parkstraat 45

8.00: Registration

8.30-9.15: Plenary Lecture (Lecture Hall A - Max Weber):

Chair: Dan Larhammar (Uppsala, Sweden)

Anna Wargelius (Bergen, Norway) - *"Towards functional understanding of reproduction in Atlantic salmon - gene editing and genome wide association studies*" (PL4)

LECTURE HALL - A - MAX WEBER

Novel Hormones and Receptors in the Animal Kingdom *(morning session)*

Organizers: *Joao Cardoso (Faro, Portugal), Deborah Power (Faro, Portugal)*

SOTA lectures:

9.20: Gáspár Jékely (Tübingen, Germany) - "Large-scale combinatorial deorphanization of GPCRs in the annelid *Platynereis*" (S17)

9.50: Maria-Dolors Piulachs (Barcelona, Spain) - "Regulation of insect oogenesis. More than an endocrine interplay." (S18)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Jerome Delroisse (Mons, Belgium) - "The anatomy of neuropeptide gene expression in a pentaradial bilaterian – the starfish *Asterias rubens* (Phylum Echinodermata)" (O41)

11.10: Esther Odekunle (London, UK) - "Molecular and neuroanatomical characterization of vasopressin/oxytocin-type signalling in an echinoderm" (O42)

11.30: Isabel Beets (Leuven, Belgium) - "An evolutionary conserved neuropeptidergic network underlying *C. elegans* behavioral plasticity" (O43)

11.50: Kristien Van Camp (Antwerp, Belgium) - "In search for neuropeptides in the zebrafish brain by LC-MS" (O44)

12.10: Heather Marco (Rondebosch, South Africa) - "The first characterisation of a crustacean neuropeptide receptor: the putative GPCR for red pigment-concentrating hormone of *Daphnia pulex*" (O45)

12.30: Group picture

12.35-14.30: Lunch and Poster Session 4 -

(*GCE Board meeting during Lunch - Raadzaal SW 00.113*)

Comparative Genomics and Evolution *(afternoon session)*

Organizers: *Dan Larhammar (Uppsala, Sweden), Frans Schuit (Leuven, Belgium)*

SOTA lectures:

14.30: Nils Wierup (Malmö, Sweden) - "Pancreatic Islet Hormones in Vertebrates" (S19)

15.00: Dean Semmens (London, UK) - "Genomic/transcriptomic identification of neuropeptides in echinoderms yields new insights into neuropeptide evolution" (S20)

ORAL presentations:

15.30: Bernard Peers (Liège, Belgium) - "Transcriptional landscape of the major pancreatic cells reveals conserved expression patterns amongst distant vertebrate species" (O46)

15.50-16.10: Afternoon Break

16.10: Harshavardhan Budamgunta (Antwerp, Belgium) - "Micro peptides as a new class of bio-active peptides in higher eukaryotes" (O47)

[Amir Fallahshahroudi (O48): last minute withdrawal]

16.30: Aniruddha Pandit (Glasgow, UK) - "DINeR- A Database for Insect Neuropeptide Research" (O49)

16.50: Dan Larhammar (Uppsala, Sweden) - "Efforts to identify gnathostome-cyclostome orthologs by conserved synteny" (O50)

19.00-….: Social Banquet (Faculty Club)

LECTURE HALL - B - AV 00.17

Omics and Physiology of Insect Neuropeptides

Organizers: *Shireen Davies (Glasgow, UK), Miriam (Vinnie) Altstein (Bet Dagan, Israel), Julian Dow (Glasgow, UK), Jozef Vanden Broeck (Leuven, Belgium)*

Morning session

SOTA lectures:

9.20: Ronald J. Nachman (College Station, Texas - USA) - "Mimetic analogs of neuropeptides as rational tools in the development of novel pest arthropod management strategies" (S21)

9.50: Reinhard Predel (Köln, Germany) - "What can we expect from insect neuropeptidomics? An update" (S22)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Miriam Altstein (Bet Dagan, Israel) - "Insect Neuropeptides: A Basis for the Design of a Novel Group of Insect Control Agents: The PK/PBAN Family as a Case Study" (O51)

11.10: Yoshiaki Tanaka (Tsukuba, Japan) - "A molluscan neuropeptide elevenin regulates the body color via a G protein-coupled receptor Nl-A42 in the brown planthopper *Nilaparvata lugens*" (O52)

11.30: Sven Zels (Leuven, Belgium) - "The sulfakinin signalling system regulates feeding and digestive processes in the migratory locust, *Locusta migratoria*" (O53)

11.50: Gerd Gäde (Rondebosch/Cape Town, South Africa) - "Beetles of the superfamily *Scarabaeoidea* are a rich source for novel peptides of the adipokinetic hormone family" (O54)

12.10: Heleen Verlinden (Leuven, Belgium) - "Adipokinetic hormone and its receptor in the desert locust, *Schistocerca gregaria*" (O55)

12.30: Group picture

12.35-14.30: Lunch and Poster Session 4 -

(*GCE Board meeting during Lunch - Raadzaal SW 00.113*)

Afternoon session

SOTA lectures:

14.30: Angela Lange (Mississauga/Toronto, Canada) - "The involvement of Rhopr-CRF/DH in feeding and reproduction in *Rhodnius prolixus*" (S23)

15.00: Neil Audsley (Sand Hutton, UK) - "G-protein coupled receptors as targets for next generation pesticides" (S24)

ORAL presentations:

15.30: Elwyn Isaac (Leeds, UK) - "Neuropeptide control of crop function of the dipteran pests, *Delia radicum* and *Drosophila suzukii*" (O56)

15.50-16.10: Afternoon Break

16.10: Zita Liutkeviciute (Vienna, Austria) - "Oxytocin-like signalling in ants" (O57)

16.30: Heinrich Dircksen (Stockholm, Sweden) - "Cell-specific distribution of three ion-transport-peptide splice forms in the central and peripheral nervous system of larval and adult *Drosophila*" (O58)

16.50: Jean-Paul Paluzzi (Toronto, Canada) - "CAPA neuropeptides in the mosquito, *Aedes aegypti*: anti-diuretic actions and cellular distribution" (O59)

17.10: Julian Dow (Glasgow, UK) - "Signals for cells: signalling and transport in the insect Malpighian tubule" (O60)

19.00-….: Social Banquet (Faculty Club)

*This Symposium has been organised by the nEUROSTRESSPEP consortium (nEUROSTRESSPEP.eu, "*Novel biocontrol agents for insect pests from neuroendocrinology*" - funded by H2020 Research and Innovation Action, grant agreement:* No. 634361).

LECTURE HALL - C - AV 02.17

Functions of Steroids, their Receptors and Binding Proteins *(morning session)*

Organizer: *Frank Claessens (Leuven, Belgium)*

SOTA lectures:

9.20: Marcel Schaaf (Leiden, The Netherlands) - "Molecular mechanisms of Glucocorticoid Receptor action in zebrafish" (S25)

9.50: Vanessa Dubois (Lille, France) - "Anabolic effects of androgens on skeletal muscle in mice and men" (S26)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Matthew Fuxjager (Winston-Salem, North Carolina -USA) - "Androgens, muscle, and athleticism: uncovering mechanisms of physically elaborate reproductive behavior" (O61)

11.10: Ferran Jardi (Leuven, Belgium) - "Testosterone Regulation of Physical Activity Behavior: Mechanisms of Action" (O62)

11.30: Farida Khammar (Algiers, Algeria) - "Testosterone modulation of adrenocortical activity and adrenal immunolocalisation of the androgen receptors in the Saharan gerbil *Gerbillus tarabuli*" (O63)

11.50: Ewa Szwejser (Cracow, Poland) - "The role of estrogen receptors and aromatase in carp leukocyte activation" (O64)

12.10: Michaël Laurent (Leuven, Belgium) - "Sex hormone-binding globulin (SHBG) regulation of androgen and estrogen bioactivity: of mice and men" (O65)

12.30: Group picture

12.35-14.30: Lunch and Poster Session 4 -

(*GCE Board meeting during Lunch - Raadzaal SW 00.113*)

Regulation of Thyroid Hormone Action at Multiple Levels *(afternoon session)*

Organizers: *Veerle Darras (Leuven, Belgium), Samantha Richardson (Bundoora, Australia)*

SOTA lectures:

14.30: Deborah Power (Faro, Portugal) - "Insights into the role of the thyroid receptors in skin maturation during flatfish metamorphosis" (S27)

15.00: Samantha Richardson (Bundoora, Australia) - "Evolution of transthyretin from uricase to T3 distributor to T4 distributor" (S28)

ORAL presentations:

15.30: Michelle Leemans (Paris, France) - "Mixtures suspected to impede neurodevelopment and metabolism affect thyroid hormone signaling in *Xenopus laevis*" (O66)

15.50-16.10: Afternoon Break

16.10: Yugo Watanabe (Melbourne, Australia) - "Role of corticotropin-releasing hormone in the thyroidal activity in avian life stage transitions" (O67)

16.30: Nele Bourgeois (Leuven, Belgium) - "Intracellular thyroid hormone availability in the chicken embryo: an *in situ* localization study of transporters and deiodinases" (O68)

16.50: Sander Raymaekers (Leuven, Belgium) - "Type 2 deiodinase in the developing song system of the zebra finch: a path to neuroplasticity?" (O69)

17.10: Pieter Vancamp (Leuven, Belgium) - "The developing cerebellum in need of thyroid hormones: a crucial role for monocarboxylate transporter 8" (O70)

19.00-….: Social Banquet (Faculty Club)

LECTURE HALL - D - AV 01.12

Model Organisms in Comparative Endocrinology and Neurobiology *(morning session)*

Organizers: *Bart Braeckman (Ghent, Belgium), Lieve Moons (Leuven, Belgium)*

SOTA lectures:

9.20: Alessandro Cellerino (Jena, Germany) - "From the bush to the bench: the annual fish *Nothobranchius furzeri* as a model organism for neurobiology of aging" (S29)

9.50: Florian Raible (Vienna, Austria) - "Biochemical identification of the annelid brain hormone reveals an ancient role of sesquiterpenoids in regulating animal reproduction" (S30)

10.20-10.50: Morning Break

ORAL presentations:

10.50: Pedro Palma (Faro, Portugal) - "Unveiling STC and PTHrP actions on metabolism: a metabolomics approach using 1H-NMR" (O71)

11.10: Elisabeth Eppler (Basel, Switserland) - "Model systems in diabetes mellitus research: what can we learn from different species and methodological approaches?" (O72)

11.30: Matthias Van Hiel (Leuven, Belgium) - "Towards dIfferential neuropeptide expression upon trypanosome infection in tsetse flies" (O73)

11.50: Luca Fancsalszky (Leuven, Belgium) - "*C. elegans* as a model to unravel neuropeptidergic control of learning and memory" (O74)

12.10: Patrick Laurent (Brussels, Belgium) - "Systematic genetic analysis of dense core vesicle biology in *C. elegans*" (O75)

12.30: Group picture

12.35-14.30: Lunch and Poster Session 4 -

(*GCE Board meeting during Lunch - Raadzaal SW 00.113*)

Recent Advances in Comparative Endocrinology & Neurobiology - Young Investigator Symposium - 1 *(afternoon session)*

Organizers: *Bert De Groef (Bundoora, Australia), Elisabeth Marchal (Leuven, Belgium)*

ORAL presentations:

15.30: Weigang Cai (London, UK) - "Localisation of the expression of a calcitonin-type neuropeptide in echinoderm" (Y1)

15.50-16.10: Afternoon Break

16.10: Luis Alfonso Yanez Guerra (London, UK) - "Functional characterization of a tachykinin-like neuropeptide signaling system in an echinoderm" (Y2)

16.30: Rik Verdonck (Leuven, Belgium) - "A look into the brain transcriptome of *Schistocerca gregaria* during early behavioural gregarisation" (Y3)

16.50: Sifang Liao (Stockholm, Sweden) - "*Drosophila* insulin-like peptide 1 (DILP1) is transiently expressed during non-feeding stages and reproductive dormancy" (Y4)

17.10: Esther Gill-Mansilla (Vienna, Austria) - "Combining MALDI imaging and µCT to localize neuropeptides in ant brains" (Y5)

19.00-….: Social Banquet (Faculty Club)

Thursday, August 25:

LECTURE HALL - A - MAX WEBER

Endocrinology and Chronobiology

Organizers: *Florian Raible (Vienna, Austria), Horst-Werner Korf (Frankfurt, Germany)*

SOTA lectures:

[Davide Dulcis (S31): last minute withdrawal]

9.00: Martina Pfeffer (Frankfurt, Germany) - "Melatonin and its role in the circadian system" (S32)

ORAL presentations:

9.30: Martin Zurl (Vienna, Austria) - "Elucidating the function of the melatonin biosynthesis pathway in the marine bristleworm *Platynereis*" (O76)

9.50: Michaela Fredrich (Frankfurt, Germany) - "Melatonin-dependent rhythmicity in cell proliferation and apoptosis in the hippocampus of adult mice" (O77)

10.10: Moran Homola (Frankfurt, Germany) - "Impact of melatonin receptor deficiency on ecto-5'-nucleotidase mRNA levels in the mouse prosencephalon" (O78)

10.30: Mamta Tripathy (Delhi, India) - "GDF9 in ovary of *Hemidactylus flaviviridis*: molecular characterization, phylogenetic analysis, stage specific expression and gonadotropic regulation" (O79)

10.50: Shona Wood (Manchester, UK) - "The role of the circadian clock in seasonal timing" (O80)

11.10-11.40: Morning Break

11.20-11.40: General Assembly of ESCE members (Business meeting)

11.40-12.25: Closing Lecture (Lecture Hall A - Max Weber):

Chair: Horst-Werner Korf (Frankfurt, Germany)

Takashi Yoshimura (Nagoya, Japan) - "*Universality and diversity in the photoperiodic signal transduction in vertebrates*" (PL5)

12.25-13.00: Closing Ceremony

13.00-14.00 Lunch

14.00-19.00: Leopoldina Symposium on Seasonal Rhythms (post-conference activity)

LECTURE HALL - B - AV 00.17

Molecular Evolution, Structures and Functions of GPCRs - 2

Organizers: *Christian Gruber (Vienna, Austria), Heleen Verlinden (Leuven, Belgium)*

SOTA lectures:

8.30: Kathleen Van Craenenbroeck (Gent, Belgium) - "Interaction between dopamine D2-like receptors and μ opioid receptor" (S33)

9.00: Patricia Pietrantonio (College Station, Texas - USA) - "Are GPCRs back to insect taste perception? A kinin mimetic elicits aversive behavior in mosquito *Aedes aegypti* and inhibits the sugar taste neuron" (S34)

ORAL presentations:

9.30: Joao Cardoso (Faro, Portugal) - "Expansion of the Secretin-GPCR members in the molluscs" (O81)

9.50: Shi Tian (London, UK) - "Urbilaterian origin of paralogous GnRH and corazonin neuropeptide signalling pathways" (O82)

10.10: Gabriella Köblös (Budapest, Hungary) - "Identification and functional characterization of the pheromone biosynthesis activating neuropeptide receptor isoforms from *Mamestra brassicae*" (O83)

10.30: Jorge Ronderos (La Plata, Argentina) - "The GPCR allatotropin/orexin family: an ancestral conserved mechanism of signals, as a probable alternative system of phylogenetic molecular markers" (O84)

10.50: Guy Smagghe (Gent, Belgium) - "Potential interactive amino acids in the *Tribolium castaneum* sulfakinin receptors for ligand binding" (O85)

11.10-11.40: Morning Break

LECTURE HALL - C - AV 02.17

Stress and Adaptation

Organizers: *Gudrun De Boeck (Antwerp, Belgium), Patrick Kestemont (Namur, Belgium)*

SOTA lectures:

8.30: Patrick Prunet (Rennes, France) - "Plasticity of the corticotrope axis reactivity in rainbow trout exposed to stressors." (S35)

9.00: James Carr (Lubbock, Texas - USA) - "Glutamate receptor regulation of tectal CRF release" (S36)

ORAL presentations:

9.30: Maithé Corbani (Montpellier, France) - "V1B and CRF1 receptors involved in stress associate as heterodimers in the living pituitary to form assemblies with functional synergism" (O86)

9.50: Benoît Bernard (Namur, Belgium) - "Temperature shift impairs osmoregulatory capacities during the smoltification phase of two Atlantic salmon (*Salmo salar* L.) strains" (O87)

10.10: Sebastien Baekelandt (Namur, Belgium) - "Neurophysiological stress response of pikeperch *Sander lucioperca* juveniles to intensive culture conditions" (O88)

10.30: Nadezhda Goncharova (Sochi, Russian Federation) - "Individual and age-related differences in stress responsiveness of HPA are associated with features of vasopressinergic and melatoninergic regulation" (O89)

10.50: Nataly Gruntenko (Novosibirsk, Russian Federation) - "Stress response in *Drosophila melanogaster*: biogenic amines, juvenile hormone, 20-hydroxyecdysone and insulin signalling are involved" (O90)

11.10-11.40: Morning Break

LECTURE HALL - D - AV 01.12

Recent Advances in Comparative Endocrinology & Neurobiology - Young Investigator Symposium - 2

Organizers: *Bert De Groef (Bundoora, Australia), Elisabeth Marchal (Leuven, Belgium)*

ORAL presentations:

9.30: Zulvikar Syambani Ulhaq (Chuo-Ku, Japan) - "Localization and possible function of brain aromatase in zebrafish eye" (Y6)

9.50: Jelena Periz Stanacev (Antwerp, Belgium) - "Transcriptional profiles of the endocrine system during embryo-larval development of the zebrafish" (Y7)

10.10: Kaat Kehoe (Antwerp, Belgium) - "Prolyl carboxypeptidase: a potential biomarker for obesity and diabetes mellitus?" (Y8)

10.30: Lakshmi Vasudevan (Gent, Belgium) - "Implementation of new tools to study the interaction between G protein-coupled receptors (GPCR primers)" (Y9)

10.50: Ye Hwa Jin (Stirling, UK) - "Expression pattern of *nanos* and *piwi* genes during ontogenic development in Nile tilapia *Oreochromis niloticus*" (Y10)

11.10-11.40: Morning Break

Poster sessions (Monday and Wednesday)

The posters will be displayed during the entire period of the conference (Monday morning - Thursday morning). The posters should be displayed on the numbered boards according to their PO-abstract number.

Presenters are requested to be at their poster during one of the four following sessions:

- Monday, August 22, 13.30-14.30: **posters PO1 - PO22**;
- Monday, August 22, 17.30-18.30: **posters PO23 - PO44**;
- Monday, August 22, 18.30-19.30: **posters PO45 - PO67**;
- Wednesday, August 24, 13.30-14.30: **posters PO68 - PO88**.

LECTURE HALL - A - MAX WEBER

Leopoldina Symposium on seasonal rhythms *(post-conference activity):*

Organizer: *Horst-Werner Korf (Frankfurt, Germany)*

14.00: Hans-Peter Zenner (Tübingen, Germany) - Opening Address

14.10: Takashi Yoshimura (Nagoya, Japan) - "Towards understanding the mechanism of seasonal time measurement" (L1)

14.40: Andrew Loudon (Manchester U.K.) - "Genetic and cellular mechanisms involved in the generation of long-term seasonal cycles" (L2)

15.10: Valerie Simonneaux (Strasbourg, France) - "What makes a mammal a seasonal breader?" (L3)

15.40: David Hazlerigg (Tromsö, Norway) - "Gestational photoperiod programmes offspring reproductive development via the fetal pituitary gland" (L4)

16.10-16.30 Afternoon Break

16.30: Paul Pévet (Strasbourg, France) - "Synchronization of seasonal functions by photoperiodic changes with or without melatonin" (L5)

17.00: Horst-Werner Korf (Frankfurt am Main, Germany) - "Signaling pathways to and from the pars tuberalis" (L6)

17.20: Josephine Arendt (Guildford UK) - "Non-24h sleep wake disorder in relation to different light conditions" (L7)

17.50: Gilles Vandewalle (Liege, Belgium) - "Seasonal variation in human brain function" (L8)

18.20: Anna Wirz-Justice (Basel, Switzerland) - "Seasonal rhythms in affective disorders" (L9)

18.50: Concluding Remarks

Leopoldina
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ABSTRACTS

PLENARY SPEAKERS AND LECTURES

Pierre Leopold

Pierre Léopold graduated from the University of Nice and trained Drosophila developmental genetics as a post-doctoral fellow at the University of California, San Francisco (UCSF). He then obtained a CNRS starting grant to return to France where he created his own group. He has a current appointment at the University of Nice (Institute of Biology Valrose) as a Research Director Exceptional Class for the National Institute of Health and Medical Research (INSERM). Over the recent years, he has developed a research interest for the control of tissue growth using Drosophila as a model organism. His work primarily focuses on the understanding of systemic mechanisms controlling organismal growth. More recently, his laboratory has demonstrated the importance of organ-organ communications in the mechanisms of growth coordination and the determination of final organ size.

PL1 Growth coordination mechanisms during Drosophila development

Pierre Leopold

Univ. Nice/CNRS/Inserm, NICE, France

Body size is an intrinsic property of organisms linked to their developmental program to produce fit individuals with proper proportions. Final size is the result of genetic determinants as well as sophisticated mechanisms adapting size to available resources. Classical regeneration and transplantation experiments have established that different body parts grow according to autonomous programs, challenging the concept of systemic, harmonious growth. Therefore, coordination mechanisms must ensure that all parts have reached an appropriate final size before animals stop growing. Recent advances making use of physiological and genetic approaches have started unravelling some of the cross talks contributing to body growth coordination. In flies, the “coordination hormone”Dilp8 plays a major role in these processes. This relaxin-like hormone is produced by tissues upon growth perturbation. It couples the program of developmental transitions with organ growth by acting on its membrane receptor Lgr3, activating a neural circuitry that is only partially elucidated. Interestingly, animals lacking Dilp8 or Lgr3 present defects in bilateral symmetry, a sign that this novel hormonal system participates in the developmental control of organ growth coordination. In this presentation, I will discuss our recent research aimed at understanding the mechanisms allowing organ/organ growth coordination both in response to abnormal growth (injury/neoplasm) and in the course of normal development (control of developmental stability).

Marc Caron

Marc G. Caron is a native of Quebec Canada, holds a Ph.D. from the University of Miami. He is a James B. Duke Professor of Cell Biology at Duke University Medical Center. He has also hold joint appointments in Neurobiology and Medicine. He was previously associated with the Howard Hughes Medical Institute at Duke University for more than 20 years (1981-2004). His long-standing research interests have been in the mechanisms and regulation of G protein-coupled receptors (GPCR) and on the mechanisms of neurotransmission as controlled by neurotransmitter transporters. Some of his notable accomplishments have been the purification and cloning of GPCRs as well as the identification and multiple functions of components of the desensitization machinery of GPCRs. His work with monoamine transporters has led to a better appreciation of their role in the control of neurotransmission and the development animal models of abnormal neurobiological function including disorders such as schizophrenia and depression. His recent investigations have led to the notion that GPCRs, like the dopamine D2 receptors, can signal not only through G proteins but also through the ability of barrestins to scaffold distinct signaling complexes, a concept referred to as functional selective/biased signaling. His lab is currently leveraging this concept for the development of more specific and effective therapeutic approaches for psychiatric disorders. Caron has authored more than 650 publications, has received numerous recognitions such as a Doctorate Honoris Causa from the Universite de Montreal (2008); The Linda and Jack Gill Center for Biomolecular Science Award, Indiana Univ. (2011); The Lieber Prize for Schizophrenia Research, Brain and Behavior Research Foundation (NARSAD) (2013), election as a Fellow of AAAS (2013) and a Member of the American Academy of Art and Sciences (2015), to name a few.

PL2 Physiological Consequences of GPCR Functional Selectivity and Therapeutic Approaches.

Marc G Caron

Duke University, DURHAM NC, United States of America

G protein-coupled receptors (GPCRs) represent the largest family of receptors in the human genome and are one of the most common targets of pharmaceutical drugs. It is now firmly established that GPCRs engage downstream signaling pathways not only through canonical G protein activation but also through the ability of β-arrestins to scaffold distinct intracellular signaling complexes. β-arrestins and GPCR kinases were originally associated with desensitization of G protein-mediated signaling. For those GPCRs for which this concept of 'functional selectivity/biased signaling' has been studied, invariably each signaling pathway has the ability to mediate distinct physiological responses. Synthetic GPCR ligands have been found that can selectively affect each pathway, but how *in vivo* functional selectivity is achieved is still poorly understood. The brain neurotransmitter dopamine (DA) mediates its effects through GPCRs (D1R and D2R subtypes) and through both G proteins and β-arrestins (Urs et al., 2011; 2012). D2Rs are the main target of antipsychotics (ADPs) for the management of schizophrenia and other symptoms in humans. These symptoms are thought to be associated with an over action of DA in the caudate/striatum but a deficit in cortical areas of the brain. Current ADPs that primarily block D2Rs are not effective at alleviating deficits in cortical DA. To examine the potential *in vivo* relevance of D2R biased signaling, we developed novel functionally selective ligands (Allen et al., 2011). When tested in pharmacological mouse models of striatal or cortical DA dysfunction, one of these ligands, UNC9994A, exerts its antipsychotic-like properties by acting as a D2R antagonist in the striatum but a D2R agonist in the cortex. These properties, which are dependent on both β-arrestin and GPCR kinase (GRK2), can be demonstrated by both behavioral and electrophysiological approaches. Interestingly, this neuronal selective action of UNC9994A is determined by the markedly higher levels of GRK2 and β-arrestin2 in the cortex vs the striatum. Since *in vivo* most GPCRs are activated by single ligands, our results highlight the importance the cellular complement of signal transducer proteins like β-arrestins and GRKs, as the most important determinant of GPCR functional selective signaling. Therefore, leveraging the new concept of GPCR biased signaling should help in the development of more efficacious and selective therapeutic approaches.

Charlotte Cornil

Charlotte Cornil is a Research Associate of the Belgian Science Foundation (Fonds pour la Recherche Scientifique ; F.R.S.-FNRS) and Adjunct Associate Professor at the University of Liege. She received her PhD from the University of Liège in 2004 and conducted her post-doctoral research in the Department of Brain and Psychological Sciences at Johns Hopkins University.

Her research focuses on the analysis of the neuroendocrine and neurochemical mechanisms that mediate the activation and sexual differentiation of reproductive behaviors using birds and mice as animal models with a particular interest in the role of membrane- vs nuclear-initiated estrogen signaling.

She is the author of more than 55 original papers, reviews or book chapters. She currently directs the lab of Behavioral Neuroendocrinology, at the GIGA Neurosciences of the University of Liège.

PL3 The dual action of neuroestrogens on sexual behavior

Charlotte Cornil

ULg, LIEGE, Belgium

Neuroestrogens (estrogens produced by brain testosterone aromatization) play a key role in the activation of many behaviors including male sexual behavior. The effects of estrogens on behavior are typically associated with long-term (seasonal) changes in the circulating concentration of testosterone and are mainly considered to result from the transcriptional activity of their liganded nuclear receptors (estrogen receptor alpha and beta). Accordingly, the genomic effects of neuroestrogens would prime the neural circuits involved in behavior regulation which could then be switched on and off by neurotransmitter systems in a more rapid manner conveying information from the social environment such as the presence of a sexual partner or predator. However, it is now clearly accepted that beside their genomic mode of action, estrogens are also capable to induce much faster effects through the activation of membrane-associated receptors. For a long time, it was thought that these actions were only mediated by novel estrogen receptors with a distinct molecular structure compared to the classical nuclear receptor. However, recent evidence revealed that nuclear receptors can themselves translocate to the membrane where they interact with other receptors to signal non-genomically. Our recent investigations in quail revealed an interesting dichotomy in the control of male sexual behavior by membrane- and nuclear-initiated signaling of estrogens: acute membrane-initiated effects control sexual motivation while the capacity to display the copulatory sequence depends on a long-term exposure to both testosterone and estrogens. The mechanism underlying the acute effects of neuroestrogens on sexual motivation depends on an ER-beta-mediated transactivation of metabotropic glutamate receptor 1. In parallel, our work on the acute regulation of brain aromatase activity led to the observation that brain estrogen synthesis is also rapidly regulated *in vivo* in a region- and context-dependent manner. Fast changes in sexual motivation could thus result from rapid fluctuations in local estrogen concentration, presumably at the synaptic level. Indeed *in vitro* studies suggest that only synaptic aromatase is sensitive to acute changes in neuronal activity. Together, these results support the idea that some aspects of acute regulation of male sexual behavior depend not only on classical neurotransmitter systems, but also on fast and spatially confined changes in brain estrogen concentration. The existing literature and recent data from our group also suggest that this acute regulation by estrogens of the motivational aspects of behavior could be generalized to other systems.

This work was supported by a R01 NIH/MH50388 grant and a grant from the Special Funds for Research from ULg. CAC is a F.R.S.-FNRS Research Associate.

Anna Wargelius

Anna Wargelius (MSc, PhD) Principal Scientist (1183) at the Institute of Marine Research. Gene expression pattern and level reflects to a significant extent the ongoing anatomical, morphological and physiological activities in a tissue and/or organism. Her field of interest is exploring how the genome and environment regulate global, epigenetic, local and spatial gene expression in fish and how these processes regulate key life history situations such as early development reproduction and growth. Recently she has identified a single locus in the salmon genome which largely predicts time of maturation for salmon at sea. This finding will allow us to further investigate how a single locus in the genome exerts its action in the animal. She can also investigate how this trait can be overrun by environmental conditions since it is known that both light and temperature severely affects time of puberty in salmon.

These studies will work as explanatory models how a genetic predisposition can be modulated by environmental triggers. She is also utilizing gene editing technologies in Atlantic salmon to be able to explore; molecular mechanisms related to the reproductive system, in particular germ cell specification and early sexual development, with the main aim to produce germ cell free salmon. Both aims explore fields which can result in sterility models for aquaculture that could solve major bottlenecks related to un-wanted sexual maturation and genetic interactions with wild populations. They are also currently exploring the functionality of fatty acid metabolism genes in salmon, a key area for development of sustainable feed for the aquaculture industry.

PL4 Towards functional understanding of reproduction in Atlantic salmon - gene editing and genome wide association studies

Anna Wargelius

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Recent biotechnological innovations have allowed the development of new approaches to apply genetic engineering to non-model organisms, including the Atlantic salmon. Key innovations include the development of next generation sequencing which allows fast and cost effective analysis of whole genomes, transcriptomes and epigenomes. Studies on gene functions became feasible for many, following the introduction of the CRISPR-Cas9 methodology, which can specifically target and mutate genes in any organism, thus also allowing studies on the genetics of key traits. We have explored both methodologies with the aim to target two major problems in today's salmon aquaculture: (*i*) escaped fish and (*ii*) early maturation.

(*i*) Genetic introgression mediated into wild populations by farmed salmon escapees is a major concern, and is currently one of the factors limiting the expansion of the Norwegian salmon industry. To address this problem, we are investigating the possibility to induce sterility by vaccination in salmon. Exploring the testicular transcriptome of immature and maturing salmon and comparing it to other tissues enabled us to select suitable vaccine targets. We have utilized CRISPR-Cas9 technology to elucidate the function of candidate genes in the development and survival of germ cells in salmon. Due to the long generation time of salmon we have had to analyze complete loss of function in F0. To avoid analysis of mosaic individuals, we simultaneously induced CRISPR-Cas9-mediated mutations in the *albino* (*alb*) and in the target gene. We observed that complete loss of pigmentation indicated bi-allelic disruption of *alb* but also of the second gene that was targeted. This methodology allowed producing germ cell-free salmon, in which we could show that Atlantic salmon, like loach and goldfish, has a germ cell-independent sex determination system. We are also following growth and performance of these fish.

(*ii*) Precocious maturity in salmon farms leads to increased susceptibility to disease and hypo-osmoregulatory problems. Also early maturity increase risk of genetic interactions with wild fish, since escaped early maturing fish are more likely to survive until maturation and spawn in the wild. Currently the problem with early maturation is partially controlled by using artificial light regimes. However, both increasing sea water temperatures and increased use of closed farming systems in the growth phase, can increase the incidence of early maturation. To unravel genetics behind early maturity at sea, we have re-sequenced pools of salmon returning to spawn after 1 or 3 sea winters in 6 rivers in Western Norway. The study revealed one major selective sweep, which covered 74 significant SNPs in a 370 kb region of chromosome 25. Genotyping domesticated fish narrowed the haplotype region to four SNPs covering the *vgll3* gene, that could explain 33-36% phenotypic variation. This study demonstrates that a single locus plays a highly significant role in governing age at maturity in this species. We are now exploring the functionality of the *vgll3* gene in salmon using CRISPR-Cas9 technology. In conclusion, the use of new biotechnological tools has enhanced significantly and will keep doing so our knowledge on the life cycle biology of salmon.

Takashi Yoshimura

Takashi Yoshimura is currently running three independent laboratories. He is a Professor of Animal Physiology in the Institute of Transformative Bio-Molecules (WPI-ITbM) and the Graduate School of Bioagricultural Sciences, Nagoya University, Japan. He is also a Visiting Professor in the National Institute for Basic Biology, Japan.

Work in Yoshimura laboratory focuses on understanding the molecular mechanism of seasonal time measurement in vertebrates. The uniqueness of his research lies in the use of various vertebrate species such as Japanese quail, chicken, hamster, mouse, salmon and medaka. By applying functional genomics approach, he has uncovered the signal transduction pathway regulating seasonal reproduction in vertebrates. He is currently applying chemical biology and forward genetics approaches to understand the mechanism of vertebrate seasonal time measurement.

He currently serves as board member of Japanese Society of Chronobiology and Society for Research on Biological Rhythms, editorial board of Journal of Biological Rhythms, and fellow of Royal Society for Biology (FRSB).

PL5 Universality and diversity in the photoperiodic signal transduction in vertebrates

Takashi Yoshimura

Nagoya University, NAGOYA, Japan

Animals living in temperate zone use changes in day length to adapt to seasonal changes in environment, but mechanisms underlying seasonal (photoperiodic) time measurement are not fully understood. Japanese quail is an excellent model for the study of these mechanisms because of its rapid and dramatic response to changes in photoperiod. We have demonstrated that local thyroid hormone catabolism within the mediobasal hypothalamus (MBH) by thyroid hormone-activating enzyme (type 2 deiodinase: DIO2) regulates photoperiodism. Functional genomics analysis in quail demonstrated that long day stimulus induces thyrotropin (thyroid stimulating hormone: TSH) production in the pars tuberalis (PT) of the pituitary gland, which triggers DIO2 expression in the ependymal cells of the MBH. In mammals, nocturnal melatonin secretion provides an endocrine signal of the photoperiod to the PT that contains melatonin receptors in high density. We have also demonstrated the involvement of TSH signaling pathway in mammals by using the TSH receptor null mice. Well known function of TSH derived from pars distalis (PD) of the pituitary gland is stimulation of thyroid gland. However, the mechanisms by which PT- and PD-TSH exert distinct functions within the body remained mystery. We found TSHs from two anatomical sources undergo different glycosylation and this tissue-specific glycosylation imparts different functions on a single hormone. In fish, the regulatory machinery for seasonal reproduction, from light input to neuroendocrine output, has been recently demonstrated in the coronet cells of the saccus vasculosus (SV). I would like to discuss the universality and diversity of signal transduction pathways that regulate vertebrate seasonal reproduction.

Yoshimura et al., (2003) Nature 426, 178-181. Nakao et al., (2008) Nature 452, 317-322. Ono et al., (2008) Proc. Natl. Acad. Sci. USA 105, 18238-18242. Nakane et al., (2013) Nat Commun. 4, 2108. Ikegami et al., (2014) Cell Reports 9, 810-809.

INTERNATIONAL GCPR SYMPOSIUM

Within the context of CECE2016, a joint ***International GPCR Symposium*** has been organized with focus on the study of G protein-coupled receptors (GPCRs/7TM).

Symposium organizers:

Marc Parmentier (ULB, Brussels), Jan Steyaert (VUB, Brussels), Paul Proost, Johan Thevelein, Jo Van Damme, Jozef Vanden Broeck (KU Leuven), and Silvano Sozzani (Brescia, Italy).

This international meeting is organized in the frame of the Belgian Interuniversity Attraction Poles programme (BELSPO - IAP P7/40) "G protein-coupled receptors: from structures to functionally validated targets" coordinated by Marc Parmentier.

G1 Structure of the human adenosine A2a receptor bound to an engineered G protein

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MRC Laboratory of Moelcular Biology, CAMBRIDGE, United Kingdom

G protein-coupled receptors (GPCRs) activate intracellular signalling proteins (G proteins and arrestins) in response to extracellular signalling molecules. GPCRs are highly dynamic proteins, which rapidly interchange between different conformational states. In order to understand the molecular mechanisms of GPCR activation, and to facilitate efficient drug design, structures of GPCRs in all conformational states are required. Unsurprisingly, ternary complexes, involving native signalling proteins, have proved most difficult to crystallise due to their instability.

We have developed an engineered G protein, mini-G_s, which is significantly more stable in complex with GPCRs than native G_s. Mini-G_s is particularly stable in short chain detergents and thus suitable for crystallisation of GPCR-G protein complexes by vapour diffusion. Additional components, such as nanobodies, are not required for maximal stability. We have purified complexes of engineered Gs with both the adenosine A_{2a} receptor ($A_{2a}R$) and β_1 adrenergic receptor (β_1AR) and determined the structure of wild type human $A_{2a}R$ bound to engineered Gs at 3.4 Å resolution by X-ray crystallography. I shall discuss the structure of the $A_{2a}R$-mini-Gs complex and the implications for activation of GPCRs, with particular reference to β_1AR.

This work was funded by a grant from Heptares Therapeutics Ltd and by core funding from the Medical Research Council.

Carpenter & Tate (2016) Engineering a minimal G protein to facilitate crystallisation of G protein-coupled receptors in their active conformation. *Submitted*. Carpenter et al. (2016) Structure of the adenosine A2A receptor bound to an engineered G protein. Nature, *In press*.

G2 What are they waiting for? - Tethered agonism in GPCRs

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In classical pharmacology agonists bind to their respective receptors by specific interaction and induce structural changes followed by cellular responses. However, some G protein-coupled receptor (GPCRs), such as rhodopsin and protease-activated receptors (PARs), have their agonists already covalently bound and are parts of the receptor proteins, respectively. Recent studies add adhesion GPCRs and glycoprotein hormone receptors (GPHRs) to the group of GPCRs activated by integral agonists. In contrast to rhodopsin and PARs, adhesion GPCRs and GPHRs exhibit large ectodomains (ECDs) which bind a number of different proteins and other extracellular molecules. It seems that these large size ECDs are required to integrate a multitude of extracellular signals, such as protein ligand binding, cell-cell contacts and even mechanical forces, into uniform intracellular signals. Upon extracellular ligand binding, the intramolecular agonist of those receptors is exposed or isomerizes and induces structural changes in the 7-transmembrane helix domain triggering G-protein activation. The existence of activating structures integrated in receptor molecules challenges our current pharmacological definition of an agonist. We summarized and discussed the specifics of tethered agonist pharmacology which add a number of new features of the already broad signaling abilities of GPCRs and may find useful applications in designer GPCRs.

G3 Natural biased agonism at Angiotensin II-type 1 receptor

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Hyperactivity of the renin-angiotensin-aldosterone system (RAAS) through the Angiotensin II type 1 receptor (AT1-R) axis constitutes a hallmark of hypertension. Recent findings indicate that only a subset of AT1-R signaling pathways is cardio-deleterious and their selective inhibition by biased ligands promotes therapeutic benefit. So far, only synthetic biased ligands have been described and whether natural RAAS peptides exhibit functional selectivity at AT1-R remains unknown. In this study, we systematically determined efficacy and potency of AngII, III, IV and 1-7 in AT1-R expressing HEK293T cells on activation of cardiodeleterious G-proteins and cardioprotective β-arrestin2. AngIII and AngIV fully activate similar G proteins than AngII, the prototypical AT1-R agonist, despite weaker potency of AngIV. Interestingly, Ang1-7 which binds AT1-R, fails to promote G protein activation but behaves as a competitive antagonist for AngII/Gi and /Gq pathways. Conversely, all RAAS peptides act as agonists on the AT1-R/β-arrestin2 axis but display biased activities relative to AngII as indicated by their differences in potency and AT1-R/β-arrestin2 intracellular routing. Importantly, we reveal Ang1-7, a known Mas receptor specific ligand, as an AT1-R biased agonist, selectively promoting β-arrestin activation while blocking the detrimental AngII/AT1-R/Gq axis. This original pharmacological profile of Ang1-7 at AT1-R, similar to that of synthetic AT1-R biased agonists, could in part contribute to its cardiovascular benefits. Accordingly, in vivo, Ang1-7 counteracts the phenylephrine-induced aorta contraction which was blunted in AT1-R knockout mice. Collectively, these data suggest that Ang1-7 natural biased agonism at AT1-R could fine-tune the physiology of the RAAS.

G4 Coexpression of CCR7 and CXCR4 during B cell development controls CXCR4 responsiveness and bone marrow homing.

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G protein-coupled receptors (GPCRs) constitute one of the largest families of plasma membrane proteins involved in cell signaling. Besides their canonical role in signaling, GPCRs can also act as allosteric modulators of one another through receptor oligomerization. However, little is known about the role of GPCR oligomerization in physiological processes. By using chemokine receptors and B cell development as a model system, we unveil a novel role of CCR7 as selective endogenous allosteric modulator of CXCR4. The CXCL12-CXCR4 axis plays a key role in the retention of stem cells and progenitors in dedicated bone marrow niches. When lymphopoiesis progresses to more differentiated stages, B cells lose their responsiveness to CXCL12 despite the continuous expression of CXCR4. B cells regain their sensitivity to CXCL12 as they further differentiate into plasma cells, but the mechanism involved in this transient loss of CXCR4 responsiveness was not identified. We show that during the last stages of their development in bone marrow, B cells undergo changes in the relative expression of chemokine receptors with a two-fold downregulation of CXCR4 and an upregulation of CCR7. We show that expression of CCR7 in mature B cells is involved in the selective inactivation of CXCR4, and that mature B cells from CCR7-KO mice display higher responsiveness to CXCL12. Accordingly, CCR7-KO mice have significantly higher number of mature B cells in bone marrow, confirming that CCR7 modulates CXCR4-dependent signaling and bone marrow homing. Finally, we provide evidence that this regulation does not require CCR7 signaling nor the scavenging of G proteins by CCR7, but most likely the formation of CXCR4-CCR7 heteromer in which CXCR4 is selectively impaired in its ability to activate G protein complexes. These results constitute the first indication that chemokine receptor heteromerization plays a role in a physiological process.

G5 Fructose-1,6-bisphosphate couples glycolytic flux to activation of Ras downstream in the GPCR-controlled glucose sensing network of yeast

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The yeast *Saccharomyces cerevisiae* contains an elaborate network of glucose-sensing mechanisms, of which a major system controls the activity of the cAMP-PKA pathway. The latter controls proliferation, fermentation, reserve carbohydrate levels, stress tolerance and other cellular properties. A major player in this system is the glucose-sensing G-protein coupled receptor, Gpr1, that activates cAMP production through a monomeric Galpha protein homolog, Gpa2. This GPCR system shows an unusual dependency for activation of cAMP synthesis on uptake and partial metabolism of glucose in glycolysis. The mechanism involved has remained elusive. Yeast adenylate cyclase is not only activated by the Galpha protein, Gpa2, but is also dependent for its activity on a second type of G-proteins, the Ras proteins. These are well known from mammalian cells as the most widespread oncogenes. The yeast and mammalian *RAS* genes are closely related. They can be functionally exchanged and overactive RAS oncogene products expressed in yeast also cause aberrant control of cell proliferation and viability. Yeast cells and mammalian cancer cells also share the unusual characteristic of favoring fermentation of sugar over respiration in the presence of oxygen. In cancer cells this is known as the Warburg effect. It is unclear whether it plays a role in cancerogenesis or stimulation of tumor growth. Here we demonstrate a direct molecular link between fructose-1,6-bisphosphate (Fru1,6bisP), the most elaborately controlled intermediate of the glycolytic fermentation pathway, and the Ras proteins. A yeast mutant (*tps1*Δ) with overactive influx of glucose into glycolysis and hyperaccumulation of Fru1,6bisP, shows glucose-induced hyperactivation of Ras followed by apoptosis. A putative binding site for Fru1,6bisP with strongly conserved positively charged residues is present in the Ras activator Cdc25 and its mammalian homolog Sos1. Their mutagenesis abolishes glucose-induced apoptosis and restores growth in the *tps1*Δ mutant. We show that glucose also triggers activation of Ras and its downstream targets MEK and ERK in mammalian cells and that Fru1,6bisP helps to dissociate the Sos1/Ras complex *in vitro*. Our results reveal an evolutionary conserved mechanism that couples glycolytic flux to activity of Ras and thus explain how glycolytic flux is coupled to the activity of adenylate cyclase. They also show that combined deregulation of glycolysis and overactivation of Ras triggers apoptosis in yeast. Our results suggest that Fru1,6bisP acts as metabolic messenger for the rate of glycolytic flux and explain why genetic modifications stimulating Fru1,6bisP synthesis or breakdown act as activators or suppressors of cancerogenesis, respectively.

Conrad M., et al. (2014) Nutrient sensing and signaling in the yeast Saccharomyces cerevisiae. FEMS Microbiology Reviews 38, 254-299.

G6 Two for one, one for two: Myoinhibitory Peptide and Sex Peptide Receptor

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G protein-coupled receptors (GPCRs) represent the largest family of membrane proteins essential for intercellular communications in a plethora of biological processes. By combining pharmacological and molecular genetic analyses in *Drosophila melanogaster*, a genetically amendable animal model, we uncover molecular mechanisms underlying a functional pleiotropy of GPCR and its ligand. One GPCR of our interest is the sex peptide receptor (SPR), which was initially identified as a receptor for sex peptide (SP), the seminal protein evoking the post-mating responses in females. Although biochemical and genetic evidences supporting SPR as a physiologically relevant SP receptor were highly compelling, it was evident that SPR has an additional ligand(s) besides SP, because SPR occurs in a much larger number of animal genomes than SP does. The subsequent studies of others and ours identified myoinhibitory peptides (MIP) as potent ligands for SPR. According to a sequence comparison, MIP shares little relationship with SP. Unlike SP, MIP is not involved in the post-mating responses. Instead, both MIP and SPR are essential to stabilize sleep. Furthermore, *mip* coincides with *spr* broadly in most of the lophotrochozoan and ecdysozoan species, indicating MIP represents an evolutionarily ancestral ligand for SPR. Recently, we uncovered MIP signals satiety besides sleep. *Mip* mutant is hardly satiated after feeding and becomes obese. Unlike *mip* mutant, however SPR-deficient mutant has normal satiety responses, suggesting that MIP signals through a receptor(s) other than SPR. With these studies that examined and compared *in vivo* functions of SPR and its natural ligands, we uncovered SPR signals through two functionally and structurally unrelated ligands, SP and MIP, and MIP signals through two different receptors, each of which regulates sleep and feeding behavior, respectively.

Oh, et al., 2014 A homeostatic sleep-stabilizing pathway in Drosophila composed of the Sex Peptide receptor and its ligand, the Myoinhibitory Peptide. PLoS Biol. 12, e1001974. Min et al., 2016 Identification of a Peptidergic Pathway Critical to Satiety Responses in Drosophila. Curr. Biol. 26, 814.

G7 The pleiotropic allatoregulatory neuropeptides and their receptors

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Juvenile hormones (JH) are highly pleiotropic sesquiterpenoids regulating post-embryonic development and reproduction in insects. The circulating JH titer in the haemolymph of insects is influenced by enzymatic degradation, binding to JH carrier proteins, uptake and storage in target organs, but evidently also by rates of production at the site of synthesis, the *corpora allata* (CA). The multiple processes in which JH is involved alongside the critical significance of JH in insect development emphasize the importance of elucidating the mechanisms controlling JH production. Production of JH in CA cells is regulated by different factors: by neurotransmitters, such as dopamine and glutamate, but also by allatoregulatory neuropeptides originating from the brain and axonally transported to the CA where they bind to their G protein-coupled receptors (GPCRs). Different classes of allatoregulatory peptides exist which have other functions in addition to the regulation of JH production. In this presentation, we will discuss the physiological roles and modes of action of allatotropins and allatostatins, as well as their recently characterized GPCRs.

The AT receptors (ATRs) of insects are related to vertebrate orexin receptors. Although no clear similarity exists between the insect and vertebrate peptide ligands, their precursors do possess similar regions. Compared to most other neuropeptide receptors, it took relatively long before the first ATR was characterized. This is probably because this signaling system remains absent in the most studied invertebrate models, *i.e. Drosophila melanogaster* and *Caenorhabditis elegans*.

Three types of allatostatins (ASTs) have been identified in insects so far. They have been named A, B and C-type allatostatins, or in line with more recently proposed nomenclature these peptides are also known as FGLa/ASTs, MIP/ASTs and PISCF/ASTs, respectively. They are widely pleiotropic peptides, which are present in many insect orders, but their function as inhibitors of JH production is only evident in some insect groups. An activity attributed to all ASTs is the myoinhibition of visceral muscles including gut and oviduct.

Applications in pest control based on intervening with the regulation of JH might emerge from the identification and validation of these neuropeptide receptors as novel candidate insecticide targets. The discovery of new insecticide targets is extremely important in mankind's continuously ongoing arms' race against pest species. This may address the existing and increasing problems with product selectivity and with the evolution of insecticide resistance in pest populations.

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G8 Protons as second messenger regulators of G protein signaling

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In response to environmental stress, cells generate pH signals that serve to protect vital cellular components and reprogram gene expression for survival. These processes are conserved in yeast, plants and mammals. We have shown recently that G protein alpha subunits, the principal transducers of G protein-coupled receptor (GPCR) signals, are pH sensors. Our structure-based calculations and biophysical investigations revealed that G-alpha subunits contain networks of pH-sensing sidechains buried between their Ras and helical domains, and that proton binding induces changes in conformation to suppress signaling. The GPCRs likewise contain spatially conserved networks of buried ionizable groups. These networks are likely relevant to receptor function because they connect the ligand binding pocket of the receptor (outside the cell) to the nucleotide-binding pocket of the G protein (inside the cell). We propose that agonist and G protein binding facilitate the formation of electrostatic networks and promote important structural rearrangements that are diagnostic of receptor activation. These structural signatures may be used to predict ligands that stabilize the activated receptor state.

Isom DG, Sridharan V, Dohlman HG. Biochemistry. 2016 Jan 26;55(3):534-42. Isom DG, Dohlman HG. Proc Natl Acad Sci U S A. 2015 May 5;112(18):5702-7. Isom DG, Sridharan V, Baker R, Clement ST, Smalley DM, Dohlman HG. Mol Cell. 2013 Aug 22;51(4):531-8.

G9 Unraveling the function of GPR37 in the brain: more than a Parkinson's disease-associated receptor

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GPR37 is an orphan G protein-coupled receptor highly expressed in the brain. Despite the precise function of this orphan receptor is still unknown, a number of evidences indicate its participation in several physiological and pathological (i.e. Parkinsons disease) conditions. Here, we demonstrate that GPR37 and A_{2A} receptor ($A_{2A}R$) co-distribute and co-assemble into functional interacting complexes both in heterologous expression systems and in the striatum. Interestingly, GPR37 deletion promotes $A_{2A}R$ subsynaptic redistribution and cell surface targeting in the striatum. Furthermore, the GPR37 knock-out mice show enhanced $A_{2A}R$ agonist-mediated catalepsy. Overall, our results demonstrate a key role of GPR37 in the striatal $A_{2A}R$ synaptic targeting and function in behaving animal.

G10 Developing anti-inflammatory drugs for infectious diseases

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Infectious diseases are usually accompanied by inflammation in the affected tissues. This inflammatory response is necessary for the capacity of the host to control invasion and consequent establishment of infection, and it also provides necessary signals for an ensuing adaptive immune response. In the absence of inflammation, infections can become out of control and lead to death of the host, as seen in neutropenic patients. On the other hand, there is now much evidence to show that inadequate (insufficient, excessive, misplaced or altered) inflammatory responses may lead to disease following infection. For example, in bacterial sepsis, excessive production of mediators of inflammation, especially in the systemic circulation, causes systemic inflammation and deatyh. During influenza infection, excessive neutrophilic infiltration in the lung may cause pulmonary damage, prevent adequate lung function and consequently death. Here, we will discuss the concept that modifying inflammatory responses during infection may lead to development of novel therapies for infectious disease. Any anti-inflammatory compound to be used in the context of infection should in general be used with an anti-microbial drug, when available. Focus will be given to the role of chemoattractant molecules in driving tissue inflammation and damage, and the potential for blocking their G-protein coupled receptor during infection. Examples of the potential of blocking GPCRs in the context of dengue, influenza and zika will be discussed.

G11 Natural nitration of CXCL12 reduces its signaling capacity and chemotactic activity in vitro and abrogates intra-articular lymphocyte recruitment.

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The chemokine CXCL12/stromal cell-derived factor-1 is important for leukocyte migration to lymphoid organs and inflamed tissues and is involved in tumor development. *In vitro,* CXCL12 activity is strongly regulated by proteolytic processing. However, limited information is available on *in vivo* posttranslationally modified CXCL12. Therefore, natural CXCL12 from stromal cells stimulated with leukocytes and inflammatory agents was purified. CXCL12 with a nitration on Tyr^7, designated [3-NT^7]CXCL12, was detected. CXCL12 and [3-NT^7]CXCL12 were chemically synthesized to evaluate the biological effects of this modification. [3-NT^7]CXCL12 recruited b-arrestin 2, phosphorylated the kinases Akt and ERK1/2 and bound to glycosaminoglycans and the G protein-coupled chemokine receptor CXCR4 similar to CXCL12. However, it showed a reduced ability to enhance intracellular calcium concentrations, to generate inositol triphosphate and to induce monocyte and lymphocyte chemotaxis *in vitro*. Moreover, nitrated CXCL12 failed to induce *in vivo* extravasation of lymphocytes to the joint. In summary, nitration on Tyr^7 is a novel natural posttranslational regulatory mechanism on CXCL12 under inflammatory conditions which may affect the CXCR4-mediated inflammatory and tumor-promoting activities of CXCL12.

G12 Chemokine receptor activation - progress towards novel therapeutic principles

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The chemokine system mediates leukocyte migration during homeostatic and inflammatory processes. Traditionally, it is described as redundant and promiscuous, with a single chemokine ligand binding to different receptors and a single receptor having several ligands. The actual interaction of chemokines with their cognate receptors occurs in a multi-step pattern. In the initial steps the chemokine core interacts with extracellular receptor regions (with the receptor N-terminus being essential for this process). Later steps involve docking of the chemokine N-terminus into the main binding pocket of the receptors. In addition to the complexity in the chemokine:receptor interaction and recognition pattern, multiple small molecule antagonists and agonists have been presented in the chemokine system with allosteric action via anchorage deep in the main receptor binding pocket and in certain cases also with extracellular receptor regions.

Signaling of chemokine receptors occurs via two major routes, G protein- and β-arrestin-dependent, which can be preferentially modulated depending on the ligands or receptors involved, as well as the cell types or tissues in which the signaling event occurs. The preferential activation of a certain signaling pathway to the detriment of others has been termed signaling bias and can accordingly be grouped into ligand bias, receptor bias, and tissue bias. Bias has so far been broadly overlooked in the process of drug development. The low number of currently approved drugs targeting the chemokine system, as well as the broad range of failed clinical trials, reflects the need for a better understanding of the chemokine system. Thus, understanding the character, direction, and consequence of biased signaling in the chemokine system may aid the development of new therapeutics.

Here we present data on the extracellular receptor requirements for chemokine binding and subsequent receptor activation across the entire family of endogenous chemokine receptors and describe how conserved aromatic residues in ECL2 interact with aromatic residues in the main binding pocket to create an aromatic cluster needed for receptor activation. We furthermore describe the molecular requirements for the action of allosteric small molecule antagonists and agonists among CC-chemokine receptors and define an allosteric receptor interface that controls this. Using receptor mutagenesis, we create biased receptors with impaired arrestin recruitment and maintained G protein activation and vise versa in CCR5 and describe how this affect chemokine and small molecule binding and how it affects the HIV fusion process. Using this principle, we present evidence for small molecule agonists, biased towards G protein-activation., are excellent inhibitors of HIV cell entry without internalization of CCR5. Finally, we describe the structural basis for signaling bias in CCR7, where differences in CCL19 and CCL21 interaction with CCR7 explain ligand bias and differential expression of the two chemokines and the glucoaminoglycan content contribute to the tissue bias observed in CCR7.

G13 Oncogenic potential of HCMV-encoded chemokine receptors

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Pathogenic herpesviruses alter cellular signaling after viral infection through expression of viral G protein-coupled receptors (GPCRs). These viral GPCRs show highest homology to chemokine receptors, which are known to regulate the immune system but are also involved in the development of cancer. We have shown that several viral GPCRs, including the HCMV-encoded chemokine receptor US28, signal in a constitutive manner and hijack proliferative signaling pathways[1]. US28 stimulates cell proliferation through activation of the IL-6-STAT3 axis. US28 expression promotes tumour formation in a xenograft model and is expressed in tumour specimen of glioblastoma patients. Moreover, US28 induces neoplasia in the intestine of US28 expressing transgenic mice via activation of beta catenin. Recently, we have identified llama-derived antibody fragments, nanobodies, which effectively bind and modulate oncogenic signaling of US28. Taken together, by modulating inflammatory and proliferative signaling pathways, viral GPCRs may effectively rewire cellular signaling networks and contribute to tumour progression. Insight into these mechanisms is crucial for the treatment of virus-associated pathologies.

Vischer HF, Siderius M, Leurs R, Smit MJ. (2014) Herpesvirus-encoded GPCRs: neglected players in inflammatory and proliferative diseases? Nat Rev Drug Discov;13:123-39.

G14 Chemerin and its receptors in leukocyte trafficking, inflammatory diseases, metabolism and cancer.

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Chemerin was isolated as the natural ligand of the G protein-coupled receptor CMKLR1/ChemR23. Chemerin acts as a chemotactic factor for leukocyte populations expressing CMKLR1, particularly immature plasmacytoid dendritic cells, but also immature myeloid DCs, macrophages and natural killer cells. Chemerin is expressed by epithelial and non-epithelial cells as an inactive precursor, present at nanomolar concentrations in plasma. Processing of the precursor C-terminus is required for generating bioactive forms of chemerin, and various proteases mediate this processing, including neutrophil serine proteases and proteases from coagulation and fibrinolytic cascades. CMKLR1-expressing cells are recruited in human inflammatory diseases. In animal models, both pro-inflammatory and anti-inflammatory roles of chemerin have been reported. Two other receptors for chemerin were described, GPR1 and CCRL2, but their functional relevance is still largely unknown. Both chemerin and ChemR23 are also expressed by adipocytes, and chemerin is now considered as an adipokine regulating lipid and carbohydrate metabolism. Using mouse and zebrafish genetic models, we study the role of chemerin and its receptors in inflammatory and tumoral contexts.

STATE OF THE ART LECTURES

S1 The effect of GnIH on the signaling pathways leading to the activation of GnRH neurons triggered by kisspeptin and VIP

You Lee Son, Kazuyoshi Tsutsui

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Gonadotropin-inhibitory hormone (GnIH) was initially isolated from Japanese quail as a hypothalamic neuropeptide that directly inhibits gonadotropin secretion from the anterior pituitary. GnIH peptides have since been identified in all vertebrate classes, and their inhibitory effects on gonadotropin release has been demonstrated. Therefore, the discovery of GnIH has broken new ground in the field of reproductive neuroendocrinology. Despite the well-demonstrated physiological significances of GnIH as a negative regulator of reproduction, the molecular mechanism of GnIH action has not been fully understood. On the basis of GnIH neuronal fiber distribution and the existence of GnIH receptor, it has been suggested that gonadotropes and gonadotropin-releasing hormone (GnRH) neurons are the major target cells of GnIH action. Following the successful demonstration of the inhibitory intracellular pathways of GnIH action in gonadotropes, we recently investigated the potential signal transduction pathway that conveys the inhibitory signal of GnIH in GnRH neurons. Although GnRH is the final output that regulates reproduction by stimulating gonadotropin secretion from the pituitary, GnRH neuronal functions are finely tuned by stimulatory and inhibitory afferent inputs in the brain. Therefore, our study focused on the interactions of these inputs to GnRH neurons. We specifically investigated whether GnIH inhibits the stimulatory signal transduction pathways in GnRH neurons triggered by kisspeptin and vasoactive intestinal polypeptide (VIP). We examined the effect of GnIH on GnRH release induced by kisspeptin and VIP from hypothalamic explants, then performed the in depth analysis of signal transduction pathways of GnIH and its interactions with kisspeptin and VIP on the second messenger system, mitogen-activated protein kinases (MAPK) activation, and gene expression in an immortalized GnRH neuronal cell line, GT1-7. GnIH effectively suppressed the stimulatory effect of kisspeptin on GnRH release from hypothalamic tissue. However, GnIH had no inhibitory effect on kisspeptin stimulation of serum response element (SRE) and nuclear factor of activated T-cells response element (NFAT-RE) activities, and extracellular signal-regulated kinases (ERK) phosphorylation, indicating that GnIH may not directly inhibit kisspeptin signaling in GnRH neurons. On the other hand, GnIH eliminated the stimulatory effect of VIP on GnRH release as well as VIP-induced signaling pathways, cAMP-response element (CRE) activity, p38 and ERK phosphorylation, and c-Fos expression. The use of pharmacological modulators clearly demonstrated that GnIH inhibits VIP signaling *via* the adenylate cyclase (AC)/cAMP/protein kinase A (PKA)-dependent pathway in GnRH neurons, consistent with the inhibitory mechanism of GnIH action on GnRH-induced signaling in gonadotropes. Therefore, our results suggest that GnIH exerts its inhibitory effect by specifically acting *via* the AC/cAMP/PKA pathway in its target cells, GnRH neurons and gonadotropes.

S2 The Roles of Estradiol and Specific Z-Chromosome Genes in Masculinization of the Zebra Finch Song System

Juli Wade

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Sex differences in brain and behavior exist across diverse vertebrate species. In many cases they are permanently organized by steroid hormones in development. The zebra finch song system is highly sexually dimorphic. Only males sing, and the brain regions that control the learning of these vocalizations in juveniles and the production of the behavior in adulthood are far larger in males compared to females. Administration of estradiol in the first few weeks after hatching has potent, but incomplete, masculinizing effects on structure and function of the song system, which is consistent with a role for this steroid among other factors in masculinization. However, consistent sex differences in circulating hormone levels, neural estrogen synthesis, and song system estrogen receptors have not been identified. Attempts to inhibit masculinization by treating juvenile males with aromatase inhibitors and estrogen receptor blockers have been largely unsuccessful, with a few exceptions, raising questions about the natural role of estradiol in males. Therefore, we have been investigating additional factors that may be important for masculinization of the song circuit, focusing on Z-chromosome genes (males = ZZ; female = ZW). Dosage compensation is limited in birds, and we and others have detected specific Z-genes that exhibit increased expression in the song system of developing males compared to females. We are particularly interested in the idea that estradiol exposure and expression of specific Z-chromosome genes might work in concert to masculinize structure and function of the song system. Recent work in my lab has focused on two Z-genes: TrkB (the high affinity receptor for BDNF) and tubulin specific chaperone protein A (TBCA), which is important for microtubule formation. We have demonstrated that inhibition of each of these genes within a song control region diminishes masculinization of neural structure. However, indications of interactive or additive effects of these Z-genes with estradiol are limited. Thus, TrkB and TBCA appear to play roles in masculinization of song circuit morphology, but processes normally regulated by estradiol remain unclear. Supported by NIH R01-MH096705.

