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Book of Abstracts

INVITED SPEAKERS- KEYNOTE AND PLENARIES

KEYNOTE LECTURE

LIGHT AND SLEEP SIGNALLING TO THE MOLECULAR CLOCKWORK

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By studying how circadian rhythms and sleep are regulated by light we demonstrated that there exists a "3rd class" of photoreceptor within the eye based upon a small number of photosensitive retinal ganglion cells (pRGCs) that utilise the blue light sensitive photopigment melanopsin (OPN4). Whilst there has been remarkable progress in understanding the mechanisms that generate circadian rhythms, the molecular pathways whereby the pRGCs entrain circadian biology and sleep has remained poorly understood. The suprachiasmatic nuclei (SCN) are the site of the primary circadian pacemakers within the mammalian brain. Until recently, the model for entrainment involved a simple linear pathway whereby glutamate release from the pRGCs resulted in Ca^{2+} influx and raised intracellular cAMP in SCN neurones, which in-turn resulted in CREB phosphorylation and the transcription of two key clock genes, *Per1* and *Per2*. This signal then advanced or delayed the molecular clockwork. However, an important feature of entrainment is that circadian responses to light are limited – as typified by jet-lag. Full recovery from jet-lag requires a day for every time-zone crossed. We addressed this issue and have identified and characterized a key role for Salt Inducible Kinase 1 (SIK1) and the CREB-regulated transcription co-activator 1 (CRTC1) in clock re-setting. However, our more recent and unpublished findings have shown that light entrainment also involves the parallel activation of a Ca^{2+}-ERK1/2-AP-1. Thus both CRE and AP-1 regulatory elements drive light-induced clock gene expression. In addition, whilst light activation of the Ca^{2+}-ERK1/2-AP-1 signalling pathway increases *Per1* and *Per2* expression, sleep/wake behaviour alters the effects of light on the clock. Our proposed mechanism suggests that adenosine acts as a signalling molecule that encodes wake duration. Adenosine acts via inhibitory A1 receptors on the SCN to inhibit the Ca^{2+}-ERK1/2-AP-1 signalling, which in turn, reduces the expression of *Per1* and *Per2.* Thus sleep/wake history, encoded by adenosine, limits the phase shifting effects of light upon the circadian system, altering sleep/wake timing. These new pathway will be presented and placed into an ecological and potentially therapeutic context.

PLENARY 1

What Wild Animals Can Tell Us About the Physiology of Stress

Professor Michael Romero

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Despite stress being the subject of intense study for many decades, there are still numerous unresolved aspects of stress physiology that prevent us from an integrated understanding. Until recently, biomedicine had settled on a standard model of stress that was inspired by human and pre-clinical studies using laboratory animals. Twenty-plus years of studies on wild animals, however, have exposed serious weaknesses in that biomedical model. For example, stress responses vary during different life-history stages and many of the presumed benefits of stress hormone release do not appear to occur in free-living animals. These challenges to the traditional biomedical model of stress led to the proposal of several alternative stress models, one of which is the reactive scope model. Reactive scope presumes that stress regulators (e.g. the classic stress hormones, glucocorticoids and catecholamines) exist in four ranges. The predictive homeostasis describes the range of hormone titers needed to cope with predictable environmental changes. Reactive homeostasis describes the higher range need to cope with unpredictable environmental changes. Combining the predictive and reactive homeostasis ranges forms the normal reactive scope of the animal. Hormones in the reactive homeostasis range, however, start creating wear-and-tear that results in a narrowing of reactive scope. If hormone titers exceed the reactive scope, the hormones enter the homeostatic overload range and will start initiating pathology. Similarly, if hormone levels drop below the predictive range, homeostatic failure will ensue. The reactive scope model is proving useful for interpreting the stress physiology of wild animals, and appears to be applicable in many contexts.

PLENARY 2

THE COMPLEX IMPACT OF NATURAL LIGHT ON PHYSIOLOGY AND BEHAVIOR: MAN IS BUT A WORM?

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Natural light is highly complex in its spectrum and intensity, changing over time and depending on location. Organisms have evolved a multitude of light receptors that sense, interpret and subsequently help to adjust the organisms’ physiology and behavior to the information conveyed by light.
As humans, we are very “vision-centric”, however, work from multiple labs over the past decades has provided continously increasing evidence that animals and humans possess a varying number of photoreceptors that are likely involved in non-viusal processes. A particularly prominent group are Opsins, many of which are expressed in tissues outside the eyes, but nevertheless constitute functional light receptors, at least in tissue culture.
We use the marine bristle worm *Platynereis dumerilii* , as well as medaka and zebrafish, to study the function of these non-visual Opsins. We determined the action and absorbance spectra for several *Platynereis* light receptors and compare these data with the spectra and intensities reaching the animals at their natural habitat at different times. Together with gene knock-out functional analyses, this allowed us to identify a light receptor that conveys summer versus winter time information. The light information sensed by this Opsins significantly impacts on the expression of neurotransmitter enzymes, such as *tyrosine hydroxylase*, and preprohormones and – as a likely consequence- alters behavior.

PLENARY 3

FINDING MISSING LINKS IN THE EVOLUTION OF NEUROPEPTIDE SIGNALING

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Until recently, much of our knowledge of the evolution and comparative physiology of neuropeptide signalling has been based on studies of vertebrates and protostomian invertebrates, which include arthropods (e.g. *Drosophila melanogaster*), nematodes (e.g. *Caenorhabditis elegans*) and molluscs (e.g. *Aplysia californica*). However, advances in transcriptome/genome sequencing have expanded the range of phyla in which a comprehensive analysis of neuropeptide signalling systems is now feasible. This is important because it is providing a basis for reconstructing the evolutionary history of neuropeptide signalling and revealing relationships between neuropeptide systems in different phyla. In this lecture I will illustrate this with reference to findings from phyla that occupy an "intermediate" phylogenetic position with respect to vertebrates and protostomes – echinoderms and hemichordates (collectively the Ambulacraria). For example, studies on ambulacrarians have provided important new insights into the evolution of neuropeptide-S/CCAP-type, GnRH/Corazonin-type and achatin-type neuropeptide signalling systems.

Reconstructing the evolution of neuropeptide signalling systems at a molecular level is also providing a framework to address evolutionary questions at the level of organismal physiology and behaviour. Thus, to what extent are the physiological roles of neuropeptide signalling systems conserved between phyla? Relevant to this question, I will illustrate how experimental research on an echinoderm model, the starfish *Asterias rubens*, is providing new insights into the comparative physiology of neuropeptide signalling in the unique context of a pentaradial body plan.

Looking ahead, a long-term objective of research on neuropeptide signalling will be to reconstruct the evolution of neuropeptide function in the animal kingdom so that we gain understanding of not only what neuropeptides do but also why they do what they do.

PLENARY 4

HORMONAL PHEROMONES IN TELEOST FISHES

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Communication and behaviour are tightly linked, and fishes use several sensory channels to communicate. Chemical communication is the most ancient form of communication, but its role is often ignored because the substances involved are rarely known. Among teleost fishes, the endocrine system appears to be tightly linked to chemical communication as hormones and their metabolites are released and perceived by the olfactory system of conspecifics. This causes changes in behaviour, hormonal responses and physiology that help the synchronization of reproduction. Less well understood is the involvement of chemical communication in territorial behaviour and as a modulator of aggression. We will discuss the recent advances in teleost fish chemical communication and interactions with the endocrine system, with emphasis on cichlids as a model system.

Acknowledgement: The research leading to these results has received funding from the European Union's Horizon 2020 research and innovation programme (grant agreement n° 746507 and from the Foundation for Science and Technology of Portugal (FCT) through project UID/Multi/04326/2013.

PLENARY 5

ENDOCRINE DISRUPTION OF THE THYROID AXIS: FROM CORAL FISH AND AMPHIBIAN METAMORPHOSIS TO HUMAN BRAINS

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Thyroid hormones govern major developmental processes in all vertebrates, with the most spectacular examples being amphibian and flatfish metamorphosis. Thyroid signaling shows strong evolutionary conservation, the most biologically active form of the hormone, tri-iodothyronine (T3), being exactly the same molecule across all the major vertebrate groups.
Thyroid hormones contain iodine. As such they are the only complex halogenated molecules synthesized by chordates, making thyroid signaling particularly vulnerable to interference by xenobiotics containing other halogens, bromine, chlorine and fluorine. Many authors have hypothesized that this vulnerability to endocrine disrupting chemicals (EDCs) is contributing, along with climate change, to the decline of numerous species. Of particular concern are declines in amphibian, coral fish and migratory bird populations. Examples will be given of how thyroid hormone disruption is documented as interfering with the major physiological and developmental processes in each of these categories.
The same mechanisms that underlie this vulnerability can also be exploited to screen chemicals for their thyroid disrupting potential. As all the major components of thyroid signaling (deiodinases, receptors and transporters) are present from the earliest stages of development, early Xenopus embryos can be used to determine those chemicals in the environment have the capacity to interfere with thyroid hormone production, distribution or action.
Multiple processes in vertebrate brain development implicate regulation by thyroid hormone: stem cell proliferation, migration, differentiation, synaptogenesis and myelination. This dependence provides a highly plausible link between thyroid hormone disruption, especially in the prenatal period, and the increased incidence of neurodevelopmental disease in currently observed in humans. The evidence for this latter point will be discussed briefly.

PLENARY 6

THE EVOLUTIONARY ORIGIN AND FUNCTION OF TRH AND OTHER NEUROPEPTIDE RELEASING FACTORS REVISITED

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Neuropeptide releasing factors play an important role in the control of homeostasis. In humans, deficiencies in hypothalamic thyrotropin-releasing hormone (TRH) signaling underlie defects in growth, weight gain, reduced appetite, and neurological disorders. The control of thyroid hormone levels in the hypothalamus-pituitary-thyroid axis is a prime function of TRH that underlies several of its effects. However, mounting evidence indicates that TRH also regulates growth in ways we don't yet properly understand, for instance through appetite. TRH is highly conserved in vertebrates but seems to have no or minor effects on TSH production in fish and amphibians, which raises the question as to what the ancient function of TRH might have been. Although evolutionarily conserved G-protein coupled receptors for TRH have been identified across proto- and deuterostomian animals, a functional equivalent of the TRH neuropeptide in protostomians remained elusive until recently. Elaborate *in silico* and receptor deorphanization studies recently identified TRH-like peptides in deuterostomian invertebrates and in all major phyla of the Ecdysozoa and Lophotrochozoa. Two *C. elegans* TRH-1 neuropeptides not only activate orthologous TRH receptors of *C. elegans*, but also human and *Platynereis* TRH-receptors *in vitro*. In addition, *C. elegans* TRH-1, like mammalian TRH, regulates growth as shown by analysis of *trh-1* mutants, generated by CRISPR/Cas9 editing. The growth-stimulatory effect of TRH-1 signaling through its TRH receptor, is food-dependent, suggesting that TRH may also modulate feeding or energy metabolism in *C. elegans*. The conservation of TRH and its receptor, the glycoprotein receptor as well as thyrostimulin hormone subunits, makes the genetically amenable model *C. elegans* highly suitable to further dissect TRH's modulatory functions in the control of growth and energy balance. Similarities between the TRH systems in vertebrates and worms suggest that their common ancestor most likely used an ancestor molecule of TRH to regulate growth.

PLENARY 7

A DISCUSSION ON THE NEURAL CONTROL OF EGG LAYING IN TWO INSECT MODELS: THE LOCUST AND KISSING BUG

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Coordination of muscles of the reproductive system in both male and female insects is critical for successful mating and the eventual production of viable eggs. This coordination involves a variety of neurons and their neuroactive chemicals. We have described these events in detail in the locust, *Locusta migratoria,* and shown that female reproductive tissues are under central neuronal control, with the neural substrate expressing an array of neurpeptide and amine phenotypes. Central pattern generators (CPGs) are involved in oviposition, and the CPGs that control digging of the ovipositional hole, initial egg retention in the lateral oviducts and then sperm release onto the eggs, are synchronized.

More recently, we have been examining the female reproductive systems of the medically-important kissing bug, *Rhodnius prolixus*. This obligate blood-feeder, a major vector for Chagas disease, takes a very large blood meal once in each instar, and this blood meal triggers endocrinological events associated with growth, development and reproduction. In the adult female, neurohormones are released that control short-term changes in physiology associated with nutrient storage and distribution and that also influence the number of eggs produced and deposited. The muscles of the reproductive system are also under neuronal control, with the neurons utilizing a variety of neuropeptides and amines to influence muscle contraction, ensuring successful mating and egg-laying.

These two models will be discussed, illustrating commonalities in the neurochemical architecture used for coordinating the parts.

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POSTERS

P-1

THE ROLE OF CRUSTACEAN CARDIOACTIVE PEPTIDE AND ITS RECEPTORS IN THE MOULTING PROCESS OF THE DESERT LOCUST, SCHISTOCERCA GREGARIA

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The desert locust, *Schistocerca gregaria*, is a swarming hemimetabolous pest insect that can form a serious threat to agriculture in the poorest parts of the world, thereby contributing to famine and health problems. A profound knowledge of the desert locust's physiology and the underlying molecular processes will help us in the quest towards more selective insecticides. Since the moulting process is crucial in an insect's life cycle, we are interested in the molecular pathways regulating this process. However, the neuroendocrine system that orchestrates the moulting process has mainly been studied in Holometabola. One key regulator in the neuropeptidergic cascade that initiates the actual ecdysis (shedding of the old cuticle) in Holometabola, is crustacean cardioactive peptide (CCAP). Since very little is known about the neuropeptidergic cascade initiating ecdysis in Hemimetabola, our research focuses on CCAP and its receptor (CCAPR) in the hemimetabolous insect *S. gregaria.*

The different *Schgr*CCAPRs, which are G protein-coupled receptors (GPCRs), were characterized using cell-based functional receptor assays. After confirming that *Schgr*CCAP activates the *Schgr*CCAPRs, we investigated the downstream signalling pathway by expressing the different *Schgr*CCAPRs in cultured cells. While no effect was observed on the cAMP signalling pathway, application of *Schgr*CCAP to the CCAPR-expressing cells resulted in an increase of the intracellular Ca^{2+} concentration. Furthermore, we functionally characterized the *Schgr*CCAPRs and the *Schgr*CCAP precursor *in vivo.* Using the RNA interference (RNAi) technique, we investigated their role in ecdysis. RNAi-mediated silencing of the *Schgr*CCAPRs and/or the *Schgr*CCAP precursor in fourth and fifth nymphal instars resulted in high mortality rates at the expected time of ecdysis, when compared to control locusts. Since, CCAP and its receptors seem to play a crucial role in moulting, they constitute promising molecular targets for the development of novel insecticides to combat this harmful locust species.

P-2

THE ROLE OF MELATONIN IN THE TEMPORAL EXPRESSION OF MOLT-RELATED GENES IN THE BLUE-CRAB, CALLINECTES SAPIDUS: A TEMPORAL EVALUATION

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Molting is a cyclic process comprised of 5 stages, essential for the growth of crustaceans. Ecdysteroids and the molt inhibiting hormone (MIH) stimulate and inhibit molting, respectively; however, the effect of melatonin on this process is still an open question. Here, we evaluated in *Callinectes sapidus*: 1) the temporal gene expression of ecdysone receptor isoform 1 (*CasEcR1*) and MIH (*CasMIH)* in hepatopancreas (HP) and eyestalk (ES) in premolt and intermolt stages; 2) the daily variation of hemolymph melatonin levels in premolt and intermolt stages; 3) the effect of daily injections of melatonin on the expression of *CasEcR1* and *CasMIH* in HP and ES of intermolt animals. Animals were individually kept at 22°C ± 2, under a photoperiod of 12:12 light:dark cycle (lights on at 7 AM, 400 lux). The animals were sacrificed, and the tissues removed at 8, 16, and 24 h (clock time). Our data show that *CasMIH* and *CasEcR1* display similar, molt stage independent, oscillatory profile in HP. In ES of premolt animals, the oscillatory pattern of these genes was similar to HP; however, in intermolt an antiphase relationship between the two genes was observed. For the first time we showed the presence of melatonin in *Callinectes sapidus*. Interestingly, the levels of melatonin exhibited a temporal variation, peaking at 8 AM, only in premolt animals. Daily administration of melatonin (10^{-7} moles/crab) at 12 PM during 7 days inhibited the expression of *CasMIH* and *CasEcR1* in HP and ES in comparison to the control group. Taken altogether, our results demonstrate a circadian oscillation of molt cycle related genes, which can be influenced by environmental and endogenous factors. The oscillatory levels of melatonin in premolt, and melatonin-induced inhibition of molting related genes suggest that this hormone exerts important effects on molt cycle, acting as a positive regulator of this process.

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P-3

PRENATAL BISPHENOL A (BPA) EXPOSURE ALTERED AROMATASE, BDNF EXPRESSION AND INDUCED ANXIETY AND STRESS-LIKE BEHAVIOR IN F1 OFFSPRING IN A SEX-SPECIFIC MANNER

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Exposure to Bisphenol A (BPA) in animal models and humans has been observed to have long-lasting effects on the brain development, and the exposure levels were positively associated with symptoms of depression and anxiety. Since the role of estrogen on the early developmental events like synaptogenesis, neural differentiation and maintenance has been well studied; it has been hypothesized that the presence of BPA in the fetal developing environment will perturb the organization of the brain leading to altered neurobehavioural outcomes. In the following study human exposure equivalent concentrations of BPA (25 mg/kg-bw/day, 2.5 mg/kg-bw/day and 250µg/kg-bw/day) were administered to gestating rats covering critical developmental stages of early brain development from gestational day (GD) 9-21 through water. The pups were weaned on postnatal day (PND) 21, further the neurobehavioural phenotyping: open field test, elevated plus maze test, light dark preference test and novel object recognition test were carried out in the F1 offspring during PND 21-26. Results obtained from behavioural analysis showed a significant increase in anxiety-like and stress behaviour in F1 generation male and female pups. However, there was no significant variation observed in memory patterns. In addition to elucidate the underlying molecular mechanism gene and protein expression studies were carried out. The expression of CYP19A1 and BDNF genes were significantly downregulated in males whereas in the case of females prenatal exposure to BPA led to increased expression of both the genes. Further, intracellular protein levels of p-Akt, p-MEK and p-ERK showed an increased expression in males, while gestational exposure to BPA suppressed the expression of p-Akt, p-MEK and p-ERK in the females. Together this study suggests that long-term impact of BPA on neurobehaviour is mediated by dose and sex-specific organisation of the brain during critical stages of early development.
Key words: BPA, prenatal exposure, BDNF, anxiety and stress-like behaviour.

P-4

EXPRESSION OF STEM CELL MARKERS IN BETA CELLS OF THE HUMAN

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The purpose of this study was to assess the expression of embryonic stem cell and pancreas-specific markers in insulin producing beta cells in adult human islets, including Nanog, Oct4, Sox2, menin, Ngn3, FZD2 and PDX-1. An indirect immune staining was applied on archival formalin-fixed human pancreatic samples. Expression of the three beta cells precursor markers menin, Ngn3 and PDX-1 were identified in the islets, always co-localized with insulin. Expression of Nanog, Oct4 and Sox2 were distinctly identified in the islets of the human pancreas. Oct4 and Sox2 expression was always co-localized with insulin, indicating that all insulin secreting beta cells do express these two markers. Nanog was mainly co-expressed with insulin in beta cells, but there was a small number of cells in the islets expressing Nanog, but not insulin, demonstrating that Nanog might be expressed by the others endocrine cells or early precursors or tissue-specific stem cells. A majority of islet cells also expressed FZD2, the WNT5a receptor, which has been shown to be expressed in islet precursor cells. These findings demonstrate that the three embryonic stem cell markers Nanog, Oct4 and Sox2, as well as FDZ2, are expressed in the islets of the human pancreas. The precursor markers of beta cells such as menin, Ngn3 and PDX-1 are always localized with insulin producing beta cells as well. Consequently, the presence of embryonic stem cell markers in the human adult pancreas may offer a new principle for regenerating and replacing ageing or damaged beta-cells.

P-5

A COMPARATIVE APPROACH TO MEASURE SEX AND AGE-RELATED DIFFERENCES IN SHOULDER MORPHOLOGY AND BODY SIZE

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In the human scapula, a significant sex dimorphism has been recently described by our group (Mathews et al., BMC Musculoskelet Disord. 2017;18:9), which was in line with the scarce data in similar populations. However, when it comes to correlation with body size, the database is still weak, particularly for women and smaller individuals. For instance, previous studies from our group (e.g. Häusler and co-workers University of Zurich 2001; J Hum Evol 2004;46:433-65; 2007;53:383-405) in 100 skeletons with emphasis on small-bodied individuals (65 men, 35 women) originating from Europe, Asia and Africa, have revealed contradictory results for the ratio of glenoid to body size depending on the calculation method. Furthermore, in the elderly, potential osseous shrinking processes have to be considered. Thus, there is a demand to compare the individual scapula size with body size in a larger data set with special emphasis on aged and female individuals. In this multimodality study, we systematically explore the glenohumeral joint using morphological and CT-based measurements, and compare the data with donor body size using different methodological approaches. CT scans including the shoulder girdle and arms were performed prior to dissection. Glenoid size was determined on subsequently isolated scapulae and on 3D-CT reconstructions of the glenohumeral joint according to the method of Friedman as described (Mathews et al. 2017). Body length was measured by CT and additionally, body size determined from femur length and femur head diameter, respectively, and both methodological approaches were compared and correlated with the glenoid size to establish a data set to extrapolate glenoid and body size in the elderly. This study is one of the first to combine dissection with anatomical measurements and radiological CT data to systematically correlate the scapula and glenoid size with the body size as a basis for endocrinological studies in physiology and pathological state.

P-6

OSMOTIC STRESS TRANSCRIPTION FACTOR 1b (Ostf1b) TRIGGERS HYPEROSMOTIC RESPONSES IN THE CHLORIDE CELLS OF GILLS IN JAPANESE MEDAKA

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Euryhaline teleosts are able to maintain their body fluid osmolality by the movement of water and ions such as Na^+ and Cl^- in the osmoregulatory tissues of the gill, kidney and intestine in response to changes in external salinity. Osmotic stress transcription factor 1 (Ostf1) was first identified as signal transducers that trigger effectors such as ion transporters and channels in the gill of tilapia transferred from freshwater (FW) to seawater (SW) (Fiol and Kultz, 2005). However, the function and signaling pathway of Ostf1 have not been fully elucidated. In the present study, we have attempted to understand the functional characterization of Ostf1b which strongly expresses in response to hyperosmotic stress, in euryhaline Japanese medaka (*Oryzias latipes*). Quantitative real-time PCR showed the rapid increase of Ostf1b in gill after transfer of medaka from isotonic brackish water (300 mOsm) to SW (1000 mOsm). The Ostf1b mRNA expression reached to 20 folds at 2h after the transfer and gradually declined to baseline levels within 12h. On the other hand, transfer from SW to isotonic water does not increase mRNA expression of Ostf1b. To determine the osmotic stressor specificity of induction of Ostf1b, medaka were exposed to other types of osmotic stress (500 mOsm of NaCl, Urea, Mannitol, and Glucose) for 2h. As a result, Ostf1b expression was induced only in the exposure to hyperosmotic NaCl solution. To identify the localization of Ostf1b mRNA in the gill, we performed in situ hybridization (ISH) in medaka after transfer from isotonic water to SW. Our ISH observation showed Ostf1b mRNA was mainly localized in the chloride cells which are characterized by an excess of Na^+,K^+-ATPase expression. These results suggest that Ostf1b plays a key role in the functions of chloride cells in the immediate response to high salinity.

P-7

MORPHO-FUNCTIONAL ACTIVITY OF PINEAL GLAND IN RELATION TO PHOTOPERIODIC REGULATION OF REPRODUCTION IN WISTAR RATS

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Pineal gland participates in the regulation of diurnal and seasonal periodicity of physiological functions, including reproduction, by changing the amplitude and duration of melatonin secretion. We investigated the effects of standard illumination LD 12:12 and constant darkness DD from the prenatal period on pineal morphology, reproduction rates and rates of growth and development of Wistar rats. Results indicated that parturition in all animals from both groups started on the same day and were completed on the same day. In DD the number of stillborn rats was more than 2 times higher than in LD. On the 25 days after birth, the number of surviving animals was slightly lower in the DD group than in LD. The opening of the eyes and the timing of sexual development of both males and females passed less intensively in DD rats than those in LD ones. In rats kept in DD during prenatal development and further for 3 months, an increase in the number of pinealocytes and their morphometric parameters compared to those in rats from LD. In addition, multinucleolar cells were found in the pineal of rats from DD. Nevertheless, it is erroneous to assume that in DD the synthesis of melatonin significantly increases. In DD rats there is a smoothing of the rhythm of melatonin secretion per day - the daily portion increases and the night portion decreases in comparison with the LD regime, but the total concentration of the hormone per day in DD does not exceed that in LD.
The study was carried out under state order (project № 0221-2017-0052) using the equipment of the Core Facility of the Karelian Research Centre of the Russian Academy of Sciences and according to EU Directive 2010/63/EU for animal experiments with the special permission of Local Ethic Committee of Institute of Biology.

P-8

ARE THE PLEUROPODIA OF INSECT EMBRYOS ORGANS FOR ECDYSONE BIOSYNTHESIS?

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Where ecdysone comes from during insect embryogenesis is not yet fully understood. A hypothesis was proposed that ecdysone, or a similar steroid hormone, is produced by paired glandular organs called the pleuropodia. These are appendicular structures that transiently appear on the first abdominal segment in embryos of species from almost all insect “orders”. The pleuropodia always degenerate before hatching. Perhaps because they are missing in the fruitfly *Drosophila* and other models such as the silkworm *Bombyx*, the pleuropodia escaped attention in the recent decades. Whether they indeed produce ecdysone or have another function is unclear. We studied the development of the pleuropodia in the embryos of the locust *Schistocerca gregaria* and by RNA-seq isolated the genes that they express at several ontogenetic stages. We show that the pleuropodia do not specifically upregulate the genes for biosynthesis of ecdysone. Instead, the pleuropodia are highly enriched in transcripts for enzymes capable to digest chitinous cuticle and proteins playing a role in immune defense.

P-9

FlyAtlas2 – NEW VERSION OF THE DROSOPHILA EXPRESSION ATLAS

Dr Sue Krause

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FlyAtlas2.org is a recent developed resource that will useful to the Drosophila community as shown by its valued predecessor, FlyAtlas.org (with over 1700 citations since 2007), for studying the expression of Drosophila melanogaster genes in adult and larval tissues (1, 2). FlyAtlas2 utilizes a new set of expression data based on RNA-seq and miRNA-seq rather than the Affymetrix microarray technology (3). Additionally, the data for somatic tissues are available for both male and female adult flies, aiding in sexual dimorphism studies. The web interface allows one to inspect the RNA-seq reads alongside the annotated Drosophila genome on the UCSC browser (external) and can link to FlyAtlas.org for comparison between the RNA-seq data and microarray data. In FlyAtlas2.org, we can analyse the expression pattern of the different families of neuropeptides and their receptors. Here, we will show the comparisons of the FlyAltas dataset to the FlyAtlas2 dataset. We will highlight some of differences in the male and female somatic transcriptomes in the adult fly tissues.

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P-10

CAN A SINGLE EXPOSURE EVENT OF 17α-ethinylestradiol AND/OR CLOFIBRATE INFLUENCE SOME WEEKS LATER EITHER ESTROGENIC OR LIPIDIC PATHWAYS IN BROWN TROUT LARVAE?

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In early life stages of fish, lipids accumulated in the yolk-sac are the unique energetic source before the first feeding and, consequently, any interference on the lipidic homeostasis may lead to irreversible consequences during larval development. Evidence shows that human pharmaceuticals can affect the intracellular transfer and lipid metabolism in fish, causing hypertriglyceridemia or disturbances in lipid distribution. Exposure to those compounds may occur acutely and reach high levels. Later effects of such acute events are often less studied than the immediate ones.

This study aims to infer the possible interferences caused by an estrogenic hormone (ethinylestradiol – EE2), a hypolipidemic compound (clofibrate – CLF) and the mixture of both, on a selection of target genes related with estrogenic (estrogen receptor α – ERα and vitellogenin A – VtgA) and peroxisomal/lipidic (peroxisome proliferator-activated receptor αBb – PPARαBb and fatty acid binding protein 1 – Fabp 1) pathways in brown trout (*Salmo trutta fario*). As a model of single exposure event to study later impacts, eyed-stage embryos were injected with 1 µL of dimethyl sulfoxide (DMSO) – solvent control, 0.5 ng/egg of EE2, 200 ng/egg of CLF and with a mixture of EE2 0.5 ng/egg + CLF 200 ng/egg (Mix). Larvae with about four weeks after hatching (at 10 ºC) were sampled and mRNA levels were quantified by real-time polymerase chain reaction.

VtgA mRNA expression increased in EE2 injected larvae, while Fabp1 expression decreased after EE2, CLF and Mix. PPARαBb and ERα mRNA expressions were not affected by any treatment. Overall, a single injection of both EE2 and/or CLF interfered with the lipid metabolism during early stages of brown trout development. The results justify exploring further deregulations in distinct lipidic pathways.

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P-11

TEMPORAL EXPRESSION OF GONADAL REPRODUCTIVE MARKERS AND STEROID HORMONE LEVELS IN ATLANTIC BLUEFIN TUNA, Thunnus thynnus, CAUGHT IN THE MEDITERRANEAN SEA DURING SPAWNING AND POST-SPAWNING SEASON

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Atlantic bluefin tuna (*T.thynnus*) is a prized pelagic fish species representing an important worldwide economic fishery resource. Despite the increasing number of studies, many aspects of the basic biology of tuna are still lacking. In this study, we investigate the variation of the expression of a set of reproductive signals controlling gametogenesis in fish sampled at spawning and post-spawning stages. Focusing on the ovary, variations of mRNA levels of genes playing pivotal role in egg envelope formation, oocyte hydration, fatty acid uptake and intracellular transport, were analyzed. In the testis, the expression of markers responsible for sperm binding and fatty acid uptake, were analyzed. Results showed that signals involved in ovarian maturation and oogenesis were more expressed at reproductive stage, significant decreasing in post spawning stage. On the contrary, in the testis, lack of significant changes of mRNA levels were found between the reproductive and the post reproductive events. Plasma levels of steroid hormones, E2 and T closely correlated with gonadal development and reproductive season. In both male and female fish, E2 levels significantly varied between spawning and post- spawning seasons, with the highest levels measured at spawning. T levels remained almost constant in males in both seasons. In contrast, a significant decrease of T was observed in post-spawning females. Concerning P, no significant variation was observed between the two sampling periods neither in male nor in female. This study contributes to increase the knowledge on the basic reproductive biology of bluefin tunas. Integration of these results with recently evidence relative to molecular features of body growth, can contribute to gain knowledge on the reproductive physiology of bluefin tuna for the conservation of natural stocks.

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P-12

THE TRANSCRIPTION FACTOR GATAe IS REQUIRED FOR THE MORPHOLOGY, FUNCTION AND NEUROPEPTIDE RESPONSE OF THE DROSOPHILA RENAL TUBULE

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The development and homeostasis of an organ is a complex process which involves several programmed events, including changes in cell shape and adhesion, proliferation, differentiation and maintenance. These events are defined by the expression and combination of specific Transcription Factors (TFs). The Malpighian Tubules (MTs) of *Drosophila melanogaster* are an important model for tubulogenesis, and for 'omics and integrative physiology.

In this study, we silenced the expression of the TF *GATAe* in the MTs, and examined the defects in tubule function and morphology. Larval, pupal and adult tubules were dissected and immunostained with antibodies to the specific MT cell types, as well as kinin receptors [1] to confirm the morphological phenotypes. In addition, Ramsay secretion assays [2] were performed to investigate the impact of *GATAe* RNAi on kinin signaling. Given the role of MTs in fluid homeostasis, *GATAe* MT RNAi flies were subject to desiccation assays.

GATAe RNAi in only the MT Stellate Cells (SCs) results in a significant reduction of number of cells, as confirmed with ClC-a and LKR antibody markers [1,3]. Consequently, these tubules show significantly reduced secretion rates in response to Kinin which acts specifically on SCs in *D. melanogaster* [4]. *GATAe* RNAi was also targeted to the Principal Cells (PCs), inducing strong defects in the morphology of the tubules in pupal and adult stages.

Altogether, our results show that *GATAe* is critically required in the MTs. In the SCs, *GATAe* is required for a normal response to Kinin, as SCs numbers are significantly reduced in *GATAe* RNAi (SC) tubules.

In the PCs, *GATAe* is required to maintain the correct architecture of the MTs.

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P-13

TDAG8 ACTIVATION RESPONSE BY PROTONS DIFFERS ACCORDING TO SPECIES

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Human and mouse OGR1, GPR4, TDAG8 are proton-sensing GPCRs activated with increasing extracellular proton concentration (pH decrease). We reported that the OGR1, GPR4 homologous (zOGR1, zGPR4) present in zebrafish are also proton-sensing GPCRs like human OGR1, GPR4. This result suggests that the activation response of OGR1 and GPR4 by protons may be preserved evolutionarily. With TDAG8, however, it is not yet clear whether the proton activation response is evolutionarily preserved.

We aimed to clarify whether chicken, Xenopus laevis, zebrafish TDAG8 homologous is also activated by proton.

