ECCN10 PRAGUE
JUNE 22-24 2022

# PROGRAM

**Wednesday June 22nd**

Albertov 6, The lecture hall (Velká geologická posluchárna)

8:45 – 9:15 **Registration**
9:15 – 9:40 **Conference opening**

Pavel Němec – Welcome and opening remarks
Hans J. ten Donkelaar – From ECCN1 to ECCN10

9:40 – 10:40 **Keynote talk**
José M. Martín-Durán – The evolution of the bilaterian nervous system

10:40 – 11:10 **Coffee break**

11:10 – 12:30 **Developmental & functional studies**

Ali Murat Elagoz – Cephalopod approach of evolving a complex brain: morphological and molecular characterization during octopus development

Ronen Segev – Finding the homolog of the hippocampus in the teleost brain: evidence from physiology of representation of space in the goldfish

Andrea Messina – Quantities in the brain: behavioural and neurobiological evidence in zebrafish

Bruno Mota – The fractal cortex: Multi-scale surface-preserving analysis indicates all cortices are approximations of a single universal shape

12:30 – 14:30 **Lunch**

14:30 – 16:00 **Variation of telencephalic development that paved the way for neocortical evolution I.**

Kei Yamamoto **–** Re-interpretation of pallial regionalization in vertebrates

Nerea Moreno – The lateral and ventral pallium at the amniote-anamniote transition: insights from anurans and reptiles

Loreta Medina – Evolution of the amygdala: contributions of the pallium, the subpallium and the telencephalon-hypothalamic transition

Zoltan Molnár – In search of common developmental and evolutionary origin of the claustrum and subplate

16:00 – 16:30 **Coffee break**

16:30– 18:00 **Comparative transcriptomics approaches in brain evolution I.**

Maxwell E.R. Shafer – A tale of two fish: how gene family evolution underlies cell type diversification

Lora Sweeney – Evolution, development, and function of motor circuits

Justus Kebschull – Brain region evolution by duplication-and-divergence -- lessons from the cerebellar nuclei

Botanical garden of the Faculty of Science
19:00 **Welcome reception**

**Thursday June 23rd**

Albertov 6, The lecture hall (Velká geologická posluchárna)

9:00 – 10:00 **Keynote talk**

Onur Güntürkün – A new view on the avian pallium

10:00 – 10:30 **Coffee break**

10:30 – 12:00 **Brain complexity evolution and its drivers**

Robert Barton – The evolutionary dynamics of mammalian brain and body size

Kristina Kverková **–** The evolution of brain processing capacity in land vertebrates

Pavel Němec – Neuronal numbers in avian pallium: A reliable proxy of bird intelligence

Suzana Herculano-Houzel – Life histories of warm-blooded animals depend on the brain, not the body

12:00 – 14:00 **Lunch**

14:00 – 16:00 **Molecular evolution of sensory systems I.**

Joel Clinton Glover – Molecular underpinnings of vestibulospinal organization and function

Bernd Fritzsch – Making a brainstem and a cochlea require Lmx1a/b

Demian Burguera – Evolutionary dynamics of olfactory receptor genes in ray-finned fish

Ivan Manzini – Information processing and metamorphic changes within the olfactory network of *Xenopus laevis*

16:00 – 16:30 **Coffee break**

16:30– 18:00 **Poster session**

**Friday June 24th**

Albertov 6, The lecture hall (Velká geologická posluchárna)

9:00 – 10:00 **Keynote talk**

Ornella Bertrand – From small to big: How did complex brains emerge in placental mammals?

10:00 – 10:30 **Coffee break**

10:30 – 12:00 **Comparative transcriptomics approaches in brain evolution II.**

Amit Zeisel – Transcriptomics-based molecular neuroanatomy and cross-species comparison of goldfish telencephalon

Maria Antonietta Tosches – Cell type profiling in salamanders identifies innovations in vertebrate forebrain evolution

Bjørn André Bredesen-Aa – Molecular atlas of the adult zebrafish forebrain at single-cell resolution reveals homologies with terrestrial vertebrates

Rodrigo Senovilla-Ganzo – Conserved cell types in the early embryonic brain across vertebrates

12:00 – 14:00 **Lunch**

14:00 – 15:30 **Variation of telencephalic development that paved the way for neocortical evolution II.**

Elia Benito-Gutierrez – Development of a telencephalic-like region in the amphioxus brain through spatially restricted proliferation

Victor Borrell – Evolution of cortical progenitor cells: much to gain, much to lose

Roberto Toro – Mechanical morphogenesis and the development and evolution of the brain

15:30 – 16:00 **Coffee break**

16:00 – 17:30 **Molecular evolution of sensory systems II.**

Zbynek Kozmik – Eye evolution: a Pax6 gene perspective

Zuzana Musilová – Deep-sea fishes have evolved extraordinary vision using multiple rhodopsin genes

David Lagman – Evolution of genes important for nervous system function in vertebrates with a focus on vision and long-term memory

Dan Larhammar – Dynamic evolution of transient receptor potential vanilloid (TRPV) ion channel family with numerous gene duplications and losses

17:30– 18:00 **Closing ceremony**

Bastion Prague Restaurant

19:30 **Banquet dinner**

# LIST OF POSTERS

**P1 Yuri Ignatyev** Deciphering spinal neuron heterogeneity across vertebrate evolution and frog metamorphosis

**P2 Muhammad Tibi** Molecular cell type taxonomy of the wild bat amygdala

**P3 Eneritz Rueda-Alaña** Evolution of pallial high-order sensory processing circuits

**P4 Sara Jiménez** The medial pallium in anurans: subdomains and connectivity

**P5 Ruth Morona** Expression of Satb2 in adult brain of representative non-mammalian vertebrates: insight from evolution

**P6 Eleni Voukali** Adult neurogenesis in birds and mammals as indicated by cerebrospinal fluid proteomes

**P7 Zuzana Konvičková** How does the common barbel (*Barbus barbus*) see? The effect of the whole-genome duplication on vision

**P8 Manuel A. Pombal** Functional development of ocular movements in lamprey

**P9 Samuel Marashli** Auditory brainstem responses: A key tool for understanding auditory sensory processing and abnormalities across species

**P10 Pierre Estienne** Encephalization in teleost fishes: yet another way of allowing complex behaviors?

**P11 Seweryn Olkowicz** Mosaic evolution shapes the neuronal composition of the avian brain

**P12 Francesco Dionigi** Extremely high numbers of brain neurons in weakly electric fish

**P13 Yicheng Zhang** Cellular scaling rules for amphibian brains

**P14 Rahul A. Bhaskaran** Quantitative whole-brain mapping of neuronal and glial cell type distributions in birds and reptiles

**P15 Patrik Stehlík** Neuron numbers in hornbills, woodpeckers, and coraciiform birds

**P16 Fabien Knoll** A proxy for brain-to-endocranial cavity index in non-neornithean dinosaurs and other extinct archosaurs

**P17 Karina Santiago Gonzalez** Hippocampal immediate early gene induction and HPA-axis activation in response to acute stress in chickens

**P18 Trinh Anh-Tuan** Neural circuit architecture and computations underlying sensory processing and learning in the zebrafish paleocortex

**P19 Daniel Divín** Chromosomal rearrangements are responsible for altered neuroinflammatory regulation in parrots

**P20 Eva Landová** Emotional response to spiders, fearful snakes, and disgusting animal stimuli: fMRI study

**P21 Ester Desfilis** Neuronal plasticity in the forebrain of juvenile swine (*Sus scrofa domesticus*)

# KEYNOTE ABSTRACTS

### Onur Güntürkün – A new view on the avian pallium

For about a century, bird brains were seen as small, non-cortically organized systems that casted a dim prospect on the cognitive abilities of their beholders. Within the last two decades, this view has changed dramatically. My talk will concentrate on discoveries of the last few years that demonstrate that avian neuron numbers are not only much higher than expected by brain size, but also mostly allocated to associative areas in corvids. In parallel, birds developed the ability to cut down metabolic demands of their neurons by a factor of three. This not only makes a brain with so many neurons affordable, but may also provide cellular computational properties that are out of reach for mammals. Lastly, birds even developed a sophisticated cortex within their sensory pallial areas – possibly independent from mammals. Thus, avian neuroscience is currently continuing to draw completely new views on the evolution and organization of the bird pallium.

**Ornella Bertrand – From small to big: How did complex brains emerge in placental mammals?**

Mammals have the largest brains among vertebrates, but questions remain about the origin of their intelligence. We used new high-resolution computed tomography (CT) scans of Paleogene fossils of the USA and France alongside previously published endocasts. Contrary to previous hypotheses stating that relative brain size increased continuously over time, our results show that following the end-Cretaceous extinction, body mass increased at a faster rate leading to a decrease in relative brain size in early placental mammals. Ten million years after the extinction during the Eocene, greater encephalization independently emerged in several mammalian lineages.