S3 Neuropeptide signalling in echinoderm reproduction and development

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Research on neuropeptide signalling has largely focused on vertebrates and selected protostomian invertebrates (insects, molluscs etc.) and until recently relatively little was known about the biology of neuropeptides in deuterostomian invertebrates (e.g. echinoderms), which occupy an “intermediate”position in animal phylogeny with respect to vertebrates and protostomes. Our research focuses on echinoderms (e.g. starfish, sea urchins, sea cucumbers) as model systems to investigate the evolution and comparative physiology of neuropeptide signalling.

The common European starfish *Asterias rubens* is our main experimental model system and analysis of the neural transcriptome of this species has enabled identification of forty transcripts encoding neuropeptide precursors. On-going studies are investigating the expression patterns of these neuropeptide precursor transcripts in larval and adult starfish as a basis for investigation of the physiological roles of neuropeptides in echinoderms.

Relevant to reproductive biology, a relaxin-like gonad-stimulating peptide (RGP) that triggers spawning in starfish has been identified. Our analysis of the expression of the RGP precursor in *A. rubens* using mRNA *in situ* hybridization has revealed that RGP is expressed by cells located in the body wall epithelium that surrounds a cavity containing the optic cushion (“eye”) at the tips of starfish arms. This is interesting because it suggests that sensory inputs may stimulate release of RGP from these cells to trigger spawning in starfish.

Relevant to development, we have used mRNA *in situ* hybridization to investigate the expression of neuropeptide precursor genes during the pre-metamorphic larval stages of *A. rubens*. Three of the neuropeptide precursors analysed (L- and F-type SALMFamide, NGFFYamide) are expressed in both the bipinnaria and brachiolaria stages of larval development, whereas expression of five other neuropeptide precursors analysed (vasopressin-, GnRH-, TRH-, CRH- and calcitonin-type) is only detected in brachiolaria larvae. Analysis of the patterns of neuropeptide precursor expression has provided interesting insights into the physiological roles of neuropeptides in starfish larvae. For example, both CRH-type (ArCRHP) and calcitonin-type (ArCTP) precursors are expressed in the attachment complex of brachiolariae, but with non-overlapping patterns of expression. Thus, ArCRHP is expressed in the brachia whereas ArCTP is expressed in or near the adhesive disk, parts of the attachment complex that respectively mediate temporary and then permanent attachment of the larva to the substratum prior to initiation of metamorphosis. These findings suggest that ArCRH and ArCT may have distinct roles associated with larval attachment prior to metamorphosis in starfish.

In conclusion, our analysis of neuropeptide expression in larval and adult starfish is providing a basis for new experimental approaches to investigate the physiological roles of neuropeptides in reproduction and development.

Semmens et al. 2016 Transcriptomic identification of starfish neuropeptide precursors yields new insights into neuropeptide evolution. Open Biol. 6, 150224. Mita et al. 2009 A relaxin-like peptide purified from radial nerves induces oocyte maturation and ovulation in starfish PNAS 106, 9507.

S4 Different neuropeptides convergently activate egg maturation in mosquitoes

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Reproduction in mosquitoes is a relatively quick and highly regulated sequence of behavioral and physiological processes that result in the production of eggs. This is because females of most species steal a blood meal from a vertebrate host to acquire amino acids for yolk production, but some do not and instead mobilizes teneral reserves for this purpose. Both strategies depend on the release of neuropeptides from brain neurosecretory cells. We determined roles for two such neuropeptides, ovary ecdysteroidogenic hormone (OEH) and insulin-like peptides (ILPs) with bioassays of species that blood feed for egg production and others that do not. OEH is the primary factor activating production of ecdysteroid hormone by ovaries in all species. ILPs also activates this process and other key processes associated with egg production. Receptors for OEH and ILP3 were identified through bioinformatics as related receptor tyrosine kinases. Subsequent work showed they signal through the insulin pathway, and the effects on ovary ecdysteroid production are enhanced through TOR and calcium signaling. Research support from the National Institutes of Health (RO1AI33108 to MRB and MRS) and the Georgia Agricultural Experiment Station.

S5 Osmoregulation and stress tolerance in Drosophila melanogaster

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Insects are exposed to multiple stressors across a range of environments. Desiccation tolerance and survival is dependent on fluid homeostasis by fluid transporting epithelia including the Malpighian tubules. Fluid transport by insect Malpighian tubules is modulated by diuretic neuropeptides [2, 3] which have also recently been shown to affect desiccation and starvation tolerance in the insect genetic model *Drosophila melanogaster* [1, 5].

However, in addition to this, a novel role for Malpighian tubules in cold tolerance has been recently demonstrated, which occurs via homeostatic control of water and ion balance and is modulated by the capa neuropeptide [4, 5]. Altogether, this current evidence suggests that at least three diuretic neuropeptides (capa, DH44, kinin) perform functions in environmental stress tolerance via the Malpighian tubules.

Thus, Malpighian tubules, and diuretic neuropeptides, have much wider implications for physiology and behaviour beyond osmoregulation.

[1] Cannell E, *et. al.*, Peptides. 2016.

[2] Coast GM, Garside CS. Ann. NY Acad. Sci. 2005;1040:1-8.

[3] Halberg KA, *et. al.*, Nat. Commun. 2015;6:6800.

[4] MacMillan HA, *et. al.*, Sci. Rep. 2015;5:18607.

[5] Terhzaz S, *et al.*, PNAS, 2015;112(9):2882-7.

S6 Molecular physiology of hormonal actions on body fluid ionic and acid-base homeostasis in zebrafish

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Fish have developed sophisticated mechanisms of ionic and acid-base regulation for maintaining body fluid homeostasis. Many hormones have been proposed to control the ionic and acid-base regulation mechanisms in fish; however, most of the proposed actions lack convincing cellular and/or molecular evidence. Given the advantages in terms of genetic database availability and manipulation, zebrafish is an emerging model for research into regulatory and integrative physiology. Different types of ionocytes were found to transport ions through various sets of ion transporters, and the molecular mechanisms of ionocyte proliferation and differentiation have also been dissected, providing a competent platform with which to precisely study the ion transport pathways and ionocytes targeted by hormones, including isotocin, prolactin, cortisol, stanniocalcin-1, calcitonin, endothelin-1, vitamin D, parathyorid hormone 1, catecholamines, the renin-angiotensin-system, estrogen-related receptor α, and calcitonin gene-related peptide, which have been demonstrated to positively or negatively regulate ion transport through specific receptors at different molecular levels (transcriptional, translational, or posttranslational) or at different developmental stages of ionocytes (proliferation or differentiation). The knowledge obtained using zebrafish answered many long-term contentious or unknown issues in the field of fish iono-/osmoregulation. The homology of ion transport pathways and hormone systems also means that the zebrafish model informs studies on mammals or other animal species, thereby providing insights into related fields.

S7 State-of-the-art lecture: Mechanisms of locust phase change: parallels and differences across several different lineages.

Stephen Rogers

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Locusts are grasshoppers (Orthoptera: Acrididae) that can undergo a reversible transformation between a lone-living solitarious phase and a gregarious phase that is adapted for a life of swarming migration. Phase change encompasses extensive changes in behaviour, physiology and / or morphology, but this ability appears to have evolved independently in several different Acridid lineages. Differences and parallels in the mechanisms inducing phase change in different locust species are compared. The immediate trigger for gregarisation is the presence of stimuli from other locusts, which may be tactile, olfactory or visual. These induce a rapid reconfiguration of behaviour within a few hours or days of sustained stimulation. There is now extensive evidence that the early stages of gregarisation are mediated by biogenic amine signalling, but precise mechanisms differs between species. serotonin, for example, has a critical role in gregarising the desert locust (*Schistocerca gregaria*); in the migratory locust (*Locusta migratoria*) dopamine and octopamine have been implicated as having key roles, whereas in the Australian plague locust (*Chortoicetes terminifera*) both octopamine and serotonin have roles in different stages of the gregarisation process. Recent progress in understanding how behavioural change is initiated and consolidated, including a putative role for neuropeptides are discussed. In the longer term, phase change entails extensive changes in gene expression, which fundamentally alters the physiological substrate on which these mechanisms of phenotypic plasticity operate.

S8 Serotonin promotes the development and regeneration of spinal cord motor neurons in zebrafish

Antón Barreiro-Iglesias

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In contrast to mammals, including humans, zebrafish regenerate spinal cord motor neurons following a complete spinal cord injury. Previous work has shown that, during regeneration in adult zebrafish, developmental signals are re-deployed. We have shown that during spinal cord development, diffuse serotonin promotes motor neuron generation from progenitor cells, leaving interneuron numbers unchanged. Pharmacological manipulations and receptor knockdown indicate that serotonin acts at least in part via serotonin 1A receptors in motor neuron progenitor cells. In zebrafish adults, serotonin is supplied to the spinal cord mainly by spinal-projecting axons from the brain. After a complete spinal cord injury, serotonergic axons degenerate caudal to the lesion but sprout rostral to it. Toxin-mediated ablation of serotonergic axons rostral to the injury site impaired regeneration of motor neurons only there. Conversely, intraperitoneal serotonin injections doubled numbers of new motor neurons and proliferating progenitors caudal to the lesion. Regeneration of spinal intrinsic serotonergic interneurons was unaltered by these experimental manipulations. Serotonin selectively promotes the development and adult regeneration of motor neurons in zebrafish (Barreiro-Iglesias et al., 2015).

Barreiro-Iglesias A, Mysiak KS, Scott AL, Reimer MM, Yujie Y, Becker CG, Becker T. Serotonin promotes development and regeneration of spinal motor neurons in zebrafish. Cell Reports. 2015, 13(5): 924-932.

S9 Comparative GPCR structure and sequence analyses in GPCRdb and in ligand design

David Gloriam

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This talk will give an overview of GPCR structures and comparative structure/sequence analyses of receptor mechanisms and ligand design.

Today, more than 140 structures have been reported for 36 unique GPCRs across the classes A, B, C and F. The transmembrane ligand binding sites largely overlap, but display a variation for the depth of pocket penetration. The first insights into the structural mechanisms of GPCR signalling have come from complexes of the β_2-adrenoceptor to Gs, and opsin to β-arrestin and a Gt peptide. These and nanobody complexes have uncovered common conformational changes upon receptor activation.

The structural templates have also served as Rosetta stones for the alignment of receptor protein sequences, in particular across the GPCR classes and for transmembrane helices containing bulges or constrictions. Structure-based sequence alignments and generic residue numbers let us to map and infer common conserved evolutionary and functional properties. Furthermore, it opens up for comparative analyses to identify the characteristic sequence signatures underlying ligand binding, G protein coupling and stabilisation of an active receptor conformation.

The GPCR database, GPCRdb.org provides structure statistics (overview), browsing (template selection) and superposition (comparison). It has extracted over 6000 ligand interactions from structure complexes, and made them available for pharmacophore generation. Phylogenetic trees and site searches are based on structure-based sequence alignments that can be focussed to any receptor segment or (generic) residue set. Finally, new tools for mutation and construct design serves to equip molecular pharmacologists and structural biologists, respectively.

S10 Pharmacology of nature-derived neuropeptide ligands

Christian Gruber

The University of Queensland, BRISBANE, Australia

The diversity in nature has long been and still is one of the biggest resources of pharmaceutical lead compounds and many natural products often exhibit biological activity against unrelated biological targets, thus providing us with starting points for pharmacological analysis. Natural peptides of great number and diversity occur in all organisms from plants to microbes to man. Examples for such rich and yet largely untapped libraries of bioactive compounds are animal venom peptides, invertebrate peptide hormones or plant defense peptides. Our goals are to discover and characterize novel oxytocin- and vasopressin neuropeptide analogs in invertebrates, study their function, determine their pharmacological activity, and use them as probes to design peptides to develop ligands for human G protein-coupled receptors. Using an interdisciplinary approach by combining physiology, pharmacology and peptide chemistry, we are aiming to generate selective, potent and stable peptide ligands and may be useful for the treatment of a wide range of challenging, but yet untreated diseases.

[1] Gruber et al., Curr Pharm Des, 2010; 16: 3071-3088. [2] Gruber & Muttenthaler, PLoS One, 2012; 7(3): e32559. [3] Koehbach et al., PNAS, 2013; 110: 21183-21188. [4] Gruber et al., Future Med Chem, 2012; 4: 1791-1798.

S11 RNA interference based antiviral immunity in insects.

Niels Wynant, Dulce Santos, Jozef Vanden Broeck

KU Leuven, LEUVEN, Belgium

Invertebrates rely on the innate immune system to combat viruses, as they do not possess an antibody-based adaptive immune system. In recent years, evidence accumulated highlighting the importance of small regulatory RNAs in protecting invertebrates against viruses. These small RNAs don't code for proteins but trigger a sequence-specific gene silencing response, acting at the post-transcriptional level via a process called RNA interference (RNAi). Although RNAi is a very ancient mechanism, it exhibits some characteristics of the mammalian adaptive immune system, such as its systemic nature, specificity and immunological memory. In this state of the art lecture, recent progress on the role of different types of small RNAs in invertebrate antiviral immunity is summarized.

S12 The cytokine-serum amyloid A-chemokine network

Mieke Gouwy, Mieke De Buck, Paul Proost, Sofie Struyf, Jo Van Damme

Rega Institute, LEUVEN, Belgium

Serum amyloid A (SAA) is, like C-reactive protein (CRP), an acute phase protein and can be used as a diagnostic, prognostic or therapy follow-up marker for many diseases. Increases in serum levels of SAA are triggered by physical insults to the host, including infection, trauma, inflammatory reactions and cancer. The order of magnitude of increase in SAA levels varies considerably, from a 10- to 100-fold during limited inflammatory events to a 1000-fold increase during severe bacterial infections and acute exacerbations of chronic inflammatory diseases. This broad response range is reflected by SAA gene duplications resulting in a cluster encoding several SAA variants and by multiple biological functions of SAA. SAA chemoattracts monocytes, lymphocytes, and granulocytes via its G protein-coupled receptor formyl peptide receptor like 1/formyl peptide receptor 2 (FPRL1/FPR2). We demonstrated that the SAA1α isoform also chemoattracts monocyte-derived immature dendritic cells (DCs) in the Boyden and µ-slide chemotaxis assay and that its chemotactic activity for monocytes and DCs was indirectly mediated via rapid chemokine induction. Indeed, SAA1 induced significant amounts (≥5 ng/mL) of MIP-1α/CCL3 and IL-8/CXCL8 in monocytes and DCs in a dose-dependent manner within 3 h. However, SAA1 also directly activated monocytes and DCs for signaling and chemotaxis without chemokine interference. SAA1-induced monocyte migration was nevertheless significantly prevented (60-80% inhibition) in the constant presence of desensitizing exogenous MIP-1α/CCL3, neutralizing anti-MIP-1α/CCL3 antibody, or a combination of CCR1 and CCR5 antagonists, indicating that this endogenously produced CC chemokine was indirectly contributing to SAA1-mediated chemotaxis. Further, anti-IL-8/CXCL8 antibody neutralized SAA1-induced monocyte migration, suggesting that endogenous IL-8/CXCL8 acted in concert with MIP-1α/CCL3. This explained why SAA1 failed to synergize with exogenously added MIP-1α/CCL3 or SDF-1α/CXCL12 in monocyte and DC chemotaxis. In addition to direct leukocyte activation, SAA1 induces a chemotactic cascade mediated by expression of cooperating chemokines to prolong leukocyte recruitment to the inflammatory site. Further, we demonstrated that SAA1α more potently chemoattracts neutrophils *in vivo* than *in vitro*. In contrast to $CD14^+$ monocytes, no rapid (within 2 h) induction of IL-8/CXCL8 or MIP-1α/CCL3 was observed in purified human neutrophils after stimulation of the cells with SAA1α or lipopolysaccharide (LPS). Moreover, IL-8/CXCL8 induction in monocytes by SAA1α was TLR2-mediated and was inhibited by association of SAA1α with high density lipoprotein (HDL). This indicates that the potent chemotactic response of neutrophils towards intraperitoneally injected SAA1α is indirectly enhanced by rapid induction of chemokines in peritoneal cells, synergizing in a paracrine manner with SAA1α. Indeed, we observed direct synergy between IL-8/CXCL8 and SAA1α, but not LPS, in chemotaxis and shape change assays with neutrophils. Furthermore, the selective CXCR2 and FPR2 antagonists, SB225002 and WRW4 respectively, blocked the synergy between IL-8/CXCL8 and SAA1α in neutrophil chemotaxis *in vitro*, indicating that for synergy their corresponding GPCRs are required. Additionally, SB225002 significantly inhibited SAA1α-mediated peritoneal neutrophil influx. Taken together, endogenous (e.g. IL-1β) and exogenous (e.g. LPS) inflammatory mediators induce primary chemoattractants like SAA, that synergize in an autocrine (monocytes) or a paracrine (neutrophils) fashion with secondary chemokines induced in stromal cells.

*De Buck M, Berghmans N, Pörtner N, Vanbrabant L, Cockx M, Struyf S et al, Serum amyloid Aalpha induces paracrine IL-8/CXCL8 via TLR2 and directly synergizes with this chemokine via CXCR2 and formyl peptide receptor 2 to recruit neutrophils. J Leukoc Biol 2015;98:1049-1060.

S13 The vertebrate immune system as a target of endocrine disrupting compounds

Helmut Segner

University of Bern, BERN, Switzerland

Endocrine disruption refers to the ability of environmental substances to interfere with endogenous hormone metabolism and signalling, thereby disrupting the physiological target systems which are regulated by the hormones. To date, emphasis has been given to effects of endocrine-disrupting compounds (EDCs) on reproduction and sexual development. However, there exists increasing evidence that physiological systems other than these “classical targets”are at risk by EDCs - what is to be expected given the pleiotropic nature of hormonal activities. Here, we discuss the available evidence that environmental EDCs are able to modulate and disrupt the immune system of vertebrates, and whether this has adverse consequences for the immunocompetence of the exposed organisms. Particularly for estrogen- and thyroid-active compounds, immunodisruptive effects have been reported for principally all vertebrate classes. This observation leads to the next questions, that are the question on the underlying mechanisms mediating the EDC effects on the immune system, and whether these mechanisms are evolutionary conserved. Finally, the question arises whether the immunomodulating activities of EDCs pose indeed a risk to wildlife populations by increasing their susceptibility to disease at EDC concentrations as they can be found in the environment.The presentation will discuss our current state of knowledge on potential immunodisruptive effects of EDCs.

S14 Mixtures of environmental xenobiotics affect endocrine signalling and brain development

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Endocrine disrupting chemicals were officially named 25 years ago at Wingspread conference. Since then many chemicals have been shown to be harmful to wildlife and human health with a strong focus on steroid disruption. Characteristics of EDCs that have been established include action at low doses, timing of exposure with fetal exposure being a major vulnerable window. Moreover research on EDCs examines single molecules whereas a more realistic exposition paradigm would invlove mixtures.

Thyroid hormone (TH) signaling is conserved across vertebrates and can be seen as bridging the environment to gene expression networks. Our focus is on TH actions on early brain development. Data suggest a causal link between mixtures of EDCs affecting TH signalling and the increasing incidence of human neurodevelopmental disorders, such as Autism Spectrum Disorders (ASD). Thus, it is increasingly urgent to screen chemical mixtures that could be exerting “environment x gene”interactions, thereby contributing to the exponential rise in neurodevelopmental disorders with their enormous socio-economic costs for individuals and society.

To study effects of ubiquitous environmental chemical mixtures found in humans including pregnant women, we took advantage of an assay using transgenic xenopus to screen thyroid disrupting potential of 15 chemicals tested as singletons or in mixture. Application of the mixture of the 15 chemicals together, at concentrations reported in amniotic fluid, induced a significant and dose-dependent increase in T_3 dependent transcription. RT-qPCR analysis on the dissected brain tissue from the mixture-exposed *Xenopus* embryos revealed modifications of TH related genes including *thrb*, *klf9* and especially the deiodinases (*dio1, 2, 3*). Using a locomotor tracking system we observed that tadpoles exposed to increasing concentrations displayed severely and significantly reduced mobility. In order to study the mixture impact on neurogenesis we further subjected the amniotic mixture exposed embryonic brains to immuno-histochemistry. We observed increased proliferation within the developing brain and modification of cell fate when exposed to the mixture. Taken together these results show that a mixture of chemicals found in human at legal levels affect T_3 signalling at a critical moment for a optimal brain development.

S15 Mechanisms that underlie nutrition-associated "metabolic shifting" of growth hormone

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Growth hormone (GH) regulates two disparate metabolic processes: growth promotion (anabolic) and the breakdown of stored lipid (catabolic). Trout hepatocytes are particularly useful for studying switching between these two processes because they are a principal site of IGF-1 production and lipid storage. GH stimulates IGF-1 production in cells from fed fish but not in cells from fasted fish, whereas GH stimulates lipolysis [as measured by mRNA and functional expression of hormone sensitive lipase (HSL)] in cells from fasted fish but not in cells from fed fish. Fed fish display pronounced growth correlated with activated JAK2, STAT5, and Akt, whereas fasted fish display retarded growth and enhanced HSL expression in association with activated ERK and PLC/PKC. In hepatocytes from fasted fish, GH activates the PLC/PKC and MEK/ERK pathways, whereas the JAK-STAT and PI3K-Akt pathways are deactivated. Blockade of the PLC/PKC and MEK/ERK pathways inhibit GH-stimulated lipolysis and mRNA expression, whereas blockade of the JAK-STAT and PI3K-Akt pathways have no effect on the activation of lipolysis or HSL expression. By contrast, in cells from fed fish, GH activates the JAK-STAT and PI3K-Akt pathways, whereas the PLC/PKC and MEK/ERK pathways are deactivated. Blockade of the JAK-STAT and PI3K-Akt pathways inhibit GH-stimulated IGF-1 production, whereas blockade of the PLC/PKC and of the MEK/ERK pathways have no effect on IGF-1 production. Serum from fed fish rescues IGF-1 production in cells from fasted fish, whereas serum from fasted fish inhibits IGF-1 production in cells from fed fish. Moreover, insulin and IGF-1 block GH-stimulated activation of PLC/PKC and HSL expression in cells from fasted fish and can rescue IGF-1 production in cells from fasted fish concomitant with activation of JAK-STAT, PI3K-Akt, and ERK. These findings indicate that the metabolic response of GH results from the differential linkage of GH to signaling pathways and that nutritional state, via insulin and IGF-1, modulates GH-signal pathway-response linkage. (Supported by NSF grant IOS 0920116)

S16 Insulin-like peptides in Rhodnius prolixus: the vector of Chagas disease

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Insulin-like peptides (ILPs) are functional analogs of insulin and have been identified in many insect species. The insulin/insulin-like growth factor (IGF) pathway is a conserved regulator of metabolism, and in insects, as well as in other animals, it can modulate physiological functions associated with growth, development and metabolism of lipids and carbohydrates. Although the signaling components of the pathway are well conserved throughout evolution, the number of peptides and receptors differ considerably between species. The presence of insulin-like immunoreactivity in neurosecretory cells in the brain of *Rhodnius prolixus* has been previously reported and a link between ILPs and the release of ecdysteroids was suggested.

In the present study, we have identified one ILP and one IGF and investigated their involvement in *R. prolixus* metabolism and growth. We have identified the peptides within the *R. prolixus* genome and have cloned their cDNA sequences. Expression profile analyses showed that the ILP transcript is predominantly present in the brain while the IGF is distributed among a variety of tissues, mostly in the fat body, the dorsal vessel and the central nervous system. Using RNAi, we have knocked-down the expression of both transcripts separately and examined the effects on metabolism and growth. We observed that the absence of the ILP transcript increased the levels of lipids and carbohydrates in the hemolymph, while the lipid content in the fat body was increased. At the same time, the carbohydrate level was decreased in the fat body and the leg muscles, indicating that this peptide is involved in energy homeostasis. The absence of the IGF transcript resulted in defective molting of fifth instars into adults. Compared to the control, insects lacking IGF display abnormal morphological features such as smaller wings and reduced body size. Further experiments are being conducted on the physiological significance and downstream signaling of both peptides.

This work was supported by NSERC.

S17 Large-scale combinatorial deorphanization of GPCRs in the annelid Platynereis

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Neuropeptides, representing the largest class of neuromodulators, commonly signal by G-protein-coupled receptors (GPCRs). While the neuropeptide repertoire of several metazoans has been characterized, many GPCRs are orphans. We developed a strategy to identify GPCR-peptide pairs using combinatorial screening with complex peptide mixtures. We screened 126 neuropeptides against 87 GPCRs of the annelid Platynereis and identified ligands for 19 receptors. We assigned many GPCRs to known families and identified conserved families of achatin, FMRFamide, RGWamide, FLamide, and elevenin receptors. We also identified a ligand for the Platynereis ortholog of vertebrate thyrotropin-releasing hormone (TRH) receptors, revealing the ancient origin of TRH-receptor signaling. We predicted ligands for several metazoan GPCRs and tested predicted achatin receptors. These receptors were specifically activated by an achatin D-peptide, revealing a conserved mode of activation. Our work establishes an important resource and provides information about the complexity of peptidergic signaling in the urbilaterian.

S18 Regulation of insect oogenesis. More than an endocrine interplay.

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Oogenesis is a crucial process in the animal kingdom to ensure the continuity of the species. Thus, it is not surprising that it is finely regulated. During the process of oogenesis, the ovarian follicle gets a series of signals that initiates competence, which will ultimately lead to maturation and oviposition. Insects are good models to study oogenesis as they have developed very different strategies to regulate it, and one of the most basic has been the design of different ovarian types. Two main types can be distinguished among insects: the panoistic and the meroistic. The panoistic is common in phylogenetically basal species, whereas the meroistic type predominates in distal insect groups. To study insect oogenesis we currently use the cockroach *Blattella germanica* as a model, a basal species with panoistic ovaries. The knowledge of this species can allow comparing its oogenesis with that of more modified species, like *Drosophila melanogaster* (with meroistic ovaries). In *B. germanica*, the juvenile hormone is the main reproductive hormone, involved in vitellogenesis control and oocyte maturation. We will review the role of juvenile hormone and its signaling pathway, the function of ecdysone, which is necessary for chorion formation in adult cockroaches, and is also involved in the first steps of oogenesis, and the role of the Insulin pathway, which regulates tissue growth as well as vitellogenesis through the control of juvenile hormone synthesis. Finally, we will comment the involvement of these hormones in the signaling pathways (Hippo, Notch) that typically regulate insect oogenesis, in general.

S19 Pancreatic Islet Hormones in Vertebrates

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The islets of Langerhans are micro endocrine organs intermingled within the exocrine pancreas. In humans and rodents the islets contain 5 distinct cell types, i.e. glucagon producing alpha-cells, insulin-producing beta-cells, somatostatin-producing delta-cells, pancreatic polypeptide-producing PP-cells and ghrelin-cells. Other cell types have been described in certain species, e.g. gastrin cells and EC-cells. In addition, the islets harbor a plethora of more or less well-characterized peptide hormones, e.g. IAPP, PYY, NPY, CGRP and CART. Notably, large species variations with respect to expression pattern of these peptides are at hand. In addition many of the peptides show a dynamic regulation and the expression pattern changes during fetal development, diabetes development, and pregnancy. From a clinical perspective islet peptides are attractive since they to varying extent regulate islet hormone secretion and thus targeting peptides and their receptors can be a therapeutic avenue for diabetes.

S20 Genomic/transcriptomic identification of neuropeptides in echinoderms yields new insights into neuropeptide evolution

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Neuropeptides are evolutionarily ancient mediators of neuronal signalling that regulate a broad range of physiological processes and behaviours in animals. Neuropeptide signalling systems have been particularly well studied in the vertebrates and in protostomian invertebrates including *Drosophila melanogaster* and *Caenorhabditis elegans*. However, recent advances in genomics and transcriptomics have led to an increasingly wide range of phyla becoming accessible for molecular analysis.

The echinoderms (e.g. starfish, sea urchins and sea cucumbers) are a phylum of marine animals, which together with the hemichordates (e.g. acorn worms), form a sister clade to the chordates (e.g. vertebrates, urochordates and cephalochordates). As deuterostomian invertebrates, the echinoderms are of particular interest because they occupy an "intermediate"position in animal phylogeny, bridging the gap between model protostomian invertebrates (e.g. *D. melanogaster* and *C. elegans*) and the vertebrates.

Thus, the generation and analysis of echinoderm genome and transcriptome data has begun to yield important insights into the evolutionarily ancient origins of a number of neuropeptide signalling systems. For example, the identification of a neuropeptide-S (NPS)-type receptor in the sea urchin *Strongylocentrotus purpuratus* and subsequent analysis of gene structure and contiguity has provided the foundation to functionally characterise a bilaterian family of neuropeptides that include NPS-type peptides in tetrapod vertebrates, crustacean cardioactive peptide (CCAP)-type peptides in protostomian invertebrates and NG peptides in deuterostomian invertebrates.

Recently, we have generated and analysed a neural transcriptome dataset from the common European starfish *Asterias rubens* and have identified 40 neuropeptide precursors. This is the most comprehensive identification of neuropeptide precursor proteins in an echinoderm species to date, yielding new insights into the evolution of a number of neuropeptide signalling systems. Interestingly, these include kisspeptin-type and melanin-concentrating hormone (MCH)-type precursors, which are the first to be discovered in a non-chordate species. Moreover, tachykinin-type, somatostatin-type, pigment-dispersing factor (PDF)-type and corticotropin-releasing hormone (CRH)-type precursors are the first to be discovered in the echinoderm/ambulacrarian clade of the animal kingdom.

We are using mass spectrometry (LC-ESI-MS/MS) to determine the structures of the mature neuropeptides derived from these precursor proteins, analysing extracts of *A. rubens* nerve cords. Using this information, we are developing antibodies to the neuropeptides for immunocytochemical analysis of their expression, providing an anatomical framework for *in vitro* and *in vivo* pharmacological analysis of neuropeptide function in starfish.

S21 Mimetic analogs of neuropeptides as rational tools in the development of novel pest arthropod management strategies

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Insect neuropeptides regulate critical processes in insects, though they are unsuitable as tools to the development of pest control agents due to poor biostability and/or bioavailability. Peptidomimetic analogs can overcome these limitations and either over-activate or block critical neuropeptide-regulated functions. Stereochemical/conformational aspects of insect neuropeptides were exploited to design/discover mimetic analogs with enhanced biostability and selectivity. These classes include the insect kinins (IK), tachykinin-related peptides (TRP), and the pyrokinin-like (PK-like) peptide superfamily.

Biostable analogs of the diuretic insect kinins demonstrate antifeedant properties in various insects, including aphids as well as disease vectors such as *Rhodnius prolixus*, which spreads Chagas' disease during the act of taking blood meals that are approximately 10 times its body weight. A biostable insect kinin analog significantly inhibits blood feeding and prevents ecdysis. In aphids, oral delivery of biostable kinin analogs demonstrates antifeedant activity and leads to significant induction of mortality.

Diapause hormone (DH), a PK superfamily member, has been shown to terminate the pupal diapause of heliothines. A potent mimetic prevents the onset of pupal diapause when injected into heliothine larvae, inducing them to commit a form of ecological suicide. Novel amphiphilic analogs can also induce the same behavior following topical delivery to pupae programmed to undergo diapause. Mimetic analogs provide leads for the generation of an agent capable of disrupting diapause in economically important lepidopteran pests.

Insects are such successful survivors due to their great resistance to environmental stress. CAP2b peptides, PK-like superfamily members, regulate diuresis and/or antidiuresis in various insects and are critical mediators of desiccation resistance and cold tolerance. Analogs have been shown to inhibit resistance to desiccation and modulate cold tolerance in flies. Evaluation of a series of mimetic analogs on five expressed insect receptors for PK-like peptides led to the identification of new biostable agonists/antagonists for PK and DH receptors, as well as the first antagonist (selective) of the CAP2b class; providing exciting new tools to endocrinologists.

Selectivity of mimetic analogs is an important issue for development of control agents that target pest arthropods while sparing beneficial species. A specific sequence sub-class of the TRP family has been identified that selectively activates the TRP receptor of the parasitic mite *Varroa destructor* over the analogous receptor in the beneficial host honey bee *Apis mellifera*. This motif can serve as a seed sequence with which to design mimetic agonists/antagonists that specifically target the mite TRP receptor and disrupt the systems they regulate. Biostable TRP analogs show potent aphicidal properties that match or exceed that of some commercially available products.

The use of rationally-designed combinatorial, heterocyclic libraries has been validated as a route to the discovery of non-peptide agonists/antagonists of insect neuropeptides. Non-peptide neuropeptide mimetics may offer benefits of greater stability, enhanced bioavailability, and lower synthetic costs over pseudopeptide mimetics in the development of novel, selective and environmentally friendly arthropod management agents in the future.

S22 What can we expect from insect neuropeptidomics? An update

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Neuropeptidomics is the analysis of neuropeptides within an organism, tissue or even single cell. Due to the large number of neuropeptides in the nervous system, their often cell-specific expression, cell specific processing and modifications, it is often difficult to define the role of neuropeptides in a neuronal or hormonal network. Mass spectrometry is the method of choice if information about mature peptides is needed. The availability of comprehensive genomic or transcriptomic data facilitates peptidomic analyses considerably and makes mass spectrometry even attractive for beginners. Proper sample preparation is, however, still the most crucial step to obtain the necessary data. Here we present an overview of methods that are used for a quick and comprehensive mass spectrometric analysis of nerve tissues or peptidergic neurons in insects. Key applications are discussed in detail.

S23 The involvement of Rhopr-CRF/DH in feeding and reproduction in Rhodnius prolixus

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Feeding and reproduction are interrelated processes in the blood-feeding insect *Rhodnius prolixus*. Nutrition is a determinant of mating motivation, nutrient allocation toward egg production, and oviposition. The messengers behind these interactions are yet to be fully identified and explored. Corticotropin-releasing factor (CRF) is a neuropeptide involved in the stress response in mammals. In *R. prolixus*, a CRF-related peptide (Rhopr-CRF/DH) is a diuretic hormone, released from neurosecretory cells found in the mesothoracic ganglionic mass. Rhopr-CRF/DH is also found in cell bodies throughout the central nervous system, including medial neurosecretory cells in the brain, suggesting it has other potential neurohormonal roles. The G protein-coupled receptor for Rhopr-CRF/DH is present in digestive tissue, including Malpighian tubules, but also in the reproductive system. Thus, Rhopr-CRF/DH may have multifaceted roles in *R. prolixus*, associated with feeding-related events and reproduction. Rhopr-CRF/DH is released at feeding to control diuresis, but as a neurohormone it may also feed-back onto reproductive processes in the female adult. We artificially elevated Rhopr-CRF/DH titres in the haemolymph to study its influence on feeding behavior and reproductive success. Experiments were carried out to determine the effects of injected Rhopr-CRF/DH on satiety and on egg-laying. The preliminary results demonstrate that Rhopr-CRF/DH alters feeding behaviour by inducing premature satiety, and alters reproduction by decreasing egg-laying capacity and interfering with the timing and duration of oviposition.

This work was supported by NSERC.

S24 G-protein coupled receptors as targets for next generation pesticides

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There is a growing need for the discovery and development of new pesticides with novel modes of actions due to the loss of existing products through removal from the market and development of resistance and the desire for products with more favourable environmental and toxicological profiles.

The G-protein coupled receptors (GPCRs), which mediate essential physiological and behavioural responses to hormones and neurotransmitters and environmental stimulants, have great potential as targets for the development of next generation pesticides.

Based on the Insecticide Resistance Action Committees' (IRAC) mode of action classification insect GPCRs are an under resourced target for pesticides. Currently the octopamine receptor agonists are the only class recognized by IRAC that act specifically on a GPCR, and these are limited to use as veterinary drugs for the control of ectoparasites.

The potential of GPCRs as pesticide targets has received increased attention over the last few years and investigations utilising RNA interference or screening for small molecule ligands have identified candidate receptors for future exploitation.

The myosuppressin, corticotropin-releasing factor-like diuretic hormone and/or the PISCF-allatostatin receptors were identified from the genome databases of the spotted winged *Drosophila* (*Drosophila suzukii*) and the pea aphid (*Acyrthosiphon pisum*) and structurally characterised. Functional receptor assays have been developed to screen for potential lead insecticidal compounds.

S25 Molecular mechanisms of Glucocorticoid Receptor action in zebrafish

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Upon a stressful stimulus, vertebrate organisms activate their stress axis and produce glucocorticoid hormones like cortisol. These hormones control the stress response by regulating a wide range of systems, like our metabolism, growth, immune system and behavior. The effects are mediated by the glucocorticoid receptor (GR), which acts as a ligand-activated transcription factor. In our laboratory we use the zebrafish as an in vivo model system to unravel the molecular mechanism of GR action. First, using transcriptome analysis we found two distinct clusters of genes regulated by GR. The first cluster is regulated under basal conditions and contains mainly genes involved in cell cycle control and apoptosis. The second cluster is regulated upon increased activation of GR and consists mainly of genes involved in glucose metabolism and proteolysis. Second, we studied the anti-inflammatory action of GR using tail fin amputation as an inflammation model. Transcriptome analysis revealed the glucocorticoid treatment has a dramatic effect on the amputation-induced gene regulation. Almost the entire transcriptional response was inhibited. Both neutrophils and macrophages migrate towards the wounded site upon amputation, and only the migration of neutrophils is inhibited by glucocorticoids, whereas macrophage migration is unaffected. Further studies showed that the glucocorticoid resistance of macrophages must be due to a transcriptional pathway that was not detected by our (whole body) analysis. Third, we performed a forward-genetic screen using as readout the glucocorticoid-induced decrease in POMC expression in the pituitary gland, which is important for stress axis feedback. As a result of this screen three zebrafish mutants were identified that are resistant to glucocorticoid suppression of the stress axis. Genetic identification of two of the mutants showed mutations in the adenomatous polyposis coli (apc) and the wd repeat domain 82 (wdr82) gene. Both genes have not previously been associated with glucocorticoid feedback of the HPA axis, and we are currently investigating the molecular mechanism behind their involvement.

S26 Anabolic effects of androgens on skeletal muscle in mice and men

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The increase in skeletal muscle mass and strength in response to androgens is well-documented in humans. Therefore, androgens could potentially be exploited in several patient groups suffering from muscle wasting, such as frail elderly, immobilized and chronically ill patients. However, the lack of knowledge regarding the exact mechanism of androgen action in muscle, as well as their many effects on other organ systems, currently limits their use in a routine clinical setting.

To gain insight into muscle androgen action, several mouse models have been developed. In contrast to humans, however, the anabolic effects of androgens are less unambiguous in rodents. For example, the perineal skeletal muscles bulbocavernosus (BC) and levator ani (LA) are highly androgen responsive and depend on androgens for their development and maintenance, whereas the limb skeletal muscles show a lower responsiveness to androgen withdrawal and administration.

Mice with a global knockout of the androgen receptor (ARKO) have helped to determine the effects of androgens on skeletal muscle. The ARKO mice developed by our group display lower lean body mass, complete absence of BC/LA, and reduced limb muscle mass together with a reduction in limb muscle strength. To further decipher the underlying mechanisms, we subsequently developed cell-type specific ARKO models, interrogating the role of AR signaling in satellite cells (satARKO) or myocytes (mARKO). Our results demonstrate that none of these muscle-specific ARKO models fully reproduces the muscle phenotype of the global ARKO, suggesting that the myocyte lineage is not the sole target for androgen action in muscle. For example, in satARKO mice LA mass was lower than in control mice but decreased further upon orchidectomy. In addition, residual AR positive cells co-expressing the fibroblast-lineage marker vimentin were present in satARKO muscle, suggesting that muscle-resident fibroblasts could be involved in the indirect effects of androgens on muscle.

In recent years, concerns about adverse effects of androgens on other organs such as the prostate have stimulated the development of tissue-selective AR agonists, the so-called selective AR modulators (SARMs), which are proposed to have desired anabolic effects in muscle and bone while avoiding e.g. prostate hyperplasia. The processes by which SARMs induce muscle hypertrophy are, however, not fully elucidated. The satARKO model was used to investigate the mechanism of action of SARMs, with a focus on GTx-024, a SARM that was reported to increase muscle mass in elderly men and cancer patient with muscle wasting. In orchidectomized satARKO mice, GTx-024 was still able to restore LA muscle weight, suggesting that GTx-024 action on muscle is, at least in part, indirect via non-muscle AR pathways. Thus, just like androgens, the SARM GTx-024 has a dual mechanism of action in skeletal muscle, with both muscle AR and non-muscle AR pathways contributing to its anabolic effect.

S27 Insights into the role of the thyroid receptors in skin maturation during flatfish metamorphosis

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In flatfish metamorphosis thyroid hormones (THs) promote the change from a symmetric pelagic larva to an asymmetric benthic juvenile with both eyes on the upper, ocular side of the body. Asymmetric pigmentation develops as the skin matures and accompanies the shift in body axis symmetry during metamorphosis. In the present study we tested two hypotheses; i) that skin maturation is TH responsive, and 2) that asymmetry in the pigmentation of the skin results from modified TH responsiveness due to asymmetric receptor distribution. Skin from the future ocular and abocular skin was dissected from Atlantic halibut (Hippoglossus hippoglossus) larval stages 7 to 9A-C (n = 5 per stage) and sequenced on a GS-FLX platform (Roche Life Sciences, USA). After quality filtering approximately 70% of the initial reads from skin (1,200,186) were used for the assembly using MIRA V3. Functional annotation was established using Blast2GO and KEGG. Abundant transcripts included genes involved in pigmentation, melanocyte differentiation and melanosome transport. Quantitative PCR (qPCR) revealed that elements of pigmentation, melanocyte differentiation and melanosome transport developed an asymmetric expression as metamorphosis advanced. In particular, dopachrome tautomerase and tyrosine related protein 1 had a significantly ($p < 0.01$) divergent expression between ocular and abocular skin at the climax of metamorphosis. TH receptors (TRs) had a variable expression in skin during metamorphosis and the relative transcript abundance was TRβ > TRαB > TRαA. The transcript abundance of all the TR isoforms increased significantly ($p < 0.01$) during metamorphosis but TRβ was most significantly modified ($p < 0.05$) between ocular and abocular skin at the climax of metamorphosis and also relative to the other TR isoforms. Deiodinase 3 (the TH inactivating enzyme) was the only deiodinase significantly ($p < 0.05$) changed during metamorphic climax, but no asymmetry at the level of skin was found. Overall, it appears that during metamorphosis asymmetry in some elements of TH signaling orchestrate the divergent phenotype of ocular and abocular skin in flatfish. Acknowledgements: This project was supported by FP7 (LIFECYCLE No. 222719). RNA was in receipt of a PhD fellowship (BD/69209/2010) from the Science Foundation of Portugal. We thank H Smárádóttir from providing the Atlantic halibut samples.

S28 Evolution of transthyretin from uricase to T3 distributor to T4 distributor

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Transthyretin is a homo-tetrameric protein, well-known as a thyroxine (T4) distributor protein in the blood and cerebrospinal fluid of mammals. We (and others) revealed that mammals were the exception, as transthyretins from fish, amphibians, reptiles and birds bind the active form of thyroid hormone (T3) with higher affinity than the pro-hormone T4. Since the amino acids in the binding sites of human and chicken transthyretins are identical, another region of the protein must be responsible for determining ligand preference. The region of transthyretin that changed most during evolution is the N-terminal region. The N-terminal region has changed from longer (in fish, amphibians, reptiles, birds), to intermediate (in marsupials), to shorter (in eutherians). Transthyretins with longer N-terminal regions bind T3 with higher affinity than T4, whereas those with shorter N-terminal regions bind T4 with higher affinity than T3. This hypothesis was confirmed by analysis of chimeric human/crocodile and crocodile/human transthyretins (1). The mechanism for the shortening of the N-terminal regions is a stepwise shift in the intron 1 - exon 2 splice site in the 3' direction. Distributing the pro-hormone T4 could allow a tighter regulation of tissue/cell specificity of activation to T3, which would be particularly important in the eutherian brain that is characterised by the extensive myelination of the corpus callosum. Myelination is regulated by thyroid hormones.

We used the highly conserved vertebrate transthyretin amino acid sequence to search for open reading frames that could code for transthyretin-like proteins (TLPs) in the genomes of non-vertebrates. More than 80 putative TLP genes were identified, with representatives in all kingdoms. Analyses using signal-peptide prediction programs divided TLPs into three groups: cytoplasmic, periplasmic and peroxisomal (2). TLP genes from a plant, a worm and bacteria were shown to be expressed. These genes were cloned and recombinant proteins were shown to be tetramers (similarly to vertebrate transthyretins). However, TLPs did not bind thyroid hormones. The x-ray crystal structure of Salmonella TLP was determined and shown to be superimposable over those of vertebrate transthyretins (3). The only difference in structure is in the binding site: in transthyretins the thyroid hormone binding sites are deep and negatively charged, whereas the equivalent sites in TLPs are shallow and positively charged. We identified TLP as a 5-hydroxyisourate hydrolase involved in the oxidation of uric acid to allantoin. This was confirmed experimentally for Salmonella TLP (3) and has been subsequently confirmed by others for TLPs from many other species. Furthermore, Salmonella required TLP to survive the high uric acid environment of the digestive tracts of hens (4).

Preliminary synteny analyses suggested that duplication of the invertebrate TLP gene gave rise to the vertebrate transthyretin gene, most probably as part of a whole genome duplication event. The change from TLP to transthyretin was probably dependent on two amino acid substitutions in the active site of TLP (5). However, further minor mutations were probably required to optimise T3 binding (6).

1.Prapunpoj et al 2006 FEBS J 273:4013-23 2.Hennebry et al 2006a Prot Struct Funct Bioinf 64:1024-45 3.Hennebry et al 2006b J Mol Biol 359:1389-99 4.Hennebry et al 2012 PLoS One 7(12):e46675 5.Cendron et al 2011 J Mol Biol 409:504-12 6.Richardson (2015) Front Endo 5:245.

S29 From the bush to the bench: the annual fish Nothobranchius furzeri as a model organism for neurobiology of aging

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Neurobiological research in aging vertbrates is hampered by the lifespan of available model organisms. Mice and zebrafish have lifespan of several years and life-long interventions in these species are unpractical. African annual fishes from the genus Nothobranchius are small teleosts that inhabit temporary water bodies subject to annual desiccation due to the alternation of the monsoon seasons. Nothobranchius furzeri, the most popular laboratory species, is the vertebrate species with the shortest lifespan recorded in captivity. In the laboratory, its lifespan is between 4 and 12 months and is coupled to rapid age-dependent functional decline and expression of cellular and molecular changes (such as lipofuscin accumulation, gliosis and stem cell exaustion) comparable to those observed in other vertebrates, including humans. The recent development of transgenesis in this species makes it possible to insert specific constructs into their genome, and the establishment of transgenic lines is facilitated by their very rapid generation time, which can be as short as one month. Using this species we identified novel conserved genes linked to neuronal stem cells and revealed a role for the microRNA miR-29 in the regulation of iron homeostasis during aging.

S30 Biochemical identification of the annelid brain hormone reveals an ancient role of sesquiterpenoids in regulating animal reproduction

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Animals require molecular signals to determine when to divert resources from somatic functions into reproduction. This decision is vital in animals that reproduce in an all-or-nothing mode, such as bristle worms: Females committed to reproduction spend roughly half their body mass for yolk and egg production; following mass spawning, the parents die. An enigmatic brain hormone activity is long known to suppress reproduction. Whereas this brain hormone is commonly assumed to be a neuropeptide, we now identify that its active component is a sesquiterpenoid compound. The identified substance suppresses transcript levels of the yolk precursor Vitellogenin both in cell culture and *in vivo*, directly inhibiting a central energy-costly step of reproductive maturation. We reveal that sesquiterpenoids are ancient animal hormones present in marine and terrestrial lophotrochozoans, and that several agonists of that pathway suppress annelid vitellogenesis in cultured cells. Our findings challenge current views of animal hormone evolution, and indicate that non-target species and marine ecosystems are susceptible to commonly used insect larvicides.

S31 Photoperiod-dependent neurotransmitter switching in the adult brain affecting stress response

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Bipolar disorder is a neuropsychiatric condition associated with altered function of specific neurotransmitters, including serotonin and dopamine. Although it is generally believed that neurotransmitters expressed by differentiated neurons are fixed, our findings demonstrate that photoperiod regulates the number of dopamine- and somatostatin -expressing neurons in the adult mammalian hypothalamus.

We found that altered light exposure affects behaviorally relevant transmitter expression in the mature brain as well as the level of corticotrophin-releasing factor in cerebral spinal fluid.

We identified populations of interneurons in the adult rat hypothalamus that switch between dopamine and somatostatin expression in response to exposure to short- and long-day photoperiods. Changes in postsynaptic dopamine receptor expression matched changes in presynaptic dopamine. Pharmacological blockade or ablation of these dopaminergic neurons led to anxious and depressed behavior, phenocopying performance after exposure to the long-day photoperiod. Induction of newly dopaminergic neurons through exposure to the short-day photoperiod rescued the behavioral consequences of lesions. Natural stimulation of other sensory modalities may cause changes in transmitter expression that regulate different behaviors.

Induction of neurotransmitter plasticity by alterations in ambient illumination raises the possibility of activating other sensory modalities to regulate transmitter expression in specific brain nuclei otherwise accessible only by invasive procedures. Activity-dependent induction of neurotransmitters in circuits in the adult brain could have clinical benefit for treatment of bipolar disorder.

S32 Melatonin and its role in the circadian system

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Melatonin, synthesized at night by the pineal organ, is an endocrine signal in the circadian system. Exogenous melatonin has been shown to entrain circadian rhythms in physiology and behavior. However, the role of endogenous melatonin with regard to circadian and diurnal rhythms is still poorly understood, since mice with deletions in the melatoninergic system (be it melatonin deficiency or the lack of melatonin receptors) do not display any obvious defects in their rhythmic behavior.

To study the role of endogenous melatonin we analyzed locomotor activity rhythms of melatonin-deficient (C57BL) and melatonin-proficient (C3H) mice as well as of melatonin-proficient mice with targeted deletion of the MT1, the MT2 or both receptors under entrained- and free-running conditions as well as after experimental jet lag. We used recently established methods to characterize differences in the timing (chronotype) and stability (accuracy of daily reproduction) of circadian activity.

We found that mouse strains with defects in melatoninergic system reproduce their daily rhythms with less accuracy than those with an intact system. Rhythmic oral application of melatonin in the dark period significantly increased the rhythm stability in C57Bl mice, while application of a MT1,2 receptor antagonist decreased the stability of the rhythms in C3H mice. Their respective chronotypes, however, remained unaltered. After jet lag experiments we could show that in melatonin-deficient mice and in mice lacking functional MT2 receptors, re-entrainment after the phase advance was significantly slower as compared to melatonin-proficient animals with intact MT2 receptors. The endogenous melatonin signal facilitates re-entrainment of the circadian system by acting upon MT2 receptors, whereas for the stability of rhythmic diurnal behavior both melatonin receptors are necessary. Interestingly, the strain specific chronotype was not affected by either application of external melatonin, deletion or antagonistic blockade of the MT-receptors.

Thus, melatonin does affect the rigidity of the phase locks that occur between external and internal rhythmic processes. The true biological, selective role of a 'tight' vs. 'loose' phase lock remains to be established.

S33 Interaction between dopamine D2-like receptors and μ opioid receptor

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For a long time it was widely accepted that G protein-coupled receptors (GPCRs) exist and function as monomers. This paradigm has begun to shift since many studies indicate that GPCRs function either as dimers or higher-structure oligomers. These oligomers can expand the diversity of intracellular responses. Knowledge about oligomer-specific signaling can provide new opportunities in drug discovery.

The dopaminergic and opioid peptide system show an impressive co-distribution in many nuclei of the brain, which suggests intermolecular receptor-receptor interaction. Indeed, evidence from *in vivo* studies indicates that there exists a cross-regulation between the dopamine $D_{2\text{-like}}$ receptors ($D_{2\text{-like}}R$) D_2R and D_4R, and μ opioid receptors (μOR). This interaction is believed to play a role in morphine-induced tolerance and dependence.

In our studies, we first identified heterodimerization of $D_{2\text{-like}}R$ (i.e., D_2R and D_4R) and μOR by co-immunoprecipitation, bioluminescence resonance energy transfer (BRET) assay and proximity ligation assay (PLA). Next, we investigated the functional role of μOR-$D_{2\text{-like}}R$ dimerization. Therefore, we studied ERK phosphorylation and a cross-desensitization was observed in HEK293 cells: other functional assays are ongoing. Then, in striosomes of rat caudate putamen this cross-desensitization was also detected: μOR immunoreactivity, agonist binding density and μOR coupling to G proteins are up-regulated by continuous morphine treatment. In contrast, co-treatment with morphine and the D4R agonist PD168,077 fully counteracts these adaptive changes in μOR.

Now, we are developing new tools to specifically target the heterodimer in vivo. Heterobivalent ligands are particularly useful to study the behavior of a specific GPCR heterodimer (without any receptor modification) and to demonstrate the existence of receptor heteromers in native tissue.

Heterobivalent ligands with a spacer of optimal length are envisaged to exhibit a potency that is greater than that derived from the sum of its two monovalent pharmacophores and may allow selective targeting of certain heteromeric subtypes. The bivalent ligands are used as molecular probes to assess possible therapeutic advantages of modulating heterodimeric receptors instead of the protomers.

S34 Are GPCRs back to insect taste perception? A kinin mimetic elicits aversive behavior in mosquito Aedes aegypti and inhibits the sugar taste neuron

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Insect kinins (leucokinins) are relatively short, multifunctional neuropeptides that act as neurotransmitters and neurohormones. In the mosquito vector *Aedes aegypti* (L.) endogenous aedeskinins are diuretic, as they stimulate fluid secretion from the renal organs and hindgut contractions. We had previously analyzed the recombinant kinin receptor function in CHO-K1 cells and developed specific antibodies against it that localized the receptor in the mosquito excretory system. Now, insect kinin peptidomimetics designed to be protease resistant were tested in sucrose solutions offered as drops to starved females of the mosquito *Ae. aegypti*. One of these kinin analogs, 1728, had proven equally or more potent that the endogenous kinins on tick and mosquito kinin recombinant receptors. Upon contact of the female leg tarsi and proboscis with 1mM analog 1728 in sucrose solution, females rejected the solution and either flew-, walked- or jumped-away, with a median time in contact with the solution of 6 s, significantly shorter than for the control sucrose solution. Capillary feeder assays showed the mosquitoes eat significantly less of diets containing analog 1728 at 600 µM and 1mM. Electrophysiological recordings from long labellar sensilla in the proboscis showed the kinin analog 1728 at 1mM was the most potent in inhibiting the sucrose taste neuron, and inhibition occurred within milliseconds. We determined by immunohistochemistry that the kinin receptor is expressed in contact chemosensory neurons in prothoracic tarsi and in sensory neurons and accessory cells of long labellar sensilla in the distal labellum in the proboscis. Receptor silencing by RNAi eliminated the aversive behavior elicited by analog 1728, implicating the kinin receptor in the aversive response. Our findings point to a modulatory role of sugar taste perception by the kinin signaling system in mosquitoes. Considering that the kinin receptor is a GPCR, and that a kinin peptidomimetic elicited an aversive behavior, more attention should be focused on understanding the role of GPCRs in feeding regulation in mosquito vectors of pathogens. This may lead to the discovery of novel mosquito antifeedants.

H. Kwon, M. A. Agha, R. C. Smithc, R. J. Nachman, F. Marion-Poll, and P. V. Pietrantonio. 2016. Leucokinin mimetic elicits aversive behavior in mosquito *Aedes aegypti* (L.) and inhibits the sugar taste neuron. PNAS doi: 10.1073/pnas.1520404113.

S35 Plasticity of the corticotrope axis reactivity in rainbow trout exposed to stressors.

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The corticotrope axis (hypothalamic-pituitary-interrenal, HPI axis) is part of a suite of adaptive responses when fish is exposed to stressors and develop fast reflexive actions to cope with these challenging situations. Stressors are perceived in the hypothalamus via imputs from nervous system and then transmitted by hormone cascade of the HPI axis to produce cortisol. Such cascade involve not only hormones directly regulating HPI axis activity (CRH, ACTH) but also negative feed-back regulation involving corticosteroid receptors located in the HPI axis. Thus, recent studies demonstrated such regulation involves not only glucocorticoid receptors but also mineralocorticoid receptors. Modulation of the HPI axis reactivity by external (ex. environmental) or internal (ex. genetic) factors may indicate interference with key regulatory mechanisms at one or several levels of this corticotrope axis. It is therefore important to establish which are these key regulatory mechanisms which underlie plasticity of the HPI axis reactivity when fish are exposed to acute stressors as such plasticity has functional implications for animal health. These issues have been studied in rainbow trout exposed to different kinds of stressors or to the same stressors but with different genetic backgrounds. Reactivity of the HPI axis was assessed within by measuring increase of plasma cortisol levels after a standardized acute confinement stress. These results illustrate the large plasticity of the HPI axis reactivity which probably involves environmental (life history) and genetic interactions. Moreover, we have also analyzed in these different experiments expression of the genes encoding for the major regulators of the HPI axis at the level of the brain, pituitary and interrenal gland. Whether these genes would be involved in HPI axis plasticity will also be discussed.

S36 Glutamate receptor regulation of tectal CRF release

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The stress related neuropeptide corticotropin-releasing factor (CRF) modulates visually guided prey capture but its precise role within the circuitry of the optic tectum (OT) is not known. The elemental circuit of the OT consists of retinotectal fibers releasing glutamate in tectal layer 9 and activating pyramidal cells in layer 6 whose axons travel to the brainstem to engage prey capture. This signal from retinotectal fibers is amplified by pear shaped cells in layer 6 whose axons ascend to layer 9. CRF is produced by cells in layers 6 and 8 of the OT but whether glutamate activates or inhibits these tectal CRF neurons is not known. We examined glutamate receptor regulation of CRF release from tectal explants of the South African Clawed frog (*Xenopus laevis*). Juvenile frogs were anesthetized in MS222 and tectal explants incubated in Earle's Basic Salt Solution with 95% O2/5% CO2 gassing containing a basal (5 mm) concentration of K^+ followed by a depolarizing (60 mM) concentration of K^+. CRF released into the incubation was measured by homologous radioimmunoassay. Glutamate inhibited depolarization- induced CRF release from *X. laevis* tectal explants in vitro at a concentration of 10^{-4} M. Agonists for kainate and AMPA ionotropic receptors had no effect on basal or depolarization-induced CRF release. Removal of Mg^{2+} from the incubation medium had no effect on glutamate inhibition of evoked CRF release. Interestingly, NMDA, an agonist for the Mg^{2+} sensitive ionotropic receptors, dramatically stimulated both basal and depolarization-evoked CRF release. We so far conclude that glutaminergic regulation of tectal CRF release is complex and involves stimulatory control by NMDA receptors and inhibitory control by an as of yet unidentified metabotropic glutamate receptor, possibly type II or II metabotropic receptors. These data also suggest a more complex role for CRF than previously thought, as NMDA receptors play an integral excitatory role at the retinotectal synapse. Studies on glutamate metabotropic receptor regulation of tectal CRF release are ongoing.

ORAL PRESENTATIONS

O1 FoxO directs the timing of larval-pupal metamorphosis through ecdysteroid signaling in the red flour beetle, Tribolium castaneum

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Steroid hormone 20-hydroxyecdysone (20E), the major developmental hormone in insect, controls all the developmental transitions including ecdysis and metamorphosis. In our study, silencing of Forkhead Box class O (FoxO) delayed the metamorphosis from larva to pupa in the red flour beetle, *Tribolium castaneum*. And the FoxO-silenced pupae turned black and died in the middle pupal stage. Moreover, knockdown of FoxO delayed ecdysone pulse, down-regulated the expression of several Halloween genes that are involved in ecdysone biosynthesis and delayed the expression of the ecdysone response gene, *hormone receptor 3* (*HR3*). Further, applying 20-hydroxyecdysone (20E) to *T. castaneum* after treated with dsRNA against FoxO partially eliminated the delayed developmental timing. Thus, FoxO directs the timing of larval-pupal metamorphosis via regulating ecdysone biosynthesis.

O2 The neuroendocrine consequences of being male and female at the same time

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While ultimately the outcome of successful reproduction - fertilisation of eggs and the production of surviving offspring - is relevant for how these processes evolve, a thorough understanding of the underlying, proximate mechanism is essential if one want to interpret evolutionary outcomes properly. Comparing neuroendocrine processes across different species, with different sexual systems, is one way of uncovering similarities and differences in how they have evolved to regulate their reproductive processes. I will present some experimental work on hermaphroditic snails that will illustrate that it is relevant to consider the mode of sexual system when addressing the neurobiology of reproduction. I will show that, on the one hand, hermaphroditic animals regulate their male and female reproduction via largely non-overlapping neurobiological wiring and neuroendocrine substances, which is not necessarily the case in separate sexed species. On the other hand, because both regulatory systems are present in each individual, this also offers opportunities for “hijacking”their partner’s reproductive system in such a way that their own reproductive success is enhanced.

O3 Effect of Plag1 deficiency on gene expression in the hypothalamo-pituitary-gonadal axis in male mice

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Pleomorphic adenoma gene 1 (PLAG1) is a zinc finger transcription factor which is thought to play a role in embryonic growth. In addition, *Plag1*-deficient mice have reduced fertility of unknown cause. In order to determine at which level of the hypothalamo-pituitary-gonadal axis PLAG1 exerts its effects on fertility, this study aimed to investigate the expression of PLAG1 and to identify the genes affected by PLAG1 deficiency in each of these three tissues in mouse. Using X-gal staining, *Plag1* expression was detected in adult murine testes, pituitary gland and hypothalamus. Signal was detected in multiple cell types in the seminiferous tubules of the testes, but the presence of PLAG1 in the Leydig cells could not be confirmed due to endogenous galactosidase activity. *Plag1* was also expressed in multiple cell types throughout the anterior pituitary gland, and in multiple neurons of the hypothalamus, including the gonadotropin-releasing hormone (GnRH) neurons. Transcriptome analysis of all three tissues was conducted using RNA sequencing, and gene expression was compared between adult male *Plag1* knock-out (KO; *Plag1-/-*), heterozygous (*Plag1+/-*) and wild-type (WT; *Plag1+/+*) mice (*n*=3 per genotype). A total of 245 genes were upregulated and 132 genes were downregulated in KO testes compared to WT. Genes involved in spermatogenesis and androgen synthesis were typically downregulated, while some involved in immunity were mostly upregulated in the KO testes. In the KO pituitary gland, a total of 714 genes were upregulated and 558 genes were downregulated. Most notably, we observed an overall upregulation of cell proliferation genes. Finally, in the KO hypothalamus, a total of 82 genes were upregulated and 178 genes were downregulated potentially affecting a number of processes, including an upregulation of steroid biosynthesis. The mRNA expression levels of the pituitary gonadotropins, hypothalamic GnRH and their receptors, however, were unaffected in the KO tissues, indicating that the effect of *Plag1* deficiency may not directly impact the HPG axis at these levels. It is likely that that most of the fertility issues seen in male *Plag1* KO mice are caused by effects on spermatogenesis, androgen synthesis and/or immunity in the testes.