TDAG8 expression vectors derived from the above species were prepared. Human TDAG8 (hTDAG8) activates the reporter (CRE-Luc) through Gs / cAMP pathway as the extracellular pH decreases. Therefore, each TDAG8 expression vector was introduced into HEK 293 cells together with the reporter vector, stimulated under pH 7.7 to 6.6, and its reporter activity was measured to evaluate activation of the receptor.

hTDAG8 and Xenopus TDAG8 (xTDAG8) were activated by an increase in the extracellular proton concentration. On the other hand, the degree of activation of chicken TDAG8 and zebrafish TDAG8 (zTDAG8) was weak. In order to investigate the receptor region generating this difference in the activation responses, chimeric receptors between hTDAG8 and zTDAG8 were prepared and the activation responses by protons were compared. The result indicates that the difference is at least existed in the N terminal extracellular region.

The result in this study suggests that protons may not be an evolutionarily conserved agonist to TDAG8. It was reported that TDAG8 of mouse and zebrafish plays a similar role in blood cell differentiation. The results of this study indicate that further studies are necessary to determine whether the true ligand of TDAG8 in vivo is protons.

P-14

ACTIVATION OF OVARIAN CANCER G PROTEIN-COUPLED RECEPTOR 1 BY METAL DIFFERS AMONG SPECIES

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Ovarian cancer G protein-coupled receptor 1 (OGR1) is known as a proton-sensing GPCR. In addition, human and mouse OGR1 has been reported that they also are activated by metals in addition to protons. Thus, human and mouse OGR1 is a unique GPCR that is activated by protons and metals. We reported that zebrafish OGR1 is also activated by protons and metals, like human and mouse OGR1. In that study, we also noticed that the activation pattern of zebrafish OGR1 by the metals is somewhat different from that of human and mouse. It suggests that the activation pattern of OGR1 by metals may be different among the animal species.

We aimed to clarify whether OGR1 homologous from various species also sense and are activated by protons. In addition, we also clarify the activation pattern of these homologs by metals.

OGR1 homologous from porcine, rat, chicken and Xenopus were cloned and ligated into the expression vector. These receptors were expressed in HEK 293 cells and the activation of the receptors was measured as SRE promoter activity.

Extracellular protons activated all OGR1 homologs tested, like human and mouse OGR1. On the other hand, the activation pattern of these receptors by metals was different among the species. The extracellular region of the receptor is involved in the activation pattern by the metals.

This study revealed that protons were evolutionarily conserved ligand for OGR1, since all of the tested OGR1 in this study was activated by protons. On the other hand, the activation pattern of the homologs by metals varied among the species. OGR1 may change the response to metals according to their environment where they live.

P-15

AMINO ACID SENSING SYSTEMS IN TELEOST GASTROINTESTINAL TRACT

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Regulation of digestion and feed intake are complex mechanisms that ensures proper intake, absorption and allocation of substrates for growth and body homeostasis. While it is well acknowledged that nutrient sensing is a key player in the control of digestive function and appetite in mammals, there is little information in teleosts, the largest vertebrate group with more than 25000 species.

In mammals, the presence of AA and peptides in the gastrointestinal tract (GIT) lumen is monitored by sensory systems based on members of the G protein-coupled receptors (GPCRs) family A and C. The lysophosphatidic acid receptor 5 (LPAR5) belongs to family A and responds to peptides. Members of family C include monogamous receptors for L-Glu such as the metabotropic glutamate receptors (mGluRs) while promiscuous receptors calcium-sensing receptor (CasR), GPCR family C subtype 6A (GPRC6A) and taste receptors (T1Rs) respond to L-αAAs.

Based on the hypothesis that nutrient sensing systems are conserved throughout vertebrate phylogeny, we currently investigate the AA and peptide sensing GPCRs homologues in Atlantic salmon (*Salmo salar*), a key commercial species in global aquaculture. *In silico* analysis revealed that Atlantic salmon GPCRs family A and C primary structure is well conserved within vertebrates. Salmon LPAR5 share > 50% AA sequence similarity with other vertebrate homologues. Other nutrients sensors identified in the salmon genome include GPCR6A, five T1R1 transcripts, two T1R3, six GPR6A, thirteen mGluR1, seven mGluR4 and three CasR transcripts. We explore the different transcript sequences, starting by family C, using cloning, qPCR and spatial and temporal expressions profile to understand how each AA potentially affect feed intake and digestion.

P-16

INSECT PEPTIDE HORMONES AND NEUROPEPTIDES: POTENTIAL INSECTICIDE TARGETS

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New insecticides are needed for continued control of arthropods, from agricultural pests to vectors of disease, as resistance to insecticides and non-target toxicity are ongoing challenges. The receptors of peptide hormones and neuropeptides, many of which are G-protein coupled receptors (GPCRs), represent a diverse but relatively unexplored array of targets for the development of new insecticide classes to circumvent existing forms of insecticide resistance. Some peptide hormones and neuropeptides are arthropod-specific and targeting these receptors may identify molecules that have reduced off-target toxicity. These peptides coordinate numerous biological processes in insects, ranging from critical physiological functions to behavior. One such is neuropeptide F (NPF), which in insects has been demonstrated to regulate food-searching and food-acceptance behaviors, among other functions. We therefore identified genetic sequences for NPF receptors through homology searches of available insect genome and transcriptome databases and confirmed these by sequencing of PCR amplified cDNA. Receptors were cloned and expressed in reporter cell lines, and pharmacological profiles were characterized in response to predicted peptide ligands. In addition, potential non-peptide analogs were examined to determine the activity at these receptors and to explore their potential to disrupt normal signaling. This investigation provides a foundation for the exploration of peptide hormone and neuropeptide receptors as viable insecticidal targets. Current progress on the characterization of NPF receptors in pest insects will be discussed.

P-17

TEMPERATURE AFFECTS MUSCULOSKELETAL DEVELOPMENT AND MUSCLE LIPID METABOLISM OF GILTHEAD SEA BREAM (SPARUS AURATA): IN VIVO AND IN VITRO APPROACHES

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World population is expected to considerably increase by 2050 with uncertainty in the food supply, where aquaculture may have a relevant role satisfying the growing need of fish. Moreover, researchers are urged to evaluate fish thermal tolerance and health consequences, since climate change is a challenge humanity is facing. The aim of this study was to determine whether rearing temperature (19-control, 24 or 28ºC) may influence musculoskeletal development and muscle lipid metabolism in gilthead sea bream juveniles. The expression of growth hormone (GH)/insulin-like growth factors (IGFs) axis-, osteogenic-, myogenic- and lipid metabolism-related genes in vertebra bone and white muscle was analysed, together with proliferation/differentiation and osteogenic genes expression in primary cultured bone-derived mesenchymal stem cells obtained from the same animals. Increasing temperature to 28ºC significantly down-regulated bone mRNA levels of *igf-1*, *igf-2*, the *igf-1ra* receptor, and the binding proteins *igfbp-4* and *igfbp-5*, whereas the main osteogenic genes analysed were unaltered. Similarly, in muscle, the expression of *igf-1*, *igf-1ra and igfbp-1*, and that of the myogenic regulatory factors *myod1* and *mrf4* was significantly affected upon temperature increase compared to the control condition. Furthermore, in this tissue, the expression of the lipases *lipa* and *lpl-lk* resulted significantly enhanced, whereas *β*-oxidation markers *cpt1a* and *cpt1b* were significantly down-regulated in fish maintained at elevated temperatures. Regarding the *in vitro* studies, up-regulated expression of the extracellular matrix markers *on*, *op* and *ocn* was found, together with a gradual decrease in mineralization along with temperature. Overall, these results reveal that increasing temperature outside optimum appears to induce in this species unfavourable musculoskeletal growth and development, through modulating the expression of different members of the GH/IGFs axis, and probably osteogenic genes in prolonged exposure, and to accelerate the utilization of lipids as an energy source, albeit with little efficiency. Supported by MICINN-AGL2014-57974-R and Generalitat de Catalunya-2014SGR-01371.

P-18

REGULATION OF INSULIN-LIKE PEPTIDES (ILPS) SECRETION BY CHOLINERGIC MUSCARINIC SYSTEM IN TENEBRIO MOLITOR LARVAE

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Cholinergic system is one of crucial systems involved in the regulation of physiological processes both in vertebrates and invertebrates. In vertebrates, it regulates, for example, reproduction, development, immunity and metabolism. This system acts on cells *via* two types of receptors, one of which are muscarinic (mAChRs) receptors belonging to G-protein coupled receptors. In mammals, 5 classes of mAChRs are distinguished (M_1–M_5) whereas, in insects, they are divided into two types: A and B. The available data suggest that in insects, muscarinic receptors are involved in the regulation of behaviour and locomotion. Additionally, mAChRs also affect the functioning of such neuro-endocrine processes as the secretion of juvenile and protoracotropic hormones. Studies has shown that they are expressed in cells of retrocerebral complex which release insulin-like peptides (ILPs) into haemolymph. In our study, we investigated the influence of mAChRs agonists (acetylcholine, carbachol and pilocarpine) and antagonists (atropine and scopolamine) on the insulin-like peptides level in *Tenebrio molitor* larvae haemolymph.
Our experiments with immunenzymatic ELISA test showed that tested compounds affect significantly the level of ILPs in haemolymph. An application of agonists increased the level of these neuropeptides only two hours after the injection. The strongest effect was observed in the case of acetylcholine application; when insects were treated with antagonists, the opposite effect was noticed. Nevertheless, the significant lowering of ILPs level was observed after an hour since the injection. The effect was definitely weaker after two hours. Changes in ILPs level in the haemolymph correspond too changes of such biochemical parameters as concentration of total sugar in haemolymph and glycogen content in fat body.

P-19

INSULIN LIKE GROWTH FACTORS ON THE NUTRITION AND ERYTHROPOIESIS AFTER PARATHYROIDECTOMY – THERE IS A LINK BETWEEN PTH AND IGFS ON KIDNEY FAILURE-

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Hyperparathyroidism shows the effects to many target organ besides kidney and bone at kidney failure. We reported cardiac function improved after PTX (Parathyroidectomy) on hyperparathyroidism of kidney failure. More over improvement of nutritional state and erythropoiesis are observed after PTX.

This study was performed to clarify interaction between PTH and GH (Growth Hormone), IGFs (Insulin-like growth factors) and Insulin-like growth factor binding protein on nutritional improvement and erythropoiesis.

Twelve hemodialysis patients (Primary renal disease:Chronic glomerulonephritis 9cases Nephrosclerosis 3cases, male 6 female 6, mean age 49.1yrs . mean duration of dialysis therapy is 14.3years.

GH(Growth Hormone), IGF-I,IGF-II and IGF-binding protein 3 are investigated on Pre and Post PTX. Duration of observation on pre and post PTX is 12months on each. As the same, TP, Alb, UN, Cr, Ca, iP, and body weight) are studied each on Pre and Post PTX.

The relation between iPTH and GH, IGF-I, IGF-II, IGF-binding protein 3 was studied. And the shift of nutritional factors (TP, Alb, Hematocrit, body weight gain) and the change of GH,IGF-I,IGF-II,IGF-I /IGF-BP3 ratio levels were compared to pre and post PTX.

TP,Alb,Ht,BW showed increase significantly($p<0.01$) on post PTX compared to pre PTX.

IGF-I levels increased with value 226±93ng/ml on post PTX significantly ($P=0.05$) compared to the value 166±48ng/ml on pre PTX. Comparison between on Pre and Post PTX, GH, IGF-II and IGF-BP3 levels were not observed.

Positive correlation between increase of BW gain and the IGF-I level was observed ($P=0.012$) on post PTX. The negative correlation between iPTH

levels and IGF-I/IGFBP3 ratio was observed significantly ($P=0.008$). After PTX, IGF-I level, especially IGF-I /IGFBP3 ratio increase and then elevation of albumin, Ht and BW levels are shown. There is an interaction between PTH and IGF-I/IGF-BP3 ratio at nutritional state and erythropoiesis of kidney failure patients.

P-20

ANALYSIS OF MOLECULAR MECHANISM OF ELEVATION OF INTRACELLULAR CALCIUM CONCENTRATION IN GONADOTROPH CELL LINE BY EXTRACELLULAR PROTONS

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It is well known that the locally induced pH reduction affects physiological and pathophysiological functions in the body, but its molecular mechanism is unclear. Proton-sensing GPCR is a G protein-coupled receptor activated by a decrease in extracellular pH. At present, GPR68 (OGR1), GPR4, GPR65 (TDAG8) and GPR132 (G2A) have been reported as the proton-sensing GPCRs. These receptors are activated by a decrease in extracellular pH and leading to increase intracellular calcium concentration, production of cAMP, and activation of small G protein. In this study, we found that a transient increase of intracellular calcium concentration in gonadotroph-derived cells (LbetaT2) when they are stimulated by extracellular protons.

We aimed to clarify whether OGR1 is involved in the elevation of intracellular calcium concentration of Lbeta2 by the increase of extracellular protons.

The change in the intracellular calcium concentration were monitored using Fura2. To examine that the transient increase of intracellular calcium concentration by extracellular protons was mediated though OGR1, we used YM-254890, the Gq family inhibitor, and CuCl2, an OGR1 antagonist.

A decrease in pH caused a transient increase in intracellular calcium concentration in LbetaT2 cells. The increase of calcium concentration was completely inhibited by the addition of YM-254890. Furthermore, this increase was also completely inhibited by the addition of CuCl2.

The results of this study revealed that the transient increase of the intracellular calcium concentration in LbetaT2 by a decrease pH is caused via OGR 1. In hormone-producing cells such as gonadotrophs, it is known that intracellular calcium signaling pathway is involved in its hormone synthesis and secretion. We are now checking how the rise in intracellular calcium concentration by OGR1 is affected to the hormone synthesis and secretion of LbetaT2.

P-21

MECHANISMS AND EFFECTS OF PREMATURE INDUCTION OF GROWTH HORMONE PRODUCTION DURING CHICK EMBRYONIC DEVELOPMENT

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Normal differentiation of growth hormone (GH)-producing cells in the chicken pituitary occurs on embryonic day (e) 14, and differentiation can be induced prematurely by treatment with corticosterone (CORT) on e11. CORT induction of GH promoter activity occurs with 1727bp of the 5'-flanking sequence of the chicken GH gene. This region contains two predicted Pit-1 sites and a predicted glucocorticoid-responsive region (GCRR). Potential transcription factor binding motifs in the GCRR include ETS-1 and a degenerate glucocorticoid response element half site (dGRE). Mutation of the ETS-1 or dGRE sites abolished CORT responsiveness of the GH reporter, indicating these sites are necessary for CORT responsiveness. Chromatin immunoprecipitation indicated that CORT increased recruitment of Pit-1 to the proximal Pit-1 site and glucocorticoid receptor (GR) to the GCRR and the distal Pit-1 site. In contrast, CORT resulted in dissociation of Ets-1 from the GCRR. Inhibitors of ERK1/2 signaling suppressed CORT induction of GH, and Ets-1 can be directly phosphorylated at threonine-38 (pT38) by ERK1/2 activation. Furthermore, CORT induced ERK1/2 activity in embryonic pituitary cells, and the level of pT38 on Ets-1 was increased in CORT-treated pituitary cells, indicating that the increase in Ets-1 pT38 levels resulting from CORT treatment is mediated by ERK1/2 signaling and that this phosphorylation event is necessary for glucocorticoid induction of GH during embryonic development. CORT injection into embryonated eggs on e11 increased body weight of the resulting chickens after hatch in a gender-dependent fashion. CORT did not affect body weight of females. However, CORT-treated males were heavier than controls at 4, 5 and 6 weeks of age, due in part to an increase in breast muscle mass. We conclude that CORT treatment during embryonic development stimulates premature GH production via ERK1/2 signaling and phosphorylation of Ets1 and increases subsequent body weight and breast muscle weight during posthatch growth.

P-22

NEUROPEPTIDE ANALOGUES AND DROSOPHILA STRESS TOLERANCE: FIRST STEPS TOWARDS DEVELOPING TARGET-SPECIFIC PEST CONTROL

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Drosophila suzukii is a serious pest of soft fruit worldwide. With current management programs for *D. suzukii* based primarily on trapping, in addition to the global over-dependence on broad-spectrum pesticide application more generally, a strong imperative exists for more effective, environmentally-friendly and targeted methods of control. One promising avenue of control involves neuropeptide analogue-based insecticidal agents to selectively reduce the fitness of target pest insects, whilst minimising detrimental environmental impacts. Central to the regulation of physiological and behavioural processes, neuropeptides play a vital role in cold and desiccation survival.

The current study investigated the effect of biostable kinin analogues, as well as CAP2b and pyrokinin analogues active on a heterologous insect CAP2b receptor, on desiccation, starvation and cold stress tolerance of the pest species *D. suzukii* and the closely related non-pest species *D. melanogaster*.

Results revealed the CAP2b analogues to be of most interest in significantly impacting the pest target when under conditions of desiccation stress. However, these peptides enhanced desiccation stress survival in relation to control groups, suggesting that they may be operating as antagonists/inhibitors. A focus on incorporation of native *Drosophila* CAP2b and insect kinin sequences in future analogue development may yield pure agonists that can reduce desiccation stress survival in the pest flies.

P-23

dsRNA COMPLEXATION WITH BLOCK COPOLYMERS ENABLES PEST CONTROL BY ORAL APPLICATION

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Without significant advances in crop-protection, it is likely that food production will not be able to sustain an ever-increasing world population. Unfortunately, environmental concerns and the increasing frequency of pesticide resistance means our current arsenal of insecticides is unlikely to be sufficient. As a result, it is important to consider alternatives to existing chemical pesticides. Biopesticides derived from natural sources with new modes of action, such as peptide/protein toxins, hormone analogues and double-stranded RNA (dsRNA) mediated gene silencing by RNA interference (RNAi) have been proposed as alternatives. RNAi has a number of advantages over conventional chemical insecticides, such as selectivity for a target pest, however, the efficiency varies from species to species, and its wide-scale use is currently limited by its rapid degradation upon ingestion and suffers from poor uptake from the gut.

By complexing dsRNA to specific block copolymers containing a complexing and stabilising block, we can increase the protection of the dsRNA to degradation from extracellular nucleases and increase RNAi efficiency. Specifically, we have produced well-defined diblock copolymers comprising poly(2-hydroxypropyl methacrylamide) –b-poly((dimethylamino)ethyl methacrylate) (PHPMA-b-PDMAEMA) which are subsequently quaternised. We have investigated their complexation with dsRNA targeting the gut vATPase. Phenotypic observations of the increased stability and penetration was undertaken for both complexed and naked dsRNA targeting the expression of the *Drosophila suzukii* gene. It was found that complexation protected the dsRNA from gut nucleases and strongly enhanced the lethality of the dsRNA, resulting in 75% mortality when incorporated into the larval diet. Feeding of the complex to *Drosophila melanogaster* had no adverse effects on larval and pupal development of this related fruit fly, demonstrating the advantageous selectivity of this complexed dsRNA. Our approach has the potential to greatly improve the efficacy of dsRNA as an oral bioinsecticide.

P-24

FMRFamide-like PEPTIDES REGULATE MUSCLE CONTRACTIONS IN TENEBRIO MOLITOR AND ZOPHOBAS ATRATUS BEETLES

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Neuropeptides are very important components in the regulation of physiological processes occurring both in vertebrates and invertebrates. They are very diversified class of molecules. In insects, we distinguish around 30 different neuropeptide families that are involved in control of various processes, starting from regulation of feeding, through reproduction, ending up with regulation of circadian rythm in dipterans. One of the biggest and most diverse neuropeptide family is the family of FMRFamide-like peptides (FaLPs). The first peptide from this group was isolated in 1977 from the nervous system of the clam, *Macrocallista nimbosa*. FMRFamide itself was never found in insects until today, however, *N* terminally extended bioanalogues of this neuropeptide were found in different insects. The exact physiological role of this group of peptides is still unclear.

The aim of this study was to evaluate the myotropic properties of FMRFamide-related peptides in two beetles *Tenebrio molitor* and *Zophobas atratus*. Based on transcriptomic approach we analyzed brain and retrocerebral complex transcriptome to find the precursor sequence of FaLPs in both species together with FaLPs receptor sequence. We also tested synthetic peptide Zopat-FMRF-I (NSNFLRFa) to assess its ability to modulate the contractile activity of the heart, oviduct, ejaculatory duct and hindgut in *Zophobas atratus* and *Tenebrio molitor* beetles.

Transcriptome analysis detected the precursor sequence of FaLPs for both beetles and FaLPs receptor sequences. In the precursor six extended FMRFamide like peptides has been found. Analysis of transcriptome showed the sequence of FaLPs receptor, which as expected belongs to the G protein coupled receptors family (GPCR). Tested peptide showed concentration dependent and organ specific myotropic properties. For example, Zopat-FMRF-I exerted negative chronotropic effect on the heart whereas it increases the contraction frequency of hindgut, but only in high concentrations tested (10^{-6}-10^{-5}M).

Obtained results suggest that FMRFamide-like peptides are potent modulators of muscle contractions in beetles.

P-25

SEASONAL CHANGES OF INOTOCIN IN A BIPARENTAL BEETLE, LETHRUS APTERUS

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Inotocin, an insect neurohormone, is a member of the oxytocin/vasopressin family, which is a highly conserved group of neuropeptides. Its orthologs appear to be involved in a wide range of processes in other taxa including regulation of social behaviour such as grouping behaviour, individual recognition and parental care. We identified the sequences of the genes coding for inotocin and its receptor in the genome of a beetle species, *Lethrus apterus*, which has well-developed biparental care. Based on conserved structure and functions across the peptide family, we hypothesized that inotocin may have a role in the regulation of social behaviour and parental care in this species. To investigate this question, samples were collected from two different natural populations during the periods of mate choice and parental care in the breeding season to examine whether season and sex influence the expression of inotocin or its receptor. Head and thorax samples were taken from males and females at each sampling time. After RNA isolation and reverse transcription from each sample, the relative gene expression of inotocin and its receptor was measured by real-time quantitative PCR. Data were normalised by two previously evaluated reference genes. Changes and differences in the expression levels of inotocin and its receptor were analysed using mixed-effects linear models. We found that expression levels of both molecules were significantly higher in the course of parental care than in the period of mate choice, however, no consistent difference was found between the sexes. Interestingly, receptor expression levels in head samples were significantly higher compared to thorax samples. Our results are in line with the hypothesis that inotocin may be involved in the regulation of parental care of *Lethrus apterus*.

P-26

DINeR- TWO YEARS ON: MORE DATA, MORE FEATURES

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Insect neuropeptides are responsible for regulating a variety of functions, including development, metabolism, mating, water and ion homeostasis and reproduction.

The Database for Insect Neuropeptide Research (DINeR) has been created to provide a consolidated, comprehensive and standardized resource for neuropeptide information in insect species. *DINeR* is an intuitive and user-friendly, 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.

The data includes comprehensive sequence information for each neuropeptide family including location and known functionality. Visual representations of sequence alignments per order and cladograms per neuropeptide provide information on conservation, phylogeny and evolution of neuropeptides across class Insecta.

The database is updated when studies and data are made publicly available. At the time of the last database update in April 2018, the curated data included 53 families of neuropeptides from 540 different insect species. Approximately 5200-neuropeptide isoform amino acid sequences in FASTA format and over 200 records of physiological functionality have been recorded. Newer neuropeptide families such as Anti-diuretic Factor (ADF), Allatostatin triple C (AstCCC) and Tryptopyrokinins (tPK) and their identified peptide sequences have been recorded. DINeR is also a repository for images of receptor-binding assays of the neuropeptides and more images are being uploaded on a periodic basis.

New DINeR features include updated search functionality options including search by order or by common name. The calculated molecular weight of each isoform peptide sequence is now displayed in the results. The administrative section of DINeR has also seen many improvements; including more accurate data upload features and privacy settings for super-users, administrators and consortium members.

DINeR is an output of the H2020 nEUROSTRESSPEP programme dedicated developing peptide-based bioinsecticides. It is available at the official project website: http://www.neurostresspep.eu

P-27

PHYLOGENETIC AND GENOMIC ANALYSIS OF KISSPEPTIN-TYPE SIGNALLING IN ECHINODERMS

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The neuropeptide kisspeptin is a regulator of sexual maturation and reproductive function in mammals and other vertebrates. Investigation of the phylogenetic distribution of kisspeptin-type receptors indicates that the evolutionary origin of kisspeptin sigalling can be traced to the common ancestor of bilaterian animals. However, little is known about the kisspeptin signalling in invertebrates. Here we have investigated the occurrence of genes encoding kisspeptin-type precursors and receptors in a deuterostomian phylum – the echinoderms. Analysis of transcriptome sequence data from the starfish *Asterias rubens* has revealed transcripts encoding a precursor protein (ArKPP) that comprises two kisspeptin-like peptides (ArKP1 and ArKP2) and nine kisspeptin-type receptors (ArKPR1-9). Analysis of genome sequence data enabled identification of genes encoding kisspeptin-type precursors and receptors in the starfish *Acanthaster planci* and the sea urchin *Strongylocentrotus purpuratus.* Furthermore, phylogenetic analysis of echinoderm kisspeptin-type receptors reveals four distinct clades: Clade 1 that includes ArKPR1, clade 2 that includes ArKPR2-4, clade 3 that includes ArKPR5-7 and clade 4 that includes ArKPR8-9. Interestingly, genomic analysis of *A. planci* and *S. purpuratus* reveals that genes in the same clade are typically located on the same scaffold, which is indicative of local gene duplication having given rise to expanded gene families in each of four receptor clades. Furthermore, experimental studies have revealed that ArKP2 acts as a ligand for the clade 1 receptor ArKPR1, whilst ArKP1 acts as a ligand for the clade 4 receptors ArKPR8 and ArKPR9 (see also abstract by Semmens et al.). In conclusion, analysis of the occurrence of genes encoding kisspeptin receptors in echinoderms has revealed an expanded family of receptors by comparison with the single kisspeptin receptor in humans and other mammals. Our findings provide new insights into the evolution of kisspeptin signalling in the animal kingdom.

P-28

CHARACTERIZATION OF GONADOTROPIN-RELEASING HORMONE (GnRH) GENES FROM CARTILAGINOUS FISH: EVOLUTIONARY PERSPECTIVES

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The neuropeptide gonadotropin-releasing hormone (GnRH) plays an important role in the control of reproductive functions. Vertebrates possess multiple GnRH forms that are classified into three main groups, namely GnRH1, GnRH2 and GnRH3. In order to gain more insights into the *GnRH* gene family in vertebrates, we sought to identify which paralogs of this family are present in cartilaginous fish. For this purpose, we searched the genomes and/or transcriptomes of three representative species of this group, the small-spotted catshark, *Scyliorhinus canicula,* the whale shark, *Rhincodon typus* and the elephant shark *Callorhinchus milii*. In each species, we report the identification of three *GnRH* genes. In catshark and whale shark, phylogenetic and synteny analysis showed that these three genes correspond to *GnRH1*, *GnRH2* and *GnRH3*. In both species, *GnRH1* was found to encode a novel form of GnRH whose primary structure was determined as follows: QHWSFDLRPG. In elephant shark, the three genes correspond to GnRH1a and GnRH1b, two copies of the *GnRH1* gene, plus *GnRH2*. 3D structure prediction of the three catshark GnRH-associated peptides (GAPs) revealed that GAP1 and GAP2 peptides exhibit a helix-loop-helix (HLH) structure. As for all other GAP3 described, no typical 3D HLH structure was observed for catshark GAP3. RT-PCR analysis showed that *GnRH1*, *GnRH2* and *GnRH3* genes are differentially expressed in the catshark brain. *GnRH1* mRNA was predominant in the diencephalon while *GnRH2* and *GnRH3* mRNAs were most abundant in the mesencephalon and telencephalon, respectively. Taken together, our results show that the *GnRH* gene repertoire of the vertebrate ancestor was entirely conserved in the chondrichthyan lineage but that the *GnRH3* gene was probably lost in holocephali. They also suggest that the three *GnRH* neuronal systems previously described in bony vertebrates are also present in cartilaginous fish.
(*Work supported by ANR NEMO*)

LATE BREAKING POSTERS (P-29 to P-41)

P-29

UNRAVELING THE ROLE OF A SOMATOSTATIN-LIKE NEUROPEPTIDE SYSTEM IN C. ELEGANS

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Behavioral plasticity is one of the most remarkable features of nervous systems. To date, we know that neuropeptides - a diverse group of neuron-secreted messenger molecules - are key modulators of behavioral plasticity, but their mode of action remains poorly understood. With its well-defined nervous system and the availability of powerful genetic tools, the nematode *C. elegans* offers a well-suited model system to unravel the functions of neuropeptides in behavioral modulation. Despite its small nervous system of 302 neurons, *C. elegans* shows a variety of complex behaviors. The *C. elegans* genome encodes over 250 bioactive peptides and 150 putative neuropeptide receptors. Using phylogenetic and reverse pharmacology approaches, we have identified a neuropeptide system that is related to somatostatin signaling in vertebrates. In an *in vitro* calcium mobilization assay, somatostatin-related neuropeptides dose-dependently activated *C. elegans* somatostatin-like receptors at nanomolar concentrations. Vertebrate somatostatin is a cyclic peptide that is involved in neuroendocrine regulation as well as in sensory processes such as nociception, motor and cognitive functions including learning and memory. By combining expression analyses with genetic and behavioral assays, we are further investigating the role of somatostatin-like signaling in

P-30

STANNIOCALCIN AND PARATHYROID HORMONE RELATED PROTEIN PROMOTE GLUCONEOGENESIS AND LIPID METABOLISM IN EUROPEAN SEA BASS LIVER

Pedro Palma, Dr Bruno Louro, Dr Patricia Pinto, Professor Deborah Power, Dr Pedro Guerreiro, **Professor Adelino Canario**

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Stanniocalcin 1 (STC1) and parathyroid hormone-related protein (PTHrP) are best known, respectively, as hypocalcemic and hypercalcemic hormones in teleost fishes. In the present study we tested the hypothesis that STC1 and PTHrP have a metabolic role in fish. Juvenile European sea bass (*Dicentrarchus labrax*) were IP-injected with the pro-PTHrP treatments, i) PTHrP(1-34) or ii) a combination of PTHrP(1-34) together with STC1 antiserum, or the pro-STC1 treatments iii) PTHrP(7-34) - a PTHrP antagonist -, or iv) PTHrP(7-34) together with STC1, or v) a saline (control). Liver samples were collected 6h and 24h later. Liver of pro-STC1 groups had increased concentrations of the branched-chain amino acids alanine, glutamine and glutamate suggesting their mobilization for degradation and gluconeogenesis. The STC1 treatment decreased the concentrations of succinate, fumarate and acetate, indicating a slowing of the citric acid cycle. Pro-PTHrP groups had decreased concentrations of glucose, erythritol and lactate, indicative of gluconeogenesis from lactate, and hepatic glucose export to peripheral tissues. Taurine, TMA, TMAO and carnitine concentrations changed in opposite directions in the pro-STC1 versus the pro-PTHrP groups. STC1 appears to stimulate lipogenesis and PTHrP appears to activate lipolysis/β-oxidation of fatty acids. Transcriptome analysis revealed treatments caused enrichment carbohydrate, amino acid and lipid metabolic processes. KEGG analysis of DE genes revealed enrichment with genes related to intermediary, amino acid and lipid metabolism, and indicating that PTHrP was associated with lipolysis while STC1 stimulated lipogenesis. Overall, the results from metabolomics and transcriptomics were concordant, supporting the hypothesis that PTHrP and STC1 modify liver energy metabolism promoting gluconeogenesis and potentially have an antagonistic role on lipid metabolism.

This work received funds from Portuguese Foundation for Science and Technology (FCT) through projects PTDC/MAR/121279/2010 and UID/Multi/04326/2013 and fellowships to PFSP (SFRH/BD/103185/2014), BL (SFRH/BPD/89889/2012) and PISP (SFRH/BPD/84033/2012).

P-31

β2-adrenoceptor AGONISTS INDUCE PROLIFERATION AND LIPID METABOLISM OF GILTHEAD SEA BREAM CULTURED MUSCLE CELLS

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β_2-adrenoceptors are a subtype of G-protein coupled receptors whose activation in mammalian skeletal muscle causes hypertrophy, through increased protein synthesis and decreased degradation. Use of β_2-agonists as dietary additives has been successful as means to optimize livestock production, but similar information on fish species is scarce. This work evaluates on gilthead sea bream (*Sparus aurata*) primary cultured muscle cells the effects of the β_2-agonists noradrenaline (NA), formoterol (FOR) and salmeterol (SALM) to determine its potential use in the aquaculture industry. Activation of signaling pathways, cellular development and gene expression of relevant growth-related molecules were analyzed in day 4 myocytes. The three agonists increased either cAMP levels or PKA phosphorylation, plus TOR phosphorylation, demonstrating that these fish cells are β_2-sensitive. Furthermore, the percentage of proliferating cell nuclear antigen (PCNA)-positive cells increased together with *pcna* mRNA levels, while the gene expression of the myogenic factor *myf5* was significantly down-regulated; thus, suggesting enhanced proliferation of cells committed to the muscular linage. With regards to insulin-like growth factors, β_2-agonists treatments up-regulated *igf-1* and *igf-2* mRNA levels, proposing an additional anabolic effect through their local muscular production. Moreover, specifically incubation with SALM up-regulated the gene expression of the lipases *hsl* and *lipa* and the β-oxidation marker *cpt1a*, and all three agonists increased mitochondrial dehydrogenase *hadh* mRNA levels; a situation that agrees with enhanced lipolysis (supported by increased glycerol released into the media) and fat oxidation capacity. Overall, these data suggest that a condition of hyperplastic growth with a favorable protein/fat ratio profile is taking place upon treatments; therefore, advising that β_2-agonists (especially SALM) may be considered good candidates to be included in the diet of such important aquaculture species as gilthead sea bream to optimize its growth and flesh quality. Supported by MINECO (AGL2012-39768, AGL2015-70679-R, AGL2014-57974-R), Generalitat de Catalunya (2014SGR-01371) and European Union (LIFECYCLE FP7-222719).