**José M. Martín-Durán – The evolution of the bilaterian nervous system**

A centralized nervous system with a brain and nerve cords is arguably one of the most fascinating biological phenomena. Yet how such organ system evolved is contentious. In this talk, I will show how the study of the diversity of neural anatomies in often neglected invertebrate lineages has transformed our views on the origin and diversification of bilaterian nervous systems.

# INDEX OF ABSTRACTS

# ORAL PRESENTATION AND POSTER ABSTRACTS

## Comparative transcriptomics approaches in brain evolution

**(i) Invited talks**

Maxwell E.R. Shafer, Ahilya N. Sawh, Alex F. Schier

Biozentrum, University of Basel

**A tale of two fish: how gene family evolution underlies cell type diversification**

Hundreds of cell types form the vertebrate brain, but it is largely unknown how similar cellular repertoires are between or within species or how cell type diversity evolves. To examine cell type diversity across and within species, we performed single-cell RNA sequencing of ~130,000 hypothalamic cells from zebrafish (*Danio rerio*) and surface- and cave-morphs of Mexican tetra (*Astyanax mexicanus*). We found that over 75% of cell types were shared between zebrafish and Mexican tetra, which diverged from a common ancestor over 150 million years ago. Shared cell types displayed shifts in paralog expression that were generated by sub-functionalization after genome duplication. Expression of terminal effector genes, such as neuropeptides, was more conserved than the expression of their associated transcriptional regulators. Species-specific cell types were enriched for the expression of species-specific genes, and characterized by the neo-functionalization of expression patterns of members of recently expanded or contracted gene families. Comparisons between surface- and cave-morphs revealed differences in immune repertoires and transcriptional changes in neuropeptidergic cell types associated with genomic differences. The single-cell atlases presented here are a powerful resource to explore hypothalamic cell types, and reveal how gene family evolution and shifts in paralog expression contribute to cellular diversity.

Lora Sweeney

Institute of Science and Technology Austria (ISTA)

**Evolution, development, and function of motor circuits**

A hallmark of the nervous system is its rich cell-type diversity, its intricate connectivity and its coordinated patterns of activity. Behavior largely is an emergent property of this complexity. Thus, to understand behavior, we must parse neurons' molecular, cellular and functional properties. This task has proven especially challenging for motor circuits which exhibit readily apparent output in motor activity but heterogenous populations of neurons. In the Sweeney lab, to parse motor circuit complexity, we apply a novel approach harnessing the unique behavioral switch from swimming to walking during Xenopus frog metamorphosis and the vertebrate evolution. I will present on our ongoing efforts to dissect motor circuit cell-type composition in the developmental, evolutionary and behavioral context. Our approach has the potential to deepen our understanding of the origin of motor complexity and its relationship to motor circuit composition and output in tetrapods.

Justus Kebschull

Dept. of Biomedical Engineering, Johns Hopkins University, Baltimore, USA

**Brain region evolution by duplication-and-divergence -- lessons from the cerebellar nuclei**

How have complex brain regions, circuits, and cell types evolved from simple origins? Here we investigate brain region evolution at cell-type resolution in the cerebellar nuclei, the output structures of the cerebellum. In recent work, we applied single-nucleus RNA sequencing in chickens, mice, and humans, STARmap spatial transcriptomic analysis in chicken and mice, and whole-CNS projection mapping in mice. Our work revealed a conserved cell type set containing three classes of region-invariant inhibitory neurons and two classes of region-specific excitatory neurons. This cell type set forms an archetypal cerebellar nucleus that was repeatedly duplicated to create new regions, and thus cerebellar output channels. In excitatory neurons, duplication was accompanied by divergence in gene expression and shifts in projection patterns. By contrast, inhibitory neurons maintained their gene expression signatures. Interestingly, the excitatory cell class that preferentially funnels information to lateral frontal cortices in mice becomes predominant in the massively expanded human Lateral CN. Our data provide the first characterization of CN transcriptomic cell types in three species and suggest a model of brain region evolution by duplication and divergence of entire cell type sets. We are now working to extend these findings to tetrapods and to query connectomic cell types using cellular barcoding techniques MAPseq and BARseq in different species.

**(ii) Contributed talks**

Muhammad Tibi[1], Stav Biton[2], Hannah Hochgerner[1], Zhige Lin[1], Shahar Givon[2], Osnat Ophir[1], Ronen Segev[2], Amit Zeisel[1]

1 Faculty of Biotechnology and Food Engineering, Technion, Haifa, 2 Department of Life Science, Ben-Gurion University of the Negev

**Transcriptomics-based molecular neuroanatomy and cross-species comparison of goldfish telencephalon**

With more than 26,000 species, teleost fish represent the evolutionary most successful and diverse group of living vertebrates, making them suited for uncovering mechanisms of brain evolution. However, to this date the teleost forebrain remains poorly understood in terms of both comparative anatomy and functional organization. The field lacks molecular definitions that allow to distinguish homologous and divergent characteristics in relation to tetrapods and mammals where knowledge is most advanced. We combined single-cell RNA-sequencing (scRNA-seq) with spatial transcriptomics to generate an unbiased cartography and molecular atlas of the goldfish (Carassius auratus) forebrain. We characterized dozens of GABAergic, glutamatergic and non-neuronal transcriptional cell types in the goldfish telencephalon and mapped their approximate locations using complementary spatial transcriptomics data. In parallel, spatial transcriptomics revealed an unbiased molecular division of the telencephalon based solely on gene expression, with notable deviations from traditional annotation. In addition, we conducted cross-species cell-types comparison with mouse and zebrafish telencephalon and found several analogous regions. Our study can enhance our understanding of the evolution of forebrain in teleost and identify paralogous neuroanatomical regions between the teleost and mammalian brain.

Maria Antonietta Tosches

Department of Biological Sciences, Columbia University, New York, NY, USA

**Cell type profiling in salamanders identifies innovations in vertebrate forebrain evolution**

The evolution of advanced cognition in vertebrates is associated with two independent innovations in the forebrain: the six-layered neocortex in mammals and the dorsal ventricular ridge (DVR) in sauropsids (reptiles and birds). How these novelties arose in vertebrate ancestors remains unclear. To reconstruct forebrain evolution in tetrapods, we built a cell type atlas of the telencephalon of the salamander *Pleurodeles waltl*. Our molecular, developmental, and connectivity data indicate that parts of the sauropsid DVR trace back to tetrapod ancestors. In contrast, the salamander dorsal pallium is devoid of cellular and molecular characteristics of the mammalian neocortex, yet shares similarities with entorhinal cortex and subiculum. Our findings chart the series of innovations that resulted in the emergence of the sauropsid DVR, and the mammalian six-layered neocortex

Bjørn André Bredesen-Aa[1], Francisca Acuna-Hinrichsen[1], Astha Gupta[2], Annette Bogdoll[2], Benedikt Nilges[2], Nachiket Kashikar[2], Bram Serneels[1], Nathalie Jurisch-Yaksi[3], Emre Yaksi[1]

1 Kavli Institute for Systems Neuroscience, Norwegian University of Science and Technology, Trondheim, Norway, 2 Resolve Biosciences GmbH, Monheim Am Rheim, Germany, 3 Department of Clinical and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

**Molecular atlas of the adult zebrafish forebrain at single-cell resolution reveals homologies with terrestrial vertebrates**

Zebrafish is extensively used for investigating fundamental principles underlying the assembly and the function of the vertebrate brain, most commonly for the study of sensory-motor computations in larval zebrafish midbrain and hindbrain. Recent studies have shown that juvenile and adult zebrafish can perform cognitively demanding tasks, which is partly attributed to the maturation of forebrain networks, ancestral to mammalian cortico-limbic structures. Nonetheless, the molecular cytoarchitecture and cell types of adult zebrafish forebrain and its homologies to terrestrial vertebrates are yet to be discovered. In order to identify distinct regions and cell types of the adult zebrafish forebrain, we generated a serial-section transcriptomic atlas for 99 genes at subcellular resolution, and by aligning sections we created a high resolution three-dimensional atlas. For validation and to predict homologies, we integrated our molecular atlas with single-cell RNA sequencing data from zebrafish and several terrestrial vertebrates. Our results revealed multiple excitatory and inhibitory neuron types and non-neuronal cells across the forebrain. We observed that some of these cell types are dispersed widely while others are spatially organized. We also identified novel and anatomically distinct forebrain regions with predicted homologies to mammalian cortical and sub-cortical structures. Finally, we match several of these molecularly identified zebrafish forebrain regions with functional maps based on calcium imaging of ongoing brain activity. Our results highlight the evolutionary conservation and divergence of the anatomy and the cytoarchitecture of zebrafish forebrain with the rest of terrestrial vertebrates.