O4 Sexual motivation is driven through kisspeptin neuron activity

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In female rodent species, sexual behavior is exquisitely orchestrated by sex steroids in order to coincide with ovulation and thus to ensure the highest possible chance of fertilization. At present, the underlying neural network coordinating this synchronization is still poorly understood. In the present study, we focused in particularly on kisspeptin neurons because of their well-known role in stimulating GnRH secretion and thus reproductive physiology. We found that kisspeptin neurons in the anteroventral periventricular area (AVPV) were specifically activated upon mating in female mice. Furthermore, we observed that sexual behavior was impaired by kisspeptin gene knockout (Kiss-KO) or acute ablation of kisspeptin neurons in the AVPV, but restored by a single injection with kisspeptin. Optogenetic stimulation of AVPV kisspeptin neurons also reliably triggered sexual behavior in female mice, further confirming that kisspeptin neurons are part of the neural network regulating female sexual behavior. Since AVPV kisspeptin neurons can also be activated by male odors, these neurons could actually be part of a neural network governing sexual motivation. Indeed, Kiss-KO females failed to show a mate preference for the male, which could however, be induced by a single injection with kisspeptin. Finally, we determined whether AVPV kisspeptin neurons are exclusively activated by male odors or by other sensory modalities received during mating. Removal of the vomeronasal organ, but not ablation of the main olfactory epithelium, completely eliminated the ability of male odors to activate AVPV kisspeptin neurons. Importantly VNO removal also profoundly affected sexual behavior indicating that female sexual behavior depends entirely on a functional VNO projection pathway. These results thus confirm that kisspeptin neurons are part of a motivational neural pathway that is triggered by male olfactory cues, detected and processed by both the main and accessory olfactory system, ultimately leading to the female adapting the lordosis posture necessary for the male to fertilize the female. Since AVPV kisspeptin neurons also control ovulation via the activation of GnRH neurons, our data establish these cells as a central hub in the neural network governing the orchestration of sexual behavior with ovulation in female mice.

O5 Permanent deiodinase type 2 deficiency alters local thyroid hormone levels, disturbs development and strongly reduces fertility

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Introduction: Vertebrate development and reproduction are both thyroid hormone (TH) dependent processes and TH bioavailability within each tissue is tightly controlled by deiodinating enzymes. To decipher the function of deiodinase type 2 (Dio2), the main TH-activating enzyme in zebrafish, we generated two mutant lines.

Methods: KO was accomplished by zinc finger nuclease technology and *dio2* gene disruption was verified by enzyme activity tests. Phenotypical analysis of Dio2KO zebrafish was carried out in embryos and larvae, using morphometric analysis, scoring of swim bladder volume, locomotor assay and immunohistochemistry, as well as during adulthood, by means of quantitative PCR, histology and clutch size counting.

Results: We have generated two homozygous Dio2KO zebrafish lines. The first line is characterized by an in-frame deletion of 9 base pairs, resulting in the elimination of 3 conserved amino acids. The other line possesses an insertion of 4 base pairs, leading to the introduction of a premature stop codon. Both mutations permanently and specifically abolish Dio2 activity and the resulting mutant phenotypes are equally severe. Levels of active TH are strongly reduced in all tissues tested and downregulation of the inactivating enzyme Dio3 and TH receptor transcript levels points towards a severe hypothyroid status. Measurements within the first 7 days post fertilization (dpf) show a disturbed overall early development of Dio2KO zebrafish: body length, absolute and relative eye and ear size are all significantly reduced. Swim bladder inflation is delayed in Dio2KO larvae, which correlates to an impaired locomotor behaviour. Also vision is affected in Dio2KO larvae as tested by light response and confirmed by a reduction in retinal photoreceptor number during early-larval stages. Linear growth of Dio2KO fish is obviously retarded starting from early-larval stages until adulthood (4 months). By the age of 7 months, male Dio2KO fish are still significantly smaller than wild-type fish. At this stage, the weight of female Dio2KO fish is however significantly increased. This weight gain may point to fertility problems, since Dio2KO zebrafish have a very limited time window of reproductive activity. They become sexually mature at a later stage than wild-type fish, i.e. around 5 months compared to 3 months, and also the duration of their reproductive period is shortened to 2 months instead of 1.5 years. Dio2KO females not only have problems with egg deposition, resulting in strongly reduced clutch sizes, but histological analysis of ovaries reveals a time shift in oocyte production. At 4 months, Dio2KO fish possess significantly more primary oocytes than wild-type fish and the number of mature oocytes is significantly reduced. The fact that fertilization percentages are low suggest that also Dio2KO males may have fertility defects.

Conclusion: Our Dio2KO zebrafish represent the first non-mammalian model with permanent deiodinase deficiency. Our results demonstrate that Dio2, and hence active TH, is essential at early life stages, to assure normal development, motility and vision, but also during adulthood for crucial processes such as reproduction.

O6 Neurohormones that regulate molluscan and echinoderm reproduction

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The molluscan and echinoderm groups contain species that have both positive (eg. aquaculture) and negative (eg. pests) impacts on humans. Methods that target reproduction capacity holds potential for manipulating their populations. We have utilised whole genomes, transcriptomes and secretomes to build a broad picture of the type and distribution of neurohormones across these groups, from gastropod, bivalve and cephalopod for mollusc, and asteroid and holothuroid for echinoderm. Subsequent functional bioassays have defined the identity of some that regulate reproduction processes, including gonad maturation, spawning and mating. Those hormones include the egg-laying hormone, buccalin and the APGWamide. We show that: 1) in abalone, the egg laying hormone can induce broadcast spawning, 2) in oysters, gonad maturation can be advanced through adminisration of buccalin, and 3) in helminth-infected freshwater molluscs *Biomphalaria*, there is a significant decrease in reproductive neurohormone abundance. Our comparative analysis of radial nerves in echinoderms between reproductive and non-reproductive stages provides us a list of candidate neurohormones that regulate their reproduction. One sea cucumber neurohormone displays features which indicate that it may have been co-opted for a pheromone role. Our findings have greatly enhanced our understanding of reproductive neurohormone function in molluscs and echinoderms.

O7 Functional and pharmacological characterization of the ecdysis triggering hormone receptor in the desert locust, Schistocerca gregaria

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One of the main characteristics that defines insects is the exoskeleton, a rigid armour protecting the animal's body. Periodic production of a new exoskeleton and shedding of the old one are necessary to allow growth. This process, called moulting, is tightly regulated by the ecdysteroid hormone family. A pulse in ecdysteroid titre will trigger the onset of moulting and activate a peptide-mediated signalling cascade that regulates the actual ecdysis sequence at the end of the moult. In Holometabola, it is known that the ecdysis triggering hormone (ETH) is a key regulator in this cascade. However, in Hemimetabola very little is known about the cascade initiating ecdysis. Therefore, our research focuses on ETH and its receptor (ETHR) in the desert locust, *Schistocerca gregaria. S. gregaria* is a voracious, swarming pest that can ruin crops and harvests in some of the world's poorest countries. Knowledge of the peptide-mediated signalling cascade regulating ecdysis, may be of great interest for the development of more target-specific insecticides combating this harmful pest insect.

We pharmacologically characterized an ETHR from *S. gregaria*. To investigate the downstream signalling pathway, the receptor was expressed in CHO-PAM28 and HEK293 cells. An increase in Ca^{2+} concentration could be observed in CHO-PAM28 cells after application of ETH peptides. In HEK293 cells, expressing *SgETHR*, addition of ETH peptides increased cyclic AMP levels. The data suggest that this locust ETH receptor displays dual coupling properties to G_s or G_q like proteins. Additionally, we investigated the role of *SgETHR* and *SgETH* in ecdysis, using the RNA interference technique (RNAi). RNAi-mediated silencing of *SgETHR* and *SgETH* in the fourth and fifth nymphal stage resulted in high mortality rates at the expected time of ecdysis, when compared to control animals.

O8 Species specificity of relaxin-like gonad-stimulating peptides in starfish

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Relaxin-like gonad-stimulating peptide (RGP) of starfish *Patiria* (= *Asterina*) *pectinifera* is the first identified invertebrate gonadotropin to trigger final gamete maturation. Recently, chemical structures of RGP were identified in several species of starfish. Three kinds of RGP molecules are found in the class Asteroidea. *P. pectinifera* RGP (PpeRGP) is a heterodimeric peptide with a molecular weight of 4,740, comprising an A-chain of 24 amino acids (aa) and a B-chain of 19 aa, with disulfide cross-linkages of one intra-chain and two inter-chain disulfide bonds. The chemical structure of PpeRGP is conserved among starfish of the order Valvatida beyond species. In contrast, the chemical structures of RGP identified in *Asterias amurensis* and *Aphelasterias japonica* of the order Forcipulatida are quite different from that of PpeRGP, although the cysteine motifs of *A. amurensis* RGP (AamRGP) and *A. japonica* RGP (AjaRGP) coincide exactly with that of PpeRGP. Molecular weights of AamRGP and AjaRGP are 5,156 and 5,117, respectively. The coding DNA sequence (CDS) of AamRGP consists of 330 bp with an open reading frame (ORF) encoding a peptide of 109 aa, including a signal peptide (26 aa), B-chain (20 aa), C-peptide (38 aa) and A-chain (25 aa). The AjaRGP CDS is composed of 342 bp with an ORF encoding a peptide of 113 aa, a signal peptide (26 aa), B-chain (20 aa), C-peptide (42 aa) and A-chain (25 aa). The amino acid identity levels between AamRGP and AjaRGP are 84% for the A-chain and 90% for the B-chain. In contrast, the amino acid sequences of AamRGP and AjaRGP are not quite the same as that of PpeRGP, with homology levels of 58% and 58% for the A-chain, 73% and 68% for the B-chain, respectively. The chemical structure of RGP in AamRGP is exactly the same as that in *Astropecten scoparius* (the order Paxillosida), *Astropecten polyacanthus* (the order Paxillosida), and *Echinaster luzonicus* (the order Spinulosida). The chemical structure of *Coscinasterias acutispina* RGP (the order Forcipulatida) is consistent with that of AjaRGP. In cross-experiments using *P. pectinifera*, *A. amurensis*, and *A. japonica* ovaries, AamRGP and AjaRGP can induce each species of ovaries. Neither AamRGP nor AjaRGP induce oocyte maturation and ovulation in the ovary of *P. pectinifera*, although the PpeRGP is active in ovaries of *A. amurensis* and *A. japonica*. This suggests that the AamRGP and AjaRGP partly act species specificity.

O9 The role of SGPP-5 in the reproductive cycle of the desert locust, Schistocerca gregaria.

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In 1987, the first member of a new family of serine protease inhibitors was discovered in the crayfish *Pacifastacus leniusculus*. These pacifastins like peptides belong to a family of Arthropoda-specific inhibitors, of which the endogenous functions haven't been fully detected so far. Nevertheless, there are indications that these peptides may play a role in several regulatory pathways. In the desert locust, *Schistocerca gregaria*, there is evidence that SGPP-5, the fifth precursor of the pacifastin like protease inhibitors, is involved in the regulation of adult reproductive physiology.

Analysis of the reproductive organs as well as the investigation of copulation and post-copulation effects after an RNAi-mediated SGPP-5 knockdown revealed some remarkable effects. SGPP-5 knockdown led to an increased oocyte size in RNAi-treated females compared to the control group. In experimental males, injection of the dsRNA caused a higher gonadosomatic index. While there was no difference in clutch or egg size after egg deposition, we observed a higher percentage of hatchlings in the control group in comparison to the RNAi-treated animals. Also the cuticular coloration appeared to differ. Unlike the control group, in which most of the hatched nymphs were black, the first instar larvae of RNAi-treated females appeared to be green. Also, the morphology of eggs deposited in the experimental group was different from controls. The observed effects point at the existence of a regulatory link between this peptide precursor and the reproductive cycle in adult *S. gregaria*.

O10 Nervous control of reproduction in Octopus in the time of Genomics

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Cephalopods genomics has been promoted after 2011 and efforts achieved during these years provided the first cephalopod genome ever sequenced (i.e. *Octopus bimaculoides,* Albertin et al., 2015).

Many questions concerning the study of the reproductive physiology of cephalopods remain without answers (Di Cristo, 2013). In these animals some systems have provided spectacular models for solving general problems; however serious technical difficulties continue to hinder advance in our understanding of the undoubtedly complex control of reproduction in these “advanced invertebrates”. New genomic and transcriptomic approaches have opened new windows to understanding some key steps of neurohormonal control of reproduction in these animals. Among these, looking for a putative optic gland hormone, the possible existence of an egg laying hormone, the hormonal role of the gonads, the role of olfaction and chemotactile stimuli and sex recognition are just some of the issues that urgently require investigation.

Albertin CB, et al. 2015. The octopus genome and the evolution of cephalopod neural and morphological novelties. Nature, 524(7564):220-4. Di Cristo C. 2013. Nervous control of reproduction in Octopus vulgaris: a new model. Invert Neurosci. 13(1):27-34.

O11 Effects of androgens on osmoregulatory mechanisms in Atlantic salmon (Salmo salar L.)

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The anadromous lifecycle of salmonids entails migratory transitions between freshwater (FW) and seawater (SW) habitats. Such migrations require a remodeling of key ion transporting mechanisms in osmoregulatory organs. Here we will present molecular and physiological changes in the gill and intestine in sexually maturing Atlantic salmon adults and post-smolts. We further address the effects of androgens on hyper-osmoregulatory mechanisms by *in vivo* administering testosterone, T (75 µg/g body weight), 11-ketoandrostenedione, OA, (25 µg/g), or T+OA (T; 75 µg/g +OA 25 µg/g) to immature, seawater-acclimated post-smolts. Androgen-treated post-smolts displayed several-fold increases in gill *nka-a1a*, *cftr-II* and *h-atpase* β subunit mRNA levels, all of which are transcripts typically up-regulated in freshwater gill epithelia. Conversely, the expression of typical seawater transporter genes, such as gill *nka-a1b, nkcc1a* and *cftr-I,* as well as the number of Nka-a1b positive ionocytes (ICs), and Nka-a1b protein levels were not affected by androgen treatment. Androgen-treated post-smolts displayed 35-65 fold increases in the abundance of gill Nka-*a1a* positive ICs, which were located mostly on the primary filaments. The presence of gill androgen receptors (*ara*1 and *ara*2) and up-regulation of *ara*2 transcripts in gills of T and T+OA treated post-smolts may allow for direct androgen action on gill epithelia. Maturing salmon displayed loss of intestinal barrier function, observed as a reduction in trans-epithelial resistance (TER) and structural lesions of intestinal epithelia viewed by histological analysis. Mature fish compensated by increasing the active pumping mechanisms, as observed by increased short circuit current (SCC). Our findings show that maturing salmon in seawater shifts towards freshwater characteristics in gill and intestinal epithelia and experiences minimal osmoregulatory perturbations.

O12 Convergent signaling of neurohypophysial hormones on epithelial ion regulation in cephalopods

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Cephalopods were proved to control pH homeostasis in extracellular body fluids via efficient acid-trapping mechanism in the unstirred boundary layer of the epithelium surface; however, its functional basis of how the pH modulation links to neuronal hormone signaling is not understood so far. In this study, we utilized embryos of cephalopod cuttlefish (*Sepia pharaonis*) to examine expressions of neurohypophysial hormones (pro-sepiatocin and sepiatpcin) and respective receptor (sepiatocin receptor, *str*) under CO_2-induced acidic perturbation. RNA *in situ* hybridization images showed that, on one hand, pro-sepiatocin and sepiatocin were both expressed in neurons of optic lobe region. On the other hands, *str* was observed in mental epithelium, the dominant sites for maintaining intact homeostasis during larval stages. Moreover, pro-sepiatocin and *str* were up-regulated accompanied with those acid-base regulation genes in epithelium (e.g. *vha*, *nbc*, *nhe3*, *rhp* and *nka*) under CO_2-acidified condition. The present work inferred that the convergent evolved features of neurohypophysial hormone signaling could regulate intact homeostasis in molluscan cephalopods during their oviparous development as well.

O13 Mapping water transport in insect renal systems.

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Fluid homeostasis is a prerequisite for organismal survival; in the majority of insects this process is directed by specialized set(s) of columnar organs known as the Malpighian (renal) tubules (MTs). In *Drosophila*, osmoregulation is driven by (trans-cellular) ion transport across the epithelium, orchestrated by the co-ordinated activities of principal (PC) and stellate (SC) cell populations. PCs transport K^+, powered by a v-type H^+ ATPase, establishing a favourable electrochemical gradient for Cl^- influx. This Cl^- influx in turn generates an osmotic pressure for water to 'follow' through. Cl^- and aquaporin (water-specific) channels have been identified in the SCs as opposed to PCs. The process of Cl^- (and subsequent water) influx, is modulated in response to organismal fluid volumes and environmental physiological factors via neuro-endocrinal signals, specifically Dromekinin and the biogenic amine Tyramine. However, neither this two-cell model and/or specificity of diuretic neuropeptides is conserved throughout insecta. Using high molecular weight fluorescently conjugated dextrans we have been able to map the flux of water through disparate cell types in a variety of insects. In keeping with previous findings delineating specific neuropeptide responsive cells within differing insect species, we have been able to manipulate secretion levels in these insect renal systems using specific neuropeptides. Describing the variant water-transport systems in specific insects, and the diuretic neuropeptides that may modulate them, can provide us with greater insight into how we might then restrictively perturb the neurophysiology of pest species.

O14 Unraveling of the corazonin circuit identifies novel roles for this signaling system in Drosophila

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Various environmental factors can challenge the homeostasis in animals, thus evoking stress responses. A multitude of physiological and behavioral mechanisms have evolved to counter stress and at the organism level these involve several hormones and neuropeptides. Corazonin (CRZ) is one such neuropeptide that has been proposed to be involved in stress responses in *Drosophila* and other insects. However, the neural circuit underlying this stress signaling is unknown. In this study, we map the CRZ receptor (CRZ-R) expression in the nervous system using various Gal4 lines. The CRZ-R is expressed by several neurons in the *Drosophila* central nervous system (CNS). In particular, CRZ-R is expressed in the median neurosecretory cells in the brain, hugin/pyrokinin producing cells in the suboesophageal ganglion (SOG) and CAPA neurons in abdominal neuromeres (Va neurons) of adult flies. CAPA peptide signaling has recently been implicated in mediating recovery from desiccation and cold stress and hugin/pyrokinin cells in the SOG regulate food intake. Here we explore the effects of knocking-down CRZ in CRZ-producing neurons (DLPs) and CRZ-R in various subpopulations of neurons on feeding, metabolism and various stresses. We also corroborate our results using CRZ-R null mutants. Our results delineate a neural circuit that coordinates various stress associated behaviors in *Drosophila.*

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O15 Neurohormonal control of diuresis: discoveries from the blood-gorging bug, Rhodnius prolixus

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Rhodnius prolixus, the kissing bug, is an obligatory blood-feeding insect and one of the vectors for the parasite *Trypanosoma cruzi* that causes Chagas disease in humans. Interest in *R. prolixus* as a model insect has been reinvigorated by the sequencing of its genome. *Rhodnius prolixus* takes a huge blood meal, which is followed by a rapid post-feeding diuresis. Here we examine the neurohormones controlling this diuresis.

Serotonin is a diuretic hormone (DH) in *Rhodnius* and works in concert with a member of the CRF-related family of insect neuropeptides, via GPCRs. Serotonin and the *R. prolixus* CRF-related DH (Rhopr-CRF/DH) have potent biological activity on anterior midgut and Malpighian tubules, and work synergistically during rapid post-feeding diuresis in *R. prolixus*.

An anti-diuretic peptide (Rhopr-CAPA-2) and its cognate GPCR are important mediators in the cessation of rapid post-feeding diuresis in *R. prolixus*, inhibiting serotonin-stimulated secretion by Malpighian tubules, but not Rhopr-CRF/DH-stimulated secretion. Rhopr-CAPA-2 appears to prevent the synergism between the diuretic hormones serotonin and Rhopr-CRF/DH.

The interplay between diuretic and antidiuretic hormones in *R. prolixus* results in a remarkable diuresis; a diuresis that has evolved to eliminate excess water and salts from a massive blood meal in a very rapid way. The parasite, *T. cruzi*, is transmitted via the excreted fluid during this diuresis, and therefore these DHs control the transmission of Chagas disease.

This work was supported by NSERC

O16 Expression and functional analysis of neuropeptides in ticks

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Ticks are blood-feeding ectoparasites belonging to a group of invertebrate vectors that transmit numerous pathogens (encephalitis virus, *Rickettsia*, *Borrelia*, *Anaplasma*, *Babesia*, etc.). During feeding of the tick these dangerous pathogens are transmitted from the salivary glands or gut into the vertebrate hosts including humans and cause serious diseases. To determine regulatory pathways involved in transmission of these pathogens, we used various techniques to identify neuropeptides and their receptors expressed in peptidergic neurons and endocrine cells in the in the brain (synganglion), salivary glands, gut and reproductive organs of ticks. We found that several neuropeptides are expressed in central neurons innervating specific secretory cells in the salivary glands, gut, accessory glands and ovaries. Additional regulatory peptides are produced by various types of endocrine cells in the midgut. We observed dramatic changes in expression and release of neuropeptides from these cells during development. RNAi approaches showed that specific neuropeptides control vital functions during feeding, digestion and reproduction of ticks. These data indicate that the tick nervous and endocrine systems produce diverse neuropeptides that control important physiological functions associated with transmission of pathogens.

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Šimo L, Sonenshine DE, Park Y, Žitnan D (2014) Nervous and sensory systems: structure, function, genomics and proteomics. In: Sonenshine DE, Roe RM (eds) Biology of ticks. Oxford Univ Press, pp. 309–367.

O17 Unravelling mouse behaviour upon optogenetic activation of the superior colliculus

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From a long history of pharmacological and electrical stimulation experiments, it is known that the midbrain superior colliculus (SC) mediates a wide behavioural spectrum from orientation to defensive movements, such as escape and freezing.

Here, we set out to investigate whether optogenetic stimulation of the SC could reproduce some of these known behaviours in mice. Activation of the fast spiking Channelrhodopsin (ChR)2 mutant (L132C/T159C), transduced in the right SC, caused a repeatable counter-clockwise turning when 100 ms 473 nm light pulses were used. This movement contralateral to the stimulated side corresponds to an orienting movement, since it represents a movement towards a point in the left visual field. By increasing the pulse duration and light power, defensive behaviours could be elicited, ranging from freezing to jumps and even panic-like running at the highest light power. This corroborates data from pharmacological and electrical stimulation, indicating that the behavioural response mediated by the SC is a continuum ranging from orientation to more defensive reactions for weak to stronger stimuli, respectively.

To further increase the stimulus strength and duration, we used Stable Step-Function Opsin (SSFO), a slow ChR2 mutant (C128S/D156A), which upon blue light stimulation opens its cation channel for up to 30 minutes. This allowed prolonged stimulation of the right SC with a single light pulse, reducing the risk of brain heating and tissue damage due to extended laser exposure. Just after light stimulation, animals started running in a wild and undirected manner for 10 - 20 seconds. Then, after quieting down, the mice began turning counter-clockwise, and these specific turns were interspaced with short periods of immobility. Over time, prominence of these latter behaviours decreased and by 30 minutes after the light pulse, animals behaved like before the optogenetic stimulation. Thus, SSFO-mediated collicular stimulation induced the entire spectrum of SC-mediated behaviour, and each behaviour had a specific time window and duration. Notably, the escape response was short, despite the prolonged nature of the stimulation. This could indicate differential response properties of the neuronal pathways mediating escape and orientation.

In summary, these data indicate that optogenetics is a reliable tool to investigate the SC, with results similar to electrical and pharmacological stimulation. Future research will use specific advantages of optogenetics, such as cell-type specific expression, to elucidate the role of distinct neuronal populations in collicular behaviours.

O18 Behavioral phenotyping of neuropeptidergic mutants in C. elegans experience-dependent salt chemotaxis

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Behavioral responses to stimuli are not always hardwired in the nervous system. Behavior is highly flexible and adaptive, and the brain's ability to learn and remember from experience allows making predictions on future events and adjusting choices appropriately. Neuropeptides and their receptors are important regulators of cognitive processes as they are able to directly modulate neuronal activity on large spatial and temporal scales. Although mounting evidence points towards an instrumental role of neuropeptide-mediated neuromodulation in behavioral plasticity, their modulatory mechanisms in the underlying neural circuits remain poorly understood. Several adaptive behaviors are manifested in *Caenorhabditis elegans* nematodes including experience-driven modulation of salt chemotaxis, a type of associative learning in which normal chemotaxis towards salt is modulated by pre-exposure to this substance in the absence of food. We have optimized a high resolution video-tracking platform of *C. elegans* navigating well-defined soluble compound gradients that enables us to quantitatively evaluate salt learning behaviors. Several navigational mechanisms are known to contribute to *C. elegans* locomotion during chemotaxis depending on the (attractive) substance. When a worm navigates gradients in its environment, it modulates the ongoing pattern of runs, reversals, and sharp turns in order to near preferred conditions. The analysis of behavioral responses such as turn rates and run lengths of naive and conditioned worms in time can offer a quantitative and dynamic insight into the magnitude and lifetime of conditioned behavior as well as its molecular regulation. It is therefore an important tool for the phenotyping of experience-dependent chemotaxis behavior of diverse mutants impaired in neuropeptidergic signaling, the underlying pathways and neural circuits of which are currently being further investigated.

O19 Orexigenic response of Neuropeptide Y to varying nutritional status in the tadpole brain of frog Euphlyctis cyanophlyctis.

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Orexigenic role of NPY has been well established in the food and feeding control of mammals. Inspite of this nothing is known about its feeding circuit in anuran brains. However, there is lot of information about the role of NPY in stimulating feeding in juvenile and adult amphibians, but the involvement of NPY in the frog brain has not been studied till date. Therefore in the present study, an attempt has been made to study the role of NPY peptide in frog *Euphlyctis cyanophlyctis* in response to varying nutritional conditions. Intracranial administration of 2-Deoxy-D-glucose (2DG; 16ppm/g body weight, a metabolic antagonist of Glucose) to premetamorphic tadpoles (Stage 38) there was a significant increase in the NPY immunoreactive cells in olfactory bulb suggesting its plausible role in the processing of sensory and appetite related information to the brain. NPY-like immunoreactive cells and fibres were significantly higher in the hypothalamic regions, in the nucleus preopticus and raphe nucleus in frog fasted for 5 days, than those frogs that had been fed normally. An extensive increase of NPY immunoreactive cells and fibres was also observed in hypothalamus and pituitary of tadpoles injected with 2DG as compare to glucose injected group (16 ppm/g body weight). Our results suggest that NPY is involved in controlling satiety center in the tadpole brain of frog *Euphlyctis cyanophlyctis.*

Tuinhof, et al., 1994. J Chem Neuroanat 7:271–283. D'Aniello, et al., 1996. Cell Tissue Res; 9 285:253. Cresp, et al., 2004. J. Neuroendocrinology. 16 279–288. Shimizu, et al., 2013. Peptides 46 102–107. Crespia, et al., 2005. Comparative Biochemistry and Physiology, 141 381– 390

O20 Neuroprotective effects of growth hormone in the green iguana neuroretina

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It has been described that growth hormone (GH) is expressed in several extrapituitary tissues, where it may be involved in cell survival, anti-apoptotic and proliferative effects through autocrine/paracrine mechanisms. In reptiles, however, there is little information regarding these GH actions, so we analyzed the expression and distribution of GH in different iguana tissues, with emphasis in the eye, and studied its potential neuroprotective role in retinas that were damaged by the intraocular administration of kainic acid (KA). It was found by western blotting (WB) that GH-immunoreactivity (GH-IR) was expressed mainly as two isoforms (15 and 26 kDa, under reducing conditions) in cornea, vitreous, retina, crystalline, iris and sclera, in varying proportions. The small variant might be the result of post-translational modifications since, by RACE5', we could not find mRNAs with alternative splicing that might lead to this variant. Also, two bands for the growth hormone receptor (GHR)-IR were observed (70 and 44 kDa, respectively) in the same tissues. By immunofluorescence, GH-IR was found in neurons present in several layers of the neuroretina (inner nuclear [INL], outer nuclear [ONL] and ganglion cell [GCL] layers) as determined by its co-existence with NeuN, but not in glial cells. In addition, GH mRNA was amplified in the retina and its sequence was identical to pituitary GH mRNA. On the other hand, GHR, IGF-I, GHRH, PACAP, TRH and SST mRNAs were also expressed in the retina, suggesting that the synthesis and secretion of retinal GH may be regulated through autocrine/paracrine mechanisms by these neuropeptides in this tissue. KA administration induced retinal excitotoxic damage, as determined by a significant reduction of the cell density and an increase in the appearance of apoptotic cells in the INL and GCL. In response to KA injury, both endogenous GH and Insulin-like growth factor I (IGF-I) expression were increased by 70±1.8% and 33.3±16%, respectively. The addition of exogenous GH significantly prevented the retinal damage produced by the loss of cytoarchitecture and cell density in the GCL (from 4.9±0.79 in the control, to 1.45±0.2 with KA, to 6.35±0.49 cell/mm^2 with KA+GH) and in the INL (19.12±1.6, 10.05±1.9, 21.0±0.8 cell/mm^2, respectively) generated by the long-term effect of 1 mM KA intraocular administration. The co-incubation with a specific anti-GH antibody, however, blocked the protective effect of GH in GCL (1.4±0.23 cell/mm^2) and INL (11.35±1.06), respectively. Furthermore, added GH induced an increase of 90±14% in the retinal IGF-I concentration and the anti-GH antibody also blocked this effect. These results indicate that GH and GHR are expressed in the iguana eye and may be able to exert, either directly of mediated by IGF-I, a protective mechanism in neuroretinas that suffered damage by the administration of kainic acid. Taken together, these studies suggest that ocular GH may be expressed locally to regulate processes of cell survival, and be involved in the ability of reptilian retina to regenerate after damage.

O21 RNA trafficking in moth pheromone gland cells

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Due to limited RNA editing common to human and high mammals, it is currently accepted that one gene is translated into one single RNA strand that becomes a string of codes for one single protein. Insects, however, display a very odd range of protein complexity, very large number of chemosensory proteins mainly from cells in the pheromone gland with extensive posttranslational modifications and certainly specific physiological and temporal variations. We used genomic, RNA and protein information sequenced at the individual level in various tissues from the silkworm moth *Bombyx mori* to show that specific RNA editing events generate expression of multiple chemosensory protein (CSP) variants from one single CSP gene (Xuan et al., 2014; Picimbon, 2014a,b, 2016; Liu et al., 2016). For CSP1, CSP2, CSP4 and CSP14, we found no variation in genomic DNA, but a high number of mutations in mRNA and protein sequences. These mutations are not random. In contrast to Human A>I mutations, *Bombyx* base mutations are located in coding region of the gene. In addition, most of the mutations are non-synonymous and pinpointed on some key functional structures of the protein. This strongly suggests that CSP-RNA editing leads to new proteins with new functions. The extremely high density of natural mutations in a moth such as *B. mori* is illustrative of a high diversity of RNA and/or protein editing mechanisms that remain to be found in specific compartments of the pheromone gland-producing tissue cell type.

Xuan N. et al. 2014; PLOS ONE, 9(2), e86932. Picimbon J.F. 2014a; Gene Technology 3, 112. Picimbon J.F. 2014b; RNA & Disease 1, e240. Picimbon J.F. 2016; Journal of Clinical and Experimental Pathology 6, 3. Liu et al. 2016; PLoS ONE 11(5), e0154706.

O22 Role of receptor CXCR2 in the pathogenesis of experimental septic arthritis

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Introduction: Septic Arthritis is the joint disease which occurs when pathogens invade the joint causing infection. The main microorganism involved in that pathology is *Staphylococcus aureus*. The disease results in high mortality and morbidity, about 50% of patients have irreversible loss of joint function. The most prominant cell involved in immune response to bacterial infections is the neutrophil. This cell is the first to arrive at the site of inflammation, thereby helping to combat infection by means of several enzymes and mediators. The CXC chemokines that signals via CXCR2 activate neutrophils and promote their adhesion to the endothelium. The objective of this work was to investigate the role of chemokine receptor CXCR2 in inflammation caused by *S. aureus* in an experimental model of septic arthritis.

Methods and Results: Experimental septic arthritis was induced by intra-articular injection of *S. aureus* (10^7 CFU; 10 µL) in C57/Bl6 mice (5-6 mice/group). Mice were treated orally with an alosteric inhibitor (DF2156A) of CXCR2 receptor 1 h after the injection of bacteria and daily for 7 days or treated intra-articularily every two days for 7 days. The oral treatment with DF2156A presented decreased number of total cells and neutrophils into the inflamed joint when compared to non-treated arthritic mice. This reduced cellular migration in DF2156A-treated mice was associated to a lower TNF-α and IL-1β in inflamed tissue. Furthermore, DF2156A-treated mice had decreased articular damage and reduced hypernociception compared to vehicle-treated mice. On the other hand, DF2156A-treated group showed slightly increased in bacterial load at the joint when compared with non-treated mice. In a similar manner, the local treatment with DF2156A increased the bacterial load compared to non-treated mice. Purified human neutrophils stimulated with CXCL8 increased the killing of *S. aureus* compared to non-stimulated neutrophils.

Conclusions: The blockage of CXCR2 prevents the accumulation of neutrophils in the joint and decreases the articular inflammation, tissue damage and dysfunction following *S. aureus* infection. However, CXCR2-binding chemokines are very important to control the bacterial load by neutrophils. Thus, the use of CXCR2 antagonists in the context of *S. aureus*-induced septic arthritis must be carefully investigated to avoid the loss of bacterial control.

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O23 Mouse P2Y4 nucleotide receptor is a negative regulator of cardiac adipose-derived stem cell differentiation and cardiac fat formation

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Cardiac adipose-derived stem cells (cASCs) have the ability to differentiate into multiple cell lineages giving them a high potential for use in regenerative medicine. Cardiac fat tissue still raises many unsolved questions related to its formation and features. P2Y nucleotide receptors have already been described as regulators of differentiation of bone-marrow derived stem cells but remain poorly investigated in cASCs. We defined here the $P2Y_4$ nucleotide receptor as a negative regulator of cardiac fat formation and cASC differentiation.

Higher expression of $P2Y_4$ receptor in cardiac fat tissue was observed compared to other adipose tissues. $P2Y_4$-null mice displayed a higher mass of cardiac adipose tissue specifically. We therefore examined the role of $P2Y_4$ receptor in cASC adipogenic differentiation. An inhibitory effect of UTP, ligand of $P2Y_4$, was observed on the maturation state of differentiated cASCs, and on the expression of adipogenesis-linked genes and adiponectin, a cardioprotective adipokine. Higher adiponectin secretion by $P2Y_4$-null adipocytes could be linked with cardioprotection previously observed in the heart of $P2Y_4$-null ischemic mice. We realized here left anterior descending artery ligation on simple and double knockout mice for $P2Y_4$ and adiponectin. No cardioprotective effect of $P2Y_4$ loss was observed in the absence of adiponectin secretion. Additionally, $P2Y_4$ loss was correlated with higher expression of UCP-1 and CD137, two markers of brown/beige cardiac adipocytes.

Our data highlight the $P2Y_4$ receptor as an inhibitor of cardiac fat formation and cASC adipogenic differentiation, and as a potential therapeutic target in the regulation of cardioprotective function of cardiac fat.

O24 Molecular determinants for constitutive activity of GPR101, an orphan GPCR associated to X-linked acrogigantism syndrome (X-LAG)

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GPR101 is an uncharacterized orphan (without known ligand) G protein coupled receptor (GPCR). Recently, a clinical research study showed that GPR101 is strongly associated to X-linked acrogigantism syndrome (X-LAG). People suffering from the disease develop pituitary tumors and growth hormone (GH) hypersecretion that leads to gigantism. It is inheritable or occurs sporadically. This syndrome is caused by a microduplication of Xq26.3 containing 4 genes. Among these genes, only GPR101 has been found to be overexpressed in the pituitary tumors of X-LAG patient. GPR101 has been described as coupled to Gs-cAMP pathway and is characterized by a very high level of constitutive activity. The precise mechanism by which GPR101 contributes to pituitary tumor initiation and growth is currently not known. The lack of mechanistic insight into GPR101 function precludes its validation as a drug target. In order to better understand the link between GPR101 and X-LAG, we analyzed the signaling pathways of GPR101 and several mutants. We confirmed a robust increase of cAMP levels upon transfection of increasing concentration of WT GPR101 in HEK293 cells. In addition, we also observed a strong basal association with arrestins with a luciferase complementation assay. We completed our study with an examination of receptor coupling to other pathways and G proteins. With immunohistochemistry and FACS analysis, we determined that the receptor was massively present in intracellular endosome-like structures. Furthermore, we applied targeted mutagenesis to understand the mechanisms for the receptor high constitutive activity. Collectively, our results will help us to understand the role of this receptor in GH regulation and to validate it as a drug target for people suffering from pituitary dysfunction. This insight at the molecular level is an important step to link pharmacology of GPR101 with physiological functions.

O25 Using BRET biosensors to detect direct G protein activation

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G-protein-coupled receptors (GPCRs) are seven-transmembrane receptors involved in all major biological processes in Metazoa. GPCRs act as conductors sensing extracellular signals converting them into intracellular signals. Thereby, several signaling cascades are induced leading to various physiological responses. This class of receptors can bind a diverse range of signaling molecules including hormones, neurotransmitters, paracrines, gustatory and odorant compounds. Since these receptors are involved in important processes, such as reproduction, growth and development, feeding and stress tolerance, they have been proposed as targets for the development of next generation pesticides.

Since the characterization of the first insect neuropeptide receptor, the tachykinin-like receptor in *Drosophila melanogaster* 25 years ago, multiple insect GPCRs have been cloned and deorphanized. A lot of effort has been made to unraveling the signaling pathways. However, until now these studies have mostly been restricted to the detection of Ca^{2+} and cAMP concentrations. Increased Ca^{2+} concentrations may point at activation of the $G_{\alpha q}$ subunit. Elevation or decrease of cAMP concentrations may suggest that $G_{\alpha s}$ or $G_{\alpha i}$ are involved, respectively.

Using bioluminescence resonance energy transfer (BRET) biosensors, we are now able to examine the GPCR signaling pathway at the heterotrimeric G protein level sensing the direct activation of the G_{α} subunit. We performed BRET analyses with a selection of neuropeptide receptors in combination with 10 different G_{α}-biosensors, representing all major G protein families.

O26 Ligand specificity of insect neuropeptide G protein-coupled receptors.

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Neuropeptides make up a very diverse class of extracellular signaling molecules that usually exert their action by interacting with signal-transducing membrane receptors. Most of these receptors belong to the family of G protein-coupled receptors (GPCRs). In insects, neuropeptides are master regulators of a diverse array of physiological processes, such as metamorphosis, metabolism, diuresis, reproduction and behavior. These ligand/receptor couples are therefore very interesting targets in designing a new generation of insecticides that, given rational design, will have a more specific mode of action.

We have focused on the *in vitro* characterization and comparison of neuropeptide-receptor interactions using different insect species. The red flour beetle*, Tribolium castaneum*, the pea aphid, *Acyrthosiphon pisum* and the swarm-forming locusts, *Locusta migratoria* and *Schistocerca gregaria,* were used as pest insect models, while the honey bee *Apis mellifera* was chosen as a benefical insect model.

Using an aequorin luminescence assay in CHO-WTA11 cells, we have established the ligand specificity of several neuropeptide GPCRs, thereby showing potential as targets in more selective insecticide design.

O27 In Locusta migratoria, bacterial PAMPs make GBP activating the hemocytes whereas Angiotensin Converting Enzyme regulates pro-inflammatory peptides

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In *Locusta migratoria*, classical antimicrobial peptides as cecropin, attacin and defensin are agents that are seemingly missing in their innate immune armory. Instead, these locusts count upon a very efficient cellular immune response. Following immune challenge a GBP cytokine of the growth blocking peptide family is released from multiple tissues except the hemocytes. As a result the circulating hemocytes become activated and obtain adhesive traits. This, in combination with the vast number of cells that become sacrificed during the process of phagocytosis, nodulation and melanization, end in a steep decrease in total circulating hemocyte count. Circulating hemocyte stem cells, not the presumed pericardial hematopoietic organ which better can be called phagocytotic organ, assure the hemocyte replenishment.

L.migratoria Angiotensin converting enzyme or Locmi-ACE, of which the expression by macrophage-like hemocytes strongly enhances post immune challenge with bacterial but not fungal PAMPS, in its turn is essential for the final processing of into the hemolymph secreted peptides. Surprisingly some of these peptides originate from precursors as HEMOCYANIN/HEXAMERIN, SPARC, TRAP-α and PACIFASTIN which all have known, though not well specified, immune related functions. In addition to the role of Locmi-ACE in the final activation/maturation of such peptides, this enzyme, as is the case in mammals, by its vast peptide degrading activity, diminishes the side effects of pro-inflammatory (cytokine-) peptides that are recruited into the hemolymph by an LPS challenge.

Duressa TF, Boonen K, Huybrechts R, (2016) *submitted* Gen. Comp.Endocrinol; Duressa TF, Boonen K, Hayakawa Y, Huybrechts R.(2015) Peptides, 74, 23-32; Macours N, Poels J, Hens K, Francis C, Huybrechts R.(2004) Int Rev Cytol.239, 2004, 47-97.

O28 Interferon gamma peptidomimetic targeted to interstitial myofibroblasts attenuates renal fibrosis after unilateral ureteral obstruction in mice

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Renal fibrosis cannot be adequately treated since anti-fibrotic treatment is lacking. Interferon-γ is a pro-inflammatory cytokine with anti-fibrotic properties. Clinical use of interferon-γ is hampered due to inflammation-mediated systemic side effects. We used an interferon-γ peptidomimetic (mimγ) lacking the extracellular IFNγReceptor recognition domain, and coupled it to the PDGFßR-recognizing peptide BiPPB. Here we tested the efficacy of mimγ-BiPPB (referred to as "Fibroferon") targeted to PDGFßR-overexpressing interstitial myofibroblasts to attenuate renal fibrosis without inducing inflammation-mediated side effects in the mouse unilateral ureter obstruction model. Unilateral ureter obstruction induced renal fibrosis characterized by significantly increased α-SMA, TGFß1, fibronectin, collagen I and collagen III protein and/or mRNA expression. Fibroferon treatment significantly reduced expression of these fibrotic markers. Compared to full-length IFNγ, anti-fibrotic effects of Fibroferon were more pronounced. Unilateral ureter obstruction-induced lymphangiogenesis was significantly reduced by Fibroferon but not full-length IFNγ. In contrast to full-length IFNγ, Fibroferon did not induce IFNγ-related side-effects as evidenced by preserved low-level brain MHC II expression (similar to vehicle), lowered plasma triglyceride levels, and improved weight gain after unilateral ureter obstruction. In conclusion, compared to full-length IFNγ, the IFNγ-peptidomimetic Fibroferon targeted to PDGFβR-overexpressing myofibroblasts attenuates renal fibrosis in the absence of IFNγ-mediated adverse effects.

O29 The role of the CXC chemokine CXCL4 and its variant CXCL4L1 in monocyte differentiation

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Macrophages are a heterogeneous population of innate myeloid cells involved in health and disease. Under inflammatory conditions, macrophages can differentiate from peripheral blood monocytes under the influence of various growth factors, cytokines or infectious agents. The best defined polarization types are M1 and M2. It has been described that chemokines, in particular the platelet-derived CXC chemokine CXCL4, are also involved in polarization and survival of monocytes. Indeed, CXCL4 induces the polarization into an M4 phenotype with a unique transcriptome. M4 macrophages produce high levels of metalloproteinases, do not express the scavenger receptor CD163 and lose their phagocytic capacity. In this study we compared the effect of the CXC chemokines CXCL4 and its variant CXCL4L1, which strongly differs from CXCL4 in angiostatic potential, on the differentiation of monocytes. $CD14^+$ monocytes were cultured for 6 days in the presence of various concentrations of CXCL4 and CXCL4L1. M-CSF (30 ng/ml) was used as a control survival and differentiation factor. The expression levels of several genes (cytokines and matrix metalloproteinases) and surface receptors were analyzed by qPCR and flow cytometry, respectively. Cytokines released in the extracellular milieu were measured by specific ELISA. In contrast to M-CSF and CXCL4, CXCL4L1 did not induce monocyte survival. CXCL4L1-treated human $CD14^+$ monocytes showed a different transcriptional profile compared to CXCL4-polarized macrophages. In contrast to CXCL4, CXCL4L1 (10 µg/ml) stimulated the release of CXCL8 and CCL2 (10- fold and 3-fold increase above M-CSF-induced levels, respectively). On the other hand, CXCL4 enhanced production of CCL22 (> 20-fold) compared to CXCL4L1- and M-CSF-stimulated $CD14^+$ monocytes. Furthermore, unlike CXCL4, CXCL4L1 increased expression of the inflammatory chemokine receptors CCR2, CCR5 and CXCR3. We can conclude that both CXCL4 and its variant CXCL4L1 exert a direct, but distinct effect on monocytes yielding differently polarized macrophage phenotypes.

O30 CD40 mediated hepatitis in TNF-receptor 1 gene knockout mice

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Tumour necrosis factor-alpha (TNF) plays an important role in liver inflammation. CD40:CD40 ligand (CD40L) is a key receptor-ligand signalling pair involved in the adaptive immune response. CD40:CD40L interactions play a pivotal role in the pathogenesis of autoimmune diseases including autoimmune hepatitis, primary biliary cirrhosis, inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis. While a few studies have already used CD40-CD40L immune activation or deactivation in different experimental autoimmune mouse models, this approach has not been applied so far to address the course of CD40 mediated hepatitis in TNF-receptor 1-depleted ($TNFR1^{-/-}$) mice. As shown previously, CD40 activation leads to sickness behaviour syndrome characterised by weight loss, sleep and depression. These effects were shown to be blocked by administration of the TNF inhibitor etanercept.

In the study presented here we assessed the extent of inflammation and course of hepatitis in mice devoid of the TNFR1 mediated signalling pathway. $TNFR1^{-/-}$ adult male C57BL/6J mice aged 10-14 weeks and their wildtype (wt) littermates were given a single intra-peritoneal injection of CD40 agonist monoclonal antibody (CD40 mAB) or as antibody control, rat IgG2a isotope control, respectively. Body weight and food consumption were recorded daily. Serum Alanin-Aminotransferase sALT was analysed as a marker for hepatic tissue damage. Confirming other reports, sALT was strongly elevated in CD40-activated wt mice as compared to isotype control-injected animals. In CD40 mAB-treated $TNFR1^{-/-}$ mice, the increased sALT was significantly less pronounced compared to the wt mice, but still significantly higher than in the isotype control-treated animals. Quantitative analysis of tissue inflammation was performed by cell-counting on visual fields of haematoxylin-eosin-stained histological liver sections from 3 CD40 mAb- and 2 isotype-injected $TNFR1^{-/-}$ and wildtype mice, respectively, with regard to hepatic intraparenchymal infiltrates, perivascular clusters (>100 cells) and necrosis infiltrate. Compared to wt mice, $TNFR1^{-/-}$ mice showed much less intraparenchymal infiltrates and perivascular clusters upon CD40 activation. Gene microarray comparing 3 animals/group revealed TNFR1-depletion in CD40 stimulated mice to be associated with upregulation of metabolic and detoxification pathways, while immunological parameters such as complement cascades and cytokine-receptor interactions were downregulated. We conclude that immune activation and development of liver inflammation in CD40:CD40L interactions depend on TNFR1 mediated signalling pathways.

Gast H, Müller A, Lopez M, Meier D, Huber R, Dechent F, Prinz M, Emmenegger Y, Franken P, Birchler T, Fontana A. Brain Behav Immun 2013; 27:133-44.

O31 The COOH-terminal GAG binding CXCL9(74-103) peptide inhibits CXCL8-induced neutrophil extravasation and monosodium urate crystal-induced gout in mice

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The ELR$^-$CXC chemokine CXCL9 is characterized by a long, highly positively charged COOH-terminal region, absent in most other chemokines. Several natural leukocyte- and fibroblast-derived COOH-terminally truncated CXCL9 forms missing up to 30 amino acids were identified. To investigate the role of the COOH-terminal region of CXCL9, several COOH-terminal peptides were chemically synthesized. These peptides display high affinity for glycosaminoglycans (GAG) and compete with functional intact chemokines for GAG binding, the longest peptide, i.e. CXCL9(74-103), being the most potent. The COOH-terminal peptide CXCL9(74-103) does not signal through or act as an antagonist for CXCR3, the G protein-coupled CXCL9 receptor, and does not influence neutrophil chemotactic activity of CXCL8 *in vitro*. Based on the GAG binding data, an anti-inflammatory role for CXCL9(74-103) was further evidenced *in vivo*. Simultaneous intravenous injection of CXCL9(74-103) with CXCL8 injection in the joint diminished CXCL8-induced neutrophil extravasation. Analogously, monosodium urate crystal-induced neutrophil migration to the tibiofemural articulation, a murine model of gout, is highly reduced by i.v. injection of CXCL9(74-103). These data show that chemokine-derived peptides with high affinity for GAGs may be used as anti-inflammatory peptides: by competing with active chemokines for binding and immobilization on GAGs, these peptides may lower chemokine presentation on the endothelium and disrupt the generation of a chemokine gradient, thereby preventing a chemokine from properly performing its chemotactic function. The CXCL9 peptide may serve as a lead molecule for further development of inhibitors of inflammation based on interference with chemokine-GAG-interactions.

O32 Inter-laboratory OECD validation of the Xenopus Embryonic Thyroid Signalling Assay

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The Xenopus Embryonic Thyroid signalling Assay (XETA) was designed as a screening assay to provide information on the potential of a test substance to alter the normal functions of the thyroid system. The XETA provides a rapid (72h) way to measure the response of embryonic stage tadpoles to potential thyroid disrupting chemicals, allowing a efficient method for screening thyroid disruptors. In addition to serving as a quick screen for thyroid active chemicals, XETA, could serve as a potential alternative method to the *in vivo* Amphibian Metamorphosis Assay (AMA - OECD TG231). The AMA test is based on the study of the metamorphosis of tadpoles after three weeks of exposure to a given chemical, and includes histological examination of the thyroid gland. XETA could provide an alternative test that can be performed quickly, providing information that would be useful for screening large number of molecules or testing environmental samples that couldn't be stored or sampled in large quantities.

The objective of the validation study is to establish the relevance of the assay by assessing its sensitivity to detect disruption of the thyroid system by compounds active at different points within the thyroid system. The validation is intended to determine the performance and transferability of the assay across a range of both experienced and naïve laboratories.

XETA utilizes free-living X. laevis embryonic-stage animals (stage 45 up to stage 47) in a multi-well format to detect modulation of thyroid receptor signaling by potential thyroid active chemicals. The assay is transcriptional-based, and uses a transgenic tadpole line containing the THbZIP genetic construct to detect the activity of Thyroid active molecules that work through various mechanisms. The phase one ring test experiments gave the expected results for the chemicals chosen. A statistical approach was determined and a great consistency of the results was observed between laboratories. The XETA Phase I results demonstrate that the assay provides reasonable sensitivity with the chemicals tested and is reproducible, with a few exceptions, across replicates and labs.

The XETA phase II started in fall 2015 and active chemicals with modes of action that were not covered in phase I are tested. Estradiol was selected as an inert chemical to challenge the assay and the statistical analysis procedure. The XETA protocol has been modified in accordance to lesson-learned during the phase one.

O33 Long term dietary exposure of adult common sole (Solea solea) to the flame retardants PBDEs - impact on thyroid and reproductive status

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Polybrominated diphenyl ethers (PBDEs) have been the focus of several studies relative to their potential health impacts on humans and wildlife. These molecules have been included in the list of persistent organic pollutants (POPs) of the United Nations Stockholm Convention in 2009 and are also included in the list of priority substances of the European Water Framework Directive. In view of their physicochemical properties and fate in the environment (persistence, bioaccumulation, long-range transport), their presence has been documented worldwide and in all environmental compartments (atmosphere, soil, sediment and biota). Moreover, PBDEs are suspected of having endocrine disrupting properties.

In this study, we investigated the effects of a long term (1 year) dietary exposure of adult common sole (*Solea solea*) to a mixture of environmentally relevant dose of PBDEs (BDE-47, BDE-99, BDE-100, BDE-153, BDE-209). Biometric parameters (weight, length, Fulton's condition index, gonadosomatic index -GSI), plasma levels of thyroid (triiodothyronine -T3,- thyroxine -T4) and steroid hormones (estradiol -E2- in females, 11-ketotestosterone -11-KT- in males) were measured during the first individual sexual maturation.

Our results showed no differences in biometric parameters and 11-KT plasma levels in males between the control and PBDE-exposed groups during the experiment. In contrast, in females, a difference in condition index and GSI was observed with lower values in the PBDE-exposed group compared to the control group during the reproductive period.

Concerning thyroid hormones, plasma levels were similar in both males and females. T3 plasma levels remained stable with similar concentrations in control and PBDE-exposed groups throughout the experiment. On the other hand, plasma levels of T4 showed high variations throughout the experiment and higher levels were measured in the PBDE-exposed group compared to the control group at the beginning of the exposure period.

Our results indicate that PBDEs seem to be able to interact with and affect thyroid as well as reproductive endocrine axis in adult sole exposed to an environmental relevant mixture.

O34 Development of a zebrafish embryo test for environmental risk assessment of endocrine disrupting pharmaceuticals using nano-injection

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Pharmaceutical companies are obligated to perform an environmental risk assessment for each new drug that is launched on the market. The mandatory tests for potential endocrine disrupting compounds require a lot of time and are not consistent with the 3R principle. Therefore, the goal of this study is to develop a zebrafish embryo test, which is not considered an animal test according to the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting (ED) modes of action (MoAs). We focus on the following MoAs: estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism and aromatase inhibition. For every MoA one compound was chosen as a model. Since many ED compounds are lipophilic, aqueous exposure is often difficult to achieve. Therefore we are developing a new method in which the compounds are first dissolved in a solvent and then injected into the yolk of zebrafish embryos before they reach the 64-cell stage. An important validation aspect in the development of this new method is to compare the biological responses following nano-injection to those following aqueous exposure, which is currently routinely applied in the Fish Embryo Acute Toxicity test (OECD TG 236). After an evaluation based on several endpoints, rapeseed oil was chosen as a solvent, because it caused the least mortality and no sublethal effects, and 0.5 nl was injected after which the embryos were monitored until 120 hours post fertilization. Using a profile based on multiple biological responses, including survival, hatching, heart rate, morphological deviations, swimming behaviour, length, GFP expression, gene transcription, … we are trying to create a classifier that can discriminate among the 5 MoAs.

Aquatic exposure to the compounds was characterised first. We observed some similarities and some differences in the responses among compounds following aqueous exposure, which can aid in discriminating among the five MoAs. Aqueous exposure to the AR agonist 17β-trenbolone, the AR antagonist flutamide and the ER agonist 17α-ethinylestradiol caused (sub)lethal effects at the morphological and physiological level. The ER antagonist fulvestrant and the aromatase inhibitor letrozole caused no (sub)lethal effects at maximum water solubility. If mortality occurred it always happened during the first 24h. Both agonists showed some sublethal effects, while no sublethal effects were observed after exposure to flutamide. Exposure to 17α-ethinylestradiol caused dose-dependent skeletal malformations of the spine. Cranial malformations following 17α-ethinylestradiol exposure were only significant around the LC_{50} concentration and above. For 17β-trenbolone heart rate was already significantly decreased after exposure to concentrations below the LC_5. Growth was decreased below the LC_{50} concentration for 17β-trenbolone, while for the other two compounds the decrease was only significant around the LC_{50} concentration. Swim bladder inflation and swimming behaviour were impaired for both agonists at sublethal concentrations.

Although we already observed differences between the MoAs on a morphological and physiological level which can help to distinguish among the MoAs, we are currently adding more specific endpoints (e.g. gene transcription and GFP expression) to further increase the discriminating potential.

O35 Reproductive and developmental impairment by low concentrations of environmental contaminants with hormone-like activity in fish using Omics approach

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Contaminants of emerging concern (CECs) present in the environment have been associated with a number of health concerns in humans and animals. However, more information is needed on the mechanisms by which contaminants cause adverse health effects in order to accurately assess the risk, and come up with appropriate preventive measures. Our previous studies provided evidence, linking environmental contaminants with female bias in wild fish population downstream of municipal wastewater treatment plants. The findings suggest disruption of gonadal development by compounds with hormone-like activity. However, contaminants that have endocrine disruptive properties, lack high degree of specificity for the endogenous hormone receptors. Therefore, overlap of specificity can result from higher concentrations of the contaminants. In animals that are seasonal breeders, overlap of specificity can also be resulted from changes in the receptor levels for endogenous hormones that control reproduction and development. To investigate the adverse effects of contaminants, we performed controlled laboratory experiments using zebrafish embryos, adult goldfish and fathead minnows exposed to low environmentally relevant concentrations of a selected number of chemicals detected in the river system, individually and as mixtures. A systems approach was used to investigate physiological, morphological, transcriptional and metabolic disruption. Our studies were designed to investigate neuroendocrine impairment in the adult fish undergoing seasonal variation under controlled laboratory conditions by low concentrations of contaminants individually and in mixture. Multiple parameters including morphometric, neurogenesis, metabolomics and targeted transcriptomics were used in this study to investigate how environmental contaminants impair normal seasonally related reproduction, development and metabolism. Gene expression pattern in the brain, liver and gonads were investigated to elucidated mechanisms by which brain-pituitary gonadal axis are disrupted. Tissue samples were also used for metabolite extraction using quantitative ^{1}H-NMR spectroscopy and mass spectrometry to determine metabolic profile in fish exposed to environmental contaminants. To obtain transcriptomic profile, we exposed fathead minnows to environmental contaminants individually and in mixture, using FHM 8 × 15K arrays. To investigate neurogenic disruption, we investigated different brain regions in terms of hypothalamic progenitor pool and timing of neuronal birth. The overall results provide novel information on adverse cellular effects of CECs individually and in mixture in fish. Environmental contaminants disrupt reproduction in fish at multiple levels by 1) altering production of hormones involved in the brain-pituitary-gonadal axis; 2) altering hormone responsiveness by affecting expression of hormone receptors; 3) altering energy metabolism whereby affecting energy investment required during different stages of gonadal development in male, and especially in female fish; 4) altering hypothalamic neurogenesis via a mechanism involving aromatase; 5) changes in neurogenesis linked to contaminants altered behaviour in developing zebrafish. The present study provides a framework for better understanding of the mechanisms of health disruption by CECs. Using 'Omics' approach we were able to identify pathways that are targeted by environmental contaminants. The results can be used to develop better tools to assess the environmental impact of contaminants, and to elucidate the mechanisms of health impacts in fish and other vertebrate species. Funded by NSERC grant to HRH.

O36 Ghrelin Modulates Digestive Enzymes and Glucose Transporters in Goldfish Gut and Hepatopancreas in vitro via the GHS-R1a Receptor, and PLC-PKC and AC-PKA Signaling Pathways

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Ghrelin is an important peptide hormone mainly synthesized by the gut, primarily involved in maintaining energy balance. In mammals, it has been demonstrated that the metabolic functions of ghrelin include the modulation of digestive enzymes and glucose transporters. However, very little is known about these aspects of ghrelin in fish. Therefore, the first aim of this study was to determine the localization of some important digestive enzymes (sucrase-isomaltase, aminopeptidase a, trypsin and lipoprotein lipase) in relation to ghrelin in the gut and hepatopancreas of goldfish (*Carassius auratus*) using immunohistochemistry. Our second objective was to determine whether ghrelin modulates the expression (gene and protein) of the above-mentioned digestive enzymes, as well as of two key glucose transporters (glucose transporter 2, glut-2, and sodium-glucose linked transporter 1, sglt-1) using an *in vitro* approach. Cultured gut and hepatopancreas sections were exposed to ghrelin (0.1, 1 and 10 nM) for 30, 60 and 120 minutes, and mRNAs encoding the above mentioned genes, and proteins itself were quantified by RT-qPCR and Western blot, respectively. Finally, the specificity of ghrelin in the modulation of digestive enzyme expression, as well as the involvement of the PLC-PKC and AC-PKA intracellular signaling pathways in those effects, were studied by preincubating the tissues with selective inhibitors: the GHS-R1a ghrelin receptor antagonist [D-Lys^3]-GHRP-6, the PLC inhibitor U73122 and the PKA inhibitor H89. Results show the presence of ghrelin and the digestive enzymes sucrase-isomaltase, aminopeptidase a, trypsin and lipoprotein lipase colocalizing in some intestinal and hepatopancreas cells. *In vitro* exposure to ghrelin resulted in a significant upregulation of *sucrase-isomaltase* mRNAs in gut at 30 and 120 min, and in hepatopancreas at 30 min, while a downregulation was observed in gut at 60 min. Treatment with ghrelin also led to a concentration-dependent induction of *aminopeptidase a* in the gut, and of *lipoprotein lipase* in both tissues. Most of the observed upregulatory effects of ghrelin on digestive enzyme mRNA expression did also extend to protein level. Furthermore, ghrelin-evoked inductions of digestive enzyme expression were abolished by preincubation with the GHS-R1a ghrelin receptor antagonist [D-Lys3]-GHRP-6, and most of them also by the PLC and PKA inhibitors, suggesting that GHS-R1a is mediating such ghrelin effects via the PLC/PKC and AC/PKA intracellular signaling pathways. Ghrelin upregulated *glut-2* and *sglt-1* mRNA expression in a time- and concentration-dependent manner. Overall, these results demonstrate that ghrelin exerts important regulatory effects on enzymes and transporters involved in the digestion and absorption of nutrients. The effects discovered here suggest that ghrelin, in addition to its orexigenic actions, assist in the assimilation and partitioning of nutrients, which solidify a key role for ghrelin in maintaining energy balance in fish.

O37 Endocrines and microbes get mosquitoes through feast and fast

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Mosquitoes emerge as larvae into aquatic habitats of varying food quantity, and development through each of the four instars begins with a feast and ends with a fast, which persists through the pupal stage. During the last instar feast, teneral reserves are laid up for adult differentiation and survival. As adults, mosquitoes emerge into an aerial terrestrial habitat and must fast until nectar or blood is obtained. The yellow fever mosquito, *Aedes aegypti*, can withstand prolonged fasting in all stages, and our work has shown that first instar larvae must acquire a gut microbial community for development to continue. However, axenic or starved larvae can persist in any instar for a long time and still be stimulated to molt by ecdysteroid hormone treatment. Current efforts seek to understand how gut microbes influence the endocrine regulation of molting and to identify neuropeptides that activate ecdysteroid production and nutrient storage in larvae. The ability of female mosquitoes to survive and still engage in host seeking in a fasting state is a key component in the transmission of pathogens. Previously, we showed that adipokinetic hormone, a glucagon homolog, and insulin-like peptides have opposing roles in nutrient apportionment in non-blood females. Our interest now is to understand how gut microbes influence the endocrine regulation of nutrient storage in blood fed females simultaneously provisioning a large egg clutch. These nutrients are needed for the fast between blood meals and used as well by infective pathogens for development and proliferation. We suspect the endocrine mechanisms that regulate nutrient dynamics are conserved and highly dependent on gut microbe interactions through all mosquito life stages. Research support from the National Institutes of Health (RO1AI33108 to MRB and MRS) and the Georgia Agricultural Experiment Station.