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CALCITOX-METAMORPHOSIS IN INSECTS: OVERLOOKED ASPECTS IN HORMONAL SIGNALLING

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In hemimetabolous insects with their gradual development the Juvenile Hormone (JH) titre never drops to zero. In contrast, in holometabolous insects such drastic drop occurs at the onset of the last larval instar. It triggers the spectacular drastic morphological and physiological changes, e.g. from caterpillar to butterfly in Lepidoptera. How does *absence* of JH signal? In other words, which molecular mechanisms are inhibited by high titres of JH which are instrumental to maintaining the larval stage? A partial answer was already (unintentionally) given almost 20 years ago by Roullet et al. (1999) and Luft et al. (1999), vertebrate electrophysiologists working with rodent models, not with insects. They showed that all-*trans*-farnesol, a precursor of all JHs and itself a compound with moderate JH activity, is a rapid open channel blocker of all types of high voltage-activated calcium channels. JHs likely use a similar type of membrane receptor. Another breakthrough came from Cai et al. (2014) and other Chinese research teams who identified a GPCR-type plasma membrane receptor for 20E (named ErGPCR-2) that facilitates the entry of Ca^{2+}. Both data urge to abandon the contemporary erroneous view that only nuclear receptors for JH and 20E, without major links to the Ca^{2+} homeostasis system really matter. No hormone can pass the plasma membrane without starting signalling there. Thus, the full mode of action of JHs and Ecdysteroids can only be understood if their link with the Ca^{2+}-homeostasis system is taken into account. Farnesol and its JH-esters are formed by the mevalonate biosynthetic pathway which is evolutionarily ancient, present in all eukaryotes, and multifunctional. Its major (undervalued) function likely resides in being part of the Ca^{2+}-homeostasis controlling system. The picture emerges that endogenous farnesol-like sesquiterpenoids act as horseshoe-shaped flexible chemical valves that can restrict the untimely entry of Ca^{2+} through selected transmembrane proteins.

P-33

REPRODUCTIVE DYNAMICS OF SWORDFISH (XIPHIAS GLADIUS) IN THE MEDITERRANEAN SEA: NEW INSIGHT FROM FOURIER TRANSFORM INFRARED MICROSPECTROSCOPY (FTIRM) ASSAY

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Swordfish (Xiphias gladius) is an important commercial species with an extensive seasonal migration and a circumglobal distribution. In the last twenty years, catches have decreased by almost 50% and too many juveniles are caught before they reach gonadal maturity. According to the most recent stock assessment of the International Commission for the Conservation of Atlantic Tunas (ICCAT), the Mediterranean swordfish stock is overfished and suffering overfishing. In this light, comprehensive information on gonad development such as the time of spawning and the size at maturity became necessary to determine their reproductive potential in the Mediterranean Sea.

To clarify the seasonal maturation dynamics and reproductive biology of swordfish in the central Mediterranean Sea (Sardinia and Sicily), were monitored by the histological analysis of gonadal development in 116 females caught by the domestic longline fishery fleet over 7 different months in two consecutive year.

The relation between the size and maturity was analysed using the least squares estimation procedure and the size at first maturity was estimated to be 139.2±2.1. The monthly distribution of females at different ovarian maturation stages (previtellogenic, vitellogenic and spawing) established by ovarian histological examination and Gonadal index calculation, showed that reproductive potential increased steadily from June to reach a maximum around July, and then decreased remaining very low from September to May.

The topographical distribution of lipids, proteins, phosphates and carbohydrates within oocytes at different developmental stages was assessed by FTIRM, providing macromolecular characterization of vitellogenin vesicles, beyond macromolecular changes of cortical alveoli and oil droplets during maturation phase. Concomitantly, macromolecular properties of Zona Radiata in oocytes at previtellogenic stages were compared to that of late vitellogenic stage and mature oocytes. An increase in width and changing in its protein, carbohydrates and lipids content was found along the oocyte maturation process.

In conclusion, such results provide a better understanding of the reproductive biology of this endangered species with remarkable improvements towards a sustainable stock management.

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P-35

LIGHT AND ELECTRON MICROSCOPIC CHARACTERIZATION OF THE PUTATIVE NEUROHEMAL ORGAN OF THE SUPRAPHARYNGEAL GANGLION ('BRAIN') IN THE EARTHWORM EISENIA ANDREI

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A characteristic capillary bed was identified in the earthworm brain with gross anatomical and histological methods. The dorsal midsagittal blood vessel of the pharynx entered the brain and ramified to capillaries from which a single blood vessel, which ran to the prostomium, collected the blood. Close to the capillary bed medium sized neurons were located and their relatively short axons formed close contacts with capillary walls. These neurons proved to be peptidergic ones stained with a specific monoclonal antibody raised against both insect periviscerokinins (PVK) and pyrokinins (PK) so-called CAPA peptides. Two distinct groups of earthworm CAPA homologues XPRLamides and FVRIamides were identified in the whole central nervous system of *E. andrei*. The spatial expression pattern of the XPRLamides and FVRIamides coding genes in the brain was determined by in situ hybridization techniques that revealed that only FVRIamides expression was characteristic of brain neurons. Ultrastructural observations showed that the perikarya contained numerous dense granules that often occurred in clusters separated by rough endoplasmic reticulum cisternae. A few dilated cisternae of smooth endoplasmic reticulum, well developed Golgi complex and a few autophagic bodies typically appeared. Cytoplasm was rich in free ribosomes, mitochondria and glycogen rosettes. High number of granules was seen in axon hillock and the end foot of neurons was tightly packed with dense and grey granules. Exocytosis from perikarya seems to be a release mechanism for only some granules, however, most of them released through synapses or was liberated extrasinaptically from neural profiles to the neuropile. Omega profiles, thought to be characteristic of neurosecretory cells, frequently occurred also in the end foot of neurons attached to capillary walls. Based on the cytological characteristics of the stained neurons we can propose that the identified capillary bed of the earthworm brain with connecting neurons form a neurohemal organ in earthworms that functions as storage-and-release centre for neurohormones. The physiological and phylogenetic significances of the identified structure warrant further investigations.

P-36

RESPONSE OF THE MUSCULOSKELETAL SYSTEM TO FASTING AND REFEEDING IN GILTHEAD SEA BREAM (SPARUS AURATA)

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Gilthead seabream (*Sparus aurata*) is one of the most important species in Mediterranean aquaculture in terms of production. Given its importance, the aim of this work was to broaden the knowledge of this species' musculoskeletal system regulation during fasting (21 days) and refeeding (2, 5, 24 hours and 7 days), to help further consolidate this industry. The expression of relevant genes of the growth hormone (GH)/insulin-like growth factor (IGF) axis and other growth regulators in skeletal muscle and bone were studied using real-time quantitative PCR. Moreover, western blot analyses were performed to determine signaling and regulatory proteins levels during the experimental trial. Fasting caused a significant down-regulation of key growth-promoting genes from the GH/IGF axis such as *igf-1* and its downstream molecules like *tor*, myogenesis and osteogenesis regulatory factors (i.e. *myf5; mrf4* and *osteonectin; osteocalcin*, respectively) and most of the proteolytic systems-related genes. Nevertheless, catabolic genes such as the GH-receptor *ghr2*, some proteolytic molecules (i.e. *n3*, *murf1* and *mafbx*) and the osteoclastic cathepsin *ctsk* increased, suggesting a metabolic switch in order to cover the nutritional requirements. In contrast, refeeding reversed the effects caused by fasting in most of the evaluated genes, indicating a return to an anabolic state in both, muscle and bone. With regards to protein expression the IGF-1 signaling molecules AKT and TOR were significantly activated at the onset of refeeding, whereas the osteoclastic CTSK that increased with fasting was decreased. Furthermore, CTSD in muscle and matrix metalloproteinase MMP9 in bone showed a similar pattern decreasing with fasting without postprandial recovery. Interestingly, the expression of few muscle regulatory genes was also detected in bone (i.e. *myod2*), suggesting that both tissues respond to nutritional status in a coordinated way to regulate proper synchronic body growth. Supported by funds from the MINECO (AGL2014-57974-R; AGL2015-70679-R) and Generalitat de Catalunya (2014SGR-01371; XRAq).

P-37

THE NEURAL STEM CELL NICHE IN THE ADULT HYPOTHALAMUS: A COMPARATIVE STUDY IN THE MOUSE, RAT, GRAY MOUSE LEMUR (MICROCEBUS MURINUS) AND HUMAN

Dr Giuliana Pellegrino[1], Ms Claire Trubert[1], Dr Jérémy Terrien[2], Dr Fabien Pifferi[2], Ms Danièle Leroy[1], Ms Anne Loyens[1], Dr Martine Migaud[3], Dr Marc Baroncini[4], Dr Claude-Alain Maurage[4], Dr Christian Fontaine[4], Dr Vincent Prévot[1], **Dr Ariane Sharif[1]**

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The adult brain contains niches of neural stem cells that continuously add new neurons to selected circuits throughout life. Besides the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus, which have been extensively studied in various mammalian species including humans, a third neurogenic niche has recently been identified in the adult hypothalamus of several animal models, mostly rodents. In order to evaluate whether a neural stem cell niche also exists in the adult hypothalamus in humans, we performed multiple immunofluorescent stainings to assess the expression of a panel of neural stem/progenitor cell (NPC) markers (Sox2, nestin, vimentin, GLAST, GFAP) in the human hypothalamus in comparison with the mouse, rat and a non-human primate species, the grey mouse lemur (*Microcebus murinus*). Our results show that the adult human hypothalamus contains four populations of cells co-expressing the five NPC markers: i) a ribbon of small stellate cells that lines the third ventricular wall behind a hypocellular gap and is similar to that found along the lateral ventricles, ii) ependymal cells, iii) tanycytes, which line the floor of the third ventricle in the tuberal region, and iv) a population of small stellate cells in the suprachiasmatic nucleus. In the mouse, rat and mouse lemur hypothalamus, co-expression of NPC markers is essentially restricted to tanycytes and these species lack a ventricular ribbon. Altogether, we identify in the adult human hypothalamus four distinctive cell populations harbouring an antigenic profile of neural stem cells, three of which appear specific to humans.

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CHARACTERIZATION OF CORAZONIN RECEPTOR IN THE ORIENTAL FRUIT FLY WITH A ROLE IN LARVAL-PUPAL TRANSITION AND ECDYSIS BEHAVIOR

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Corazonin (CZ) is a highly conserved neuropeptide hormone of widespread distribution in insects and it has diverse physiological functions. Here, we identified and cloned the *Bactrocera dorsalis* genes that encode CZ and its receptor CZR. Mature *BdCZ* has 11 residues with a unique Ser^{11} substitution (instead of the typical Asn) and a His in the evolutionary variable position 7. The *BdCZR* cDNA encodes a putative protein of 608 amino acids with 7 putative transmembrane domains, typical for the structure of G-protein-coupled receptors. When expressed in Chinese hamster ovary (CHO) cells, the *BdCZR* exhibited a high sensitivity and selectivity for CZ ($EC_{50} \approx 52.5$ nM). With qPCR, the developmental stage and tissue-specific expression profiles in *B. dorsalis* demonstrated that both *BdCZ* and *BdCZR* were highly expressed in the larval stage, and *BdCZR* peaked in 2-day-old 3rd-instar larvae, suggesting that the *BdCZR* may play an important role in the larval-pupal transition behavior. Immunochemical localization confirmed the production of CZ in the central nervous system (CNS), specifically by a group of three neurons in the dorso-lateral protocerebrum and eight pairs of lateral neurons in the ventral nerve cord. qPCR analysis located the *BdCZR* in both the CNS and epitracheal gland (EG), containing the Inka cells that are the source of ecdysis-triggering hormone (ETH). Importantly, dsRNA-*BdCZR*-mediated gene-silencing caused a delay in larval-pupal transition and blocked ecdysis behavior, and this phenomenon agreed with a delayed expression of the ETH, tyrosine hydroxylase and dopa-decarboxylase genes. Finally, injection of CZ in head-ligated larvae could rescue the effects. These findings provide a new insight into the roles of CZ signaling pathway components in *B. dorsalis* and support an important role of CZR in larval-pupal transition and ecdysis behavior.

Keywords: Neuropeptide; Corazonin; Corazonin receptor; *Bactrocera dorsalis*; Expression pattern; RNA interference; Larval-pupal transition; Ecdysis behavior

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CORAZONIN SIGNALING IS REQUIRED IN THE MALE FOR SPERM TRANSFER IN THE ORIENTAL FRUIT FLY BACTROCERA DORSALIS

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Corazonin (Crz) is a widely distributed neuropeptide (or neurohormone) in insects with diverse physiological functions. The present study aimed to reveal the functions of Crz and its receptor (CrzR) in the regulation of sexual behavior and fertility in male *B. dorsalis*. Tissue-specific expression analyses showed that the *BdCrz* transcript was most abundant in the central nervous system (CNS), and the *BdCrzR* transcript was most abundant in both the fat body and CNS. Immunochemical localization confirmed that three pairs of Crz-immunoreactive neurons are located in the dorso-lateral protocerebrum region of male adult brain. Importantly, RNAi-mediated Crz knockdown lengthened mating duration in males, and knockdown of Crz or CrzR strongly decreased male fertility in the following three days, while the courtship behavior and mating efficiency were not affected. The reduced number of sperm in the reproductive organs of mated females indicated that Crz knockdown in males reduced sperm transfer. The findings of this study indicate that Crz contributes to the reproductive physiology of the oriental fruit fly *B. dorsalis* by regulating sperm transfer in male adults.
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Keywords: Corazonin, male adult, reproduction, sperm transfer, mating duration

P-40

ECDYSIS TRIGGERING HORMONE SIGNALING (ETH/ETHR-A) IS REQUIRED FOR THE LARVA-LARVA ECDYSIS IN BACTROCERA DORSALIS (DIPTERA: TEPHRITIDAE)

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Insects must undergo ecdysis for successful development and growth, and the ecdysis triggering hormone (ETH), released by the Inka cells, is a master hormone in this process. In this study, we determined the sequence of the ETH precursor and receptors in an agriculturally important pest insect, the oriental fruit fly *Bactrocera dorsalis* (Hendel). We identified two functionally distinct splice receptor isoforms: ETHR-A and ETHR-B, and when expressed in Chinese hamster ovary (CHO-WTA11) cells, they exhibited a high sensitivity to the two mature peptides BdETH1 and BdETH2. The *BdETH* transcript was detected in the tracheal tissue of the larvae. Inka cells were identified with immunohistochemical antibody staining against *Drosophila melanogaster* ETH1, and *in situ* hybridization with specific DNA probes. Selective RNA silencing of *ETH* or *ETHR-A*, but not of *ETHR-B*, caused developmental failure at ecdysis. The dsRNA-treated larvae displayed tracheal defects and could not shed the old cuticle followed by death. Our results demonstrated that ETH, via activation of ETHR-A but not ETHR-B, plays an essential role in regulating the process of larva-larva ecdysis in *B. dorsalis*.

Key words: *Bactrocera dorsalis*, Ecdysis-triggering hormone, Ecdysis, larva development, RNAi

P-41

THE ECDYSIS TRIGGERING HORMONE RECEPTOR ISOFORM B IS RESPONSIBLE FOR JUVENILE HORMONE-DEPENDENT VITELLOGENESIS IN A MAJOR PEST INSECT, BACTROCERA DORSALIS

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Ecdysis triggering hormone (ETH), released from the Inka cells, is a master hormone in regulating the ecdysis process in insects. ETH acts through two alternatively spliced variants ETHR-A and ETHR-B. We previously have demonstrated the role of ETHR-A in the ecdysis process in the larval stages the oriental fruit fly (*Bactrocera dorsalis*) that is one of the most important invasive pest insects in agriculture worldwide (Shi et al., 2017). In this study, we found that ETHR-B has a separate role in the female adult stage. In the female, expression of ETH was confirmed in the Inka cells at the tracheae by immunostaining and also *in vitro* exposure to ETH stimulated isolated adult corpora allata (CA). Temporal expression patterns of *ETH* and *ETHR-B* coincided with the time for ovarian development which occurs between day 10 and 15 after eclosion, while *ETHR-A* expression was undetectable. Elevated transcript levels of the Halloween genes *Spook* and *Shade*, and the vitellogenin genes *Vg1*, *Vg2* and *Vg3* also occurred at days 10-15 and an elevation of *JHAMT* was found on day 15. In addition, RNAi of ETH and ETHR, reducing the transcript levels by 62% and 56% of the control, respectively, resulted in decreased mRNA levels of *JHAMT* and *Vg2*, and most importantly decreased the JH titer and egg production. The RNAi phenotypes in the females were rescued by injection of either 20-hydroxyecdysone (20E) or the juvenile hormone (JH) mimetic methoprene. The data suggest ETH and ETHR-B, regulated upstream by 20E, are the direct signal for activation of JHAMT-mediated production of JH in the CA, which promotes ovarian development.

Key words: *Bactrocera dorsalis*, Ecdysis-triggering hormone, Juvenile hormone, 20-hydroxyecdysone, Vitellogenin, Reproduction, RNAi

P-43

ASSESSMENT OF SURVIVAL, REPRODUCTION AND FORAGING BEHAVIOR IN BUMBLEBEES (BOMBUS TERRESTRIS) BY EXPOSURE TO BENZETHONIUM CHLORIDE, A NON-PEPTIDYL AGONIST OF MYOSUPPRESSIN

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In this project we investigated for the potential side-effects of benzethonium chloride (Bztc) that is a non-peptidyl mimetic analogue of myosuppressin (TDVDHVFLRFamide) (Lange et al., 1995), on the buff-tailed bumblebee (*Bombus terrestris*), an important generalist pollinator of wild plants and many agricultural crops. We assessed for effects against survival, food intake, reproduction and foraging behaviour. The tests were done with queen-less bumblebee micro-colonies at standardized conditions (Mommaerts et al., 2010). The bumblebee workers were exposed via the drinking sugar water, starting from Bztc at 0.1, 1, 10, 100 to 1000 µg/ml. This work on environmental risk assessment with beneficial insects as pollinators was performed? in the frame of the development of neuropeptide analogues as novel insecticide agents.

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Mommaerts V, Reynders S, Boulet J, Besard L, Sterk G and Smagghe G, Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology* 19:207-215 (2010).

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EARLY CAREER RESEARCHER RAPID POSTER PRESENTATIONS

5B-P1

UNDERSTANDING WATER HOMEOSTASIS OF A PEST BEETLE

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Despite being the most species-rich group of insects, there is a lack of molecular studies in beetles (Order Coleoptera). This has hindered advancement of management techniques. *Hylobius abietis*, the large pine weevil, is a serious pest of European conifer forest. Following clearfelling of trees, large populations of *H. abietis* develop in the tree stumps. The emerging adults then feed on replanted seedlings, causing death due to girdling. Currently, chemicals are regularly used to control these pests but are economically costly as well as raising environmental and operator health concerns.
Beetles typically obtain water from their diet and therefore water homeostasis is tightly regulated. The Malpighian tubules serve a vital role in maintaining water and ion balance and are under the control of several neuropeptides. Understanding how this organ responds to neuropeptides will aid in analysing the importance of Malpighian tubules and water homeostasis in *H. abietis*.
Fluid secretion rate of *H. abietis* Malpighian tubules was measured using the modified Ramsay assay (Dow *et al,* 1994). The tubules were isolated intact from the adult female beetles and a cut was introduced in the suspended tubule to allow the tubule to secrete. Synthetic diuretic neuropeptides were synthesized based on the *Drosophila melanogaster* sequences. Basal and stimulated fluid secretion rates were measured before and after addition of these diuretic neuropeptides.
Data from these experiments suggest that Drome-DH44 modulates *H. abietis* tubule secretion rates and so could be an endogenous regulator of tubule function in this species. Drome-CAPA reduces secretion rate. However, Drome-DH31 did not affect secretion rates.

5B-P2

POSSIBLE INVOLVEMENT OF SEX STEROIDS IN OOCYTE DEVELOPMENT OF THE SCLERACTINIAN CORAL, ACROPORA TENUIS

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Scleractinian corals engage in mass spawning during the selected moon phase once a year. Since mass spawning of *Acropora tenuis* occurs around the full moon in May and June in Okinawa, Japan, it is possible that vitellogenesis of this species occurs rapidly towards full moon of these months. Although some past researches have paid attention to physiological roles of sex steroids in corals, their involvement in the processes of oocyte development in coral is not known. The aim of this study was to detect sex steroids including estradiol-17β (E2), testosterone (T), and progesterone (P) from branches of *A. tenuis*, and to clarify relationship between oogenesis and these steroids. Two coral branches were collected every month from the tagged *A. tenuis* in coral reefs around Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Japan. One branch was fixed for observations of histology, immunohistochemistry (vasa & vitellogenin), and in-situ hybridization (vasa and vitellogenin receptor), while another branch was frozen in liquid nitrogen for steroid hormone measurement using Liquid Chromatography – Mass Spectrometry (LC/MS). Histological observation revealed that oocytes developed steadily towards and disappeared after the spawning moon phase (full moon). Vitellogenin and its receptor showed strong signals in the oocytes and their surrounding tissues respectively. These results suggest that vitellogenesis actively underwent in each oocyte until gametes were released. Contrarily, vasa signal was strongly observed in oogonia and spermatogonia and became faint with the progress of vitellogenesis and spermatogenesis. LC/MS analyses revealed that three sex steroids were successfully detected. E2 could be detected in coral branches for three months before spawning but diminished after spawning, although this fluctuation was not evident in T and P. It is concluded that E2 detected in coral branches is related to vitellogenic processes in *A. tenuis*.

Keyword: coral reproduction, oocyte development, vitellogenesis, sex steroids, liquid chromatography mass spectrometry

5B-P3

CHARACTERIZATION OF A CHOLECYSTOKININ/SULFAKININ SIGNALLING SYSTEM IN A LOPHOTROCHOZOA, THE OYSTER CRASSOSTREA GIGAS

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Extended knowledge of the repertoires of neuropeptides and G Protein-coupled receptors (GPCRs) in the oyster *Crassostrea gigas* offers the opportunity to identify ligand/receptor pairs in a Lophotrochozoan animal. In *C. gigas*, two GPCRs (Cragi-CCKR1 and Cragi-CCKR2) displaying homologies and phylogenetic proximity with both the vertebrate Gastrin/cholecystokinin (G/CCKR) and the insect sulfakinin (SKR) receptors were characterized. A unique transcript encoding the precursor for two peptides exhibiting only subtle similarity with vertebrate CCKs and SKs was considered as the oyster CCK/SK homologue. The predicted peptides (Cragi-CCK1: pEGAWDY**DY**GLGGG**RF**a; Cragi-CCK2: F**DY**NFGGG**RWa**) share the C-terminal RF(W)amide with the SKs and the DY motif common to the CCK/SK peptide family. The Y residue of this conserved motif being often subjected to sulfatation. The presence of a second tyrosine residue in the sequence of Cragi-CCK1 suggests the possibility of a di-sulfated peptide. Therefore, Cragi-CCK1 peptides were synthesized in the disulfated form ([Y^6S-Y^8S] Cragi-CCK1), in the monosulfated forms ([Y^6S] Cragi-CCK1 and [Y^8S] Cragi-CCK1) and in the non-sulfated form (Cragi-CCK1). Cragi-CCK2 was synthesized in its likely sulfated form ([Y^3S] Cragi-CCK2) and in a non-sulfated form (Cragi-CCK2). To investigate the actual coupling of Cragi-CCKRs with these peptides, we challenged Cragi-CCKRs expressed in a mammalian (HEK) cell line with the oyster CCKs. Using a calcium mobilization assay, only Cragi-CCK1 peptides (except [Y^6S] Cragi-CCK1) activated both receptors in a dose dependent manner. Non-sulfated and specific sulfated peptides were characterized by mass spectrometry from visceral ganglia extracts indicating that they represent physiologically active forms. Expression of Cragi-CCK and Cragi-CCKR genes in various tissues and in relation with the nutritional status suggests the involvement of oyster CCK signalling in the regulation of feeding processes consistent with the myotropic activity of Cragi-CCK peptides on oyster hindgut.

5B-P4

PHYSIOLOGICAL AND BEHAVIOURAL EFFECTS OF AN ACUTE STRESS EVENT IN BROWN TROUT JUVENILES

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Stressful events, including food deprivation, transport or temperature changes, are responsible for triggering multiple neuroendocrine responses in fish under natural or experimental conditions. In general, fish are able to respond properly to challenging aggressions, although physiological and behavioural adaptations are required to re-establish homeostasis. The period needed for establishing this post-stress, and the parameters that best define the recovery of fish welfare, are essential aspects to be considered before an experiment. Thus, the proposals with this study were: 1) to monitor during 42 days the stress induced in brown trout (*Salmo trutta fario*) juveniles (1 year-old) exposed to an acute transport (2 h) between facilities, using biochemical, physical, nutritional and behavioural parameters; and 2) to define a minimum required acclimation period before assays. For those purposes, fish were monitored daily, and at least 6 animals were sampled at 12 hours, 7, 14, 28 and 42 days post-transport.

During the acclimation, fish show a preference for one area of the tank, and the fin beat amplitude and swimming movements decreased. Time to reaction to food decreased, until be nearly instantaneous, and food intake increased gradually. Glucose levels were relatively stable along the assay, as well as plasma cortisol concentrations. Cortisol was positively (linearly) correlated with the amount of pigmented skin (analysed at defined body areas), which decreased from day 14 on, compared to 12 hours and day 7. Both cholesterol and triglycerides decreased at the 7th and 14th days, compared to 12 hours, and then increased until the 42nd day.

Overall, we concluded that: (1) the functional stabilization of juveniles occurred between the 14 and 28 days post-transport; (2) a multi-parameter approach (behavioural, biometrical, biochemical…) is required for a systematic evaluation of the acclimatization process under experimental conditions.

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5B-P5

EFFECTS OF FEED RESTRICTION AND INTERMITTENT FASTING ON HEPATIC GENE EXPRESSION IN RED JUNGLEFOWL

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Caloric restriction and intermittent fasting procedures are routinely used in the rearing of broiler breeder chickens around the world to reduce obesity-related pathologies and infertility. While daily feed restriction is generally appreciated as the welfare-friendlier approach in poultry, intermittent fasting procedures are often described as beneficial for health and general well-being during weight loss and obesity prevention in mammals. Previous studies in restricted and intermittently fasted broiler breeders suggest that fasting procedures incur huge day-to-day fluctuations in liver mass that are largely attributed to increased glycogen stores and to a smaller extent increased lipid stores. This is not surprising but suggests that understanding the hepatic response to various forms of feed restriction may be crucial to better understand why intermittent fasting has so far appeared largely detrimental in poultry but beneficial in mammals.

To avoid the confounding effect of the extreme selection pressure exerted on broiler-type chickens we reared 24 chickens of the undomesticated breed Red Junglefowl under ad libitum feeding conditions (AL), calorie-restricted daily to 70% of AL intake (CR) or fed on a 2:1 intermittent fasting procedure (IF), i.e. two fully fed days preceded each fasting day. After 4 weeks on the varying feeding regimens liver samples were collected and gene expression was analyzed in a microarray. As expected, both CR and fasting during IF led to a general down-regulation of glycolytic enzymes and an upregulation of enzymes involved in fatty acid uptake, beta-oxidation and ketogenesis. There were also large differences in the expression of enzymes that add or subtract to the cytosolic acetyl-CoA pool, which would suggest hepatocytes are actively reducing this pool under fasting conditions and maximizing it under ad libitum conditions in a similar manner to what has recently been described in mammals.

5B-P6

EFFECTS OF VASOPRESSIN V1B RECEPTOR BLOCKADE ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA) IN OLD MONKEYS WITH DEPRESSION-LIKE AND ANXIETY-LIKE BEHAVIOR UNDER STRESS EXPOSURE OR VASOPRESSIN ADMINISTRATION

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Expanding the range of stress factors effecting on the human body in modern society and a sharp increase in the incidence of stress-dependent pathology determines the relevance of the problem of identification of persons with increased vulnerability to stress and the development of scientific approaches aimed at its prevention. Previously, we presented data indicating that the ACTH response to acute stress in old female rhesus monkeys with DAB is significantly higher compared to the ACTH response to similar effect in old females of the same species, but with the usual standard behavior (SB), which is presumably based on an increase in the vasopressinergic regulation of HPA responsiveness. In addition, in older monkeys with DAB in response to vasopressin administration the ACTH secretion was significantly higher than in older monkeys with SB. These results gave us reason to assume that in old macaques with DAB the activity of V1b receptors located on adenohypophysis corticotrophs is increased. The purpose of this study was to investigate effects of preliminary administration of a selective antagonist of vasopressin V1b receptors (Nelivaptan, SSR149415, the Netherlands) on ACTH and corticosteroid secretion in response to the tests with insulin-induced hypoglycemia and vasopressin injection in old female rhesus monkeys with DAB. In our studies, it was established that in old monkeys with DAB Nelivaptan administration (intravenous at a dose of 1.1-1.7 μg/kg body weight) leads to inhibition of the ACTH concentration increase induced by hypoglycemia or vasopressin injection. At the same time, the rises in cortisol and dehydroepiandrosterone sulfate concentrations did not change or increased. Effects of Nelivaptan prove that previously identified in old monkeys with DAB disorders of stress HPA reaction may occur due to excessive activation of Vlb receptors located on the pituitary corticotrophs, and the use of the V1b receptor antagonists is promising for its prevention.

CONTRIBUTED ORAL PRESENTATIONS

1A-O1

INTERACTION OF STARFISH GONADOTROPIN WITH ITS RECEPTOR: EFFECT OF CHIMERA RELAXIN-LIKE GONAD-STIMULATING PEPTIDES

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Relaxin-like gonad-stimulating peptide (RGP) of starfish is the first identified invertebrate gonadotropin to trigger final gamete maturation. RGP stimulates ovarian follicle cells to produce maturation-inducing hormone, 1-methyladenine (1-MeAde). In this sense, RGP is functionally analogous to the vertebrate luteinizing hormone. RGP is a heterodimeric peptide comprising an A- and B-chains with disulfide cross-linkages of one intra-chain and two inter-chain disulfide bonds. Recently, three orthologous of RGP molecules were found in the class Asteroida; PpeRGP of *Patiria pectinifera*, AamRGP of *Asterias amurensis*, and AjaRGP of *Aphelasterias japonica*. The chemical structure of AamRGP is close to that of AjaRGP, although the amino acid sequences of AamRGP and AjaRGP are quite different from that of PpeRGP. Cross-experiments among *P. pectinifera*, *A. amurensis*, and *A. japonica* showed that PpeRGP could induce oocyte maturation and ovulation in ovarian fragments of *A. amurensis*, and *A. japonica*. In contrast, neither AamRGP nor AjaRGP induced spawning in the ovary of *P. pectinifera*. This is possibly due to species specificity of an RGP receptor. Therefore, chimera RGPs, in which each A- and B-chains had been exchanged, were used to examine the interaction of RGP with its receptor. PpeRGP derivatives (Aam A-chain/Ppe B-chain and Aja A-chain/Ppe B-chain) replaced with the A-chain of either AamRGP or AjaRGP were failed to induce spawning in the *P. pectinifera* ovaries. On the contrary, the B-chain replaced PpeRGP derivatives (Ppe A-chain/Aam B-chain and Ppe A-chain/Aja B-chain) could induce oocyte maturation and ovulation in *P. pectinifera* ovaries but their spawning activities were relatively lower than that of PpeRGP. These results suggest that A-chains of RGP are important for spawning activity. It may be possible that the A-chain of RGP interacts with its receptor protein in follicle cells to produce 1-MeAde.

1A-O2

CHARACTERIZATION OF NEUROPEPTIDE-S/CCAP-TYPE SIGNALLING IN AN ECHINODERM REVEALS ROLES IN REGULATION OF FEEDING AND LOCOMOTION

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Neuropeptides in deuterostomian invertebrates that have an Asn-Gly motif (NG peptides) have been identified as orthologs of neuropeptide-S (NPS) in vertebrates and crustacean cardioactive peptide (CCAP) in protostomian invertebrates. Here we have characterised an NG peptide (NGP) signalling system in an echinoderm, demonstrating that the neuropeptide NGFFYamide is the ligand for an NPS/CCAP-type receptor in the starfish *Asterias rubens*. Using mRNA *in situ* hybridization, NGFFYamide precursor-expressing cells were revealed in the radial nerve cords, circumoral nerve ring, coelomic epithelium, apical muscle, stomach and tube feet of *A. rubens*, indicating that NGFFYamide has a variety of physiological roles in starfish. One of the most remarkable aspects of starfish biology is their feeding behaviour, where the stomach is everted out of the mouth over the soft tissue of prey. Previously, we reported that NGFFYamide triggers retraction of the everted stomach in *A. rubens* and here we show that *in vivo* injection of NGFFYamide causes a significant delay in the onset of feeding on prey. To investigate roles in regulation of other aspects of starfish physiology we examined the *in vitro* effects of NGFFYamide and found that it causes relaxation of apical muscle preparations and induction of tonic and phasic contractions of tube feet. Furthermore, analysis of the effects of *in vivo* injection of NGFFYamide on starfish locomotor activity revealed that it causes a significant reduction in mean velocity and distance travelled. Importantly, experimental studies on mammals have revealed that NPS is an anxiolytic that suppresses appetite and induces hyperactivity in mammals. Therefore, our discovery that NGFFYamide inhibits feeding and locomotion in starfish is interesting because it indicates that the NPS/NGP-type signalling system is an evolutionarily ancient regulator of feeding and locomotion in deuterostomes.

1A-O3

CHARACTERISATION OF SOMATOSTATIN-TYPE SIGNALLING IN AN ECHINODERM

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The neuropeptide somatostatin (SS) is an important regulator of hormone release in mammals that inhibits secretion of growth hormone, insulin and glucagon. SS also acts as a modulator of neurotransmission in the brain. SS signalling has been characterised in non-mammalian vertebrates and a homologue of SS, allatostatin-C (ASTC), has been characterised in protostomian invertebrates; e.g. causing inhibition of juvenile hormone synthesis in insects.