Senovilla-Ganzo, Rodrigo[1]; Rueda-Alaña, Eneritz[1]; García-Moreno, Fernando[1,2,3]

1 Achucarro Basque Center for Neuroscience, Leioa, Biscay, Spain. 2 IKERBASQUE Foundation, Bilbao, Biscay, Spain. 3 Faculty of Medicine/Odontology, UPV/EHU, Leioa, Biscay, Spain

**Conserved cell types in the early embryonic brain across vertebrates**

**Organogenesis**, also known as *phylotypic* period, is the most evolutionarily conserved developmental stage when comparing morphology and whole-body transcriptome across vertebrates. During this ***phylotypic*** stage, body axes are defined and cell types are given their spatial identity. Due to its physiological importance, these **segmentation** and **patterning** genes (e.g. HOXs) are thought to be **evolutionarily conserved**. However, across vertebrates, there is yet no evidence of transcriptomic conservation in essential organs as the **brain**, nor identification of homologue **neural cell types** in its initial neurodevelopmental ***bauplan*** Thus, by performing single cell RNAseq and in situ hybridization assays, we obtained cellular and molecular atlases of the early developing brains of five vertebrate species: chicken, gecko, mice, zebrafish, and human. These single cell atlases allowed us to identify equivalent neuroanatomical identities that naturally segment different vertebrate early brains and the genes that pattern these regions. Secondly, to unbiasedly evaluate the transcriptional conservation of these cell types across species, we performed three complementary methods: correlation of gene specificity indexes, datasets integration ("RPCA") and label transference. All approaches proved a high transcriptional conservation of equivalent morphogenic organizers and neuromeric identities among these vertebrate species, specially at transcription factor level. These results confirm the existence of a common phylotypic brain as well as the conservation of homologue neural cell types mastering its underlying bauplan. Therefore, this bauplan conservation is essential to stablish the foundations for assembling vertebrate brain structures, but also it sets the diversity boundaries within which these brains were allowed to evolve. Such an important constrain that early embryonic vertebrate brain has barely changed despite 500 million years of evolution.

**(iii) Contributed posters**

**P1**

Yuri Ignatyev[1], Stavros Papadopoulos[1], Tzi-Yang Lin[2], Leonid Peshkin[3], Elly M Tanaka[2], Mariano I Gabitto[4,5], Lora B Sweeney[1]

1 IST Austria, Klosterneuburg, Austria, 2 Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Vienna, Austria, 3 Harvard Medical School, Department of Systems Biology, Boston, MA, 4 Allen Institute for Brain Science, Seattle, WA, 5 University of California, Berkeley, Department of Statistics, Berkeley, CA

**Deciphering spinal neuron heterogeneity across vertebrate evolution and frog metamorphosis**

Vertebrate motor behaviour is orchestrated by neural circuits in the spinal cord. We take a novel cross-species evolutionary approach using single-cell sequencing to elucidate the conserved and divergent features of neuronal cell-type diversity for limb-, as compared to tail-based, movement. We elucidate the evolutionary conserved neuronal makeup of limb circuits by comparing amphibians (*Xenopus laevis*), the most ancient tetrapod, with mammals (*Mus musculus*). For *Xenopus laevis*, we generated a comprehensive single-cell atlas of spinal cord neurons at limb stages (NF54). For *Mus musculus*, we took advantage of existing single-cell data of developing mouse spinal cord (Delile et al., 2019).

We first assessed the conservation in spinal cell-type composition between frog and mouse. Using existing knowledge and unbiased differential expression analysis, we resolved a common spinal neuron cell-type architecture. This conserved architecture reveals a core set of marker genes for major neural classes in both species. This high degree of conservation supports a molecular and cellular base state for tetrapod circuits that spans 360 million years of vertebrate evolution. Next, we examine divergence between frog and mouse spinal circuits. We reduce cross-species batch effects by performing data integration based on mouse-frog gene homology. We find neuron classes that are species-specific, representing candidates for differences in tetrapod behaviour. We validate our findings with in-situ hybridization.

We next capitalized on frog metamorphosis to characterize how cell-type heterogeneity scales with the transition from swimming to walking. We expanded our single-cell characterization of neural composition across metamorphosis, asking (i) how neural diversity develops in the frog and scales with this dramatic change in behaviour and (ii) whether limb circuit formation results from the emergence of new neural types or the expansion of the existent ones.

**P2**

Tibi M.[1], Nissan Y.[2], Hochgerner H.[1], Ophir O.[1], Rachum A.[2], Mano A.[2], Yovel Y.*[2], Zeisel A.*[1]

1 Technion, Haifa, Israel , 2 TAU, Tel-Aviv, Israel

**Molecular cell type taxonomy of the wild bat amygdala**

In the wild, social animals are presented with social inputs from their environment representing various opportunities and threats. The amygdala is a brain region involved in many innate social behaviors and plays a major role in regulating reward and fear responses to those environmental stimuli. We use the Egyptian fruit bat (Rousettus aegyptiacus) as a unique wild animal model that uses social communication for navigation and foraging. However, the molecular building blocks of the amygdala in wild animals remains poorly understood. Single-cell transcriptomics can provide detailed insights into the molecular repertoire of tissue with high resolution. Here, we performed a single-cell RNA-sequencing of the amygdala in juvenile Egyptian fruit bats. We identified unique molecular marker sets of cell type profiles for excitatory and inhibitory neurons as well as non-neuronal cells, including glial and immune cells. To reveal the similarities and differences in amygdala in mammals, we conducted a bat-mouse cross-species comparison of amygdala cell types based on their gene expression profile. By using the wild bat as an animal model, we can uncover the evolutionary homology and the molecular signature in brain cells in the amygdala of wild animals.

## Variation of telencephalic development that paved the way for neocortical evolution

**(i) Invited talks**

Elia Benito-Gutierrez

Department of Zoology, University of Cambridge, Downing Street, CB2 3EJ, Cambridge, UK.

**Development of a telencephalic-like region in the amphioxus brain through spatially restricted proliferation**

The evolutionary origin of our brain is still a mystery. Even the most basally branching vertebrates, such as lampreys or hagfishes, emerge in evolution already possessing a highly regionalised brain. Down the tree of life, ascidians are the sister group of vertebrates, but their development includes a catastrophic metamorphosis that results in the elimination of the brain. Amphioxus, instead, lies at the base of the entire chordate tree and maintains all chordate features for life, including the only invertebrate dorsal brain available for study today. Our lab has been investigating how the amphioxus brain develops and to our surprise we have found that it develops very slowly. Consequently, some of the brain regions are not fully specified until adulthood. We have found this is the case of the pars anterodorsalis: PAD, which is similar in expression profile and neuronal composition to the vertebrate telencephalon.
We have observed the amphioxus brain connectivity substantially changes during the first six months of development and that this might be supported by sustained proliferation of neuronal progenitors at the ventricular zone, which unlike in vertebrates seems to be sparsely constant throughout development and in adulthood. Early in development, we have been able to track some of these neuronal progenitors, confirming there is a spatial-temporal sequence of neuronal maturation, probably linked to the maturation of their target organs. This is the case for the serotonergic neurons of the retina-like region and many hypothalamic cell types, which are formed by short and localised bursts of proliferation, thereby setting the base for differentiation of specific neuronal types when needed later in development and contributing to regionalise the brain.

Victor Borrell

Institute of Neuroscience, CSIC-UMH, San Juan de Alicante, Spain

**Evolution of cortical progenitor cells: much to gain, much to lose**

The human brain is the result of millions of years of evolution, when genetic mechanisms were selected driving the amplification of neural stem cells during embryonic development, which gave rise to a dramatic expansion and folding of the cerebral cortex. This process was secondarily reversed in some lineages like rodents, leading to smaller brains with a smooth cortex. The genetic bases of these complex evolutionary dynamics remain unclear, but they can inform us on developmental processes derailed in human brain malformation and disease. While brain expansion in the recent human lineage is in part explained by the emergence of some new genes, mounting evidence points at the differential regulation of conserved genetic mechanisms as being central in the evolution of neurogenesis and brain size. I will discuss our recent findings on the small non-coding RNA MIR3607, which promotes the amplification of neural stem cells by activating beta-Catenin signaling via blockade of APC expression. Importantly, MIR3607 is highly expressed in germinal layers of the embryonic cortex of primates and carnivores, where it promotes progenitor amplification, but this was secondarily lost in rodents, leading to smaller progenitor pools and, consequently, to the reduction in size and folding of their cortex. Our findings demonstrate the central importance of mechanisms regulating gene expression in the evolution of embryonic cortical development, and its consequences on cortex size.

Roberto Toro, Katja Heuer

Institut Pasteur, Paris, France

**Mechanical morphogenesis and the development and evolution of the brain**

During the short period of brain development, nature is able to build the only system we know capable of producing cognition, language, creativity, and consciousness. The neocortex – the outermost layer of the mammalian cerebrum – appears to be the biological substrate of these abilities. Its development requires not only the precise placement and wiring of billions of cells, but also the implementation of mechanisms to ensure a viable cognition despite sometimes dramatic perturbations. Today, this remarkably complex organisation is thought to be genetically encoded, and further refined by activity-dependent processes. We propose that mechanical morphogenesis – the capacity of homogeneously growing elastic tissue to produce complex shapes – can also play an important role. Out of homogeneous growth, mechanical morphogenesis can induce the segregation of the neocortex into mechanical and geometric modules – the neocortical folds. Through the feedback of physical forces on developing tissue, these modules can influence the differentiation and wiring of the neocortex, having a causal role on neocortical development, and providing adaptable and robust units for its evolution.