O38 Drosophila melanogaster Adipokinetic hormone regulates food intake and expression of other neuropeptide regulators of fly metabolism

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Mammalian homeostasis of storage and circulating energy substrates is maintained by the antagonistic action of glucagon and insulin hormones. Similarly, insect energy homeostasis is regulated by the anabolic insulin-like peptides and by the catabolic glucagon-like Adipokinetic hormone (AKH). Upon secretion from the neuroendocrine system, AKH induces the mobilization of storage lipids and/or of glycogen, thus increasing the circulating energy substrates in the insect haemolymph. Our recent study of the *Drosophila melanogaster* AKH-specific mutant *AkhA* has shown that deficiency for this hormone results in adulthood-specific onset of obesity coupled with hypoglycemia. Here, we will present results of our loss-of-function and gain-of-function analyses of additional physiological roles of *Drosophila* AKH. Our work shows that AKH regulates fly metabolism at several levels, including feeding and metabolic rate control, and expression of endocrine regulators of fly metabolism. AKH-regulated genes include, for example, regulators of feeding like *CCHamide-2* and *neuropeptide F*, and metabolic regulators like *corazonin* and *limostatin*. Importantly, AKH also controls the expression of central as well as peripheral *insulin-like peptides.* Altogether, our study shows that AKH is a pleiotropic regulator of energy homeostasis and neuroendocrine signaling in *Drosophila* adults.

O39 Effects of reproductive dormancy on metabolism and genome-wide transcriptome in Drosophila melanogaster

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All organisms have evolved mechanisms to cope with environmental stress. A wide range of species responds to adverse conditions by entering dormancy. In *Drosophila melanogaster* dormancy (diapause) occurs in adults and is characterized by arrested reproduction, reduced food intake, altered metabolism, and drastically extended lifespan. To get insight into the underlying molecular mechanisms we performed a genome-wide analysis of transcript changes in diapausing female flies. Gene ontology and pathway analysis of over four thousand differentially regulated genes identify genes regulating metabolism, stress, immunity and protein synthesis. A more detailed annotation indicates downregulation of insulin and target of rapamycin signaling, activation of immune signaling, JNK and JAK/STAT pathways, as well as effects on ecdysone, juvenile hormone and peptide hormone pathways. We found that also male flies diapause, albeit in a more shallow state. Diapause in *D. melanogaster* is reminiscent of dormancy in other invertebrates and hibernating mammals, and our study provides a proof of principle that *D. melanogaster* is an excellent model for genetic analysis of mechanisms underlying dormancy.

O40 Use of primary cultured adipocytes to better understand adipogenesis, lipid metabolism and fat accumulation in fish

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Over the past few years, the traditional view of adipose tissue as a simply reservoir for energy storage has changed. Besides its classical function in the regulation of energy balance, adipose tissue is a highly active endocrine organ that plays a critical role modulating several physiological processes such as appetite and lipid metabolism. Furthermore, adipose tissue is responsible for the secretion of a variety of bioactive mediators named adipokines, which exert an impact on whole-body metabolism and homeostasis. Thus, dysregulation of adipose tissue functionality is associated with a range of related diseases such as insulin resistance and obesity. Although this field is extensively studied in mammals, there is little information available about the complex role of adipose tissue in other vertebrates such as fish. In this framework, our research group established and characterized primary adipocyte cells cultures from gilthead sea bream (*Sparus aurata*) and rainbow trout (*Oncorhynchus mykiss*) in order to study adipose tissue metabolism and adipogenesis in fish. It has been demonstrated that fish species share a significant amount of genetic identity with humans, and show similar metabolic pathways and organ systems. Adiponectin and leptin are two important adipokines that regulate adipogenesis and adipose tissue dynamics. Previous *in vitro* results have shown that insulin up-regulates adiponectin mRNA levels, while it exerts a negative modulation on trout adipocyte adiponectin receptors gene expression. Moreover, we have also demonstrated that leptin stimulates lipolysis and decreases fatty acid uptake in these adipocytes, supporting the anti-adipogenic function previously suggested in mammals. In addition, our studies have established that this fish cellular model can be successfully used as an *in vitro* screening tool for testing drugs and other compounds. Precisely, we have demonstrated that environmental endocrine disruptors such as tributyltin (TBT) and triphenyltin (TPT) enhance adipocyte differentiation and act as potential obesogens through the activation of peroxisome proliferator-activated receptor gamma (PPARγ) protein expression and the up-regulation of adipogenic genes (i.e. fatty acid synthase, FAS). Finally, we have also recently confirmed the anti-adipogenic role of two vegetal compounds, caffeic acid and hydroxytyrosol as well as the estrogenic effect of genistein, another vegetal ingredient currently incorporated in fish feeds. In summary, *in vitro* studies using this adipocyte cell system, can be used not only to study fish metabolism and adipogenesis, but also can serve as an adequate screening tool to characterize different compounds and to make suggestions for further analyses in an *in vivo* scenario to increase our knowledge on lipid metabolism and fat accumulation in fish. Supported by MINECO AGL2014-57974-R and DECO 2014SGR-01371.

O41 The anatomy of neuropeptide gene expression in a pentaradial bilaterian - the starfish Asterias rubens (Phylum Echinodermata)

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Neuropeptides are ancient neuronal regulators of physiology and behaviour in eumetazoans. Neuropeptide systems are well characterised in vertebrates and some protostomian invertebrates (e.g. *Drosophila, C. elegans*) but relatively little is known about neuropeptide expression and function in deuterostomian invertebrates, such as echinoderms. As a chordate sister lineage, echinoderms can provide important insights into the evolution of neuropeptide systems. Furthermore, echinoderms provide a unique anatomical perspective on neuropeptide function because they exhibit pentaradial symmetry as adult animals. We are using the starfish *Asterias rubens* as a model echinoderm system to investigate neuropeptide evolution and function. By analysing neural transcriptome sequence data, we have recently identified forty neuropeptide precursor transcripts in *A. rubens*. Here we have analysed the anatomical expression patterns in *A. rubens* of twelve of these neuropeptide precursor transcripts, focusing on precursors of neuropeptides that belong to known bilaterian neuropeptide families: Asterotocin (vasopressin/oxytocin-type), NGFYYamide (NPS/CCAP-type), GnRH, corazonin, TRH, CCK, orexins, luqin, tachykinin, calcitonin and CRH. Using mRNA *in situ* hybridization methods, expression of all of these precursors was revealed in cells of the circumoral nerve ring and radial nerve cords, with some expressed in both the ectoneural and hyponeural parts (e.g. TRH) and others only in the ectoneural part (e.g. tachykinin). Expression of the GnRH-type precursor appears to be restricted to the nerve ring and cords, but expression of all the other precursors was detected in one or more other regions of the body, including the digestive system and body wall. Expression of numerous precursors was observed in the evertible cardiac stomach, indicating that multiple neuropeptidergic systems are required for control of the unusual extraoral feeding behavior of starfish. Our data provide an anatomical framework for investigating the conservation/divergence of bilaterian neuropeptide function in the context of the highly derived echinoderm bauplan.

This work was supported by a BBSRC grant awarded to Maurice R. Elphick.

O42 Molecular and neuroanatomical characterization of vasopressin/oxytocin-type signalling in an echinoderm

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Vasopressin/Oxytocin (VP/OT)-type peptides are a highly conserved bilaterian family of neuropeptides that exert their effects via co-evolved G-protein coupled receptors. The VP/OT-type signaling system is involved in regulation of a variety of processes, including osmoregulation, reproduction and social behavior. However, our knowledge of VP/OT-type signaling is largely based on studies of vertebrates and protostomian invertebrates (e.g. *C. elegans*). Deuterostomian invertebrates (urochordates, cephalochordates, hemichordates, echinoderms) occupy an "intermediate"phylogenetic position, bridging the evolutionary gap between protostomes and vertebrates. Therefore, studies on deuterostomian invertebrates could provide important insights into the evolution of VP/OT-type neuropeptide function in the animal kingdom.

Here we have investigated VP/OT-type signaling in an echinoderm - the common European starfish *Asterias rubens*. Analysis of neural transcriptome sequence data identified a transcript encoding the precursor of a VP/OT-type neuropeptide ("asterotocin"; CLVQDCPEG-NH_2) and LC-MS-MS confirmed the presence of this peptide (with a disulphide bridge) in *A. rubens* nerve extracts. Analysis of neural transcriptome sequence data identified a VP/OT-type receptor as a candidate receptor for asterotocin, which was then cloned and sequenced. Heterologous expression of this receptor in CHO cells expressing aequorin enabled luminescence-based demonstration that asterotocin is a ligand for this receptor (EC_{50} = 3.824 x 10^{-8}M).

Having characterized the molecular components of the VP/OT-type signaling pathway in *A. rubens*, we then employed use of mRNA *in situ* hybridization and immunocytochemistry (using novel antibodies) to map the expression of asterotocin and its receptor in this species. Expression was observed in the major components of the starfish nervous system - the circumoral nerve ring within the central disc and the radial nerve cords that branch out from the nerve ring into each arm. In these structures, asterotocin and its receptor are expressed by cells in the epithelium of the ectoneural layer, with stained processes in the underlying neuropile. Asterotocin-expressing cells are also observed in the locomotory tube feet, body wall and cardiac stomach, and immunostained processes of these cells are present in the basal nerve ring of the tube foot disc, the sub-epithelial nerve plexus of the body wall and the basiepithelial nerve plexus of the cardiac stomach.

Investigation of the pharmacological effects of asterotocin in starfish has revealed that it triggers cardiac stomach relaxation (*in vitro*) and eversion (*in vivo*). Detailed analysis of asterotocin and asterotocin receptor expression in the cardiac stomach indicates that asterotocin may exert its relaxing effect by acting on asterotocin receptor-expressing processes located within the basiepithelial nerve plexus. Thus, asterotocin may cause cardiac stomach relaxation indirectly by stimulating neural release of another signaling molecule that then acts as a muscle relaxant.

O43 An evolutionary conserved neuropeptidergic network underlying C. elegans behavioral plasticity

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Neuropeptides are key modulators of adaptive behaviors and represent one of the largest groups of neural messengers. While many neuropeptides are shown to regulate behavioral plasticity, the neuromodulatory actions and evolutionary origin of these effects are much less well understood. To unravel the cellular and molecular mechanisms underlying this neuropeptidergic control, we use the nematode model *Caenorhabditis elegans* relying on its well-defined nervous system and the availability of many targeted genetic tools. Despite the broad diversity of more than 120 peptide precursor genes in the *C. elegans* genome, ligands for only a handful of neuropeptide receptors have been characterized so far. Using *in vitro* reverse pharmacology, we are performing a large-scale deorphanization of all putative *C. elegans* neuropeptide G protein-coupled receptors (GPCRs). More than 150 peptide GPCR candidates are expressed in heterologous cell systems and screened with a library of all known and predicted *C. elegans* peptides. We have identified a diverse neuropeptidergic network including many evolutionary conserved pathways such as tachykinin, myoinhibitory peptide, neuromedin, neuropeptide Y, and neuropeptide FF related signaling systems. Phenotypic and expression studies in *C. elegans* reveal key functions of these neuropeptide pathways in the experience-dependent modulation of feeding behavior, decision-making, learning and memory. Our results provide a scaffold to further unravel the intertwined peptidergic modulation of adaptive behaviors.

O44 In search for neuropeptides in the zebrafish brain by LC-MS

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(Neuro)peptides are small messenger molecules that are derived from larger, inactive preproproteins or peptide precursors by the highly controlled action of processing enzymes. After processing, bioactive peptides can be stored in dense core vesicles prior to their release within the nervous system or peripheral organ systems where most of them will act through G-protein coupled receptors to govern a wide variety of physiological processes and behaviour (such as feeding, locomotion and reproduction) in response to internal and external stimuli. Obviously, detailed knowledge on the actual peptide sequences, including the potential existence of truncated versions or presence of post-translation modifications, is of high importance when studying their function.

A peptidomics approach therefore aims to identify and characterize the endogenously present peptide complement of a defined tissue or organism using liquid chromatography and mass spectrometry. While the zebrafish *Danio rerio* is considered as an important aquatic model, either in the domain of ecotoxicology or rather as general vertebrate model in a medical context, very little is known about their peptidergic signaling cascades. We therefore set out to biochemically characterize endogenously present (neuro)peptides from the zebrafish brain by LC-MS. This peptidomics setup yielded 56 different neuropeptides in addition to various truncated versions.

This archive of identified endogenous peptides will aid future research in (neuro)endocrinology in this important model organism. The methodology that was developed in this study will allow us to study the changes in peptide expression in response to changes in the organism or the environment using differential peptidomics or molecular imaging techniques such as immunocytochemistry or MALDI imaging. As such, our peptidomics data is likely to aid further functional elucidation of defined neuropeptidergic signalling systems.

O45 The first characterisation of a crustacean neuropeptide receptor: the putative GPCR for red pigment-concentrating hormone of Daphnia pulex

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The Adipokinetic hormone (AKH)/Red pigment-concentrating hormone (RPCH) family of peptides are well-known neuropeptides of invertebrate animals. Members of this peptide family share sequence structure similarities and are generically named for the first identified biological functions in insects and decapod crustaceans, viz. mobilising lipids (metabolism) and pigment granule movement (epidermal blanching), respectively. Although RPCH has been sequenced from many decapod crustacean species, the RPCH receptor has never been sequenced or characterised to date. In contrast, AKH peptides and the AKH receptor have been elucidated from insects; the AKH receptor was characterised as a G protein-coupled receptor (GPCR), and the intracellular transduction pathways were investigated in many insects.

The elucidation of the whole genome of a branchiopod crustacean, the water flea *Daphnia pulex* in 2010, made it possible to search for orthologous genes for the RPCH peptide, as well as its GPCR. We shall report on our findings related to the water flea RPCH and its receptor.

Information pointed to a putative mature RPCH octapeptide in *D. pulex* with the primary sequence of pGlu-Val-Asn-Phe-Ser-Thr-Ser-Trp amide (= Dappu-RPCH), which differs from the decapod RPCH in three positions (amino acids number 2, 6 and 7). We cloned the Dappu-RPCH preprohormone from a German colony of *D. pulex*; confirmed the amino acid substitutions, and demonstrated in an *in vivo* assay that this RPCH is not active in a decapod crustacean. The biological relevance of RPCH in the microscopic crustaceans is not known.

We further identified the putative GPCR for Dappu-RPCH from the genome, confirmed the sequence after amplification of cDNA templates using RACE, and found that the open reading frame encodes a protein of 451 amino acids with typically seven transmembrane domains. This provided us with the unique opportunity to gather information about the ligand binding and activation requirements of a crustacean GPCR. Thus, this is the first characterisation of a crustacean peptide GPCR. To achieve our aims, we expressed the Dappu-RPCH receptor in a vertebrate cell line with a bioluminescence reporter system, and tested a selection of insect AKH peptides, as well as an alanine-substitution series of Dappu-RPCH peptide analogues at various concentrations against the expressed receptor in an *in vitro* cellular assay. Our preliminary dose-response data reveal that Dappu-RPCH binds efficiently to the Dappu-RPCH receptor (EC_{50} in the nM range) and the selected insect AKHs also bind efficiently to the expressed crustacean receptor in the same dose range; differences in receptor binding and activation become visible, however, in the pM range. Additional information on the relative importance of each amino acid in the Dappu-RPCH peptide for receptor activation was obtained from cellular assays with the Ala-analogue series: we shall present evidence that amino acids in positions 1 to 5, and 8 of the ligand, as well as the C-terminal amidation of Dappu-RPCH are critical for activating the Dappu RPCH receptor. Our data will be discussed in view of insect AKH receptors.

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O46 Transcriptional landscape of the major pancreatic cells reveals conserved expression patterns amongst distant vertebrate species.

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Defining the transcriptome and the genetic pathways of pancreatic endocrine and exocrine cell types is crucial for elucidating the molecular attributes of disorders such as diabetes. The comparison of these transcriptomes amongst distant vertebrate species highlight the genes under strong evolutionary constraints which have maintained their selective expression in pancreatic cells due to their crucial function in these cells.

We took advantage of zebrafish transgenic lines to isolate by FACS the major pancreatic cell types and we obtained highly purified preparations of endocrine α-, β- and δ cells as well as the exocrine acinar and ductal cells. Transcriptomic profiling by RNA-seq identified the transcriptomic signature of each cell type and highlighted novel cell-specific markers including transcription factors, signaling pathways components and lincRNAs. By performing interspecies comparisons, we identified hundreds of genes with conserved enriched expression in endocrine or in exocrine cells amongst human, mouse and zebrafish. This list includes many regulatory genes known as crucial for the differentiation and the function of endocrine and exocrine cells, but also pinpoints previously unrecognized regulators. While the transcriptomic signature of pancreatic endocrine and exocrine cells is well conserved amongst vertebrates, the signatures distinguishing the endocrine cell subtypes, and notably alpha (glucagon+) and beta (insulin+) cells, are much less conserved between zebrafish, mouse and human, with only a few genes displaying conserved cell subtype specific expression. This suggests that endocrine cell subtype identity may be determined by few regulatory genes. The function of some identified endocrine regulators is presently addressed through CRISPR mutagenesis.

In conclusions, this study provides the molecular signature of the major zebrafish pancreatic cell types and identifies sets of genes with selective and evolutionary conserved expression in pancreatic endocrine or exocrine cells from fish to mammals, likely important in pancreas physiology and relevant to pancreatic diseases such as diabetes and pancreatic cancer.

O47 Micro peptides as a new class of bio-active peptides in higher eukaryotes

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Introduction Micropeptides are small non-classical peptides that are encoded by short open reading frames. These peptides are translated by sORFs less than 100 Amino acids (AA). In contrast to other bioactive peptides, micropeptides are not cleaved from a large precursor protein. They lack the N-terminal signal sequence and are released into cytoplasm immediately after translation. These peptides are quite conserved. The coding sequences of micropeptides are conserved across very large evolutionary distances. This is demontstrated by the recently identified *sarcolamban* that shows very strong conservation, from flies to human. Until recently, in many genome annotation projects, it was assumed that genes do not code for translation products shorter than 100 AA, so small translation products were neglected in most studies. Recent evidence suggests that micropeptides might play key roles in development. In this study, we aim to analyze the presence and functionality of micropeptides in *Drosophila*. Results are validated by ribosome profiling data, where multiple sORFs appear to be translated.

Methods The challenge with detecting micropeptides by mass spectrometry lies with their low abundance and also by their size. Size wise, most micropeptides are too small to be detected by classical (shotgun or gel based) proteomics, but they are on average larger than a typical peptide and so are easily missed in a peptidomic analysis. Despite recent advances in mass spectrometry, the dynamic range can still be insufficient to identify micropeptides in the sample, especially since they have to be detected in extracts that usually contain highly abundant metabolites and protein degradation products. Therefore we set to optimize different stages of peptidomic workflow in *Drosophila* at 3 levels:

1) Sample preparation, we compared different existing methods from literature to extract the micropeptides from the sample.

2) Chromatography: Different set-ups were tested, in which the aim was to reduce the co-elution of low abundant peptides with other highly abundant compounds.

3) In MS, we set up a middle down proteomics method in which we focused on the peptides of range between 3-15 kDa, using alternative fragmentation methods such as HCD and ETD. In addition, we evaluated different identification strategies that are adapted for large peptides.

Preliminary results

We created a novel method where different solvents were used to elute the peptides from the column, which in turn significantly reduce the dynamic range of peptidome. Because of varying elution strengths of solvents, the elution pattern of a peptide is different when using different solvents. Combining different solvents results in relatively orthogonal separation, improving the identification rate for (low abundant) natural peptides. The results are still under evaluation but it seems that by applying all the above techniques we do not only identify most of the known bioactive peptides in the fruit fly but we also have good indications for the presence of micropeptide(s) that were predicted based on sequence analysis and ribosome profiling data.

O48 QTL mapping for traits associated with stress neuroendocrine reactivity in chicken

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Animal domestication has provided us with a model to study genetic determinants of complex phenotypic traits. We recently have compared physiological stress response of two breeds of chickens, namely; Red Junglefowl (RJF) which is the main ancestor of all domesticated breeds of chickens and the commercial *White Leghorn* (WL). It was shown that the RJF reacted stronger both behaviorally and physiologically to the restraint stress, but also recovered faster. The levels of corticosterone (CORT), pregnenolonle and DHEA were significantly different between the breeds. The aim of the current study was to find QTLs involved in observed physiological differences between the breeds using the 12^{th} generation of an advanced intercross line (AIL) between RJF and WL. The plasmic levels of CORT, pregnenolone, DHEA and aldosterone were measured before and after restraint stress using HPLC/MSMS. Immediately after blood sampling the birds were culled and hypothalamus and adrenal gland were dissected out. The expression levels of 46 known genes involved in physiological stress response were measured using Taqman qPCR in hypothalamus and adrenal. A full genome scan of all individual AIL birds was also conducted for determining the genetic map. The result showed that expression levels of glucocorticoid receptor (GR) in the brain was an important negative determinate of CORT response. Expression level of several genes including Heat shock factor 1 (HSF1), benzodiazepine receptor 1, GABA receptor 2 and HSD11B2 were also significantly associated with CORT response. QTL analysis revealed several loci regulating plasmic steroids and gene expression in both brain and adrenal. One QTL on chromosome 5 (at 1085 cM) was found for CORT response. The same genomic location was also involved in regulation GR and FOS in hypothalamus and HSF1, FOS, and DAX1 in adrenal glands. Further genetic analysis suggested that the region has been subject of positive selection during chicken domestication. The region has been sequenced in parental line and is being analyzed.

O49 DINeR- A Database for Insect Neuropeptide Research

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Introduction:

Insect neuropeptides are responsible for regulating a variety of functions, including development, metabolism, mating, water and ion homeostasis, reproduction and aggression. Neuropeptides also play an important role as neuromodulators in circuits of the central nervous system. The number and functions ascribed to neuropeptides in different species has dramatically increased, requiring systematic curation. Moreover, historically, researchers have inadvertently named neuropeptides based on observed function in a particular species without considering homologs that may have different functions. This creates confusion when neuropeptides are named differently.

As a rapidly growing field, insect neuroendocrinology requires a consolidated, comprehensive and standardised resource for managing neuropeptide information.

We have created the Database for Insect Neuropeptide Research or *DINeR* , a web-based database-application used for search and retrieval of neuropeptide information of various insect species detailing their isoform sequences, physiological functionality and images of their receptor-binding sites.

Methods:

Using a PERL Web Framework front-end, a PostGreSQL backend and current website design technologies, *DINeR* is designed to be intuitive, accessible and user-friendly in its operation.

Additionally, we have adopted the system proposed by Coast and Schooley (2011), with a few exceptions, to address erroneous nomenclature of neuropeptides.

Results:

The curated data includes 48 families of neuropeptides from around 400 different insect species. Approximately 1400 FASTA formatted, neuropeptide isoform amino acid sequences and over 200 records of physiological functionality have been recorded based on published literature. Also available are images of receptor-binding assays of the neuropeptides.

The data also includes comprehensive information on each neuropeptide including their location and known functionality. Graphical representations of the multiple sequence alignments of the neuropeptide amino acid sequences along with cladograms across species are displayed, to annotate conserved motifs across species with respect to each neuropeptide.

As this is an ongoing project, more data will be added progressively. These data will be continuously curated, ensuring a comprehensive and standardised resource for the scientific community.

DINeR is part of the H2020 nEUROSTRESSPEP grant dedicated to identifying greener integrated pest management solutions. It is available at the official project website:
http://www.neurostresspep.eu

Coast G. and Schooley D., 2011. Toward a consensus nomenclature for insect neuropeptides and peptide hormones. Peptides, 32, 620-631.

O50 Efforts to identify gnathostome-cyclostome orthologs by conserved synteny

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The early stages of vertebrate evolution involved two rounds of genome doubling called 2R. It has been intensely debated whether one or both of these took place before the cyclostome-gnathostome divergence (CGD). One gene family strongly supports CGD after 2R, namely the visual opsins [1], possibly because the opsin duplicates diversified rapidly after 2R and therefore give robust orthology between lamprey and gnathostome genes. However, many other gene families display ambiguous phylogenies. Ortholog-paralog distinctions are worsened by differential losses in the two lineages and by technical challenges to assemble the sea lamprey, *Petromyzon marinus*, genome (the chromosomes are numerous, the genome has a high content of the bases C and G, the genome contains abundant repeated elements, and the level of heterozygosity is high). The problems to resolve the 2R and CGD events are further aggravated by a third tetraploidization in the lamprey lineage implicated by the genome assembly of the Arctic lamprey, *Lethenteron camtschaticum* (also called Japanese lamprey) [2] that may have led to an increased rate of evolution. In addition to sequence-based phylogenies, orthology-paralogy relationships may be investigated by comparison of synteny between evolutionary lineages. The genome assembly for *L. camtschaticum* makes it possible to address this question. By investigating the constellation of gene neighbours near the NPYR (neuropeptide Y receptor) genes, we recently proposed the identities of four lampreys NPYRs. Although the ancestral vertebrate had no less than 7 NPYR genes [3-4], lampreys and humans have only four and seem to (incidentally) have retained the same four genes: subtypes 1, 2, 4 and 5 [5]. We are now using the same approach investigate orthology-paralogy relationships for other peptide and receptor gene families and especially to see if some of these provide support for a 3R event in lampreys.

1) Lagman, D. et al. BMC Evol. Biol. 2013:13, 238. 2) Mehta, T.K. et al. Proc. Natl. Acad. Sci. U.S.A. 2013:110, 16044–16049. 3) Larsson, T. A. et al. Genomics 2009:93, 254-260. 4) Larhammar, D. and Bergqvist, C.A. Front. Neurosci. 2013:7, 27. 5) Xu, B. et al. Gen. Comp. Endocrinol. 2015:222, 106-115.

O51 Insect Neuropeptides: A Basis for the Design of a Novel Group of Insect Control Agents: The PK/PBAN Family as a Case Study

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Insect neuropeptides (Nps) are prime targets in the search for novel insecticides, since they regulate many physiological and behavioral processes. Their blockers (antagonists) may disrupt and interfere with the normal growth, development and behavior of insects, and can yield, therefore, receptor-selective, insect specific control agents. The chemical nature of Nps enables them to be used as the basis for the design of a generic group of non-toxic environment friendly insect control agents - an approach that has been applied to human Nps in the pharmaceutical industry.

In the course of our research we have developed a novel generic strategy which enables development of simple and cost effective Np antagonist-based insecticide prototypes. The strategy, which is based on a rational design approach of small molecule antagonists based on a known Np agonist, was applied to one of the major insect Np families the Pyrokinin (PK)/Pheromone Biosynthesis Activation Neuropeptide (PBAN) family, known to regulate many key functions in insects (associated with mating, feeding, development and defense). Currently we have highly potent molecules which inhibit PK/PBAN mediated functions and are, metabolically stable and highly bio-available through the cuticle. In addition, two receptors that mediate the PK/PBAN bioactivity have been cloned, stably expressed in an insect cell line and characterized structurally and functionally. The expressed receptors can be used as high throughput assay for discovery of insect control agents from various libraries. Our achievements on the above issue will be presented.

O52 A molluscan neuropeptide elevenin regulates the body color via a G protein-coupled receptor Nl-A42 in the brown planthopper Nilaparvata lugens

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Body-color polymorphism is a widespread phenomenon in insect species. The brown planthopper (BPH), *Nilaparvata lugens* (Stål) displays a polymorphism for body color in wild populations, but its endocrine mechanism exhibiting color polymorphism remains to be explored. We employed comprehensive RNA interference (RNAi) screening of neuropeptides and G protein-coupled receptors (GPCRs) for neuropeptides to explore their functions, and found that a neuropeptide elevenin (Nl-elevenin, KVNCRKFVYAPVCRGVAA) and an orphan G protein-coupled receptor, Nl-A42, are involved in the regulation of body color. Injection of dsRNA against the genes encoding Nl-elevenin precursor or Nl-A42 at 3rd or 4th nymphal stage caused blackish body color of adult in a dose-dependent manner. Several neuropeptides are reported to regulate the body color in other insects, but knockdown of the genes encoding other neuropeptide GPCRs or neuropeptides did not have any effect on the body color in *N.lugens*. Calcium assay with aequorin indicated that the Nl-A42 heterologously expressed in HEK293 cells exhibited responses to synthetic Nl-elevenin peptide from 10^{-9} M. A related peptide Nl-ETH and Cys^{4}Ala-mutated Nl-elevenin had no response to the expressed Nl-A42. These results support the previous report that Nl-A42 belongs to the family of receptors for *Platynereis* L11/elevenin peptide (Bauknecht and Jékely, 2015). This is the first report of a physiological function of elevenin-like peptides in insects.

Bauknecht P and Jékely G.(2015) Cell Rep. 12(4):684-93.

O53 The sulfakinin signalling system regulates feeding and digestive processes in the migratory locust, Locusta migratoria

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The fundamental physiological processes of feeding and digestion are essential to acquire sufficient building blocks and energy to fuel anabolic processes, such as growth, development and reproduction. A rigid regulatory system is required to make sure that these processes are performed in a spatially and temporally controlled environment. The regulation of food uptake and digestion is coordinated by the nervous and endocrine systems, in part by the action of neuropeptides. One of the multiple neuropeptides involved in the regulation of feeding and digestion in insects is sulfakinin (SK). SK is a sulfated insect neuropeptide that is homologous to vertebrate cholecystokinin and gastrin. SK inhibits food uptake and influences intestinal contractions in various insects. However, multiple effects of SK on the regulation of feeding and digestion have only been reported in the holometabolous fruit fly, *Drosophila melanogaster*, so far. In order to expand the knowledge on the sulfakinin signalling system and its control of feeding and digestion, an in-depth characterization of the function of SK in the migratory locust, *Locusta migratoria* was performed. The size of *L. migratoria* allows for straightforward physiological testing by means of peptide injections and microdissection. *L. migratoria* also displays a potent RNAi response, which enables the opportunity to perform (partial) loss-of-function experiments. Multiple experiments were performed to gain insight into the regulation of feeding and digestion by SK in *L. migratoria*. A tissue distribution analysis using qRT-PCR revealed the expression pattern of a putative SK receptor. Injection of SK provided confirmation that it can function as an inhibitor of food uptake in *L. migratoria*. Furthermore, SK injections caused a significant downregulation of digestive enzyme activity in gut secretions and mediated the removal of partially digested material and digestive enzymes from the gastric caeca. The sulfated tyrosine residue was revealed as an indispensable structural requirement for biological activity of SK in *L. migratoria*. Finally, an RNAi knockdown of the receptor transcript suggested a potential interaction between the putative SK receptor and SK peptide *in vivo.*

O54 Beetles of the superfamily Scarabaeoidea are a rich source for novel peptides of the adipokinetic hormone family

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All insects investigated to date, synthesize peptides of the so-called adipokinetic hormone (AKH) family in neurohemal organs called the corpora cardiaca. The AKH peptide family is named after the first fully characterized members and their most prominent functions, amongst others, the release of diacylglycerides into the hemolymph of locusts to supply metabolic fuel for locomotory needs. From an evolutionary point this interesting peptide family is not restricted to arthropods but has already evolved in molluscs. In arthropods, it is noteworthy that with respect to primary structure of the AKH family peptides, there is very little diversification detected in the Crustacea to date. In contrast, the biodiversity of AKHs in insects is large and more than 60 members have been structurally characterized. It is not surprising then that the most species-rich Order of insects, the Coleoptera (beetles), contributes 15 different AKHs. Ten percent of all beetle species, thus about 35 000, comprise the superfamily Scarabaeoidea, which contains such environmentally and thus economically important families as the dung beetles. Our knowledge of AKHs in this large superfamily is still in its infancy. To broaden our data base we have analyzed the CC of eight species from Scarabaeoidea by bioassay and mass spectrometry for their complement of AKH peptides. We investigated and report here on species from the more basal families (Lucanidae, Geotrupidae) and different subfamilies and tribes from the Scarabaeidae.

Two species of the family Geotrupidae, *Trypocoris vernalis* and *Typhaeus typhoeus*, and two species of the subfamily Cetoniinae of the family Scarabaeidae, *Mausoleopsis amabilis* and *Cetonia aurata*, synthesize the octapeptide pGlu-Leu-Asn-Tyr-Ser-Pro-Asp-Trp amide, which was known before to occur in a member each of Geotrupidae and Cetoniinae and is denoted Melme-CC. The species *Kheper bonelli*, a member of the subfamily Scarabaeinae of the Scarabaeidae contains two octapeptides known previously from two other Scarabaeinae species and code-named Scade-CC-I (pGlu-Phe-Asn-Tyr-Ser-Pro-Asp-Trp amide) and Scade-CC-II (pGlu-Phe-Asn-Tyr-Ser-Pro-Val-Trp amide). The Scarabaeinae *Circellium bacchus*, a large flightless dung beetle, also contains Scade-CC-II but in addition, has a novel octapeptide code-named Cirba-AKH with the sequence: pGlu-Phe-Asn-Phe-Ser-Ala-Gly-Trp amide. The other scarab beetle investigated, *Euoniticellus intermedius*, has only one octapeptide, Euoin-AKH, and this is a novel sequence: pGlu-Ile-Asn-Phe-Thr-Thr-Gly-Trp amide. The lesser stag beetle, *Dorcus parallelipipedus*, from the basal family Lucanidae has two AKH members: one is the well-known and possibly ancestral scarab AKH code-named Melme-CC and the other is a novel member denoted as Dorpa-AKH with the sequence pGlu-Val-Asn-Tyr-Ser-Pro-Val-Trp amide.

We believe that the species radiation in the superfamily Scarabaeoidea may very likely also be reflected in the number of unique AKH sequences, and we expect numerous novel structures to be elucidated from this superfamily during the next few years.

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O55 Adipokinetic hormone and its receptor in the desert locust, Schistocerca gregaria

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Adipokinetic hormones (AKHs) are a family of neuropeptides that consist of 8-10 amino acids and have an N-terminus blocked by pyroglutamate and a C-terminus blocked by an amide group. AKHs show significant structural similarity with the red pigment concentrating hormones (RPCHs) of crustaceans and thus these neuropeptides were grouped in the AKH/RPCH family. AKH and RPCH are arthropod homologues of the vertebrate gonadotropin-releasing hormone (GnRH) family.

AKH is a central regulator of energy metabolism in insects. It is secreted by the *corpora cardiaca* into the hemolymph in conditions of intense skeletal muscle activity during energy demanding processes (such as flight) and regulates the mobilisation of energy-rich metabolic substrates from the fat body. AKH can mobilise three different energy sources, partly depending on the process requiring energy and sometimes even on the insect's life stage: stored glycogen can be broken down to trehalose, lipids can be converted to diglycerides and proline can be used as an energy source as well. AKHs also inhibit energy demanding anabolic processes, such as protein and lipid biosynthesis, during periods of intense muscle activity. Besides its main role as the pivotal energy regulating neuropeptide, AKH fulfils a number of other functions as well. AKH exerts its action through AKH receptors, which are rhodopsin-like G protein-coupled receptors that are related to vertebrate GnRH receptors.

In this study, we investigated the transcript levels of three AKH precursor genes and the AKH receptor gene in different tissues and during different physiological conditions in the desert locust. We also studied the ligand-receptor interactions and performed RNAi experiments.

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O56 Neuropeptide control of crop function of the dipteran pests, Delia radicum and Drosophila suzukii

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The spotted wing drosophila, *Drosophila suzukii*, and the cabbage root fly, *Delia radicum,* are important pests of European agriculture causing substantial economic damage. There is now an urgent need to develop new control agents with novel modes of action to replace conventional insecticides that have been compromised through the development of resistance. In order to identify new insecticide targets, we have focused attention on the neural control of crop and midgut physiology of *D. radicum* and *D. suzukii.* Myosuppressin (MS, TDVDHVFLRFamide) is an insect neuropeptide known to inhibit contractions of insect muscle needed for the movement of food within the digestive tract. We have identified MS in the crop nerve bundle of both *D. suzukii* and *D. radicum* by direct tissue peptide profiling using MALDI-TOF mass spectrometry and have visualized by immunocytochemistry the presence of MS-like peptides in the nerve bundle and in nerve fibres that spread over the surface of the crop and innervate the proventriculus and anterior region of the midgut. MALDI-TOF of the nerve bundle from *D. radicum* also identified short neuropeptide F $^{4-11}$ (sNPF$^{4-11}$, SPSLRLRFamide), a peptide that shares the C-terminal LRFamide sequence with MS.

Using a bioassay for crop contractions, we show that MS is a potent inhibitor of the spontaneous muscle activity of both fly species with an IC_{50} of 3 x10^{-9} M and 4.4x10^{-8} M for *D. suzukii* and *D. radicum,* respectively. sNPF$^{4-11}$, which was also present in the nerve bundle of *D. radicum,* did not display any inhibitory activity when applied to the crop of this insect at concentrations up to 10 $^{-4}$ M. Benzethonium chloride (Bztc), a non-peptidyl agonist of myosuppressin peptides, also inhibited crop contractions in both insect species with IC_{50} values of 1.0 x10^{-5} M and 7.2 x10^{-6} M for *D. suzukii* and *D. radicum*, respectively.

These results indicate a key role for myosuppressin in the regulation of adult crop function in two dipteran pests and form the foundation for the rational design of potent non-peptidyl agonists targeting the myosuppressin receptor of the crop in order to disrupt gut physiology.

O57 Oxytocin-like signalling in ants

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The oxytocin/vasopressin-like (OT-like) signalling system comprises G-protein coupled receptors (GPCR) and their ligand peptide. It is one of the oldest and best-studied peptidergic signaling system, and in evolution it is considered to date back more than 600 million years. In invertebrates similar to the vertebrates, OT-like signalling appears to be important for water homeostasis, reproduction, learning, memory and behaviour [1]. To date its biological function in insects has only been studied in the beetle *Tribolium castaneum* where it has been implicated in water retention [2; 3] even though insects constitute the largest and most diverse group of organisms on earth making up at least half of the global species diversity. In recent years the genomes of different ant species have been sequenced and the putative OT-like precursor and receptor were discovered [4]. It was the first evidence that OT-like signalling system exists in social insects and possibly regulates individual physiology and social organization in ant colonies. For our study we have chosen two ant species of the genus *Lasius* that are closely related genetically, but significantly differ in their ecology and colony structure [5].

Following pharmacological characterization of the ligand-receptor pair *in vitro*, our aim was to quantify expression levels of both the receptor and the inotocin peptide precursor in different parts of the body and developmental stages in ants using quantitative PCR. In contrast to beetles where both genes exhibit the highest expression in the larvae stage [3], the expression of the receptor and precursor in early broods (eggs and larvae) of ants is about an order of magnitude smaller compared to pupae or worker. Next we dissected organs of gaster of workers, queens and males and showed that in most of the cases the highest expression of receptor exists in nervous system, crop, fat body and testis while it is very low in Malpighian tubules, ovaries, glands and midgut. Using immunostaining of ant brains, the inotocin was found in two cell bodies - the subesophageal ganglion - as described for other insects [2]. qPCR data also confirmed that precursor of inotocin is mainly expressed in the head.

These findings indicate that the expression patterns of inotocin and its receptor can be different in distinct insect species and provide further hints about diverse biological functions of this important neuropeptide signalling system. These preliminary outcomes will be important for further functional studies to elucidate the role of inotocin signalling for individual and group level behaviour in ant societies.

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[1] Gruber CW. Exp Physiol. 2014; 99(1):55-61. [2] Aikins MJ et al., 2008; Insect Biochem Mol Biol. 38(7):740-8. [3] Stafflinger et al., 2008; Proc Natl Acad Sci USA. 105(9):3262-7 [4] Gruber CW, Muttenthaler M. PLoS One. 2012; 7(3):e32559. [5] Cremer S et al., 2008; PLoS One. 3(12):e3838.

O58 Cell-specific distribution of three ion-transport-peptide splice forms in the central and peripheral nervous system of larval and adult Drosophila

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First discovered as antidiuretic hormone in locusts, ion transport peptide (ITP, a member of the crustacean hyperglycemic hormone family) has been found in all investigated insects. In *Drosophila melanogaster*, three splice-forms derive from a single 5-exon gene; they share the identical first half of the peptide but are very different in the C-terminal parts, i.e. a short form ITP (73aa) and two longer forms ITPL1 and ITPL2 (both 87aa). As localised using splice-form specific antibodies, ITP occurs in 3-4 groups of neurons throughout the central (CNS) and in distinct putative sensory neurons of the peripheral nervous system (PNS), whereas the ITPL1/L2 occur only in putative PNS sensory neurons (so-called lbd-neurons) innervating alary muscles of the heart. In larvae, ITP is only co-localised with allatostatins and CCAP in hindgut innervating neurons, but in adults also with CCAP in heart neurons and other neuropeptides such as sNPF, NPF in CNS neurons. One large ITP-neuron type is a typical pars lateralis neurosecretory neuron innervating the corpora cardiaca (incl. adipokinetic hormone-cells) and corpora allata intrinsic cells. These neurosecretory ITP-neurons also transiently innervate muscles to the so-called ptilinum, a structure used by pharate adults during head eclosion. In adults only, an ITP-interneuron is identified as a ('evening') clock-neuron in a similar position but different from the ('morning') pdf-clock neurons. Interestingly, one long splice-form, ITPL1, also occurs in the larval non-neuronal Inka-cells. These cellular distributions of itp-gene derived splice-form-specific innervation patterns of very different target structures suggest several important functions associated with moulting, homeostasis and heart beat control apart from at least one distinct interneuronal function in the biological clock.

O59 CAPA neuropeptides in the mosquito, Aedes aegypti : anti-diuretic actions and cellular distribution

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Insects rely on various hormones to maintain ionic and osmotic homeostasis of their haemolymph. These hormones act upon the excretory system, which is composed of the Malpighian tubules (MTs) and hindgut. One such iono/osmoregulatory hormone is a peptidergic factor belonging to the CAPA family that is produced following processing of the precursor polypeptide encoded by the *capability* gene. In the mosquito *Aedes aegypi*, an important vector of various diseases including Zika virus, peptidomic studies have revealed the structures of the endogenous CAPA peptides but their physiological roles have not been fully elucidated. In adult stage mosquitoes, a diuretic action for CAPA peptides was determined using doses at or above the mid-nanomolar range. Later studies on larval-stage mosquitoes have identified an anti-diuretic function for the CAPA peptides at femtomolar doses, inhibiting serotonin-stimulated fluid secretion by MTs. To better understand this potentially complex regulatory mechanism, we have investigated the regulation of fluid secretion by isolated MTs using various known diuretic factors and have also tested the activity of an endogenous CAPA neuropeptide. Moreover, combining immunohistochemistry and fluroscent *in situ* hybridiztion, we have confirmed cell-specific expression of CAPA neuropeptides in the nervous system and have identified CAPA-like immunoreactivity associated with gut innervations in the adult stage mosquito. Finally, we have isolated the elusive CAPA receptor in *A. aegypti* and have examined its transcript expression profile in all post-embryonic developmental stages and in various adult-stage tissues. Our findings help to better understand the physiological functions regulated by the CAPA peptides in the mosquito and, more generally, clarify the interplay between the various regulators of fluid and ion secretion by isolated insect MTs.

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O60 Signals for cells: signalling and transport in the insect Malpighian tubule

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Insects are the most successful Class of life on Earth. Scaling arguments suggest that terrestrial insects are in constant danger of desiccation, yet their excretory system operates at a very high rate. The mechanism and control of osmoregulation are thus mission-critical to an insect's success. Our understanding of these processes has undergone a step-change in the last decade, at least in part through the combination of genetic, molecular and physiological approaches in Drosophila melanogaster.

Here we review the current state of understanding of renal function in Drosophila, and the extent to which it can be applied to the broader phylogeny of insects.

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1: Chintapalli VR, et.al. PNAS 2015 Sep 15;112(37):11720-5. 2: Halberg KA, et.al. Nat Commun. 2016 Apr 13;7:11266. 3: Halberg KA, et.al. Nat Commun. 2015 Apr 21;6:6800. 4: Cabrero P, et.al. PNAS 2014 Sep 30;111(39):14301-6. 5. Chintapalli VR, et.al. PLoS One. 2013 Oct 29;8(10):e78066.

O61 Androgens, muscle, and athleticism: uncovering mechanisms of physically elaborate reproductive behavior

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In diverse species, androgenic hormones organize and activate masculine reproductive behavior. These effects are usually studied at the level of the brain, with special attention to the neural circuitry that underlies appetitive and consummatory components of sexual behavior. However, in many animals, motor command is also an important factor that influences the sexual interactions between individuals, including the way in which males compete with rivals, court females, and copulate with mates. Yet, little is known about how androgenic hormones modulate reproductive motor skills, especially by acting outside of the brain. To investigate this issue, I study the hormonal basis of a physically elaborate courtship display. I use the golden-collared manakin (*Manacus vitellinus*) as a model system, as males of this tropical bird rapidly and repeatedly snap their wings together above their back to help solicit copulations from females. Results suggest that androgenic activation of androgen receptor (AR) in the wing muscles mediates the fine motor skills required to perform these so-called “wing-snaps.”Through additional work using RNA-Seq, I further demonstrate the functional effects of muscular AR that likely support this behavior. For example, androgenic stimulation of manakin skeletal muscle increases the expression of genes that enhance fuel metabolism, ion trafficking across the muscle cell membrane, and filament contraction. These effects are strongest in the skeletal muscles that play a prominent role in driving the wing-snap, which suggests that androgens prime muscle performance for courtship in a tissue-specific manner. Furthermore, comparative analyses show that the relative abundance of AR expressed in the wing muscles co-evolves with species differences in the physical complexity of courtship displays. Because they have one of the most complex courtship routines, golden-collared manakins also maintain some of the highest AR levels in their wing musculature compared to a variety of other taxa. Overall, these studies not only indicate that androgens act on skeletal muscles to mediate complex sexual motor skills, but they also imply that androgen-muscle relationships evolve to support sexually selected behavioral displays.

O62 Testosterone Regulation of Physical Activity Behavior: Mechanisms of Action

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The beneficial effects of exercise on the musculoskeletal system are widely recognized. In this line, androgens promote the engagement to physical activity in rodents and increase self-perceived energy in hypogonadal men. However, the mechanism by which androgens stimulate physical exercise behavior remains unclear. Our aim was to answer three specific questions about how androgens influence exercise: 1) does testosterone (T)-induced stimulation of activity depend in part on its aromatization into estradiol (E2)? 2) to what extent are the effects of androgens exerted through central (motivation) independent from peripheral (muscle) actions? and 3) are brain dopaminergic (DA) pathways implicated in the T regulation of activity? For this purpose, we determined wheel running (WR) and home-cage activity (HCA) in two male mouse models of androgen deficiency: androgen receptor knockout (ARKO) and orchidectomized (ORX) mice. Global ARKO mice displayed a 67 and 24% reduction in WR and HCA, respectively, compared to WT. Similarly, ORX diminished by 80 and 50% the intensity of WR and HCA. Treatment with T was twice as effective as dihydrotestosterone (DHT) (226% vs. 106%) in restoring WR after ORX. The role of T aromatization into E2 in promoting WR was confirmed by treating ARKO mice with T and DHT. As expected, DHT-treated ARKO mice showed no response compared to placebo, while T induced a dramatic enhancement (250%) of WR. The involvement of a neuromuscular dysfunction in the two models was ruled out by observing no alterations either in the rotarod and grip strength test nor in the compound muscle action potentials or motor neuron counts. To corroborate the implication of central DA pathways, mice were treated with low-dose amphetamine (0.75 mg/kg). Amphetamine induced a rapid and transient hyperlocomotion response in WT animals, which was more pronounced in ARKO mice, suggesting a dopamine dysregulation. Similarly, an enhanced response to amphetamine was observed in ORX animals, which was completely abolished by T but not DHT. We conclude that T regulates physical activity behavior in male mice centrally trough a dual model of action (androgenic and estrogenic) although the effects of E2 appear to dominate. If the psychological effects of T on exercise behavior are confirmed in humans, it would represent an additional benefit of T replacement therapy in aging men with sarcopenia, dysmobility and low T.

O63 Testosterone modulation of adrenocortical activity and adrenal immunolocalisation of the androgen receptors in the Saharan gerbil Gerbillus tarabuli

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Adrenal corticosteroids were known to modulate gonadal activity in several mammals, but reciprocal effects of sexual hormones on the hypothalamo-pituitary-adrenal (HPA) axis have received much less attention particularly among desert Mammals. In this work we investigated, in *Gerbillus tarabuli* living in the Algerian Sahara desert, the effects of gonadectomy and testosterone replacement, performed during the breeding season (from mid winter to late spring) on adrenocortical activity and plasma ACTH. Thirty nine adult males obtained from the Béni Abbès area (wilaya of Béchar, 30°7' N, 2°10' W) in january-february, were divided into three groups: control (n=13), bilaterally castrated (50d; n=13) and castrate treated twice daily by 75µg testosterone enanthate/40µl sesame oil/100g p.c. (7d, n=13). After euthanizing animals, adrenal glands were quickly removed, weighed and right ones were fixed in 10% formalin and used for androgen receptor (AR) immunohistochemistry; semi quantitative evaluation of immunostaining was appreciated in each cortical zona (number of marked cells/total cells per 100 cells field). Blood was collected into heparinized tubes for cortisol assay and into EDTA tubes for ACTH, aldosterone and androstenedione assays. Cortisol and ACTH were analyzed by electrochemiluminescence method, androstenedione by ELISA and aldosterone by RIA. Intra and inter-assay coefficients of variation were less than 8% for all hormones according to the kit providers. Sensitivity was respectively 0.018µg/dl for cortisol, 1pg/ml for ACTH and 0.021ng/ml for androstenedione.

In control gerbils, the AR immunostaining was detected into glomerulosa (33%) and fasciculata (30%) zona whereas reticularis cells were weakly immunostained. Orchidectomy increased the AR immunostaining in glomerulosa (+33% *vs* control; $p>0.05$) and fasciculata (+73% *vs* control; $p<0.05$) and make it appeared in the reticularis (45% *vs* less than 5% in control). Testosterone replacement restores the control status in AR immunostaining in fasciculata (- 44%, $p<0.05$) and reticularis (less than 6%) but not in glomerulosa which showed more labeling (59%). Androgen deprivation induced an increase in relative adrenal weight (+32%; $p<0.01$) and plasma cortisol concentrations (+94%; $p>0.05$), whereas plasma androstenedione, aldosterone and ACTH concentrations decreased (-37%; -57% and -40% respectively; $p>0.05$). Except for plasma ACTH concentrations which were unchanged, testosterone replacement in castrate restores the adrenal weight and hormonal control status.

Changes induced by orchidectomy in adrenal AR localization suggested that, in the Saharan gerbil *Gerbillus tarabuli*, testicular androgens modulate the adrenal corticosteroidogenesis probably by inhibiting glucocorticoids either directly *via* their specific receptors or *via* the hypothalamic pituitary axis. These results suggest the involvement, at least in part, of testicular androgens as endogenous mechanism, in the seasonal activity of pituitary-adrenal axis. This regulation, in association with exogenous factors, leads to the success of reproduction of this species living in a harsh environment.

O64 The role of estrogen receptors and aromatase in carp leukocyte activation.

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Next to the effects exerted on various physiological processes such as sexual differentiation and reproduction, it is well documented for mammals that estrogens are important immunoregulatory hormones. Estrogen-induced immunoregulation can be mediated via nuclear (ERs) or membrane (GPR30) receptors that are expressed differentially on/in leukocyte populations. Moreover, estrogens can work without direct receptor interaction by interfering with the synthesis or the metabolism of steroid hormones e.g. by changing the activity of aromatase cytochrome P450 which is critical for the conversion of C19 steroids to estrogens.

In teleosts, the expression of both estrogen receptors as well as aromatase in the immune system is less explored and their involvement in the regulation of the immune response is poorly understood. Therefore, the present study aimed to measure the expression of estrogen receptors and aromatase in the lymphoid organs and in different populations of leukocytes from common carp. Furthermore we checked the effects of their agonists/antagonists on leukocyte activity.

We found that all three genes for estrogen receptors *erα, erβ* and *gpr30,* as well as two genes encoding aromatase: *cyp19a* and *cyp19b,* were constitutively expressed in lymphoid organs and in different populations of carp leukocytes. Interestingly, in monocytes/macrophages PMA-stimulation up-regulated *cyp19a* expression, while LPS-stimulation up-regulated *cyp19b* expression. Moreover upon restraining stress the cyp19a and b expression was modified both in carp leukocytes and in organs of HPI axis.

Furthermore, we found that 17β-estradiol (E2) and G1 (GPR30 agonist) increased the PMA-induced production of reactive oxygen species in carp monocytes/macrophages. Interestingly, this reaction was reversed with G15 or wortmannin pretreatment but not with ICI 182:780. These data reveal that the GPR30 receptor is involved in E2-induced up-regulation of monocyte/macrophage activity.

In summary, estrogen receptors and aromatase are expressed in carp leukocytes and lymphoid organs and they participate in the regulation of the immune response.

O65 Sex hormone-binding globulin (SHBG) regulation of androgen and estrogen bioactivity: of mice and men

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Sex hormone-binding globulin (SHBG) is the high-affinity binding protein for androgens and estrogens. According to the free hormone hypothesis, SHBG modulates the bioactivity of sex steroids by limiting their diffusion into target tissues. Still, the in vivo physiological role of circulating SHBG remains unclear, especially since mice and rats lack circulating SHBG post-natally. To test the free hormone hypothesis in vivo, we examined total and free sex steroid concentrations and bioactivity on target organs in mice expressing a human *SHBG* transgene. SHBG increased total androgen and estrogen concentrations via hypothalamic-pituitary feedback regulation and prolonged ligand half-life. Despite markedly raised total sex steroid concentrations, free testosterone was unaffected while sex steroid bioactivity on male and female reproductive organs was attenuated. This occurred via a ligand-dependent, genotype-independent mechanism according to in vitro seminal vesicle organ cultures. These results provide compelling support for the determination of free or bioavailable sex steroid concentrations in medicine, and clarify important comparative differences between translational mouse models and human endocrinology.

O66 Mixtures suspected to impede neurodevelopment and metabolism affect thyroid hormone signaling in Xenopus laevis

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Endocrine disrupting chemicals (EDCs) have been shown to be harmful to wildlife and human health. Most of the work done on EDCs remained on single molecules studies whereas a more realistic exposition paradigm should be done with mixtures. This discrepancy gives fuel for inaction regarding legislation and risk assessment. A starting point of European EDC-MixRisk project is analyses of epidemiological data already available from two large European pregnancy cohorts (SELMA and Life Child). Over all aim with these studies is to identify prenatal EDC mixtures that are associated with adverse health outcomes in offspring children. In a pilot study mixtures of EDCs were found to be associated to health outcomes in three domains: one associated to growth and metabolism (mixture G), one associated to neurodevelopment (mixture N), and another one associated to sexual development (mixture S). The endocrine disrupting potential of these three mixtures are being assessed by a battery of *in vivo* and *in vitro* bioassays. In Pr Demeneix's lab, mixes G and N were screened for thyroid hormone disruption using one week old transgenic *Xenopus laevis* embryos harboring a TH/bZIP-eGFP construct (XETA assay). Our results show that the two mixtures G and N exhibit thyroid disrupting effects at any concentration tested, high concentrations tested (100x an 1000x more than found in the SELMA cohort) with a T3 co-treatment and also low concentration (0.1x and 1 fold concentration measured in SELMA) exhibit slight but significant effect when tested alone. Due to crucial need of accurate amounts of thyroid hormones during embryonic development and normal brain development, we tested assessed behavior of tadpoles exposed to the two mixtures. The alteration of mobility of stage 45-48 tadpoles in response to both mixes were as well investigated using a tracking system. The mixture G did not affect the mobility of the tadpoles. However, a 3-day co-application of the mixture N with T3, significantly increased the mobility compared to the control group of tadpoles exposed to T3 only. Gene expression in brain of exposed tadpoles to mixture N is being investigated by qPCR. Combining all the results from the *in vivo* and *in vitro* studies involved in this project will promote better risk management for EDCs and their mixtures.

O67 Role of corticotropin-releasing hormone in the thyroidal activity in avian life stage transitions

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Thyroid hormones and corticosteroids act as developmental hormones, responsible for several metabolic and developmental processes in vertebrate life stage transitions such as amphibian metamorphosis and hatching in precocial birds. Since both groups of hormones often increase simultaneously and act synergistically in vertebrate life stage transitions, corticotropin-releasing hormone (CRH), stimulator of both adrenocorticotropic hormone (ACTH) and thyroid-stimulating hormone (TSH) release in species of non-mammalian classes, could play a role in the neuroendocrine coordination of the transitional processes by regulating both the adrenal and thyroidal axes. The dual function of CRH affect the timing of amphibian metamorphosis; however, it is not known whether CRH controls other vertebrate life stage transitions as well, such as in avian species. We first investigated the effect of daily *in ovo* injection of 2 µg CRH from embryonic day 10 to 18 on hatching time in a precocial bird, the chicken. CRH treatment was found to accelerate average hatching time by 8 hours. We then investigated the TSH-releasing capacity of CRH in an altricial species, the zebra finch (*Taeniopygia guttata*). The cellular localisation of type 2 CRH receptor (CRHR2) mRNA, shown to be involved in the TSH-releasing effect of CRH in chickens and amphibians, was determined by *in situ* hybridisation, combined with immunohistochemical staining of pituitary thyrotropes. Our study showed that CRHR2 mRNA is present in the zebra finch thyrotropes, similar to what was previously found in chicken and frog pituitary. Furthermore, isolated zebra finch pituitaries stimulated with 100 nM CRH showed increased secretion of TSH-like activity as measured in a thyroid bioassay. These results suggest that the dual role of CRH exists in both precocial and altricial avian species and may be a key mediator of avian life stage transitions, such as hatching in precocial birds. We speculate that in altricial birds, the TSH-releasing activity of CRH may be involved in the control of fledging.

O68 Intracellular thyroid hormone availability in the chicken embryo: an in situ localization study of transporters and deiodinases

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Introduction Thyroid hormones (THs) are known to be important in vertebrate development. Abnormal TH levels can lead to major developmental deficits, such as mental retardation. To understand the processes that underlie these deficits, we study the influence of THs on early development using the chicken embryo as a model. In order to analyze -and influence- the supply of THs to specific tissues and cells, the key regulators of TH availability need to be located. We present here the localization of several of these regulators throughout embryonic development, namely L-type amino acid transporter 1 (*LAT1*) and its partner 4F2 cell-surface antigen heavy chain (*4F2hc*), organic anion transporting polypeptide 1C1 (*OATP1C1*), monocarboxylate transporters 8 (*MCT8*) and 10 (*MCT10*) and TH-activating type 2 deiodinase (*DIO2*).

Methods & Results White Leghorn eggs were incubated for 6, 10 or 18 days and the entire body (E6, E10) or selected organs (E18) were sampled and fixed. Cryosections were analyzed by *in situ* hybridisation (ISH) allowing to visualize regions with strong mRNA expression. LAT1 expression was additionally analyzed at the protein level by immunohistochemistry (IHC).

We found a stage-dependent expression of the different target genes in several embryonic organs. At different stages of eye development expression was found in the lens (*LAT1*), ciliary body (*LAT1*, *MCT10*) and retina (*LAT1*, *MCT8*, *DIO2*). At E6 *MCT10* was found in liver and *DIO2* in heart and at E18 *MCT8* was present in kidney and testis. At all tested stages, both *MCT10* and *LAT1* were present in the pancreas. The latter had a pattern similar to the preproprotein for *glucagon*, indicating its presence in glucagon-producing α cells. Furthermore, structures such as the feather buds (*LAT1*, *MCT8*, *DIO2*) and the beak (*DIO2*) also seem to depend on THs for their development. All regulators were found in the embryonic chicken brain, distributed among neurons, glia and -above all- both barriers: the choroid plexus and blood-brain barrier (BBB). Interestingly, the spinal cord extends the patterns found in brain with *LAT1-DIO2* co-expression in blood vessels and *MCT8* located in grey matter. We also studied the expression pattern of *4F2hc*, the auxiliary protein predicted to guide LAT1 to the plasma membrane. This partner was clearly present in *LAT1*-expressing structures, such as eye and pancreas, in line with the proposed interaction.

Additionally, through IHC of brain sections, we were able to show the presence of the LAT1 protein at both luminal and abluminal membranes of the BBB endothelial cells.

Conclusion The strong and stage-dependent expression of an arsenal of TH-regulating genes suggests organs such as eye, pancreas and especially brain, to be highly under control of THs for their development. This is similar to what has been found for other vertebrates, valuing the chicken embryo, with its easy *in ovo* manipulation, as an important model in studies of THs and vertebrate development.

O69 Type 2 deiodinase in the developing song system of the zebra finch: a path to neuroplasticity?

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The zebra finch (*Taeniopygia guttata*) song system consists of several interconnected brain nuclei that show a marked difference in size and development between males and females. While the young zebra finch brain already reaches adult size by 20-30 days, the song nuclei in young males continue to show a high degree of neuroplasticity during the song learning period until they reach adulthood and their song crystallises. Although a lot of research has been conducted on how these changes occur, the precise molecular mechanisms remain poorly understood. Thyroid hormones (THs) have been associated with neural development in several animals, but surprisingly have been mostly overlooked in songbirds. We hypothesised that THs play a role in the development of the song nuclei and consequently in the song learning process. We therefore performed *in situ* hybridisation for type 2 deiodinase (*DIO2*), the most important TH-activating enzyme in the brain, on both male and female zebra finch brains at multiple time points during the song learning process: 10, 20, 30, 40, 50, 60, 90 and 120 days post hatch (dph). These time points span the duration of the sensory phase (20-60 dph), the sensorimotor phase (30-90 dph) and the crystallisation phase (90-120 dph) of song learning. As female zebra finches do not learn to sing, they provide a good reference for the same brain regions with presumably minimal plastic changes.

Our results show that *DIO2* is expressed in the endothelial cells of the blood-brain barrier throughout the entire brain in both sexes at early stages (10-20 dph), correlating with the intense neuronal proliferation and differentiation at those stages. However, while the expression levels gradually become undetectable after 30 dph in almost the entire brain, *DIO2* expression remains strong up to 60 dph in the endothelial cells in the nuclei Area X of the striatum, HVC (used as a proper name) and RA (robust nucleus of the arcopallium) of the male brain. The 30 dph time point marks the onset of the sensorimotor phase, in which the birds start producing their own song and several song nuclei start to increase in volume. By the time the song crystallises, *DIO2* expression recedes entirely. In females however, *DIO2* expression is limited to non-existent in the entire brain from 30 dph onwards. These results indicate there is a high degree of local thyroid hormone activation during the period for song learning in males and suggest that THs have a direct or indirect role in the development of the song nuclei. We can conclude that during the majority of both the sensory phase and the sensorimotor phase, THs are activated specifically in the endothelial cells of several song nuclei in males. This could either influence the development of the blood vessels, increasing neurotrophin expression and the influx of nutrients in the region, or the active TH could be transported to the surrounding neural tissue and induce plasticity through activation of TH-responsive genes.