To gain insights into the evolution of SS/ASTC-type signalling, it is of interest to investigate the occurrence and functions of this signalling system in deuterostomian invertebrates that are "evolutionary intermediates" of vertebrates and protostomes.

Here we have characterised SS-type signalling in an echinoderm – the starfish *Asterias rubens*. Two cDNAs encoding precursors of SS-type peptides were cloned and sequenced, ArSSP1 and ArSSP2, and the structures of the neuropeptides derived from these precursors (ArSS1 and ArSS2) were determined using mass spectrometry. Three SS-type receptors were identified (ArSSR1-3) and ArSS2 was identified as the ligand for ArSSR1 and ArSSR2. Analysis of the expression of ArSS1 using mRNA *in situ* hybridisation and immunohistochemistry revealed expression in the nervous system, digestive system and tube feet. Furthermore, *in vitro* pharmacological tests revealed that ArSS1 causes dose-dependent contraction of cardiac stomach, tube foot and apical muscle preparations. Experiments investigating the expression and pharmacological actions of ArSS2 in *A. rubens* are on-going.

This is the first study to functionally characterise SS-type signalling in an echinoderm, providing new insights into the evolution and comparative physiology of SS/ASTC signalling in the animal kingdom.

1B-O1

CIRCADIAN CLOCK, SEASONALITY AND HORMONES IN INSECT MODEL, PYRRHOCORIS APTERUS

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Many organisms deal with daily and seasonal changes using their circadian clock and photoperiodic timer. The interplay between circadian clock, photoperiodic timer and hormonal signaling is poorly understood in insects, because Drosophila melanogaster has only very weak diapause phenotype. Therefore, we have established the Linden bug, Pyrrhocoris apterus, an insect with robust photoperiodically-regulated reproductive arrest, as a new model for insect chronobiology. Our previous results indicate connection between juvenile hormone (JH) signaling and circadian clock proteins in peripheral tissues. JH is primarily reproductive and metamorphosis-regulating factor. Often, action of JH is balanced and adjusted by ecdysteroid signaling. Here, we will discuss influence of JH and ecdysteroids on circadian clock as was revealed by our microsurgical experiments, RNAi silencing of selected genes and application of hormone-mimicking compounds.

1B-O2

LUNAR-RELATED CHANGES IN TRANSCRIPT LEVELS OF GONADOTROPIN-RELEASING HORMONES AND GONADOTROPINS: POSSIBLE ROLES OF MOONLIGHT AND MELATONIN

Mr. Kodai Fukunaga, Ms. Natsuki Ohta, Ms. Fumika Yamashina, Dr. Yuki Takeuchi, Dr. Hiroki Takekata, **Professor Akihiro Takemura**

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Most marine organisms inhabiting coral reefs repeat lunar-related gametogenesis at a one-month interval. Their gametes develop toward and are released around the specific lunar phase. To date, little is known about how the lunar-related gametogenesis is regulated endogenously. The present study aimed to clarify the expression profiles of gonadotropin-releasing hormones (*gnrh1* and *gnrh2*) in the diencephalon and gonadotropin *β*-subunits (*fshβ* and *lhβ*) in the pituitary of the honeycomb grouper *Epinrphelus merra*, which repeats gametogenesis toward the full moon (FM) during the spawning season. Effect of melatonin treatment on their abundance was also evaluated. When females were collected every week from May to June, gonadosomatic index (GSI) increased toward FM in June and then dropped to basal levels around the last quarter moon (LQM). Concomitantly, vitellogenic oocytes in an ovary developed toward FM and disappeared afterwards. Ovulatory follicles could be observed in the ovary around LQM, suggesting that spawning occurred between these lunar phases. Real-time quantitative polymerase chain reaction (qPCR) analyses revealed that *fshβ* and *lhβ* increased toward FM, while *gnrh1* and *gnrh2* peaked around LQM and the first quarter moon (FQM), respectively. It is suggested that gonadotropins play a role in gametogenesis and fluctuate with lunar phase. Intraperitoneal treatment with melatonin resulted in decrease in GSI as well as disappearance of vitellogenic oocytes in the ovary. In addition, this treatment lowered *fshβ* and *lhβ*, suggesting that melatonin has a negative impact on gametogenesis through alternation of gonadotropin levels. It is concluded that “brightness at night” is related to fluctuation of hormones at the HPG axis through melatonin.

1B-O3

ALTERNATE MELATONIN AND GLUCOCORTICOIDE TREATMENT FACILITATES THE RECONSTITUTION OF RHYTHMIC CLOCK GENE EXPRESSION IN COLON CANCER CELL LINES

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The circadian system regulates many physiological processes such as cell proliferation, apoptosis and metabolism in mammalian tissues and organs. This goal is achieved via the oscillatory activity of the endogenous clock in the SCN of the hypothalamus which transduces time information into hormonal and neural outputs to peripheral tissues. Malignant tumors and cancer-bearing hosts often exhibit altered circadian rhythms. These alterations consist of amplitude damping, phase shifts and/or the appearance of ultradian rhythms. Melatonin, synthesized at night by the pineal organ, encodes the phase and the length of the dark period and thus transduces photoperiodic information. The hormone and its agonists have been shown to entrain circadian rhythms in physiology and behavior.

To investigate whether a rhythmic melatonin signal is able to reconstitute the circadian rhythm in arrhythmic cancer cells, we stimulated different colon cancer cell lines, SW480, SW620, HT29 and RKO cells, for 3 consecutive days with either 1nM Melatonin (Mel) for 12h in the designated dark period, or with 100nM Dexamethasone (Dex) for 12h in the designated light period or with a combination of Mel given during the dark and Dex given during the light period. Unstimulated cells were used as control. After the 3-day stimulation period, we analyzed the expression of the clock gene Period1 (Per1) mRNA and protein levels at 4 different timepoints: 6h, 12h, 18h and 24h. Alternate application of Mel/Dex significantly reconstitutes a rhythmic expression of Per1 mRNA and protein in colon cancer cell lines, SW480 and SW620, which express both melatonin receptors (MT1 and MT2). RKO and HT29 cells, which only express the MT1 receptor, did not respond to the Mel/Dex treatment. Our data show that the alternate glucocorticoide and melatonin stimulation can reconstitute rhythmic Per1 expression in colon cancer cell lines. Furthermore, the melatonin signal seems to facilitate the reconstitution of rhythmic clock gene expression by acting upon MT2 receptors.

1C-O1

EFFECTS OF ETHYNILESTRADIOL ON THE ONTOGENESIS OF KISSPEPTINS AND GNRHS SYSTEM IN EUROPEAN SEA BASS

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The reproductive function is essential for the conservation of species. In vertebrates, this function is controlled by the brain pituitary gonadal axis (BPG). At the brain level, kisspeptin (Kiss) and gonadotropin-releasing hormone (GnRH) systems are known to be the main actors of the reproductive function. Moreover, they are largely influenced by the estrogenic environment.
In European sea bass (*Dicentrarchus labrax*), there is two kisspeptin (Kiss1 and Kiss2) and three GnRH isoforms (GnRH1, GnRH2 and GnRH3), which are expressed early during the larval development and for which we already described the setting up.

In the present study, we used a synthetic estrogen, the 17α-ethynilestradiol (EE2), to evaluate the impact of estrogen-like endocrine disruptors on the ontogenesis of GnRH and Kisspeptin systems in sea bass. Using a combination of real time quantitative PCR and *In Situ* Hybridization (ISH) techniques, we investigated the effects of EE2 exposition (0,5 and 50nM) on gene expression as well as on localization of kisspeptin and GnRH neurons from 0 to 10-days post hatching (DPH).

Our results showed that the expression of the different target genes was not significantly affected by EE2 exposition in total larvae. Regarding the cell localization, GnRH1 and kisspeptin expressing neurons do not seem to be affected by EE2 exposition. However, we observed a decrease in staining of GnRH2 and GnRH3 expressing neurons between control and exposed fish larvae.

Our results demonstrated that estrogenic compounds, like EE2, are able to disrupt the ontogenesis of GnRH2 and GnRH3 systems in the brain of European sea bass. An early exposition to estrogen-like endocrine disruptors during the larval development of sea bass could have effects on brain development and/or, on a long-time range, on the reproductive function.

1C-O3

OCEAN ACIDIFICATION IMPACTS THE REPRODUCTIVE PROCESS OF THE EUROPEAN SEA BASS

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Anthropogenic CO2 emissions are absorbed by the oceans, leading to decreasing pH and carbonate levels, a process known as ocean acidification (OA). Fish are considered effective acid-base regulators able to cope with increasing PCO2 by restoring plasma and tissue pH. As a result, fish exposed to PCO2 predicted by IPCC (Intergovernmental Panel on Climate Change) scenarios rarely show effects on growth and survival. However, the acid-base compensatory response leads to sub-lethal impacts on fish behavior and physiology.
To date few studies focused on OA impacts on reproduction despite the fact that this function is critical for population replenishment, is energetically expensive and sensitive to environmental constraints.
In this context we have investigated the OA effects on reproduction-related processes in European sea bass (*Dicentrarchus labrax*) chronically exposed (since 2 days post hatching) to three PCO2 conditions; present condition, (control, C, PCO2 = 590 µatm, pH = 8); low acidification (LA, 2050 IPCC scenario, PCO2 = 980 µatm, pH = 7.8); and high acidification (HA, 2100 IPCC scenario, PCO2 = 1520 µatm, pH = 7.6).

We have observed an earlier sexual maturation in females exposed to HA condition compared to C group, as shown by sexual steroids plasma profiles. This leads to an advanced spawning period of HA females.
OA also impacts fish gamete quality. Notably, males reared in acidified water show an earlier decrease of spermatozoa motility at the end of the breeding period.

Altogether, our findings suggest OA will lead to an advanced breeding season for the European sea bass, which means that larvae would have to cope with an unfavorable environment at least in terms of food availability.

Therefore, our data show that the projected increase in PCO2 during this century would impact the reproductive process of marine fishes, with likely consequences for population dynamics.

1C-O4

THE YIN AND YANG OF METAZOAN ENDOCRINE SYSTEMS IN THE ANTHROPOCENE EPOCH

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Metazoan endocrine systems encompass an intricate network of signaling cascades. When disturbed, episodes of disruption occur leading to developmental and physiological impairment. Over the past decades a flurry of studies disclosed the harmful effects resulting from the environmental exposure to Endocrine Disrupting Chemicals (EDCs), particularly in aquatic environments. EDCs act in many cases as high-affinity ligands of Nuclear Receptors (NRs). This ability to be ligand-activated is of crucial ecological relevance. Several studies have revealed NR exploitation (agonist/antagonist) by EDCs, with dire endocrine outcomes. Importantly, a variable NR gene repertoire has been appointed to Metazoan lineages with unknown repercussions in endocrine disruption. Decisively also, is whether orthologous receptors remained functionally stable over evolutionary time scales and with what consequences in endocrine disruption processes. Outside a handful of model species our knowledge remains sparse. Here, I will examine the power of comparative genomics to unravel the role of evolutionary processes to perceive endocrine disruption events in the Anthropocene Epoch.

2A-O1

THE GASTRIC CAECUM OF LARVAL AEDES AEGYPTI: REGIONALIZATION OF ION-MOTIVE ATPases, AND MODULATION OF ION TRANSPORT BY SEROTONIN AND NEUROPEPTIDES

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Gastric caeca (GC) are outpocketings of the anterior midgut of the larvae of some insects. In larvae of the yellow fever mosquito, Aedes aegypti, two ATPases, vacuolar-type H^+-ATPase (VA) and Na^+/K^+-ATPase (NKA), were hypothesized to be the main drivers of active ion transport and nutrient resorption across the GC. There is a striking regionalization of these ATPases along the GC: VA is expressed along the apical membrane throughout the length of the GC, with expression on both the apical and basal membrane along the distal third, whereas NKA is expressed only on the basal membrane of the proximal two-thirds of the GC. We provide the first measurements of H^+, K^+, and Na^+ fluxes across the distal and proximal GC, and have shown that fluxes differ in the two regions, consistent with regionalization of ion transporters. Ion fluxes across the GC decline rapidly in isolated gut preparations, as do basolateral membrane potential and transepithelial potential (TEP). Stimulation with serotonin (5-HT) restores both the TEP and active accumulation of H^+, K^+ and Na^+ in the GC lumen. Additionally, 5-HT restores the basolateral membrane potential (V_b) and H^+, K^+ and Na^+ fluxes across the distal GC, but has no effect on V_b or ion fluxes across the proximal GC. We also show the effects of neuropeptides, diuretic hormone 31 (DH31) and leucokinin (LK), on ion fluxes along the GC. DH31 restores K^+ fluxes across the distal GC, and LK restores Cl^- fluxes across the proximal GC. LK also restores the basolateral membrane potential in cells of the proximal GC, but not distal GC. Taken together, our data reveal similarities in the expression of ion transporters across the GC and Malpighian tubules, and suggests an important role for the GC in osmoregulation. These findings provide a platform for investigating the neuroendocrine regulation of the GC.

2A-O2

KISSPEPTINS AND GnRHs ONTOGENESIS IN THE BRAIN OF THE EUROPEAN SEA BASS, DICENTRARCHUS LABRAX

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In teleosts, as in vertebrates, the reproductive function is under the control of the brain pituitary gonadal (BPG) axis. In the brain part, the main 'actors' controlling this axis are the kisspeptins (Kiss) and the gonadotropin-releasing hormones (GnRH). These peptides are produced by different neuronal populations well described in adult brains. In sea bass (*Dicentrarchus labrax*), two kisspeptins (Kiss1 and Kiss2) and three GnRH isoforms (GnRH1, GnRH2 and GnRH3) have been characterized and cell localizations described in the adult brain. In contrast, their ontogenesis and particularly for Kiss neurons, are still poorly investigated. In teleost, the brain is still maturing after hatching and it is during this period that the Kiss and GnRH neurons are taking place.

In this study, we focused on the setting up of Kiss and GnRH neurons in the brain of the European sea bass. The description of ontogenesis was performed using a combination of quantitative real time PCR (qPCR) and *in situ* hybridization (ISH) techniques permitting to describe the dynamic of gene expression and cell localization during larval development.

Our results showed an expression of all the target genes as early as 4-days post hatching (DPH). Moreover, we observed different patterns of gene expression during the larval development: *gnrh2*, *gnrh3* and *kiss2* mRNA levels decreased whereas for *gnrh1* and *kiss1* mRNA levels increased. Regarding the brain localization, at 4DPH, *gnrh2* expressing cells were detected in mesencephalon, *gnrh3* and *kiss2* expressing cells were detected in prosencephalon. The first *kiss1* expressing cells were detected at 50DPH in the habenular area.

Our results demonstrate that the brain 'actors' of the BPG axis are taking place during larval development. This suggests that, in addition to their known implication in the control of reproduction, these peptides seem to play a role during larval development.

2A-O3

EARLY EVOLUTION OF GROWTH HORMONE RECEPTOR/PROLACTIN RECEPTOR IN SEA LAMPREY AND ITS FUNCTIONAL ROLE IN METAMORPHOSIS AND SEA WATER EXPOSURE

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A truncated GHR/PRLR like gene from sea lamprey was previously identified. This study has completed full sequence of the GHR/PRLR gene from sea lamprey and identified its functional roles in metamorphosis and sea water exposure. This gene includes 9 exons, encoding 629 amino acids. It shares the highest identity (35%) with coelacanth PRLR, 28% with spotted gar PRLR, and generally around 25% with GHRs and PRLRs from other species. It has the conserved TSXW and WSNWS motifs in the extracellular part, and the intracellular “box 1” and “box 2” motifs, as well as a conserved tyrosine residue closed to the C-terminus. Phylogenetic analysis suggests that the lamprey possess an ancestral gene encoding a common GHR/PRLR that diverged to give rise to distinct GHRs and PRLRs later in the course of vertebrate evolution.

Expression of lamprey GHR/PRLR mRNA was high in the gill, brain and kidney, moderate in gut, heart, and pituitary gland, and low in liver and muscle. In the gill, the *ghr/prlr* expression was progressively elevated during metamorphosis, and upregulation became significant in the latest stage (stage 7) compared with ammocoetes; higher *ghr/prlr* expression was observed in downstream-migrating post-metamorphic juveniles. In seawater (SW) exposure experiments, ammocoetes could not tolerate SW and had the lowest *ghr/prlr* expression in gill; *ghr/prlr* expression in gill was significantly higher in juveniles exposed to SW than in gill of ammocoetes in fresh water. These findings indicate that GHR/PRLR plays a role in growth and development of lamprey as well as in the acquisition of SW tolerance. (Supported by NSF grant IOS 1558037 to SDM and MAS)

2A-O4

DISTRIBUTION OF UROTENSIN II RECEPTOR IN THE AFRICAN CLAWED FROG PROVIDES INSIGHTS INTO NOVEL FUNCTIONS OF UROTENSIN II

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Urotensin II (UII) exhibits diverse physiological actions including vasoconstriction, locomotor activity, osmoregulation, and immune response via the UII receptor (UTR) in mammals. However, in non-mammals the function of the UII-UTR system remains unknown. We investigated the distribution of UTR expression in various tissues of the African clawed frog, *Xenopus laevis,* in order to identify target sites of UII. We found novel tissues (spleen and lung) that UTR is expressed by RT-PCR analysis. An immunohistochemical study showed that UTR was expressed in the spleen-resident macrophages and the chondrocytes of the lung. Because tissue-resident macrophages are produced by the differentiation of monocytes, we isolated leukocytes from peripheral blood using Percoll gradient centrifugation and then investigated the potential immune function of UII using leukocytes. Migration assays showed that UII and UII-related peptide (URP) enhanced migration of leukocytes, and that UII effect was inhibited by the UTR antagonist Urantide. Treatment of isolated leukocytes with UII increased the expression of several cytokine genes including tumor necrosis factor-α, interleukin-1β, and macrophage migration inhibitory factor, and the effects were abolished by Urantide. These results suggest that in amphibian leukocytes the UII-UTR system is involved in the activation of leukocyte migration and cytokine gene expression. Furthermore, the distribution of the UTR protein in the cartilage of the lung suggested a novel function for UII/URP. We revealed that UTR was expressed in chondrocytes of various hyaline cartilages, such as the interphalangeal joint and the sternum, and further showed that UTR expression in chondrocytes is common among tetrapods. Treatment of isolated *Xenopus* sternum with UII increased the expression of type2 collagen (Col2) which is produced by chondrocytes. Therefore, UTR may be involved in the formation of the cartilaginous matrix, such as in the production of the Col2.

2A-O5

TRANSCRIPTIONAL REGULATION OF THE CRHR2 GENE IN THE THYROTROPES OF THE CHICKEN PITUITARY GLAND

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Corticotropin-releasing hormone (CRH) is a well-known corticotropic factor in all vertebrate species. Additionally, CRH has been shown to act as a thyrotropic factor in some non-mammalian species, such as chicken and bullfrog. This is mediated by type 2 CRH receptor (CRHR2) expression on the thyrotropes in the pituitary gland. However, the response elements (REs) and their corresponding transcription factors (TFs) that control *CRHR2* expression in thyrotropes has not been investigated. Since thyrotrope-specific expression of thyrotropin-□ is synergistically stimulated by the co-expression of PIT1 and GATA2, we hypothesised that in non-mammalian vertebrate species like chicken, *CRHR2* expression would be controlled by the same TFs, in other words that these TFs would also be expressed in the thyrotropes of chickens and their REs present in the chicken *CRHR2* gene promoter. We used luciferase reporter assays to determine the effects of GATA2 on the activation of the putative chicken *CRHR2* promoter and the location of its REs. We show that GATA2 activated the promoter region of chicken *CRHR2*. A deletion analysis showed that essential GATA2 RE(s) are located between bp □116 and □198 upstream of the *CRHR2* start codon. Our results further indicate that steroidogenic factor 1 (SF1) suppresses GATA2-induced promoter activity, and therefore may play a role in restricting *CRHR2* expression in gonadotropes, which also express GATA2. In addition, the *CRHR2* promoter contains a conserved PITX1 RE in non-mammalian vertebrates, but the effect of PITX1 on CRHR2 promoter activation remains to be determined.

2B-O1

HOMOLOGUES OF THE VERTEBRATE CALCITONIN-LIKE SYSTEM IN MOLLUSCS AND THEIR POTENTIAL ROLE IN SHELL MINERALIZATION

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The shell is a natural calcified structure and the exoskeleton of many terrestrial and marine invertebrate species. This biomineralized structure is essential for survival and sustains and protects soft tissues and may also serve as a reservoir for minerals that contribute to whole animal homeostasis. The shells of marine molluscs are mostly composed of calcium carbonate crystals (CaCO3) within an organic protein matrix and shell growth and repair results from the secretory activity of the mantle. The increase in the number of available mollusc genomes, mantle transcriptomes and mantle/shell proteomes have shed light on candidate shell-forming genes and matrix proteins. However, the process by which the shell is produced and repaired still remains unclear and is proposed to be species-dependent. Recently, sequence homologues of the calcitonin system (peptide and receptors) that play a key role in vertebrate calcium metabolism and skeletal mineralization and promote calcium bone deposition have been described in molluscs. We hypothesize that the role of the calcitonin system emerged early during evolution and its function in calcium homeostasis has been conserved. In the present study we characterize the vertebrate calcitonin-like system (peptides and receptors) in different molluscs and in other invertebrates and compare it with the system in vertebrates. Homologues in sequence and structure were found but receptor gene number varied suggesting that gene evolution of this system was distinct across species. Evidence about the function of the calcitonin-like system in the shell-secreting mantle of the marine bivalve, the Mediterranean mussel (*Mytlilus galloprovincialis*), will be discussed.

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2B-O2

ZINC DEFICIENCY-INDUCE DOWNREGULATION OF Wnt/β-CATENIN SIGNALING PATHWAY

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WNTs are secreted signaling molecules which control cell differentiation and proliferation. They are known to play essential roles in various developmental processes. Wnt genes have been identified in a variety of animals, and it has been shown that their amino acid sequences are highly conserved throughout evolution. In view of this, using carp, *Cyprinus carpio* as animal model, cloning and expression analysis of *wnt5, wnt11* and *wnt8* in different tissues, at various developmental stages, during reproductive cycle, and after Zinc induction where the concentration of Zn in the testis increased in tandem with the progression of spermatogenesis. Staining of testicular cells with a Zn-specific fluorescent probe revealed that Zn accumulates in germ cells, particularly in the mitochondria of spermatogonia and spermatozoa. Using an *in vitro* testicular organ culture system for the *Cyprinus carpio*, production of a Zn deficiency by chelation with N,N,N′,N′-tetrakis (2-pyridylemethyl)ethylenediamine (TPEN) caused apoptosis of the germ cells. However, this cell death was rescued by the addition of Zn to the cultures. Furthermore, an induced deficiency of Zn by TPEN chelation was found to inhibit the germ cell proliferation induced by 11-ketotestosterone (KT), and Testosteron (T) a fish specific androgen, an inducer of spermatogonial stem-cell renewal. We also investigated the effects of Zn deficiency on steroidogenic genes, *wn*t and germ cell markers and observed that TPEN treatment of *Cyprinus carpio* spermatogonial stem cells down regulated. Our present results thus suggest that Zn is an essential trace element for the spermatogenesis through *wnt* signaling pathway.

2B-O3

THYROID HORMONE SIGNALING-RELATED GENE EXPRESSION IN THE HIND LIMBS OF THE DIRECT-DEVELOPING FROG *ELEUTHERODACTYLUS COQUI* AND *XENOPUS TROPICALIS*

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Direct development is a novel reproductive mode that has evolved independently in at least ten anuran lineages. Direct-developing frogs, including the Puerto Rican coquí, *Eleutherodactylus coqui*, hatch from terrestrial eggs as miniature adults. While a portion of their embryonic development resembles metamorphosis in several respects, many characters develop in a different sequence compared to those in metamorphosing frogs. For example, both limb growth and tail resorption in metamorphosing frogs occur following thyroid gland formation and well after hatching. In contrast, limbs in direct-developing frogs begin to form early in embryogenesis and well before the thyroid, suggesting that their development is thyroid hormone (TH) independent. Thus, changes in thyroid hormone provisioning, metabolism, or action may underlie the evolution of direct development. Embryonic expression of thyroid hormone receptors (TRs) and deiodinases in *E. coqui* suggest that the last third of limb growth is mediated by TH. These TR expression dynamics approximate those in the developing limb of metamorphosing *Xenopus laevis*, which is dependent on TH. TH concentrations also increase in the *E. coqui* embryo after thyroid gland formation, although there are maternal THs present. To further explore the hypothesis that limb development prior to thyroid gland development is TH independent, we sequenced the whole hind limb transcriptome of *Xenopus tropicalis* at NF 53, NF 56 and NF 58.5 and the morphologically equivalent stages of *E. coqui*. We predict that genes that are endogenously regulated by TH in *X. tropicalis* will increase in expression in *E. coqui* only after formation of the thyroid gland. This is the first RNAseq experiment to describe limb development in *X. tropicalis* and in a direct-developing frog. Results shed light on the evolution of direct development and the role of thyroid hormone signaling in amphibian life history evolution.

2B-O4

Na+/ H+ EXCHANGER REGULATORY FACTOR SIP1 MODULATES RENAL STONES FORMATION

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Nephrolithiasis is one of the most common kidney diseases with poorly understood pathophysiology. *Drosophila melanogaster* has been established as a model organism for renal diseases because of its genetic and functional similarities of *Drosophila* Malpighian tubules with the human kidney. Here, we inhibited the function of Sip1 (SRY-interacting protein 1) gene, which is a homolog of human Na^+/H^+ Exchanger Regulatory Factor (NHERF1). Deficiency of this gene led to abundant stone formation, whose solubility increased with increasing pH of the bathing solution. No stones accumulated in mutant tubules after feeding flies with allopurinol (an inhibitor of xanthine oxidase, and hence purine metabolism), suggesting that the stones were composed of uric acid. Co-transport of Na^+/H^+ in the tubule is mediated by Nhe2; which expressed in epithelial tissues and has specialized role in ion transport in tubules. Taken together, our results further increase the understanding of human kidney stone disease, which may lead to the identification of novel approaches to treatment.

Keywords: *Drosophila*, Nephrolithiasis, Malpighian tubules, Sip1, uric acid stones

2B-O5

ALTERATIONS IN THE EXPRESSION OF ESTROGEN RECEPTOR ALPHA IN THE BRAIN, OVARY AND SHELL GLAND AND REPRODUCTIVE PERFORMANCE OF JAPANESE QUAIL: MODULATION BY THE GONADAL STEROIDS AND STRESS

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Estrogens play an important role in the growth and differentiation of reproductive tissues via specific receptors (ER). ERα is expressed predominantly in the ovary and uterus of females. We tested the hypothesis that ERα in the chicken brain, ovary and shell gland are regulated during water deprivation stress in response to estrogen treatment. Immature chickens were divided into four groups. First group served as control (C), second water deprived for two days (WD), third treated with estradiol benzoate (E) and fourth treated with estradiol benzoate followed by water deprivation during last two days of treatment (E+WD). The dose of estradiol benzoate administered for 15 days was 0.5 mg/100g. ERα expression decreased on water depriving the immature chicks while treatment of estradiol during water deprivation increased their expression in all these tissues. Immuno-fluorescent studies established that water deprivation treatment down-regulated ERα in granulosa cells, brain POA area and in the tunica mucosa of the immature chicks. Estradiol administration in the immature chick exhibit predominant upregulation of ERα in these tissues compared to the controls. Plasma estradiol significantly increased in E and E+WD group while decreased on water deprivation. Plasma corticosterone decreased significantly in E and E+WD group while increased in WD. It appears that administration of estradiol in stress decreases corticosterone induced sensitivity mediated by an increased expression of ERα in brain, ovary and shell gland. The predominant ERα expression in the brain, ovary and shell gland after estradiol administration is consistent with an important biological role mediated by ERα in ovary and shell gland even during stress.

2C-O1

THE NEUROENDOCRINE CONTROL OF BEETLE RENAL FUNCTION AND ITS EVOLUTIONARY ORIGINS

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Insects have severe challenges in osmoregulation due to a high surface to volume ratio. This is particularly pronounced in species that inhabit osmotically hostile environments, such as the genetic model organism and global pest species, *Tribolium castaneum* (red flour beetle). However, the cellular mechanisms that underpin renal function and control in Coleoptera – the largest insect Order – is largely unknown. Here, we apply a combination of traditional physiological approaches and advanced genetic and molecular techniques to identify and characterise the neuroendocrine signals that control renal function in *Tribolium*. Using whole transcriptome shotgun sequencing (RNASeq) on dissected renal (Malpighian) tubules (MTs), we identified a gene, *TC34462*, that is most highly expressed in the MTs, and which encodes a receptor that shows high sequence homology to the vertebrate CRF receptor. Immunolocalization of the *TC34462* protein show that the receptor localizes to a small-nucleated cell-type in the main segment of the tubules, which when stimulated with the putative receptor ligands (DH47/37) results in a dramatic increase in primary urine production. Furthermore, we demonstrate that this increased fluid flow is mediated by DH47/37-induced changes in K^+-flux through this specialized cell-type. These data suggest that renal function is regulated in a fundamentally different way than all other insects. To test if the mode of action is conserved across coleopteran evolution, we mapped renal tissue architecture across the major beetle families. Application of fluorescently labelled analogues of both DH37 and DH47 neuropeptides localize to the same small-nucleated cell-type across the more 'advanced' beetle families, yet show a more generalized reactivity in the basal groups. Our data thus provide an unparalleled overview of renal function and control in the largest animal group on the planet – the beetles.

2C-O2

A COORDINATED TRANSCRIPTOMIC AND PEPTIDOMIC APPROACH FOR IDENTIFICATION OF NEUROPEPTIDES AND THEIR RECEPTORS IN THE LARGE PINE WEEVIL, HYLOBIUS ABIETIS, A MAJOR FORESTRY PEST

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Hylobius abietis (Linnaeus), or large pine weevil (Coleoptera, Curculionidae), is a pest of European coniferous forests. We have assembled a transcriptome of *H. abietis* in order to gain information about neuropeptide-modulated processes and the overall functional physiology of this species by identifying genes, which encode neuropeptides or peptide hormones, and their putative G-protein coupled receptors (GPCRs).

Illumina paired-end sequencing technology was performed on RNA extracted from whole adults, gut and central nervous system tissue samples. The sequence reads were used to assemble the *de novo* transcriptome, following which data analysis was performed for transcriptome completeness, annotation, gene ontology and functional assignment along with KEGG pathway analysis. Bioinformatics pipelines were created using popular tools and techniques for prediction and identification of neuropeptides and their receptors. A combination of MALDI-TOF and Q-Exactive Orbitrap mass spectrometry were used as part of a peptidomic analysis to confirm the identified neuropeptides.

Using the aforementioned pipelines and techniques, precursor sequences and mature peptides for a total of 41 putative neuropeptide families were identified in *H. abietis,* and these included Adipokinetic hormone (AKH), Pyrokinin, CAPA and DH31 among others. Neuropeptide F, which has not been yet identified in the model beetle *T. castaneum*, was identified. Using alignment as well as non-alignment methods, 25 putative neuropeptide receptor-encoding transcripts were identified. The sequence reads have been submitted to the NCBI sequence read archive repository (SRA accession: SRP133355), with the intent of making the transcriptome publicly available. This can be used to further the understanding of neuropeptide-modulated physiology and behaviour in *H. abietis*; and to develop specific neuropeptide-based tools for *H. abietis* control.

This work is an output of the H2020 nEUROSTRESSPEP programme dedicated developing peptide-based bio-insecticides. It is available at the official project website: http://www.neurostresspep.eu

3A-O1

ANALYSIS OF THE ROLE OF THE Mc4r SYSTEM ON DEVELOPMENT, GROWTH AND PUBERTY OF MEDAKA

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In vertebrates, puberty is a vital transitional period in reproductive physiology, being regulated by the hypothalamus-pituitary-gonad axis. In teleost fish of the genus *Xiphophorus* (platyfish and swordtails) the onset of puberty is regulated by a single locus, which encodes the melanocortin 4 receptor, *mc4r*. Mc4r belongs to the class A of GPCRs and is known to be involved in energy homeostasis. Wild populations of *Xiphophorus* are polymorphic for early and late-maturing individuals. Copy number variation of different *mc4r* alleles is responsible for the difference in puberty onset.

Medaka (*Oryzias latipes*) is a close relative to *Xiphophorus* and a well-established model for genetic modification, which is impossible in the live-bearing *Xiphophorus*. The role of Mc4r in puberty in medaka is still unknown.

We demonstrate by molecular phylogeny and synteny conservation analysis on Mc4r system genes (*mc4r*, *mrap2*, *pomc*, *agrp*) and on certain genes (*leptin*, *ghrelin*, *kisspeptin*) known to be involved in energy homeostasis and puberty, that the Mc4r system in medaka and *Xiphophorus* is generally conserved, but individual genes have considerably diverged in protein sequences and some genes are even missing in either fish.

RT-qPCR during development revealed that all Mc4r system genes are expressed before hatching, except for AgRP, which is strikingly upregulated after hatching, correlating with the initiation of feeding behavior. Mc4r system genes are mainly expressed in brain. By *in situ* hybridization we found that *mc4r* and *mrap2* are co-localized in the pre-optic region and hypothalamus in the adult brains.

Comparing growth and puberty between wild-type and *mc4r* knockout medaka revealed that absence of Mc4r does not change puberty timing but significantly delays hatching. Knockout females have shorter body length at puberty compared to wild-type females. In conclusion, the Mc4r system may correlate with growth rather than puberty in medaka, reminiscent to the situation described in zebrafish.