**(ii) Contributed talks**

Ali Murat Elagoz[1], Daniela Aparacio[1], Dries Janssen[1], Sofia Maccuro[1], Marie Van Dijck[1], Ruth Styfhals[1,2], Astrid Deryckere[1], Eve Seuntjens[1]

1 Laboratory of Developmental Neurobiology, Department of Biology, KU Leuven, Belgium, 2 Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Naples, Italy

**Cephalopod approach of evolving a complex brain: morphological and molecular characterization during octopus development**

Cephalopods are exceptionally intelligent invertebrates that exhibit a morphological and behavioural complexity similar to vertebrates. The cephalopod nervous system is comparable to the mammalian brain in terms of neuronal number and richness in behavioural output. Nevertheless, the last common ancestor between cephalopods and mammals was a small worm-like marine organism that existed approximately 600 million years ago. Studying cephalopod molluscs presents an opportunity to understand the genetic drivers of neural development that evolved convergently with vertebrates. While the adult anatomy of *Octopus vulgaris* brain has been substantially described, the morphological, cellular and molecular features of its embryonic brain development remain unclear. Recently, we characterized the developing octopus brain morphologically using conventional histological techniques and gene expression analysis in 2D . We discovered that the neuronal progenitor cells are located in lateral lips, a neurogenic zone surrounding the eyes, and newly born neurons display long-distance migration into the centralized brain, reminiscent of vertebrate neurogenesis. To further elaborate on the morphological characterization of octopus embryonic neurogenesis in 3D, we have carried out multiplexed *in situ* hybridization chain reaction for specific neuronal and glial markers. Moreover, we have identified differentially expressed transcripts of genes which are part of the evolutionarily conserved growth factor signalling pathways during embryogenesis based on QuantSeq 3' mRNA-Seq. We used multiome data to assess which neurogenic cell types express specific signalling pathway genes at a certain developmental embryonic stage. Finally, we have started to evaluate the role of these signalling pathways in octopus neurogenesis by using small molecule inhibitors and activators.

Kei Yamamoto

Paris-Saclay Institute of Neuroscience (NeuroPSI), CNRS, Université Paris-Saclay

**Re-interpretation of pallial regionalization in vertebrates**

Comparative neuroanatomists have long tried to identify the neocortex homolog in the non-mammalian pallium (dorsal telencephalon). The current consensus assumes that all vertebrates possess three or four pallial subdivisions inherited from their common ancestors: dorsal pallium (DP), medial pallium (MP), lateral pallium (LP)/ventral pallium (VP). These subdivisions largely depend on the description of Holmgren (1922, 1925) where the DP (called general pallium by Holmgren) corresponds to the mammalian six-layered neocortex. Here, I question the ancestry of the pallial subdivisions, based on recent findings as well as reinterpretations of previous publications.

In the absence of six-layered cortical structures in non-mammals, the vertebrate DP has been defined as the pallial area receiving sensory thalamic inputs (= thalamorecipient pallium). However, the topological localization of thalamorecipient areas within the pallium varies widely in different vertebrate groups (i.e. in the area proposed to be VP in birds, in the proposed MP of amphibians) and is not restricted to the "dorsal pallium".

By taking into account the pallial organization of teleosts, the presence of pallial subdivisions becomes even more ambiguous. Our recent data strongly suggests that the similar connectivity patterns of the tectofugal visual pathways in amniotes and teleosts have evolved independently and have not been inherited from their common ancestors (Bloch et al., eLife 2020).

Pallial organization of the common ancestor of vertebrates may have been relatively simple, and distinct subdivisions may have independently evolved in each vertebrate lineage. Thus, topological position may not be a critical factor for determining pallial functions. In other words, any part of the pallium would have the potential to evolve functional properties similar to the mammalian neocortex.

Jiménez Sara, Morona, R; López, J.M; Lozano, D; and Moreno Nerea

Department of Cell Biology, Faculty of Biology, University Complutense, Madrid, Spain

**The lateral and ventral pallium at the amniote-anamniote transition: insights from anurans and reptiles**

The pallium, which in mammals gives rise to the cerebral cortex and subcortical structures, is present in all vertebrates and represents one of the most functionally and structurally complex areas of the brain. It is composed of different histogenetic domains, with distinct specification programs during development, and different functional involvement of its derivatives in the adult. In particular, in amniotes the lateral and ventral regions of the pallium are involved in olfactory, visual, somatosensory and auditory processing, thus its study in non-mammalian vertebrates is fundamental to understand its functional implications, as well as its evolutionary relationships. In the present study, we have analyzed conserved markers of the latero-ventral region, by immunohistochemistry and in situ hybridization techniques, in the pallium of the amphibian Xenopus laevis (an anamiote tetrapod) and in the turtle Trachemys scripta (an amniote vertebrate). Despite the lack of a layered organization in the lateral pallium of Xenopus, this region was defined in this model based on the expression of Reelin and Satb2, as in the lateral cortex and pallial thickening of Trachemys, both derived from the lateral pallium. In the case of the ventral pallium in Xenopus, which constitutes the main olfactory processing center, it was identified by the expression of markers such as Tbr1, MeCf2, ErbB4, Fez2 and Lhx2/9. Therefore, in both models it was possible to demarcate the boundaries and derivatives of the lateral and ventral region of the pallium, as well as their involvement in olfactory processing, providing evidence, at this point of the anamnio-amniote transition, on their similarities, but also new acquisitions and evolutionary singularities, in particular related to the evolution of the cortex arrangement in tetrapods.

Loreta Medina, Abellán A, Morales L, Pross A, Metwalli AH, Freixes J, Desfilis E

Universitat de Lleida & IRBLleida, Lleida, Spain

**Evolution of the amygdala: contributions of the pallium, the subpallium and the telencephalon-hypothalamic transition**

The amygdala is a complex telencephalic structure critical for control of emotions, social behavior and cognition. Studying how this brain structure evolved can help to better understand its structural and functional organization, and to identify variations in neural mechanisms that are behind different adaptations in the regulation of essential behaviors. The amygdala has been identified in different vertebrates. However, its extension in reptiles and birds is highly controversial due to the great divergence of their telencephalon during evolution. To solve this problem, our group is using an evolutionary developmental neurobiology approach, which allows distinction of fundamental brain divisions shared by all vertebrates, and identification of derived cell groups. This approach has allowed distinction of pallial and subpallial divisions of the sauropsid amygdala, expressing distinct combinations of transcription factors during development, which produce glutamatergic or GABAergic neurons, respectively. In the subpallial amygdala, we also identified dorsal striatal, ventral striatal, pallidal and preoptic derived cell subpopulations comparable to those found in the centromedial extended amygdala of mammals. These appeared to include the GABAergic cell subpopulations that have been involved in the on/off systems, which regulate the fear response in the mammalian central amygdala. Remarkably, we also found another telencephalic division near the frontier with the hypothalamus, which produces a large subpopulation of glutamatergic cells for the medial extended amygdala in mammals and sauropsids. In chicken (but not mouse), this new telencephalic division also appears to produce a subset of glutamatergic cells for the central extended amygdala. These findings raise questions on how these glutamatergic cells of the extended amygdala interact with the GABAergic cells to regulate emotions and social behaviors, and open new venues to study their evolution.

Zoltan Molnár

Department of Physiology, Anatomy and Genetics, University of Oxford, Parks Road, Oxford OX1 3PT, United Kingdom

**In search of common developmental and evolutionary origin of the claustrum and subplate**

The claustrum is a thin, bilateral sheet of gray matter located between the insular cortex and the striatum. The subplate contains some of the earliest generated neurons of the cerebral cortex and has important developmental functions to establish intra- and extracortical connections. Gene expression patterns, such as those of Nurr1/Nr4a2, have suggested that the rodent subplate and the persistent subplate cells in Layer 6b and the claustrum might have similar origins. Moreover, the birthdates of the claustrum and Layer 6b are similarly precocious in mice. These observations prompted our speculations on the common developmental and evolutionary origin of the claustrum and the subplate. Here we systematically compare the currently available data on cytoarchitecture, evolutionary origin, gene expression, cell types, birthdates, neurogenesis, lineage and migration, circuit connectivity, and cell death of the neurons that contribute to the claustrum and subplate. Based on their similarities and differences we propose a partially common early evolutionary origin of the cells that become claustrum and subplate, a likely scenario that is shared in these cell populations across all amniotes.