O70 The developing cerebellum in need of thyroid hormones: a crucial role for monocarboxylate transporter 8

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Introduction Thyroid hormone (TH) supply to the developing vertebrate brain needs to be strictly regulated, and intracellular TH uptake by various transmembrane TH transporters is an essential step. The monocarboxylate transporter 8 (MCT8) has gained particular interest in this context, because mutations in the human gene coding for MCT8 result in a pathological condition accompanied by profound neurological deficits. Although altered TH tissue levels are the likely cause of this phenotype, the precise underlying molecular and cellular mechanisms leading to these deficits remain to be elucidated. We used the chicken embryo as a model to study the impact of MCT8 deficiency on cerebellar development. This highly TH-sensitive brain region is a common model to study corticogenesis because of its rather simple cyto-architectural organisation. Moreover, Purkinje cells (PCs), the sole output neurons of the cerebellum, strongly express MCT8 throughout embryonic development, and probably rely on this transporter for appropriate TH supply. Disruption of TH signalling in this cell type may lead to structural and functional deficits and ultimately impaired cerebellar functioning.

Methods & Results We first injected and electroporated an RNAi construct into the cerebellar *anlage* at E3, predominantly targeting PCs. *In vivo* knockdown of *MCT8* was confirmed using *in situ* hybridisation. At E6, this resulted in downregulation of *Nestin* and *RORα*, two TH-responsive markers for neural stem cells and early differentiating PCs, respectively. Disrupted differentiation was further confirmed by diminished expression of the PC-specific marker LHX1/5 at sites of transfection. EdU-labelling of granule cell precursors at E10 showed markedly reduced proliferation. As a result, the thickness of the external granular layer was reduced. In addition, a lowered PAX6 signal suggested that the signal for early inward migration of post-mitotic granule cells was affected. These dysregulated processes are likely due to incorrect intercellular cross-talk between MCT8-deficient PCs and granule cell precursors. PC differentiation was also affected at a later stage (E18), since *MCT8* knockdown resulted in smaller dendritic trees. Concomitant with this, Sholl analysis demonstrated reduced dendritic tree complexity as illustrated by the decreased number of branching points.

Conclusion In summary, our results indicate a pivotal role for MCT8 in both early and late embryonic cerebellar development, especially for PC differentiation and granule cell proliferation. We have shown that the underlying mechanisms are at least in part regulated by a TH signalling cascade. As such, our *in vivo* MCT8 silencing approach is a valuable research strategy to unravel the precise cellular mechanisms of TH-dependent brain development.

O71 Unveiling STC and PTHrP actions on metabolism: a metabolomics approach using 1H-NMR

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Stanniocalcin (STC) and parathyroid hormone-related protein (PTHrP) are the two main calcitropic hormones in teleost fishes and the mammalian homologues have multiple functions. Mammals have two isoforms of STC and one of PTHrP, but teleost fishes have twice as many isoforms because of the extra round of genome duplication. The corpuscles of Stannius are unique to fish and are the gland that produces the endocrine factor STC1. In contrast, STC1 in mammals is not produced by a specific gland and has a wide tissue distribution and a paracrine action. PTHrP is expressed in many tissues both in fishes and in mammals. STC1 binding has been associated with the mitochondria and recently a possible role in gluconeogenesis has been identified in rat and fish kidney. The present work tested the hypothesis that STC1 and PTHrP have a metabolic role in European seabass, Dicentrarchus labrax, by analyzing the liver temporal metabolomics response profile to the hormones. Duplicate groups (n=10) of sea bass were given intraperitoneal injections of 1) control 0.9% saline vehicle, 2) 0.5 µg/g PTHrP(1-34), 3) 0.5 µg/g PTHrP(7-34) - a PTHrP antagonist, 4) 0.5 µg/g PTHrP(7-34) together with 2.5 µg/g native STC1 and 5) 1µl/g STC1 antiserum together with 0.5 µg/g PTHrP(1-34). Metabolites from liver samples were extracted in 0.6 M of perchloric acid and identified and quantified using Proton Nuclear Magnetic Resonance (1H-NMR) Spectroscopy. Multivariate analysis, metabolite enrichment and pathway analysis were performed using the web-based platform MetaboAnalyst. There were very significant changes in metabolite profiles in all treatment groups relative to the control. Changes were more pronounced after 24h of treatment compared to 6h. At 24h, treated groups showed significant changes in important metabolites that are involved in energy metabolism, such the amino acids valine, glutamine and alanine as well as the intermediate of the citric acid cycle fumarate. Overall, the groups treated with PTHrP antagonist, or with the PTHrP antagonist and STC1 had relatively higher concentrations of branched-chain amino acids and alanine, which is an important gluconeogenic substrate, suggesting a stimulatory role in gluconeogenesis and the reverse for PTHrP. In conclusion, we confirm that STC1 and PTHrP modify liver metabolite concentrations in sea bass liver, a finding which suggests an antagonistic regulatory role for the two hormones in pathways related to energy metabolism. Future studies will target specific metabolic pathways to test the hypothesis that STC1 and PTHrP have a direct stimulatory role on specific metabolic pathways. Acknowledgements: This study received national funds from FCT - Foundation for Science and Technology through projects PTDC/MAR/121279/2010 and UID/Multi/04326/2013 and doctoral grant SFRH/BD/103185/2014 to PSP.

O72 Model systems in diabetes mellitus research: what can we learn from different species and methodological approaches?

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Diabetes mellitus together with cardiovascular disease is a leading cause of death worldwide. Aside known risk factors for diabetes mellitus such as obesity and genetic predisposition, chronic inflammation has been detected to be an indispensable feature in the pathogenesis of diabetes mellitus. Different types of diabetes mellitus exist, and different model systems in diverse species are applied in research and treatment. We discuss the use of different species and morphofunctional approaches applied in current research for optimised treatment options such as pharmacological and immunomodulatory treatment as well as islet transplantation and generation of beta cells derived from pluripotent stem cells.

O73 Towards dIfferential neuropeptide expression upon trypanosome infection in tsetse flies

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As vectors of trypanosomes tsetse flies are an economically and medically important species since they cause sleeping sickness in humans and nagana in livestock. Upon infection with trypanosomes tsetse flies show behavioral changes, such as increased probing and higher feeding rates. Nearly all physiological processes, including behavior is regulated or modified by neuropeptides. The recent release of the tsetse fly genome allowed the construction of a detailed in silico neuropeptide database, as well as an in-depth mass spectrometric analysis of the most important neuropeptidergic tissues of this insect species. As in vivo peptides can be modified, cleaved, or even mispredicted, mass spectrometric confirmation of predicted peptides is a vital step in the functional characterization of neuropeptides. Using our setup we detected 51 putative bioactive neuropeptides encoded by 19 precursors: adipokinetic hormone (AKH) I and II, allatostatin A and B, capability/pyrokinin (capa/PK), corazonin, calcitonin-like diuretic hormone (CT/DH), FMRFamide, hugin, leucokinin, myosuppressin, natalisin, neuropeptide-like precursor (NPLP) 1, orcokinin, pigment dispersing factor (PDF), RYamide, SIFamide, short neuropeptide F (sNPF) and tachykinin. In addition, propeptides, truncated and spacer peptides derived from seven additional precursors were found, and include the precursors of allatostatin C, crustacean cardioactive peptide, corticotropin releasing factor-like diuretic hormone (CRF/DH), ecdysis triggering hormone (ETH), ion transport peptide (ITP), neuropeptide F, and proctolin. The majority of the identified neuropeptides are present in the central nervous system, with only a limited number of peptides in the corpora cardiaca-corpora allata and midgut. Next, we will compare the neuropeptide content of these neuropeptidergic tissues in infected and non-infected flies using differential peptidomics.

O74 C. elegans as a model to unravel neuropeptidergic control of learning and memory

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Learning and memory are essential biological functions with evolutionary conserved molecular mechanisms. As studying the effects of genes in the human brain is extremely challenging, we need to turn to model organisms. In contrast to the anatomic complexity of mammalian brains, the nematode *Caenorhabditis elegans* has a 'mini-brain' consisting of 302 neurons with a complete connectome. Worms and humans share many similarities in their repertoire of neurotransmitters and neuronal modulators and in their genes required for neuronal development. Additionally, the worm has a lot of other advantages such as a transparent body, invariant cell lineage and fast life cycle. Despite the anatomic simplicity of its nervous system, *C. elegans* is capable to identify a broad range of environmental cues including food and chemicals via neuron-specific detection and shows aversive or attractive behavioral responses. It can associate nutrients with odours, tastes, or even food pathogenicity.

Neuropeptides represent one of the largest groups of intercellular signaling factors in the nervous system. In *C. elegans*, they are derived from more than 120 precursor genes encoding over 250 bioactive peptides. Presumably, all *C. elegans* neurons produce and secrete neuropeptides with possible signaling activity. These neuropeptides can act as ligands for evolutionary conserved G protein-coupled receptors (GPCRs), regulating a variety of physiological and disease-related signaling pathways. Accumulating evidence implicates neuropeptides in the regulation of learning and memory processes, but little is known on mechanisms underlying this neuropeptidergic control. In the genetic model *C. elegans*, many neuropeptide receptors that are linked to learning and memory in vertebrates have been evolutionary conserved. Using *C. elegans'* extensive genetic toolbox, we are studying the molecular and cellular mechanisms underlying neuropeptide-mediated learning and memory.

O75 Systematic genetic analysis of dense core vesicle biology in C. elegans

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Neuropeptides are ancient, potent, and ubiquitous regulators of behaviour e.g. they control sleep, pain, and appetite. They are synthesized in the cell body, sorted to dense core vesicles (DCVs), and then trafficked to and captured by release sites. Appropriate stimulation evokes DCV exocytosis, with kinetics that depends on neuron type. Studies in endocrine cells link DCV cell biology to multiple pathophysiologies. However, DCV cell biology is poorly understood, particularly in neurons.

C. elegans has proven useful to delineate molecular mechanisms for synaptic release. It offers similar opportunities to investigate DCV biology. Confirming this, recent *C. elegans* work is uncovering mechanisms for DCV biogenesis[1-4]. In *C. elegans*, we can quantify YFP-tagged neuropeptide distribution and secretion in a specific neuron, in vivo. Using a candidate gene approach, this rich feature set was quantified for >200 alleles, allowing us to group mutants by phenotypic similarity using hierarchical clustering methods. The method succeded to groups alleles/genes with similar functions and provides a unique framework for future forward genetic approach. It also revealed several new gene involved in DCV biogenesis, trafficking and exocytosis.

1 Hoover, C. M. et al. Genetics 196, 745–765 (2014). 2 Sumakovic, M. et al. J. Cell Biol. 186, 897–914 (2009). 3 Ailion, M. et al. Neuron 82, 167–180 (2014). 4 Speese, S. et al. J. Neurosci. 27, 6150–6162 (2007).

O76 Elucidating the function of the melatonin biosynthesis pathway in the marine bristleworm Platynereis

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Whereas daily (circadian) oscillators and their output signals are intensely studied, the molecules that mediate the function of oscillators with longer period lengths are less well investigated. In vertebrates, melatonin is a used as a physiological signal of darkness, and plays a central role in both circadian physiology and yearly reproductive cycles. In invertebrates, the function of melatonin and its biosynthesis pathway remain controversial. In order to shed light on the function of the melatonin pathway in invertebrates, we us the marine bristle worm *Platynereis dumerilii.* This worm possesses at least two light-entrained molecular oscillators - a circalunar clock regulating maturation and spawning, and a circadian clock regulating daily locomotor behaviour.

Intriguingly, unlike insect and nematode model species, *Platynereis* possesses clear orthologs of the two enzymes Aanat and Hiomt that catalyse the final steps of melatonin synthesis in vertebrates. Systematic qPCR assays showed that transcript levels of *Platynereis hiomt* are regulated both in a daily fashion and by the circalunar clock, compatible with a function of this pathway in either of the two timing mechanisms. To assess the functional requirement of the pathway, we have generated two mutant strains in which the *hiomt* gene carries deletions that severely truncate its predicted open reading frame.

We have assessed circadian clock function by tracking the distance that wild-type or mutant worms move in a defined arena under free-running conditions. Under the tested conditions, the circadian locomotor activity of mutants is indistinguishable from wild-type animals. Similarly, a first molecular characterisation revealed no major aberrations in the regulation of a set of putative circadian clock target proteins. Together, these data argue against a major impact of the melatonin pathway for circadian regulation in this species. In contrast, reproductive peaks of mutant populations differ from those of wild-type animals. Along with first molecular analyses, this suggests that the function of the melatonin biosynthesis pathway in the bristle worm is rather tied to the regulation of reproduction than to circadian control.

O77 Melatonin-dependent rhythmicity in cell proliferation and apoptosis in the hippocampus of adult mice

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The hippocampus is subjected to diurnal/circadian rhythms on both the morphological and molecular level. Cell proliferation in the adult hippocampus is regulated by melatonin and should be accompanied by apoptosis to ensure proper tissue maintenance and function. We studied the hippocampus of male adult mice to investigate whether cell proliferation and/or apoptosis follow a diurnal rhythm controlled by melatonin signaling. Melatonin-proficient C3H/HeN (C3H) mice and melatonin-proficient mice with targeted deletion of both melatonin receptors (MT1/2KO) were adapted to a 12h light 12h dark cycle and sacrificed at ZT00, 06, 12 and 18. Immunohistochemistry for Ki67, a nuclear marker for all active stages of the cell cycle, served to measure cell proliferation. Apoptosis was measured on the basis of immunohistochemistry for activated caspase-3, a marker for cells in the execution phase of apoptosis. At each ZT proliferating cells, apoptotic cells and the “total”number of cells (stained by Hoechst) were counted within the subgranular zone (SGZ), the granule cell layer (GCL), the polymorphic layer (PL), the molecular layer (ML) and the Cornu Ammonis (CA). Additionally, Ki67 was co-visualized with DCX, S100β, Iba1, OLIG2, or CD31 and activated caspase-3 with DCX, NeuN, S100β, Iba1, CNPase, OLIG2, or CD31 by immunofluorescence double staining at ZT06 and ZT18 for identification and quantification of proliferating and apoptotic cell types. In all regions and genotypes analyzed, we found both ongoing cell proliferation and apoptosis at all 4 ZTs. The total number of cell nuclei, including dividing and non-dividing did not change significantly in the course of the day. Cell proliferation and apoptosis in the PL, ML and CA were constitutive and showed no rhythm. In the SGZ and GCL ZT-dependent changes in cell proliferation and apoptosis were found exclusively in melatonin-proficient mice with functional melatonin receptors. In the SGZ and GCL of C3H mice, the number of Ki67 immunoreactive cells was significantly lower at ZT00 and ZT06 as compared to ZT12 and ZT18. Apoptosis followed ZT-dependent changes in the SGZ and GCL of C3H mice with an increase at ZT06 as compared to the other ZTs. Proliferating and apoptotic cells were found in nearly all cell types residing in the hippocampal regions analyzed. The number of proliferating cells immunoreactive for DCX showed ZT-dependent changes: it was significantly increased at ZT18 as compared to ZT06, in fact exclusively in the SGZ of C3H mice. Conversely, the number of apoptotic cells immunoreactive for DCX was significantly decreased at ZT18 as compared to ZT06, again, exclusively in the SGZ of C3H mice. Other cell types proliferating or undergoing apoptosis did not show ZT-dependent changes, neither in the SGZ nor in the other hippocampal regions analyzed. Our results indicate that ZT-dependent changes in cell proliferation are counterbalanced by ZT-dependent changes in apoptosis exclusively in the SGZ and GCL of adult melatonin-proficient mice with functional melatonin receptors. Melatonin signaling appears to be crucial in both generation and timing of ZT-dependent changes in proliferation and apoptosis of immature neuronal cells.

O78 Impact of melatonin receptor deficiency on ecto-5'-nucleotidase mRNA levels in the mouse prosencephalon

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Ecto-5'-nucleotidase (eN) is the major extracellular adenosine-producing ecto-enzyme in mouse brain. Via the production of extracellular adenosine, eN participates in multiple physiological processes, such as wakefulness, inflammation, nociception and neuroprotection, and is related to many diseases, such as multiple sclerosis, epilepsy and cancer. The mechanisms regulating the expression of eN are therefore of considerable neurobiological and clinical interest. By comparing melatonin-proficient with melatonin-deficient mouse strains, we demonstrated a modulatory effect of melatonin in the temporal regulation of eN mRNA levels. We further analyzed the melatonin receptor subtype involved in this regulation by investigating the temporal patterns of eN mRNA in melatonin-proficient transgenic mice lacking either the melatonin receptor subtype 1 (MT1 KO) or both melatonin receptor subtypes (MT1 and MT2; MT1/2 KO) and the corresponding wild type (WT) controls. By means of radioactive in situ hybridization, we demonstrated a strong reduction in eN mRNA levels in both MT1 and MT1/2 KO as compared with WT controls suggesting a stimulatory effect of melatonin on eN mRNA levels. Apparently it is primarily the MT1 receptor subtype which mediates this response, since the eN mRNA levels were reduced in MT1 KO and MT1/2 KO to a similar extent. Our results further suggest a role for melatonin in setting the maximum of eN mRNA levels to the night-time. It seems that this effect is mediated via the MT2 receptor subtype, as eN mRNA levels peaked in the middle of the night in both the WT and MT1 KO mice, while in the double MT1/2 KO, the elevated night-time eN mRNA levels were abrogated and the peak was shifted toward day-time. Interestingly, day-time locomotor activity (during the resting phase) was significantly higher in MT1/2 KO but not in MT1 KO, as compared to WT. This conforms to the suggested role for melatonin and MT2 in the regulation of sleep. The here presented impact of melatonin on eN expression suggests melatoninergic signaling as an interface between the purinergic system and the circadian system.

O79 GDF9 in ovary of Hemidactylus flaviviridis: molecular characterization, phylogenetic analysis, stage specific expression and gonadotropic regulation

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Growth differentiation factor 9 (GDF9) belonging to transforming growth factor-β superfamily is an oocyte-specific factor and is reported to be obligatory for ovarian folliculogenesis in mammals. Female mice lacking *gdf9* are infertile with follicles arrested at the primary stage. Ewes with homozygous mutation of the gene are infertile while those carrying heterozygous mutation have an increased ovulation rate. Nonetheless, the role of *gdf9* in ovarian folliculogenesis has remained unexplored in reptiles despite their phylogenetic importance. Present study is the first attempt to gain an insight into the expression pattern and potential role of *gdf9* in the ovarian functions of reptiles. The complete sequence of gene was attained from ovarian transcriptome sequencing data of the wall lizard. A 1329bp long ORF was detected coding for 443 amino acid long peptide. *H. flaviviridis* GDF9 consists of 22aa long signal peptide and 103aa long mature protein. Twenty four phosphorylation sites and 6 potential N-glycosylation sites were predicted in the protein. This study also aimed to analyse differential expression of *gdf9* in the ovary of wall lizard during different reproductive phases. q-RT PCR showed that expression of the gene increased progressively from regressed through early recrudescence phase and reaches peak level in late recrudescence phase beyond which there is a decline in expression during breeding. To understand the gonadotropic regulation of *gdf9* expression, wall lizards were given 3, 7, 11 injections of ovine-FSH (30ug/injection/lizard on alternate day). Maximum expression of gdf9 was observed in ovary of lizards treated with 11 injections of oFSH. Further, i*n vitro* experiment was performed to substantiate the direct role of FSH in regulation of *gdf9* expression. Different stages of ovarian follicles (early growing, previtellogenic and early vitellogenic follicles) isolated during breeding phase were incubated in medium containing oFSH for 12 hours. A marked stimulation of gdf9 expression was observed in all stages of follicles. In conclusion, observations of the current study suggest that *gdf9* is involved in growth of ovarian follicles and its expression is upregulated by FSH.

O80 The role of the circadian clock in seasonal timing

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There is powerful evidence that photoperiodism in seasonal organisms is driven by the circadian clockwork. The external co-incidence hypothesis Bunning proposed states that the circadian clock sets a photo-inducible phase, which when exposed to light generates a long-photoperiod (LP) response. Lengthening photoperiod (LP) stimulates thyroid hormone (TH) conversion in ependymal tanycytes lining the 3rd ventricle of the hypothalamus, thus timing breeding seasons. TH is activated on LP by thyroid-stimulating hormone (TSH) from the adjacent pituitary pars tuberalis (PT), driven by the transcriptional co-activator EYA3, which operates as a TSH on-switch. Rhythmic melatonin (MEL) signals are proposed to drive a circadian oscillation in the PT of Eya3, leading to elevation of Eya3 12h after MEL onset. This only occurs in the absence of continued MEL-signalling, and is de-repressed on LPs (i.e. external co-incidence).

Using next generation sequencing methods we set out to establish how the circadian clock may interact with the seasonal clockwork by asking if other elements of the seasonal transcriptome obey the external co-incidence hypothesis. We collected the PT of seasonal sheep at 4 hourly intervals in both long (LP) and short photoperiods (SP) and identified over 700 transcripts as under photoperiod-control. In SPs, transcription is circadian-organised across the day. In LP, the peak-phase of transcript expression is highly organised to the photo-inducible phase in early morning, concurring with the “Bunning” model. In both LP and SP, the pattern of gene expression is consequent on the long-term history of exposure to multiple repeated melatonin-cycles. Remarkably, very few genes are acutely regulated by melatonin exposure, emphasising the critical importance of long-term history effects in this key melatonin target site. To further investigate this we undertook bisulphite sequencing and found a dominant role for photoperiodic history in driving patterns of DNA methylation. These data now suggest that epigenetic regulation underpins the output of the melatonin signal and that these modifications develop as an accumulated response to many repeated melatonin cycles (i.e. history effect). The importance of the core circadian clock in driving these complex set of changes and its role in the maintenance of photoperiodic history remains to be established.

O81 Expansion of the Secretin-GPCR members in the molluscs

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Mollusca are the second largest phylum of animals and analysis of the available gastropod and bivalve genomes suggests that their gene repertoire and organization is more similar than the nematodes and insects to the deuterostomes. Therefore, studies of mollusc genomes can contribute significantly to understand metazoan genome evolution. Particularly since it is likely that features have been retained in mollusc genomes that may have been lost or diverged in other protostomes. The recent increase in the number of available mollusc genomes and transcriptomes provides a unique opportunity to extend knowledge and understanding about the evolutionary origin of important metazoan endocrine gene families and to characterise conserved and species-specific functions. In this context we characterised homologues of the Secretin-GPCRs in the molluscs by exploring available genome and transcriptome data from different species. Mollusc homologues of the vertebrate Secretin-GPCR family, including the newly identified nematode and insect receptor Cluster A and Cluster B, but the insect DH31R/HecR and DH44R were absent. The different molluscs analysed had a variable number of receptors that is congruent with lineage specific gene family expansion during the evolution of the bivalves. Analysis of RNASeq data indicated that duplicates of the Secretin-GPCRs were expressed in the mantle of the Mediterranean mussel (*Mytillus galloprovinciallis,* SRP063654) and hard-shelled mussel (*Mytilus coruscus*) and their role as novel molecular targets in bivalve mantle function will be discussed.

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O82 Urbilaterian origin of paralogous GnRH and corazonin neuropeptide signalling pathways

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Gonadotropin-releasing hormone (GnRH) is a key regulator of reproductive maturation in humans and other vertebrates. Homologs of GnRH and its cognate receptor have been identified in invertebrates - for example, the adipokinetic hormone (AKH) and corazonin (CRZ) neuropeptide pathways in arthropods. However, the evolutionary relationships and origins of these signalling systems remain unclear. Here we have addressed this issue with the first identification of both GnRH-type and CRZ-type signalling systems in a deuterostome - the echinoderm (starfish) *Asterias rubens*. We have identified a GnRH-like neuropeptide that specifically activates an *A. rubens* GnRH-type receptor and a novel neuropeptide that specifically activates an *A. rubens* CRZ-type receptor. With the discovery of these ligand-receptor pairs, we demonstrate that the vertebrate/deuterostomian GnRH-type and the protostomian AKH systems are orthologous and the origin of a paralogous CRZ-type signaling system can be traced to the common ancestor of the Bilateria (Urbilateria).

O83 Identification and functional characterization of the pheromone biosynthesis activating neuropeptide receptor isoforms from Mamestra brassicae

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The pheromone biosynthesis activating neuropeptide (PBAN) regulates pheromone production in many moth species, including *Mamestra brassicae*. PBAN activates specific G-protein-coupled receptors (GPCR) located on the surface of pheromone-producing cells in the pheromone gland (PG) to trigger an influx of extracellular calcium as the second messenger. Herein we report isolation, cloning and characterization of three PBAN receptor isoforms from *M. brassicae* (Mambr-PBANR). A partial cDNA encoding Mambr-PBANR was amplified from PG tissue by reverse transcription-PCR using degenerate oligonucleotide primers. To obtain full-length cDNA sequences, we performed 5'- and 3'-rapid amplification of cDNA ends (RACE). Three variants of Mambr-PBANR designated PBANR-A, -B and -C isoforms were identified. The deduced amino acid sequences of the Mambr-PBANRs show high homology to other receptors within the PBANR family of GPCRs, in particular to heliothine PBANRs [eg. 99% with *Mythimna* (*Pseudaletia*) *separata* PBANR-A, 97% with *Mythimna* (*Pseudaletia*) *separata* PBANR-B and -C, 97%, 97% and 94% with *Heliothis virescens* PBANR-A, -B and -C, respectively, and 88%, 85% and 84% with *Bombyx mori* PBANR-A, -B and -C, respectively].

For functional characterization, the full length coding sequences of the Mambr-PBANR A, B and C isoforms were transiently expressed in cultured *Trichoplusia ni* cells and Sf9 cells. Confocal microscopic studies demonstrated specific binding of rhodamine red-labelled pheromonotropin (PT) (RR10CPBAN) ligand to all three Mambr-PBANR isoforms. RR10CPBAN binding did not trigger ligand-induced internalization in cells expressing PBANR-A, but did in cells expressing the PBANR-B and -C isoforms. When co-expressed with a *Drosophila melanogaster* arrestin homolog (DmKurtz-mCherry), fluorescent chimeras of the PBANR-B and -C isoforms co-localized with the fly protein following stimulation with Mambr-PT (18 amino acid long fragment) but not with an unrelated peptide, indicating that Mambr-PT is an active ligand. To gain insights into the potential biological functions mediated by the Mambr-PBANRs, we analyzed their tissue expression patterns. In conclusion, all three isoforms were amplified from the *M. brassicae* PG, with the PBANR-C transcript being the most abundant form.

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O84 The GPCR allatotropin/orexin family: an ancestral conserved mechanism of signals, as a probable alternative system of phylogenetic molecular markers.

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GPCRs constitute a widely distributed family of proteins mediating the activity of numerous cell-cell messengers. These kind of receptors seems to be ancient, appearing early in evolution. Among them, the Allatotropin/Orexin family, characterized mainly in chordates and insects, conforms a highly conserved group of receptors associated to peptides involved in myoregulatory processes in invertebrates, as well as complex functions in vertebrates involving stimulation of food consumption, regulation of sleep and wakefulness, etc. A basic feature that characterizes GPCRs is the presence of the aminoacids DR or ER associated to the transmembrane 3 (TM3). In a great quantity of GPCR families, this sequence is followed by tyrosine (Y). The allatotropin/orexin family is characterized by the presence of tryptophan (W) constituting the sequence DRWY. In the present study, we analyze the molecular phylogeny of the orexin receptors, focusing mainly in Chordata. Furthermore, we found that the TM3 associated sequence DRWYAI is highly conserved from Placozoa to Chordata and might be considered as a molecular “signature”of the family. We analyzed more than 200 sequences of Chordata proteins obtained from the GeneBank including Chondrichthyes, Actinopterygii and Sarcopterygii. Only those sequences containing the DRW pattern associated to TM3, also including the seven transmembrane domains and the amino and carboxyl terminals were selected. Once the selection was finished, the sequences were aligned by the Clustal Omega algorithm and post analyzed by the JalView software. The probable phylogenetic relationships were studied by Neighbourg - Joining and Maximum Likelihood methodologies by the Mega 6.06 software. Finally, a detailed phylogenetic analysis was performed for Orexin receptor type 2 in the groups detailed above. As a first approach to analyze the probable quality of Orexin receptors as molecular markers, we went deeper in the phylogenetical relationships in Acantomorpha. The Neighbourg - joining analysis of the total sequences including Orexin receptors type 1 and 2 show that both kind of receptors diverge completely to form a clearly defined subfamily. Furthermore, the accepted assumption about the phylogenetic relationship about Tetrapoda, which is actually considered as a member of the clade Sarcopterygii is represented. The Maximum likelihood analysis clearly grouped Acantomorpha showing the different orders in separately clades, showing also the corresponding species grouped by families in the orders Perciformes and Cyprinodontiformes. Regarding Sarcopterygii, *Latimeria* sp. is grouped with the only species of Amphibia included in the analysis, appearing both of them as a sister group of Sauropsida, which in fact includes reptilian and birds. Looking further, Crocodilia appears as the sister group of birds, being both of them closely related with Testudines. Squamata, including lizards and snakes, is shown as the outer group. Finally, a first approach to understand the origin and phylogenetic relationships of Orexin receptors shows that it represents with high fidelity the phylogenetical relationships inside Chordata, even at the level of Family. The sequences DRWYAI, associated to TM3 and VTNYFIVNLS, to TM2, are 100% conserved in all the species analyzed and could be considered as a “signature”for Orexin receptors in Chordata.

O85 Potential interactive amino acids in the Tribolium castaneum sulfakinin receptors for ligand binding

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Insect sulfakinin receptors (SKRs) are G-protein coupled receptors, structurally and functionally homologous to the human cholecystokinin receptors. We value SKRs as a potential target for pest management given their important endocriological roles in insects. Our previous molecular modeling illustrated the potential interaction between sulfakinin and SKRs in the red floure beetle, *Tribolium castaneum*. This provides us primary information to determine the interactive amino acids in SKRs that are active in ligand (sulfakinin) binding. In the present study, two *T. castaneum* SKRs, TcSKR1 and TcSKR2, are subjected to alanine screening via site-directed mutagenesis. The interaction between mutated SKRs and sulfakinin ligand is then evaluated in an in vitro GPCR reporter assay. With this strategy, we can determine the potential interactive amino acids in TcSKRs for sulfakinin binding. This will enlarge our knowledge of the mechanism of SK-SKR interaction.

O86 V1B and CRF1 receptors involved in stress associate as heterodimers in the living pituitary to form assemblies with functional synergism

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Stress and anxiety are mainly monitored by the hypothalamo-pituitary adrenal axis, with 2 major neuropeptides, vasopressin and CRH, orchestrating the physiological response to external inputs reaching the central nervous system through the body sensors. Secreted from the hypothalamus, these neurohormones, via the V_{1B} receptor for the vasopressin and the CRF1 for the corticoliberine, stimulate ACTH synthesis and secretion at the anterior pituitary, this later hormone stimulating the adrenals to release corticosteroids and catecholamines from the adrenals. We have shown in models cells that these receptors can associate as heterodimers displaying functional synergism with both second messenger crosstalk and new pharmacological properties of the heterodimers. However, so far, dimers have not been shown directly in native tissues and structural data about dimerization interface deduced from crystals are only available for few receptors. Here, we address the question of V_{1B}/CRF_1 dimerization in native tissue and of which receptors regions support the physical interaction between the 2 partners. First, using fluorescent double knock-in mice co-expressing V_{1B}-EGFP and CRF_1-mCherry, we show that the 2 receptor subtypes are indeed expressed in the same cells of the pituitary lobe. Each specific ligand can induced the simultaneous internalization of the 2 receptors indicating close proximity. Fluorescence Lifetime Imaging Microscopy, validated on HEK293 model cells, confirmed that V_{1B}/CRF_1 heterodimers occur in the living pituitary of control animals.

By using site-directed mutagenesis of receptors of each the vasopressin and CRH family, BRET and pharmacological studies, we showed that V1B/CRF1 is a unique receptor couple and pinpointed 4 amino-acids in the CRF_1 transmembrane domain 4 (TM4) that mediate V_{1B}/CRF_1 receptor heterodimerization and display a distinctive pattern of signalling. Drawn from our present results and supported by recent X-ray analyses of GPCR structure from the literature, a model is proposed for the V_{1B}/CRF_1 heterodimer, which involves TM4, but also TM5. As a clinical goal of our study, we propose that new pharmacophores regulating V_{1B}/CRF_1 heterodimerization could constitute good pharmacological tools with anxiolytic properties.

O87 Temperature shift impairs osmoregulatory capacities during the smoltification phase of two Atlantic salmon (Salmo salar L.) strains.

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Temperature is known to be a crucial factor influencing the smoltification but anthropogenic use of water systems may cause temperature fluctuations between rivers and tributaries. Based on Belgian field data, we simulated the Atlantic salmon downstream route finishing with a seawater challenge to investigate the impact of a 5°C temperature shift during smoltification on hypo-osmoregulatory capacities of migrating smolts. Three temperature conditions were tested (no, early and late temperature shift). We also addressed to what extent osmoregulatory processes are influenced by genetic background through the comparison of two allochthonous strains used in Belgium for restocking purposes, Loire-Allier (France) and Cong (Ireland). For juveniles reared without temperature shift, differences were noticed in smolting peak timing (defined by means of gill Na^+/K^+ATPase activity) and maximum intensity (17.7 µmol ADP mg^{-1} protein h^{-1} vs 14.8 µmol ADP mg^{-1} protein h^{-1}) between the strains as well as in plasma sodium and potassium concentrations. In both conditions with a temperature shift, gill Na^+/K^+ATPase activity was negatively influenced, as well as plasma osmolality and ion concentrations in both strains. After the salinity tests, we noticed that the temperature shift significantly impacted hypo-osmoregulatory abilities of the smolts with higher osmolality in smolts subjected to the temperature shift. Predictably circulating levels of GH and IGF-1 changed over the smolting period but plasma levels of T3, T4, GH and IGF-1 do not permit to explain the observed modifications in hypo-osmoregulatory abilities. Confirmation under natural conditions is ongoing in a three-year-long field study in the Belgian Meuse and one of its tributary, the Ourthe. Osmoregulatory markers tend to support our lab results but differences in hormonal levels seem to emerge.

O88 Neurophysiological stress response of pikeperch sander lucioperca juveniles to intensive culture conditions

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High mortality and impairment in growth rate during the young developmental stages of pikeperch (*Sander lucioperca*) are among the major bottlenecks for its development in aquaculture. These failures may be related to high stress responsiveness since the rearing conditions for pikeperch are not yet optimized. High secretions of catecholamines and cortisol are involved in the first line of the integrated response to stress in fish. Moreover, the activation of several brain monoamine neurotransmitters, including dopamine and serotonin is also involved in the primary responses to stress. The objective of this study was to characterize the physiological stress response of percid fish in intensive culture conditions using pikeperch as experimental model. In a screening experiment, eight factors considered as relevant for the welfare of pikeperch according to the literature were compared in two modalities using a fractional multifactorial design (2^{8-4}). Each experimental unit represented a combination of sixteen variants including grading, stocking density (15 vs 30 kg/m^3), food type (floating vs sinking), light intensity (10 vs 100 lux), light spectrum (red vs white), photoperiod (long vs short), dissolved oxygen (60 vs 90%) and temperature (21 vs 26°C). Fish sampling occurred on days 36 and 63 in order to better describe short and long-term stress responses. The available results on day 63 showed that growth rate was significantly affected by the type of food with the highest values for fish receiving sinking food, while mortality rate was increased by the interactions of high light intensity with low temperature and with high density. Dual associations between white spectrum, long photoperiod and high density induced higher cortisol level in plasma while glucose level was mainly influenced by interactions of food type and temperature with various husbandry factors. In terms of neurotransmitters, low light intensity associated with white spectrum increased significantly the concentrations of brain serotonin and tryptophan, while 5HIAA concentration was decreased by the interaction between low light intensity and high temperature. DOPAC level was significantly increased with high temperature. The available results indicate that light characteristics (*e.g.* low light intensity, red spectrum and short photoperiod) in combination with appropriate food type may be considered as directive factors influencing the stress response in pikeperch and thereby their welfare status. Further analyses are still undergoing such as dopamine and melatonin concentrations in brain and expression of stress key genes (GR1, MR …) in order to better describe the profiles of stress responses.

O89 Individual and age-related differences in stress responsiveness of HPA are associated with features of vasopressinergic and melatoninergic regulation

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Studies of the stress responsiveness of the hypothalamic-pituitary-adrenal axis (HPA), the key adaptive system, of individuals, which differ in age and behavioral response, is one of the most promising approaches in searching for biomarkers of increased vulnerability to the negative effects of stress and accelerated/premature aging.

Purpose - investigation of the reaction of HPA in response to acute stress (AS) and its vasopressinergic and melatoninergic regulation in young and old individuals of healthy standard adaptive behavior (SB) and individuals of maladaptive depression-like and anxiety-like behavior (DAB).

Methods: We used 14 young (6-8 years) and 14 old (21-30 years) female rhesus monkeys, half of which were animals with SB while the others were animals with DAB. All animals were subjected to the restraint for 2 hours along with the AVP test and blood sampling at night time to study the secretion of melatonin (M). Besides, two animals, young and old ones with DAB, were subjected to the AVP V1b receptor antagonist (SSR149415) for 1 hour prior to the AVP injection.

Results: Young animals with DAB and SB showed the similar rises in the levels of ACTH and cortisol (F) in response to AS. At the same time, the levels of ACTH in response to the AVP test were significantly higher in young animals with DAB compared with young animals with SB and were inversely correlated with the night M level. At aging, the monkeys with SB demonstrated the decrease in ACTH secretion rise in response to AS and AVP test, while the animals with DAB demonstrated the tendency of increasing of ACTH reaction to AS and AVP injection. As a consequence, it has resulted in development of the marked intergroup differences in HPA response to AS and AVP test for old animals with higher ACTH levels, lower F levels, and higher the molar ratio F to dehydroepiandrosterone sulfate, that is important biomarker of aging, in old animals with DAB. At aging, M levels decreased in the monkeys with SB and positive correlated with the area under the curve of ACTH response to AS and AVP test. At the same time, the monkeys with DAB demonstrated with aging only a tendency to decrease of M levels and the absence of any correlation M level with ACTH response. Preliminary administration of a V1b receptor antagonist to two monkeys with DAB led to decrease of the ACTH response to AVP test and increase of F response, which are more significant in the old animal.

Conclusions: Thus, adult female rhesus monkeys differ in their HPA responsiveness to AS and AVP injection which is more expressed in old monkeys being associated with the features of their behavior. Young and old animals with DAB demonstrate more pronounced vulnerability of HPA to the stress exposure and accelerated aging that is apparently conditioned by disturbances in vasopressinergic and melatoninergic regulation of HPA stress reactivity.

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O90 Stress response in Drosophila melanogaster: biogenic amines, juvenile hormone, 20-hydroxyecdysone and insulin signalling are involved

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In insects, biogenic amines, dopamine (DA) and octopamine (OA), and gonadotropins, juvenile hormone (JH) and 20-hydroxyecdysone (20E), are the main components of neurohormonal stress-reaction. Their levels sharply increase under unfavorable conditions, contributing to adaptation. Individual stress-resistance depends on expression of genes controlling the background level of DA. DA controls JH titer by either stimulating or inhibiting its synthesis and degradation depending on the developmental stage and number of D_1 and D_2-like receptors in *corpus allatum* (a specialised endocrine gland that synthesises JH) and the fat body (the location of JH-esterase synthesis); there is a negative feedback in this regulation. 20E controls JH metabolism *via* DA metabolic system - a rise in 20E titre increases DA content in young females and decreases it in mature females, thus leading to a rise in JH levels in both.

The signaling pathway of insulin/insulin-like growth factor is also involved in the regulation of stress resistance. This pathway in *D. melanogaster* includes eight insulin-like peptides, DILP1-8, a transcription factor of the Forkhead box class O family (dFOXO), an insulin-like receptor (InR), and the fly ortholog of mammalian insulin receptor substrates (CHICO). In females of *D. melanogaster* the insulin/insulin-like growth factor signaling pathway (IIS) regulates dopamine metabolism (*via* the system of JH metabolism) and interacts with JH in the control of reproduction and stress resistance. The strong hypomorph mutation of DILP6 results in a reduction in JH-hydrolysing activity and in increased fecundity, activity of enzymes that produce dopamine - alkaline phosphatase (ALP) and tyrosine hydroxylase (TH) and the intensity of their response to stressor (stress reactivity). Treating the mutant females with the JH inhibitor, precocene, restores the activity and stress reactivity of ALP and TH as well as fecundity to levels similar to those in the control flies. The hypomorph mutation of dFOXO leads to the increase of OA synthesis, JH degradation level and its stress-reactivity together with decrease of stress resistance and DA synthesis. The JH application restores OA and DA metabolism. The suppression of InR in the *corpus allatum* causes an increase in DA level and JH-hydrolysing activity and alters the activity of ALP and TH as well as modulates DA, ALP, TH and JH response to heat stress and decreases the fecundity of the flies; the JH application restores both DA metabolism and fecundity. The null mutation of the gene of a substrate of insulin-like receptor, *chico1*, in the heterozygous state affects JH, dopamine and octopamine metabolism, and leads to the decrease in heat stress resistance and fecundity of *D. melanogaster* females. The results of our study suggest a negative feedback system in the interaction of JH and IIS in *Drosophila*.

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YOUNG INVESTIGATOR SYMPOSIUM

Y1 Localisation of the expression of a calcitonin-type neuropeptide in echinoderm

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Calcitonin belongs to a family of neuropeptides that exert effects by activating secretin-type G-protein coupled receptors. Calcitonin lowers blood calcium levels and acts to protect against calcium loss from the skeleton during periods of calcium mobilization. Analysis of the phylogenetic distribution of calcitonin-type peptides and their receptors indicates that the evolutionary origin of this signalling system can be traced to the common ancestor of the Bilateria. Furthermore, it has been discovered that calcitonin-type peptides act as diuretic hormones in insects. However, little is known about the physiological roles of these peptides in other invertebrates. We are using the starfish *Asterias rubens* (phylum Echinodermata) as a model system to investigate the evolution and comparative physiology of neuropeptides. As deuterostomian invertebrates, echinoderms bridge the “evolutionary gap”between protostomian invertebrates (e.g. insects) and the vertebrates. Furthermore, the pentaradial symmetry of echinoderms provides a unique context for investigation of neuropeptide function. Here we report the cloning and sequencing of a cDNA encoding an *A. rubens* calcitonin-type neuropeptide precursor (ArCTP). Analysis of the expression of ArCTP in *A. rubens* using mRNA *in situ* hybridization revealed that it is expressed by cells in the ectoneural and hyponeural regions of the radial nerve cords and circumoral nerve ring and in the digestive system (cardiac and pyloric stomach; pyloric caecae). To enable investigation of the expression of the mature peptide (ArCT) derived from ArCTP, we generated antibodies to the C-terminal region of ArCT. Consistent with the distribution of ArCTP transcripts, ArCT-immunoreactive (ir) cells were labeled in both the ectoneural and hyponeural regions of the radial nerve cords and circumoral nerve ring as well as in the digestive system (cardiac and pyloric stomach; pyloric caecae). Furthermore, immunocytochemistry revealed the immunostained processes of cells expressing ArCT. For example, a dense plexus of immunostained fibres is present in the neuropile of the ectoneural region of the nerve cords and in the longitudinal nerve and basal nerve ring of the tube feet. Immunostained processes are also present in the innervation of the ampullae that control protraction/retraction of tube feet and in the innervation of the body wall-associated pupulae, thin-walled finger-like projections that facilitate gas exchange. Collectively, these data suggest that ArCT may be involved in neural control of a wide variety of physiological processes in starfish, including feeding and digestion, locomotor activity and gas exchange. Our data provide a basis for investigating the physiological roles of ArCT in *A. rubens*, which may provide novel insights into the evolution of calcitonin function in the animal kingdom.

Y2 Functional characterization of a tachykinin-like neuropeptide signaling system in an echinoderm

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Neuropeptides are neuronal signalling molecules that regulate a broad spectrum of physiological processes. The tachykinins (TK; e.g. substance-P) are an evolutionarily ancient family of neuropeptides that have been identified in several animal phyla and have important roles in, for example, intestinal motility, smooth muscle contraction and nociception. Recent analysis of transcriptome sequence data has identified the first candidate TK-type neuropeptide precursor to be discovered in an echinoderm - the starfish Asterias rubens (ArTKP). Echinoderms are of particular interest because as deuterostomes they are more closely related to vertebrates than the majority of other invertebrates. Therefore, experimental studies on echinoderms can provide important insights into the evolution of neuropeptide function in the animal kingdom. Here we have used a variety of techniques to characterize ArTKP and neuropeptides derived from it.

A cDNA encoding ArTKP was cloned and sequenced and analysis of A. rubens nerve extracts using mass spectrometry enabled determination the structures of two TK-like peptides derived from ArTKP, which we refer to as ArTK1 and ArTK2. Analysis of the expression of ArTKP transcripts in A. rubens using mRNA in situ hybridization revealed expression in cells in the ectoneural region of the radial nerve cords and circumoral nerve ring and in the digestive system, including the peristomial membrane, oesophagus, cardiac stomach and pyloric duct. We generated antibodies to ArTK1 and immunocytochemical analysis of ArTK1 expression in A. rubens revealed patterns of immunostaining in the radial nerve cords and circumoral nerve ring that are in accordance with the expression of ArTKP transcripts. In the digestive system, ArTK1-immunoreactive nerve processes were revealed in the basiepithelial nerve plexus but only in distinct zones, primarily in the “roof”of the cardiac stomach, in the pyloric stomach and in the pyloric duct.

Preliminary in vivo pharmacological experiments revealed that injection of synthetic ArTK2 into starfish triggers mouth opening. These findings indicate that one of the functions of TK-like peptides in starfish may be to regulate their unusual extraoral feeding behaviour, which involves eversion of the cardiac stomach out of the open mouth over prey.

Y3 A look into the brain transcriptome of Schistocerca gregaria during early behavioural gregarisation

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In the desert locust, the transition from solitarious to gregarious behaviour is initiated through two separate sensory pathways that signal the presence of conspecifics: a cephalic pathway that integrates visual and olfactory cues, and a thoracic pathway where the most effective stimulus is the repeated touching of the hind femora. Both pathways initiate a serotonin-dependent behavioural change. In this study, we applied several different stimulus regimes and compared both their behavioural effects and the transcriptomic changes they evoked in the brain.

Fifth instar solitarious-phase locusts were stimulated for 2 hours with one of several different stimuli that mimic high population density. Next, their behaviour in the presence of conspecifics was quantified in an arena assay. Immediately afterwards, their brains were dissected for RNA extraction, followed by sequencing of all transcripts on Illumina HiSeq2000. The transcriptomic data were analysed for transcripts that show stimulus condition-specific differences in expression.

As expected, all stimulus paradigms caused a significant increase in both activity and attraction towards conspecifics. The extent of this behavioural shift differed, however, between the different stimulus paradigms. Transcriptional responses occurred as early as ten minutes after the onset of stimulation. The expression of several genes involved in neuroendocrine signalling responded in a stimulus-specific manner. Analysis for transcripts that respond to both gregarising sensory pathways will provide new insights in the neuroendocrine underpinnings of the earliest stages of behavioural gregarisation.

Y4 Drosophila insulin-like peptide 1 (DILP1) is transiently expressed during non-feeding stages and reproductive dormancy

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The insulin/insulin-like growth factor signaling pathway is evolutionarily conserved in animals, and is part of nutrient-sensing mechanisms that control growth, metabolism, reproduction, stress responses, and also affects lifespan. In *Drosophila,* there are eight insulin-like peptides (DILP1-8). Six of these have been investigated in some detail, whereas the expression and functions of DILP1 and DILP4 still remain enigmatic. We show here that *dilp1* transcript and DILP1 peptide are transiently expressed in insulin producing cells (IPCs) of the brain from early pupa until a few days into adult life. This corresponds to non-feeding stages and periods of extensive development in the pupa, as well as metabolic remodeling and ovary maturation in the newly-eclosed flies. In adult female flies where diapause can be triggered by low temperature (11°C) and short days (10L:14D), within a time window of 0-10h post-eclosion, the *dilp1*/DILP1 expression remains high for more than 9 weeks. The *dilp1* mRNA level is elevated in *dilp2,3,5* and *dilp6* mutant flies, indicating systemic feedback regulation. Furthermore, we show that the DILP1 expression in IPCs can be regulated by short neuropeptide F, juvenile hormone and presence of larval adipocytes. In male *dilp1* mutant flies lifespan is extended and the resistance to starvation is reduced, whereas in female *dilp1* mutants early oviposition is reduced. In summary, DILP1 is expressed in non-feeding stages and in diapausing flies, is under feedback regulation and appears to play sex-specific functional roles. Thus, DILP1 seems to play some roles distinct from that of other DILPs.

Y5 Combining MALDI imaging and µCT to localize neuropeptides in ant brains

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Neuropeptides play a determining role in biological processes. These small molecules and their associated signaling systems are involved in metabolism, development, reproduction, behavior and learning. The origin of these compounds has deep roots in metazoan evolution and likely emerged at very early stages of nervous system development (1).

In the animal kingdom, insects, which appeared around 479 million years ago (2), represent the most diverse group in terms of numbers of described species. This taxon has an enormous significance with respect to ecology, biology and economy. In fact, insects participate in ecosystem processes, act as vectors of diseases, parasite other organisms and impact agriculture. Besides, they are being used as model organisms in different fields of life science, such as genetics and biochemistry. Ants are particular interesting due to their social colony organization and unique castes systems. For instance, individuals share identical genes but exhibit very different morphology also affecting brain structure and size (3).

In this study, we combined MALDI mass-spectrometry imaging (MSI) and micro-computed tomography (µCT) of the leafcutter ant *Atta sexdens* for neuropeptide localization in brain. MALDI-IMS provides spatial distribution and relative quantification data of known-molecular weight compounds and has been a useful tool in honeybee neuropeptide analysis (4). Additionally, µCT is a non-invasive scanning tool to acquire the detailed 3D anatomy of a sample. (5). We integrated the spatial distribution of neuropeptides from different planes of the ant brain into an accurate 3D anatomy model. Furthermore, histological staining combined with the model provided additional information of the neuropeptide brain patterns and the associated tissue. The first example of combining these two state-of-the-art techniques, MALDI-IMS and µCT, for micro tissue samples will allow studying ant brain anatomy and co-localized neuropeptide function in the future.

(1) Mirabeau and Joly 2013, Proc. Natl. Acad. Sci. U.S.A 11022):E2028-37. (2) Misof et al. 2014, Science 346:763-767. (3) Gronenberg, 2008 Myrmecol. News 11:25-36. (4) Pratavieira et al. 2014, J. Proteome Res. 13:3054–3064. (5) Smith et al. 2016, Sci. Rep. 6:21768.

Y6 Localization and Possible Function of Brain aromatase in Zebrafish Eye

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Aromatase is the key enzyme for aromatization of testosterone to estradiol (E_2). Although E_2 is generally known as a circulating hormone derived from the gonad, it is also recognized as neuroestrogen having a crucial role in development of central nervous system (CNS). Previous studies in zebrafish have shown that brain aromatase (*cyp19a1b*) is expressed in the eye. However, its distribution and function in the eye have not been revealed yet. Therefore in this study we aim to localize brain aromatase and to elucidate possible functions of brain aromatase in zebrafish eye. Immunohistochemistry using specific antiserum to zebrafish brain aromatase showed that immunoreactivity of brain aromatase (irAroB) was localized in retina layers of adult fish including inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL). When fish were exposed to E_2, the intensity of staining was increased in those layers and additional positive reaction was appeared in the ganglion cell layer (GCL). The irAroB was specifically expressed possibly in ganglion cells (GC), amacrine cells (AC), horizontal cells (HS) and bipolar cells (BP). Interestingly, irAroB was also detected in lens epithelial cells (LEC), and this result was also confirmed by RT-PCR. In order to elucidate possible functions of brain aromatase, effects of morpholino targeting brain aromatase (AroB MO) were investigated. The irAroB in retinal layers was decreased in 120 hpf larvae injected with aroB MO but not with inverted AroB MO (negative control). Injection of 5 ng AroB MO decreased the diameter of the eye at 48 hpf, and the effect was partially reversed by co-incubation with 1 μM of E_2. Apoptosis in the eye detected by acridine orange staining at 24 hpf was significantly increased by AroB MO. Furthermore, immunohistochemistry for acetylated tubulin demonstrated that AroB injection decreased the diameter of optic nerve, which was reversed by incubation with 1 μM of E_2. Taken together, estradiol produced by brain aromatase in the eye may be required for development of the eye possibly by regulating apoptosis and axonogenesis.

Keywords: brain aromatase, zebrafish, eye development.

Y7 Transcriptional profiles of components of the endocrine system during embryo-larval development of the zebrafish

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The zebrafish has many advantages as a model to study vertebrate development as well as disruptions of normal development. A thorough understanding of the fundamental biology of the species is a prerequisite for the interpretation of the scientific findings. Although at the morphological level many aspects of zebrafish embryonic development have been described, surprisingly little is known about the role of the endocrine system in early development. Several studies have reported that thyroid and steroid hormones play an important role in various stages of development, but available information is fragmented.

Our study was designed to provide detailed baseline information about the activity of the thyroid and steroid hormone system during normal zebrafish embryonic and larval development, which will serve as gold standard. In this study, we describe the timing of the normal embryonic activation of the hormone synthesis machinery, as well as the hormone and receptor profiles, during the early stages of vertebrate development, which has never been done so far. We isolated zebrafish RNA at 25 time points between 0 and 32 days of zebrafish development covering all embryonic and larval stages. The expression levels of genes coding for thyroid and steroid biosynthetic enzymes and receptors were measured using QPCR. This allows us to construct very detailed time-dependent profiles of mRNA expression of receptors and enzymes involved in synthesis of these hormones.

In order to achieve a comprehensive overview, we selected genes coding for the key enzymes involved in steroid hormones biosynthesis from the cytochrome P450 superfamily (cyp19a1a, cyp19a1b, cyp11c1, cyp21a2, cyp11a1, cyp11a2, cyp17a1,) and the hydroxysteroid beta dehydrogenases (hsd3b1, hsd3b2, hsd17b1, hsd17b3, hsd20b, hsd11b2) as well as two enzymes involved in cholesterol biosynthesis (hmgcrα, hmgcrβ). For the thyroid system we analysed expression of genes involved in the hypothalamic pituitary thyroid axis (thyrotropin releasing hormone, thyrotropin, thyrotropin receptor), thyroid hormone biosynthesis (thyroperoxidase, thyroglobulin, sodium/iodide symporter), transport (thransthyretin) and activation (deiodinases 1, 2, 3a and 3b). Also, expression of steroid receptors was analysed including 7 nuclear receptors (estrogen receptor 1, estrogen receptor 2a and 2b, progesterone, androgen, mineralocorticoid and glucocorticoid receptors) and 6 membrane receptors (membrane progesterone receptor a and b, gonadotropine releasing hormone, follicle stimulating hormone, luteinizing hormone/choriogonadotropin and G protein coupled estrogen receptors). Relative to the thyroid system, we analysed expression of thyroid hormone receptor alpha and beta.

The obtained results will improve our fundamental understanding of the role of thyroid and steroid hormones during embryonic and larval development and will be broadly applicable to the zebrafish research community.

Y8 Prolyl carboxypeptidase: a potential biomarker for obesity and diabetes mellitus?

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Background : Obesity and diabetes are metabolic diseases causing a major concern for society. Recent findings indicate that the enzyme prolyl carboxypeptidase (PRCP) plays a role in energy homeostasis by changing the biological activity of peptides involved in the regulation of food intake. Studies have shown that the plasma concentrations of PRCP may be used to reflect metabolic conditions in individuals with obesity and diabetes mellitus[1,2]. Unfortunately, PRCP activity measurements were not included or not properly performed.

Aims: The purpose of this study was to investigate PRCP activity in obesity and diabetes and to discover novel PRCP peptide substrates linked to these metabolic diseases. Pyroglutamated apelin-13 ((pyr)-apelin-13), ghrelin, enterostatin and obestatin were investigated since these are feeding-regulating peptides with potential cleaving sites (-Xxx-Pro/Ala-Xxx) for PRCP.

Material and methods: Human patients were categorized based on their BMI (<25, 25-29.9, 30-39.9, >40 kg/m^2 ; n=20 per group). Diabetes was induced in Wistar rats (n=8 per group) by intravenous streptozocin (65 mg/kg) combined with intraperitoneal nicotinamide injection (230 mg/kg). PRCP activity in human and rodent serum was measured via a RP-HPLC activity assay[3]. The kinetic parameters of recombinant human PRCP for potential peptide substrates were determined using isothermal titration calorimetry and mass spectrometry.

Results: We observed that PRCP activity is significantly increased in serum of obese patients and that this activity is positively correlated with body weight (r=0.392), visceral and subcutaneous fat (r= 0.376), waist (r=0.385) and hip (r=0.360) circumference. Furthermore, we found that the serum PRCP activity is significantly higher in streptozotocin-nicotinamide-induced diabetic Wistar rats versus controls. The search for novel PRCP substrates revealed that in the reaction conditions used enterostatin, ghrelin and obestatin were not hydrolyzed, but that PRCP removed the C-terminal Phe from (pyr)-apelin-13.

Conclusion: Serum PRCP activity is associated with obesity in humans and diabetes in rats thus a potential role for PRCP as biomarker is conceivable. The finding that (pyr)-apelin-13 is a novel PRCP substrate *in vitro* adds evidence to the hypothesis that PRCP plays a role in the pathophysiology of obesity.

(1) Xu S. et al., Clinical Chemistry. 2012, 58, 1110-1115. (2) Tabrizian T. et al., Cardiovascular & Hematological Agents in Medicinal Chemistry. 2015, 13, 113-123. (3) Kehoe K. et al., Analytical Biochemistry. 2013, 443, 232-239.

Y9 Implementation of new tools to study the interaction between G protein- coupled receptors (GPCR dimers)

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Recent findings have indicated that G protein- coupled receptors not only exist as monomers but can possibly also interact with each other to form homo- and heterodimers and even higher order oligomers. But what remains as a grey area is the functional implication of such interactions. This still needs to be explored. The GPCR dimers and particularly the heterodimers have “mysterious”effects on signaling and this could impact its responsiveness to treatments as well. Thus, there is a growing need to understand the interaction between GPCRs and then dig into the signaling pathway that arises as a consequence of the formation of GPCR dimers.

In this view, Protein Complementation methods such as split fluorescent protein i.e. Bimolecular Fluorescence Complementation (BiFC) or split luminescent protein i.e. Bimolecular Luminescence Complementation (BiLC/NanoBiT) seem to be promising for the in-depth analysis of the capacity of the two GPCRs to interact with each other. In this approach, the two GPCRs under investigation are coupled to a split protein and upon the dimerization of the two GPCRs, the two split protein fragments come together to form the functional protein.

Our research group focuses on Dopamine D2 receptor (D2R) and Mu-opioid receptor (MOR) and techniques such as Co-immunoprecipitation and Bioluminescence Resonance Energy Transfer 1 (BRET1) indicate an interaction between them. In addition to this, we sought to prove the interaction between the two receptors by Protein complementation technique. Consequently, D2R and MOR were cloned to split fragments of Venus. While analyzing the interaction via BiFC using HEK293 cells transfected with D2R and MOR fused to split fragments of Venus, a high autocomplementation was detected.

Thus, in search of an alternative method, we came across a complementation technique introduced by Promega, based on split fragments of Nanoluciferase (NanoBiT). To analyze the applicability of this assay, we cloned D2R and MOR to the Large (LgBiT) and Small (SmBiT) fragments of Nanoluciferase. The results indicated that D2R fused to SmBiT + MOR-fused to LgBiT showed a significant increase in the signal as compared to the negative control which is MOR-LgBiT + Halotag-SmBiT. Halotag-SmBiT is ubiquitously expressed all over the cell and serves as a good negative control when expressed with MOR-LgBiT.

Our long term goal is to understand the influence of dimerization on the downstream pathway (through β-arrestin recruitment or G-protein coupling) when the dimers are stimulated by their respective agonists. This can possibly be studied by BRET1 between the BiFC coupled dimers and β-arrestin/G-protein fused to *Renilla* luciferase or by FRET between BiFC coupled receptors and β-arrestin/ G-protein fused to a fluorophore. Thus, the second approach (FRET) would also help us to find an answer to the question whether there is a co-internalisation of the dimers when stimulated by an appropriate agonist of the interacting partner.

Y10 Expression pattern of nanos and piwil genes during ontogenic development in Nile tilapia oreochromis niloticus

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Primordial germ cells (PGCs) are set aside from somatic cells, migrate into prospective gonadal sites during early development and give rise to germ cell lineage. There is interest within the Aquaculture sector to explore viable means to disrupt the natural PGC development which will ultimately induce sterility. If successful, PGC disrupted animals will not sexually mature which has a range of economic as well as environmental benefits. PGCs are specified by maternally provided determinants in fish, flies, worms and frogs, while it is independent to maternal information and affected by inductive signals from surrounding tissues in mammals and most animal species (see review Extavour and Akam, 2003). In addition, maternally deposited germ genes are known to have important roles in migration, maintenance and/or survival of PGCs in other teleosts species, but their functions vary between species. As a preliminary study to find potential target genes for sterilisation, ontogenic expression pattern of selected known germ genes in the literature and their tissue distribution were investigated in Nile tilapia to confirm their potential role in regulating PGCs.

To investigate the mRNA expression patterns of nanos and piwil genes during ontogenic development, embryos or larvae were sampled throughout development from unfertilized eggs to hatched larvae. Total RNAs were extracted and cDNAs were synthesized after DNase I treatment. The mRNA expression levels of nanos1a, 1b, 2 and 3, piwil1 and 2 were analysed by qRT-PCR and normalised using the geometric mean of elf1a, ß-actin and GAPDH expression. For the tissue screen of nanos and piwils, total RNAs were extracted from brain, pituitary, eye, heart, intestine, spleen, kidney, liver & gonads of both male and female adult tilapia and used for cDNA synthesis after DNase I treatment. The tissue distribution was analysed by RT-PCR and gel electrophoresis.

In Nile tilapia, there were 4 nanos genes and 2 piwil genes (Bellaiche et al., 2014; Xiao et al., 2013). The ontogenic expression pattern revealed that nanos1a, 1b and 3 and piwil1 and 2 are maternally transferred while nanos2 appeared to not be expressed in eggs. The zygotic transcription of nanos1a and nanos1b was initiated at neurula and gastrula stages, respectively, and they were distributed in various tissues of adult tilapia. On the other hand, nanos3, piwil1 and 2 showed gonad (ovary and testis) specific expression and nanos2 showed testis specific expression. Most of the maternal transcripts of nanos3, piwil1 and 2 were degraded between blastula and gastrula stages, indicating the period of maternal to zygotic transition (MZT). In contrast to nanos1a and 1b, none of nanos3, piwil1 and 2 showed distinct zygotic transcription by hatching stage. In summary, this result suggests that among 4 nanos and 2 piwil genes, the maternal transcripts of nanos3, piwil1 and 2 are considered to play a role in migration, maintenance and/or survival of PGCs during ontogenic development in Nile tilapia. This study has helped rationalise the targets to be taken forward into functional disruption studies with the aim to develop interventions that will induce sterile fish by disrupting normal PGC development.

LEOPOLDINA SYMPOSIUM

L1 Towards understanding the mechanism of seasonal time measurement

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Animals living in temperate zones use changes in day length to adapt to seasonal changes in the environment. It is well established that the circadian clock is involved in seasonal (photoperiodic) time measurement. However, the mechanism of how the circadian clock measures day length remains unknown.