3A-O2

THE KISSPEPTIN SIGNALLING SYSTEM: INSIGHTS FROM AN ECHINODERM

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Kisspeptins are neuropeptides that trigger hypothalamic secretion of gonadotropin-releasing hormone (GnRH), leading to the release of gonadotropins from the pituitary. Kisspeptins are key regulators of reproductive maturation, with mutations in the kisspeptin receptor causing delayed puberty in humans. Kisspeptin signalling systems have been identified throughout the vertebrates and experimental studies in non-mammalian vertebrates have provided evidence of a conserved role in regulating reproductive maturation. In 2013, a comprehensive analysis of neuropeptide signalling systems across the Bilateria revealed the presence of kisspeptin-type receptors in non-chordate deuterostomes (echinoderms, hemichordates) and in lophotrochozoans (molluscs, annelids), but with loss in some lineages (urochordates, ecdysozoans). Interestingly, the analysis also revealed the presence of four genes encoding putative precursors of kisspeptin-type peptides in the cephalochordate *Branchiostoma floridae*. Recently, we reported the discovery of a precursor encoding two kisspeptin-type peptides (ArKP1-2) in an echinoderm – the starfish *Asterias* rubens. These were the first kisspeptin-type peptides to be identified outside of the chordate branch of the animal kingdom. We have determined the structures of ArKP1-2 using mass spectrometry (LC-ESI-MS/MS) and generated antibodies to ArKP1. Immunohistochemistry has revealed that ArKP1 is expressed in the nervous system, digestive system (e.g. cardiac stomach, pyloric stomach, pyloric ducts), tube feet and body wall associated structures in *A. rubens*. We have also identified nine candidate kisspeptin-type receptors in *A. rubens*, indicating that the kisspeptin signalling system is more “complex” than in the vertebrates. Furthermore, we have discovered that ArKP1-2 are ligands for at least three of the nine kisspeptin-type receptors. The characterisation of a kisspeptin signalling system in the starfish *A. rubens* has provided a framework to investigate the function of kisspeptin signalling in an invertebrate for the very first time and provide important new insights into the role of kisspeptins in the unique context of a decentralised nervous system and pentaradial body plan.

3C-O1

MOLECULAR GENETIC ANALYSES OF SEX PEPTIDE RECEPTOR AND ITS SIGNALING PEPTIDE LIGANDS IN MULTIPLE BEHAVIORAL PARADIGMS

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The small signaling peptide is the most ancient type of signaling molecules essential for the intercellular communication. The G-protein coupled receptor (GPCR) is the principal receptor that mediates actions of signaling peptides and critical for a plethora of biological processes, including metabolism, sleep-wake cycle, and reproduction. The sex peptide receptor (SPR) is a GPCR receptor for sex peptide (SP), the seminal fluid protein that induces the post-mating responses such as elevated egg-laying and strong mating refractoriness observed in the mated female Drosophila melanogaster. In addition to SP, SPR is also highly sensitive to other unrelated neuropeptides, myoinhibitory peptides (MIP). Unlike SP, MIP does not occur in the seminal fluid nor induces the post-mating responses. Instead, MIP and SPR in the brain regulate sleep by consolidating the sleep state. Also, MIP is crucial for the satiety induction. Mip mutant overfeeds and becomes obese. Unlike sleep behavior, however, the MIP-dependent satiety induction does not require SPR, suggesting that MIP signals through another yet-unknown receptor. Recently, we observe that MIP in the ventral nerve cord renders females engage in mating more readily, and it does so again SPR-independently. Together, our high resolution molecular genetic analyses of SPR and its peptide ligands SP and MIP uncover the presence of unexpected complex interactions between GPCR and its ligands.

3C-O2

NEUROPEPTIDES AND THEIR RECEPTORS IN THE TWO-SPOTTED CRICKET GRYLLUS BIMACULATUS

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The two-spotted cricket *Gryllus bimaculatus* is one of the important experimental hemimetabolous insects. Also, this species *G*. *bimaculatus* is one of the oldest insects which have been used to identify the neuropeptides. Because of its recent emergence for the experimental availabilities of their transcriptional knockdown by RNA interference (RNAi), we are investigating endocrine control using this species. For further investigation on endocrine controls in many biological processes, we here report the identification of neuropeptides and bioactive peptides in this species using RNA sequencing analyses.

The comprehensive identification using RNA-sequencing analyses were performed using several organs including brain, midgut and so on isolated from *G*. *bimaculatus*. Most identified neuropeptides from RNA-sequencing data were similar to those of the closely related orthopteran species, *Locusta migratoria* and *Schistocerca gregaria.* In the identified neuropeptides, it is intriguing that insulin like peptide is only one copy in the database. Also, although most invertebrate neuropeptides and bioactive peptides have been identified, tachykinin-related peptides were not observed in the data.

Further, we also report the identification of the GPCRs, which are possible receptors for those peptides. We finally discuss here the comparative analyses of those GPCRs using other insect GPCRs

4B-O1

STRESSOR EFFECTS ON THE VISUAL ATTENTION TO FOOD IN HUMANS

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Neuroanatomical and physiological studies support the idea of two (or more) pathways relaying stressor information to CRF neurons in the paraventricular nucleus (PVN); anticipatory stressors that reach the PVN through limbic system pathways and so-called reactive stressors that ascend from the brainstem via ventral noradrenergic pathways. While both anticipatory and reactive stressors have been reported to modulate food intake, there has been little work comparing anticipatory and reactive stressor influences on eating behavior. We examined the influence of an anticipatory stressor (Trier-social stress test, TSST) and a reactive stressor (cold-pressor test, CPT) on visual attention to food images in human participants. Sixty participants were divided equally between control, TSST, or CPT groups. We measured salivary cortisol levels before and after stressor exposure to gauge activity of the HPA axis. Following stressor exposure participants performed an eye-tracking test (using a standardized picture database (Food-pics). We analyzed three metrics in balanced pairs of food and non-food images: saccade latency, gaze duration, and saccade bouts. Salivary cortisol was elevated over baseline in both stressor groups. Preliminary ANOVA analysis of stressor treatment (between groups) and image type (within groups) with harmonic mean replacement of missing data revealed no statistically significant main or interaction effects of the stressors on any of the three eye movement variables. We did find a statistically marginal trend for CPT reducing ($p=0.07$ main effect, post-hoc $p=0.03$ for CPT relative to control) gaze duration on food images with our preliminary ANOVA analysis. There was a main effect of image type, with participants spending more time looking at food images across all treatment groups. Thus far, analyses suggest both stressors types were effective in elevating salivary cortisol, that food images are viewed more intently than non-food images, and that neither anticipatory or reactive stressor exposure robustly affect visual attention to food images.

4B-O3

KNOCKDOWN OF THE THYROID HORMONE TRANSPORTER MCT8 IN RETINAL PRECURSOR CELLS DISRUPTS CHICKEN RETINAL DEVELOPMENT IN A CELL-SPECIFIC AND PERSISTENT WAY

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Thyroid hormones (THs) coordinate cellular processes during vertebrate neurodevelopment, including those that shape the neuroretina of the eye. Transmembrane transporters thereby ensure cellular uptake of THs prior to their action on gene transcription. The TH-specific monocarboxylate transporter 8 (MCT8) is widely involved in brain development, but information on a possible role in retinal development is lacking. As retinal cyto-architecture and developmental events are evolutionary conserved, we tested this hypothesis in the chicken embryo. MCT8 was knocked down in retinal precursor cells by electroporating a pRFP-MCT8-RNAi vector or an empty control vector into the retinal neuro-epithelium at embryonic day 4 (E4). Both vectors co-expressed red fluorescent protein enabling visualisation of transfected cells. EdU-pulse labelling at E6 revealed a reduction in retinal precursor cell proliferation, resulting in cellular hypoplasia and a thinner retina at E18, two days prior to hatching. Cell counts using cell type-specific markers showed a predominant loss of photoreceptors and horizontal cells, the major cell types that are generated around the stage of electroporation. The proportion of a given cell type within the transfected population was however unaltered, implying that the loss of cells was not due to reduced cell differentiation, but a consequence of earlier proliferation defects. In addition, poorly formed sublaminae in the inner plexiform layer suggested synaptogenesis was impaired. Immature photoreceptors displayed delayed migration at E6, but had reached the photoreceptor layer by E18. However, MCT8 deficiency increased differentiation into short-wavelength-sensitive cones at the expense of medium- and long-wavelength-sensitive cones, potentially interfering with colour detection in posthatch chickens. Altogether, our results echo findings in hypothyroid and TH receptor beta-knockout mice, and indicate that MCT8-dependent TH uptake is needed for normal retinal development.

4A-O1

DISCOVERY OF A NEUROPEPTIDE Y/SHORT NEUROPEPTIDE F RELATED SIGNALLING SYSTEM IN ECHINODERMS

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Neuropeptide Y (NPY) regulates a variety of physiological processes in mammals; for example, acting as a potent vasoconstrictor and stimulating food intake. Orthologs of NPY named neuropeptide F (NPF) have been identified in protostomian invertebrates and a related neuropeptide (short neuropeptide F; sNPF) has been characterised in arthropods. To gain insights into the evolution of NPY/NPF/sNPF-type neuropeptides and their cognate G-protein coupled receptors (GPCRs), here we investigated the occurrence of these signalling systems in a deuterostomian "evolutionary intermediate" of vertebrates and protostomes – the starfish *Asterias rubens* (phylum Echinodermata). A cDNA encoding an *A. rubens* NPY/NPF-type precursor was cloned and sequenced and the structure of the mature peptide derived from this precursor was partially confirmed using mass spectrometry - Pyr-DRSKAMQAERTGQLRRLNPRF-NH_2 (ArNPF). Furthermore, a GPCR that is activated by ArNPF was identified and, interestingly, phylogenetic analysis revealed that this receptor is more closely related to sNPF receptors than to NPY/NPF-type receptors. On-going analysis of genomic sequence data may provide further insights into the relationships of this neuropeptide signalling system in starfish (and other echinoderms) with NPY/NPF/sNPF-type signalling systems in other taxa. To investigate the physiological roles of ArNPF in starfish, we have examined its expression in *A. rubens* using mRNA *in situ* hybridisation and immunohistochemistry. This revealed a widespread pattern of expression, with labelled cells or processes present in the nervous system (radial nerve cords, circumoral nerve ring), water vascular system (tube feet), digestive system (cardiac stomach, pyloric stomach) and body wall-associated structures (apical muscle, papulae). These anatomical data provide a framework for investigation of the pharmacological actions of ArNPF in *A. rubens*. In conclusion, this is the first study to characterise a NPY/NPF/sNPF related signalling system in an echinoderm, providing new insights into the evolution and comparative physiology of NPY/NPF/sNPF-type signalling in the animal kingdom.

4A-O2

GNRH-LIKE SIGNALING INTEGRATES LUNAR PHASE AND FOOD INTAKE TO REGULATE GROWTH, REGENERATION AND SEXUAL MATURATION IN PLATYNEREIS

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In vertebrates, hypothalamic gonadotropin-releasing hormone (GnRH) is key in triggering sexual maturation, promoting the release of gonadotropins from the pituitary gland. The plethora of different functions that its homologs play in the animal kingdom yet complicates the understanding of the ancestral role of GnRH-like signaling at the dawn of Bilateria. We investigated this aspect in the marine bristle worm, *Platynereis dumerilii*, a species where sexual maturation is tightly regulated by both metabolic state and lunar cycle. Platynereis GnRH-like system entails four GnRH/AKH/Crz-like preprohormones, as well as three GnRH-like receptors. Binding studies on the receptors showed that two of them are specific for the same ligand, whereas the third binds all the other three peptides, showing potential signal redundancy. All four preprohomones are expressed in specific and distinct neuronal clusters in the worm's brain, upregulated in sexually mature animals, as well as after feeding. Furthermore, all preprohormones and one receptor are also regulated by circalunar phase. We then generated a TALEN-mediated homozygous knockout of *GnRH1*, the gene that showed the most remarkable expression changes. *GnRH1-/-* animals exhibit a significant delay in maturation, reduced growth and attenuated regeneration. Amount of food up-take is however unaffected. We thus interpret that *Platynereis* GnRH-like signaling serves as a hub to coordinate energy expenditure with lunar timing to regulate growth, reproduction and sexual maturation. As these processes are widespread for marine animals and likely evolutionarily ancient, a coupling role of GnRH-like peptides in energy homeostasis and allocation with sexual maturation, growth and development with both environmental cues and metabolic state likely represents a basic function for this preprohormone system.

4B-O4

GLUCOCORTICOID NEGATIVE FEEDBACK IN REGULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA) IN NONHUMAN PRIMATES WITH VARIOUS TYPES OF ADAPTIVE BEHAVIOR: INTERGROUP, AGE-RELATED AND CIRCADIAN DIFFERENCES

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The glucocorticoid-mediated negative feedback (GNF) is the most important mechanism in regulating HPA activity in both basal and stress conditions. Its central sensors are mineralcorticoid and glucocorticoid receptors expressing in various brain structures. Aging disrupts the process of adaptation to stressful effects due to inadequate HPA response to stress exposure. The HPA disturbances of various severities in response to the same stress exposure are formed in different individuals depending on features of their adaptive behavior and the most pronounced HPA disorders demonstrate old monkeys with depressive-like and anxiety-like behavior (DAB). However, the role of possible disturbances in GNF mechanism underlying individual features of HPA stress responsiveness during aging is not understood yet. The aim of study was to investigate GNF features of Macaca mulatta monkeys, females of different age, with DAB and control standard behavior (SB). The test with fludrocortisone demonstrated significant intergroup differences in the GNF sensitivity with resistance in animals with DAB, more pronounced in old monkeys with DAB. These intergroup differences were accompanied by higher plasma cortisol and ACTH levels in DAB monkeys in the afternoon and in the evening. The inhibitory effect of dexamethasone (DEX) in young animals with SB was determined by the time of DEX administration. It was higher with DEX administration at 09.00 compared with DEX administered at 15.00. This rhythm was not maintained in young animals with DAB and in old animals regardless of behavioral characteristics. With the DEX injection at 15.00 there was a tendency to a greater sensitivity of animals with DAB to the inhibitory effect of DEX compared to SB monkeys of the corresponding age. Thus, these data allow to consider monkeys with DAB as individuals with higher disturbances in GNF, which can lead to neuroendocrine dysregulation and underlie disturbed stress induced HPA responsiveness in old monkeys with DAB.

4B-O5

STRESS ELICITED BY ENTOMOPATHOGENIC NEMATODES AND ADIPOKINETIC HORMONE ACTIVITIES IN THE INSECT BODY

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The role of adipokinetic hormone (AKH) in the firebug *Pyrrhocoris apterus* adults, and the fruit fly *Drosophila melanogaster* larvae infected by the entomopathogenic nematode (EPN) *Steinernema carpocapsae* was examined in this study. Beside the ordinary insects, loss-of-function individuals (*Pyrrhocoris - iAkhr* (AKH receptor), *Drosophila - Akh[1]* (AKH itself)) were employed. It was found that co-application of EPN and AKH significantly enhanced the insect mortality beyond rates observed in EPN-only treatment and resulted in metabolism intensification, as carbon dioxide production significantly increased compared to control- and EPN-treated insects, respectively. Interestingly, while EPN application increased *Akh* gene expression and AKH level in the corpora cardiaca, and AKH level in haemolymph of *Pyrrhocoris*, similar changes in AKH characteristics of *Drosophila* were minimal. Furthermore, level of haemolymph lipids and carbohydrates was generally increased by EPN treatment, however, those changes were lower in the loss-of-function individuals. In contrast, haemolymph protein content dropped after EPN infection in both control and *Akh[1] Drosophila* larvae, but this decline was more intense among the *Akh[1]*. Mutation decreased also anti-oxidative capacity in *Akh[1]* larvae, however, their post-infection increases were similar as in controls, suggesting that antioxidant response in *Drosophila* involves mechanisms also beyond AKH. Thus, the outcomes of the present study demonstrate involvement of AKH into the anti-stress reaction elicited by the nematobacterial infection. The exact mechanism by which AKH acts is unknown, but results suggested that the increase of metabolism and nutrient amounts in haemolymph might play a role.

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4B-O6

THE ROLE OF ECDYSTEROIDS AND THEIR RECEPTOR COMPLEX IN THE REPRODUCTIVE PHYSIOLOGY OF THE FEMALE DESERT LOCUST, SCHISTOCERCA GREGARIA

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Studies over the past few decades have shown the importance of ecdysteroids in the post-embryonic development of several insects. Not only are these hormones important regulators of the moulting process, they also seem to play a crucial role in reproduction. Unfortunately, most studies focussing on the role of ecdysteroids in reproductive physiology have been conducted in holometabolous insects with meroistic ovaries. Our research focusses on ecdysteroid biosynthesis and their action in the female reproductive physiology of the hemimetabolous insect, *Schistocerca gregaria*. This insect species with panoistic ovaries is a voracious, swarming pest that can ruin crops and harvests in some of the world's poorest countries. Knowledge of the ecdysteroid biosynthesis and signalling pathways in this pest insect may therefore be useful for development of more selective insecticides combating this harmful locust species.

We investigated the *in vivo* roles of both the ecdysone receptor complex, which is a heterodimeric nuclear receptor complex containing ecdysone receptor (EcR) and retinoid-X-receptor/ultraspiracle (RXR/Usp) proteins, and the ecdysteroid synthesis enzymes (*Halloween* genes), more specifically Spook (Spo), Phantom (Phm), Disembodied (Dib) and Shade (Shd), in the reproductive physiology of female *S. gregaria*. After analysing the tissue and temporal distribution profiles of the gene transcripts of interest during the first gonadotropic cycle of adult female locusts, we applied the RNA interference (RNAi) technique to investigate the role of these genes of interest in ovarian maturation, fertility and fecundity. We discovered a crucial role of ecdysteroid synthesis and signalling genes in ovulation and oviposition of *S. gregaria.* Silencing the *Halloween* genes affected ovarian maturation by preventing the proper formation of interfollicular tissue, while silencing the ecdysone receptor complex resulted in impaired chorion formation. We also found evidence for a feedback of ecdysteroids on juvenile hormone biosynthesis by the corpora allata.

4B-O7

TRANSCRIPTOMICS AS A TOOL FOR THE SWORDFISH XIPHIAS GLADIUS CONSERVATION AND STOCK MANAGEMENT

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The swordfish *Xiphias gladius* is a large, highly migratory and valuable commercial species which has been recently put through a stock recovery plan by the International Commission for the Conservation of Atlantic Tunas (ICCAT). As a part of such conservation effort, Next Generation Sequencing (NGS) represents a suitable approach in order to elucidate molecular pathways involved in growth, metabolism and reproduction of this species. Here, by leveraging the power of Illumina sequencing, ovary mRNA from mature and immature females were sequenced in order to gain insights into the transition towards sexual maturity to highlight unknown pathways potentially implicated in such transition.

The final swordfish transcriptome assembly was made up of 100.869 sequences, of which 30.398 with a Gene Ontology (GO) annotation and 25.151 unigenes. Moreover, differential expression analysis followed by GO and KEGG pathway enrichment analyses revealed that most of the genes involved into key biological functions underlying reproductive maturation such as ovarian steroidogenesis, RNA/DNA processing and lysosome formation/maturation, in addition to transport and lipid metabolism, were up-regulated in mature ovaries. Interestingly, genes involved in the circadian rhythm, would deserve particular attention since were found as differentially expressed.

The present study provides the first de novo assembly of the swordfish transcriptome as well as delivering a wealth of molecular information which will facilitate future studies on this species. Moreover, molecular pathways involved in the transition from immature to mature ovary are described as well as novel genes with potential autocrine/paracrine roles. In conclusion, such results provide a better understanding of the reproductive biology of the swordfish with remarkable improvements towards a sustainable stock management throughout the next years.

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5A-O1

ANGIOTENSIN CONVERTING ENZYME DOWN REGULATOR OF THE INNATE IMMUNE RESPONSE: A STUDY USING THE INSECT, LOCUSTA MIGRATORIA

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Angiotensin converting enzyme down regulator of the innate immune response: a study using the insect, *Locusta migratoria*

Somatic ACE, a Zn-dependent carboxydipeptidase within the Renin-Angiotensin-Aldosterone-System plays an important role in short and long term blood pressure regulation. Accordingly, the use of ACE inhibitors for the treatment of hypertension is common practice. Insects also encode a functional one-domain sACE orthologue but experience no blood pressure issues. In addition, the other RAS components as angiotensinogen and renin remain undisclosed in their genomes. Earlier, we reported the enhanced expression of ACE by hemocytes of *Locusta migratoria* when challenged with bacteria but could not disclose the significance of this finding. Using full inhibition of ACE by Captopril and RNAi in combination with differential mass spectroscopy, we evidenced the involvement of ACE in either appearance and clearance of circulating peptides in LPS challenged locusts. We further focused upon two ACE dependent peptides that originated from known immune active precursors as hexamerin and hemocyanin. Although devoid of apparent antimicrobial activity, these peptides were shown to respectively be uncompetitive (Locmi- antimelanin-I) and non-competitive (Locmi-antimelanin-II) inhibitors of the primary immune enzyme Phenoloxidase (PO). In healthy insects, only inactive Prophenoloxidase circulates. An immune challenge induced serine protease activates PPO into PO that in turn initiates the formation of melanin and simultaneously evokes the appearance of intermediate ROS used for microbial killing. In insects, this PO apart from melanisation corroborate with the cellular and humoral line of defense. Using ethanol activated locust PO we calculated an inhibition constant for Locmi- antimelanin-I and ii of 558 µM and 149 µM respectively. Seen involvement of Lom-ACE in the appearance in circulation of both Locmi-antimelanin I and II, down-regulators of PO, we can conclude that the post-inflammatory action of ACE, as illustrated in mammals and human, is evolutionary conserved and probably precedes its role in blood pressure regulation.

5A-O2

EFFECTS OF SEAWATER AND FRESHWATER CHALLENGE ON THE GH/IGF-I SYSTEM AT DIFFERENT LEVELS OF THE BRAIN-PITUITARY- GLANDULAR AXIS IN THE SALINITY-TOLERANT BLACK-CHINNED TILAPIA (SAROTHERODON MELANOTHERON)

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Both adenohypophyseal hormones prolactin (PRL) and growth hormone (GH) as well as insulin-like growth factor (IGF)-1 are known to be involved in the physiological osmoregulation in fish in adaptation to varying salinities. The IGFs have been also ascribed roles in fish physiology during development, reproduction and immune regulation. However, main emphasis in the investigation of osmoregulatory responses has been laid on the endocrine, liver-derived IGF-1 route and local regulation within the liver and osmoregulatory organs where endocrine and auto/paracrine IGF-1 and IGF-2 reacted in an organ-specific manner (Link et al., Mol Cell Endocrinol 2010;327:40-6). Nevertheless, few studies have investigated the impact of salinity changes on the GH/IGF-axis at different levels of the brain-pituitary-glandular axis in a particularly salinity-tolerant species (Panfili et al., Aquat Living Resour 2004;17:65-74). Therefore, this study is investigating the effects of increased salinity – seawater (SW) and retransfer into freshwater (FW) on the gene regulation of PRL, GH, IGF-1, IGF-2, and the GH receptor (GHR1). A mixed population of sexually mature 14-month old black-chinned tilapia *Sarotherodon melanotheron* heudelotii were transferred from FW to SW and maintained for one wk in SW, then retransferred back to FW and maintained for one wk in FW. Tissue sampling was performed at 4 h, 1 d, 2 d, 3 d and 1 wk after transfer from FW to SW and at 4 h, 1 d, 2 d, 3 d and 1 wk after retransfer to FW. For quantitative analysis of mRNA expression, TaqMan real time PCR assays were created for the target genes PRL, GH, IGF-1, IGF-2, and the reference genes 18S ribosomal RNA, ß-actin and elongation factor EF1. This ongoing investigation should give us a better understanding of the role of the GH/IGF axis within the brain-pituitary-glandular axis and increased and reduced salinity's impact on fish osmoregulation outside the osmoregulatory organs.

5C-O1

MASS SPECTROMETRY IMAGING REVEALS DISTINCT COMPARTMENTALIZATION IN NEUROENDOCRINE TISSUE OF AN INSECT

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Neuropeptides are signaling molecules regulating physiological functions in multicellular organisms. Many neuropeptides are produced in neurosecretory cells of the central nervous system (CNS) and released from neurohemal organs into the circulatory system. In insects, one of the major neuroendocrine tissues is the retrocerebral complex (RCC) which is constituted from different parts: a glandular part of the *corpora cardiaca* producing and releasing adipokinetic hormones (AKHs), a neurohemal part of the *corpora cardiaca* which mostly stores and releases neuropeptides produced in the brain and gnathal ganglion, and the endocrine *corpora allata* which synthesize and release juvenile hormones. All of these tissues are crossed by axons from neurosecretory cells of the CNS. In our study, we optimized a protocol for the detection of neuropeptides using Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI). Unlike immunohistochemistry or many MS approaches, MALDI-MSI allows for the simultaneous visualization of the distribution of numerous molecules in a single tissue section. Using our optimized protocol, we studied the special distribution of neuropeptides at a 15µm spatial resolution in 14 µm tissue sections of the RCC of the American cockroach *Periplaneta americana*. We detected more than 100 peptides with around 75 mature neuropeptides from 16 precursor genes in a single tissue section; thus obtaining for the first time a nearly complete coverage of insect neuropeptides by MSI. Based on the optimized protocol and differential distribution of neuropeptides in the RCC we were able to reconstruct the compartmentalization of the RCC and to detect differential neuropeptide processing. Thus, using our protocol, it is possible not only to investigate neuropeptide distributions but also to analyze differences in the neuropeptidome, e.g. in response to insecticide exposure or environmental stress in general.

5C-O2

CRUCIAL ROLE OF THE ADIPOKINETIC HORMONE/GONADOTROPIN RELEASING HORMONE SYSTEM IN POSTEMBRYONIC DEVELOPMENT IN THE DESERT LOCUST

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Adipokinetic hormone (AKH) is a highly researched insect neuropeptide that induces the mobilization of carbohydrates and lipids from the fat body at times of high physical activity, such as flight and locomotion. As a naturally occurring ligand, AKH has undergone quite a number of amino acid changes throughout evolution, and in some insect species multiple AKHs are present. They consist of 8-10 amino acids and have an N-terminus blocked by pyroglutamate and a C-terminus blocked by an amide group. AKH acts by binding to a rhodopsin-like G protein-coupled receptor, which is a homolog of the vertebrate gonadotropin-releasing hormone receptor.
In the present study, we provide molecular evidence that in the desert locust, *Schistocerca gregaria*, AKHs, which are released from the corpora cardiaca during energy demanding processes, will bind to their receptor in the locust fat body, thereby triggering lipid release. Our research shows that the AKH signalling system also plays a role in the regulation of locust reproduction.
Locust swarms threaten the livelihood of people living in some of the world's poorest countries. In gregarious locusts, energy availability can be a crucial determinant allowing flight and migration. Development of strategies that interfere with this energy demanding process may therefore lead to novel opportunities for combatting devastating locust swarms.
Acknowledgements: We gratefully acknowledge the Horizon 2020 project of the European Commission (nEUROSTRESSPEP), the Research Foundation of Flanders (FWO-Flanders) and the Interuniversity Attraction Poles programs (Belgian Science Policy Grant (P7/40)) for financial support.

6B-O1

EXPLORING RELATIONSHIPS AMONG EXPOSURE TO AVIAN MALARIA, BACTERIAL KILLING ABILITY, AND CIRCULATING CORTICOSTERONE IN A HAWAIIAN HONEYCREEPER

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Avian malaria is a commonly occurring vector-borne disease caused by *Plasmodium* blood parasites that typically causes only minor symptoms in most species. The Hawaiian honeycreepers, however, which have evolved without blood parasites for millions of years, often experience high mortality following inoculation with *Plasmodium*. Remarkably, the Hawaii Amakihi has maintained stable populations in low elevation habitats, where malaria transmission by the cold-intolerant *Culex* mosquito is prevalent. Recent studies suggest that resident low elevation Amakihi populations have evolved malaria tolerance, whereas high elevation populations remain susceptible. The mechanisms by which low elevation Amakihi tolerate malaria remain unknown. As unchecked propagation of parasites in the blood of susceptible honeycreepers appears to be the main cause of mortality in infected birds, we expected that changes in immunity likely contribute to Amakihi tolerance. Additionally, since corticosterone and testosterone have been implicated in immune function modulation, we hypothesized that hormones may also play a role. In this study, we investigated correlations between exposure to avian malaria and 1) the functional capacity of whole-blood to limit the spread of the microbe *E. coli*, and 2) levels of an established immunomodulatory hormone, corticosterone. We sampled for baseline corticosterone and bacterial killing capacity (BKC) in wild Amakihi in low elevation (malaria-exposed) or high elevation (malaria-unexposed) habitats. While baseline corticosterone was found to be consistent across elevations ($P = 0.80$), a negative relationship between body fat and corticosterone was found for low elevation birds only (low elevation: $P = 0.01$, high elevation: $P = 0.27$). Although BKC did not significantly differ across elevations ($P = 0.14$), the coefficient of variation (CV) in BKC in high elevation birds ($CV = 48.6$) was found to be more than twice that of low elevation birds ($CV = 22.3$).

6B-O2

CHARACTERIZATION AND NUTRITIONAL REGULATION OF GHRELIN AND ITS RECEPTORS IN GILTHEAD SEA BREAM (SPARUS AURATA)

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Ghrelin is a peptide hormone that regulates different physiological processes in vertebrates (feeding, metabolism, intestinal activity and growth hormone (Gh) secretion). However, specific actions of this hormone have been reported to change among species. The objective of this work was to characterize *preproghrelin* and Ghrelin receptors (*ghsrs*) sequences, and to study their responses to fasting and refeeding in gilthead sea bream (*Sparus aurata*) juveniles. Structural and phylogenetic analysis of the *S. aurata preproghrelin* was accomplished, and its expression analyzed in main tissues. Fish were fasted for 21 days and then refed and sampled at 2, 5, 24 h, and 7 days afterwards. Plasma levels of Ghrelin, Gh and insulin-like growth factor (Igf-1), and *preproghrelin*, *ghsrs* and members of the Gh/Igf-1 axis gene expression were determined in key tissues. *preproghrelin* and its receptors presented a good conservation among fish, being highly expressed in stomach and pyloric caeca, and in the pituitary and brain, respectively. Fasting caused a growth recession while increased Ghrelin plasma levels, which rapidly recovered at 5 h postprandial. Gh plasma levels were also increased by fasting, and slowly recovered after refeeding. Contrarily, circulant Igf-1 followed an inverse pattern, suggesting important changes in the Gh/Igf ratio by nutritional status. In agreement with the plasma levels, fasting up-regulated *gh* expression in pituitary until 1 day after refeeding, when lowest *igf-1* expression in the liver was observed. Moreover, the expression profile of hepatic Gh receptors resulted to be inverse to that if circulating Gh levels. Altogether, this study demonstrates the important role of Ghrelin concerning nutritional status, suggesting an orexigenic function in gilthead sea bream, and an interaction with pituitary Ghsrs and Gh/Igf-1 axis to promote optimal growth in this species. Supported by funds from the MINECO (AGL2014-57974-R and AGL2015-70679-R) and Generalitat de Catalunya (2014SGR-01371 and XRAq).

6B-O3

NITRIC OXIDE REGULATES Na+/K+-ATPase FUNCTION IN FISH BRAIN

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The neuronal circuitries of brain and its ion transporters contribute to the physiological homeostasis in fishes. The sensitivity of these neuronal groups to environmental challenges further implies its plasticity and contribution to stress adaptation. As a Na transporter that provides the driving force for many other transport systems, Na^+/K^+-ATPase (NKA) is vital for Na^+ and K^+ homeostasis of neuronal cells. Nitric oxide (NO), a gasotransmitter, is involved in ion transport in many peripheral tissues of fishes. But, its role in ion transport has not yet been investigated in fish brain. We investigated the response of Na transporter to NO in fish hypoxia-stressed fish brain. The results indicate that NKA has differential sensitivity to SNP and L-NAME and that showed a reversal pattern when the fish was exposed to hypoxia stress. Likewise, mRNA expression of brain nkaα1 isoforms viz. nkaα1a, nka α1b and nkaα1c showed a switching after hypoxia-stress. Furthermore, the analyses of NKA protein abundance, kinetics and phosphorylation status also revealed reversed patterns upon hypoxia stress. Overall, our data provide evidence for differential sensitivity of NKA function to NO and that further supports the hypothesis that NO regulates NKA-driven ionic signals in fish brain (Supported by grants from iCEIB Project, Govt. of Kerala, UGC SAP DRS II and DBT BioCare project).