**References:**

Bruguier et al., (2020) J Comp Neurol.2020;528:2956–2977.
Hoerder-Suabedissen et al., (2022) bioRxiv preprint doi: https://doi.org/10.1101/2022.05.20.492804

Bruno Mota[1], Yujiang Wang[2]

1 metaBIO Lab, Instituto de Física, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil 2 CNNP Lab (www.cnnp-lab.com), Interdisciplinary Computing and Complex BioSystems Group, School of Computing, Newcastle University, Newcastle upon Tyne, UK

**The fractal cortex: Multi-scale surface-preserving analysis indicates all cortices are approximations of a single universal shape**

The morphological complexity of the mammalian cerebral cortex has long puzzled scientists, with cortices across and within species exhibiting a large diversity of shapes and sizes. Such diversity is not arbitrary, however. The mammalian brain folds into stereotypical, hierarchically-organized structures such as lobes and major gyri. Altough aualitative and quantitative regularities in cortical scaling have been often suggested and observed, there is no known systematic way of characterizing such shapes univocally. Briefly, among all potential gyrified shapes, there is no broadly-accepted criteria to determine which correspond to possible cortices, rather than (say) walnuts or coral surfaces. We present here a new way of expressing the shape of a cortex explicitly as the hierarchical composition of structures of different spatial scales. As one successively removes sulci and gyri smaller than a specified scale, the cortices of 11 primate species are gradually coarse-grained, or ``melted'', into lyssencephaly. We show that this process, in all cases, occurs along a common scale-free morphometric trajectory, indicating that these cortices are not only approximately fractal, but also approximations of the same archetypal fractal shape. These results imply the existence of a single universal gyrification mechanism that operates in a scale-free manner on cortical folds of all sizes, and that there are surprisingly few effective degrees of freedom through which cortical shapes can be selected for by evolution. As an example, we use this new understanding to demonstrate that the aging process affects cortical morphology very differently in large and small scales. To our knowledge, this is the most general description of the brain's shape that is at the same time mechanistically insightful and in full agreement with empirical data across species and individuals.

**(iii) Contributed posters**

**P3**

Eneritz Rueda-Alaña[1,2], Enrique Vázquez[3], Rodrigo Senovilla-Ganzo[1,2], Juan Manuel Encinas[1,2,4], Fernando García-Moreno[1,2,4]

1 Achucarro Basque Center for Neuroscience. Leioa, Bizkaia, Spain, 2 Department of neuroscience, Faculty of Medicine and Odontology, University of the Basque Country (UPV/EHU). Leioa, Bizkaia, Spain, 3 Genomics Unit, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain, 4 Ikerbasque, The Basque Science Foundation. Bilbao, Bizkaia, Spain

**Evolution of pallial high-order sensory processing circuits**

Understanding the appearance of the neocortex in evolution requires the study of its stereotyped sensory processing circuits and their formation during embryonic development. These circuits appear in sensory structures of the telencephalon of different amniote species, which suggests their homology. However, their evolutionary history is still a matter of debate. If they were homologous they would have evolved from a common ancestral circuit that was already present over 300 mya. To study the evolution of these circuits, we aim to compare their development in different pallial regions of selected amniote species. Only circuits generated in an equivalent fashion can be considered homologous.

**P4**

Jiménez Sara, Morona, R; López, J.M; Lozano, D; and Moreno Nerea

Department of Cell Biology, Faculty of Biology, University Complutense, Madrid, Spain

**The medial pallium in anurans: subdomains and connectivity**

In all vertebrates, the most dorsal region of the telencephalon constitutes the pallium, which is further subdivided into different subdomains. Particularly in mammals, the medial pallium gives rise to the hippocampus and associated cortical structures. Although this region is structurally different among amniotes, its functions attributed to spatial memory and social behavior are highly conserved. Thus, the aim of the present study is to analyze in the anuran amphibian Xenopus laevis, the only anamniote tetrapod, the expression pattern of conserved markers of this region, as well as the different connectivity of the possible distinct subdomains. Based on the combined expression of Lhx2, Prox1 and Er81 we have identified two medial pallial subregions in Xenopus, further corroborated by the expression of LMO4, among other markers. These subdomains showed differential connectivity between them, supporting their distinction. The dorsal region mainly presented intratelencephalic connections in contrast to the ventral one, additionally related to extratelencephalic brain centers. This anatomical organization is compatible with that described in amniotes; this, together with the functional involvement in anurans of this region in spatial memory tasks, supports the existence of a basic program in the specification and functionality of the medial pallium in vertebrates, or at least in the base of tetrapods. Furthermore, it shows that the anatomical differences found in different vertebrates may be due to divergences and adaptations during evolution.

**P5**

Morona, R; López, J.M; Lozano, D; Jiménez S; Moreno N

Department of Cell Biology, Faculty of Biology, University Complutense, Madrid, Spain

**Expression of Satb2 in adult brain of representative non-mammalian vertebrates: insight from evolution**

Cross-species comparative studies of the structure and function of the Satb2 transcript have shown that this transcription factor possess highly conserved functional domains, regulatory mechanisms, and post-translational modifications. However, data about its distribution are scarce, especially in non-mammalian vertebrates. Therefore, we analyzed the pattern of expression in the adult brain of specimens at key evolutionary points of vertebrate diversification (the lungfishes, as most basal sarcopterygian model and the closest living relatives of tetrapods; the amphibian anuran, the only anamniote tetrapod, and the turtles representing an amniote model with a simple cortical organization, although with some shared features with mammals). Due to the basic phylogenetic importance of these groups, the distribution of Satb2 provided us valuable information on brain organization in the common ancestor for tetrapods. Significant differences were found in the pallium, where only the turtle showed expression in the dorsal cortex, whereas expression in the lateral pallium is conserved in all groups. In the medial amygdala expressed Satb2 in all models, but only in tetrapods some of these cells also expressed Otp. There is a conserved expression in other regions such as in the preoptic region. Noticeably, only turtles showed expression in the thalamus, in contrast to the conserved scattered prethalamic expression in all specimens. In the hypothalamus, scattered cells were found in the suprachiasmatic nucleus and a significant population in the tuberal hypothalamus, mainly avoiding the Otp expression territory, except in tetrapods, where a small subpopulation was doubly labeled. In the midbrain, just the turtle showed scattered cells in the optic tectum, but all models expressed Satb2 in the torus semicircularis, the reticular column and the parabrachial nucleus. Finally, a prominent population of Satb2 in the lateral line nucleus was found only in *Xenopus*.

**P6**

Eleni Voukali, Michal Vinkler

Laboratory for Evolutionary and Ecological Immunology, Department of Zoology, Charles University

**Adult neurogenesis in birds and mammals as indicated by cerebrospinal fluid proteomes**

Adult neurogenesis in vertebrates occurs partly by signals received through the cerebrospinal fluid. Thus, the characterization of its molecular composition has the potential to delineate these pathways and eventually translate them into future regenerative interventions. In this comparative study, we aimed to delve into the fundamental processes of adult neurogenesis and explore its conserved regulatory pathways enriched in avian and mammalian cerebrospinal fluid proteomes. We performed literature-based data mining and extracted 24 datasets from studies using liquid chromatography-tandem mass spectroscopy that analysed cerebrospinal fluid samples from healthy adult humans (*Homo sapiens*).

**P21**

Fatma ElZahraa S. Abdel-Rahman, Loreta Medina and Ester Desfilis

Universitat de Lleida and Lleida's Institute for Biomedical Research-Dr. Pifarré Foundation (IRBLleida)

**Neuronal plasticity in the forebrain of juvenile swine (*Sus scrofa domesticus*)**

Neural plasticity is crucial throughout ontogeny for acquisition of new knowledge and skills, sensory and behavioral adaptation, and brain recovery following injury. In the juvenile and adult brains, plasticity has been related to modification of pre-existing neurons, to the production of new neurons (adult neurogenesis), and to the presence of immature neurons. In contrast to the first, the latter two were assumed to be less common in the mature brain of mammals. However, recent studies showed the presence of immature neurons throughout many pallial areas of human infants and adolescents, suggesting that the presence of these neurons is more spread than previously thought. Similar results have been found in juvenile and adult brains of other gyrencephalic mammals, such as sheep and monkey. To know whether this is a common feature of gyrencephalic brains, it is important to obtain data on the distribution of immature neurons in other mammalian species. The aim of this project was to investigate neuronal plasticity in the pallium of the gyrencephalic swine brain. We studied the distribution of doublecortin (DCX), a cytoskeletal protein expressed in immature neurons, using immunocytochemical methods. To better understand the results, parallel sections were processed for detecting different calcium binding proteins. We found abundant DCX+ cells in the subventricular zone (SVZ) of both subpallium and pallium. The pallial SVZ was related to a putative lateroventral migratory stream, which extends into dorsolateral, lateral and ventral parts of the pallium, reaching the claustroinsular region, endopiriform nuclei, piriform cortex and pallial amygdala. DCX+ cells were also found in layer II of the cingulate cortex and most areas of the neocortex. The pattern highly resembles that previously described in sheep and primates, supporting the suggestion that neural plasticity related to immature neurons is more common in gyrencephalic than in lissencephalic brains.