It has been reported that Medaka populations inhabiting higher latitudes require longer day lengths for reproduction than those inhabiting lower latitudes for reproduction. We obtained Medaka populations, including inbred strains, closed colonies and natural populations that were derived from different latitudes. When we examined the critical day length required for reproduction, Northern populations required 14 hours of light for gonadal development, while Southern populations required 13 hours of light. To identify genes that define this critical day length, we crossed different populations and obtained F1 and F2 generations. Subsequently, we performed quantitative trait loci (QTL) analysis using restriction-site associated DNA (RAD) markers and identified a significant QTL.

In addition to the above-mentioned forward genetic approach, we are currently using a chemical genetic strategy. We performed structure-activity relationship studies on the period-lengthening molecule KL001 and surprisingly discovered period-shortening molecules that target cryptochrome. In addition, we have discovered many compounds that alter circadian period both in vitro and in vivo by high-throughput chemical library screening. Because the circadian clock is involved in photoperiodism and various human diseases, we expect that modulation of the circadian clock by small molecules will contribute to photoperiodism research and form the basis for therapeutic applications.

L2 Genetic and cellular mechanisms involved in the generation of long-term seasonal cycles

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Organisms that live in seasonal environments are driven by internal year-long cycles (circannual), which in animals drive deep-rooted metabolic and reproductive rhythms. These persist in constant conditions, but in nature are synchronized by changing photoperiod, which activate reproduction and growth for the optimal time of year. We now know that these rhythms are regulated by changing activity of thyroid hormones in the hypothalamus, mediated by photoperiodic control of the de-iodinase enzymes in specialized hypothalamic tanycytes. In mammals, the nocturnally secreted pineal hormone melatonin (MEL) is sculpted by photoperiod and provides the brain with an internal representation of external photoperiod change. Recent research shows that MEL acts on specialized TSH-secreting cells (thyrotrophs) in the pars tuberalis (PT) of the pituitary, which then drive TSH-receptors in the hypothalamic tanycytes, to regulate de-iodinase activity. The up-stream molecular switch driving TSH in the PT is Eya3, a member of the retinal determining gene family. This is activated via a circadian mechanism on long summer-like photoperiods, in which rhythmic MEL signals drive a circadian oscillation in the PT of Eya3 via an E-box mediated mechanism.

We now have strong evidence that a circannual clock is also located within PT thyrotrophs. Using seasonal sheep, we have mapped expression of EYA3 (a summer-like signal) and chromogranin-A (CHGA; a winter-like signal) to show that any individual PT cell can only be in one of two states (EYA3+ve or CHGA+ve). Thus, circannual time is encoded digitally, in which cells flip from one state to another, the relative proportion of which determines circannual phase. We therefore propose a new model for the control of an endocrine tissue in which environmental information is encoded digitally, likely through binary regulation of transcriptional programmes, which can only exist in one of two states.

Our data also suggest that the PT may establish morphogenic gradients, which extend to the ME, leading to retrograde signaling, and seasonal re-modeling of neuronal terminals and the associated tanycyte end feet. Seasonal changes in GnRH accessibility to the have been shown in Japanese quail and are of a similar magnitude to those we report here, but, in contrast to our data, this occurred on opposing photoperiods (i.e., tanycyte enclosure of neuronal synapses occurred on SPs in quail, rather than on LPs in sheep). This suggests that re-modeling of the neuroendocrine synapse may be linked to the phase of the reproductive cycle rather than the prevailing photoperiod.

We propose that the PT thyrotroph acts as a seasonal calendar cell with the capacity to generate long-term rhythms in mammals, driving both hypothalamic and pituitary endocrine circuits. This may represent an ancient signaling mechanism driving adaptation in eukaryotic organisms in seasonal environments for the past 2 billion years.

L3 What makes a mammal a seasonal breader?

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In the wild, species adapt their biological functions to the predictive seasonal changes in their environmental lighting. More than 50 years ago, it was established that in mammals the annual changes in photoperiod are integrated into the nervous system via the nocturnal production of the pineal hormone melatonin. Recent findings have demonstrated that the seasonal change in the nocturnal production of melatonin drives TSH synthesis in the pars tuberalis, so that it is high in long- and inhibited in short- photoperiod. TSH released from the pars tuberalis in long photoperiod increases the local concentration of thyroid hormones (TH) in the basal hypothalamus via a regulation of deiodinases 2 and 3 in the tanycytes. We recently reported that kisspeptin neurons in the arcuate nucleus are a critical interface between the TSH/TH signal and the activity of GnRH neurons. However, kisspeptin expression is regulated by other biological cues and displays marked differences among seasonal species, indicating that another hypothalamic system operating upstream of kisspeptin/GnRH neurons may integrate the photoperiodic TSH/TH signal. We found that another peptide of the same RF-amide family of kisspeptin, RFRP-3, expressed in neurons of the dorso/ventro medial hypothamus, displays strong photoperiodic variation that are conserved among seasonal species. Our experiments in seasonal rodents demonstrate that RFRP gene expression is fully activated by TSH and is not submitted to the feedback effect of sex steroids, thus pointing these RFRP neurons as the critical gate for the hypothalamic integration of photoperiod cues. Furthermore, we report that RFRP displays remarkable sex- and species- specific differences in the regulation of reproductive activity, which may explain the various reproductive responses to photoperiod cues observed among seasonal species.

L4 Gestational photoperiod programmes offspring reproductive development via the fetal pituitary gland.

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In wild mammals, offspring development must anticipate forthcoming metabolic demands and opportunities. Within species, different developmental strategies may be employed, dependent on when in the year conception takes place. This phenotypic flexibility is initiated before birth, and is linked to the pattern of day length (photoperiod) exposure experienced by the mother during pregnancy. This depends on transplacental communication via the pineal hormone melatonin.

Here, we show that, in the Siberian hamster (*Phodopus sungorus*), the programming effect of melatonin is mediated by the pars tuberalis (PT) of the fetal pituitary gland, before the fetal circadian system and autonomous melatonin production is established. Maternal melatonin acts on the fetal PT to control expression of thyroid hormone deiodinases in ependymal cells (tanycytes) of the fetal hypothalamus, and hence neuroendocrine output. This sets the trajectory of reproductive development in pups, and has a persistent effect on their subsequent sensitivity to photoperiod. This programming effect occurs downstream of pineal melatonin production and of PT melatonin-sensitivity. Rather, tanycyte sensitivity to TSH is dramatically and persistently increased by short photoperiod exposure *in utero*.

Our results define a novel transplacental pathway for epigenetic programming of fetal brain function, and establish programmed changes in TSH receptor signal transduction as a central feature of seasonal timekeeping.

L5 Synchronization of seasonal functions by photoperiodic changes. With or without melatonin.

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In a seasonal environment, mammals time their reproductive phase so that the offspring is born in spring and summer. Two strategies have evolved to ensure accurate seasonal timing but both share a common *zeitgeber*, the photoperiod: 1) Photoperiod *controlled* seasonal rhythms and 2) photoperiod *entrained* circannual rhythms. In the former the photoperiod controls directly via a melatonin-dependant mechanism the physiological state. For example, in Syrian hamsters short photoperiod induces gonadal atrophy but after 28 weeks exposure a sexual reactivation is observed. An unknown endogenous process referred to as photorefractoriness induces sexual reactivation for the lifetime unless photorefractoriness is broken by a new exposure to long photoperiod. In short, these photoperiodic species are unable to pass a complete annual cycle without changes in photoperiod. Pinealectomy renders them unresponsive to photoperiod changes.

In contrast, when circannual species (e.g. European hamster) are kept in constant environmental conditions, repetitive complete seasonal oscillations are observed with a period of ≈ one year. These seasonal changes are endogenously driven by a still un-identified circannual clock. To keep the circannual clock synchronized with the environmental period length of one year, the photoperiodic signal acts via a circadian-based but melatonin-independent mechanism.

Considering the current concept of seasonal time keeping developed in photoperiodic species, the circannual seasonal time keeping (as determined in the European hamster) differs in fundamental details. A specific circadian organization is necessary to measure day length and the resetting of the circannual clock is melatonin-independent. Melatonin acts also in circannual species, but data favours the idea that melatonin does not act on the circannual clock.

We propose thus that in mammals two distinct processes ensure accurate seasonal time keeping. A circannual one circadian-based but melatonin-independent and a photoperiodic melatonin-dependent one. This raises the question of the nature of the melatonin-independent photoperiodic signal and of the identification of the nervous and neuroendocrine pathways involved. For both the melatonin-dependent and –independent pathway, the SCN is the site where the photoperiodic message is generated. After the SCN, there should be a specific pathway to transduce the melatonin-independent photoperiodic seasonal signal, analogous to the melatonin-dependent pathway. Research is in progress and preliminary data suggest that arcuate nuclei are involved.

In conclusion. This work hence breaks down 2 dogmas: first, contrary to what was previously considered, we show that the melatonin pathway is not the only means by which photoperiodic information can be conveyed; and second, circannual and photoperiodic species show differences in the way that they process photoperiodic information for seasonal entrainment, which was not thought to be the case. While photoperiodic species rely predominantly (but not exclusively) on the melatonin-dependent pathway, circannual species rely predominantly (but also not exclusively) on a currently uncharacterized melatonin independent pathway. Circannual resetting is thus more complex than was previously thought.

L6 Signaling pathways to and from the hypophysial pars tuberalis

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The hypophysial pars tuberalis (PT), an important interface between the hypophysial pars distalis and neuroendocrine centers in the brain, plays an essential role in the regulation of seasonal functions, such as reproduction. Photoperiodic signals provide a major input to the PT. In mammals photoperiodic signals are perceived in the retina and translated into melatonin that is produced in the pineal organ night by night and encodes the length of the night. In the PT melatonin acts upon MT1 receptors. Output signals from the PT are transmitted via two pathways. A very well studied retrograde pathway employs TSH and targets ependymal cells in the third ventricle which control a local hypothalamic T3 system. The anterograde pathway targets cells in the PD, is implicated in the control of prolactin secretion and supposedly employs small molecules as signal substances collectively denominated as “tuberalins”. Several “tuberalin” candidates have been proposed, such as tachykinins, the secretory protein TAFA and endocannabinoids (EC). The PT-intrinsic EC system was discovered in Syrian hamsters and shown to respond to photoperiodic changes. Subsequently, the EC system was also demonstrated in the PT of mice, rats and humans. Likely targets for the EC are folliculo-stellate cells that contain the CB1 receptor and are adjacent to lactotroph cells. Interestingly, the CB1 receptor is also found on corticotroph cells suggesting that, via EC, the PT might also modulate the stress response. Taken together, the results support the concept that the PT transmits its signal via a “cocktail” of messenger molecules which occur also in other brain areas and systems rather than through PT-specific “tuberalins”. An important aspect for future studies will be to determine whether (some of the) messengers of the anterograde pathway are also engaged in the retrograde pathway to the hypothalamus and median eminence.

L7 Non-24h sleep wake disorder in relation to different light conditions

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Non 24h sleep wake disorder is manifested by sleep times getting progressively later and later (rarely earlier and earlier), so the person is eventually sleeping during the day until they cycle back to a nighttime bedtime. It is a persistent or recurrent pattern of sleep disruption that is primarily due to an alteration of the circadian system or to a misalignment between the endogenous circadian rhythm and the sleep-wake schedule required by an individual's physical environment or social or professional schedule. WHO (ICD-10) refers to it as "Circadian rhythm sleep disorder, free-running type (Non-24)", and DSM-5 refers to it as "Circadian rhythm sleep-wake disorder, Non-24-hour sleep-wake type".

The main factor maintaining synchrony of the circadian system with the environment is the light dark cycle. Completely blind people with no conscious or unconscious (hypothalamic) perception of light, frequently show Non-24 with accompanying decrements in sleep, performance, social and employment abilities: effectively intermittent jet-lag over a lifetime. This problem can now be treated by appropriate administration of chronobiotics, notably melatonin or a recently approved agonist Hetlioz (tasimelteon) developed specifically for Non-24 by Vanda Pharmaceuticals.

However non-24 also occurs in otherwise healthy sighted people with insufficient exposure to light of appropriate intensity, spectral composition, timing and photoperiod. During the polar winter within the Arctic and Antarctic circles, the sun does not rise for variable periods of time in winter: the nearer to the poles the longer is the 'sundown'. In early work, with a maximum recorded lux in winter of approximately lux (standard indoor lighting), substantial phase delays of the circadian system occurred, leading to free-run, i.e. Non-24, in some cases, and notably in the absence of other strong social and other time cues. This problem resolved when the sun returned. Moreover the phase delay in winter could be eliminated by administration of a skeleton spring photoperiod of 2500 lux bright white light. Most recently a single 1h daily exposure to morning bright light (4800 lux) was sufficient to normalise phase, sleep and performance.

Night-shift work in the polar winter (and in high latitudes on some oil rig schedules) in contrast to temperate zones, leads to rapid and complete adaptation of the circadian system with improved sleep compared to day shift work. This illustrates how the circadian delay to the night shift schedule provides a more appropriate phase relationship between desired sleep time (much later with night shift) and phase. Difficulties arise when attempting to readapt back to dayshift in winter and subjects can free-run for weeks during this time. In summer this problem does not arise. However the polar summer (24h daylight) is not without problems: exposure to bright sunlight in the evenings also leads to phase delays. However in this case avoidance of light and appropriate bedtimes in darkened rooms is the answer.

Since circadian desynchrony is now associated with increased risk of some major diseases, as well as poor sleep and performance (in shift workers), there is good reason to maintain synchrony as far as possible. Whilst skeleton light treatment in dim light conditions is efficient, it is intrusive. We have investigated the effects on sleep and phase of a full 10-12h photoperiod of extra bright white light (5000 K) compared to extra blue enriched light (17000 K) in alternate months, provided throughout the Antarctic base of Halley during the winter. It was clear that both treatments were effective (blue was slightly better than white) and that the critical feature was the average maximum daily light exposure (1631 ± 487, white, 2068 ± 485, blue).

L8 Seasonal variation in human brain function

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Although seasonal variations in some aspect of human physiology have been identified, little is known about potential seasonal variations in human brain physiology. We investigated annual rhythms of brain activity via two cross sectional experiments conducted under strictly controlled conditions, free of in-lab seasonal cues in young healthy volunteers. The first experiment used functional Magnetic Resonance Imaging (fMRI) recordings of cognitive brain responses to two different tasks following 4.5 days of controlled in-lab conditions. The second experiment used repeated recordings of Transcranial Magnetic Stimulation (TMS) evoked EEG responses during 29h of sleep deprivation conducted under constant routine conditions, and sought for relationships with concomitant cognitive performance assessments.

The data show that fMRI cognitive brain responses vary significantly across seasons but, surprisingly, the pattern of the rhythm was task-dependent, revealing a previously unappreciated process-specific seasonality in human cognitive brain function. Brain responses to a sustained attention task had maximum and minimum responses around summer and winter solstices, respectively, while for a working memory task, maximum and minimum responses were observed around autumn and spring equinoxes. In the second experiment, we observed an earlier daily peak, during short as compared to long photoperiod, in cortical excitability, a basic feature of brain function, sustaining ongoing cognition, and in fast EEG frequencies related to inhibitory neuronal function. In addition, computational modeling further revealed that these changes were likely triggered by an earlier peak in the variation in the activity balance of GABA/glutamate receptors. The latter results speak for a role of the balance between inhibition and excitation in seasonal encoding in human, as suggested in rodents.

These yearly dynamics in brain and neuronal activity may represent some of the means through which human beings cope with season changes to maintain cognitive performance and brain function.

L9 Seasonal rhythms in affective disorders

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Daily and seasonal rhythms in the symptoms and occurrence of psychiatric illness, in particular affective disorders, have been documented since earliest times: diurnal variation of mood, phase shifts of sleep-wake cycles with clinical state, higher occurrence of mania in summer and depression in spring. The associated search for biochemical markers indicated that e.g. monoamines and their metabolites undergo circadian and seasonal changes in plasma, CSF, or CNS. Thus, humans possess the neural mechanisms to respond to environmental cues of light and dark and daylength, even though in our modern society these are largely diminished in amplitude.

The diagnosis of autumn-winter Seasonal Affective Disorder in the eighties (i.e. not an epidemiological cluster of illness incidence, but an individual pattern of recurrence) was a key moment in chronobiological research. Here was a human manifestation of dysfunction that could be understood in terms of the neurobiology of seasonal behaviour in mammals, which led to a treatment postulated to restore euthymia with a simulated spring day. Light therapy has since become the treatment of choice for winter depression, as well as proving efficacious in non-seasonal affective disorder, bipolar illness, dementia, sleep-wake cycle disturbances in many psychiatric diagnoses as well as in internal medicine. Light (and dark) therapy are direct examples of translational medicine: It is to be hoped that their potential will be adequately recognised and implemented, in spite of the lack of patentability and difficulty to be incorporated into treatment guidelines in a pharmacologically-oriented medical paradigm.

POSTERS

PO1 Allatostatin-C and allatotropin regulate hypostome activity by an antagonistic mechanism involving calcium in hydra sp.

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Allatotropin (AT) and allatostatin-C (AST-C) are neuropeptides described by its ability to stimulate (AT) or inhibit (AST-C) the secretion of juvenile hormone (JH) in insects. This neuropeptides also act as myoregulators exerting their functions through G protein-coupled receptors (GPCR) activating different signaling cascades. We have previously showed the presence of AT-like peptides in invertebrates other than insects (flatworms and cnidarians) suggesting that this peptide is widely distributed in nature playing an ancestral function related with visceral muscle activity regulation.

Here, we analyze the existence of an antagonistic relationship between AT and AST-C on the hypostome in cnidarians (*Hydra* sp) and its probable mechanism mediated by calcium.

Materials and methods. Individuals of Hydra sp. were obtained from a colony maintained in dechlorinated water at 20±2°C with a 12:12 hour light/dark period and were fed with Artemia salina. For physiological assays, specimens were starved during the 48 hours previous to the experiment. All the experiments were performed with groups comprised by 7 individuals. Physiological assays: Hydroids were divided in experimental groups: Controls (C) and treated with: 10^{-6}M AT (AT); 10^{-6}M AT plus 10^{-6}M AST-C simultaneously (AT+ASTC) or 10^{-6}M AT plus 10^{-6}M ASTC and 1 µM of Thapsigargin (TG) that inhibits the Ca^{2+} ion pump protein of the sarco(endo)plasmic reticulum (AT+ASTC+TG). Hydroids were examined individually under a binocular microscope and recorded with a digital video camera. A time-lapse was recorded for each experiment by taking a picture every 3 seconds during 15 mins. The length of hypostome was measured at different time points during the 15 min of exposition to each treatement by using the GNU Image Manipulation Program (GIMP) software. Differences between treatments were analyzed by multifactorial ANOVA.

Results. As previously describes the hypostome reacts to AT. Now, our results showed that this reaction is dose-dependent, generating a significative increment in their length with doses higher to 10^{-12}M ($p \leq 0.05$). When hydroids were treated with AT and AST-C peptides together, a statistically significant decrease of the effect of AT was observed ($p \leq 0.05$). This effect was manteined five minutes, then the length of the hypostome increased to values similar to those produced by the addition of AT alone. When TG was involved in the treatment (AT+ AST-C+TG), the effect of AST-C was not evident, showing the hypostome values similar to those reached when the treatment was performed only with AT.

Conclusions. Our results confirms that AT acts as a myoregulatory peptide in *Hydra* sp, inducing a reversible and dose-dependent increase in the length of the hypostome. Furthermore, they show that the AST-C peptide would also have a myoregulatory effect probably antagonizing the activity of AT. AST-C acts via somatostatin-like receptors that are mainly inhibitors, acting through a decrease of cytosolic Ca^{2+} availability. The use of TG to avoid the decrease of cytosolic Ca^{2+} caused that AST-C treatment failed, suggesting that AST-C antagonizes AT by a reduction in the cytosolic Ca^{2+} availability. Taking together, these results suggest that the Allatotropin/Orexin and Allatostatin/Somatostatin regulatory systems could pertain to ancestral mechanisms regulating the behavior in animals.

PO2 Modulation of gene expression and activity of the adrenal cortex by the daily and seasonal light in the D'Man sheep

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The aim of our study is to highlight the role of light on the adrenocortical function supported by many genes that regulate the biosynthesis and rhythm of cortisol. The investigation was conducted on 28 adult D'man race sheep high at the experimental station of El-Meniaa (30 ° 34 'North Latitude, 02 ° 52' East Longitude, Altitude 379m), maintained in their natural temperature and light.

During the equinoxes and solstices, blood samples were collected every 15 min during 25 hours; animals are then sacrificed. The adrenal collected are stored; either part in the aqueous bouin (for the histomorphometric study), the other part is frozen at -80 ° C (for the molecular study). Cortisol is measured by radioimmunoassay technique (RIA).

The results show a rhythmic expression of genes studied, with a strong expression of: Clock, Bmal1, 3βhsd and Star during the night phase and an important expression of Per2 and Reverb during the daytime phase. Furthermore there are variations in the thickness of the cortex (more developed in light phase than in the dark phase) for all solstices and equinoxes. The adrenal cortex also present morphometric changes during the year characterized by: a maximum in summer / spring and a minimum in winter / autumn. The glucocorticoid activity is characterized by a seasonal pattern (high cortisol at the summer solstice and low at the winter solstice). Finally, we report the existence of significant correlations between the light factor, gene expression, structure and adrenal glucocorticoid activity of the adrenal. Given these results, it seems that the daily and seasonal light has a powerful effect on the timing of genes expressed in the adrenal cortex, manifested by changes in the histological appearance of the adrenal glucocorticoid and its activity, that would be an adaptive mechanism of the D'man sheep to environmental conditions.

Keywords: adrenal cortex, gene clocks, cycle light / darkness, D'man Sheep, Sahara.

PO3 Growth signals modulation by IGFs in gilthead sea bream myocytes

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IGF-I and IGF-II are essential hormones for stimulating and modulating growth and IGFs investigation may represent an important index of biological growth. To study the response of gilthead sea bream (*Sparus aurata*) cultured myocytes to the administration of IGFs (at 100 nM concentration), we determined the mRNA levels of 19 genes including members of the IGFs system, myogenic regulatory factors (MRFs), AKT and target of rapamycin (TOR) signaling pathways and developmental markers such as proliferating cell nuclear antigen (PCNA) and myosin heavy chain (MHC) at two different incubation times (6 and 18 h). Then, to work more deeply into the signaling pathways, we analyzed the protein level of AKT, TOR and PCNA. Both IGFs stimulate growth, supported in this study by increased expression of IGF-I and the binding protein IGFBP-5. IGF-I reduced the expression of the IGF-I receptors (IGF-IRs) and IGF-II increased the expression of IGF-IRb. Moreover, IGF-I down-regulated the expression of Myf5, while it enhanced the expression of MHC, suggesting increased differentiation into myotubes. On the other hand, IGF-II showed a more important role in proliferation as indicated by increased PCNA at the protein level. Moreover, the protein expression of AKT increased after IGFs treatments. IGF-II up-regulated the gene expression of TOR as well as its activation by phosphorylation. Taken together, these results provide evidence for the importance of IGFs on the control of muscle development and growth in gilthead sea bream. Supported by MICINN (AGL2012-39768, AGL2015-70679-R), Generalitat de Catalunya (XRAq, 2014SGR-01371).

PO4 Sexual differentiation in isopods

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In crustaceans, the androgenic gland (AG), thanks to the synthesis of the androgenic gland hormone (AGH), controls the differentiation of the primary and secondary male sexual characters. This gland, first discovered in Amphipods by Charniaux-Coton in 1954, was also described in Decapods and Isopods. The sequence of the AGH was however first deciphered in the terrestrial isopod *Armadillidium vulgare*, more than 40 years after the discovery of the AG, and have been found more recently in decapods. Since then, sequences of two different receptors of the AGH have been identified, both in decapods. The first one, called Insulin-like Growth Factor Binding Protein (IGFBP), is a circulating receptor, which seems highly conserved and which displays an ubiquist tissular expression. The second one, called Insulin-like Receptor (IR), is a membrane-anchored receptor with a tyrosine-kinase domain.

We successfully identified these receptors in isopods in transcriptomics libraries, in particular in the model species *A. vulgare*. Phylogenetics analyses revealed different levels of conservation of these two receptors in Crustaceans. New transcriptomes are currently being generated to extend the in silico analyses to the Isopods scale.

Furthermore, the AGH and receptor gene expression has been investigated during post embryonic development of *A. vulgare* as well as in the different tissues of adults. In situ approaches will also enable to characterize interactions between the hormone and its receptors.

Finally, this hormone, as well as its receptors, might be the targets of virulence factors of the feminizing Alphaproteobacteria *Wolbachia*, which infects numerous terrestrial isopod species. Experimental injection of *Wolbachia* into *A. vulgare* males revealed unexpected increase of AGH gene expression in gonads. This huge effect is not even inhibited by siRNA compared to uninfected controls. The effect of this endogenous endocrine disruptor on the AGH receptors will now be investigated in situ using FISH and immunocytochemistry. As feminizing effects of exogenous endocrine disruptors like Bisphenol A (BPA) have also been described on isopods, we started a comparative analysis of BPA and *Wolbachia* effects on the sexual differentiation of *A. vulgare*.

PO5 In vivo growth stimulation affects in vitro cell response

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Growth in fish is regulated through the growth hormone (GH) - insulin-like growth factors (IGFs) axis, and by its downstream signaling pathways, which are responsible for converging the inputs from the different agents implicated in growth, such as the genetic, environmental, nutritional and endocrine factors. These signaling pathways in turn regulate cell proliferation and differentiation, protein synthesis, metabolism and also the gene expression of some myogenic transcription factors. Several *in vivo* strategies have been used as successful approaches to stimulate growth in different fish species, as is the case of GH long-term treatment or moderate-sustained swimming. The main goal of this work was to characterize if these *in vivo* interventions can modify the *in vitro* development of cultured myocytes in order to improve knowledge in fish growth regulation. To achieve this objective, juveniles of gilthead sea bream (*Sparus aurata*) under a prolonged GH treatment or forced to perform moderate-sustained swimming were used as fish models. Then, primary cultures of muscle satellite cells extracted from these experimental fish were performed. Cell proliferation by means of immunocytochemistry and gene expression of myogenic regulatory factors and members of the endocrine GH/IGF-I axis and AKT-TOR signaling pathways by quantitative PCR were determined. The results indicate that the *in vivo* experience modifies the physiology of the cells, especially in the case of the GH treatment, since myocytes derived from GH-injected fish showed increased proliferative capacity when compared with those of control fish. Moreover, the positive effect of exercise on cultured cells was less remarkable but showed a similar tendency towards myogenesis stimulation. Altogether, the present work provides new insights about the factors involved in muscle growth regulation, and demonstrates how whole-body interventions have an important effect at the cellular level.

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PO6 Claudin-3 is selectively expressed in the olfactory system of postnatal and adult mouse

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The olfactory system is continuously renewing in mammals as in non-mammalian species, and thus, of particular interest with respect to continuous axonal growth and synaptic reorganization. The olfactory system comprises unmyelinated axons, which grow from the sensory neurons leaving the sensory epithelium as fila olfactoria, wrapped in glial cells, termed olfactory ensheathing cells (OECs), and surrounded by a perineural cell sheath, which is continued by the meningeal sheath after traverse of the olfactory fila through the cribriform plate to enter the olfactory bulb. Claudin-3 is a constitutive tight junction protein, which has been detected in choroid plexus epithelium of human, rat and mouse. Furthermore, it has been detected in the rat olfactory region of the nasal cavity and in several developing and malignant tissues, which suggests a role for anchorage-dependent and anchorage-independent cell migration. We here report in mouse the specific expression and localization of claudin-3 not only in the nasal cavity (as previously described for rat), but also in the olfactory bulb and nerve by immunohistochemistry and Western blot. Furthermore, other tight junction proteins (claudin-1, claudin-2, claudin-4, claudin-5 and occludin) were investigated to specify the distinct expression of claudin-3. Laminin was detected to differentiate the basal lamina of the olfactory epithelium and the blood vessels, and glial fibrillary acidic protein (GFAP) as a glial marker known to be expressed also by OECs. Claudin-3 was continuously present in the fila olfactoria from the olfactory epithelium via the cribriform plate to the olfactory nerve and the glomerular cell layers of the main olfactory bulb as well as in the accessory olfactory bulb. In contrast, immunoreactivity for GFAP as astrocyte marker was abundant within the olfactory bulb, particularly the glomerular cell layer, and was still found in the olfactory nerve layer as marker for OECs accompanying the fila olfactoria. We suggest that the abundant presence of claudin-3 in the olfactory system, particularly the axons, which we present here for the first time, may play a role at the interface of the central and the peripheral nervous system, both as a barrier and for promotion of axonal growth.

Wolburg H, Wolburg-Buchholz K, Sam H, Horvát S, Deli MA, Mack AF. Histochem Cell Biol 2008;130:127-40. Steinemann A, Galm I, Chip S, Nitsch C, Maly IP. Front Neuroanat. 2016;10:16.

PO7 Purification of two vitellogenesis-inhibiting hormones from Trachysalambria curvirostris and isolation of cDNAs encoding their precursors

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Vitellogenesis is an essential physiological event in the reproduction of oviparous animals. Various nutritive materials including carbohydrates, proteins, lipids, minerals, and vitamins destined for utilization in embryonic development are accumulated in the oocytes during this process. In crustaceans, vitellogenesis is negatively regulated by a neuropeptide, vitellogenesis-inhibiting hormone (VIH), which is synthesized in and secreted from the X-organ/sinus gland complex in the eyestalk. Until now, the intensive researches have been conducted to search VIH in the commercially important penaeid shrimp species, whereas the Southern rough shrimp *Trachysalambria curvirostris* has never been used as an experimental animal. In order to characterize multiple VIH molecules from *T. curvirostris*, one hundred eighty six sinus glands were dissected under stereo microscope and subsequently peptides were extracted. The extract was applied to reversed-phase HPLC (RP-HPLC). Measurement of MALDI-TOF mass spectra of all the peak fractions recovered from RP-HPLC revealed that two fractions contained candidate molecules for VIHs, since their molecular weights ranged from 8,000 to 9,500 Da. The two fractions were separately subjected to N-terminal amino acid sequence analysis, which identified more than 51 amino acid residues. The two peptides showed considerable sequence similarity to VIHs characterized from other penaeid shrimp species. In this study, the two peptides were designated as Trc-VIH-I and -II, respectively. Since these sequences were not complete, cDNA clones encoding the Trc-VIH-I and -II precursors were cloned by RT-PCR coupled with 5´- and 3´-RACE. The Trc-VIH-I cDNA consisted of 648 bp including a 5´-untranslated region (UTR) (66 bp), an open reading frame (ORF) (327 bp), and a 3´-UTR (195bp). The Trc-VIH-II cDNA consisted of 646 bp including a 5´-UTR (63 bp), an ORF (354bp), and a 3´-UTR (229 bp). The mature Trc-VIH-I and -II were thought to consist of 72 amino acid residues containing six conserved cysteine residues and possess an amidated C-terminus. The mature Trc-VIH-I and -II showed sequence identities 63.0% and 74.3% to Liv-SGP-G (one of six VIHs in *Litopenaeus vannamei*), 59.3% and 72.9% to Maj-SGP-VII (one of six CHH/VIHs in *Marsupenaeus japonicus*) and 58.6% and 66.7% to Mej-SGP-III (one of three VIHs in *Metapenaeus joyneri*), respectively. In RT-PCR analysis of tissue-specific gene expression of Trc-VIH-I and -II, both transcripts were detected only in the eyestalk. This is the first report that VIH cDNAs have been cloned from a shrimp species belonging to the genus *Trachysalambria*. Therefore, these amino acid sequences provide us for understanding molecular evolution of VIH in penaeid shrimps.

PO8 Expression pattern of nanos and piwil genes during ontogenic development in Nile tilapia oreochromis niloticus

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Primordial germ cells (PGCs) are set aside from somatic cells, migrate into prospective gonadal sites during early development and give rise to germ cell lineage. There is interest within the Aquaculture sector to explore viable means to disrupt the natural PGC development which will ultimately induce sterility. If successful, PGC disrupted animals will not sexually mature which has a range of economic as well as environmental benefits. PGCs are specified by maternally provided determinants in fish, flies, worms and frogs, while it is independent to maternal information and affected by inductive signals from surrounding tissues in mammals and most animal species (see review Extavour and Akam, 2003). In addition, maternally deposited germ genes are known to have important roles in migration, maintenance and/or survival of PGCs in other teleosts species, but their functions vary between species. As a preliminary study to find potential target genes for sterilisation, ontogenic expression pattern of selected known germ genes in the literature and their tissue distribution were investigated in Nile tilapia to confirm their potential role in regulating PGCs.

To investigate the mRNA expression patterns of nanos and piwil genes during ontogenic development, embryos or larvae were sampled throughout development from unfertilized eggs to hatched larvae. Total RNAs were extracted and cDNAs were synthesized after DNase I treatment. The mRNA expression levels of nanos1a, 1b, 2 and 3, piwil1 and 2 were analysed by qRT-PCR and normalised using the geometric mean of elf1a, ß-actin and GAPDH expression. For the tissue screen of nanos and piwils, total RNAs were extracted from brain, pituitary, eye, heart, intestine, spleen, kidney, liver & gonads of both male and female adult tilapia and used for cDNA synthesis after DNase I treatment. The tissue distribution was analysed by RT-PCR and gel electrophoresis.

In Nile tilapia, there were 4 nanos genes and 2 piwil genes (Bellaiche et al., 2014; Xiao et al., 2013). The ontogenic expression pattern revealed that nanos1a, 1b and 3 and piwil1 and 2 are maternally transferred while nanos2 appeared to not be expressed in eggs. The zygotic transcription of nanos1a and nanos1b was initiated at neurula and gastrula stages, respectively, and they were distributed in various tissues of adult tilapia. On the other hand, nanos3, piwil1 and 2 showed gonad (ovary and testis) specific expression and nanos2 showed testis specific expression. Most of the maternal transcripts of nanos3, piwil1 and 2 were degraded between blastula and gastrula stages, indicating the period of maternal to zygotic transition (MZT). In contrast to nanos1a and 1b, none of nanos3, piwil1 and 2 showed distinct zygotic transcription by hatching stage. In summary, this result suggests that among 4 nanos and 2 piwil genes, the maternal transcripts of nanos3, piwil1 and 2 are considered to play a role in migration, maintenance and/or survival of PGCs during ontogenic development in Nile tilapia. This study has helped rationalise the targets to be taken forward into functional disruption studies with the aim to develop interventions that will induce sterile fish by disrupting normal PGC development.

PO9 A rare case of insulinoma - What can we learn from the sweetener?

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Intro

An insulinoma is an insulin secreting tumour typically arising from the beta cells of the islet of Langerhans within the pancreas. This pancreatic islet cell tumour was first identified in 1927 and the first surgical resection of an insulinoma was carried out in 1929. Although a rare tumour, with an incidence of 4 people per million in the general population, it is the most common neuroendocrine tumour. Insulinomas have a female predominance and a peak incidence between 30-60 years. Surgical resection is the mainstay of insulinoma treatment, as the majority of cases are benign, this provides a cure in 90% of individuals with the diagnosis. Patients unfit for surgery or with a diagnosis of malignant insulinoma are medically managed with a high carbohydrate diet and anti-hypoglycaemic agent.

Case

A 94 year old female patient presented to hospital with recurrent episodes of symptomatic hypoglycaemia. She described and demonstrated Whipple's triad (hypoglycaemia <2.5mmol/L, neuroglycopenic symptoms, reversal of symptoms on administration of glucose); a triad unique to insulinoma.

Initial imaging with an abdominal ultrasound & CT abdominal-pelvis proved unremarkable, but a subsequent MRI of the pancreas confirmed a 12mm lesion in the body of the pancreas suggestive of an insulinoma. The diagnosis of insulinoma was confirmed following specific blood tests after a period of patient fasting. A blood glucose level of 1.9mmol/L, alongside a raised insulin level (14.5 µU/mL) and a significantly raised c-peptide level (1169) confirmed an engogenous hyperinsulinism.

Results

Due to the patient's age, opinion and performance status, she was not a candidate for surgical intervention. The decision was made to medically manage her insulinoma with diazoxide. Diazoxide acts by inhibiting insulin release from the pancreas, therefore increasing blood glucose by secondary effect. The diazoxide dose was guided by patient weight and effectively raised and maintained the patients' glucose levels within normal limits.

A reported, common side effect of diazoxide therapy is fluid retention. This patient was unfortunately no exception. Within approximately one month of treatment she developed a drug induced congestive cardiac failure. Despite reducing the dose of diazoxide, the patient unfortunately developed fatal pulmonary oedema.

Conclusion

This case highlights important factors to consider when medically managing insulinoma in elderly patients; of which there is little literature and few published case reports. Following this iatrogenic mortality our aim is to raise awareness and caution when prescribing diazoxide. We recommend a baseline echo prior to initiating treatment, the use of prophylactic loop diuretics and diazoxide dose titration to ensure the patient is on the lowest therapeutic dose, limiting the risk of potentially fatal side effects.

PO10 Effect of cortisol treatment on the expression of IMPA1 gene homologues in the gill and kidney of the European eel (Anguilla anguilla).

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The European eel (*Anguilla anguilla*) is able to osmoregulate over a wide range of environmental salinities. Both “sexually maturing”silver and “sexually immature”yellow eels are able to successfully acclimate to both seawater (SW) and hyper SW environments. Cortisol is a major osmoregulatory hormone released from inter-renal gland that regulates the expression of a range of genes encoding a variety of ion transporters and intracellular proteins that are essential for osmotic homeostasis during SW migration. Recent studies have implicated a role for the cyclic alcohol inositol as an important cellular osmolyte that helps to maintain cell volume and fluid balance when fish are exposed to hypertonic conditions. Myo-inositol monophosphatase 1 (IMPA1) is a key enzyme responsible for the cellular production of myo-inositol *de novo* and three isoforms (IMPAs1.1, 1.2, and 1.3) exhibit tissue-specific expression in the eel. Only IMPA1.1 was found to be salinity sensitive with the expression of this isoform being up-regulated in the kidney, fin, oesophagus and gill when fish were transferred to SW. In this study we investigated the effect of cortisol administration on the expression of the three IMPA1s homologues in the osmoregulatory tissues from FW-acclimated yellow eels. qPCR analysis revealed that only the IMPA1.1 isoform was significantly up-regulated by cortisol in the gill, while in the kidney the expression of IMPA1.1 appeared highly variable between individuals in the cortisol-treated group so mean levels were not significantly different from controls. No impact of cortisol was seen on the expression of IMPA1.2 or IMPA1.3 in either tissue suggesting a potentially different physiological role of these homologues in the eel.

PO11 Prolyl carboxypeptidase: a potential biomarker for obesity and diabetes mellitus?

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Background : Obesity and diabetes are metabolic diseases causing a major concern for society. Recent findings indicate that the enzyme prolyl carboxypeptidase (PRCP) plays a role in energy homeostasis by changing the biological activity of peptides involved in the regulation of food intake. Studies have shown that the plasma concentrations of PRCP may be used to reflect metabolic conditions in individuals with obesity and diabetes mellitus[1,2]. Unfortunately, PRCP activity measurements were not included or not properly performed.

Aims: The purpose of this study was to investigate PRCP activity in obesity and diabetes and to discover novel PRCP peptide substrates linked to these metabolic diseases. Pyroglutamated apelin-13 ((pyr)-apelin-13), ghrelin, enterostatin and obestatin were investigated since these are feeding-regulating peptides with potential cleaving sites (-Xxx-Pro/Ala-Xxx) for PRCP.

Material and methods: Human patients were categorized based on their BMI (<25, 25-29.9, 30-39.9, >40 kg/m^2 ; n=20 per group). Diabetes was induced in Wistar rats (n=8 per group) by intravenous streptozocin (65 mg/kg) combined with intraperitoneal nicotinamide injection (230 mg/kg). PRCP activity in human and rodent serum was measured via a RP-HPLC activity assay[3]. The kinetic parameters of recombinant human PRCP for potential peptide substrates were determined using isothermal titration calorimetry and mass spectrometry.

Results: We observed that PRCP activity is significantly increased in serum of obese patients and that this activity is positively correlated with body weight (r=0.392), visceral and subcutaneous fat (r= 0.376), waist (r=0.385) and hip (r=0.360) circumference. Furthermore, we found that the serum PRCP activity is significantly higher in streptozotocin-nicotinamide-induced diabetic Wistar rats versus controls. The search for novel PRCP substrates revealed that in the reaction conditions used enterostatin, ghrelin and obestatin were not hydrolyzed, but that PRCP removed the C-terminal Phe from (pyr)-apelin-13.

Conclusion: Serum PRCP activity is associated with obesity in humans and diabetes in rats thus a potential role for PRCP as biomarker is conceivable. The finding that (pyr)-apelin-13 is a novel PRCP substrate *in vitro* adds evidence to the hypothesis that PRCP plays a role in the pathophysiology of obesity.

PO12 Identification, bioactivity and cellular localization of relaxin-like gonad-stimulating peptide in the starfish Asterias rubens

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The peptide hormone relaxin has important roles in human reproductive biology, causing softening of the cervix and relaxation of the uterus prior to childbirth. Interestingly, evidence that relaxin-type peptides may have an evolutionarily ancient role in regulation of reproductive processes has been obtained with the discovery of a relaxin-like gonad stimulating peptide (RGP) that triggers gamete release in the starfish *Patiria pectinifera* (PpeRGP). Here we have investigated the bioactivity and anatomical expression of RGP using the European starfish *Asterias rubens* as a model experimental system.

Analysis of *A. rubens* neural transcriptome sequence data enabled identification of a transcript encoding the RGP precursor, which was confirmed by cDNA cloning and sequencing. Investigation of the bioactivity of synthetic AruRGP and synthetic PpeRGP revealed that both polypeptides induced oocyte maturation and ovulation in *A. rubens* ovarian fragments, but the concentration required to induce spawning in 50% of ovarian fragments differed. The EC_{50} for AruRGP was 1.33 nM whereas for PpeRGP the EC_{50} was ~10 fold higher (14 nM), consistent with sequence differences.

To gain new insights into the physiology of RGP, mRNA *in situ* hybridization was used to map expression of RGP precursor transcripts in *A. rubens*. Cells expressing RGP were detected in the radial nerve cords, circumoral nerve ring and tube feet. Furthermore, strong expression was also detected in a band of cells in the body wall epithelium lining a cavity at the tips of each arm, which contains the terminal tentacle and the optic cushion.

Because RGP was originally isolated from starfish nerve cords there has been an assumption that the nerve cords are the physiological source of RGP for hormonal control of spawning. Our findings have revealed alternative sources. In particular, the discovery of RGP-expressing cells in the arm tips of *A. rubens* is important because these cells are located in close proximity to sensory organs such as the optic cushion. Thus, environmental cues (e.g. changes in day-length, changes in water temperature and/or the presence of gametes released by conspecifics) may trigger release of RGP by the RGP-expressing cells in the arm tips to mediate hormonal control of spawning in starfish.

PO13 The role of target of rapamycin (TOR) in body size and adult structure development in the red flour beetle, Tribolium castaneum

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Adult structure development is an important process during insect growth. Here in the red flour beetle, *Tribolium castaneum*, silencing of target of rapamycin (TOR) decreased the pupal mass and size, as well as the adult structures' size, such as the size of wings, legs, gin traps and mandible. The observed effects were nutrition-independent, because the food consumption rate of the TOR-silenced insects did not differ from the controls. In addition to the interference on adult structures size, some abnormal effects were also observed during insect development. Injured pupae, larval-pupal and pupal-adult intermediates were the most phenotypes observed in TOR-silenced insects, and they died finally. Only a small number of the treated insects became adults, though of smaller size. Thus, TOR plays an essential role in the insect development, especially for the body size and adult structures development.

PO14 Drosophila insulin-like peptide 1 (DILP1) is transiently expressed during non-feeding stages and reproductive dormancy

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The insulin/insulin-like growth factor signaling pathway is evolutionarily conserved in animals, and is part of nutrient-sensing mechanisms that control growth, metabolism, reproduction, stress responses, and also affects lifespan. In *Drosophila,* there are eight insulin-like peptides (DILP1-8). Six of these have been investigated in some detail, whereas the expression and functions of DILP1 and DILP4 still remain enigmatic. We show here that *dilp1* transcript and DILP1 peptide are transiently expressed in insulin producing cells (IPCs) of the brain from early pupa until a few days into adult life. This corresponds to non-feeding stages and periods of extensive development in the pupa, as well as metabolic remodeling and ovary maturation in the newly-eclosed flies. In adult female flies where diapause can be triggered by low temperature (11°C) and short days (10L:14D), within a time window of 0-10h post-eclosion, the *dilp1*/DILP1 expression remains high for more than 9 weeks. The *dilp1* mRNA level is elevated in *dilp2,3,5* and *dilp6* mutant flies, indicating systemic feedback regulation. Furthermore, we show that the DILP1 expression in IPCs can be regulated by short neuropeptide F, juvenile hormone and presence of larval adipocytes. In male *dilp1* mutant flies lifespan is extended and the resistance to starvation is reduced, whereas in female *dilp1* mutants early oviposition is reduced. In summary, DILP1 is expressed in non-feeding stages and in diapausing flies, is under feedback regulation and appears to play sex-specific functional roles. Thus, DILP1 seems to play some roles distinct from that of other DILPs.

PO15 Short neuropeptides F regulate physiology of reproductive organs in Tenebrio molitor males

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Neuropeptides in insects are responsible for regulation of most physiological processes, including development, reproduction or visceral organ functioning. Among the many neurohormones produced by the nervous system, there are peptides related to vertebrate neuropeptides. These include short neuropeptides F (sNPF). Previous studies in different insect species indicated the pleiotropic activity of these neuropeptides in various tissues. They have gonadotropic activity, regulate feeding, affect the contractility of the visceral muscles as well as the heart. Thus far, exact physiological function of these peptides in the beetles was not studied in details.

The aim of the present study was to evaluate the role of sNPF in the regulation of reproduction events in the males of *Tenebrio molitor*. We injected chemically synthesized Trica-sNPF (SGRSPSLRLRFa) and its truncated form Trica-sNPF$_{(4-11)}$ (SPSLRLRFa) into 7-day old males and measured sperm number in the testes, soluble protein concentration and dry mass in testes and accessory glands. In addition, we evaluated the influence of peptides on ejaculatory duct movement.

The study showed that tested peptides influence various processes in male reproduction system in different manner. Trica-sNPF$_{(4-11)}$ reduces the dry mass of testes and accessory glands and decreases the protein concentration in these organs 24h after injection. Trica-sNPF decreases the soluble protein concentration in the testes but it increases protein content in accessory gland. Only one of tested peptides Trica-sNPF$_{(4-11)}$ influences the sperm number measured 24h after injection. It increases the total sperm number whereas longer peptide did not change this parameter. Both peptides stimulate the ejaculatory duct contractions in low concentrations (10^{-12}-10^{-11} M).

Obtained results suggest that short neuropeptides F are regulators of reproduction in *T. molitor* males. Trica-sNPF influences the functioning of accessory gland whereas its shorter form Trica-sNPF$_{(4-11)}$ affects testes.

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PO16 Towards unravelling the neuropeptidergic regulation of associative long term memory

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Understanding how the brain processes environmental stimuli into adaptive behaviours is still an open challenge for neuroscientists. Since the ability to learn from prior experiences evolved early in evolution, the “mini-brain” of the small roundworm *Caenorhabditis elegans* has proven to be ideally suited to address fundamental neurobiological questions. Among all *de novo* synthesised proteins that play a crucial role inestablishing long-term memory (LTM), the cAMP response element-binding protein (CREB) is one of the best known. Direct *in vivo* monitoring of the activation of CREB-dependent GFP reporters during associative memory formation eliminates the large variability in results that is typical for current behavioural assays and promises high throughput screening of hundreds of genes. This technique is used to determine the involvement of neuropeptides and their receptors in memory formation and retrieval, enabling a fast and reliable screening paradigm. The obtained information on the underlying neuropeptidergic pathways will shed light on the cellular and molecular networks that encode and integrate environmental and internalcues into long-term memory.

PO17 Transcriptional profiles of components of the endocrine system during embryo-larval development of the zebrafish

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The zebrafish has many advantages as a model to study vertebrate development as well as disruptions of normal development. A thorough understanding of the fundamental biology of the species is a prerequisite for the interpretation of the scientific findings. Although at the morphological level many aspects of zebrafish embryonic development have been described, surprisingly little is known about the role of the endocrine system in early development. Several studies have reported that thyroid and steroid hormones play an important role in various stages of development, but available information is fragmented.

Our study was designed to provide detailed baseline information about the activity of the thyroid and steroid hormone system during normal zebrafish embryonic and larval development, which will serve as gold standard. In this study, we describe the timing of the normal embryonic activation of the hormone synthesis machinery, as well as the hormone and receptor profiles, during the early stages of vertebrate development, which has never been done so far. We isolated zebrafish RNA at 25 time points between 0 and 32 days of zebrafish development covering all embryonic and larval stages. The expression levels of genes coding for thyroid and steroid biosynthetic enzymes and receptors were measured using QPCR. This allows us to construct very detailed time-dependent profiles of mRNA expression of receptors and enzymes involved in synthesis of these hormones.

In order to achieve a comprehensive overview, we selected genes coding for the key enzymes involved in steroid hormones biosynthesis from the cytochrome P450 superfamily (cyp19a1a, cyp19a1b, cyp11c1, cyp21a2, cyp11a1, cyp11a2, cyp17a1,) and the hydroxysteroid beta dehydrogenases (hsd3b1, hsd3b2, hsd17b1, hsd17b3, hsd20b, hsd11b2) as well as two enzymes involved in cholesterol biosynthesis (hmgcrα, hmgcrβ). For the thyroid system we analysed expression of genes involved in the hypothalamic pituitary thyroid axis (thyrotropin releasing hormone, thyrotropin, thyrotropin receptor), thyroid hormone biosynthesis (thyroperoxidase, thyroglobulin, sodium/iodide symporter), transport (thransthyretin) and activation (deiodinases 1, 2, 3a and 3b). Also, expression of steroid receptors was analysed including 7 nuclear receptors (estrogen receptor 1, estrogen receptor 2a and 2b, progesterone, androgen, mineralocorticoid and glucocorticoid receptors) and 6 membrane receptors (membrane progesterone receptor a and b, gonadotropine releasing hormone, follicle stimulating hormone, luteinizing hormone/choriogonadotropin and G protein coupled estrogen receptors). Relative to the thyroid system, we analysed expression of thyroid hormone receptor alpha and beta.

The obtained results will improve our fundamental understanding of the role of thyroid and steroid hormones during embryonic and larval development and will be broadly applicable to the zebrafish research community.

PO18 Comparative transcriptomics of neuropeptides in vectors of Chagas' disease

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Chagas' disease is an important but neglected human disease, with 8 million people infected in Latin America, and a fifth of the population of the region at risk. The causative agent, the protozoan *Trypanosoma cruzi*, is mostly transmitted to humans by triatomine bugs. The three most important vector species of this disease are *Triatoma infestans* in southern South America, *Rhodnius prolixus* in northern South America and Central America, and *Triatoma dimidiata* in northern South America, Central America and Mexico, where *Triatoma pallidipennis* is also an important vector. Reducing the number of insects is considered the best way to control the transmission of the disease. However, the occurrence of pyrethroid insecticide resistance in triatomine populations, among other causes, increases the importance of developing new insecticide strategies to replace and/or complement neurotoxics. Neuropeptides are important chemical messengers. In insects, this signaling is mainly mediated by the interaction of neurohormone ligands with G protein coupled receptors (GPCRs). Their role in the regulation and integration of neuroendocrine signals offer numerous targets and multiple modes of action for novel insecticides, through the disruption of fundamental physiological processes. Insect stress/metabolic neurohormones as well as their role in insecticide resistance are especially interesting to study, due to the stress caused by insecticides.

This work focuses in the analysis of the neuropeptides' repertoire of *T. infestans*, *T. dimidiata* and *T. pallidipennis*. Basing on their homology with neuropeptides and receptors from other species which have already been described, we performed database searches in triatomines' normalized transcriptomes. We found 16 neuropeptides and 17 receptors in *T. infestans*, 16 neuropeptides and 16 receptors in *T. dimidiata,* and 13 neuropeptides and 14 receptors in *T. pallidipennis*, suggesting a high degree of conservation of the neuroendocrine system in triatomines at the sequence level. As a first approach to analyze the involvement of neuroendocrine system in insecticide resistance, we performed qRT-PCR amplification of *T. infestans* neuropeptide transcripts, in order to compare gene expression levels between pyrethroid resistant and susceptible populations from Argentina.

Our findings contribute to expand the knowledge on triatomines' neuroendocrine system, and enables functional studies to further understand neuroendocrine regulation and its role in insecticide resistance in these important species for public health.

PO19 Localization and Possible Function of Brain aromatase in Zebrafish Eye

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Aromatase is the key enzyme for aromatization of testosterone to estradiol (E_2). Although E_2 is generally known as a circulating hormone derived from the gonad, it is also recognized as neuroestrogen having a crucial role in development of central nervous system (CNS). Previous studies in zebrafish have shown that brain aromatase (*cyp19a1b*) is expressed in the eye. However, its distribution and function in the eye have not been revealed yet. Therefore in this study we aim to localize brain aromatase and to elucidate possible functions of brain aromatase in zebrafish eye. Immunohistochemistry using specific antiserum to zebrafish brain aromatase showed that immunoreactivity of brain aromatase (irAroB) was localized in retina layers of adult fish including inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL). When fish were exposed to E_2, the intensity of staining was increased in those layers and additional positive reaction was appeared in the ganglion cell layer (GCL). The irAroB was specifically expressed possibly in ganglion cells (GC), amacrine cells (AC), horizontal cells (HS) and bipolar cells (BP). Interestingly, irAroB was also detected in lens epithelial cells (LEC), and this result was also confirmed by RT-PCR. In order to elucidate possible functions of brain aromatase, effects of morpholino targeting brain aromatase (AroB MO) were investigated. The irAroB in retinal layers was decreased in 120 hpf larvae injected with aroB MO but not with inverted AroB MO (negative control). Injection of 5 ng AroB MO decreased the diameter of the eye at 48 hpf, and the effect was partially reversed by co-incubation with 1 μM of E_2. Apoptosis in the eye detected by acridine orange staining at 24 hpf was significantly increased by AroB MO. Furthermore, immunohistochemistry for acetylated tubulin demonstrated that AroB injection decreased the diameter of optic nerve, which was reversed by incubation with 1 μM of E_2. Taken together, estradiol produced by brain aromatase in the eye may be required for development of the eye possibly by regulating apoptosis and axonogenesis.

Keywords: brain aromatase, zebrafish, eye development.

PO20 Cellular senescence and reduced regeneration potential in aged zebrafish

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Purpose: Today, a growing number of elderly suffers from neurodegenerative pathologies, seriously impacting the well being of these patients. Intensive research efforts are therefore focused on the stimulation of neuroregeneration, a capacity that is unfortunately absent in adult mammals and remains a challenge to induce, especially in an aging environment. Zebrafish however, posses high neurogenic and regenerative abilities, yet, are also subjected to gradual aging, similar as in humans. These teleost fish are therefore ideally suited to investigate the impact of aging processes on neuroregenerative potential.

Methods: Within the young and aged zebrafish retinotectal system, - a powerful model to study regenerative capacities, - axonal regeneration was studied after applying optic nerve crush (ONC). Axonal outgrowth capacity was assessed by means of qRT-PCR for the growth-associated genes *gap43 and tuba1.* Also, growth speed and targed reinnervation of regenerating axons was visualized via biocytin tracing and quantified via morphometric analyses within the retina, optic nerve and tectum. In addition, visual recovery was assessed by longitudinally analysing the optokinetic response after axonal injury. Underlying mechanisms, such as senescence of the retinal ganglion cells (RGCs) and responding innate immune cells, were analysed via outgrowth assays within retinal explants and IHC analyses within the retino-tectal system, respectively.

Results: Zebrafish subjected to ONC show a striking age-related decline in regenerative capacity, which is accompanied by reduced retinal expression of the regeneration-associated genes *gap43 and tuba1*. Detailed morphometric and immunohistochemical analyses of the aged zebrafish retinotectal system confirm a diminished outgrowth capacity of retinal ganglion cells, resulting in a significant delay in axonal regeneration and a retarded tectal innervation. In consequence, recovery of visual function is similarly affected. More importantly, preliminary data from *in vitro* outgrowth assays suggest that aged RGCs exhibit a cutback in their intrinsic outgrowth potential. In addition, altered morphological and functional changes of innate immune cells indicate that senescent microglia might also underlie the observed decline in regeneration capacity.

Conclusions: Zebrafish form an excellent species for disease modelling and comparative regeneration research. Our findings show that cellular senescence significantly delays axonal regeneration within this small vertebrate. These studies offer insights into the impact of aging on successful neuroregeneration, thereby contributing to the development of new therapeutic strategies for successful neuroregeneration within the aged mammalian CNS.

PO21 A neuropeptide Y/neuropeptide F-like signaling system in C. elegans

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Neuropeptides represent a large and diverse group of signaling molecules that are mainly produced by neurosecretory cells. They may act as fast neurotransmitters, neuromodulators or neurohormones, thereby regulating fundamental physiological processes such as feeding, locomotion, reproduction, social behavior and learning and memory formation. The *C. elegans* genome contains at least 120 neuropeptide precursor genes encoding more than 250 bioactive peptides. The majority of neuropeptides exert their function by binding to plasma membrane-associated receptors known as G-protein coupled receptors (GPCRs), of which about 150 receptor genes have been predicted in *C. elegans*. Coupling of the putative receptors and their natural ligand(s) remains a challenging task, reflected by the small number of peptide GPCRs being deorphanized so far. We performed a large-scale deorphanization screen and thereby identified several evolutionary conserved neuropeptide systems in *C. elegans*, including a novel pathway related to mammalian neuropeptide Y (NPY) and insect neuropeptide F (NPF) signaling. In order to gain clues as to the biological function of this neuropeptide signaling system, the spatial expression of the NPY/F precursor and receptor genes was examined using fluorescent reporter constructs. These expression patterns suggest a role for NPY/F signaling in the regulation of feeding behavior and chemotaxis to water soluble attractants in *C. elegans*.

PO22 Which neuropeptidergic circuits are involved in the control of food leaving behaviour in Caenorhabditis elegans?

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Concerns about food choices that may have adverse effects on health are currently at the forefront of scientific and public debate. In a world with an epidemiological trend of increasing obesity, an in depth understanding of food choice behaviour is of undeniable importance. Food choice decision-making is regulated by a vastly complex neural and endocrine network. The main hormones and brain regions involved in this control have been described in vertebrates, but a detailed characterization of the cellular and molecular processes underlying this control circuitry has not been performed up to date. The majority of chemical and neuropeptidergic signalling pathways controlling feeding and food choice are conserved among vertebrates and invertebrates. This conservation has evoked the opportunity to study neuropeptidergic regulation of these behaviours through molecular genetics in model systems such as the nematode *Caenorhabditis elegans*. *C. elegans* has a minuscule and completely mapped nervous system that comprises just 302 neurons. Despite the anatomical simplicity of its nervous system, *C. elegans* does not lack in potential candidates for peptidergic regulation of neuronal responses. The *C. elegans* genome displays a wide diversity of neuropeptide precursor and G protein-coupled receptor (GPCR) coding sequences, many of which share similarities with other metazoans. Over 100 neuropeptide precursor genes give rise to more than 300 bioactive peptides that can serve as neurotransmitters, neuromodulators or neurohormones. In addition, over 200 potential neuropeptide GPCRs are predicted in the *C. elegans* genome. Our lab has cloned over 90% of the *C. elegans* peptide GPCR candidates in a large-scale deorphanization initiative. Using an *in vitro* reverse pharmacology approach in mammalian cells, cloned peptide GPCRs are screened with an in house *C. elegans* peptide library including all known and predicted peptides of the FMRFamide-like (FLP) and neuropeptide-like protein (NLP) families. Multiple previously unknown neuropeptide-receptor couples have been elucidated in this manner. These couples encompass several candidates that display homology to vertebrate and/or invertebrate systems regulating food choice behaviour and can thus potentially be involved in *C. elegans* food leaving behaviour. Initially, mutants of these candidate receptors and peptide precursors are screened for an influence on *C. elegans* food leaving behaviour. Subsequently, candidate receptors and neuropeptide precursors can be *in vivo* localized by means of confocal microscopy. Finally, genetic and cellular rescue analyses can be performed to unveil the cellular and molecular mechanisms behind this regulation.

PO23 Secretin Receptor Alters the Angiotensin II-induced Calcium Influx in Adrenal Zona Glomerulosa via Cross-class GPCR dimerization.

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G-protein coupled receptors (GPCRs) function as the key to transition of extracellular signaling to intracellular events. There is an increasing body of evidence to show that the dimerization and oligomerization of GPCRs are necessary for their functions and fine-tuning. Recently, secretin (SCT) and angiotensin II (ANGII) have been reported to share overlapping signaling pathways, although their receptors belong to different classes. SCT receptor (SCTR, a class B1 receptor) and ANGII receptor subtype 1 (AT1R, a class A receptor) form heteromers *in vitro* which was shown to mediate ANGII-induced water-drinking in mice, giving a good instance for functional cross-class GPCRs. Our previous data showed that ANGII-induced aldosterone release was attenuated in the SCTR knockout ($SCTR^{-/-}$) mice, but the molecular mechanisms remained unclear. We therefore asked whether the heteromer of SCTR/AT1R is necessary for this ANGII-triggered aldosterone biosynthesis and release. Since calcium and cAMP are the second messengers activated though G_q- and G_s-coupled receptors, respectively, the presence of functional AT1R and SCTR was demonstrated via candesartan-inhibited ANGII-induced aldosterone release and cAMP production treated with SCT, respectively. The primary adrenal cortical cells obtained from $SCTR^{-/-}$ showed impaired aldosterone secretion in the presence of 10 nM ANGII, compared with the wild type (WT). The Fluo4AM-loaded primary adrenal zona glomerulosa (ZG) cells from WT displayed a dose-dependent increase of fluorescent intensity in response to ANGII, but not in $SCTR^{-/-}$, indicating the absence of SCTR has altered the ANGII-induced Ca^{2+} increase in the ZG cells. With the use of synthetic transmembrane (TM) peptides, it was revealed that the heteromers of SCTR/AT1R mediated the most Ca^{2+} influx induced by ANGII. Consistently, SCTR TM2 pre-incubated primary ZG cells failed to secrete aldosterone upon ANGII stimulation, while the mutated STM2 peptide could not suppress this ANGII-induced aldosterone release. Adrenocorticotropic hormone (ACTH) and potassium chloride served as control to demonstrate that this attenuated Ca^{2+} and its subsequent aldosterone were specifically SCTR/AT1R gated and the presence of TM peptides did not alter the physiology of primary ZG cells. Our data clearly provide evidence that SCTR/AT1R heteromer play the major role in ANGII-induced aldosterone release through its mediation of ANGII-triggered Ca^{2+} influx.