6A-O1

HYPOTHALAMIC AMP-kinase PLAYS A KEY ROLE IN FOOD INTAKE AND HEPATIC METABOLISM REGULATION IN RAINBOW TROUT ONCORHYNCHUS MYKISS

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AMP-activated protein kinase (AMPK) works in the mammalian cells as an energy sensor that allows the detection of lowered cell energy status with coupling to intrinsic cell mechanisms designed to restore energy balance and leading to an increase in food intake levels. AMPK-α1 and AMPK-α2 present different functions being the α2 isoform more related to food intake regulation and glucose homeostasis in mammals. In rainbow trout, decreased values of hypothalamic AMPKα phosphorylation ratio or AMPK-α1 mRNA abundance occurs in the presence of increased levels of metabolites (glucose or fatty acids) suggesting a role of hypothalamic AMPK as an energy gauge in fish as demonstrated in mammals. Here, we demonstrate that the absence of nutrient availability induce a decrease of the phosphorylation ratio of hypothalamic AMPKα in rainbow trout. Furthermore, it was induced the inhibition of AMPK expression in rainbow trout hypothalamus by i.c.v. injection of AMPK dominant negative (DN-AMPK) adenovirus either for α1 and α2 isoforms. The presence of the injected adenovirus in the hypothalamic areas has been demonstrated by immunohistochemical analyses. The results obtained demonstrate that the inhibition of AMPK-α2 leads to a decrease of food intake levels whereas the inhibition of AMPK-α1 did not show significant effects on food intake. Furthermore, an overall decrease of metabolic parameters has been observed in plasma and liver of DN-AMPK-α2 injected fish as observed for glucose and triglycerides in plasma, hepatic glycogen, triglycerides, fatty acids levels and enzyme activities and/or mRNA expression in liver. These effects have been not so clear for de DN-AMPK-α1 group. The present study demonstrates that hypothalamic AMPK also plays an important role on energy status in fish by regulating food intake and hepatic metabolism whereas the specific role of AMPK-α1 isoform needs to be elucidated.

6A-O2

METABOLIC MODULATION OF THE FAT BODY BY ENDOCRINE SYSTEMS IN THE TWO-SPOTTED CRICKET GRYLLUS BIMACULATUS

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Modulation of activities in metabolisms along with fluctuation of nutrient requirements is vital to maintain energy homeostasis in animals. In insects, neuropeptides are secreted mostly in responses to internal nutritional states, eventually regulating their physiological events including feeding behavior to compensate for their energy shortage. To address this issue, we focused on insect adipose tissue, the fat body, and then explored which neuropeptides can stimulate the fat body in energy homeostasis using the two-spotted cricket *Gryllus bimaculatus*. RNA-sequencing data of *G. bimaculatus* fat body revealed the presence of 12 receptors for neuropeptides including allatostatin B, myosuppressin, and adipokinetic hormone (AKH). This result illuminates that those neuropeptides can modulate their metabolic activities in the fat body. To investigate how those neuropeptides would modulate their metabolisms, we next analyzed the effects of disruption of those peptidyl signals on their metabolic activities mediated by the fat body. Of those peptides, we further analyzed the effects of Adipokinetic hormone (AKH) on the metabolisms in the fat body, for example. Knockdown of AKH receptor (*AKHR*^{RNAi}) resulted in the increased transcriptional levels of lipogenic genes (*fatty acid synthase*, *stearoyl-CoA 9 desaturase*, *12 desaturase, and elongation of very long chain fatty acids protein 6*), whereas decreased levels were observed in the lipolytic genes (*hormone-sensitive lipase* and *brummer*). Concomitantly, we found that hemolymph lipid levels and fatty acid composition were significantly altered in *AKHR*^{RNAi} crickets. Taken together, endocrine systems including AKH signaling can regulate the metabolisms, particularly in the lipid metabolisms, at transcriptional levels in the fat body, eventually contributing to feeding behavior to maintain their lipid homeostasis.

6A-O3

A NUTRIENT RESPONSIVE GUT/NEURONAL AXIS REGULATES ENERGY HOMEOSTASIS IN ADULT DROSOPHILA

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The control of systemic metabolic homeostasis involves complex inter-tissue programs, which coordinate energy production, storage and consumption to maintain organismal fitness in the face of environmental challenges. The mechanisms driving such programs are largely unknown. Using *Drosophila* as a model system, we show that enteroendocrine cells in the adult intestine sense nutrients and respond by secreting the hormone Bursiconα, which signals via its neuronal receptor LGR2. Bursiconα/LGR2 restricts the use of energy by preserving systemic lipid stores through modulation of Glucagonlike Adipokinetic Hormone signaling within the fat body/adipose tissue. Impaired Bursiconα/LGR2 leads to depletion of energy stores and diminishes organismal resistance to nutrient restrictive conditions. Altogether, our work reveals an intestinal/neuronal/adipose tissue inter-communication network, which is essential to restrict the use of energy and may provide insights into the physiopathology of endocrine-regulated metabolic homeostasis.

6A-O4

EFFECTS OF CRUSTACEAN CARDIOACTIVE PEPTIDE (CCAP) AND ITS RECEPTOR ON ENERGY BALANCE IN THE TWO-SPOTTED CRICKET, GRYLLUS BIMACULATUS

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Because maintaining nutrient status is vital for animal survival, dysfunction of mechanisms in energy maintenance can easily lead to various disorders even in insects. Adipokinetic hormone (AKH) has been researched as an important regulator for energy utilization and storage for a long time. Based on this, the Crustacean cardioactive peptide (CCAP) has been introduced as a heartbeat accelerator with the function of releasing AKH from corpora cardiaca. However, the effects of CCAP itself on energy balance have not been elucidated yet, although CCAP and AKH can interplay an important role in the endocrine network associated with energy balance.

After identification of CCAP and its receptor from the two-spotted cricket *Gryllus bimaculatus*, we surprisingly found that CCAP does rarely induce the effects of AKH by the following experiments. Namely, not corresponding to the effect of AKH, lipid level in the hemolymph was not altered neither by injection of the mature peptide of CCAP or by knockdown targeting on the transcripts of the precursor of CCAP and CCAP receptor. However, opposite to the lipid balance, carbohydrate level in the hemolymph was significantly altered by both CCAP injection and knockdown.

Those current data proved that CCAP is involved in the modulation of energy balance through another endocrine route other than AKH signaling, which leads to the energy change with alteration in both lipid and carbohydrate levels. Because direct endocrine control between CCAP and AKH is only proved in the Locust, in the closely related orthopteran specie *G. bimaculatus*, this research may provide different mechanisms of energy homeostasis in extended insects through their evolution

6B-O4

MMP 2 AND MMP 9 REGULATE Na+/K+-ATPase-driven Na+ SIGNALING DURING STRESS RESPONSE IN FISH IONOCYTES

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Extra cellular matrix (ECM) is remodeled constantly in response to a host of cellular stimuli. Matrix metalloproteinases (MMPs) are a family of extracellular zinc and calcium-dependent endopeptidases involved in the degradation and remodeling of ECM components and basement membrane components, cell movement, proliferation and tissues remodeling. Among the MMPs, gelatinases that constitute MMP-2 (gelatinase A) and MMP-9 (gelatinase B), have gained the attention due to its involvement in degrading collagen, the major component of the basement membrane. The role of these gelatinases, however, in Na^+/K^+-ATPase (NKA) functions has not yet understood in fish ionocytes. We, therefore, tested the effects of inhibitors of gelatinases on in varied osmoregulatory cells of climbing perch. *In situ* and *in vitro* application of these inhibitors that produced altered MMPs distribution, modified the NKA kinetics, protein abundance, immunoreactivity, phosphorylation status and nkaα1 isoform diversity in the osmoregulatory epithelia of fish. These results indicate that ECM remodeling in fish ionocytes demands modification of NKA functions (We thank the UGC-MRP project on fish and SGS acknowledges DST for an INSPIRE Fellowship. Also supported by iCEIB project, Govt. of Kerala).

6B-O6

LOVE MADE FLESH: INFANT MASS GAIN AND POSITIVE OXYTOCIN FEEDBACK LOOPS IN GREY SEAL MOTHER-INFANT PAIRS

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Secure attachments enable parents and offspring to display appropriate care giving and receiving behaviours, maximising infant health and survival. It has been theorised that oxytocin acts in a double positive feedback loop across bonded individuals, facilitating parent-infant attachment and behaviour. This study presents the first evidence from a wild animal species that such loops exist in both individuals in mother-infant pairs by demonstrating that wild grey seal mothers with high oxytocin levels produce pups with high oxytocin levels throughout lactation. Infancy is a crucial time for growth and development, and our findings additionally provide evidence connecting the oxytocin driven mechanisms for parent-infant bonding with the energetics underlying parental care for the first time. High oxytocin infants gained mass at a greater rate without additional energetic cost to their mothers, giving a selective advantage to securely bonded mother-infant pairs. By using a wild animal system, we demonstrate that this mechanism exists in natural environments. Oxytocin loops and their associated fitness benefits may connect optimal parental or social environments with direct physiological advantages for individual development, increasing our understanding of how infants can fail to thrive during early life. Oxytocin's impact on infant mass gain as part of a positive feedback mechanism dependant on the presence of a care-giver is relevant to medical and veterinary fields, the evolution of parental care and societal understanding of how health and relationships are linked.

6B-O7

SEGMENT SPECIFIC LOCATION OF CAPA PEPTIDES EXPRESSING NEURONS IN THE CENTRAL NERVOUS SYSTEM OF THE EARTHWORM EISENIA ANDREI

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Six CAPA peptides believed to be homologous with insect periviscerokinins (PVKs) and pyrokinins (PKs), namely FVRIamides and XPRLamides, were identified in the ventral nerve cord ganglia of the earthworm *Eisenia fetida* by means of immunological methods and mass spectrometry earlier. In contrast to insects, in which PVKs and PKs are typically expressed in neurosecretory cells and released by neurohaemal organs into the haemolymph, neurosecretory cells and several interneurons were labelled in earthworms. Recent work focuses on the identification and histochemical characterization of CAPA peptides expressing neurons of the central nervous system of the earthworm *Eisenia andrei* showing that there are characteristic regional differences in the segmental distribution of PVKs and PKs in earthworms. While several labelled cells were found by means of immunohistochemistry in all ganglia, from the cerebral to the terminal one, the in situ hybridization revealed that in the cerebral ganglion no XPRLamide expressing cells were located suggesting that immunostained neurons are FVRIamide expressing ones. Most of the FVRIamide expressing neurons situated close to the capillary bed of the cerebral ganglion originated from the midsagittal blood vessel of the pharynx. These neurons were characterised by a round or polygonal, medium sized soma and a short process that passed into deeper cerebral ganglion region and reached the wall of capillaries identified as the neurohemal region of the cerebral ganglion. Omega profiles, thought to be characteristic of neurosecretory cells, frequently occurred also in the end foot of neurons attached to blood vessels. Based on the morphological characteristics of the stained neurons we could propose that midsagittal neurosecretory brain region of the earthworms is the anatomical correlates of pars intercerebralis found in polychaetes and arthropods.

6C-O1

THE QUEST FOR GREEN INSECTICIDES: CAN THE KNOWLEDGE OF THE PRIMARY STRUCTURE OF INSECT ADIPOKINETIC HORMONES HELP?

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Insects are major competitors for human foodstuff. To achieve food security for the ever-increasing human population, insecticides have to be used in large quantities. Since conventional insecticides are non-specific and not able to distinguish between beneficial and pest insects, there is a real need for so-called “green” insecticides that are target-specific and bio-degradable. Such “green” insecticides can be designed on the basis of neuropeptide-receptor interactions with the idea to develop an active peptide mimetic that is only harmful to a pest insect species. The first step in this research endeavour is to find out the status quo of specific ligands in beneficial and pest insects. The adipokinetic hormone (AKH) system is in focus here for being especially involved in regulating energy metabolism in important physiological events of insects. In this contribution, the AKH inventory in beneficial and pest insects of the orders Diptera (flies) and Coleoptera (beetles) is presented and discussed as potential mimetic leads. For example, different AKHs can be found in harmful flies, such as the Australian sheep blowfly *Lucilia cuprina* (responsible for “sheep strike”) and the Mediterranean fruit fly *Ceratitis capitata* (responsible for extensive damage on fruits especially wine in South Africa), and a beneficial fly such as the black soldier fly *Hermetia illucens* (larvae are excellent decomposers: “from waste to protein”, and are used as protein feed in aquaculture and chicken farming). We will compare this with Coleoptera, where the ancestral AKH which is found in all primitive families, the Adaephaga, also occurs in a number of Orthoptera (Ensifera and Caelifera), Hymenoptera (including the honey bee), certain Hemiptera and some families of more advanced beetles. Other beetles, however, such as non-beneficial flower scarabs, chrysomelids and cerambycids have AKHs quite different from the ancestral one.

Funding: National Research Foundation

6C-O2

THE EFFECT OF BIOSTABLE PEPTIDE ANALOGUES ON APHID STRESS TOLERANCE AND THEIR POTENTIAL AS INSECTICIDAL AGENTS

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With a global dependence on broad-spectrum insecticides, the damaging effects of which are well documented, there is increasing need for the development of greener, target-specific insecticides. One promising avenue for the development of pest-specific control involves the employment of neuropeptides. Neuropeptides are regulators of critical life processes in insects and, due to their high specificity, represent potential targets for insecticidal agents to selectively reduce the fitness of pest insects, whilst minimising detrimental environmental impacts. Fundamental to this drive is an understanding of the neuroendocrine pathways that control key physiological processes in pest/non-pest insects and the screening of potential analogues.

The current study investigated the effect of biostable kinin analogues, as well as CAP2b and pyrokinin analogues active on a heterologous insect CAP2b receptor, on aphid (*Myzus persicae* and *Macrosiphum rosae*) fitness under conditions of desiccation, starvation and thermal (cold) stress.

Results revealed the CAP2b analogues to display the most promise in reducing the relative fitness of treated aphids when under conditions of desiccation and starvation stress and thus expediting aphid mortality. By demonstrating effects of analogues of the CAP2b neuropeptide family and key CAP2b analogue structures, this research will allow development of second generation analogues towards the development of neuropeptidomimetic-based aphicidal agents.

STATE OF THE ART AND INVITED PRESENTATIONS

1A-S1

PEPTIDERGIC SIGNALLING UNDERLIES PLACOZOAN BEHAVIOUR

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Placozoans, along with poriferans (sponges), are considered as some of the earliest diverging animals. Placozoans are flat, millimeter-wide disc-shaped marine animals that can be found in a large variety of coastal ecosystems around the world, and display a large array of coordinated movements and behaviors. They lack a nervous, muscular or digestive system, and their basic body plan comprise an upper and a lower epithelia delineating a cavity containing stellate cells organized in a syncitium, with six morphologically identified types of cells. The genome of the placozoan *Trichoplax adhaerens* encodes several proneuropeptide genes and genes involved in neurosecretion in animals with a nervous system. In this project, we investigate neuropeptide signalling in *Trichoplax adhaerens*, and assess on the one hand, the specific expression of several neuropeptides and on the other hand, their effects on the animals' shape, patterns of movement and velocity. Together, the data points toward an important role for peptidergic signalling in nerveless placozoans.

1A-S2

NEW INSIGHTS INTO THE EVOLUTION OF THE MOLLUSCAN BUCCALIN SYSTEM

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The arthropod type A allatostatin (AST-A) peptide shares a common evolutionary origin with the vertebrate kisspeptin (KISS). In molluscs, it is the buccalin peptides that are thought to be the orthologous system, supported by three lines of evidence: 1) the buccalin peptides are structurally related, 2) molluscs contain AST-A-like and KISS-like receptors, and 3) there exists a similar gene synteny. Together, this suggests that the buccalin system regulates metabolism and reproduction in molluscs. The buccalin-related neuropeptides have been known for over 25 years, identified initially from the sea slug *Aplysia* where it increases the size of muscle contractions elicited by firing of the motor neurons. My group has further characterized the molluscan buccalins, from oysters to land and freshwater snails. In each species, with regards to bioactivity, buccalin can be intimately tied to reproductive processes. For example, helicid land snails transfer buccalin peptides associated with their 'love' darts during mating, likely to increase the chances of paternity success. These results are helping to develop a model for the evolution of the buccalin system.

1B-S1

HOW DISORGANIZED CLOCKS MAY DISTURB HORMONE RHYTHMS AND RESULT IN METABOLIC DISORDERS

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All mammalian species possess a circadian timing system consisting of a central brain clock and peripheral tissue clocks in organs such as pancreas, liver, and adipose tissue. The central clock in the brain is synchronized to the 24hr light/dark rhythm of the environment via retinal input, and synchronizes the peripheral clock rhythms via hormones, the autonomic nervous system and the regulation of (feeding) behaviour. According to an important chronobiological hypothesis the increased incidence of metabolic disorders, such as obesity and diabetes mellitus, in our current 24/7 society is caused by a mis-alignment of the central brain clock and the metabolic clocks in the periphery. For instance, recently we showed in rats that 2h of light-at-night acutely decreased glucose tolerance. This effect was dependent on time-of-day, light intensity and wavelength. At present it is unknown how these light effects are transmitted to the periphery, but they probably involve changes in hepatic glucose production, systemic glucose uptake and/or pancreatic insulin release. Light effects on the liver, as well as other peripheral organs, may involve hormonal, behavioral and nervous pathways. To test the possible involvement of endocrine mechanisms, we studied the effects of a denervation of the autonomic nervous input to the liver on the liver transcriptome after light-at-night using transcriptomics. However, despite the amount of data from animal models supporting the mis-alignment hypothesis, data on molecular clock rhythms in human patients are scarce. Thus, in addition, we compared diurnal gene expression profiles in subcutaneous adipose tissue between obese patients with type 2 diabetes and age-matched healthy lean control subjects using RNA sequencing. In healthy controls 8.4% (1421 genes) of the 16818 expressed genes showed significant diurnal rhythms, compared to only 1.8% (303 genes) in patients. Therefore, our data suggest that indeed disturbed (adipose) tissue rhythms may contribute to human metabolic disorders.

1B-S2

THE HORMONE MELATONIN WITHIN THE CIRCADIAN SYSTEM

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Daily rhythms are depending of a network comprising circadian clocks, synchronizing inputs, clock outputs as well as multiple peripheral clocks. In mammals, the focal point of this system is a master circadian clock (within the suprachiasmatic nuclei SCN) which adjusts timing of other clocks. It is thus the complex interactions of neural, hormonal and behavioural outputs from the SCN that drive the circadian expression of events. The nocturnal release of melatonin (MLT) by the pineal gland is tightly controlled by the SCN clock. As a major hormonal output, MLT distributes temporal cues generated by the SCN to the multitude of tissues expressing melatonin receptors. MLT is thus both a SCN clock output and internal time-giver in the circadian clocks network. MLT has been credited with many other properties and several mechanisms have been suggested. The pharmacological actions of melatonin at higher concentrations (µM and above) are thought to be mediated via interactions with specific intracellular proteins such as calmodulin or QR2. Also, at high concentrations MLT appears to be a potent-free radical scavenger. In fact, as for other hormones, MLT exerts its physiological effects principally throughout high-affinity receptors. These receptors have been reported in a large number of structures within the brain and periphery with a great variability in number, localization and density among species. The presence of MLT-receptors within the SCN, explains the chronobiotic properties of xogenous MLT. Trials in humans, have confirmed the efficacy of MLT in circadian rhythm disorders. Subtypes of MEL receptors have been characterized (MT1 and the MT2). Striking differences evident at the signal transduction pathways involved as well as at the level of the tissue distribution have been observed. The understanding of links between specific target sites for MEL, identified MEL receptor subtypes, and particular physiological actions is still a great scientific challenge. The knowledge of the cell types that contain MT1 or MT2 receptors is a key to better define the different roles of these two receptor subtypes.

1C-S1

PERINATAL SSRI MEDICATION EFFECTS ON NEUROENDOCRINE OUTCOMES IN MALES AND FEMALES

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Selective serotonin reuptake inhibitor medications (SSRIs) are prescribed to up to 10% of pregnant women to treat maternal mood and anxiety disorders. Exposure to these medications in-utero has raised concerns about altered neuroendocrine and behavioral outcomes. Recent attention has turned to perinatal SSRI effects on the hypothalamic-pituitary-adrenal (HPA) axis and social interactions suggesting that the modulation of the developing serotonergic system with SSRIs leads to aberrant neuroendocrinology and related behavior. Many women prescribed SSRIs also exhibit significant symptoms of depression and anxiety which can also markedly impact neurobehavioral outcomes. Until recently most clinical and translational research has focused on perinatal SSRI effects alone and not taken into account the additional effects of maternal affective state. My work over the past 10 years has focused on understanding the effects of perinatal SSRIs on both the mother and offspring, taking into account the effects of maternal mood in clinical population or stress in animal models. Primary findings from this research in both clinical populations and rodent models shows that perinatal SSRIs act as an endocrine disruptor on the HPA axis by altering peripheral levels of glucocorticoids and increasing corticosteroid binding globulin levels. Both perinatal SSRIs and the HPA axis are linked to social behaviors and aggressive phenotypes and, thus, my recent work has investigated the long-term impact of perinatal SSRIs on social behaviors and related changes in the HPA axis. Findings from this work demonstrate clear, sex-dependent effects of perinatal SSRIs, as well as maternal stress, on social behaviors which are related to changes in the HPA axis and hippocampal plasticity. This works points to a need to consider the risks and benefits of using SSRIs to treat maternal affective disorders and suggests that fetal sex-specific treatments may need to be considered to safely and effectively treat both mother and child.

1C-S2

INTEGRATED APPROACHES TO INVESTIGATE THE EFFECT OF PROGESTINS IN FISH AND THEIR OCCURRENCE IN THE AQUATIC ENVIRONMENT

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During the past twenty years, numerous studies have examined the effects of estrogenic compounds, notably 17α-ethinylestradiol a potent synthetic steroidal estrogen used as pharmaceuticals in contraceptive pills, because these substances were among the first identified as endocrine disrupting compounds responsible for the feminization of wild populations of fish observed worldwide. There is now evidence of the occurrence of other natural and synthetic steroids in aquatic environment. Among steroidal pharmaceuticals, the risks encountered by synthetic progestins on aquatic species have been recently pointed out but data on their occurrence and effects on fish endocrinology and physiology are missing to properly assess their hazard and risk to aquatic species. The national project ANR “PROOFS” aimed at investigating the effect of progestins on key molecular and cellular targets of the endocrine system using zebrafish *in vitro* and *in vivo* reporter gene mechanism-based assays. It also aimed at providing data on the occurrence of progestagenic activity of environmental samples and to identify substances responsible for these activities through an effect-directed analysis approach. Among the 26 pharmaceutical progestins investigated, our data revealed that they can act on several steroidal nuclear receptors. By using novel transgenic zebrafish models, we demonstrated for the first time their estrogenic activity in the developing brain and their capacity to disrupt corticosteroigenesis in interrenal cells. Finally, we reported strong progestagenic activity in waste waters and identified several pharmaceuticals as potential candidate responsible for these activities. Overall, the ANR project “PROOFS” brings novel and relevant data on occurrence and endocrine potency of progestins stressing the need to further study these emerging contaminants.

1A-I1

EVOLUTION OF NEUROENDOCRINE SIGNALLING SYSTEMS: INSIGHTS FROM A LOPHOTROCHOZOA, THE OYSTER CRASSOSTREA GIGAS

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In Eumetazoans, neuropeptides regulate a majority of biological processes and play a crucial role in the elaboration of adapted physiological and behavioural responses to environmental constrains. Until recently, knowledge on neuropeptide signalling systems was chiefly limited to well-studied vertebrate species and to ecdysozoan model species. The tremendous expansion of molecular resources in Lophotrochozoa, one of the most diverse and evolutionarily highly successful bilaterian lineage, opens up the opportunity to refine our knowledge on the origin and the evolution of neuroendocrine signalling systems via the introduction of new models at a key phylogenetic position between Ecdysozoa and Deuterostoma. In this context, the pacific oyster *Crasssostrea gigas,* one of the most important aquaculture shellfish resource worldwide, has emerged as an attractive model due to the identification, using combined data mining and/or peptidomic approaches, of extended repertoires of both neuropeptides and G protein-coupled receptors (GPCRs).

Using examples of newly characterized signalling systems in oyster (ELH/ CRH / DH44, Calcitonin /DH31…) we illustrate how pairing of receptors and ligands, determination of the spatiotemporal and the stimulus induced patterns of expression of their encoding genes provide, in a comparative context, interesting insights about evolutionary trajectories of some neuroendocrine systems.

Collaboration : Julie Schwartz, Jeremy Pasquier and Marie-Pierre-Dubos (Université de Caen Normandie), Jérôme Leprince and Benjamin Lefranc (Université de Rouen Normandie) Anne-Gaëlle Lafont and Arnaud Bondon (Université de Rennes 1)

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Key words:

Neuropeptides, GPCRs, Lophotrochozoa, evolution, reverse endocrinology.

1A-I2

DIFFERENT SIGNALLING MODES OF MYOINHIBITORY PEPTIDE IN THE POLYCHAETE *PLATYNEREIS*

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Neuropeptides, signalling molecules produced by neurons, often maintain conserved functions across animal phyla, yet at the same time can play multiple roles within a single organism. How neuropeptides maintain both diverse functions within a species and conserved functions between species remains an open question. Wamide family neuropeptides, including myoinhibitory peptide (MIP), allatostatin B, prothoracicostatic peptide and GLWamide, are an example of multi-functional yet conserved signalling molecules. Wamide neuropeptides play a role in the regulation of life-stage transitions in Cnidarians, polychaetes and insects. In the polychaete *Platynereis dumerilii*, MIP is multi-functional, inducing both early larval settlement behaviour and post-larval feeding behaviour. *Platynereis* MIP activates both a G protein coupled receptor known as MAG, and a MIP-gated ion channel (MGIC). Different mature MIP peptides from the same precursor gene differentially activate MAG and MGIC. Comparison of MAG and MGIC expression in *Platynereis* reveals that these receptors occur in different cell types, enabling MIP to target different tissues through divergent signalling pathways, thus increasing the functional diversity of this neuropeptide. The *Platynereis* MGIC receptor is paralogous to RFamide-gated ion channels found in Cnidarians and molluscs, suggesting that a possible mechanism for increased functional diversity in the evolution of neuropeptide signalling is the addition of new receptor types.

1B-S3

LONG TERM BEHAVIORAL OBSERVATIONS ON MELATONIN-PROFICIENT AND MELATONIN-DEFICIENT LAB MICE UNDER CONDITIONS OF A NATURAL ABIOTIC ENTRAINMENT

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The patterns of locomotor activity of mice are widely studied as an expression of the circadian clock system. The vast majority of these studies have been carried out in the laboratory and have revealed certain differences between mouse strains that might be attributable to the action of the melatoninergic system.
To see how the activity patterns under seminatural conditions compare to those that are obtained in the laboratory, we performed a study with two standard laboratory mouse strains (melatonin-deficient C57BL/6J and melatonin proficient C3H/HeN) in an outdoor environment. The mice were kept singly in cages equipped with a hiding box, nesting material and with food and water ad lib. These cages were placed outdoors in a garden. A wire mesh kept rats, cats, etc. out, a glass roof provided protection from rain. The activity patterns of the individual mice (n=12) were continuously registered for a period of one year, together with data on ambient temperature, light and humidity.
As compared to the lab, the two strains retained their nocturnal "locomotor identity", with a "double peak" activity pattern and a late chronotype in the C57BL/6J mice and an earlier chronotype and a "single peak" pattern in the C3H/HeN. The onset of the main activity period - and hence: the phase angle of entrainment relative to external time - was mainly determined by the evening dusk. Both strains did show a seasonal regulation with an earlier chronotype in winter and a later one in summer, however, this regulation was more pronounced in the melatonin-proficient C3H/HeN than in the melatonin-deficient C57BL/6J. In addition the C3H/HeN did show more stable locomotor patterns in summer than in winter, this effect was not observed in the C57Bl/6J. These data may point at a role of the melatoninergic system in the seasonal regulation of locomotor behavior.

1C-I3

GLYPHOSATE AND GLYPHOSATE-BASED HERBICIDE EXPOSURE DURING THE PERIPARTUM PERIOD AFFECTS MATERNAL BRAIN PLASTICITY, MATERNAL BEHAVIOR, MICROBIOME AND OFFSPRING OUTCOME

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Glyphosate is by far the most widely used herbicide. Recent work in rodents suggests that glyphosate-based herbicide (GBH) can affect steroidogenesis and a number of neurotransmitter systems, leading to alterations in behavior. Here, we investigated the effects of peripartum exposure to GBH or glyphosate alone on maternal behavior and neurobiological correlates in the rat dam and the outcome in adult offspring. Pregnant female Sprague-Dawley rats received water solution (control), glyphosate (5mg/kg/day) or GBH (Round-Up® 5mg/kg/day of glyphosate) by ingestion from gestational day (GD) 9 to post-natal day (PND) 22. This dose corresponds to 1/10 of the “No Observable Adverse Effect Level. Maternal behavior was investigated in the first week postpartum. We observed a significant increase in time spent licking and grooming offspring on PND1 in control dams compared to glyphosate and GBH dams. However, between PND2-6, GBH-dams spent significantly more time licking and grooming offspring. After weaning, brains from dams were analyzed by immunohistochemistry to characterize neuroplasticity in the hippocampus, cingulate cortex and median preoptic nucleus. There was no effect of treatment on hippocampal neurogenesis. No effect of glyphosate or GBH as evident on measures of synaptic plasticity in the cingulate cortex or median preoptic nucleus. However, the expression of synaptophysin (presynaptic vesicle marker) was significantly increased in the dentate gyrus and CA3 region of hippocampus in glyphosate treated dams. These changes were not linked to corticosterone levels nor liver toxicity at PND22. However, Glyphosate or GBH treatment at GD20 and PND22 significantly modified the intestinal microbiota of the mother using 16SrRNA sequencing methods. These findings reveal that peripartum exposure to glyphosate and GBH affect maternal neuroplasticity, behavior, and microbiome. We have preliminary results suggesting that synaptophysine expression is also altered in CA1 in adult offspring by glyphosate alone, in a sexually differentiated manner. More work is currently under progress to define the extent to these potential alterations.

Keywords: glyphosate, Round-Up, maternal care, microbiome, neuroplasticity, hippocampus, mPFC, preoptic area, neurogenesis

1C-I4

PRENATAL EXPOSURE TO ENDOCRINE DISRUPTING CHEMICALS (EDCs) AND ITS POSSIBLE EFFECT ON REPRODUCTIVE HEALTH

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There is a widespread exposure of general population, including pregnant women and developing foetuses, to the endocrine disrupting chemicals (EDCs). These EDCs are compounds, either natural or synthetic, which may alter the hormonal system by mimicking the effects of endogenous hormones. EDCs have been reported to be present in urine, blood serum, breast milk, reproductive tissues and amniotic fluid. The mode of entry differs with individuals; it may be intake of oral contraceptives and other drugs, application of personal care products and cosmetics, consumption of preserved food or exposure to plasticizers etc. This study focused on screening EDCs in the maternal blood and amniotic fluid samples. These samples were collected in glass tubes during Caesarean sections (53 full term pregnant women). EDCs from the samples were extracted and analysed in GC-MS. Nine phenolic EDCs- methyl paraben (MP), ethyl paraben (EP), propyl paraben (PP), butyl paraben (BP), p-hydroxybenzoic acid (PHBA), bisphenol A (BPA), triclosan (TCS), octyl phenol (OP) and nonyl phenol (NP), were simultaneously detected in the samples. These results show that phenolic EDCs cross the placental barrier and reach the foetus. BPA and MP were chosen to study the early developmental exposure that may contribute to determination of sex or potential adult health outcomes are being investigated using animal models like rats and zebrafish.

2A-S3

ASSESSING THE FUNCTION OF PIGMENT DISPERSING FACTOR IN AN ANNELID MODEL OF CIRCADIAN AND CIRCALUNAR TIMING

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We are interested to understand the molecular signals that mediate the output of the endogenous oscillators (molecular clocks) of the marine bristle worm *Platynereis dumerilii*. One molecule in the centre of our interest is the bristle worm correlate of arthropod pigment dispersing factor (PDF) / pigment dispersing hormone (PDH). Whereas members of the PDF/PDH family are relevant modulators of circadian physiology in arthropods, and also have roles in reproductive control, their function in animal groups outside ecdysozoans remains largely elusive

We demonstrate that *Platynereis dumerilii* possesses orthologs of both a PDF/PDH-like preprohormone (closely related to the mollusc Cerebrin family) and a *bona fide* PDF receptor. With the help of a heterologous cellular receptor activation assay, we reveal that the PDF receptor is activated by the predicted mature peptide at physiological concentrations, providing direct evidence for the existence of a functional PDF/Cerebrin system in annelids.

Using immunohistochemistry, we reveal that PDF is expressed by a complex set of neurons, including distinct clusters in the brain and along the trunk of the adult worm. The posterior expression in the brain coincides with expression of *Platynereis* circadian clock genes, consistent with a function of PDF in circadian control. To test the functional requirement of the PDF/PDFR system in *Platynereis*, we have generated mutant alleles of the *Platynereis pdf* locus. Anti-PDF immunoreactivity in the brain is completely abolished in homozygous mutants, strongly indicating that these animals are deficient in PDF/PDFR signaling. By taking advantage of distinct behavioural paradigms and automated animal tracking software, we systematically investigate homozygous pdf mutant animals in comparison to their wild-type siblings in order to assess to which extent annelid PDF is involved in timing mechanisms or other functions.

2A-S4

EVOLUTION AND FUNCTIONS OF NEUROPEPTIDE SIGNALING NETWORKS IN C. ELEGANS

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Neuropeptides represent one of the largest groups of neural messengers and are key modulators of physiology and behavior. The hypothesis that they are central and conserved regulators of adaptive behaviors is an emerging trend. However, our understanding of their modulatory actions and the evolutionary origin of these effects is limited.

To unravel cellular and molecular mechanisms of peptidergic modulation, we use the nematode model *Caenorhabditis elegans* relying on its well-defined nervous system and the availability of targeted genetic tools. The *C. elegans* genome shows a broad diversity of at least 150 peptide precursor and receptor genes, but ligands for only a handful of neuropeptide receptors have been characterized so far. Using reverse pharmacology, we performed a large-scale deorphanization screen of all neuropeptide GPCRs to map the neuropeptide-receptor network in *C. elegans*. Our results uncover an intertwined network of RFamide neuropeptide pathways with diverse functions in the regulation of behavioral states, such as arousal and sleep-like behaviors. Furthermore, we identified a broad range of evolutionarily conserved neuropeptide pathways including thyrotropin-releasing hormone, myoinhibitory peptide, and neuromedin U signaling systems. Genetic studies in *C. elegans* reveal a role of these conserved neuropeptide pathways in the experience-dependent modulation of decision-making, learning and memory. At the behavioral level, we find that neuropeptides can coordinately regulate distinct mechanisms for adapting behavior. Experience shapes the activity of peptidergic neurons in the underlying neural circuits, which modulate circuit output with specific temporal characteristics. Our results provide a scaffold to further unravel the mechanisms and evolutionary conservation of peptide actions in behavioral plasticity.