## Molecular evolution of sensory systems

**(i) Invited talks**

Joel Clinton Glover

Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo

**Molecular underpinnings of vestibulospinal organization and function**

Vestibulospinal projections mediate reflexes and corrective movements to correct for loss of balance. Prior studies in my laboratory have demonstrated that the neurons giving rise to the vestibulospinal projections are organized as part of a hodological mosaic of vestibular projection neuron groups, each with a specific segmental origin in the hindbrain. Our more recent studies have elucidated unique transcriptomic signatures for the main vestibulospinal groups, providing insight into how their functional identities may be established at the molecular level. The results of these studies contribute new information about the dorsoventral origins of the vestibulospinal neurons, and raise intriguing questions about how they evolved.

Bernd Fritzsch[1], Gabriela Pavlinkova[2], Victor Chizhikov[3], Ebenezer Yamoah[4]

1 University of Iowa, Dept of Biology & Dept of Otolaryngology, Iowa City, IA, USA; 2 Institute of Biotechnology, Czech Academy of Sciences, Vestex, Czechia; 3 Department of Anatomy and Neurobiology, Tennessee Health Science, Memphis, TN, USA; 4 Department of Physiology and Cell Biology, University of Nevada, Reno

**Making a brainstem and a cochlea require Lmx1a/b**

The vertebrate auditory system, consisting of the inner ear hair cells, projections from the spiral ganglion, and brainstem nuclei, is essential for the detection of sound and vestibular sensation. It is believed that the evolution of complex systems, such as the auditory system, depends on duplicated sets of genes that evolved in ancestral vertebrates. The contribution of duplicated genes to auditory system development, however, is poorly understood. We describe that Lmx1a and Lmx1b, which originate from the invertebrate Lmx1b-like gene, redundantly regulate development of multiple principal components of the mammalian auditory system. Combined, but not individual, loss of Lmx1a/b eliminated the auditory inner ear organ of Corti and the spiral ganglion, which was preceded by a diminished expression of their critical regulator Pax2. Innervation of the remaining inner ear vestibular organs revealed unusual sizes and was more affected compared to Lmx1 single-gene mutants. Individual loss of Lmx1 genes did not disrupt brainstem auditory nuclei or inner ear central projections. Combined loss of Lmx1a/b, however, eliminated excitatory neurons in cochlear nuclei, and also eliminates the expression of a master regulator, Atoh1, in their progenitors in the lower rhombic lip. Finally, in Lmx1a/b double mutants, vestibular afferents aberrantly projected to the roof plate in the absence of the choroid plexus. This phenotype was associated with altered expression of Wnt3a, a secreted ligand of the Wnt pathway that regulates pathfinding of inner ear projections. Thus, Lmx1a/b are required for development of the mammalian inner ear, inner ear central projections, and cochlear nuclei.

Demian Burguera, Francesco Dionigi, Kristina Kverková, Yicheng Zhang, Pavel Němec, Zuzana Musilova
Department of Zoology, Charles University, Czech Republic

**Evolutionary dynamics of olfactory receptor genes in ray-finned fish**

At the molecular level, the first step in odour perception is driven by cell surface receptors expressed in the olfactory epithelium. These olfactory receptors bind to environmental odorant molecules, inducing a cascade that convert a primary chemical signal into an electrical impulse decoded in the brain. Remarkably, each olfactory neuron expresses only one receptor gene from a usually extensive repertoire. This intricate regulation is key for the processing of smell due to the axonal convergence in a singular olfactory glomerulus of those sensory neurons expressing the same receptor. To understand how olfaction evolves in response to a plethora

of distinct environments and sensory adaptations, we study the evolution of olfactory genes in approximately two hundred species across ray-finned fish phylogeny. We further analyse the impact of observed genomic changes at the transcriptome level in a reduced set of organisms. Moreover, we identify morphological and ecological traits associated to changes in the olfactory molecular repertoire in ray-finned fish.

Ivan Manzini, Daniela Daume, Lukas Weiss, Paola Segoviano Arias, Sara Joy Hawkins, Thomas Offner, and Thomas Hassenklöver

Institute of Animal Physiology, Department of Animal Physiology and Molecular Biomedicine, Justus-Liebig-University Gießen, Gießen, Germany

**Information processing and metamorphic changes within the olfactory network of *Xenopus laevis***

In vertebrates, olfactory systems consist of an olfactory epithelium (OE; peripheral odorant detection area), an olfactory bulb (OB) in the anterior telencephalon (relay and processing center), and various higher olfactory centers (terminal processing areas). Upon the detection of odorants by olfactory receptor neurons (ORNs), the neurons typically depolarize and, in turn, send olfactory information toward the OB. After various modulatory and processing steps within the neuronal network of the OB, the olfactory information is relayed to higher olfactory centers by bulbar projection neurons. We used neuronal tracings, immunohistochemistry, calcium imaging, and behavioral experiments to examine the functional connectivity between the OE, OB, and the higher olfactory centers in larvae of *Xenopus laevis*. Thereby, we mainly focused on structural and functional changes in the olfactory network that occur during metamorphosis. During metamorphosis, the olfactory system of *Xenopus* undergoes a substantial restructuring. The larval main OE located in the so-called principal cavity (PC), associated with aquatic olfaction, is transformed into an OE capable of detecting airborne odorants. An additional OE, associated with aquatic olfaction, emerges in the newly forming middle cavity (MC). In larvae, ORNs in the PC epithelium project axons to the ventral part of the OB. These projections are gradually replaced by ORN axons from the MC epithelium. Contemporaneously, new ORNs in the remodeling PC innervate the emerging dorsal part of the OB. Our results show that the “water system”, despite the massive reconstruction, remains functional throughout metamorphosis. Also, we describe substantial wiring differences between the neuronal network of the postmetamorphic “water system” and “ air system”.

[This work was supported by Deutsche Forschungsgemeinschaft (DFG) Grant 4113/4-1.]

**(ii) Contributed talks**

Zbynek Kozmik, Miroslava Kolkova, Simona Mrstakova, Jovana Doderovic, Iryna Kozmikova

Institute of Molecular Genetics of the Czech Academy of Sciences

**Eye evolution: a Pax6 gene perspective**

A paired domain and homeodomain-containing transcription factor Pax6 is an evolutionarily conserved regulator implicated in the eye development of both vertebrates and invertebrates. Mutations of the Pax6 gene disrupt eye development in both mammals and insects while Pax6 misexpression can lead to the formation of ectopic eyes. Furthermore, Pax6 gene expression has been detected in the developing eyes of many species. Altogether, these genetic and expression studies have indicated that morphologically distinct eyes may share a similar developmental program orchestrated by Pax6, working as a ‘master control gene’. We will present an updated view of the ‘master control gene’ hypothesis taking into account additional data from vertebrates and invertebrates.

Zuzana Musilova[1], Fabio Cortesi[2]

1 Dpt of Zoology, Charles University, Prague, Czech Republic, 2 Queensland Brain Institute, Brisbane, Australia

**Deep-sea fishes have evolved extraordinary vision using multiple rhodopsin genes**

In the dim light environment, such as deep sea, fish generally obtain visual information through light-sensitive photopigments based on a single rod opsin (rhodopsin 1 = RH1). Vast majority of teleost fishes possess one or two copies of the rhodopsin gene in their genome. In the deep-sea fishes, however, extreme adaptations of rhodopsins have been found. We have recently discovered a species, silver spinyfin (Diretmus argenteus), with the highest number of visual opsins among vertebrates (two cone opsins and 38 rod opsins). Such an extraordinary set up has convergently evolved also in two other unrelated deep-sea lineages (lanternfishes and tube-eye fish) in the extreme conditions, and suggests unique mode of vision, albeit its exact mechanism remains elusive. The peak sensitivity of the spinyfin rhodopsin photoreceptors spans the blue-green light spectrum of 444 to 519 nm, having actually one of the shortest-sensitive rhodopsins known in vertebrates. In evolution, the extreme diversity of the rhodopsin genes has raised through multiple gene duplications followed by the series of amino acid mutations, which cause changes of the protein function (=sensitivity). Following the original discovery, we will present new findings on the whole genome properties of this species, as well as we have recently expanded the transcriptome data set from the new deep-sea collection of the spinyfins. As the last point, we would like to focus on differential function of vision during ontogeny when larvae need to see in the shallow while adults in the deep.

David Lagman[1], Helen J. Haines[1], Xesús M. Abalo[1,2] and Dan Larhammar[1]

1 Department of Medical Cell Biology, Science for Life Laboratory, Biomedical Centre Box 571, Uppsala University, SE-75123 Uppsala, Sweden, 2 Present address: Science for Life Laboratory, KTH Royal Institute of Technology, Department of Gene Technology, Tomtebodavägen 23, SE-171 65 Solna, Sweden

**Evolution of genes important for nervous system function in vertebrates with a focus on vision and long term memory**

Whole genome duplications (WGDs) at the base of the vertebrate lineage have over the years been shown to be responsible for the expansion of many gene families that are important in vertebrate central nervous system function. We have previously resolved the evolution of many of these genes in relation to these WGDs, especially those involved in neuronal, neuroendocrine, and visual function.