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PO24 Ghrelin Suppresses Enteric Anorectic Hormones Cholecystokinin, Peptide YY and Glucagon Like Peptide-1 in Goldfish (Carassius auratus)

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Ghrelin is the only known gut-derived peptide with an appetite stimulatory (orexigenic) role. In mammals, several observations have pointed to the existence of important interactions between ghrelin and other peptide hormones, including cholecystokinin (CCK), peptide YY (PYY) and glucagon like peptide-1 (GLP-1) that are primarily anorexigenic (appetite inhibitory). For instance, ghrelin attenuates the inhibition of food intake induced by PYY in rats, and, similarly, CCK and GLP-1 inhibits ghrelin-induced feeding stimulation in the same animal model. Nevertheless, whether ghrelin modulates other gastrointestinal peptides in fish is yet to be investigated. The objective of this study was to determine the possible modulation by ghrelin of CCK, PYY and GLP-1 in goldfish (*Carassius auratus*). For this, we first aimed to determine the localization of CCK, PYY and GLP-1 in relation to ghrelin in the goldfish gut using immunohistochemistry. Second, we tested whether ghrelin modulates the expression (gene and protein) of CCK, PYY and GLP-1 using an *in vitro* approach. Cultured gut sections were exposed to goldfish synthetic octanoylated ghrelin [1-19] (0.1, 1 and 10 nM) for 30, 60 and 120 minutes, and the levels of the above mentioned peptides were quantified by RT-qPCR and Western blot. Results show that ghrelin colocalizes CCK, PYY and GLP-1 in some endocrine cells located in the luminal border of the gut mucosa. *In vitro* exposure to ghrelin led to a significant decrease in *cck*, *pyy* and *glp-1* transcript levels, while no effects were observed at 30 or 120 min. The protein expression of PYY and GLP-1 was also downregulated by 60 min of treatment with ghrelin. Overall, results of the present study show for the first time in fish that ghrelin exerts suppressive effects on other enteric appetite-regulating hormones. This inhibition of anorexigens is likely one way by which ghrelin mediates its stimulatory effects on feeding and metabolism.

PO25 The role of muscarinic cholinergic receptors agonists and antagonists in metabolism regulation in the beetle Tenebrio molitor

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Muscarinic cholinergic receptors (mAChRs) belong to wide group of metabotropic receptors and play important role in cholinergic transmission in the central and peripheral nervous system. Based on pharmacological properties, five classes of mAChRs are distinguished in vertebrates and they are classified as M_1-M_5. Receptors are involved in the regulation of many physiological processes, such as the contractile activity of smooth muscles, activity of the heart, secretion of digestive enzymes, metabolism and functioning of the immune system.

Muscarinic receptors are also found in insects and can be divided in two classes. The first type the mAChR - type A has similar structure and pharmacological properties as mammalian receptors from the M_1 and M_3 classes. The second class mAChR - type B possess significantly different properties compared to insect mAChRs-A and mammalian mAChRs. mAChR-B receptors are over 1000-times less sensitive to muscarine and are almost completely insensitive to antagonists, such as atropine and scopolamine.

The available data suggest that in insects maAChRs are involved in regulation of behaviour and locomotion, e.g. flight induced by the wind. mAChRs also affect the functioning of neuro-endocrinic processes like secretion of juvenile or protoracotropic hormone. However, there is not information, how agonists or antagonists of mAChR affect insect metabolism.

The aim of this study was to investigate the influence of two muscarinic receptors agonists (carbachol and pilocarpine) and two antagonist (atropine and scopolamine) on the carbohydrates and lipids metabolism in haemolymph, fat body and midgut of *Tenebrio molitor* larvae.

The data indicate that carbachol affects glycogen metabolism. After injection of this agonist, the increase of glycogen content was observed, both in fat body and midgut. Similar but slighter effect was observed in case of pilocarpine. Simultaneously carbachol induced significant decrease of total sugar level in haemolymph, what suggests that fat body and midgut use the haemolymph carbohydrates for glycogen synthesis. Moreover, the changes in sugars fraction level were observed in response to carbachol and pilocarpine application. The observed effect was weaker after 24 hours of incubation, and decreased with a lowering concentration. The antagonists atropine and scopolamine influenced the level of total lipids in fat body causing decrease of its content in this tissue. No effects were observed in *in vitro* experiments. The results suggest the indirect affecting of tested compounds on insect trophic tissues, probably by influence on neuro-endocrine system which plays a key role in regulation of insect metabolism.

Obtained data suggest that cholinergic transmission *via* muscarinic receptors is involved in regulation of insect metabolism. Nevertheless, lack of activity in *in vitro* conditions suggests that the tested compounds act on trophic tissues in indirect way.

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PO26 The endocannabinoid system regulates zebrafish GnRH neuronal development

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The endocannabinoid system comprises several components, among which specific seven transmembrane-domain receptors (namely CB1 and CB2 cannabinoid receptors), their exogenous (*e.g.* Δ^9-THC) and endogenous ligands (e.g. AEA and 2-AG), and a number of biosynthetic and degradative enzymes. CB1 cannabinoid receptors are widely expressed in the brain and regulate various steps of neuronal development. In the zebrafish embryo, previous data showed that CB1 knockdown causes defects in axonal fasciculation in the anterior commissure. Since this area is particularly rich in GnRH fibers, we assessed whether CB1 receptor could regulate GnRH axonal pathfinding and fasciculation in zebrafish embryos. We therefore performed both antagonist-mediated CB1 downregulation and morpholino-mediated CB1 knockdown on GnRH3::GFP zebrafish embryos. We found that CB1 knockdown reduces the number of GnRH3::GFP positive cells in the olfactory epithelium while not changing their position, it reduces the extension of GnRH neuropil, and causes axons misrouting in the anterior commissure. Furthermore, CB1 knockdown downregulates the expression of two genes involved in axonal growth and cell migration, namely Stmn2aand Sez6 Taken together these results indicate that during early zebrafish development the endocannabinoid system is involved in the regulation of GnRH system development.

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PO27 Evolutionary origin and distribution of the gamma splice variant of the type 2 corticotropin-releasing hormone receptor in primates

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Most vertebrates have two types of corticotropin-releasing hormone (CRH) receptors, CRHR1 and CRHR2, encoded by two different genes, and several splice variants have been described for both CRHR types. Mammals express up to three distinct functional CRHR2 variants that differ in their N-terminal sequence: CRHR2α, CRHR2β and CRHR2γ. These variants result from the use of multiple promoters and alternate 5' exons. CRHR2γ has only been described in human so far, where it is mainly expressed in the brain. With a growing number of genome assemblies available, we set out to investigate the phylogenetic distribution of CRHR2γ within the primate lineage and its evolutionary history. The *CRHR2* sequences from 22 primate species were retrieved from GenBank. Sequences of CRHR2γ were available for all but one catarrhine species (Old World monkeys and apes), but not for any platyrrhines (New World monkeys), tarsiers or strepsirrhines (lorises and lemurs). With the possible exception of the chimpanzee sequence, all *CRHR2* γ1 exon coding sequences showed very high sequence similarity, with identity scores of ≥ 90% at the nucleotide level and ≥ 85% at the amino acid level. Our phylogenetic analysis suggests that an intronic 'seed' sequence consisting of a *hAT-Charlie* DNA transposon with a PIT1 and OCT1 binding site, followed by a γ1-like sequence was already present in a common ancestor of the Euarchontoglires. Before the divergence of Platyrrhini and Catarrhini, around 43 million years ago, a SINE retrotransposed between the *hAT-Charlie* element and the γ1-like sequence. After the divergence of the Platyrrhini, between 32 and 43 million years ago, a C>T mutation near the 3' end of the γ1-like sequence created a 5' donor splice site. Lastly, exaptation of the intronic γ1-like sequence required the establishment of an in-frame start codon. Our analysis does not allow us to unequivocally pinpoint the timing of this event. A start codon may have been present in a common ancestor of the primates, which was then independently inactivated by a mutation to GTG or ATA in the Lemuriformes and Platyrrhini lineages, respectively. Alternatively, the start codon may have originated in a common ancestor of the Catarrhini between 32 and 43 million years ago, and independently in the galagos (Lorisiformes).

PO28 Adipokinetic hormone receptor as regulator of energy use in the anautogenous fly, Sarcophaga crassipalpis

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Adipokinetic hormone (AKH) is a pleiotropic peptide mainly involved in fat body energy mobilization. In some flies (*Phormia regina*, *Sarcophaga crassipalpis*), bugs (*Pyrrhocoris apterus*) and cockroaches (*Periplaneta americana*) AKH was demonstrated to be also involved in the regulation of digestion. In *S. crassipalpis*, injection of this neuropeptide into liver-fed decapitated flies and into sugar-fed intact flies resulted in a dose-dependent, enhanced midgut proteolytic activity, up to the level observed in intact protein-fed flies. None of the other peptides that were present in the *corpora cardiaca* of that fly was able to generate similar, long-term effects in sugar-fed intact flies, nor in decapitated liver-fed insects. This strongly suggests a role of AKH in the regulation of digestion and consequently food-dependent reproduction. This AKH peptide, member of the GnRH family, functions via activation of the adipokinetic hormone receptor (AKHR) that belongs to the family of G protein-coupled receptors. In line with the main function of the AKH peptide, the receptor was found to be most abundantly expressed in the fat body, a known energy storing organ. Nonetheless, the AKHR is also highly expressed in the brain, foregut and hindgut. Interestingly, the receptor transcript numbers were reduced in almost all tissues examined after protein feeding. The post protein feeding decreased AKHR transcript levels suggests that this receptor works as a kind of energy safety valve. A high AKHR gene expression in sugar-fed flies should allow for mobilization of the stored energy to assure all basic physiological processes, whilst its reduced expression in the liver-fed flies would protect their stored energy supplies as to save them for future energy demanding activities. Accordingly the low AKHR level observed post liver meal would prioritize the use of the ‘new’ food-derived energy.

Bil et al. (2016) J. Insect Physiol. 89, 52–59. Bil et al. (2014), Gen. Comp. Endocrinol. 208, 49-56.

PO29 Signalling profiles of peptide ligands of evolutionary related oxytocin-/vasopressin-like G protein coupled receptors

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The nonapeptides oxytocin (OT) and arginine-vasopressin (AVP) are key mediators of a range of physiological and neurological processes, signalling via their cognate family of G protein-coupled receptors. Evolutionarily, the OT/AVP system is found in all vertebrates and a number of invertebrate species, with orthologous peptides and receptors mediating similar physiological functions that include water homeostasis, reproduction, memory and behaviour [1, 2]. However, although the biology of these neuropeptides and their GPCRs continue to be intensively studied, there remains a lack of subtype-selective ligands for the OT/AVP receptor family. Indeed, OT and AVP do not exhibit overt pharmacological selectivity for their respective receptor subtypes [3]. Consequently, the currently available tool kit targeting the OT/AVP receptors is incomplete, limiting the scope of study for this receptor family. To improve understanding of OT/AVP receptor signalling and peptide selectivity, the evolutionary conservation of this peptidergic signalling system can be exploited. The OT-/AVP-like peptide-receptor systems of insects offer simpler models to study; the insect signalling systems consist of a single receptor and single cognate peptide agonist (inotocin). The signalling profiles of these insect receptors remain to be thoroughly characterised. We present pharmacological signalling profiles of inotocin and a range of synthetic OT/AVP analogues at the OT-/AVP-like receptors from different invertebrate species. In comparison to the signalling profiles at human receptors, these findings provide insights into how OT/AVP receptor signalling may have evolved across species and potential avenues for developing more selective peptides for these receptors.

[1] Feldman et al (2016) Biol Psychiatry 79(3):174-184. [2] Gruber (2014) Exp Physiol 99(1):55-61. [3] Koehbach et al (2013) Biochem Soc Trans 41(1):197-204.

PO30 Regulatory mechanisms and signaling of G-protein coupled receptor 83

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The G-protein coupled receptor 83 (GPR83) was first described as glucocorticoid-induced receptor (GIR; also termed GPR72 and GPR73). GPR83 is highly expressed in the hypothalamic *arcuate nucleus*, a region known to be involved in the regulation of food intake and energy metabolism. Hypothalamic expression of Gpr83 is dependent on nutrient availability and is decreased in obese mice compared with lean mice. Gpr83 knock-out mice are protected from diet-induced obesity. Moreover, Gpr83 constitutes heterodimers with other G-protein coupled receptors (GPCR) important for body weight regulation such as the ghrelin receptor (GHSR). This GPR83/GHSR interaction diminishes GHSR activation by acyl ghrelin. Interestingly, hetero-oligomerization of GPR83 with GPR171 - a receptor for the peptide bigLEN - has been also confirmed. The functionality of both receptors in the heteromeric constellation are mutually influenced. Of note, the neuro-peptide PEN was recently identified as a potent GPR83 ligand. Furthermore, the N-terminal extracellular receptor region of GPR83 acts as an endogenous intramolecular antagonist and is thereby involved in receptor activity regulation. In conclusion, signaling regulation at GPR83 is complex and extended studies on GPR83 for a comprehensive understanding of the entire process are needed. Finally, because GPR83 is suggested to be related to body weight control and the fact that human obesity is increased dramatically world-wide, it should be of importance to unravel the functions of GPR83 in depth, also to find potential options for a directed receptor-activity modulation. Here we summarize current knowledge on this family A GPCR and provide also new details concerning molecular determinants of signaling regulation.

PO31 Identification and molecular characterization of a pheromone receptor candidates in the cabbage armyworm Mamestra brassicae

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Sex pheromone communication in Lepidoptera has been for long a valuable model system for studying fundamental aspects of olfaction and its study led to the establishment of environmental-friendly pest control strategies. The cabbage armyworm, *Mamestra brassicae*, is a major pest of Cruciferous vegetables in Europe and Asia. Its sex pheromone has been characterized and is currently used as lure to trap males, but nothing is known on the molecular mechanisms of sex pheromone reception in male antennae. Using homology cloning and RACE-PCR strategies, we identified the first pheromone receptor candidate in this species. The transcript was found to be specifically expressed in antennae, with a male biased expression. *In situ* hybridization experiments within the antennae revealed that the receptor-expressing cells were closely associated with the olfactory structures, especially the long trichoid sensilla known to be pheromone-sensitive. The deduced protein presented a seven transmembrane structure that is a hallmark of insect olfactory receptors and it clustered in a phylogenetic tree in the clade that groups all the Lepidoptera pheromone receptors characterized to date. Taken together, our data support the view that we identified a receptor for pheromone and open perspectives to better understand how this species detect a critical signal for reproduction.

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PO32 Localization of two crustacean female sex hormones in the kuruma prawn Marsupenaeus japonicus

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The sex differentiation in crustacean is well known to be controlled by an androgenic gland hormone. Recently, another hormone, crustacean female sex hormone (CFSH), has been discovered. This novel hormone was purified from the female eyestalk of the blue crab *Callinectes sapidus*. Gene knockdown of *C. sapidus* CFSH (Cas-CFSH) by RNA interference was shown to inhibit the appearance of the female reproductive characteristics. Therefore, it has been thought that CFSH controls female secondary sex characteristics. In order to elucidate the localization of the kuruma prawn *Marsupenaeus japonicus* CFSH (Maj-CFSH) producing cells, here we cloned two Maj-CFSH cDNAs and subsequently analyzed gene expression of the two Maj-CFSHs by *in situ* hybridization. The eyestalk and ovary were dissected from the adult prawns. All tissues were flash-frozen in liquid nitrogen, and those total RNAs were extracted. The eyestalk and ovarian cDNAs were synthesized by reverse-transcription reaction. An eyestalk CFSH (Maj-CFSH_ES) and an ovarian CFSH (Maj-CFSH_OV) cDNAs were cloned by 5'- and 3'-RACE. The Maj-CFSH_ES cDNA consisted of 1,050 bp including a 5'-untranslated region (UTR) (23 bp), an open reading frame (ORF) (735 bp), and a 3'-UTR (292 bp). The ORF was conceptually translated into a putative prepropeptide comprising 244 amino acid residues, consisting of a signal peptide (SP) (34 residues), a CFSH-precursor-related peptide (CPRP) (44 residues), a processing signal (2 residues) and Maj-CFSH_ES (164 residues). Maj-CFSH_OV cDNA consisted of 942 bp including a 5'-UTR (130 bp), an ORF (678 bp), and a 3'-UTR (134 bp). The ORF encoded a putative prepropeptide comprising 225 amino acid residues, consisting of a SP (24 residues), a CPRP (35 residues), a processing signal (2 residues) and Maj-CFSH_OV (164 residues). Although both of mature Maj-CFSH_ES and Maj-CFSH_OV showed low amino acid sequence identities to Cas-CFSH (38%), eight conserved Cys residues were observed in the two molecules. The two Maj-CFSH cDNAs were used as templates for syntheses of antisense and sense cRNA probes to detect Maj-CFSH_ES and Maj-CFSH_OV mRNAs. The eyestalk and ovary were fixed in Bouin fixative for overnight at 4°C. The fixed eyestalk and ovary were embedded in paraffin and sectioned at a thickness of 10 µm. The section was subjected to *in situ* hybridization. The antisense probe of Maj-CFSH_ES was hybridized with large neurosecretory cells around medulla terminalis and medulla interna (X-organ), and small neurons around medulla externa. No signal was detected in the negative control, in which the sense probe was employed. This result suggests that Maj-CFSH_ES is thought to be synthesized in the X-organ and then secreted from the sinus gland as well as sinus gland hormones. The Maj-CFSH_OV antisense cRNA probe was hybridized with the oogonia and immature oocytes of the mature ovary, but not with any cells of the immature ovary. This result suggests that Maj-CFSH_OV might be involved with ovarian maturation in *M. japonicus*. Biological functions of Maj-CFSH_ES and Maj-CFSH_OV are still unclear. Now, we are producing recombinant Maj-CFSH_ES and Maj-CFSH_OV in order to characterize those biological activities.

PO33 Environmental pollutants induce estrogen receptor A gene expression in loggerhead sea turtle (caretta caretta) cultured erytrocytes

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Because loggerhead turtles (*Caretta caretta*) are included on the Red List of the World Conservation Union, the efforts to conserve sea turtles and the studies on their endocrine functions have increased in recent years. For example, red blood cells (RBCs) of sea turtles express functional steroid hormone receptors in terms of response to hormonal signaling. However, the impact of environmental pollutants on nuclear steroid hormone receptor-mediated signaling is still poorly investigated in sea turtles. Here we describe the application of primary erythrocyte cultures for evaluating the effects of different environmental exogenous estrogens on the expression of *C. caretta* estrogen receptor α (ERα). In this regard, cultured RBCs were exposed to increasing concentrations of 4-nonylphenol (4-NP), Diisodecyl phthalate (DiDP), tributyltin (TBT) and Tri-m-cresyl phosphate (TMCP) for 48 h. To validate our results, 17-β estradiol (E2) was used as positive control. The metabolic cell activity as a measure of cell viability was monitored using Alamar Blue colorimetric assay while ERα mRNA levels were investigated by a real time quantitative PCR assay, optimized in the loggerhead turtles RBCs. All tested compounds were found to be toxic at 10^{-4} M compared to solvent controls. In contrast, a lack of impact on cell viability was indicated for up to 10^{-5} M of both E2 as well as all tested pollutants. In addition, our results showed significantly increased levels of ERα mRNA in a dose-dependent manner after 48 h exposure to both 4-NP and TMCP. Interestingly, the dosage-dependent effects of DiDP on ERα expression were opposite in comparison to that obtained following exposure to the other tested compounds. A significant increase of ERα gene expression was also observed with the highest concentration (10^{-6} M) of TBT. Overall, our work demonstrates the validity of primary cultures of sea turtle erythrocytes for providing new insights into reptilian cell physiology and toxicology suggesting that this approach could allow to analyze components of endocrine function of ERs and determine possible effects of exogenous estrogens in a minimally invasive manner.

PO34 Regulation of accessory gland and ejaculatory duct contractions by dromyosuppressin and short NPF

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Peptides and biogenic amines are important regulators of muscle activity in both male and female reproductive tissues, and therefore can play important roles in the reproductive success of insects.

We have investigated the role of neuropeptides in regulating contractions of the male accessory gland (MAG) and the ejaculatory duct (ED). Antibodies recognising the RFamide epitope revealed extensive peptidergic staining of neuronal processes on the surface of both the MAG and ED of the dipteran insects *Drosophila melanogaster, D. yakuba*, *D. erecta, D. simulans* and the pest species *D. suzukii*. The same antibodies also gave strong staining of a pair of rectal cells and a single cell, that we have called the ejaculatory duct cell because of its projections to the ED. In *D. melanogaster* the RFamide staining was distinct from the serotonergic neurites emanating from the male-specific cell bodies of the abdominal ganglion. *Dromyosuppressin-Gal4* was used to drive expression of green fluorescent protein (GFP) which co-localised with the RFamide immunolabel on the surface of the MAG and ED, two rectal cells and the ED cell, providing evidence that the RFamide antibodies recognised dromyosuppressin (Dms). The GFP labelling allowed the rectal and ED cells to be isolated singly under a fluorescence microscope and subjected to MALDI-TOF mass spectrometry. Molecular ions of several neuropeptides were detected and the identity of dromyosppressin and $sNPF^{4-11}$ were confirmed by fragmentation sequencing. The consequence of the release of dromyosuppressin and sNPF on contractions of the MAG and ED were investigated. Both peptides reduced the frequency of contractions in a dose dependent manner suggesting that these peptides of the rectal and ED cells are involved in regulating events important for ejaculation.

PO35 Drosha, Dicer1 and Argonaute1 in the desert locust, S. gregaria: phylogeny, transcript profiling and regulation during phase transition and feeding

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Several classes of small regulatory RNAs are currently described. Based on their origin, structure and associated effector proteins, it is possible to distinguish three main categories: micro (mi)RNAs, small interfering (si)RNAs, and piwi-interacting (pi)RNAs. In this study, we focused on miRNAs, which are a class of non-coding RNAs that play a regulatory role on gene expression, at the post-transcriptional level. They derive from endogenous long RNA precursors that are trimmed to a stem loop structure by an enzyme named Drosha. This pre-miRNA is then transported to the cytoplasm, where a Dicer enzyme cleaves it into a mature miRNA. Finally, this mature miRNA is loaded into the RNA induced silencing complex (RISC) that contains an Argonaute protein and, following Watson-Crick base pairing with a complementary transcript sequence, mRNA targets are blocked or degraded, resulting in gene silencing.

In this study, we investigated the miRNA machinery of the desert locust, as well as its regulation during two important physiological processes, namely phase transition and the feeding status. In this context, we identified *drosha*, *dicer1* and *argonaute1* transcript sequences, described to be involved in insects' miRNA pathway, in the transcriptome database of the desert locust. Subsequently, we performed protein domain predictions and phylogenetic analyses to further confirm the identity of the predicted transcript sequences. Next, tissue transcript profiling was performed, which revealed that these three components are broadly expressed among the different investigated tissues. Finally, we analysed the transcript levels of *drosha*, *dicer1* and *argonaute1* during phase transition and feeding status. For this, we assessed the expression of these components in the thoracic ganglia (pro-, meso-, and metathoracic ganglia) after short-term gregarization. Interestingly, upon crowding, a significant increase in *drosha* and *argonaute1* transcript levels was observed; and 2 hours after crowding the transcript levels returned to their basal level. Although a similar trend was observed for *dicer1*, the levels were not significantly different from these of the control locusts. On the other hand, in order to investigate differential regulation of the miRNA machinery upon food deprivation, gregarious locusts were first starved for 5 days and subsequently fed with fresh cabbage leaves. Interestingly, for *drosha*, *dicer1* and *argonaute1*, a significant decrease of the transcript levels in the midgut was observed between 30 min and 1 h after feeding.

To conclude, we identified and characterized components of the miRNA machinery in the desert locust. In addition, we obtained evidence for differential regulation of the miRNA pathway during phase transition and feeding of starved locusts.

PO36 Thyroid disruption in zebrafish (Danio rerio) larvae: different molecular initiating events leading to impaired eye development and visual functions

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The vertebrate thyroid hormone system is important for multiple developmental processes, including eye development. Thus, its environmentally induced disruption may impact important fitness-related parameters like visual capacities and behaviour. The present study investigated the relation between initiating molecular events of thyroid disruption and morphological and physiological changes of eye development of zebrafish (*Danio rerio*). Two test compounds representing different molecular modes of thyroid disruption were used: propylthiouracil (PTU) as enzyme-inhibitor within thyroid hormone synthesis, and tetrabromobisphenol A (TBBPA) as ligand of thyroid hormone receptors. Both test chemicals significantly altered transcript levels of thyroid-related genes in a compound-specific way. Despite differing molecular response patterns, both treatments resulted in similar pathological alterations of the eyes such as reduced size and pigmentation, which were concentration-dependent. The morphological changes translated into impairment of swimming activity and visual performance of the larvae: the optokinetic response was significantly and concentration-dependently decreased in both treatments, together with a significant increase of light preference of PTU-treated larvae. This study provides first evidence that thyroid disrupting compounds do not only impair morphological but also functional eye development in fish early life stages, and that the phenotypic outcomes appear to be similar for different modes of molecular action of the thyroid disruptors.

PO37 Elucidating GPCR signaling: evaluation of A3AR activation with a live-cell functional complementation assay based on β-arrestin2-recruitment

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The purinergic receptor family is distributed throughout the body and includes G-protein coupled receptors (GPCRs) that respond to purines. Tremendous research on these GPCRs has provided us with extensive knowledge on their properties based on *in vitro* and *in vivo* data. The A3 adenosine receptor (A3AR) subtype is found in healthy tissue (lung, liver, inflammatory cells), but is also highly expressed in tumour cells. Unfortunately, the fact that the natural A3AR ligand adenosine is only short-lived, precludes its clinical use. This led to the synthesis of A3AR ligands that are being evaluated in (pre)clinical studies for the treatment of a variety of diseases. These include the selective ligands IB-MECA for the treatment of hepatocellular carcinoma, and its 2-chloro analogue 2-Cl-IB-MECA for the treatment of inflammatory rheumatoid arthritis, psoriasis and dry eye disease.

The affinity and activity of new A3AR ligands is classically evaluated in binding assays and cAMP accumulation assays (A3AR being G_i-coupled). However, like all GPCRs, the A3AR also signals via G-protein independent pathways (i.e. functional selectivity). Therefore, in drug development, the possibility to selectively activate one signaling pathway, offers options with respect to A3AR selectivity and the prevention of side effects.

One type of alternative GPCR signaling pathway is the recruitment of the cytoplasmic β-arrestin 2 (βarr2) protein, leading to receptor desensitization and internalization. Commercial βarr2-assays have already been evaluated for the A3AR, such as the TransFluor®, Tango™ and PathHunter[TRADEMARK] assay (Molecular Devices, Invitrogen, and DiscoverX, respectively). However, these are associated with certain drawbacks, amongst which complicated imaging, long incubation times and/or high risk of false positives. We developed and applied a live-cell assay based on the NanoLuc® Binary Technology (NanoBit®, Promega), which is based on functional complementation of two inactive subunits of a very small and bright NanoLuc® luciferase, which are coupled to the A3AR and βarr2, respectively. When βarr2 is recruited to the GPCR following its activation, the luciferase activity is restored, which, in the presence of the substrate furimazine, generates a bioluminescent signal.

HEK293T cells stably expressing both components of the bioassay showed a very sensitive (readily showing signals at 5 nM) and concentration-dependent effect for reference agonist 2-Cl-IB-MECA, with an EC50 value of 60 nM. The newly developed assay was also successfully applied to demonstrate A3AR activation by other synthetic ligands, as well as to demonstrate antagonistic properties of compounds at the A3AR. Hence, we demonstrated its potential as a novel tool to assess ligand-induced A3AR modulation.

PO38 A role for insulin-binding protein 2 in the antiviral immune response in Bombyx mori

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Transcriptome analysis of the midgut of larvae of *Bombyx mori* established that the gene *insulin-binding protein 2* (*ibp2*) is significantly induced after infection with cytoplasmic polyhedrosis virus (CPV) (*Reoviridae*), which is characterized by a segmented dsRNA genome. IBP2 is highly homologous to IGFBP7 in vertebrates which has a tumor-suppressive function and can promote apoptosis. Also in the nematode, *C. elegans*, and the mosquito, a role for the insulin pathway has been reported in the defense against pathogens.

IBP2 is a secreted protein of 33 kDa that contains two immunoglobulin-like C2 domains and can bind insulin. Using the baculovirus expression system, IBP2 with C-terminal Myc-His-tag was produced at high levels in the extracellular medium from where it could be isolated with high purity through nickel-column affinity chromatography. The purified protein is used to investigate its capacity to control infection by a variety of insect viruses such as AcMNPV and BmNPV (*Baculoviridae*), BmCPV (*Reoviridae*), Flock-house virus (*Nodaviridae*), Macula-like virus (derived from the plant virus Maculavirus; *Tymoviridae*) and Cricket-paralysis virus (*Dicistroviridae*), in two different lepidopteran models, the silkworm *B. mori* (*Bombycidae*), and the cotton leafworm, *Spodoptera littoralis* (*Noctuidae*). For comparison, a series of other secreted proteins with putative antiviral activity (antimicrobial peptides, a secreted factor with a single von Willebrand factor type C (SVWC) domain (Vago homolog), and the Toll receptor ligand Spätzle) will be tested. In addition, the antiviral mechanism of IBP2 is investigated, including its ability to promote apoptosis and to block growth, and its effects on the functioning of the RNAi machinery.

The administration of antiviral secreted factors such as IBP2 could lead to increased resistance of silkworm larvae against viral pathogens and provides a new paradigm for the development of methods for protection of economically important insects (silkworms, honeybees) that are environmentally sustainable and safe to human health.

PO39 Implementation of new tools to study the interaction between G protein- coupled receptors (GPCR dimers)

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Recent findings have indicated that G protein- coupled receptors not only exist as monomers but can possibly also interact with each other to form homo- and heterodimers and even higher order oligomers. But what remains as a grey area is the functional implication of such interactions. This still needs to be explored. The GPCR dimers and particularly the heterodimers have “mysterious”effects on signaling and this could impact its responsiveness to treatments as well. Thus, there is a growing need to understand the interaction between GPCRs and then dig into the signaling pathway that arises as a consequence of the formation of GPCR dimers.

In this view, Protein Complementation methods such as split fluorescent protein i.e. Bimolecular Fluorescence Complementation (BiFC) or split luminescent protein i.e. Bimolecular Luminescence Complementation (BiLC/NanoBiT) seem to be promising for the in-depth analysis of the capacity of the two GPCRs to interact with each other. In this approach, the two GPCRs under investigation are coupled to a split protein and upon the dimerization of the two GPCRs, the two split protein fragments come together to form the functional protein.

Our research group focuses on Dopamine D2 receptor (D2R) and Mu-opioid receptor (MOR) and techniques such as Co-immunoprecipitation and Bioluminescence Resonance Energy Transfer 1 (BRET1) indicate an interaction between them. In addition to this, we sought to prove the interaction between the two receptors by Protein complementation technique. Consequently, D2R and MOR were cloned to split fragments of Venus. While analyzing the interaction via BiFC using HEK293 cells transfected with D2R and MOR fused to split fragments of Venus, a high autocomplementation was detected.

Thus, in search of an alternative method, we came across a complementation technique introduced by Promega, based on split fragments of Nanoluciferase (NanoBiT). To analyze the applicability of this assay, we cloned D2R and MOR to the Large (LgBiT) and Small (SmBiT) fragments of Nanoluciferase. The results indicated that D2R fused to SmBiT + MOR-fused to LgBiT showed a significant increase in the signal as compared to the negative control which is MOR-LgBiT + Halotag-SmBiT. Halotag-SmBiT is ubiquitously expressed all over the cell and serves as a good negative control when expressed with MOR-LgBiT.

Our long term goal is to understand the influence of dimerization on the downstream pathway (through β-arrestin recruitment or G-protein coupling) when the dimers are stimulated by their respective agonists. This can possibly be studied by BRET1 between the BiFC coupled dimers and β-arrestin/G-protein fused to *Renilla* luciferase or by FRET between BiFC coupled receptors and β-arrestin/ G-protein fused to a fluorophore. Thus, the second approach (FRET) would also help us to find an answer to the question whether there is a co-internalisation of the dimers when stimulated by an appropriate agonist of the interacting partner.

PO40 Altered postnatal development of the cerebellum in secretin knockout mice

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Secretin (SCT), a traditional gastrointestinal hormone, functioning as a neuropeptide has been well documented. Notably in the cerebellum, SCT's neuronal function has been extensively studied. Several mouse models have been employed to further highlight the SCT's importance in the cerebellum. For example, the Purkinje cell-specific secretin knockout (Pur-$Sct^{-/-}$) mouse model recently developed by our lab showed impairments in motor coordination and motor learning, establishing the connection between the SCT deficiency in the cerebellum and the motor abnormalities. We also found a delay on initial time point of motor reflexes in Pur-$Sct^{-/-}$ juveniles. Nevertheless, it remains unclear whether the persistent functional impairments of the cerebellum were the result of early developmental defects caused by postnatal absence of SCT. Therefore, we hypothesize here that the deletion of SCT gene in mice ($Sct^{-/-}$ mice) alters the pattern of cerebellar development, leading to persistent cerebellar dysfunctions. In multiple postnatal developing stages, SCT was strongly unregulated and SCTR had a widespread distribution in the cerebellum including Purkinje cells, granular cells and GABAergic interneurons. We also demonstrated that $Sct^{-/-}$ mice, compared with their WT littermates, exhibited 1) no overt abnormalities of the global cerebellar anatomy; 2) decreased thickness in the external granular layer (EGL) and reduced protein level of nestin (the cell progenitor marker), and 3) lowered density of Purkinje cells. Several possible explanations for the thinner EGL in $Sct^{-/-}$ mice were considered, including disrupted proliferation of GCPs, untimely exit of GCPs from the cell cycle and/or premature migration of post-mitotic granular cells, and increased apoptosis of granular cells. We found out that the premature differentiation and migration of post-mitotic granular cells as well as the increased apoptosis rate of new born granular cells could account for the decreased thickness of EGL in $Sct^{-/-}$ mice. Our findings strongly supported the hypothesis that SCT played a significant part in the postnatal development of cerebellum.

PO41 Evaluation of diverse complementation assays to target GPCR dimers.

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G protein-coupled receptors (GPCRs) are responsible for regulating a wide range of physiological functions in eukaryotic cells. An increasing amount of research shows that GPCRs can form dimers or even higher order oligomers. Although it is postulated that GPCR heterodimers play a role in neuropsychiatric disorders such as Parkinson's disease, depression and ADHD, there is an urgent need for tools that selectively target such receptor complexes in order to analyze in a detailed way their biological and possible pathophysiological functions. For this reason, the implementation of interfering (IF) compounds has the potency to gain new insights concerning the dimerization interface, as they disrupt dimerization of GPCRs of interest. Consequently, a robust screening platform needs to be developed to evaluate this interfering capacity on dimerization. For this, complementation assays are a promising technique which are used in scientific research platforms to study protein-protein interactions.

Within the current study, different complementation assays were evaluated, involving fluorescent or bioluminescent proteins. An experimental set-up with transfected HEK293 cells was conducted, with vectors coding for GPCRs fused to split fragments of Venus, *Renilla* luciferase or Nanoluciferase. When the GPCRs interact, the split fragments can reconstitute, and the resulting protein can be analyzed by measuring the fluorescence or luminescence with a multi-reader. Surprisingly, a significant background signal has been observed with most assays. Only the split version of the Nanoluciferase displays a significant signal to noise ratio. Consequently, this split version was implemented as a trustworthy method for the analysis of GPCR interactions and indeed demonstrated homodimerization of the dopamine D2 receptor (D2R) as well as heterodimerization of D2R with the muscarinic M1 receptor. These interactions were confirmed via co-IP and BRET assays.

Overall, our results present a good overview of pros and cons of the applied complementation assays used in the study of GPCR oligomerization. These findings can be extended into other research areas where protein-protein interactions constitute the main focus. Furthermore, this assay will be of major value to gain better insight in the interaction domains of GPCR dimers and the implementation of IF compounds, which are of great scientific relevance to study possible consequences of GPCR dimerization on their signaling pathways.

PO42 The evolution of neuropeptide precursors: large-scale transcriptome sequencing of ophiuroid echinoderms reveals loss and gain of neuropeptides

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Neuropeptides are the most diverse group of extracellular signaling molecules that mediate neuronal communication. They control several essential physiological processes in animals, including feeding, reproduction and locomotion. Neuropeptides are derived from larger precursor proteins, which include an N-terminal signal peptide and one or more neuropeptides bounded by mono- or dibasic cleavage sites. Some neuropeptide precursors contain a single copy of a neuropeptide, whereas others contain multiple copies of identical or non-identical, but structurally-related, neuropeptides. However, the functional significance of precursor proteins that give rise to "cocktails"of structurally related neuropeptides is not known.

Genome/transcriptome sequencing is providing new opportunities to investigate how the structure and neuropeptide complement of neuropeptide precursors have changed over evolutionary time. Here we have analysed a unique transcriptome dataset from over fifty species belonging to the class Ophiuroidea (brittle stars; Phylum Echinodermata), where phylogenetic relationships have been determined with robust phylogeny. This is the first comprehensive analysis of neuropeptide precursors in brittle stars and we have identified homologs of many neuropeptide types, including calcitonin, cholecystokinin, corazonin, gonadotropin-releasing hormone, kisspeptin, luqin, nesfatin, NG peptides (neuropeptide S), orexin, pedal peptide, pigment-dispersing factor, somatostatin, tachykinin, teneurin C-terminal associated peptide, thyrotropin-releasing hormone (TRH), vasopressin/oxytocin, bursicon (a and b), glycoprotein hormone (A2 and B5), relaxin, insulin-like growth factor, L-type and F-type SALMFamide and AN peptide. Importantly, we also discovered homologs of eclosion hormone, which are the first to be discovered in deuterostomian animals.

We generated sequence alignments to determine gains and losses of neuropeptides in multiple-copy neuropeptide precursors. Interestingly, the number of peptides within the majority of precursors remained constant across all the species examined, which share a common ancestor estimated to date from ~250 million years ago. TRH exhibited the highest variation in the number of neuropeptide copies, with numbers ranging from 16 to 20 copies. Our results provide new insights into the evolution of multiple-copy neuropeptide precursors and sets the stage to address questions on the functional significance of neuropeptide "cocktails."

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PO43 Adverse conditions on myocardium morphology and function in secretin and secretin receptor deficiency.

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Myocardial remodeling after injury is critical for prognosis and survival in heart disease patients. Secretin (SCT) has been found to increase heart rate and promote the contractility of failed left ventricle upon intravenous administration. The intra coronary SCT infusion also increased the myocardial perfusion and function. Secretin and secretin receptor genes were also found in numerous tissues including the heart, coronary arteries and brain. As an evidence of its role in renin-angiotensin-aldosterone system (RAAS), SCT mediates the central action of Angiotensin II in body water homeostasis. Based on these facts, long term effects of SCT and SCTR gene deficiency on the heart were studied using 6 month old secretin and secretin receptor double knockout (SCT/R $^{-/-}$) mice compared with same age C57BL6N as control (n = 8/ group). The SCT/R $^{-/-}$ mice were found to have significant reduction in lean heart weight (117.78 ± 3.34 mg vs 128.20 ± 1.92 mg; $p < 0.01$), heart to tibia length ratio (6.96 ± 0.20 vs 7.53 ± 0.11 mg/mm; $p < 0.01$) and significant increased apoptosis and fibrosis activity. As consequent adverse effects on cardiac function, stroke volume (SV) (24.9 ± 1.1 vs 45.1 ± 4.5 µl; $p < 0.001$) and cardiac output (CO) (11.5 ± 0.5 vs 21.3 ± 2.4 ml/min; $p < 0.01$) were significantly reduced in SCT/R $^{-/-}$ mice than control ones. Conclusively, SCT and SCTR deficiency may have notorious effect on myocardium with pro-apoptotic and pro-fibrotic action and significantly reduce the cardiac function. The knowledge gained can be applied to conserve the myocardium for improved outcome after myocardial injury in various heart diseases.

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PO44 Reproductive functions of allatostatin-C in Drosophila melanogaster

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In *Drosophila*, egg-laying is an essential and complex reproductive behavior, comprised of three major steps: egg formation, ovulation and oviposition. Previously, several neuromodulators were implicated with some of these processes. To better understand how central neurons regulate egg-laying, we perform a genetic screen with a comprehensive panel of neuromodulator Gal4 lines, and identify *allatostatin-C* (*AstC*)-*Gal4* neurons, activation of which suppresses egg-laying strongly in mated females. Consistent with this observation, *AstC-RNAi* in the nervous tissue elevates egg-laying. Our analysis in virgin females uncover that prolonged depolarization of *AstC* neurons shortly after eclosion results in smaller ovaries with fewer mature eggs than the controls. *AstC* appears to suppress egg formation by inhibiting production of the juvenile hormone (JH), because treatment of methoprene, a JH analog almost fully rescues delays in the egg maturation. Consistently, JH levels in virgin females with activated AstC neurons are much lower than those in the controls. On the other hand, activation of *AstC* neurons in the mated females results in accumulation of mature eggs in ovaries. Although the mated females with activated *AstC* neurons can ovulate and transfer an egg to the uterus, they cannot oviposit the egg. Together, these results indicate that *AstC* and neurons producing are important for the female reproduction.

PO45 Application of a new activity-based assay that allows activity profiling of synthetic cannabinoids (and metabolites) and their detection in urine.

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BACKGROUND: Synthetic cannabinoids (SCs) are the largest group of compounds currently monitored in Europe by the EU Early warning system on new psychoactive substances. The number of substances, their chemical diversity and the rate at which they emerge makes this group of compounds particularly challenging in terms of detection and monitoring. Current approaches for detection of these substances, which are typically only present at low ng/ml concentrations in biological matrices, are based on targeted, structure-based methods, such as immunoassays or MS-based methods. However, both these approaches have important limitations (e.g. lack of cross-reactivity and prior knowledge of structure required). Here, we report on the development and application of a bio-assay that is based on the activity of SCs at the cannabinoid receptors. This assay may be used to perform activity profiling of new SCs and their metabolites and as a first-line screening tool to identify positive biological samples.

METHOD: The bio-assay utilized transiently transfected HEK293T cells, in which the NanoBiT® technology (Promega) was applied. Activation of the CB1/CB2 cannabinoid receptor (fused to one part of luciferase) leads to the recruitment of β-arrestin 2 (fused to the other part of luciferase). The resulting functional complementation of luciferase can be easily monitored via luminescence. The assay was applied in a 96-well format on pure substances and urine extracts. The latter was prepared from 0.5 ml, which was deconjugated and extracted with acetonitrile and ammonium formate (10M). Following evaporation of the supernatant, the residue was redissolved in 100 µl of a 50:50 mixture methanol : serum free medium. Ten µl of the extract (or pure compound) was used in the bio-assay.

RESULTS: Stimulation with a known agonist was used to select the optimal combination of GPCR and β-arrestin 2 fusion-proteins for the bio-assay. The optimized bio-assay demonstrated a dose-dependent response at both CB1 and CB2, with activation at concentrations down to 1 ng/ml JWH-018. JWH-018-mediated CB1 activation at 50 ng/ml (estimated ED_{80} concentration) was dose-dependently blocked by co-incubation with the selective CB1 receptor antagonist Rimonabant. Different SCs and their major metabolites were evaluated on their activity at CB1 and CB2. Application of the bio-assay on genuine samples from SC users shows that the bio-assay is capable of detecting positive urine samples.

CONCLUSION: The developed bio-assay may offer better insight into the potential activity of SCs (and their metabolites) and offers the opportunity to serve as a first-line screening tool for SCs in biological matrices in an alternative way.

PO46 Selective effect of 2-isopropylthioxanthone (2-ITX) on estrogen receptor alpha and beta subtype expression in juvenile goldfish (Carassius auratus)

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This work evaluated the potential endocrine disrupting effects of 2-isopropylthioxanthone (2-ITX) at the level of estrogen receptor (ER) signaling cascade using juvenile goldfish (*Carassius auratus*) as model. 2-ITX is used as photoinitiator in UV inks applied to paper- or plastic-based packaging materials and it is well known as food contaminant due to the potential migration from the outside to the inside of non-printed surface in contact with food during the packaging process Recent studies have reported the occurrence of photoinitiators, including 2-ITX, in various environmental samples, such as indoor dust and municipal sewage sludge samples. We have firstly investigated the ligand binding efficiency of 2-ITX to the ligand binding domains of goldfish ER subtypes using a molecular docking approach. Then, we have assessed the effects of 2-ITX on hepatic ERα1, ERβ1, ERβ2 and vitellogenin (VTG) gene expression in vivo. Fish were injected with increasing doses of 2-ITX ranging from 2 to 10 µg/g BW, and results were compared to the effect of 17β-estradiol (E2) or tamoxifen, a well-known ER modulator. We observed that 2-ITX/ERs complexes have affinities in the sub-micromolar range, showing the presence of van der Waals and hydrophobic interactions. At the same time, our results demonstrated that the highest dose of 2-ITX increased hepatic ERα1 mRNA levels within 96 h of exposure but did not have any effect on both ERβ-isoforms and VTG transcripts. The latter was also confirmed at protein level. Furthermore, 2-ITX reduced the estrogenicity of E2 at both transcriptional and post-transcriptional levels, indicating a clear anti-estrogenic effect. Results from these studies collectively reveal that 2-ITX can affect the hormonal control of reproductive processes in fish by selective action on ER isotypes.

PO47 Study of biased signaling at chemokine receptors

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G protein coupled receptors (or GPCRs) are the largest family of cell surface receptors and they are implicated in various physiological and pathological processes. Classically, agonist binding on receptor induces G protein activation and desensitization by arrestins. However, recent results indicated that GPCRs are able to activate G protein independent signaling, mainly through the interaction with arrestins that are now considered as multifunctional scaffold proteins interacting with several signaling proteins. We investigated the coupling selectivity associated to chemokine receptors CCR2, CCR5 and CCR7 in response to various ligands for the different G protein subtypes by using BRET biosensors recording conformational changes associated with G protein activation. We compared these results to those obtained with functional readouts such as β-arrestin2 recruitment, cAMP accumulation and intracellular calcium mobilization. Our results showed that stimulation of CCR2, CCR5 and CCR7 with chemokines activated the three Gαi subtypes and the two Gαo isoforms with potencies that correlate with their respective binding affinities. Binding of chemokines to CCR2 and CCR5 also induced Gα12 activation but not Gα13 although they belong to the same G protein family. For each receptor, the relative rank order of potencies for the different chemokines varies slightly according the assay, suggesting that chemokines receptors exhibit subtle signaling bias. However, these biases do not follow the classical dichotomy between G protein and β-arrestin dependent pathways. This is particularly true for CCR7 activation by its natural ligands, CCL19 and CCL21, which is considered to be a prototypical example of signaling bias. We showed that CCL21 is less potent than CCL19 in both G protein activation and β-arrestin-2 recruitment assays, while they behaved similarly in calcium mobilization and ERK activation assays. In conclusion, our results show that the concept of signaling bias is much more complex than initially thought. They also indicate that the signal bias remains relatively subtle for natural ligands such as chemokines, while more overt bias has been described for synthetic small molecule agonists.

PO48 Utilizing zebrafish for new insights into the roles of chemerin in cancer biology

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Chemerin is a chemoattractant factor identified at the IRIBHM that appears to regulate leukocyte trafficking and inflammatory responses, and also displays anti-tumoral properties. However, the basic mechanism of chemerin action within tissues is largely unknown and has yet to be formally addressed in vivo.

In recent years, the zebrafish has emerged as a unique and powerful model system in which to perform immunobiological investigations. Preliminary observations in the host laboratory showed the chemerin system is well conserved from zebrafish to mammals. This suggests the zebrafish model may substantially contribute to improving our knowledge on chemerin biology and functions in vivo. In addition, several models of cancer have been established in zebrafish that may provide for unprecedented opportunities for determining chemerin functions in disease conditions.

In this project, we will perform a detailed characterization of chemerin expression patterns in zebrafish and will generate knockout lines that will be used to investigate chemerin biological functions in vivo. We will engineer zebrafish that produce active chemerin in the epidermis, and by using existing fluorescent leukocyte-reporter lines, we will conduct real-time imaging studies in transparent zebrafish embryos, allowing for an unprecedented comprehensive analysis of chemerin-induced chemotaxis in vivo. Once we achieve a basic understanding of chemerin biology, we will investigate the mechanisms by which chemerin modulates tumorigenesis in vivo. Established genetic models of melanoma will be used 1) to assess the effect of chemerin disruption, or epidermis-targeted transgenic expression, on melanoma incidence, 2) to directly observe chemerin-induced immune response during the initiation of melanoma and its progression, and 3) to investigate the metastatic potential of chemerin following transplantation of fluorescently-marked primary tumor cells in transgenic fish.

PO49 Chemerin and its receptors in cancer

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Chemerin is a leukocyte chemoattractant factor for immature dendritic cells, macrophages and NK cells, which is expressed in various human tumors and is abundant in human inflammatory fluids and tissues. It is secreted as an inactive precursor (prochemerin), present at relatively high concentrations in plasma, and requiring proteolytic processing for activity, including by neutrophil cathepsin G and elastase. Its main functional receptor is ChemR23, a G protein-coupled receptor expressed on leukocyte subsets, but GPR1 and CCRL2 have also been described as chemerin receptors. Chemerin and its receptors appear to regulate leukocyte trafficking and inflammatory responses, and display also anti-tumoral properties. However, the anti-tumoral mechanisms by which chemerin acts are poorly characterized so far.

The aim of the study is to determine the receptors, cell populations and signaling pathways involved in the anti-tumoral activities of chemerin, characterize at which steps of cancer progression they act, and determine whether this system might be considered as a valid target for therapeutic intervention in the frame of human cancer. This is being investigated by using different skin tumor models and a variety of transgenic and knock out mouse lines.

To date, we generated and characterized mouse lines overexpressing chemerin in keratinocytes. An inducible bicistronic gene encoding bioactive chemerin and GFP is driven by the promoter of the keratin K5 gene (K5-tTA:tet(o)-chemerin mice). Several lines with different chemerin expression levels were generated. In these lines, the transgene was expressed in the basal layers of the epidermis and in the epithelia of buccal cavity, oesophagus, pharynx and sinuses. As a result, chemerin levels were increased in the blood.

We investigated the role of chemerin and its receptors in tumorigenesis. For this purpose, we use two models: a model of tumor growth by grafting of B16 melanoma cells in subcutaneous and a DMBA/TPA model of skin carcinogenesis leading to the development of papillomas and squamous cell carcinomas (SCCs). We used these two models on mouse lines overexpressing chemerin in the skin and/or knock out for each receptor.

We show in these two models of tumorigenesis that chemerin have anti-tumoral properties. Part of this effect of chemerin is mediated by ChemR23. We are in the process of determining whether CCRL2 and GPR1 are also involved.

PO50 Control of WNT signaling in intestinal epithelial stem cells by the LGR5 receptor

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The leucine-rich repeat-containing G protein-coupled receptor Lgr5, belonging to a rhodopsin-like GPCR subfamily, is expressed by intestinal stem cells (CBC). Rspondins have been described as ligands for Lgr5 and its paralogues (Lgr4, 6), enhancing Wnt signaling in vitro[1,2,3]. In vivo, the intestinal phenotype of Lgr5-LacZ deficient E18.5 embryos, showing precocious Paneth cell (PC) differentiation[4], appears opposed to that of Lgr4-deficient mice[5], questioning the hypothesized receptor redundancy.

To further clarify this question, we studied the phenotype of two other transgenic Lgr5 knock-in/knockout (KO) lines (Lgr5-GFP-CreERT2[6] and Lgr5DTR-GFP[7]). KOs similarly showed ankyloglossia and premature PC differentiation. Using Wnt reporter Axin2-LacZ mice, we found an upregulation of Wnt signaling in Lgr5 KO tissues (Lgr5-GFP-CreERT2 and Lgr5DTR mouse lines) as compared to controls. Ex vivo, contrary to Lgr4-deficient samples, not only mini-guts from Lgr5 KO mice grew efficiently, they also showed higher number of protrusions as compared to controls suggesting an over-activated Wnt status of CBCs ex vivo.

Attempts to rescue in vivo the Lgr5KO phenotype by Wnt overactivation via b-catenin stabilization in Lgr5-expressing cells during gestation (Lgr5-GFP-CreERT2 x bcat exon3$^{flx/flx}$ context) did not reduce precocious PC differentiation and even worsened the phenotype in recombined Lgr5 HEs and Lgr5 KOs. Decreasing Wnt signaling during gestation using the porcupine inhibitor LGK974[7] neither rescued ankyloglossia or the intestinal phenotype of Lgr5 KOs, suggesting involvement of other cascades than the sole Wnt canonical so far explored. Compared transcriptome analysis of Facs-isolated E16.5 Lgr5^{+ve} stem cells from KO and heterozygous (HE) embryos revealed deregulation of both Wnt canonical and Wnt non canonical pathways. Experiments are under way to fully dissect the crosstalk between Lgr5 and these signaling cascades.

Our preliminary results support the idea that, in vivo, Lgr5 functions as a critical regulator of Wnt signaling to fine tune the balance between self-renew and differentiation of CBCs.

1-Carmon, Mol Cell Biol, 2012. 2-Glinka, EMBO Rep, 2011. 3-De Lau, Nature 2011. 4-Garcia, Dev Biol, 2009. 5-Mustata, EMBO Rep, 2011. 6- Barker, Nature, 2007. 7-Tian, Nature, 2011.

PO51 Role of CCRL2 chemerin receptor in the tumor development

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Chemerin is the natural ligand of the G protein-coupled receptor ChemR23 and acts as a chemotactic factor for leukocyte populations as dendritic cells, NK cells and macrophages. It is expressed by epithelial and non-epithelial cells as an inactive precursor, present at nanomolar concentrations in plasma. Processing of the precursor C-terminus is required for generation bioactive forms of chemerins. ChemR23-expressing cells are recruited in human inflammatory diseases and both pro- and anti-inflammatory roles of chemerin have been reported (Bondue et al. 2011). More recently, two other receptors for chemerin were described, GPR1 and CCRL2, but their functional relevance is largely unknown.

The aim of this study is to understand the role of chemerin receptors in the development of tumor. We have injected B16 melanoma or Lewis lung carcinoma cells in ChemR23 and/or CCRL2 knock out mice. We have shown that ChemR23 deficiency did not affect the tumor size whereas CCRL2 KO mice developped smaller tumors than control mice. We were then interested in the role of chemerin receptors in the recruitment of pro- and anti-tumoral immune cells, tumoral cell proliferation, the apoptosis/necrosis axis and angiogenesis. The recruitment of immune cells and tumor cell proliferation were not affected by the deficiency of ChemR23 or CCRL2 in tumor bearing mice. However the tumors from CCRL2 KO mice presented a larger apoptotic and necrotic areas compared to control mice suggesting a role of CCRL2 in the angiogenesis.

We are now investigating the role of chemerin and its receptors in the functions of endothelial cells isolated from murine lungs and from tumors. Endothelial cells produce chemerin and express ChemR23 and CCRL2 receptors. CCRL2 expression is upregulated when endothelial cells are stimulated with IFNg and TNFα unlike ChemR23. These preliminary results suggest that the expression of CCRL2 by endothelial cells could affect the angiogenesis in a tumoral context.

Bondue B. Chemerin and its receptors in leukocyte trafficking, inflammation and metabolism. Cytokine Growth Factor Rev, 2011

PO52 Role of leucocyte chemoattractant factors and their receptors in inflammation, regeneration and cancer of the liver in a zebrafish model

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In this project, we utilize zebrafish model system, which has become a major animal model in many research fields including inflammation and cancer, to obtain new insights into functional aspects of chemerin, a chemoattractant that appears to regulate leukocyte trafficking and inflammatory responses, while displaying anti-tumoral effects in human and mouse. We focus on characterizing the role of chemerin in liver physiology and disease, a topic that remains poorly understood so far.

PO53 Combining MALDI imaging and µCT to localize neuropeptides in ant brains

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Neuropeptides play a determining role in biological processes. These small molecules and their associated signaling systems are involved in metabolism, development, reproduction, behavior and learning. The origin of these compounds has deep roots in metazoan evolution and likely emerged at very early stages of nervous system development (1).

In the animal kingdom, insects, which appeared around 479 million years ago (2), represent the most diverse group in terms of numbers of described species. This taxon has an enormous significance with respect to ecology, biology and economy. In fact, insects participate in ecosystem processes, act as vectors of diseases, parasite other organisms and impact agriculture. Besides, they are being used as model organisms in different fields of life science, such as genetics and biochemistry. Ants are particular interesting due to their social colony organization and unique castes systems. For instance, individuals share identical genes but exhibit very different morphology also affecting brain structure and size (3).

In this study, we combined MALDI mass-spectrometry imaging (MSI) and micro-computed tomography (µCT) of the leafcutter ant *Atta sexdens* for neuropeptide localization in brain. MALDI-IMS provides spatial distribution and relative quantification data of known-molecular weight compounds and has been a useful tool in honeybee neuropeptide analysis (4). Additionally, µCT is a non-invasive scanning tool to acquire the detailed 3D anatomy of a sample. (5). We integrated the spatial distribution of neuropeptides from different planes of the ant brain into an accurate 3D anatomy model. Furthermore, histological staining combined with the model provided additional information of the neuropeptide brain patterns and the associated tissue. The first example of combining these two state-of-the-art techniques, MALDI-IMS and µCT, for micro tissue samples will allow studying ant brain anatomy and co-localized neuropeptide function in the future.

(1) Mirabeau and Joly 2013, Proc. Natl. Acad. Sci. U.S.A 11022):E2028-37. (2) Misof et al. 2014, Science 346:763-767. (3) Gronenberg, 2008 Myrmecol. News 11:25-36. (4) Pratavieira et al. 2014, J. Proteome Res. 13:3054–3064. (5) Smith et al. 2016, Sci. Rep. 6:21768.

PO54 Do fish possess a sensing system for amino acids in the gastrointestinal tract?

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The ability to recognize specific nutrients enables an adequate response and ensures their efficient utilization. However, surprisingly little is known about the nutrient sensing mechanisms in the gastrointestinal tract (GIT), particularly in teleosts. Amino acids (AA), the building blocks for proteins, and peptides are key nutrients in teleost diet and essential for a range of metabolic pathways, including growth. In mammals, the presence of AA and peptides in the GIT lumen is monitored by chemical sensory systems based on members from the superfamily of G protein-coupled receptors (GPCRs) family C and A, respectively. From family A, the lysophosphatidic acid receptor 5 (LPAR5) is responsive to peptides, while members of family C includes monogamous receptors for l-Glu, such as metabotropic glutamate receptors (mGluRs), as well as promiscuous receptors, such as calcium-sensing receptor (CasR), GPCR family C subtype 6A (GPRC6A) and taste receptors (T1Rs), that respond to different AA. When nutrients bind to these receptors, neuronal and hormonal signalling pathways are activated that mediate epithelial barrier protective functions, changes in gastric emptying and intestinal transit, release of digestive enzymes, nutrient transport, and also affect the control of food intake (hunger and satiety) and metabolism. While it is well acknowledged that the nutrient sensing system is a key player in the control of these functions in mammals, almost no information exists in teleosts, the largest vertebrate group with more than 25000 species.

Atlantic salmon is a key commercial species in global aquaculture. Understanding how specific AA affect digestion and potentially enhance feed intake, growth and fish quality is therefore of high interest for the aquaculture industry. In addition, due to an additional and relatively recent whole-genome duplication event - the salmonid-specific 4th WGD - Atlantic salmon provides an excellent model system to study the role of gene duplication and adaptation in the nutrient sensing system.

We investigated the mammalian AA and peptide sensing GPCRs homologues in Atlantic salmon in order to further characterise their role in digestion, feed intake and growth. *In silico* analysis revealed that Atlantic salmon GPCRs family A and C primary structure is remarkably conserved within vertebrates, which supports the hypothesis that nutrient sensing systems are conserved throughout vertebrate phylogeny. In adult Atlantic salmon, mRNA expression analysis by qPCR shows that the peptide sensing receptor LPAR5 is broadly distributed throughout the GIT, from pyloric caeca to hindgut, with the lowest levels of expression found in the stomach. Unexpectedly, the highest levels of mRNA expression of LPAR5 were detected in the gills. This information is fundamental for further comparative studies and to study pharmacological and biological responses of nutrient-sensing receptors in salmon. The LPAR5 receptor activity using *in vitro* cell based assays will be further investigated to understand the role of this receptor in sensing protein hydrolysates in salmon.

PO55 Development by genetic immunization of monovalent antibodies (nanobodies) behaving as antagonists of the human ChemR23 receptor

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The generation of antibodies that recognize the native conformation of G protein-coupled receptors can be a challenging task as, like most multi-membrane spanning proteins, they are extremely difficult to purify as native protein. By combining genetic immunization, phage display and biopanning, we identified two functional monovalent antibodies (nanobodies) targeting ChemR23. The two nanobodies (CA4910 and CA5183) were highly specific for the human receptor and bind ChemR23 with moderate affinity. Binding studies also showed that they share a common binding site that overlaps with that of chemerin, the natural ligand of ChemR23. Consistent with these results, we found that the nanobodies were able to antagonize chemerin-induced intracellular calcium increase. The inhibition was partial when chemerin was used as agonist and complete when the chemerin(149-157) nonapeptide was used as agonist. Engineering of a bivalent CA4910 nanobody resulted in a relatively modest increase in affinity but a marked enhancement of efficacy as antagonist of chemerin-induced intracellular calcium mobilization and a much higher potency against the chemerin(149-157) nonapeptide-induced response. We also demonstrated that the fluorescently labeled nanobodies detect ChemR23 on the surface of human primary cell populations as efficiently as a reference mouse monoclonal, and that the bivalent CA4910 nanobody behaves as an efficient antagonist of chemerin-induced chemotaxis of human primary cells. These nanobodies thus constitute new tools to study the role of the chemerin/ChemR23 system in physiological and pathological conditions.

PO56 Pharmacological characterization of two serotonin receptors in the red flour beetle, Tribolium castaneum

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Serotonin (5-hydroxytryptamine, 5-HT) plays a key role in the regulation of diverse physiological processes, such as the control of nutrition, learning and memory, and behavior. 5-HT carries out its different functions by interacting with multiple 5-HT receptors. In vertebrates, seven distinct receptor families are known (5-HT_{1-7}), some containing multiple subtypes. With the exception of 5-HT_3, which is a ligand-gated ion channel, all known 5-HT receptors are G protein-coupled receptors (GPCRs). Thus far, only five 5-HT receptor subtypes have been characterized in insects that share high similarity with mammalian 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B} and 5-HT_7 receptors. Recently also a novel 5-HT receptor class, namely 5-$HT_{8,}$ has been discovered in *Pieris rapae*. Classification of invertebrate 5-HT receptors according to the existing vertebrate families is mainly based on well conserved amino acid sequences and activated second-messenger systems for 5-HT receptors. However, pharmacological profiles from insect receptors seem to differ significantly from those of vertebrates. In the genome of the red flour beetle, *Tribolium castaneum*, four 5-HT receptors could be assigned based on sequence similarity to 5-HT receptors in other insects. We pharmacologically characterized a 5-HT_1 and 5-HT_7 receptor from *T. castaneum*. To investigate the downstream signaling pathway, both receptors were expressed in CHO-PAM28 and HEK293 cells. Since no effect was observed in CHO-PAM28 cells, we concluded that the receptors do not couple via G_q to the Ca^{2+} signaling pathway. In HEK293 cells expressing *Trica*5-HT_1 application of 5-HT decreased cAMP levels, while in cells expressing *Trica*5-HT_7 application of 5-HT increased cAMP levels. In order to pharmacologically characterize both receptors, we used CHO-WTA11 cells, which stably express the promiscuous $G_{\alpha 16}$. For both receptors, the effects of a series of known 5-HT receptor agonists and antagonists were tested, and some partial agonists and competitive antagonists were identified. When studying the *Trica*5-HT_1 and *Trica*5-HT_7 transcript levels with qRT-PCR, highest expression was observed in the brain and optic lobes.