2B-S1

PERIPHERAL AND CENTRAL EFFECTS OF THYROID HORMONES ON THE SKELETON

Professor Graham Williams

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Thyroid hormones have major effects on the skeleton. The active thyroid hormone, T3, acts via nuclear receptors expressed in all bone cell lineages including cartilage-forming chondrocytes, bone-forming osteoblasts and bone-resorbing osteoclasts. There are two T3 receptors, TR□ and TR□, and TR□ is the major functional receptor expressed in bone. I will discuss current understanding of the peripheral actions of T3 on the skeleton during growth and in adulthood. There have been considerable advances in our understanding of the physiological relationship between the control of circulating thyroid hormone levels and their relation to thyroid status in target tissues. I will present work describing this new understanding to provide a physiological appreciation of thyroid hormone regulation of skeletal development and bone maintenance. We recently identified secondary skeletal responses to central actions of thyroid hormones. Seasonal reproduction enables animals outside tropical regions to rear offspring in a favourable environment. Increasing day length triggers a hypothalamic relay involving thyrotropin, the type 2 deiodinase enzyme and T3, which activates the hypothalamic-pituitary-gonadal axis to induce reproductive competence. Photoperiod regulates calcium metabolism and the egg-laying cycle in the Japanese quail (*Coturnix japonica*), and we hypothesised that activity of this relay would have major consequences for bone mineralisation and strength. I will present data demonstrating an essential role for the central actions of thyroid hormone in medullary bone formation and the exquisite sensitivity of the quail skeleton to changes in photoperiod.

2B-S2

UNDERSTANDING GROWTH HORMONE ACTION ON THE SKELETON: THE ROLE OF SOCS2

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The functional activities of growth hormone (GH) on the skeleton are wide-ranging and can influence both linear bone growth and bone development/formation. This function is mediated by the GH receptor expressed by both the bone forming osteoblast and cartilage residing growth plate chondrocyte. Regulators of skeletal function can act both exogenously (*e.g.* physical trauma and pharmacological agents) or endogenously (*e.g.* altered autocrine/paracrine and systemic control). Examples of the latter include alterations to the GH/IGF-I axis which is a recognised critical regulatory pathway for bone growth and bone formation. Whilst the anabolic role of GH on bone growth and formation is well accepted the relative contributions of the direct or indirect effects of GH remain unclear. The GH indirect effects are via endocrine and/or local (growth plate chondrocyte or osteoblast) IGF-1 production. Various spontaneous mouse mutations in GH/IGF-I signalling have been informative. Also, the mouse genetic revolution with the creation of various transgenic and knock-out (inducible and tissue specific) strains of mice *e.g.* SOCS2 null mice, have helped us understand more fully the role of GH and IGF-I initiated pathways together with their negative feed-back loops and associated kinases and phosphatases on the skeleton. Notwithstanding the direct effects of GH on growth plate chondrocytes and osteoblasts it is likely that these two pathways function in a highly coordinated manner to promote linear bone growth and bone formation. This presentation will summarise the current state of understanding and introduce emerging insights.

2C-S1

CHEMOGENETIC AND BIOSENSOR APPROACHES TO EXPLORE AND VALIDATE POORLY STUDIED G PROEIN-COUPLED RECEPTORS

Graeme Milligan, Danielle Bolognini, Natasja Barki, Li-Chiung Lin, Tezz Quon, and Andrew Tobin

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Although G protein-coupled receptors are a major class of targets for the design of therapeutic medicines a large number of the some 400 or so non-chemosensory members of the family remain poorly studied. A substantial number of these are activated, with modest potnecy, by ligands that are often described as metabolic intermediates and in many cases there are multipe receptors that are activated by the same sets of ligands.

In the first part of my talk I will use free fatty acid receptor 2, which is normally activated by the short chain fatty acids acetate and propionate, to describe a chemogenetic strategy in which we altered the nature of ligands that can activate the receptor and, following generation of a transgenic knock-in mouse line, how we have used this approach to define functions that are mediated specifically by this receptor rather than by other receptors that are activated by short chain fatty acids or effects that reflect 'off-target' effects of such pleiotropic ligands.

In the second part I will combine the use of genome editing of expression of G protein subsets and a BRET-based biosensor strategy to define the G protein selectivity of the poorly characterised receptor GPR35 and how this is defined largely by a single amino acid in the G protein $G\alpha_{13}$.

2C-S2

SIMULATING GPCR MOTIONS AT ATOMISTIC RESOLUTION

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Recent advances have made molecular dynamics (MD) simulations a powerful tool for studying the functionality of G-protein-coupled receptors (GPCRs) [1-2]. Thanks to improvements in computational power, simulation algorithms, and potential energy functions, MD is capable of simulating GPCR motions at atomistic resolution reaching the microsecond or even millisecond timescale - a spatial and temporal resolution currently unattainable by other experimental methods. This lecture will introduce to the basics of MD as well as highlight recent success stories in applying MD to GPCR research. In addition, you will learn about the GPCRmd project (www.gpcrmd.org). A central focus of this project is to make the wealth of MD data accessible to the GPCR community (e.g. medicinal chemists, structural biologists, pharmacologists) and their research programs via intuitive visualization and analysis tools. Such a public platform aims to bridge gaps between different research disciplines in order to make GPCR research more efficient and successful. Ultimately, this lecture will present the simulation project “brainstorm@home”. This project pursues the development of a massive network of volunteers that devote idle resources from their computers to form a supercomputing platform dedicated to the study of severe brain-associated diseases. Such supercomputing platform enables to obtain atomistic insights into molecular mechanisms that underlie disease conditions in the brain at timescales otherwise not reachable. Your participation can make an important contribution towards this goal.

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2C-I1

GETTING TO THE HEART OF THE CORAZONIN RECEPTOR: G-PROTEIN COUPLED RECEPTOR DEORPHANISATION IN A CRUSTACEAN MODEL

Dr Jodi Alexander[1], Dr Andrew Oliphant[2], Dr David Wilcockson[2], Dr Neil Audsley[3], Ms Rachel Down[3], Rene Lafont[4], Prof Simon Webster[1]

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Membrane-bound G-protein receptors (GPCRs) are one of the key signalling units of eukaryotes and comprise the largest family of cellular transmembrane receptors. They are the primary mediators of neuropeptide signalling in crustaceans and play a central role in numerous physiological processes, including development, metabolism, reproduction and moulting. The diversity of crustacean neuropeptides and their cognate receptors is only now being discovered due to the increased accessibility of high-throughput genetic analyses. However, despite genetic characterisation of numerous GPCRs in crustacean genome and transcriptome analyses, few studies have functionally deorphanised the receptors and their ligands. In this work, we explore the structurally-related, functionally-diverse gonadotropin-releasing hormone paralogs, corazonin (CRZ) and red-pigment concentrating hormone (RPCH) and their G-protein coupled receptors (GPCRs) in the green shore crab, *Carcinus maenas*.

C. maenas CRZ and RPCH receptors were isolated, cloned and transiently expressed in AequoScreen® CHO cells to determine specific activation using a variety of naturally-occurring arthropod ligands. Pharmacological characterisation of both receptors indicated they were activated in a dose-dependent manner and were very sensitive to their respective ligands (EC_{50} = 0.75nM CRZR; 20 pM RPCHR), though neither receptor bound GnRH or AKH/CRZ-related peptide. Comparative studies with insect CRZ peptides suggest that the C-terminus of this peptide is important in receptor-ligand interaction.

Further molecular and biological analysis indicated that CRZR was preferentially expressed in the Y-organ (YO), and although addition of CRZ peptide to YO *in vitro* had no effect on ecdysteroid synthesis, it does appear to abrogate the action of molt-inhibiting hormone (MIH) at low nanomolar concentrations. The complete abolishment of MIH-induced repression of ecdysteroid synthesis reveals an unsuspected complexity in molt regulation of crustaceans. RPCHR was expressed in nervous tissue, dactyl tissue and ovary, and chormtaophoric bioassays confirmed its role as a red pigment concentrator. This study is one of the first to deorphanise a GPCR in a crustacean and to provide evidence for hitherto unknown and diverse functions of these evolutionarily-related neuropeptides.

2C-I2

EXPLORING NEW HORIZONS WITH ELECTRICAL IMPEDANCE SPECTROSCOPY

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Selecting appropriate cell-based assays among the many different options can be a challenging task. In addition, picking one or more endpoints to measure and correlate them with a number of cellular parameters can be difficult. Choosing for electrical impedance spectroscopy (EIS) can reduce the headaches involved with these selection procedures as it measures cellular responses in real-time, regardless of the downstream signaling cascade that is triggered.

Unlike conventional endpoint assays, impedance measurements provide a continuous and label-free approach to examine different types of biological behavior. The impedance spectrum of the studied system is calculated by recording a small electron flow caused by an alternating voltage applied across gold electrodes embedded in the bottom of a microtiter plate. In this way, it is possible for researchers to study a broad range of cellular processes in a single device, without the need for specialized pretreatments, cell manipulation or additional markers.

Although impedance spectroscopy has shown clear benefits for cellular and microbiological research, the technology was up to now not easily accessible for the biology oriented user. This will now change with the introduction of CellSine's I series.

CellSine offers broad-spectrum impedance spectroscopy capabilities in a setting tailored to the needs of biological research. The I series devices are modular and can be configured to address up to eight 96-well plates simultaneously. At present, CellSine's technology already proved its value in applications designed for cytotoxicity testing, biofilm maturation, protein-protein interactions, receptor activation, and the growth of adherent as well as suspension cells.

3A-S1

THE COMPLEX WORLD OF INSULIN-LIKE PEPTIDES: INSIGHT FROM TWO MEMBERS OF ARTHROPODA

Dr Monika Gulia-Nuss, Dr Arvind Sharma, Mr Preston Vu, Ms Blanca Guzman, Mr Jared Koler, Dr Andrew Nuss

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In vertebrates, members of insulin-like peptide (ILP) super family are important growth factors and multifunctional hormones. As many as ten different ILPs, including insulin, insulin-like growth factors (IGF), and relaxin, occur in humans and other mammals. Their functional diversity is due to differences in structure, receptor binding, and signal pathway activation. Multiple ILPs are also present in particular invertebrate species, including mosquitoes and ticks. Previously, we identified eight different ILPs and components of an insulin-signaling pathway in female *Aedes aegypti* mosquitoes. In bioassays, one ILP, ILP3, stimulated egg maturation and ovarian steroid production. This peptide showed high specific binding to ovary membranes, and expression of the insulin receptor was required for ILP3 action, as determined by RNA interference. We recently found that the expression of ILPs differ during blood and sugar feeding and during the development. We also found that knockdown of the insulin receptor (IR) in *Culex quinquefasciatus* mosquitoes completely prevented development of filarial nematode, *Wuchereria bancrofti*, to the infective L3 stage, and reduced, but did not prevent, development of another filarial nematode, *Brugia malayi*, in *Aedes aegypti* mosquitoes. We identified four ILPs in blacklegged tick, *Ixodes scapularis*. These ILPs also differ in their expression pattern in different life stages and body parts. Interestingly, IR knockdown in larval ticks affected host seeking. Our data from two different hematophagous arthropods shed light on the complexity of insulin signaling in invertebrates.

3A-S2

HAS CROSS-TALK BETWEEN GPCRS SYSTEMS IN HOST-PARASITE INTERACTIONS SHAPED THEIR EVOLUTION?

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Insect reproduction and nutrient metabolism are regulated by neuropeptides many of which act through the activation of heterotrimeric GTP-binding protein (G protein)–coupled receptors (GPCRs) in the presence of an internal or external stimuli. The GPCRs are an ancient family of membrane receptors with a conserved structure and are present from insects to vertebrates. The *Anopheles* mosquito genome contains at least 276 GPCR genes many of which are orphans and have uncharacterised functions. A unique feature of hematophagous insects such as the mosquito is the coupling between blood feeding and reproduction. In particular, the females of the *Anopheles* mosquitoes require a blood meal from a vertebrate host to induce egg production and development. Based on these characteristics we hypothesised that the peptide ligands of mammalian GPCRs that circulate in blood may regulate homologue GPCR systems in the mosquito. A study of the insect Allatostatin-type A (AST-A) system that has the same evolutionary origin as the KiSS system in vertebrates, failed to reveal AST-AR activation by the vertebrate ligand KISS peptides. However, in a recent study we identified several vertebrate peptides that could trigger vitellogenin (Vg) transcription *in vivo* and identified several vertebrate peptides that could activate *Anopheles* GPCRs. The results suggest some mammalian peptides can stimulate mosquito GPCRs but if this has modified their evolution still remains to be established.

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3B-S1

HOW THE DEVELOPMENT OF OXYTOCIN NEURONS AFFECTS SOCIAL BEHAVIOUR OF ZEBRAFISH

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Oxytocin-like neurohormones (isotocin in fish and oxytocin in mammals) have been implicated in the regulation of social behavior across vertebrates. In this study we used zebrafish, a social animal model with a well-characterized repertoire of social behaviors and a wide genetic toolbox available. We developed genetic tools to manipulate the oxytocinergic neuronal circuits and to study how loss of function of these neurons during development affects the development of social behavior. Our results indicate a role for oxytocinergic neurons in the acquisition of zebrafish sociality, a trait that emerges during the third week of development. A conditional and cell-specific ablation of these neurons at a critical developmental time window, but not during adulthood, significantly altered specific adult social behaviors in zebrafish, suggesting an unique developmental organizational rather than activational effect of the oxytocin neuronal system on a specific social behavior trait. Furthermore, using genome-editing methods (i.e. TALEN, CRISPR), we found evidence that oxytocinergic neurons may modulate distinct aspects of social behaviors through different mechanisms.

3B-S2

FROM PAIR BOND TO PARTNER LOSS: NEUROPEPTIDES ARE INDISPENSABLE

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Throughout life, the formation and cultivation of positive social relationships is vital for physical and mental health, ranging from decreased risk for cardiovascular and infectious disease to increased stress resilience as well as a reduced likelihood to develop depression and anxiety disorders. In this context, the brain neuropeptides arginine vasopressin (AVP) and oxytocin (OT) play indispensable roles as has been studied extensively in the animal model of the socially monogamous prairie vole (*Microtus ochrogaster*). For example, AVP signalling in the ventral pallidum and OT signalling in the nucleus accumbens (NAcc) facilitate mating-induced pair bonds in adults.

However, the sudden disruption of a pair bond increases the susceptibility to physical and emotional dysfunctions in both humans and animal models. For example, in pair bonded male and female prairie voles, increased anxiety-related and depressive-like behaviours are among the negative outcomes of partner loss. These emotional disturbances are caused by a separation-induced activation of the brain corticotropin releasing factor (CRF) system. Recently, we demonstrated in male prairie voles that such increased CRF signalling is suppressing the OT system in the NAcc on multiple levels. Importantly, local infusion of OT into the NAcc prevents the onset of depressive-like behaviour following partner loss.

In conclusion, these studies have important translational implications relevant to the disruptions of social bonds. Therefore, potential therapeutic strategies aiming at these neuropeptide systems are promising targets for the treatment of social loss-mediated depression.

3C-S1

EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF NEUROPEPTIDE RECEPTORS IN *BOMBYX MORI*

Dušan Žitňan, Ivana Daubnerová, Ladislav Roller, Zuzana Gáliková, David Wildridge

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We have been using in situ hybridization, Illumina transcriptome sequencing, qPCR and various transgenic approaches to investigate expression and functions of neuropeptides and their receptors during ecdysis and reproduction in the silkmoth *Bombyx mori* and the fruitfly *Drosophila melanogaster.* Ecdysis triggering hormone (ETH) produced by endocrine Inka cells is a key factor in activation of the ecdysis sequence. We found that at least four neuropeptide receptors are expressed in Inka cells that mediate ETH release during ecdysis. ETH action on its receptors (ETHR-A and ETHR-B) expressed in numerous central neurons, corpora allata (CA), H-organ and Malpighian tubules elicits complex ecdysis and post-ecdysis behavioral and physiological processes. ETH apparently controls behavioral cascade for shedding the old cuticle, but also regulates the release of juvenile hormones from the CA, dopamine from the H-organ, and processes associated with diuresis in Malpighian tubules. To examine the roles of these ETHR networks in *Bombyx*, we used electrophysiology and transgenic approaches with piggyBac vectors. These approaches revealed specific functions of individual types of neurons and neuroendocrine pathways between Inka cells, central neurons and other peripheral organs.

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3C-S2

ADOPTIVE LIGANDS AT THE RECEPTOR ORPHANAGE- DEORPHANISING GPCR/LIGAND PAIRS IN CRUSTACEANS- A STRATEGY TO REVEAL FUNCTION?

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In recent years, *de-novo* assembly and data mining of transcriptomes and genomes of arthropods has revealed a wonderfully rich and diverse collection of neuropeptide signalling molecules, and their putative (mainly G-protein coupled) receptors (GPCRs). Many of these signalling systems are undoubtedly ancient and are common to both insects and crustaceans- perhaps unsurprisingly, given the consensus that all arthropod lineages arose from a monophyletic ancestor. An inevitable consequence of the rapid expansion of arthropod neuropeptidomes is that we still know remarkably little regarding the functions of many of these neuropeptides, and this is particularly striking for crustaceans where very few ligand/receptor pairs have been deorphanised, which is mandatory if we are to address this issue. Using the aequorin cell-based Ca^{2+} reporter assay, cloned GPCRs identified from our *de novo* transcriptome assemblies of neural and non-neural tissue libraries in the green shore crab, *Carcinus, maenas*, together with qRT-PCR, *in-situ* hybridization and immunochemical approaches, we have currently deorphanised several receptor-ligand pairs-which are universal components in all arthropod peptidergic systems. Current examples include calcitonin-like diuretic hormone 31 (DH31), crustacean cardioactive peptide, (CCAP) (2), pigment dispersing hormone/factors (PDFs) (2), tachykinin related peptides (TRPs), red pigment concentrating hormone/adipokinetic hormone (RPCH/AKH) and corazonin (CRZ). In this presentation I will review our current progress (and problems!) in functionally deorphanising a variety of ligand-receptor pairs in crabs. In particular, novel and speculative functions for these will be highlighted, together with discourse on the evolution, and functional diversification of neuropeptide signaling in arthropods.

3A-I1

THE ENIGMATIC ORIGIN OF VERTEBRATE NEUROPEPTIDE SYSTEMS: GENOME DOUBLINGS INVESTIGATED IN LAMPREYS FOR THE CRH PEPTIDE FAMILY

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The ancestor of the gnathostomes, i.e. the jawed vertebrates, underwent two tetraploidizations approximately 500 million years ago. These events are referred to as 1R and 2R for the first and second round of genome doubling. However, it has been exceedingly difficult to resolve whether the cyclostome lineage (the jawless lampreys and hagfish) branched off after 1R or after 2R. Previously, we reported the evolution of the corticotropin-releasing hormone (CRH) family and concluded that the 5 members in gnathostomes arose as a result of 1R and 2R: one of the two ancestral genes was duplicated whereas the other ancestral gene gave rise to a triplet (Cardoso et al., 2016). Lampreys too were found to have 5 CRH-family genes, but the orthology of these to the gnathostome genes was unclear. Recently, a new germline genome assembly of the sea lamprey *Petromyzon marinus* was reported (Smith et al., 2018) and we have reanalyzed the evolution of the CRH family in cyclostomes by comparing the new assembly with the Arctic lamprey *Lethenteron camtschaticum* and with gnathostomes. Searches in the new sea lamprey genome assembly confirmed the presence of 5 CRH-family genes. Phylogenetic analyses show that three of the lamprey CRH-family genes belong to the gnathostome CRH1/CRH2/UCN1 clade and the remaining two cluster with the gnathostome UCN2/UCN3 clade. Comparison of the neighbouring gene regions revealed highly similar synteny between cyclostomes and gnathostomes, represented by human, chicken and spotted gar. However, detailed analyses suggest that the chromosome blocks that resulted from 1R and 2R display a few cross-wise similarities between cyclostomes and gnathostomes. The most parsimonious interpretation is that cyclostomes and gnathostomes share also the second tetraploidization, but the relationships have become obscured by crossing-over events. Also other peptide families support this scenario.

3A-I2

WHAT OXYTOCIN-LIKE SIGNALLING SYSTEM DOES IN ANTS?

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The oxytocin/vasopressin signalling system comprises G protein-coupled receptors and their endogenous peptide ligands. It appears to be important for water homeostasis, reproduction, learning, memory and behaviour. To date, its biological function in insects has only been studied in the beetle *Tribolium castaneum* and the locust *Locusta migratoria* where it has been implicated in water retention in Malpighian tubules. The first evidence that an oxytocin/vasopressin-like signalling system exists in social insects came from ant genome sequencing indicating the presence of one receptor and one neuropeptide precursor protein in all sequenced ant species. 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.

Following pharmacological characterization of the ligand-receptor pair *in vitro*, our aim was to determine the distribution and expression level of both the receptor and precursor in different parts of the body and developmental stages in ants using qPCR and immunostaining. Next we were able to generate a knock-down of the precursor in *Lasius* ants and performed *in vivo* behavioural experiments.

Our qPCR results indicate that this signalling system can be involved in male reproduction (high expression of receptor in male heads and reproductive organs) similar to other invertebrate species. On the other hand, the expression of receptor in Malpighian tubules is very low which contradict earlier findings in beetles and locusts. Additionally, high expression of receptor in some parts of digestive system and fat body, different expression in winter/summer conditions, down-regulation of the precursor after starvation and increased walking activity of knock-down ants indicate that oxytocin/vasopressin signalling in ants may be involved in food uptake, digestion, maintenance of energy status and locomotion.

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3B-I1

HOW OXYGEN AND NEUROPEPTIDE SIGNALLING REGULATE A SOCIAL BEHAVIOUR IN *C. ELEGANS*

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Social behaviour can be help animals to adapt and survive in challenging environmental conditions, where individuals protect each other through close association. *C. elegans* nematodes can display social behaviour in laboratory conditions where they form groups on bacterial food lawns, a behaviour termed aggregation. Remarkably, it has been shown that aggregation is essentially a consequence of the avoidance of high atmospheric (21%) oxygen levels. It depends on the molecules that constitute the oxygen-sensing machinery and is regulated by the circuit that mediates high O_2 avoidance. Two behavioural programmes are evoked by high oxygen, a long-lasting increase of locomotory speed and a transient escape response to rising O_2. Together, the two behaviours form the basis for worms joining and remaining in groups. While the neural circuit controlling high O_2 avoidance and aggregation appears to be fixed, both aggregation and high O_2 avoidance show extensive plasticity and are modulated by environmental context or genotype. Several forms of such plasticity are regulated by neuropeptides. For example, allelic differences in a neuropeptide Y receptor homologue determine whether *C. elegans* strains avoid high O_2 and display social or solitary behaviour. The O_2 responses can be reprogrammed by experience in some genetic backgrounds but not others. We found that neuropeptide signalling affects the tuning of O_2-evoked behavioural states and also experience-dependent plasticity. This way, neuromodulation can reconfigure behavioural strategies in different ways in reaction to sensory cues, to enable adaptive behaviour in changing environments and conditions.

3B-I2

INTERACTIONS BETWEEN HORMONES, BEHAVIOUR, AND REPRODUCTIVE SUCCESS IN PHOCID SEALS; OXYTOCIN AND SOCIAL TRANSITIONS BETWEEN SOLITARY FORAGING AND COLONY LIVING

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The neuropeptide hormone oxytocin is well known for its role in birth and lactation. A growing body of literature demonstrates the hormone additionally mediates maternal and social bonding and behaviour while acting on the hypothalamic–pituitary–adrenal (HPA) axis to buffer stress responses under certain conditions. Phocid seal species typically live in highly contrasting social environments throughout the year, living mostly solitary lives at sea foraging until they aggregate in dense colonies to breed. We investigated whether plasma oxytocin concentrations in female grey seals (*Halichoerus grpyus*) breeding annually on two Scottish colonies showed any change from when they are non-breeding, and whether such changes were associated with the maternal care given to their pups. We then used oxytocin manipulations on unrelated seals from the same colony to investigate the hormones effects on social behaviour towards individuals outside of the mother-pup dyad. Mothers with pups had significantly higher plasma oxytocin concentrations than non-breeding females on the colony. High oxytocin mothers maintained closer proximity to their pups, reducing the likelihood of separation and pup starvation, the largest cause of death for dependant grey seal pups. Oxytocin manipulations on unrelated seals had a significant impact on the amount of positive social behaviour recorded between individuals, with reduced aggression and more time spent in close proximity to each other. The elevation of oxytocin due to breeding events may therefore have additional pro-social effects, facilitating tolerance of surrounding colony occupants and enabling this species to live at both ends of the social spectrum at different times of year. Reduction of aggression towards conspecifics is often cited as a crucial step in the evolution of sociality, and through the interaction of reproductive hormones and social behaviour, aggregations of breeding individuals may waste less energetic resources on costly aggressive interactions while gaining greater reproductive success.

3C-I1

TARGETING GPCRS TO REDUCE PEST POPULATIONS

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G-protein coupled receptors (GPCRs) regulate many key physiological and behavioural processes in animals. Mutations in GPCR genes, resulting in loss of function, are the cause of many human diseases. Hence, human GPCRs have been key targets for the development of pharmaceutical drugs and represent the largest family of druggable targets; around 35% of approved drugs act on GPCRs.

In arthropod pest species, modulating GPCR activity to disrupt essential physiological or behavioural functions (e.g. growth, development, reproduction), or reduce fitness, may result in population suppression or ultimately death. Consequently, GPCRs (and their ligands) are considered prime targets for the development of next generation pesticides. However, despite the advancements made in characterising the structures and functions of invertebrate peptides and their receptors, little progress has been made towards pest management applications.

With the current problems associated with conventional pesticides (development of resistance and restrictions on use), new mode of action and more selective, environmentally benign pesticides are required.

Comparisons between invertebrate species has highlighted differences in the GPCR (and peptide) complement and in their relative activities, which could be exploited for the development of selective pesticides.

The GPCRs from the invasive pest *Drosophila suzukii* and/or the honey bee (*Apis mellifera*) have been identified and functional assays developed to compare the efficacy of native peptides, analogues and synthetic compounds as an initial screening step.

3C-I2

MULTIPLE PHYSIOLOGICAL ROLES OF MYOSUPPRESSIN SIGNALLING IN DIPTERAN INSECTS

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The FMRFamide-like peptides (FLPs) are an ancient family of invertebrate peptides with a common RFamide (Arg-Phe-NH_2) C-terminus and are known to have wide ranging roles as both neuromodulators and as circulating hormones. Insect myosuppressins are N-terminally extended FLPs with important roles in visceral muscle motility (e.g. heart, gut) in many species across the Insecta.

In lepidopteran species, hormonal myosuppressin can play an important role in the regulation of development by inhibiting the synthesis of the moulting hormone ecdysone by the prothoracic gland. We will report on recent studies investigating the multiple roles of myosuppressin signalling in gut physiology, metamorphosis and reproduction in dipteran insects.

4A-S1

NEUROPEPTIDIC REGULATION OF OOCYTE MATURATION AND SPAWNING IN THE HYDROZOAN JELLYFISH *CLYTIA HEMISPHAERICA*

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In the hydrozoan jellyfish *Clytia hemisphaerica,* oocytes are released every day upon a dark-light cue, even when the gonad is isolated from the medusa. This cnidarian experimental species is thus well suited for studying the regulation of oocyte maturation and spawning. We identified specialised neurosecretory cells in the gonad ectoderm that respond to the light cue by secreting a small neuropeptidic Maturation Inducing Hormone (MIH; Takeda et al., 2018). MIH consists of PRP/PRA/PRYamide type amidated tetrapeptides, synthesized in multiple copies from two distinct precursors polypeptides. MIH provokes spawning in both males and females at nanomolar concentrations, and so may contribute to spawning synchronisation between animals when they gather at the ocean surface at dawn. Light-triggered neuropeptide secretion by gonad MIH cells is critically mediated by an opsin photopigment, *Clytia* Opsin9. Opsin9 mutant jellyfish generated using CRISPR/Cas9 fail to spawn, a phenotype reversible by MIH (Quiroga Artigas et al., 2018). Recently we have identified an oocyte-expressed GPCR as a putative MIH receptor (MIHR; collaboration with G. Jékely). We are currently generating and analysing *MIHR* mutant medusae. Sequence comparisons allow us to propose relationships between *Clytia* MIHR and known bilaterian neuropeptide GPCR families, and to speculate on the evolutionary links between reproductive regulation in cnidarians and bilaterians.

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4A-S2

UNRAVELING THE EVOLUTION AND FUNCTIONS OF CORAZONIN SIGNALING SYSTEM: INSIGHTS FROM DROSOPHILA

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Environmental factors can challenge the homeostasis in animals, thereby evoking stress responses. A multitude of physiological and behavioral mechanisms have evolved to counter stress at the organism level. These include several hormones and neuropeptides. Corazonin (CRZ) and gonadotropin-releasing hormone are two such stress peptides that arose by gene duplication in Urbilateria. CRZ has been shown to be involved in stress responses in *Drosophila* and other insects; however, the neural circuit underlying this stress signaling is unknown. Here, we mapped the CRZ receptor (CRZR) expression in the nervous system using various GAL4 lines. We found that the CRZR is expressed by several neurons in the central nervous system. More specifically, in adult flies the CRZR is expressed in the median neurosecretory cells in the brain, hugin/pyrokinin producing cells in the suboesophageal ganglion (SEG) and CAPA-expressing neurons in abdominal neuromeres (Va neurons). CAPA peptide signaling has recently been implicated in mediating recovery from desiccation and cold stress and hugin/pyrokinin cells in the SEG are known to regulate food intake. In our study, we explore the effects of knocking down CRZ in CRZ-producing neurons and CRZR in various subpopulations of neurons on feeding, water balance and response to various stresses. We show that CRZ acts upstream of CAPA neurons to influence water balance and stress tolerance. Our results delineate a neural circuit/neuroendocrine pathway that coordinates various stress associated behaviors in *Drosophila.*

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4C-S1

MULTI-OMICS APPROACHES TO UNDERSTAND INSECT RENAL FUNCTION

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Most living species are insects, and their successful diversification depends at least in part on the ability to osmoregulate successfully across a broad range of ecological niches. Insect Malpighian tubules were first described in the 17th Century, and renal physiology has been studied intensively for 70 years, step-changes in our understanding have been brought about by the advent of genomics, transcriptomics, proteomics and metabolomics. These technologies are natural partners with (though do not obligatorily require) model organisms and transgenic technologies. This talk will review the impact of multi-omic technologies on our understanding or renal function and control in *Drosophila melanogaster* and other insects.

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4C-S2

THE GOOD AND THE BAD: DO THE NEUROPEPTIDOMES OF PEST INSECTS PROVIDE TARGETS FOR CONTROL STRATEGIES THAT DO NOT AFFECT BENEFICIAL INSECTS?

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Traditionally employed insecticides are poisons that make no difference between pests and beneficial insects or spiders. Natural predators of pests are often affected much more severely because they exist in significantly fewer numbers. As a result, in the next generation the pest populations might explode because the number of predators has been diminished. Thus, there is an urgent need to develop “greener” pesticides that target damaging insects while sparing beneficial ones. Within the project *nEUROSTRESSPEP*, we aim to fight insect pests by turning their own peptide hormones against them. This requires the identification of specific features in the neuropeptidome of the targeted pest species.

Such an approach is discussed here using the neuropeptidomes of two pest beetles, *Hylobius abietis* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). The number of observed neuropeptide precursors and the sequences of processed bioactive neuropeptides of these species are compared with the neuropeptide complements of the economically important honeybee (*Apis mellifera*) as well as neuropeptides from ground beetles (*Carabidae*) which are natural predators of many common pest insects and frequently overwinter even within cultivated fields. In addition, the neuropeptidome of collembolan species, which belong to the ecologically most relevant hexapods in soils of temperate climate regions, is considered in this overview.

4A-I1

ANTI-DIURETIC HORMONES IN INSECTS: PROGRESS ON THE FUNCTION OF CAPA GENE-DERIVED PEPTIDES IN MOSQUITOES

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During their terrestrial stage, adult *Aedes aegypti* mosquitoes face desiccation stress upon emergence from their aquatic habitat and, additionally, females must cope with insult to their haemolymph balance following intake of a blood meal. To cope with this challenge to homeostasis, insects rely on a highly efficient excretory system, including the Malpighian tubules (MTs) and hindgut, which are largely regulated by neuropeptides. This involves coordination of ion transport processes in both the MTs and hindgut along with myoactivity of the hindgut. The *capability* gene encodes two types of peptides, CAPA and pyrokinin-1 neuropeptides, which act on distinct receptors. CAPA peptides have been shown to regulate activity of MTs; however, pyrokinins are not known to regulate the MTs, but may target the hindgut eliciting myotropic actions. In this study, the activity of a mosquito CAPA peptide was measured on female MTs stimulated with various diuretic factors. CAPA inhibits secretion of MTs with a subset of diuretics, but does not influence the proportions of cations transported. The second messenger cGMP mimicked the effects seen with CAPA, while pharmacological inhibition of PKG/NOS signaling abolished its anti-diuretic activity, confirming the role of cGMP/PKG/NOS in the CAPA signaling pathway. Pyrokinins are known to influence hindgut physiology in some insects, yet their functions remain unclear in mosquitoes. Herein, we examined the expression of an *A. aegypti* pyrokinin-1 receptor within the alimentary canal (i.e. hindgut) at the transcript and protein level. Immunohistochemical staining in female mosquitoes revealed pyrokinin receptor distribution within the rectum, including specific localization to the rectal pads. Receptor expression profiles at the transcript level corroborate enrichment in the hindgut, specifically within the rectum. Given this defined receptor expression, we examined prospective physiological roles of its peptidergic ligand; however, pyrokinin-1 did not elicit myotropic activity nor did it influence ion transport (Na^+ or K^+) across the rectum. Ongoing studies are examining the coordination of both CAPA and pyrokinin-1 receptors in the adult mosquito since their peptidergic ligands, although unique, are derived from a common precursor peptide expressed in neurosecretory cells of the nervous system.