Presented here are our latest results regarding the evolution of cyclic nucleotide gated cation (CNG) channel genes in metazoans. The CNG channels play an important role in vision in vertebrates by being responsible for the hypopolarization of cone and rod photoreceptor cells after the detection of light. In vertebrates these channels are heterotetramers of three CNGA subunits and one CNGB subunit and cones and rods utilize different sets of CNGA and CNGB subunits in their respective channels. Cones use three CNGA3 and one CNGB3 subunits while rods use three CNGA1 and one CNGB1 subunit. The composition of these channels and their function in many invertebrate lineages is still not resolved. However, the role of CNG channels in phototransduction has been shown in some invertebrate lineages indicating that this role is older than bilaterians.

Using extensive datamining we have managed to identify four previously unknown CNG channel gene subtypes in several invertebrate lineages. We have also managed to, through detailed analyses of conserved synteny, accurately assign vertebrate CNG genes to related chromosomal regions providing strong evidence for an expansion in the early vertebrate WGDs. These analyses open up the door for investigation of the functions of the novel CNG genes identified in invertebrate genomes.

Finally, I will describe our current endeavor into understanding the evolution and function of genes involved in long term memory formation and maintenance in vertebrates.

Marina Morini[1,2], Dan Larhammar[3], Christina A. Bergqvist[3], Juan F. Asturiano[2], Sylvie Dufour[1]

1 National Museum of Natural History (MNHN), CNRS, Sorbonne University, Paris, France; 2 Universitat Politècnica de València, Valencia, Spain; 3 Uppsala University, Uppsala, Sweden.

**Dynamic evolution of transient receptor potential vanilloid (TRPV) ion channel family with numerous gene duplications and losses**

The transient receptor potential vanilloid (TRPV) ion channel family is involved in multiple sensory and physiological functions including thermosensing and temperature-dependent neuroendocrine regulation. We have investigated the evolution of TRPV genes in metazoans with special focus on the vertebrate whole genome duplications (WGD). Gene searches followed by phylogenetic and syntenic analyses revealed multiple previously undescribed TRPV genes. The common ancestor of Cnidaria and Bilateria had three TRP genes that became four in the chordate ancestor. Two of these were lost in the vertebrate ancestor. The remaining two genes expanded to five via the two basal vertebrate WGD events (called 1R and 2R) and one local duplication, followed by several losses. Two local duplications before the radiation of gnathostomes resulted in the repertoire TRPV1, 3, 4, 5, 7, 8, 9, of which the three latter were previously unreported. TRPV7 and 8 have been lost independently in various lineages but are still retained in prototheria (platypus), amphibians, coelacanth and a basal rayfinned fish. TRPV3 and 9 are present in extant elasmobranchs, while TRPV9 was lost in the osteichthyan ancestor and TRPV3 in the actinopterygian ancestor. The coelacanth has retained the ancestral osteichthyan repertoire of TRPV1, 3, 4, 5, 7 and 8 and gained two additional duplicates of TRPV3. TRPV2 arose in the tetrapod ancestor. Duplications of TRPV5 occurred independently in various lineages including mammals. After the teleost-specific WGD (3R) only TRPV1 retained its duplicate which was subsequently lost in some species. The salmonid-specific WGD (4R) duplicated TRPV1, 4, and 5 leading to six TRPV genes. The largest number was found in Xenopus tropicalis with no less than 15 TRPV genes. This analysis distinguishing orthologs and the numerous independently originating paralogs will be useful for future functional studies for the various TRPV family members.

**(iii) Contributed posters**

**P7**

Zuzana Konvičková[1], Zuzana Musilová[1], Veronika Truhlářová[1], Pavel Lepič[2]

1 Department of Zoology, Faculty of Science, Charles University, Czech Republic, 2 South Bohemian Research Centre of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, Vodnany, Czech Republic

**How does the common barbel (*Barbus barbus*) see? The effect of the whole-genome duplication on vision**

Teleosts outstand among vertebrates in the number of visual pigments and photoreceptor types. Such extraordinary repertoire has evolved also thanks to the whole-genome duplication that occurred in their ancestor, approximately 350 million years ago. Here we explore teleost fish species that has recently experienced subsequent whole-genome duplication - the common barbel (*Barbus barbus*). We focus on the effect of tetraploidy on the visual system. We found 13 opsin genes in the common barbel genome – an unusually high number. We further investigate opsin gene expression to test if multiple opsin genes in the genome result in different visual system function. We present opsin expression profiles of adult specimens and larvae at different developmental stages. We identified ontogenetic shifts specific for barbel, as well as shared among teleost fishes. Both copies of the opsin genes resulting from the barbel-specific whole-genome duplication are expressed in the retina at some developmental stage. We found alternative expression of the two copies of SWS1, SWS2, and RH2 opsin gene during development. We also found continuous expression of both copies of LWS opsin gene. We visualise opsin expression in adult retinae via fluorescence in situ hybridization to reveal the retinal cone mosaic and we discuss its functional consequences.

**P8**

Pombal, Manuel A.[1], Barandela, Marta[2], Jiménez-López, Cecilia[2], Núñez-González, Carmen[2], Megías, Manuel[1], Pérez-Fernández, Juan[2]*

1Departamento de Bioloxía Funcional e Ciencias da Saúde, Facultade de Bioloxía-IBIV, Grupo Neurolam, Universidade de Vigo, 36310 Vigo, Spain. 2CINBIO, Neurocircuits group, Universidade de Vigo, 36310 Vigo, Spain.

**Functional development of ocular movements in lamprey**

Our eyes are capable of sophisticated movements, evolved through new motor and perceptual needs. The first movements appeared to immobilize the image on the retina: the vestibulo-ocular reflex (VOR), which ensures eye stabilization during head movement, and the optokinetic reflex, that keeps the visual field static on the retina when shifted. Other eye movements redirect gaze to specific targets. A pupillary reflex (PR) that regulates the amount of light entering the eye is present in most vertebrates. However, the origin and evolution of the circuits involved in these processes are unclear.

Lampreys have a simple nervous system with similarities to other vertebrates, as for the visual system (VS). This facilitates dissecting the mechanisms of conserved neuronal circuits and provides valuable information on their evolution.

The lamprey VS develops stepwise during its larval period. Larvae have immature, skin-covered eyes. Some of the neural circuits involved in ocular stabilization have been described, but little is known functionally. The vestibular system interacts with the extraocular muscles to generate VOR. The optic tectum controls gaze direction and is immature in larvae. The PR is mediated by the Edinger–Westphal nucleus via the ciliary ganglion in mammals. Besides, a second slower mechanism may occur, mediated by the sphincter pupillae muscle, which causes pupil reduction depending on light intensity. A PR was reported in lampreys, but its mechanisms remain to be determined.

The aim is to study when these stabilizing movements and the neuronal circuits that control them appear, to determine their occurrence in lamprey larvae and to analyze the PR mechanisms to clarify the evolutionary origin of this visual function. Our results show that stabilizing movements appear in 60 mm long larvae and that, at least, the intrinsic mechanism of PR occurs in lampreys, what means that ocular reflexes have been present since the beginning of vertebrates' evolution.

**P9**

Samuel Marashli

Interdisciplinary Center for Neurosciences (IZN), Heidelberg University, Heidelberg, Germany

**Auditory brainstem responses: A key tool for understanding auditory sensory processing and abnormalities across species**

Sensory processing abnormalities are core to neurodevelopmental disorders (NDD). In fact, auditory hypersensitivity affects up to 66% of children with autism spectrum disorders (ASDs). Developing efficient therapies require understanding the basic sensory pathways and finding the atypical alterations of the circuit during early development.

Across different species, including frog[1], birds2, rodents3 and humans4, auditory brainstem responses (ABRs) show very high similarity of the electrophysiological properties, they are also non-invasive and easy to reproduce. Thus, they are considered essential tools in detecting hearing thresholds, latencies/amplitudes and other biomarkers of auditory sensory processing.

In our study, we used ABRs to detect the physiological response to auditory stimuli in two different rodent models of NDD; Nrxn1KO rats and Cntnap2KO mice. We performed a longitudinal ABR study, focusing on the different developmental stages of rodents (juvenile, adolescence and adult). Additionally, we tested several pharmacological compounds, which are highly relevant for auditory sensory gating, processing and filtering.

We confirmed that ABR's waves increase in amplitude and decrease in latency upon brainstem maturation. In both rodent models, there were significant changes in signal latency and differences with controls. Such differences were detected at multiple ages. The pharmacological tests altered the ABR wave's peaks in a genotype-specific manner. Our results emphasize the role of ABR as a valuable biomarker in ASD, increase our understanding of gene manipulations linked to NDD and spot the light on the evolutionary route of the ABRs and their translatability across species.