PO57 Influence of allatostatins on metabolism of Tenebrio molitor beetle

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Neuropeptides are factors regulating vital insect processes like growth, development, behavior and reproduction. One of the group of insect neurohormones are allatostatins, which were identified in cockroaches, termites, moths, locusts and crickets. Among these neuropeptides three groups of peptides are distinguished (FGL, MIP and PISCF) differing from each other structurally, but connected by allatostatic activity - inhibition of the synthesis of juvenile hormone in *corpora allata*. Many of these neurohormones show pleiotropic activities influencing on a number of physiological processes in insects, like synthesis and release of vitellogenins, synthesis of digestive enzymes or regulation of visceral muscles contractions. Thus far, in the largest insect order - beetles, little is known about the biology of these neuropeptides. The main metabolic tissue in insects is fat body, which is primary place for storing nutrients and is the site of high metabolic activity. The cells of this tissue control not only the mobilization of energy reserves - lipid and carbohydrates - but also synthesize most of the proteins and metabolites circulating in the haemolymph. Recent studies show that allatostatin also participate in the regulation of reserve substances homeostasis in the body fat. In *D. melanogaster* with muted ASTA gene there is observed increased lipid accumulation in the fat body. FGL allatostatins also affect the fat body tissue and its metabolism directly. They block the process of vittelogenin glycosylation in *B. germanica*, thereby inhibiting their synthesis and release.

The aim of this study was to evaluate the effect of allatostatic neuropeptides Dippu-AST 1 (LYDFGL-NH_2), Grybi-AST B1 (GWQDLNGGW-NH_2) and Trica-AST C (pESRYRQCYFNPISCF-OH), representing each family of allatostatins on metabolic parameters of *T. molitor* beetle, such as total lipid and protein content in fat body, total sugar content as well as qualitative and quantitative composition of particular carbohydrates fractions in haemolymph. In the experiments testing participation of these peptides in regulation of energetic and storage substances metabolism in fat body it was shown that they act in tissue- and dose-dependent manner. *In vivo* bioassays demonstrate that allatostatins regulate lipid and protein content in this tissue. Additionally, they act on protein content and whole sugar level in haemolymph by decreasing or increasing the content of several carbohydrate fractions in this tissue. The effect induced by allatostatins tested may be due to their action on the tissues or to be the result of an indirect impact on the neurosecretory system of *corpora cardiaca/ corpoca allata*.

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PO58 Monocytes and lymphocytes of patients with primary ciliary dyskinesia display no difference in chemotaxis and chemoattractant-receptor expression

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PCD is a rare, autosomal (in exceptional cases, X-linked) inherited disease caused by genetic mutations leading to structural and/or functional defects in the motile cilia. On the respiratory epithelium, cilia perform a beating motion in an organized manner such that the upper layer of the mucus, wherein harmful microorganisms and particles are captured, is transported upward. Inefficient mucus clearance due to abnormal movement of these cilia leads to recurrent respiratory tract infections in PCD patients and results in chronic pulmonary inflammation. In the early 1980's, several research groups investigated whether in addition to the ciliary motion, the chemotactic migration of PCD leukocytes is affected as well, since both processes depend on the microtubules of the cytoskeleton. Most studies focused on neutrophils since these are the first immune cells to arrive at the site of infection. However, controversial results were reported. Some investigators demonstrated defective PCD neutrophil migration, whereas others claimed that chemotaxis of neutrophils derived from PCD patients and healthy individuals is similar. In this study, we investigated whether PCD monocytes and PCD lymphocytes show altered migratory capacity. We studied the chemoattractant-receptor profile on lymphocytes and monocytes of PCD patients, in comparison with healthy controls. Finally, we evaluated chemotactic migration towards monocyte and lymphocyte attracting chemokines.

PO59 Biological evaluation of bivalent ligands for dopamine D2-like receptors and the μ opioid receptor

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G protein-coupled receptors (GPCRs) constitute the largest family of membrane proteins. A vastly unexplored functional property of GPCRs concerns their propensity to engage in oligomeric assemblies involving two or more GPCRs to form homo- and heterodimers, as well as higher order multimers. Such GPCR dimers and in particular, heterodimers, can have a profound impact on signaling. Dopamine D_2-type and μ opioid receptors, two GPCRs expressed in the brain, play a major role in pain and addiction.

In this poster we describe the evaluation of twelve different heterobivalent ligands for μ Opioid Receptor-D_2-like Receptor (μOR-D_2-likeR) to further study this receptor interaction. We determined the affinity of the bivalent ligands on μOR-D_2-likeR dimers through ligand binding assay. In addition, functional assay such as MAPK was used to asses signal transduction.

PO60 Ontogenesis of the gonadotropic axis in Dicentrarchus labrax, focus on the brain and pituitary.

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In vertebrates, the reproductive function is under the control of the brain pituitary gonadal (BPG) axis. In fish, the brain is still in development after hatching and continues to evolve during the earliest stages of larval development. For instance, in the sea bass (*D. labrax)*, brain anatomy drastically changes during the first months to finally reach the adult morphology at around 60 days post hatching (dph).

In this study, we focused on the setting up of the brain-pituitary part of the gonadotropic axis by following the main actors: kisspeptins (kiss1 and kiss2) and gonadotropin-releasing hormones - GnRHs (GnRH1, GnRH2 and GnRH3) at the brain level and gonadotropins (LH and FSH) at the pituitary level during larval development of sea bass. Ontogenesis was studied by using a combination of *in situ* hybridization and real time quantitative PCR.

Our results demonstrate a differential expression of the target genes. For Kiss2, GnRH2, GnRH3, LH and FSH, mRNAs were detected by qPCR as soon as 10 dph. Moreover, a wide number of cells expressing GnRH2, GnRH3 and kiss2 were observed in different areas of the developing brain. GnRH2 expressing cells were mainly localized in the mesencephalon and rhombencephalon; GnRH3 expressing cells were observed in the olfactory bulbs and Kiss2 expressing cells were located in the ventral part of the prosencephalon.

Our results indicate that the neuroendocrine network controlling reproduction is progressively setup during the critical period of larval development.

PO61 Angiostatic activity of two natural CXCL4L1 isoforms

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Beside their well-established role in leukocyte migration chemokines are recognized as critical regulators of tumor-related processes, such as angiogenesis, tumor cell proliferation and metastasis. Among the CXC chemokines, CXCL4L1 has been characterized as a potent anti-tumoral chemokine inhibiting angiogenesis, inducing apoptosis and attracting anti-tumoral leukocytes in different tumor models (melanoma and lung carcinoma).

Here, we determined the biological activity of the alternative spliced isoform of CXCL4L1, CXCL4L1(-4-70) in comparison with the classical isoform CXCL4L1(1-70). Both isoforms are concomitantly released by activated platelets. Since alterations to the NH_2-terminus of chemokines can have severe biological consequences, we investigated the impact of the extension with 4 NH_2-terminal amino acids on the angiostatic activity.

In vitro, the chemotactic response of endothelial cells towards VEGF and FGF-2 was inhibited in the same way by both isoforms. CXCL4L1(1-70) and CXCL4L1(-4-70) reduced FGF-2 induced phosphorylation of MAP kinases ERK-1 and ERK-2, which are essential for migration and survival of endothelial cells. In a FITC-conjugated dextran cell permeability assay, both splice variants showed a strong but comparable anti-permeable effect upon VEGF stimulation of the endothelial monolayer. Accordingly, CXCL4L1(1-70) and CXCL4L1(-4-70) treatment significantly reduced *in vivo* blood vessel growth induced by FGF-2, as demonstrated in the matrigel plug assay. However, no significant difference between both isoforms was observed.

In conclusion, the effects of CXCL4L1 on endothelial cells are not influenced by the four extra NH_2-terminal residues present in the alternatively spliced isoform CXCL4L1(-4-70). We therefore assume that both isoforms equally interact with the CXCR3B receptor on endothelial cells.

PO62 An omics strategy to unravel the role of the β2-adrenergic receptor in inflammation.

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β_2-adrenergic receptor (β_2-AR) agonists are widely applied in the clinic for treatment of respiratory disease. Although their therapeutic effect mainly results from their spasmolytic activity, β-agonists have also been attributed with anti-inflammatory properties.

We studied the effect of β-agonist cotreatment on TNF-α-induced activation of inflammatory signalling in astrocytes, focusing on Nuclear Factor-κB (NF-κB), a master regulator of inflammatory gene expression. Unexpectedly, we observed that, unlike other anti-inflammatory drugs, β-agonists did not inhibit the activity of NF-κB. Instead, β-agonists enhanced TNF-α-induced expression of a subset of prototypical NF-κB target genes. The most pronounced effect was apparent for Interleukin-6 (IL-6) and the CXC chemokines CXCL2 and CXCL3. Importantly, the β_2-agonist-induced potentiation of inflammatory responses was also recapitulated *in vivo*, in rats treated intracerebroventricularly with a combination of TNF-α and clenbuterol. Gene expression changes were furthermore reflected in a redistribution of the leukocyte subsets present in the brain. Altogether, our results suggest β-agonists can catalyze inflammatory reactions.

To gain further insight into the molecular mechanisms connecting the β_2-AR to inflammatory signalling cascades, we will use an 'omics' approach. First of all, we have developed a mammalian two-hybrid technology (KISS, for Kinase Substrate Sensor) that allows proteome-wide investigation of the β_2-AR interactome. As a proof-of-concept we show that KISS can be used to demonstrate dose-dependent recruitment of β-arrestin 2 to the β_2-AR upon treatment of cells with isoproterenol. Secondly, using the IL-6 promoter as a model, we aim to identify nuclear effectors that modulate inflammatory gene expression upon β_2-AR ligation. Using a strategy that combines DNA-affinity purification and mass spectrometric analysis, we recovered 6 known and 17 novel IL-6 promoter-binding proteins. Further research will be directed towards elucidation of the β_2-AR signalosome and its intersection with inflammatory signalling cascades.

PO63 Signaling properties of chemerin receptors ChemR23, GPR1 and CCRL2.

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Chemerin is a small chemotactic protein originally identified as the natural ligand of ChemR23. More recently, two other receptors, GPR1 and CCRL2, have been reported to bind chemerin but their functional relevance remains poorly understood. In this study, we compared the binding and signaling properties of the three human chemerin receptors and showed differences in mode of chemerin binding and receptor signaling. Chemerin binds to all three receptors with low nanomolar affinities. However, the contribution of the chemerin C-terminus to binding efficiency varies greatly amongst receptors. By using BRET-based biosensors monitoring the activation of various G proteins, we showed that binding of chemerin and the chemerin 9 nonapeptide (^{149}YFPGQFAFS^{157}) to ChemR23 activates the three $G_{\alpha i}$ subtypes ($G_{\alpha i1}$, $G_{\alpha i2}$ and $G_{\alpha i3}$) and the two $G_{\alpha o}$ isoforms ($G_{\alpha oa}$ and $G_{\alpha ob}$) with potencies correlated to binding affinities. In contrast, no significant activation of G proteins was detected upon binding of chemerin to GPR1 or CCRL2. Binding of chemerin and the chemerin 9 peptide also induced the recruitment of β-arrestin2 to ChemR23 and GPR1, but not to CCRL2. However, the propensity of chemerin 9 to activate β-arrestin2 relative to chemerin is higher when bound to GPR1. Finally, we showed that binding of chemerin to ChemR23 and GPR1 promotes also the internalization of the two receptors and the phosphorylation of ERK1/2 MAP kinases, although with a different efficiency, and that phosphorylation of ERK1/2 requires both $G_{\alpha i/o}$ and β-arrestin2 activation. Collectively, these data support a model in which each chemerin receptor displays selective signaling properties.

PO64 Role of the CXCL1 and CXCL2 in liver ischemia and reperfusion injury

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INTRODUCTION: The interruption of the blood flow (ischemia), with consequent lack of oxygen is an inherent phenomenon during surgical procedures, such as during removal of liver tumors and transplantation. Once the blood flow is restored (reperfusion), there is an increased injury initiated by ischemia. This phenomenon is known as liver ischemia-reperfusion (IR). Neutrophils are central players for the pathophysiologic changes during hepatic IR injury. Neutrophils migrate from the circulation to the liver through a gradient of inflammatory chemokines. These include CXCL1/KC and CXCL2/MIP-2, two murine analogues of human CXCL8 that are recognized by the CXCR1 and CXCR2 receptors expressed on neutrophils. Among several neutrophil-related attractants, CXC chemokine receptor 2 (CXCR2)-binding chemokines are well studied in different inflammatory contexts.

OBJECTIVES: We wish to investigate whether the blockade of chemokine receptor CXCR2 may have a beneficial role in liver inflammation and damage following IR.

METHODS: The animals were subjected to 60 minutes of no-flow ischemia followed by reperfusion for up to 24 h. The CXCR2 inhibitor reparexin (30mg/kg) was given 15 minutes before the reperfusion and every 2 hours later. Considering that few studies have examined the recruitment of neutrophils in real-time in vivo, we also evaluated this effect using LysM-eGFP mice and intravital confocal microscopy (IVM) to observe how neutrophils behave during the injury development.

RESULTS: The most prominant IR-induced liver injury and inflammation was detected 6h after the reperfusion as observed by the high levels of ALT in serum and huge neutrophil infiltration in the liver, respectively. The liver injury was followed by high expression of cytokines (TNFα, IL-6) and chemokines (CXCL2 and CXCL1). Reparexin treatment significantly reduced liver injury by reducing ALT and inflammation by preventing neutrophil influx (liver and lungs). The LysM-eGFP mice subjected to IR showed a huge infiltration of neutrophils into the liver. By evaluating the behavior of these cells, it was observed that, especially 6h after reperfusion, neutrophils move longer distances and faster. In addition, after 6h, the neutrophils are bigger and many clusters of neutrophils are formed. Moreover, they become more elongated cells with an increased axis which is a signal of cell activation. The Reparexin treatment was able to decrease the influx of neutrophils, and changed the behavior of these cells. With the Reparexin treatment, the neutrophils move shorter distances, and reparexin was able to reduce the size of the cells.

CONCLUSIONS: CXC chemokines, acting on CXCR2, have an important role during liver IR injury. Thus, drugs, such as Reparexin, developed to block the function of the CXCR2 receptor, may be effective at preventing reperfusion injury in relevant clinical situations.

HONDA, M. et al. Intravital imaging of neutrophil recruitment in hepatic ischemia-reperfusion injury in mice. Transplantation, v. 95, n. 4, p. 551-8, Feb 27 2013.

PO65 Yeast and cancer: common mechanism underlying activation of Ras by glycolytic flux

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Ras proteins are small GTPases which function as an on-off binary switch for diverse signaling pathways. Nearly 30% of human tumors have mutated forms of Ras. Both in the yeast *Saccharomyces cerevisiae* and in mammalian cells, Ras is an important regulator of cell proliferation. Mammalian Ras can functionally replace yeast Ras and hyperactive Ras oncogenes cause aberrant cell proliferation in yeast. Also the Ras regulatory proteins, GEFs and GAPs, are homologous between yeast and mammalian cells.

We used the *tps1*Δ mutant of the yeast *S. cerevisiae* as a tool to study Ras regulation. In the presence of glucose, this strain cannot grow due to a major metabolic deregulation causing hyperaccumulation of fructose-1,6-bisphosphate (Fru-1,6-bisP) and also hyperactivation of Ras leading to apoptosis. This led us to the finding that upon addition of glucose to wild type yeast cells, Fru-1,6-bisP promotes in physiological concentrations activation of Ras through the GEF factor Cdc25. This activation may also be relevant in tumor biology because of the high glycolytic flux and higher concentrations of glycolytic intermediates present in cancer cells (Warburg effect). *In vitro* studies showed that Fru-1,6-bisP helps to increase the dissociation rate of the human K-Ras/SOS1 complex. Furthermore, we were able to show that glucose addition to glucose-deprived HEK293T and Hela Kyoto cells triggers rapid activation of Ras, as well as activation of its downstream targets MEK and ERK. Our results suggest that there may be a causal link between the high glycolytic activity and rapid cell proliferation of yeast and cancer cells in glucose medium through Fru-1,6-bisP activation of Ras.

PO66 Identification of Ftr1 and Zrt1 as novel iron and zinc transceptors for activation of the PKA pathway.

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Micro-organisms are continuously exposed to fluctuations in nutrient availability. In order to cope with these fluctuations, the yeast *Saccharomyces cerevisiae* has evolved a complex system of different nutrient signaling pathways. One of these pathways is the protein kinase A (PKA) pathway, which regulates cell growth and multiple growth-correlated properties in response to nutrient availability. Re-addition of a missing nutrient to starved cells leads to rapid activation of PKA during exit from stationary phase. The nutrient is sensed at the plasma membrane by nutrient specific transporters, possessing an additional function as nutrient receptor, hence called transceptors. Four different transceptors have previously been identified for macronutrients: Gap1 for amino acids, Pho84 for phosphate, Mep2 for ammonium and Sul1/2 for sulfate. Each of these transceptors is induced when yeast cells are starved for their specific substrate. Based on this analogy, we have now identified (putative) transceptors for several micronutrients: Ftr1 for iron, Zrt1 for zinc, Ctr1 for copper and Trk1/2 for potassium. We observed a clear, transceptor dependent, PKA activation upon re-addition of the cation to starved cells. Moreover, we showed partial uncoupling of transport and signaling activity for some of these proteins, using the tools developed for identification of the macronutrient transceptors. Hence, we conclude that the PKA pathway in yeast is a general nutrient sensing pathway, able to sense a wide array of nutrients. The concept of nutrient transceptors therefore is likely also evolutionarily widespread. The latter conclusion is strengthened by recent discoveries of nutrient transceptors in other organisms, including *Drosophila*, *Arabidopsis* and mice.

Keywords: transceptor, signaling, PKA, macronutrient, micronutrient

PO67 Implication of the P2Y4 receptor in the angiogenic and cardioprotective potential of Adipose-derived Stem Cells

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Background: Adipose-derived Stem Cells (ASCs) represent nowadays the ideal source of autologous stem cell by their potential in the remodeling and in the revascularization of an ischemic heart tissue. Despite the well-known and established angiogenic properties of the ASCs in vivo, many progress still need to be made to optimize the responsiveness and the tissue rebuilding capacity of these cells after their injection. The function of the P2Y nucleotide receptors in stem cell-based therapies has been poorly investigated. Here, we have investigated the implication of $P2Y_4$ receptor, a purinergic receptor, in the differentiation capacities and angiogenic properties of ASCs.

Methods: We have analyzed the capacity of P2Y-deficient ASCs, isolated from C57BL6 mice, to differentiate into functionally active endothelial cells in two different ways; the first one consisted in an CD31 immunostaining of wild type and $P2Y_4$ KO ASCs in an EGM2 culture medium after 7, 14 and 21 days and the second way was to evaluate their ability to form endothelial network in Matrigel. The effect of UTP, ligand of $P2Y_4$, was also tested on vascular network formation from differentiated ASCs. The expression of potent angiogenic factors was quantified by qPCR and by Elisa in the ASC culture supernatant after endothelial differentiation.

Results: We have observed smaller endothelial cluster formation in the $P2Y_4$-deficient ASCs culture, suggesting a certain delay in the differentiation process. ASCs plated in Matrigel-coated well and stimulated with UTP (100µM) allowed us to observe on one hand the implication of the $P2Y_4$ receptor in the vascular network establishment and on the other hand a positive effect of the UTP on this angiogenic process. These results were associated with a low-abundance of several angiogenic factors like HGF, IGF-1, Flk-1 and vWF in $P2Y_4$-deficient ASCs culture.

Conclusion: Our data demonstrate that loss of $P2Y_4$ receptor in ASCs affects their endothelial differentiation but also decrease expression of some potent angiogenic factors. Moreover, UTP stimulation seems to play a positive role in the vascular network formation. Further analysis will be necessary to understand by which mechanisms $P2Y_4$ receptor could promote endothelial differentiation of ASCs and their angiogenic properties, and therefore could represent a potent therapeutic target in cardiac stem cell therapy.

PO68 Renal function in an emerging pest insect, D. suzukii

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Introduction

Drosophila suzukii (*D. suzukii*) is a newly emergent pest insect which targets ripening fruit. First identified in Asia, this species has spread rapidly to and within North America and Europe. *D. suzukii* infestation causes massive losses to a wide range of soft skin fruit crops, including blueberries, grapes and strawberries.

Management strategies need to be implemented quickly to prevent further spread of *D. suzukii*. Research has so far focused upon tracking *D. suzukii* infestations, trap design and effectiveness, reproductive behaviour and potential environmental ranges.

However, very few investigations have targeted *D. suzukii* with the intent of elucidating its internal physiology to determine novel targets of intervention.

Comparative studies of *D. suzukii* Malpighian tubules with the well-established model organism, *D. melanogaster,* will help in understanding the physiology of *D. suzukii*. The main role of Malpighian tubules are water and ion homeostasis (Beyenbach *et al*, 2010), and are under control of several neuropeptides.

Methods

Ramsay assay for fluid secretion of *D. suzukii* Malpighian tubules were conducted according to Dow *et al,* 1994. Basal and stimulated fluid secretion rates were measured before and after addition of neuropeptide e.g. Drome-Kinin.

Results

Drome-Kinin stimulates fluid secretion in the Malpighian tubules of *D. suzukii*, increasing basal secretion rates by approximately three-fold. Drome-DH-31, Drome-DH-44, Drome-Capa-1 neuropeptides were also tested on *D. suzukii* Malpighian tubules and results showed that these peptides also increase basal secretion rates. This suggests that the tubules of *D. suzukii* are under similar neuropeptide control to *D. melanogaster,* and that physiological similarities may exist between *D. suzukii* and *D. melanogaster* Malpighian tubules.

PO69 Rhythm of androstenedione activity in D'man sheep

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The objective of our study is to demonstrate the role of the light on adrenal cortex function. For this, an investigation is conducted on 24 adult rams of D'man breed raised in the sheep-fold of the experimental farm of El Meniaa (30° 34 ' Northern Latitude, 02°52 ' Eastern Longitude , Altitude 379m), subjected to the conditions of natural temperature and light.

During equinoxes and solstices, blood samples are carried out each 4 hours during the clear phase/dark phase cycle; the animals are then sacrificed. The taken adrenal glands are preserved in aqueous Bouin for the histomorphometric study. The plasma androstenedione is measured by radioimmunoassay (RIA) using the androstenedione marked with I125.

We report variations in thickness of adrenal cortex (more developed slightly in clear phase (0.44%, p=0.03) as in dark phase) as well to the equinoxes as with the solstices. As for the reticularis zona, it is shown slightly thicker in obscure phase, except spring. The cortex and the ZR present also a morphometric variation, in relation to the duration of the day; characterized by: a maximum in summer/spring and a minimum in winter/autumn.

The profile of secretion of androstenedione is characterized by: considerable concentrations in clear phase though, the peaks of secretion are detected during the dark phase, except for the summer solstice. The seasonal rhythm of the sexocorticoïde activity is characterized by a maximum in long days (summer/spring) and a minimum in short days (winter/autumn), following perfectly the seasonal variations of the reticular morphometry. Lastly, we report the existence of significant correlations between the structure and the activity of the ZR and the ZF confirming a common endogenous determinism: the **ACTH**.

Within sight of these results, it seems that the daily and seasonal light has a powerful effect on synchronization of the adrenal cortex, appearing by changes of the histological aspect of the adrenal gland and its secretory activity, which would be an adaptation mechanism of the sheep D'man in the environmental conditions.

Key words: Androstenedione, Clear phase/dark phase cycle, Sheep D'man, Photoperiod, Desert, Reticularis zona.

PO70 Vegetable oils: effects on growth performance, plasma parameters and lipid metabolism-related genes expression in gilthead sea bream (Sparus aurata)

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Aquaculture is the fastest growing agro-alimentary production sector nowadays; nevertheless, the consequent increase of raw material demand for aquafeeds has led to the research of novel sustainable alternatives for fish meal and fish oil (FO). Vegetable meal and vegetable oil (VO) are interesting choices; however, the presence of antinutritional factors and their diverse fatty acid (FA) profiles are far from the essential FA requirements in marine fish. Hence, their use may cause imbalances being inadequate to obtain optimum product growth and quality. The aim of this experimental trial was to study the effect of partial dietary FO substitution by different VO or combinations of them on: growth performance, plasma parameters and adipose tissue metabolism-related genes expression in gilthead sea bream. Five hundred ninety-four fish with an average initial weight of 81,79 g and 13,21 cm length were kept in 27 tanks and fed for 18 weeks with one of ten isonitrogenous (46,45%) and isolipidic (22%) extruded diets, containing a 4,64% of FO to ensure similar values of essential FA and phospholipids. Then, the different VO used were: palm oil (PO), rich in saturated FA (SFA); rapeseed oil (RO), rich in monounsaturated FA (MUFA); soybean oil (SO), rich in n-6 unsaturated FA (UFA) and linseed oil (LO), rich in n-3 UFA. The results showed significant differences among diets on growth and specific growth rate (SGR). Body weight and length followed negative correlations regarding the UFA/SFA ratio of diets. Hepatosomatic index (HSI) presented a positive correlation regarding the UFA/SFA ratio, whereas visceral fat content and mesenteric fat index (MFI) showed the opposite, suggesting different fat deposition patterns. Differences were not found in color parameters, lightness (L*), redness (a*) and yellowness (b*) in muscle or skin; however, there was a positive correlation between muscle L* and total n-6 FA content. Plasma analysis of metabolites did not show significant differences with the exception of glucose, with the highest value observed in the fish fed with the diet based only on PO. Besides, there was a negative correlation between glycerol plasma levels and increasing n-6 FA in diet. Significant differences were found in relative expression of the lipogenic genes fatty acid synthase (FAS) and lipoprotein lipase (LPL), showing higher values in response to the RO diet and lower values with the PO diet. LPL also presented a negative correlation regarding n-6 FA in diet. In conclusion, diets based on SFA obtained more desirable growth features than diets with higher amount of UFA as n-6 FA or MUFA. In this sense, with just a minimum content on FO, diets based on SFA as that containing PO resulted up to now in the best choices for gilthead sea bream nutrition in this trial. Currently, we are analyzing oxidative stress and regulatory lipid metabolism and growth-related genes expression in different tissues to better understand the effects of these VO based diets. The complete study will help us to optimize the formulation of fish feeds in this important marine aquaculture species. Supported by MINECO AGL2014-57974-R and DECO 2014SGR-01371.

PO71 Corticotrope axis activity and adrenal immunolocalisation of the estrogen receptor ERa in the Bedouin goat during anestrous and breeding season

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The reproduction of Bedouin goat living in Algerian Sahara desert is characterised by an anestrous occurring from the end of winter (mid february) to the beginning of summer (june). The aim of this data is to study the ovarian influence, including ERα mediation, on corticotrope axis activity in this species.

Plasma samples were collected twice weekly during 13 consecutive months on six non pregnant females (24.6±1.2 kg) maintained in their natural environment in Beni Abbes experimental station (in south west Algeria, 30°7' N, 2°10' W).

Hormones were evaluated by RIA (cortisol) or IRMA (ACTH) methods; biochemical parameters (cholesterol and glucose) by commercial kits and electrolytes (Na^+ and K^+) by flame photometry. Localisation of estrogen receptors ERα was performed by immunohistochemistry into the adrenocortical gland; semi quantitative evaluation of immunostaining was appreciated in each cortical zona (number of immunostained cells/total cells per 100 cells field).

Results showed that for all parameters analyzed, values were more lower during the breeding season (from early august to end november), as well as for hormonal (-15.5% for plasma cortisol, -2.9% for plasma ACTH) than for biochemical parameters (-5.6% for cholesterolemia, -7.2% for glycemia, -2.3% for natremia) ($p>0.06$), except for kaliemia which decreased (- 6.8%; $p= 0.13$) during anestrous (from 15 march to 15 june); the Na^+/K^+ ratio, which could reflect plasma aldosterone and progesterone changes, decreased also during the estrous period (-4.6%, $p=0.316$). The ERα immunostaining was detected into all cortical zona with a light predominance in fasciculata during the breeding season (+6.7%; $p=0.23$ and +7.7%; $p=0.17$ *vs* respectively glomerulosa and reticularis) whereas during the anestrous, a light predominance occurred in the reticularis (+5.7%; $p=0.39$ and +12.8%; $p=0.09$ *vs* glomerulosa and fasciculata). In another hand, the immunostaining was higher during the breeding season compared to anestrous for all cortical zona and the differences were significant only for the fasciculata (+24.2%; $p=0.002$).

These results suggest that estrogens could participate to corticosteroidogenesis modulation, particularly in the fasciculata zona as it was reported in other mammals like rat and sheep.

In order to look further, it seems necessary to assess systematically aldosterone and progesterone plasma levels throughout the year, to exclude any seasonal influence on the hormonal activity and to investigate the progesterone receptor distribution in the adrenal gland. In addition, the interrelationships of adrenal gland and gonad deserve further studies in order to better specify their implication in the adaptation of the Bedouin goat to the Saharan environment.

PO72 The role of Yellow Protein in locust sexual behaviour.

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Sexual maturation in adult males of the locusts Schistocerca gregaria and Locusta migratoria is accompanied by yellowing of the cuticle. This distinct body colouration is not found in female locusts, nor is it induced when the locusts are reared in isolated (solitarious) conditions [1]. This yellowing results from the expression of a β-carotene binding protein (Yellow Protein, YP) which is activated by juvenile hormone signalling, but the reason why it happens is still unknown [2]. We used RNA interference (RNAi) to achieve a knockdown of YP in gregarious sexually mature males of L. migratoria, which subsequently lacked the yellow colouration. We then used an array of mating assays involving these YP-knockdown males alongside normal yellow males, to unravel the role of YP. Male yellowing was found to have an influence on sexual signalling between male locusts. We found that male locusts lacking the yellow colour had a 2.5 - 3.5 times greater chance of being mounted by other males, depending on the particular assay performed. The yellow colouration of male cuticle did not, however, seem to affect male mating success with females. Alongside the yellow colouration of males, we found that male stridulation upon mounting also had an important male-deterring effect in L. migratoria, and possibly acts in synergy with yellowing of the cuticle and the anti-aggregation pheromone phenylacetonitrile [3] to prevent homosexual mounting.

[1] Pener MP, Simpson SJ, 2009. Adv. Insect Physiol. 36, 1-272. [2] De Loof A, et al., 2010. J. Insect Physiol. 56, 919-925. [3] Seidelmann K, Ferenz H-J, 2002. J. Insect Physiol. 48, 991-996.

PO73 Generation of a zebrafish mutant line for the glucocorticoid receptor by CRISPR/Cas9 genome editing to analyse glucocorticoids activities

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Glucocorticoids (GCs) are steroid hormones that play important roles during embryonic development and in a vast array of physiological processes. Their actions are mediated by the glucocorticoid receptor (Gr): the active ligand/receptor complex binds to specific glucocorticoid responsive elements (GREs), positively or negatively regulating the transcription of target genes. Besides, other genes could be transcriptionally regulated indirectly by protein-protein interactions of Gr with other transcription factors.

To study GCs functions, a Gr mutant line was so far generated by applying the CRISPR/Cas9 technology on fertilized eggs from the GCs responsive zebrafish transgenic line (ia20), in which the EGFP is located downstream of nine tandem GRE repeats. In the mutant line, an insertion of five base pairs in the first coding exon produces a premature stop-codon, located upstream of the DNA binding domain. Heterozygous fish show a fluorescence decrease with respect to *gr*+/+ age-matched control siblings. In the F2 progeny, homozygous *gr-/-* larvae are morphologically similar to control siblings, but, at 5-dpf, they display an impaired visual background adaptation (VBA), a Gr/glucocorticoid dependent-neuroendocrine response. Homozygosis was confirmed by genotyping.

The response to dexamethasone (DEX) was analysed at 5 dpf on *gr*+/+, *gr-/-* and *grs357,* a zebrafish mutant line in which the Gr cannot directly bind to DNA but maintains the crosstalk with other transcription factors. *grs357* homozygous mutants, as the mouse *GRdim/dim* mutant line in which the GRE-dependent trans-activation is not functional, do not display overt morphological phenotypes and are adult-viable, in contrast with the full *GR-/-* knockout mouse. After 24 hours of 10 mM DEX treatment, a fluorescence increase was evident only in *gr*+/+ larvae, thus confirming the loss of GRE-related transcriptional activity on *gr-/-* and *grs357* mutant lines.

In both mutant lines, basal cortisol levels were higher than in *gr*+/+ fish and did not show any change after mechanical stress. Disruption of the negative feedback on the stress response was also demonstrated by higher levels of *pomca* transcripts in mutant larvae and the lack of a down regulation after DEX treatment, as seen in *Gr*+/+ larvae. Moreover, *glucocorticoid-induced leucine zipper protein* (*gilz*), as well as *fk506-binding protein-5* (*fkbp*5), that are classically glucocorticoid-regulated genes, were less expressed in mutant larvae when compared with *gr*+/+ fish and did not show any perturbation after DEX treatment.

Since *Gr* mutant zebrafish survives at least until 2 months, likely due to maternally supplied *gr* transcripts and proteins required for a correct development, this line will provide a useful model to study the Gr functions in adult life physiology and for *in vivo* drug screening of molecules and endocrine disruptors with receptor- and tissue-specific activities.

PO74 Is coping with an intermittent Ca2+ intoxication the essence of arthropod molting and metamorphosis?

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The answer to: "Why do arthropods deposit a rigid calcareous (in crustaceans) or proteinaceous/chitinous cuticle?" is not: "Otherwise they could not protect their weak body". Indeed, this view implicates that there is a goal in evolution, which is not the case. It is more likely that arthropods use, in addition to other mechanisms of the Ca^{2+}-homeostasis system, the protein secretory activity of the Rough Endoplasmic Reticulum (RER) of epidermal cells as an ultimate strategy to extrude (toxic) excess Ca^{2+} from their body in the form of Ca^{2+}-binding/transporting proteins. The protective properties of the cuticle are a fortuitous side aspect that increases survival and fitness. This alternative interpretation necessitates that the physiology and endocrinology of molting and metamorphosis are re-interpreted, in particular in the context of their role in Ca^{2+}-homeostasis. Ecdysteroids not only promote the synthesis and secretion of cuticular proteins but of some other types of proteins as well, e.g. along with saliva, silk etc. It can be inferred that ecdysteroids may make the plasma membrane of some tissues more permeable to Ca^{2+} (=opening up of Ca^{2+}-channels). As a rescuing reaction, the RER becomes more abundant, thereby increasing the capacity to extrude more Ca^{2+} along with secretory proteins/vesicles. A high titer of JH likely has the opposite effect; it keeps the cytoplasmic Ca^{2+}-concentration low. In insects, metamorphosis is initiated when the titer of Juvenile Hormone drops to very low values. This triggers drastic, tissue-specific changes in Ca^{2+}-homeostasis. Complete absence of JH results in cell death in tissues that actively secrete proteins such as salivary- and silk glands, some types of fat body cells and the midgut. In particular in crustaceans, reuse of secreted Ca2+ can be important issue that is also subject to complex regulation.

PO75 Cross-talk of juvenile hormone, ecdysteroids and insulin during reproduction of the desert locust, Schistocerca gregaria.

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The desert locust, *S. gregaria*, is a phytophagous pest insect that can ruin harvests in a vast region, spanning Northern Africa, the Middle East and the Indian subcontinent. It has a potentially devastating ability to migrate over large distances and to undergo 'phase transition' to a gregarious population. These gregarious locusts can form marching (nymphs) or flying (adults) insect armies eating everything on their path.

More selective control strategies are needed to combat this pest in its already fragile habitat which includes some of the world's poorest countries. In this aspect, the classic insect hormones, 20-hydroxyecdysone (20E) and juvenile hormone (JH) are excellent targets.

JH, a sesquiterpenoid produced by the corpora allata (CA), is critical for metamorphosis as well as reproduction in insects. The interaction between both JH and the molting hormone 20E results in a larval-larval or larval-adult molt. The JH receptor, methoprene-tolerant (Met), has been shown to mediate JH actions during both metamorphosis and vitellogenesis, but the functional interactions between this JH receptor and the different hormonal signaling pathways for ecdysteroids and insulin-like peptides (ILPs) remains largely unknown. An RNA interference (RNAi)-based approach was employed to examine these possible functional interactions in more detail in the desert locust, *S. gregaria*, a member of the hemimetabolous insect order of the Orthoptera. By using the locust's effective and systemic RNAi response, our study has targeted several downstream factors in 20E and JH signaling during locust reproduction. Our results show there is an intricate interplay between nutritional signals and hormones, such as neuroparsins (NP) and insulin-like peptides (ILP), JH and 20E.

PO76 Juvenile hormone as a regulator of digestive enzyme synthesis in the African migratory locust, Locusta migratoria

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The insect digestive tract is a highly flexible organ, and is capable of adjusting quickly to changing environmental cues. This flexibility is often demonstrated by a very large repertoire of digestive enzymes that can be expressed in the digestive tract. However, the exact regulation of the expression of these enzymes during the insects' life cycle still remains poorly understood. Previously, using the African migratory locust as a model organism, we performed a microarray experiment four hours after protease inhibitor ingestion. These inhibitors specifically target the proteolytic enzymes present in the gut, to which the insect responds with a quick compensatory upregulation of digestive proteases. Further analyses of upregulated transcripts brought to attention the putative involvement of three closely related hexamerin-like proteins in this protease inhibitor induced response. Interestingly, since insect hexamerins have been shown to bind juvenile hormone (JH), we investigated the function of JH in the general proteolytic digestion process in *Locusta migratoria*. We approached this hypothesis in two different ways. First, by interfering with JH signalling, through the knockdown of the JH receptor*,* methoprene tolerant (*Met*). Second, by stimulating JH signalling through topical application of methoprene, which acts as a JH analogue. Our results show that JH has a stimulatory effect on the expression of three highly homologous chymotrypsin genes, while knocking down the receptor for JH has opposite effects. By demonstrating the involvement of JH in the expression of these proteolytic digestive enzymes in this primitive insect species, we hypothesize a conserved role for JH in the regulation of digestion throughout the insect kingdom, possibly acting as a link between insect development and the digestive cycle.

PO77 Defence reaction against nematobacterial infection in Drosophila melanogaster: a role for the adipokinetic hormone and adenosine

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Entomopathogenic nematodes are multicellular insect parasites which are symbiotically associated with particular species of bacteria forming together highly pathogenic complexes. These complexes represent a severe stress for the infected insect that must activate its anti-stress defence system. It is supposed that adipokinetic hormone (AKH), an important stress hormone responsible for keeping homeostasis in the insect body, and adenosine, a purine nucleotide that serves as signalling factor in anti-stress reactions at both cellular and organismal levels, play a role in defence reactions against the infection. To verify this, the *Drosophila melanogaster* mutants producing (1) defective non-functional AKH (AKH-def) and (2) impaired adenosine receptor (AdoR-def), and two nematode species *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* were used in our study. The results revealed that both AKH-def and AdoR-def flies infected by the nematodes showed significantly lower mortality (1.9 and 1.7 times, respectively) than the control with AKH- and AdoR-normal production. Further, application of external AKH by dipping of experimental larvae into the Drome-AKH solution (3 pmol Drome-AKH/µl 20% MeOH) significantly increased the mortality (up to 1.8 fold). It seems that the absence of AKH reduced the production of nutrients into the haemolymph, which inhibited development of the infection. And *vice versa* the application of AKH restored the production of energy rich metabolites and supported the infection. Indeed, the level of trehalose was significantly lower (0.6 fold) in untreated AKH-def, and higher in untreated AdoR-def (1.8 fold) *Drosophila* mutants than that in controls. In nematode treated mutants the trehalose level was significantly lower in both AKH-def (0.4 times) and AdoR-def (0.7 times). These results indicate a clear stimulatory role of AKH, and minor modulatory role of adenosine in the regulation of the trehalose level in *Drosophila* haemolymph. Similar changes, however not so considerable, were recorded for levels of glucose and also lipids; negligible changes were recorded for proteins. It is indisputable that the nematode infection is stressing for the *Drosophila* organism and that is accompanied by higher production of Drome-AKH by the corresponding cells in the ring gland (up to 1.6 fold), and by the increase of all studied nutrients (with the exception of proteins) in haemolymph (max. trehalose 2.6 times). Nevertheless, the mechanism of action of the nematode infection is not quite clear yet, because the results of the total metabolism determination monitored by carbon dioxide production suggest that more players are involved in the phenomenon.

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PO78 Gene expression dynamics in major endocrine regulatory pathways along the transition from solitary to social life in the bumblebee, Bombus terrestris

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The evolution of eusocial insects from solitary ancestors is an appealing evolutionary question not only in terms of occurrence and maintenance of various social organizations but also at the proximate level of physiology and genetics. In other words, to understand the social evolution it is critical to learn how new physiological and regulatory functions arose and how the ancestral mechanisms and underlying genetics have been co-opted to fulfil new functions in social environments. Detailed investigations into the regulatory gene networks and physiological pathways are scarce.

Bumblebees represent well suited models to provide answers to these questions because their queens undergo both, the solitary and the social stage, separated by winter diapause.

Using qRT-PCR, we characterised in detail gene expression levels of major endocrine regulatory pathways across tissues, sexes, and life-stages in the buff-tailed bumblebee, *Bombus terrestris*. More specifically, we investigated: (1) Forkhead box protein O (FOXO) and insulin/insulin-like signalling (IIS), (2) Juvenile hormone (JH) pathways, and (3) Adipokinetic hormone (AKH) signalling. We show that FOXO and insulin signalling pathways are tightly linked with diapause and reproduction. Whereas the expression *of Insulin receptor 1* (*InR-1*) is upregulated across all tissues in diapausing queens, *InR-2* and *FOXO* are highly abundant in both, virgin and diapausing queens. *InR-2* and *FOXO* are also constitutively expressed in gonads and fat body, respectively, of males, workers, and queens of all life-stages. *Insulin growth factor 1* (*IGF*-1) is queen-specific and restricted to brain, fat body, and ovaries. The expression of *methyl farnesoate epoxidase* (*MFE*), which catalyses the last step in JH synthesis, is highest in reproductive queens, especially in ovaries and remarkably also in workers' ovaries. *Vitellogenin* is ubiquitously expressed, though relatively less in males and diapausing queens. The highest values are as expected, in the fat bodies, but surprisingly high expression levels are also observed in hypopharyngeal gland and flight muscles. Unexpectedly, *transcription factor Krüppel homolog 1* (*Kr-h1*) was abundant in mated, diapausing queens, as well as in the ventriculus tissues of workers and males. As anticipated, *AKH* is strongly expressed in brains only, whereas *AKH receptor* (*AKHR*) is widespread in the investigated tissues and castes. Remarkably high *AKHR* transcript levels were recorded in the fat bodies of specimen expected to perform active flying, i.e. virgin queens, workers, and males.

In sum, our results suggest that diapause is regulated by FOXO, IIS and Kr-h1, and that both JH and IIS have gonadotropic functions. In addition, the endocrine regulation of the hypopharyngeal gland reveals a striking resemblance with the fat body across all investigated genes, sexes, and life stages. *Igf-1* is only found in queens and might be involved in adaptations to sociality.

PO79 Bioassay for determining the biological activity of crayfish gonadotropin-releasing hormone (pcGnRH)

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Gonadotropin-releasing hormone (GnRH) is a neuropeptide known to regulate and maintain reproductive functions and conserved in both vertebrate and invertebrate species. Recently, new GnRH molecule has been purified from the ovary of the American crayfish *Procambarus clarkii* and its structure was determined. The primary structure of the crayfish GnRH (pcGnRH) is pQSYHFSLGWKP-NH_2, which is different from the known forms of the vertebrate and invertebrate GnRH family. This was the first GnRH molecule from crustacean species. In this study, we examined several bioassays for determining the biological activity of pcGnRH. The pcGnRH was prepared by the chemical synthesis and applied to the following *in vivo* bioassays. First, the chemically synthesized pcGnRH and saline solution were injected into adult female crayfishes (average body weight 26.1 g). Ovaries were dissected out from the injected animals at 14 days after injection and their gonadosomatic indexes (GSI) were calculated. GSI of the synthetic pcGnRH injected group was significantly higher than that of the saline solution injected group. However, some crayfishes in the saline solution injected group had matured ovary, because only immature females could not be selected from adult crayfishes captured in the wild. Therefore, the synthetic pcGnRH and saline solution were injected into juvenile female crayfishes (average body weight 3.5 g). Although all juvenile crayfishes had immature ovary, a significant elevation with GSI in the synthetic pcGnRH injected group was not observed. This result indicates that juvenile crayfish is still not sensitive to pcGnRH. Finally, the synthetic pcGnRH and saline solution were injected into immature adult females of the freshwater shrimp *Palaemon paucidens,* which were sorted by the observation of the ovaries in live shrimps. At 12 days after injection, the hepatopancreases were dissected out and subsequently subjected to RT-PCR for the detection of vitellogenin gene expression. Clear amplified PCR bands were detected in 9 out of 10 shrimps to which the synthetic pcGnRH was injected, whereas no bands were observed in the saline injected group. Therefore, this *in vivo* bioassay using *P. paucidens* was thought to be better than using *P. clarkii* for evaluation of stimulating effect of pcGnRH on vitellogenesis. Now, we are developing the pcGnRH agonists (pcGnRH analogs) with higher activity than the native pcGnRH, which are designed based on mammalian LH-RH analogs. In the near future, biological activities of those pcGnRH analogs will be examined by *in vivo* bioassay using *P. paucidens*.

PO80 RNAi-based interactions: a latent viral infection in a lepidopteran cell line

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RNA interference (RNAi), the main antiviral immune response in insects, is a post-transcriptional gene silencing mechanism triggered by dsRNA. In brief, long dsRNA molecules are processed into short RNA duplexes by a Dicer enzyme. These duplexes are then unwound and the 'guide strand' is loaded into the RNA-induced silencing complex (RISC). Subsequently, an Argonaute protein contained within the RISC cleaves or blocks the messenger RNA with sequence homology to the guide strand, which finally results in specific gene silencing. Interestingly, several cytoplasmic RNA viruses have been reported to be persistently present in insects and insect cell lines. However, although RNAi has been demonstrated to play a role in the immunity against acute infections, it is not clear whether it plays a role in the host-pathogen equilibrium that characterizes persistent infections. In this context, making use of the *Trichoplusia ni* High Five[TRADEMARK] cell line, we set out to study whether the overexpression of key components of the RNAi machinery would result in decreased levels of a persistent viral infection. Importantly, this cell line has been demonstrated to be persistently infected with the Macula-like Latent Virus (MLV). However, upon overexpression of *Dcr-2, Ago-2 and R2D2* (siRNA pathway); and of *Ago-3 and Piwi/Aub* (piRNA pathway), it was not possible to observe a decrease in the MLV transcript levels. In addition, we investigated if a Cricket Paralysis Virus (CrPV) infection would affect the MLV levels. Noteworthy, the CrPV is able to induce an acute infection in High Five cells and is known to codify a suppressor of RNAi, namely the A1 protein. Interestingly, when infected with this virus, an increase of the MLV levels in the cells could be observed. To conclude, these results contribute to a better understanding of the RNAi-based interactions between insect hosts and persistent viral infections.

PO81 Changes in the level of insulin-like peptides and the profile of free sugars in hemolymph during ageing of Tenebrio molitor beetle

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Insulin-like peptides (ILPs) in insects are structurally and functionally analogous to vertebrate insulin. The first ILP, named bombyxin, was detected in *Bombyx mori*; ILPs were then found in many other insect species, including *Tenebrio molitor*. Insulin-producing cells were detected in the brain, *corpora allata* and subesophageal ganglion of *T. molitor* beetle. Moreover, it was demonstrated that insulin-like hormone occurs in the midgut of *T. molitor* larvae.

In insects, ILPs were shown to act through conserved insulin signaling pathway and regulate development, longevity, metabolism and female reproduction. Ablation of insulin-producing cells in *Drosophila melanogaster* resulted in an increased carbohydrate level in the hemolymph, increased lipid storage in the fat body, retarded growth and reduced fertility. ILPs are believed to be involved in the regulation of circulating trehalose levels, probably by controlling the activity of trehalase *via* different molecular mechanisms depending on the insect species.

The aim of this study was to estimate the level of insulin-like peptides (ILPs) in the hemolymph during ageing of *T. molitor* beetles, as well as to evaluate the profile of free sugars in the hemolymph.

The level of ILPs in the hemolymph of ageing beetles was determined using the ELISA method, while the variations in free sugar profile were measured by means of high-performance liquid chromatography (HPLC).

The highest level of ILPs was detected in one-week-old beetles. Generally, ILPs were found to decrease progressively with age. The study revealed also that the concentration of free sugars in the hemolymph did not change significantly with age in *T. molitor* beetles. Simultaneously, the changes in the concentration of particular sugars were detected: the level of trehalose was the lowest in one-week-old beetles and then increased with their age. The concentration of polyols, including glycerol, did not vary significantly during ageing. The glucose and saccharose concentrations decreased as the beetles grew older.

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PO82 Insect resistance to dietary protease inhibitors

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Plant protease inhibitors (PIs) can act as defensive compounds and are considered as candidates for future genetic modification of crop plants. They target digestive proteolytic enzymes in the gut of insects feeding on the plant. However, insect resistance to these antinutritional PIs is frequently observed. Several pest species evolved a remarkable flexibility in their digestive system, allowing them to compensate for inhibitor ingestion by regulating expression of their digestive enzymes. However, the manner in which these insects sense and signal the presence of ingested inhibitors remains unclear to date. The general aim of this research was to identify PI induced compensatory responses in the gut of the African migratory locust, *Locusta migratoria*. This infamous pest insect is capable of forming huge swarms that can have devastating effects on crop lands. Using two color microarray analyses we studied transcriptional changes 4 hours after oral uptake of plant protease inhibitors by locust nymphs. In total, 264 transcripts were identified to be differentially expressed as a result of the treatment. The results suggest that, during adaptation to ingested PI, fewer resources will be invested in defense, stress responses and the maintenance of structural integrity. Subsequent knockdown of a group of strongly related upregulated transcripts by means of RNA interference, when combined with PI ingestion, resulted in a stunted growth of the locust nymphs, possibly due to an inability to regulate their normal compensatory response.

PO83 Effect of Solanum nigrum glykoalkaloids on the gut, oviduct and heart activity of Tenebrio molitor.

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For the natural defense many plant species produce secondary metabolites affecting various physiological processes of herbivore insects. A lot of these compounds have been studied extensively as potential alternatives for pesticides used in agriculture for crop and stored food protection. It has been proved that alkaloids present in *Solanaceae* family decrease the contractions' frequency of tissues controlled by the endocrine system. This leads to sublethal changes such as reduced fertility, decreased body mass and the inhibition of development. These effects highly depend on species, therefore there is a necessity of investigating the effects on a wide range of species. *Tenebrio molitor (Tenebrionidae)* is a common beetle pest of stored products and also one of the model organisms in insect physiology. Moreover, there are some suggestions, that alkaloids may significantly affect endocrine regulation of insect development. Hence, they may play an important role in plant protection. Therefore, we decided to test the effects of plant extract from *Solanum nigrum (Solanaceae)* containing two major glycoalkaloids: solamargine and solasonine and also pure glycoalkaloids on the gut, the oviduct *in vitro* and semi-isolated heart. The experiments have shown differences between mode of action of extract and pure substances on examined tissues. Extract in the highest concentration inhibited heart rate. The strongest effect was observed directly after application. Solasonine acted as a long term inhibitor of heart contractions. 1% extract increased the number of oviduct contractions, but they were inhibited by pure solasonine. There were no significant changes in contraction of midgut after the examined compounds application.

Chowanski S., Adamski Z., Marciniak P., Rosinski G., Büyükgüzel E., Büyükgüzel K., Falabella P., Scrano L., Ventrella E., Lelario F., Bufo S.A. 2016. A Review of Bioinsecticidal Activity of Solanaceae Alkaloids. Toxins 8 (3) 60.

PO84 The role of short neuropeptides F in the regulation of feeding in Tenebrio molitor beetle

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Short neuropeptides F (sNPF) is the family of neuropeptides which reveals the consensus *C*-terminal sequence xPxLRLRFa. In most insects species, one gene encodes a sNPF precursor peptide, which provide several isoforms. Moreover, in many species the *N*-terminally truncated forms of sNPF have been identified. In the red flour beetle (*Tribolium castaneum*), the occurrence of Trica-sNPF (SGRSPSLRLRFa), as well as its truncated form Trica-sNPF$_{(4-11)}$ (SPSLRLRFa) were recently identified by means of mass spectrometry. In many species of insects, sNPF and their receptors are widely distributed in numerous neurons of the central nervous system and some gut endocrine cells, where their functions are very often pleiotropic. Although the members of sNPF family are involved in a wide range of processes, including regulation of growth and development, osmotic and metabolic stress response, locomotor activity, circadian rhythms, hormone release regulation, learning, olfaction and reproduction, their main function appears to lie in the regulation of nutrition behaviour and metabolism. For this reason sNPF are regarded to be functional homologues of vertebrates neuropeptides Y (NPY). The feeding-effect caused by sNPF seems to be species-specific.

The aim of this study was to evaluate the role of sNPF in the regulation of feeding in the mealworm *Tenebrio molitor*. We tested chemically synthesized Trica-sNPF (SGRSPSLRLRFa) and its truncated form Trica-sNPF$_{(4-11)}$ (SPSLRLRFa) in a feeding bioassay *in vivo*, as well as isolated hindgut bioassay *in vitro*. Moreover, we measured the changes in the profile of free sugars in hemolymph of larvae in response to the injection of Trica-sNPF as well as Trica-sNPF$_{(4-11)}$ after 2 and 24 hours, by means of high-performance liquid chromatography (HPLC).

The studies showed a statistically significant increase in the weight of larvae injected with Trica-sNPF$_{(4-11)}$ 10^{-5} M, after 2, 7 and 14 days compared with control group injected with physiological saline. The injection Trica-sNPF$_{(4-11)}$ or Trica-sNPF at a lower concentration (10^{-7} M) did not cause significant changes in weight of larvae. The hindgut *in vitro* bioassay showed that Trica-sNPF$_{(4-11)}$ in the concentration range 10^{-12}- 10^{-5} M increased the frequency contractions of the organ. The application of Trica-sNPF in the same concentrations range did not cause significant changes. The profile of free sugars in hemolymph measured 2 and 24 hours after Trica-sNPF and Trica-sNPF$_{(4-11)}$ injections showed different changes in concentration of 10^{-7} and 10^{-5} M.

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PO85 The role of Allatotropin and Allatostatin C in the female reproductive system of Rhodnius prolixus: Myoregulatory peptides and antagonistic effects

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Allatotropin (AT) and allatostatin-C (AST-C) are insect neuropeptides characterized on the basis of their function as modulators of Juvenile hormone secretion in the Corpora Allata. Our laboratory has demonstrated that AT acts as a myostimulator at the level of the midgut and aorta. It is also known in *Drosophila* that AST-C presents myoinhibitory activities in the dorsal vessel. The female reproductive system (FRS) of *Rhodnius prolixus* is composed of a pair of ovaries that communicate with a chamber (uterus) through the oviducts, to finish in the bursa. The eggs are pushed down from the ovaries by muscle contractions of the wall to be fertilized. Furthermore, a pair of spermathecae that open between the uterus and the bursa are present. The aim of this study is to analyse the myostimulatory action of AT and its possible antagonist peptide AST-C on the FRS of *R. prolixus* (Hemiptera: Reduviidae) evaluating also the differential responses of insects undergoing previtellogenic and vitellogenic states in in the following conditions: starved, mated, virgins and mature after one meal. Recently emerged adult females were starved for 20 days, (30°C+/-2°C, 30%RH). Physiological experiments were performed under a dissection microscope. In the abdomen of each insect only the FRS immersed in saline was kept and the contractions of the organs were counted and recorded by a digital video camera. We performed dose-response essays testing different concentration of AT and AST-C (from 10-14M to 10-6M), in experimental groups of vitellogenics and previtellogénics females, both virgin and mated. Moreover, the antagonistic effect of AST-C (10-6M) over the stimulatory action of AT (10-9M) was evaluated. Images were recorded for 3 minutes (150 frames) at 5, 15 and 30 minutes after each dose. Finally the frames were integrated in a movie by the use of the software *Moviemaker*. The information obtained were analysed by multifactorial ANOVA. The results show that the FRS responds to AT in a dose-dependent manner, incrementing more than 200% the frequency of contractions of the ovaries, oviducts, uterus and spermathecae, in mated females, suggesting that AT acts as a myoregulatory peptide during maturation of the reproductive system and oviposition. AST-C dose-response essay showed that in the group of mated insects this peptide acts by decreasing almost 70% the frequency of contraction in the ovaries at a dose of 10-8M. Information obtained from tests performed in two experimental groups of mated females who were fed and they were in a previtellogenic and vitellogenic state showed that AST-C generate a decrease of the frequency induced by AT in ovaries, uterus and spermathecae (mature females only) suggesting the existence of an antagonistic effect. Finally, our results suggest that both peptides are part of the endocrine system that regulates muscle activity of the FRS during ovarian development and oviposition.

PO86 Analysis of a Bacterial dsRNA Delivery System in the Desert Locust, Schistocerca gregaria

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The presence of double stranded RNA (dsRNA) molecules in eukaryote cells can induce a sequence specific degradation of mRNA through RNA interference (RNAi). Due to its high degree of specificity, RNAi has evolved into a frequently utilized tool in reverse genetics. Furthermore, this technology shows great promise for future application possibilities in insect pest management. However, several major issues remain to be resolved in order to use RNAi as an efficient pest insect control strategy. One of the most pertinent problems is the current inability of several economically important insect species, such as the desert locust *Schistocerca gregaria,* to induce a systemic RNAi response after oral delivery of dsRNA. It has been speculated that one of the causes for this inability in insects could be a reduced cellular uptake of dsRNA from extracellular environments, such as the gut lumen. In addition, the presence of dsRNA-degrading enzymes in the digestive system of the desert locust indicates that enzymatic degradation of orally administered dsRNA could hinder the induction of an RNAi response. To circumvent these obstacles and to improve the efficiency of dsRNA-induced toxicity in this insect species, we propose the use of a delivery system to protect the dsRNA and to facilitate its cellular uptake. Previous studies have shown that in *Spodoptera exigua* and *Sesamia nonagrioides*, two Lepidoptera species, a successful *knockdown* could be induced through the use of bacteria as a dsRNA-delivery system. Therefore, in this research the use of *E. coli* bacteria as a potential delivery system for dsRNA in the Orthopteran species, *Schistocerca gregaria*, will be assessed. To this end, the bacterial system will be evaluated for its ability to protect the dsRNA against degradation in the Orthopteran digestive system. Additionally, the stability of the system at different PH levels will be tested. Finally, the ability of the delivery system to facilitate cellular uptake of dsRNA and to subsequently induce a successful *knockdown* will be rigorously tested *in vivo* on *Schistocerca gregaria*.

PO87 Influence of yamamarin peptide on insects immunology - cellular and humoral aspect

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Insects settle almost every environment, so they interact with a broad range of pathogens. Because of the high risk of infection, insects evolved variety of mechanisms to keep pathogens from entering their body. The most important physical barrier is the cuticle. In case of pathogens penetrating through cuticle (e.g. through natural wounds) or entering haemocel cross the intestine, insects initiate immunological mechanisms to immobilize or remove pathogens.. It seems that the most important moment in immunological response cascade is the recognition of pathogens, which can be achieved through activation of signal transduction pathways, which leads to multiply appropriate processes divided into phagocytosis, melanization, nodulation, lysis, viruses destruction by RNAi mediation etc. Thanks to new analytic techniques many of new pepides were isolated from insects, but their role is still unknown.

One of those peptides is yamamarin (DILRGa), peptide isolated from the silkmoth *Antheraea yamamai* larvae, also known as Any-GS or growth-suppressing pentapeptide. So far, only two yamamarin functions are known: suppression of rat hepatoma cells growth and diapause maintaining in *A. yamamai* moth.

Minor state of knowledge about function of yamamarin in insects, poses the question: What is the main role of the peptide in insects?

Initial data obtained from research conducted on insects suggest that the peptide possess cardioinhibitory activity in beetles. It was also demonstrated that yamamarin might inhibit cell cycle and cellular respiration in diapausing mouth *Bombyx mori*.

Owing to that fact, the aim of our study was to recognize how the peptide influences functioning of basic mechanisms of the immune response of mealworm *Tenebrio molitor* (L.) and to verify if the peptide possess hypothetical use as a bioinsecticide.

In our studies, we estimated functioning of two kinds of responses: humoral and cellular. According to humoral response we assessed changes in activity of phenoloxidase. Total haemocyte count, phagocytic ability of haemocytes and nodulation was observed towards to cellular aspects. First research results showed, that yamamarin decreased phenoloxidase activity 24 hours after injection but had no influence when assessed an hour after injection. Beyond that fact, we observed that peptide significantly decreased number of nodules appearing after *Staphylococcus aureus Wood 46 strain* injection. Changes in haemocytes morphology and ability to conduct phagocytosis were also observed.

PO88 Understanding the biology of emerging pest insects

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Beetles are the most species-rich group among insects, yet they are under-represented in insect molecular studies. This has hindered our efforts to manage pest beetles, especially beetles which have recently been identified to cause damage to the environment.

One such beetle is the large pine weevil, *Hylobius abietis*. It is typically the most damaging insect to European conifer forests, when large populations develop in root-stumps following clearfelling. Emerging adults feed on the bark of restocked seedlings, causing plant death. Control methods currently rely on chemical intervention, with associated environmental and operator health concerns, and high economic costs.

Sequencing the *H. abietis* transcriptome will provide an underlying framework for molecular and physiological studies in this species.

Furthermore, Malpighian tubules serve a vital role in maintaining water and ion balance and are under control of several neuropeptides. Comparative physiology with other insects would help in analysing the importance of Malpighian tubules to *H. abietis*.

Methods

RNA was extracted from whole adult insects to provide an overview of RNA abundance. RNA was also extracted from the Central Nervous System (CNS) and alimentary canal, and sequencing was performed using NextSeq^{TM}500.

Ramsay assays for fluid secretion of *H. abietis* Malpighian tubules were conducted according to Dow *et al,* 1994. Basal and stimulated fluid secretion rates in isolated, intact tubules were measured before and after addition of specific selected neuropeptides.

Results

The RNA sequencing data provides a framework to identify neuropeptide sequences as well as RNA enrichment in specific tissues of *H. abietis*.

For Malpighian tubule physiological studies, our data suggests that diuretic peptides identified from other insects modulates *H. abietis* tubule secretion rates and so could be an endogenous regulator of tubule function in this species.

Dow J.A.T, Maddrell S.H.P., Gortz A., Skaer N.J.V., Brogan S., Kaiser K., 1994, The Malpighian tubules of Drosophila melanogaster: A novel phenotype for studies of fluid secretion and its control, J Exp Biol, 197, 421-428.

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