4A-I2

FUNCTIONAL DIVERGENCE OF TWO GNRH SUPERFAMILY MEMBERS IN A GASTROPOD MOLLUSK

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The gonadotropin-releasing hormone (GnRH) superfamily is an ancient group of neuropeptides consisting of several peptide families. The critical role of vertebrate GnRH in reproduction has been established, but other members of the GnRH superfamily may have taxon-specific functions. A gastropod mollusk, *Aplysia californica*, produces two members of the GnRH superfamily: an invertebrate GnRH-like molecule named ap-GnRH and an adipokinetic hormone named ap-AKH. As such, *A. californica* represents an excellent model for examining the functional diversification of the GnRH superfamily members. Both ap-GnRH and ap-AKH are neuropeptides produced in the central nervous system of *A. californica*, but ap-GnRH is primarily a coordinator of motor function, and ap-AKH is primarily a volume regulator. Neither ap-GnRH nor ap-AKH stimulates reproduction in *A. californica*. The receptor for ap-GnRH has been functionally authenticated and is more closely related to the receptor for corazonin (CRZ), suggesting ap-GnRH may be more appropriately classified as a CRZ family member of the GnRH superfamily. In sum, we have identified novel functions of ap-GnRH and ap-AKH that are distinct from those reported for arthropod CRZ and AKH. Our results highlight the extraordinary functional diversification of the GnRH superfamily members in different protostome lineages. (Supported by NSF Grant IOS-1352944 to PST)

4C-I1

RNAI-BASED BIOPESTICIDES

Guy Smagghe

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Over the past decade, RNA interference (RNAi), the sequence-specific suppression of gene expression triggered by specific dsRNA molecules, has proven to be a very promising strategy in crop protection. The main advantages of RNAi are its selectivity, as well as the lack of persistency in and damage on the environment as a whole. In this paper, we report on the promising results against pest insects such as the western corn rootworm *Diabrotica virgifera*. Also successes have been reported against other beetle pests as Colorado potato beetle, but also sucking pest insects as Asian citrus psyllids and mites. In addition, a number of challenges will be discussed that needs to be addressed to implement RNAi as a widely-used pest control strategy. One of these challenges is a variable efficiency. While some insects show a very robust, efficient and systemic RNAi response, many others show a limited or variable RNAi response. Possible causes for this variability in sensitivity are degradation of the dsRNA in the insect body, insufficient uptake into the cells, viral interactions or problems with the RNAi machinery in the cells. Also RNAi of players in the endocrine pathway can be presented to control important pest insects.

4C-I2

NEUROPEPTIDES AND THEIR RECEPTORS IN LOCUST PHYSIOLOGY

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Despite their huge biodiversity, some fundamental characteristics are shared by all animals. Insects, like other metazoans, are heterotrophs, implicating the basic need for the intake of food. Subsequently, their metabolism, growth, postembryonic development and reproduction will depend on this. Therefore, it is crucial that they can rely on physiological mechanisms controlling and integrating these essential processes. Nutrient-sensing, hormonal and neuronal signalling systems are playing an important role in this complex regulation.

In this state-of-the-art presentation, we will consider a few neuropeptide-mediated signalling pathways that are implicated in the regulation of developmental-physiological processes in insects. In particular, we will discuss recent data obtained in the locusts, *Schistocerca gregaria* and *Locusta migratoria*, which are swarming pest species that irregularly devastate the agricultural production in large areas of the world. In a physiological and neurobiological research context, they have proven to be interesting experimental model organisms. We also evaluated the potential of peptidomimetic analogues to interfere with neuropeptide receptor signalling, as well as downstream physiological processes. Moreover, RNA interference constitutes a highly efficient and robust method to silence the expression of peptide precursors and/or receptors in several insect species, including locusts.

We have identified several insect neuropeptide precursors and receptors and will report on our recent physiological and molecular biological studies that further illustrate the important role of neuropeptides and their receptors in locusts. The general aim of our work is to contribute to a better understanding of the regulation of postembryonic processes, as well as of the functional interactions between different regulatory pathways in an integrative – organismal/systemic - physiological context.

We gratefully acknowledge the EU (Horizon-2020 project nEUROSTRESSPEP), the Research Foundation of Flanders (FWO-Flanders) and the Special Research Fund of KU Leuven (C14/15/050) for financial support.

5A-S1

THE LOCAL IL-1 SYSTEM OF PANCREATIC ISLETS AND ITS EFFECTS ON INSULIN SECRETION AND GLUCOSE CONTROL

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Metabolic diseases including type 2 diabetes (T2D) are associated with activation of the innate immune system characterized by changes in cytokines and immune cells in various tissues including pancreatic islets and insulin sensitive tissues. The prototypical cytokine IL-1beta has a dual role in metabolism. Acutely, it participates in the physiology of glucose control by contributing to postprandial insulin secretion while chronically, it is implicated in beta cell dysfunction, insulin resistance and cardiovascular disease. Clinical studies in patients with T2D using IL-1 antagonists showed improved insulin secretion and glycaemia pointing to the therapeutic potential of IL-1 blockade in T2D.
Pancreatic islets locally express a functional IL-1 system consisting of the IL-1Receptor1, the agonist IL-1beta and the competitive IL-1Receptor antagonist (IL-1Ra). Of note, insulin-producing beta cells have the most abundant expression of IL-1Receptor1 compared to other tissues and are thus a privileged site of IL-1 action.
To identify the cellular source and the function of IL-1beta and IL-1Ra in mouse islets we knocked out either the agonist IL-1beta or the antagonist IL-1Ra specifically in pancreatic beta cells or in myeloid cells. We identified islet myeloid cells as main source of functional IL-1beta expression within islets while IL-1Ra is expressed in both, islet beta cells and islet resident immune cells. Deletion of IL-1Ra specifically in beta cells, but not in myeloid cells, results in diminished islet cell IL-1Ra expression and impairs glucose homeostasis, beta cell proliferation, islet growth and insulin secretion. Increased IL-1beta signalling targets proliferation genes and the E2F1-Kir6.2 pathway that regulates insulin secretion. Therefore, part of the beneficial effect of IL-1 antagonism in the treatment of T2D may occur via targeting the negative effects of IL-1beta on beta cell proliferation genes and thereby may improve both, beta cell turnover and function.

5A-S2

RECENT ADVANCES IN UNDERSTANDING THE MECHANISMS OF RNA INTERFERENCE IN INSECTS

Jozef Vanden Broeck

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To combat viruses, invertebrates such as insects rely on their innate immune system, since they do not possess an antibody-based adaptive immune system. In recent years, evidence accumulated highlighting the importance of small regulatory RNA pathways in protecting insects against viruses. These small RNAs don't code for proteins but act at the post-transcriptional level via a process called RNA interference (RNAi).

In general, RNAi is triggered by double-stranded RNA molecules, which are converted into small RNAs that then induce a sequence-dependent knockdown of the RNA target. Given the high diversity in insect species, several characteristics of the RNAi process, such as dsRNA degradation, cellular uptake and systemic signal spreading, also appear to show a species-dependence. In this invited lecture, recent progress on the mechanisms of RNAi and the role of different types of small RNAs in insect antiviral immunity is summarized.

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5C-S3

THE KISS OF DEATH: NEUROENDOCRINE CONTROL OF DIURESIS IN THE KISSING BUG RHODNIUS PROLIXUSI, THE VECTOR OF CHAGAS DISEASE

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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. *Rhodnius prolixus* takes a huge blood meal over a 15 min period, which is followed by a rapid post-feeding diuresis. The parasite is passed along to the human within the urine. Here we examine the neurohormones controlling this diuresis, and therefore that control the transmission of Chagas disease.

Serotonin is a diuretic hormone (DH) in *R. prolixus* and works in concert with a member of the corticotropin-releasing factor (CRF)-related family of insect neuropeptides, via their respective G-protein-coupled receptors (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, via cAMP, to stimulate rapid post-feeding diuresis during a 3 hour period following gorging.

An anti-diuretic hormone (ADH; 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 eliminates the synergism between the two DHs, serotonin and Rhopr-CRF/DH, thereby leading to a quick cessation of the rapid post-feeding diuresis.

The interplay between the DHs and ADH 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

5C-S4

OMICS AND THE PHYSIOLOGY OF A SUPERORGANISM: GPCR SIGNALING AND BRAIN TRANSCRIPTOMES OF THE FIRE ANT (SOLENOPSIS INVICTA BUREN) TOWARD LINKING NUTRITION AND REPRODUCTION

Professor Patricia Pietrantonio

TAMU, United States

Omics and the physiology of a superorganism: GPCR signaling and brain transcriptomes of the fire ant (*Solenopsis invicta* Buren) toward linking nutrition and reproduction
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The success of the red imported fire ant (*S. invicta*) as invasive pest is due in part to its high reproductive potential and the adaptation to cooperative social living, as non-kin queens coexist and collaborate for brood care in the polygyne form. Our research relies on social insect theory, and attempts in the long term to integrate omics, molecular physiology, endocrinology and nutritional signaling to obtain a global view of nutrient sensing pathways and their role in reproduction. We aim at answering the fundamental question: at which gene network nodes, nutrient and hormonal inputs converge? We are developing knowledge on social insects and simultaneously testing this superorganism as a model for this important question in animals because division of labor offers the unique opportunity to somewhat decouple reproductive vs. organism maintenance roles (sterile workers). Toward this long term goal we have analyzed and compared brain transcriptomes of mated dealate queens vs. virgin alate queens and distinguished changes in gene expression due to mating vs. those elicited by virgin queen reproductive maturation and disinhibition from queen pheromone. Further, we compared these transcriptomes with those of worker brain and identified differentially expressed GPCRs between queens and workers. We have mined the genome for GPCRs and G proteins, and have reannotated many GPCRs and analyzed the expression of G proteins by qRT-PCR. Some GPCRs and cognate neuropeptides appear particularly suited for investigations in social hymenopterans, and yet others remain orphan.

5A-S3

FROM RESOLVING INJURIES TO PROMOTING TUMOURS: USING ZEBRAFISH TO UNDERSTAND MICROGLIA FUNCTIONS

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Microglia are the resident immune cells of our brain. Although microglia have been intensively studied since their first detailed description almost 100 years ago, it is only now, in the era of advanced imaging technologies and transgenic cell labelling methods that we are beginning to understand the tremendous repertoire of functions these cells have. As part of the macrophage lineage, microglia are extremely sensitive to changes in their environment and their fully differentiated phenotype can only be found in their natural environment, namely the brain. This is also reflected in their gene expression profile, which clearly discriminates microglia from other residential macrophages as well as from cultured microglia. Thus, animal models are indispensable for studying microglia *in vivo* and studies in leech, mice and zebrafish have uncovered a variety of unexpected functions for microglia. We have established the zebrafish as an *in vivo* model to study microglia. In particular the opportunity to image cellular interactions at high temporal and spatial resolution *in vivo,* combined with the ability to intervene genetically and pharmacologically, make the zebrafish an excellent model.

By using a combination of genetic and pharmacological tools allied to advanced confocal live imaging, we were able to show how the responses of microglia to brain injuries in the living brain are triggered. By inducing local injuries, we were able to follow individual microglia and identified the signalling mechanisms that guide their responses. Based on this model, we identified glutamate driven Ca^{2+} transients that mediate the release of ATP/ADP, which is sensed by the P2YR12 receptor expressed on microglia. Importantly, this mechanism appears to be conserved and microglial responses to Ca^{2+} mediated ATP release have been described in many contexts since.

Currently we are focussing on the role of microglia in brain tumours. It is now clear that microglia and macrophages are present in brain tumours, but whether or how they affect initiation and development of tumours is not known. Exploiting the advantages of the zebrafish model, we showed that

macrophages and microglia respond immediately upon oncogene activation in the brain. Overexpression of human AKT1 within neural cells of larval zebrafish led to a significant increase in the macrophage and microglia populations. By using a combination of transgenic and mutant zebrafish lines, we showed that this increase was caused by the infiltration of peripheral macrophages into the brain mediated via Sdf1b-Cxcr4b signalling. Intriguingly, confocal live imaging reveals highly dynamic interactions between macrophages/microglia and pre-neoplastic cells, which do not result in phagocytosis of pre-neoplastic cells. Finally, depletion of macrophages and microglia resulted in a significant reduction of oncogenic cell proliferation. Thus, macrophages and microglia show tumour promoting functions already during the earliest stages of the developing tumour microenvironment.

5A-I1

PORCINE ISLETS AS A POSSIBLE TREATMENT FOR TYPE 1 DIABETES

Dr Claire Crossan

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The use of porcine derived islets to treat type 1 diabetes mellitus (T1DM) offers patients the potential to have their blood glucose levels controlled without the need to self-administer insulin by subcutaneous injection. The major barrier to the success of islet xenotransplantation is the destruction of the transplanted islets by the recipient's immune system. However, this can be overcome by encapsulation of the islets in alginate, which is porous enough to allow the passage of small molecules such as glucoses and hormones, but protects the islets from the host's immune system. Despite these advancements, the risk of viral zoonosis remains a hurdle in the transition of islet xenotransplantation to the clinic, as it is unclear if viral nucleic acid passing through the alginate barrier, or viruses escaping the islets through a compromised alginate barrier, may pose a risk of infection to the porcine islet recipient.
To investigate this, we screened pigs for the presence of porcine viruses considered to be zoonotic or to have zoonotic potential and compared the results obtained from islet cells and blood samples for each animal. Interestingly, islet cells were negative for all viruses tested regardless of status in the animal-derived PBMC.
In addition, we investigated the potential of the porcine endogenous retrovirus (PERV) to be transmitted from islet cells by comparing the expression profile of PERV in islets to other pig tissues and conducting in vitro experiments to assay the effectiveness of alginate as a barrier against PERV transmission. Concluding that neonatal pigs have a significantly lower PERV expression level in islets compared to adults and, thus, may represent a safer islet source for use in xenotransplantation. Also, it was determined that alginate is an effective barrier to PERV transmission but this may be negated if the material becomes compromised.

5C-I1

A PROJECT PLAN ON MOLECULAR MECHANISMS OF SEX PHEROMONE PRODUCTION AND PERCEPTION IN GRAPE BERRY MOTHS (LOBESIA BOTRANA AND EUPOECILIA AMBIGUELLA); FOSTERING ENVIRONMENTALLY SOUND PEST MANAGEMENT STRATEGIES IN VINEYARDS

Professor Adrien Fonagy

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Insects use multiple neuropeptide families to regulate physiological events, which may be applicable for selective targeting in pest control. Neuropeptides and their receptors are well suited for novel management strategies, as species often have unique neuropeptide sequences. Once these sequences are determined, similar artificial messenger substances can be developed and applied in pest control. This technique is already deployed and supports why basic research is indispensable for modern and sustainable pest mitigation.

We argue that while exposure to synthetic lures of species specific sex pheromone may be effective in agriculture, there is great potential in targeting the pathways controlling pheromone production and communication through the use of synthetic stable neuropeptide analogues or double-stranded RNAs that disrupt the finely-tuned mechanism underlying pheromonogenesis. Alternatively, a novel approach for genetically modified plants has been proposed in which the plants function as “Trojan Horses” that produce and emit seasonally relevant insect sex pheromones. These plants are irrelevant to the plant culture, are not ingested, harmless to non-target organisms, and are unable to reproduce in nature.

To exploit these possibilities, a much better understanding of the systems is indispensable: from pheromone biosynthesis activating neuropeptide (PBAN)-mediated activation of PBAN receptors through the signal transduction cascade that triggers pheromone biosynthesis, and ultimately to olfactory detection of the released pheromone. The grape berry moths, *Lobesia botrana* and *Eupoecilia*

ambiguella (Lepidoptera; Tortricidae) cause serious damage to vineyards. Despite their economic importance our understanding of the neuroendocrinology and physiology of chemical communication in both species is limited.

A well-grounded research plan, based on the current state-of-the-art, will be presented. Our approach includes identification and functional characterization of relevant neuropeptides, enzymes involved in sex pheromone production, chemosensory receptors and odorant binding proteins in males by transcriptome analysis, RNA interference strategy, immunocytochemistry, electrophysiology and pheromone blend quantification of the pest moths.

5C-I2

ADIPOKINETIC HORMONES: RECEPTOR-LIGAND STUDIES IN THE DESERT LOCUST, SCHISTOCERCA GREGARIA

Dr Heather Marco[1], Dr Heleen Verlinden[2], Dr Elisabeth Marchal[2], Prof. Jozef Vanden Broeck[2], Prof. Gerd Gäde[1]

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Adipokinetic hormones (AKHs) belong to a family of neuropeptides that consist of 8-10 amino acids with blocked termini (pyroglutamate at the N-terminus and an amide group at the C-terminus), and several other conserved structural features. As such, the AKHs show significant structural similarity with the red pigment concentrating hormones (RPCHs) of crustaceans, and thus these neuropeptides are grouped in the AKH/RPCH family. Moreover, AKH and RPCH are arthropod homologues of the vertebrate gonadotropin-releasing hormone (GnRH) family, effecting physiological actions via a G protein-coupled receptor, specifically rhodopsin-like G protein-coupled receptors that are related to vertebrate GnRH receptors.

In insects, AKH is synthesised and secreted by neurons of the corpora cardiaca into the haemolymph, where it regulates energy metabolism, especially during intense skeletal muscle activity for energy demanding processes (such as flight), by mobilising fuel substrates from the fat body: e.g. glycogen can be broken down to trehalose, and lipids can be converted to diglycerides. AKHs have been identified in all insects to date, and it is believed that the AKH ligand/receptor system may be a good target for putative peptide mimetics for species-specific pest control.

The desert locust, *Schistocerca gregaria*, synthesises three AKHs in the corpora cardiaca: a decapeptide (Locmi-AKH-I) and two octapeptides (Schgr-AKH-II and Aedae-AKH). In our study, we cloned and expressed the AKH receptor gene in a cell line to investigate ligand-receptor interactions *in vitro* via a bioluminescence system, and then we compared the same interactions via an *in vivo* assay in which locusts were injected with the ligands and fuel metabolites (lipids) were measured in circulation. Apart from the endogenous locust AKH ligands, we investigated a number of carefully selected structural variants to learn more about the AKH signalling system in a pest insect. The tests showed a good correlation.

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5C-I3

pH-responsive POLYMER MICROCAPSULES FOR TARGETED DELIVERY OF PEPTIDES AND PROTEIN BIOCIDES TO THE MIDGUT OF DROSOPHILA SUZUKII

Professor Elwyn Isaac, Dr Calum Ferguson, Dr Areej Al-Khalaf, Dr Olivier Cayre

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Drosophila suzukii (or spotted wing *Drosophila*) is an economically important pest which can have a devastating impact on soft and stone fruit industries. Biological pesticides, such as neuropeptides and protein toxins, are being sought as alternatives to synthetic chemicals to control this invasive pest, but many are subject to degradation either in the environment or in the insect gut and as a result require protection. In this study we identified a sharp change in pH of the adult midgut from neutral to acidic (pH <3), which we then exploited to develop poly(2-vinylpyridine) (P2VP) microcapsules that respond to the change in midgut pH by dissolution and release of their cargo for uptake into the insect. First, we used labelled solid poly(methyl methacrylate) (PMMA) particles to show that microcapsules with a diameter less than 15 µm are readily ingested by the adult insect. To encapsulate water-soluble biological species in an aqueous continuous phase, a multiple emulsion template was used as a precursor for the synthesis of pH-responsive P2VP microcapsules with a fluorescent (FITC-dextran) cargo. The water-soluble agent was initially separated from the aqueous continuous phase by an oil barrier, which was subsequently polymerised. The P2VP microcapsules were stable at pH > 6, but underwent rapid dissolution at pH < 4.2. *In vivo* studies showed that the natural acidity of the midgut of *D. suzukii* also induced the breakdown of the responsive P2VP microcapsules to release FITC-dextran which was taken up into the body of the insect and accumulated in the haemolymph and renal tubules. In summary, we have made smart microcapsules suitable for protecting and delivering protein and peptide toxins to the insect gut for biocontrol of *D. suzukii.*

6A-S1

MULTIFACTORIAL REGULATION OF GROWTH, METABOLISM AND REPRODUCTION IN FISH

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Hypothalamic neurohormones are key regulators of growth and reproduction by controlling secretion of pituitary luteinizing hormone (LH), growth hormone (GH) and Thyroid stimulating hormone (TSH), which in turn interact with their respective peripheral target tissues. The resulting endocrine changes regulate metabolic pathways to sustain energetically demanding processes of reproduction and growth. In seasonal breeders which include many fish species, gonadotropic phase require significant allocation of metabolic energy to sustain ovarian and testicular development. After ovulation and spermeation, energy is mainly diverted to support growth activity. In this study, emphasis is placed on the role of gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), and thyroid hormones (T3/T4) in the control of growth, reproduction and metabolism. In birds and mammals, GnIH predominantly inhibits reproduction at the level of the hypothalamus and pituitary. However, studies in fish demonstrated that GnIH both stimulate and inhibits pituitary LH and GH production as well as gonadal development, depending on the stages of reproduction. Using metabolomics approach, our findings demonstrate that GnRH, GnIH and T4/T3 are important components of multifactorial regulation of reproduction and growth in fish, and their action influence pathways involving lipid, protein and carbohydrate metabolism. The results provide a framework for better understanding of the hormonally induced changes in metabolism to energetically sustain reproduction and growth in fish and other oviparous species.

6A-S2

UPDATES ON THE CROSS TALK BETWEEN GROWTH, ENDOCRINE FACTORS, NUTRIENTS AND ENVIRONMENTAL FACTORS IN MARINE FARMED FISH

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The relationship between components of somatotropic axis and growth rates varies within and across fish species from tight to non-discernible correlations. This is probably due to a wide-range of endogenous and exogenous factors that make variable the association of growth and endocrine factors along the season and productive cycles. However, circulating GH and IGF-I are reliable growth markers over precisely defined groups of experimental fish. This is well exemplified in farmed sea bream (*Sparus aurata*), in which circulating GH and IGF-I highly reflect changes in dietary protein and lipid sources or nutrient deficiencies in specific nutrients (methionine, essential fatty acids, phospholipids, minerals, vitamins). These findings have revitalized the use of GH and IGF-I as key performance indicators of growth and nutritional condition in a main European farmed fish. Hepatic transcripts of IGF-I and GH receptors mirror changes in plasma GH and IGF-I levels in either juvenile or adult fish. Both in liver and skeletal muscle, the relative GHR-I/GHR-II gene expression ratio remains mostly unaltered during seasonal changes of growth rates in well-nourished fish. However, this gene expression ratio is highly regulated at the nutritional level, and unbalanced plant-based diets or semi-purified diets formulated for specific nutrient deficiencies down-regulate the expression of hepatic GHR-I, decreasing the GHR-I/GHR-II ratio. The same trend was found in skeletal muscle, though this gene expression signature is primarily due to the up-regulated expression of GHR-II. Conversely, impaired growth during overwintering is related to a reduced expression of GHRs, IGFs and IGFBPs in liver and skeletal muscle without changes in GHR-I/GHR-II or IGF-I/IGF-II expression ratios. Intestine is also now recognized as a major target tissue for improving growth performance, and holistic “omics” methodologies combining proteomic, transcriptomic, metabolomic and metagenomic approaches have been proven useful to restore or prevent putative drawback effects of alternative feeds on intestinal health.

6C-S1

MIMETIC ANALOGS OF INSECT NEUROPEPTIDES: RATIONAL TOOLS IN DEVELOPMENT OF NOVEL PEST MANAGEMENT STRATEGIES

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Insect neuropeptides regulate critical processes in insects, though 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.

The PRXamide family can be subdivided into classes: CAPAs, pyrokinins (PK) and ecdysis triggering hormone (ETH); and PKs further subdivided into pheromone biosynthesis activating neuropeptides (PBAN) and diapause hormone (DH). Mimetic DH analogs provide leads for development of disrupters of diapause. GPCRs of the PRXamide ligands form a homologous cluster, indicating coevolution of ancestrally related ligand-receptor partners. PK analogs have shown cross activity on other recombinant receptors of the PRXamides, including CAPAs, mediators of desiccation and cold tolerance. PK analogs with antagonist activity on a CAPA receptor increase resistance to desiccation in fruit flies/aphids, and reduce tolerance to cold stress in aphids. Although not an attribute sought for pest species, an increase in desiccation resistance may aid in protection of beneficial insects. Other agonist CAPA analogs reduce survival to desiccation stress in fruit flies/aphids via topical application.

A biostable, multi-Aib insect kinin (IK) analog shows mortality induction in aphids and a deterrent response in mosquitoes. New analogs have been identified that match or exceed the activity of that analog on tick/mosquito receptors. PEGylated analogs with the potential for enhanced topical/oral activity retain activity. Other biostable IK analogs paradoxically increase desiccation tolerance in fruit flies, perhaps via receptor desensitization.

A conformationally-restricted, cyclic analog of AKH retains activity on a recombinant locust receptor, but virtually no activity on a beneficial honey bee receptor. The structure of the cyclic analog provides insight on conformational and chemical characteristics required for selective action on pest over beneficial insects. AKH analogs incorporating moieties which enhance biostability retain significant activity on the locust receptor and in *in vivo* experiments.

6C-S2

GENETIC APPROACHES FOR INSECT CONTROL

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Insect pests represent a major threat to agro-ecosystems as well as human and animal health. The most widespread population control mechanism is still the use of synthetic insecticides, however the overuse of such compounds has caused significant environmental consequences and widespread insecticide resistance in a number of species. Molecular advances have allowed development of novel genetic control mechanisms, whereby synthetic genetic material can be transformed in to the genomes of pest species, utilising the natural mating behaviour of the insect to introduce lethal cargo through the population. One of the most developed forms of this where the transgene caused female-specific lethality, is the Release of Insects carrying a Dominant Lethal (RIDL) system, subsequently field-trialled in both *Aedes* mosquitoes and the diamondback moth *Plutella xylostella*. More recent advances in this technology have sought to harness aspects of the pest species' genetics themselves making such genetic control strategies even more selective against its targets. One such candidate effector group is using insect venoms or peptides. I will describe the advances made in both *Aedes* mosquitoes and *Plutella xylostella* utilising the 'tet-off' conditional gene expression system. One aspect is to be able to dissociate the temporal and spatial expression of the promoter from the target tissue or cells, by expression of potent secreted effector molecules such as neuropeptides and peptide toxins. This approach builds on, and adds to, our knowledge of the biological role of such peptides, offering a powerful tool for observing phenotypes associated with ectopic expression of such compounds. Such strategies are also likely to prove extremely useful in offering more effective, and specific, forms of genetic pest management.

6A-I1

EFFECTS OF THE ADMINISTRATION OF PLASTICIZER-CONTAMINATED DIETS IN MALE GILTHEAD SEABREAM: A FOCUS ON HEPATIC METABOLISM AND MUSCLE PROTEOLYTIC SYSTEM

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Many chemical compounds used in the production of plastics and resins have the ability to interact with the endocrine system, acting as Endocrine Disrupting Chemicals (EDCs) and thus representing a high-priority research area. In this study, we studied two plastic additives, the Bisphenol A (BPA) and the phthalate Di-isononyl phthalate (DiNP). While the effects of BPA exposure on physiology, metabolism and reproduction have been investigated in several experimental models, data regarding the effects induced by DiNP exposure are still scarce. In this study, the effects of a chronic administration of diets contaminated with BPA or DiNP have been analyzed in adult male gilthead seabream (*Sparus aurata*), focusing on their consequences on the modulation of hepatic lipid metabolism. The obtained results showed the ability of both compounds to alter the hepatic structure and its biochemical macromolecular composition, associated to variation at transcriptional level of genes involved in lipid biosynthesis and endocannabinoid metabolism. In addition, since gilthead seabream represents one of the most valuable species by consumers, appreciated for the fillet nutritional properties and quality, changes induced by BPA and DiNP exposure on muscle macromolecular structure and insight into its proteolytic system were provided. Results showed that BPA and DiNP can substantially change muscle composition and affect fillet tenderization. These results highlight the negative effects of exposure to plasticizers on fish physiology and metabolism, suggesting a set of biomarkers as possible innovative endpoints for the development of novel OEDCS test guidelines.

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6A-I2

RAINBOW TROUT LEPTIN SIGNALING CASCADES AND ITS IMPACT ON THE GH-IGF SYSTEM DURING FASTING

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Leptin (Lep) is an adipocyte-derived hormone in human and rodents, but in teleost fish it is produced primarily in the liver. Fasting reduces plasma Lep levels in mammals; however, long-term fasting elevates Lep levels in salmonid fish. This raise questions about the conservation and divergence of Lep function in vertebrates. A previous study in rainbow trout has shown a conservation of anorexigenic Lep action in the mediobasal hypothalamus, while divergent activity was found in Lep-regulated neuropeptides. The current study determined that Lep activated the Pi3k-Akt and Jak2-Stat3 signaling cascades in rainbow trout hepatoma cell line RTH-149, but could only activate Ekr1/2 at low dose (0.4 nM). This indicates that fish Lep can activate similar intracellular signaling pathways as in rodents. A recombinant Lep binding protein type 1 (LepBP1) attenuated the phosphorylation of Akt, Jak2 and Stat3, but had no effect on Erk1/2, suggesting that LepBP1 can serve as a negative modulator of Lep signaling. A further study examined whether or not Lep affects the GH-IGF system during food deprivation. Hepatocytes were isolated from 3-week fasted rainbow trout and treated with homologous Lep, ovine GH, or both for 5 hours. Lep activated Stat3 phosphorylation in the hepatocytes in a dose-dependent manner. Lep alone had no effect on GH receptor 2, IGF-I and IGF-II gene expression, but reduced GH stimulation of *igf-1* and *igf-2* expression at low dose (1 nM). The Erk1/2 inhibitor U0126 suppressed GH-stimulated of *igf-1* and *igf-2* expression, but high-dose Lep (10 nM) could partially reverse this suppression. Lep alone did not affect *socs3* expression; however, GH stimulated *socs3* expression ($P<0.5$), and the combination of GH and Lep (10 nM) potentiated ($P<0.01$) the stimulatory effect. These results indicate that Lep modulates the GH-IGF system and fasting-induced elevation of Lep levels may help lower *igf* expression during catabolic states.

6C-I1

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 and by feeding. 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. Their expression in the course of the insect's development has also been determined. 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.

6C-I2

FOREST PEST MANAGEMENT USING NEUROPEPTIDES: A NOVEL ANSWER TO AN OLD PROBLEM?

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As insecticide resistance becomes more widespread, and their use comes under increasing scrutiny and control, so new environmentally-friendly control agents are urgently needed. The pine weevil *Hylobius abietis* is the most economically damaging insect pest of managed conifer forests in Europe, and is typically controlled by chemical means. Adult weevils emerge from root stumps on clearfell sites to feed upon newly planted seedlings, which are rapidly killed unless protected. A range of neuropeptide analogues from the CAPA, DH44 and sulfakinin families are being tested within the Neurostresspep consortium to investigate their effect upon pine weevil physiology, and their potential as targeted control agents under field conditions. Assays indicate that any diuretic effects of the CAPA and DH44 analogues investigated were limited and readily countered by the weevils, and that these peptides would be ineffective in a forest environment. However, the antifeedant effects of some novel sulfakinin analogues were more apparent in the weevils, and may indicate a conceivable future role for such peptides to be utilised as part of an integrated pest management system.

6C-I3

FUNCTIONAL CHARACTERIZATION OF DROSOPHILA CAPA RECEPTOR WITH PEPTIDOMIMETICS AND IDENTIFICATION OF CAPA AGONISTS AND ANTAGONISTS AS POTENTIAL INSECT PEST CONTROL AGENTS

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Insect neuropeptides and their G-protein coupled receptors (GPCRs) regulate vital physiological and behavioural systems and perform essential functions in various life stages. Therefore, neuropeptidergic signalling systems may be considered as potential candidate novel targets for insect control. Insect CAPA neuropeptides critically affect water balance and have myotropic effects in a variety of insects, so disrupting CAPA signalling process could be the key to creating novel biopesticides. The *Drosophila melanogaster capa* receptor (*capaR*) is a member of the PRXamide peptide receptor family and two endogenous capa peptides (*Drm*-CAPA-1, GANMGLYAFPRV-amide and *Drm*-CAPA-2, ASGLVAFPRV-amide) both activate *Drosophila* capaR through calcium (Ca^{2+}). Previous studies have investigated the structure-activity relationship of this peptide family, helping in the design of CAPA mimetic analogues. Different CAPA peptides and peptidomimetics were designed and assessed for GPCR binding and potency to activate Ca^{2+} signalling pathways in the aequorin-based [Ca^{2+}] measurements in *Drosophila* S2 cells. We identified CAPA agonist and antagonist analogues that were evaluated for their diuretic activity in the Malpighian tubules of *Drosophila*, for their resistance to peptidase hydrolysis and their biological response in insect survival. The identification of CAPA peptidomimetics with increased biostability, specificity, and permeability could be used as potential biorational pesticides.

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