References

1-T. B. Lauridsen, C. Brandt, J. Christensen-Dalsgaard, J Exp Biol. 224, (2020)

2-S. E. Crowell et al., J Comp Physiology. 201, 803–815 (2015)

## Brain complexity evolution and its drivers

**(i) Contributed talks**

Robert Barton[1] and Chris Venditti[2]

1 Durham University, 2 University of Reading

**The evolutionary dynamics of mammalian brain and body size**

Despite decades of comparative studies, fundamental questions remain about how mammalian brain and body size co-evolved. Contradictory models to account for phenomena such as variation in brain relative to body size, variability in the scaling relationship across taxonomic levels, and purported trends for brain mass to increase through time, exist concurrently in the literature. Here we resolve these questions using a comparative method enabling us to study brain relative to body mass evolution while estimating their evolutionary rates along the branches of a phylogeny. Our analysis simultaneously accounts for scaling of brain to body size, variation in slopes and intercepts across taxa, and the so-called taxon-level effect. Our analysis suggests that brain-body scaling is related to conservation of functional parameters at the neuronal level. We show that relative brain mass predominantly reflect selection on brains and is rarely a secondary consequence of change in body mass, and we demonstrate substantial variation in rates of relative brain mass evolution. Finally, we uncover a unique adaptive trend in primate brain size not present in any other mammalian group, which set the stage for unprecedentedly rapid directional increase ultimately producing the human brain.

Kristina Kverková[1], Yicheng Zhang[1], Alexandra Polonyiová[1], Lucie Marhounová[1], Martin Kocourek[1], Seweryn Olkowicz[1], Barbora Straková[1], Zuzana Pavelková[1], Roman Vodička[2], Daniel Frynta[1], Pavel Němec[1]

1 Department of Zoology, Faculty of Science, Charles University, Czech Republic; 2 Prague Zoo, Czech Republic

**The evolution of brain processing capacity in land vertebrates**

Brains of land vertebrates vary greatly in terms of absolute and relative size, neuron numbers and proportions of neurons allocated to different brain parts. Selection for cognitive abilities is usually assumed to have shaped this diversity. However, evolutionary constraints might play an equally important role. Comparing 385 species of amphibians, non-avian reptiles, birds, and mammals, we show that the scaling of neurons with brain and body mass is rather conserved, with a handful of dramatic shifts, whereas mosaic changes in brain region sizes are more frequent. Birds and mammals independently increased the number of neurons for body mass, arriving at similar levels, with two other subsequent increases in core landbirds and primates. We suggest these convergent increases in neuron numbers coincide with the advent of endothermy, an energetically expensive mode of life. Neurons are metabolically costly and require a steady supply of energy; having large numbers of neurons thus might not be advantageous for animals with low energy intake and expenditure, such as reptiles and amphibians. Accordingly, we observed no major increase in neuron-body scaling in these groups over 325 million years. In contrast, caudate amphibians show a substantial decrease in neuron density that might be partly due to their heavy dependence on energy saving and partly due to their particularly large genomes. The overall „neuronal energy budget“ can be flexibly allocated to brain structures depending on species-specific needs, with different regions or circuits having different per neuron utility. A modest volumetric increase in a neuron-dense structure such as the cerebellum will result in a substantial number of added neurons. In fact, the cerebellum is the region that truly sets apart ectothermic and endothermic land vertebrates with the latter having on average over 100 times more cerebellar neurons for equivalent body mass but only about 10 times larger brains.

Pavel Němec[1], Seweryn Olkowicz[1], Patrik Stehlík[1], Martin Kocourek[1], Yicheng Zhang[1], Lucie Marhounová[1], Christin Osadnik[2], Eva Corssmit[3], Joan Garcia-Porta[4], Thomas E. Martin[5], Ferran Sayol[6], Louis Lefebvre[7], Daniel Sol[3]

1 Department of Zoology, Charles University, Prague, Czech Republic; 2 Department of General Zoology, University of Duisburg-Essen, Essen, Germany; 3 CSIC-CREAF (Centre for Ecological Research and Applied Forestries), Catalonia, Spain; 4 Department of Biology, Washington University in St. Louis, USA; 5 Montana Cooperative Wildlife Research Unit, University of Montana, Missoula, MT USA; 6 Centre for Biodiversity and Environment Research, Department of Genetics, Evolution and Environment, University College London, London, UK; 7 Department of Biology, McGill University, Montréal, Québec, Canada

**Neuronal numbers in avian pallium: A reliable proxy of bird intelligence**

Using the isotropic fractionator, we determined numbers of neurons and non-neuronal cells in the cerebral hemispheres, cerebellum, diencephalon, tectum and brainstem in more than 200 bird species. This unique data set allowed us to establish and compare cellular scaling rules among major bird clades. Brains of birds belonging to distantly related clades differ in relative structure sizes, neuronal densities, neuronal numbers and allocation of neurons into brain compartments. Songbirds, parrots and owls share high neuronal densities and disproportionately large numbers of neurons in the pallial telencephalon. In contrast, birds representing basal lineages, such as paleognathous and galliform birds, have lower neuronal densities, a proportionally smaller telencephalon, small telencephalic and dominant cerebellar neuronal fraction. Brains of birds situated phylogenetically in between these two groups exhibit intermediate characteristics.

Using a subset of 111 species, we tested how neuron numbers shape internal brain organization and cognitive performance. We show that the number of neurons in the pallial telencephalon is positively associated with a major expression of intelligence: innovation propensity. The number of pallial neurons, in turn, is greater in brains that are larger in both absolute and relative terms, and positively co-varies with longer post-hatching development periods. Thus, our analyses show that neuron numbers link cognitive performance to both absolute and relative brain size through developmental adjustments. Our study provides a framework linking life history with cognition, neuron numbers and brain size.

Suzana Herculano-Houzel

Department of Psychology, Department of Biological Sciences, Vanderbilt Brain Institute, Vanderbilt University

**Life histories of warm-blooded animals depend on the brain, not the body**

Animal life history is extremely diverse, ranging in gestation times of a dozen days to a dozen months; postnatal growth rates of 1% to 70% of body mass added per day; and maximal longevity of one to 100 years. The main determinant of life history is traditionally considered to be body mass, which correlates strongly with basal metabolic rate across species. However, the availability of data on the numbers of neurons that compose the brains of different species made it possible to show that both age at sexual maturity and maximal longevity are much better predicted by numbers of cortical neurons across warm-blooded species (Herculano-Houzel, 2019). Here I extend those findings to show that the rate constant of postnatal growth, as well as the time to reach near-adult body mass, also scales universally with increasing numbers of cortical neurons across mammals, rather than with adult body mass. In contrast, the maximum rate of postnatal growth increases universally with decreasing neuronal density in the mammalian cerebral cortex. Interestingly, across bird species, the postnatal growth rate constant and the time to reach near-adult body mass scale universally not with numbers of pallial neurons, as in mammals, but rather with neuronal density in the pallium, in a scaling relationship that also includes all mammals, with the sole exception of primates. I thus propose that the enormously variable neuronal composition of the pallium of warm-blooded species has biological implications that extend well beyond the cognitive sphere, constituting a key controlling factor in body development and life history.

**(i) Contributed posters**

**P10**

Pierre Estienne, Kei Yamamoto

Paris-Saclay Institute of Neuroscience (NeuroPSI), CNRS, Université Paris-Saclay

**Encephalization in teleost fishes: yet another way of allowing complex behaviors?**

Besides mammals and birds, some species of teleost fishes like wrasses and cichlids also possess relatively large brains and are capable of complex behaviors such as tool use. However, the brain morphology of teleosts is very different from that of amniotes. In amniotes, the most behaviorally complex species also have an extremely developed pallium. We thus examined whether “intelligent” teleosts possess an enlarged pallium. We measured the mass of the major brain subdivisions of ten teleost species and counted the number of cells in each of them using the isotropic fractionator.

Surprisingly, unlike in amniotes, the telencephalon only accounts for a modest part (<15%) of the total number of cells. Indeed, the proportion of cells in the five major brain structures (telencephalon, tectum, rest of the forebrain/midbrain -rFM-, cerebellum, rest of the hindbrain) is very similar across all species examined. It is also remarkable that cichlids and wrasses, which display complex behaviors, have a relatively larger telencephalon and rFM compared to other species.

Whole-brain 3D-imaging revealed the presence of an extremely large fiber tract between the telencephalon and inferior lobe (IL) in cichlids and wrasses, which likely explains the enlargement of the telencephalon and rFM in these species. The IL has evolved uniquely in teleosts and their closest relatives, and has no homolog in the tetrapod brain. It receives multisensory inputs and projects mainly to the cerebellum. The presence of a large fiber tract between the telencephalon and IL in cichlids and wrasses raises the question of the role of this circuit in higher order cognitive functions.

Thus, our results show that encephalization in teleosts is very different from that in amniotes, and raises the possibility that non-telencephalic structures may perform pallial-like functions in teleost brains.

**P11**

Seweryn Olkowicz, Yicheng Zhang, Martin Kocourek, Radek Lučan and Pavel Němec

Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

**Mosaic evolution shapes the neuronal composition of the avian brain**

Mosaic evolution of the brain in which brain regions change independently of one another is now a well-recognized phenomenon. The origins of such divergences are however poorly understood, since it is difficult to differentiate between intrinsic and extrinsic factors influencing them. To analyze possible mechanisms of mosaic brain evolution we compared numbers of neurons in a set of brain structures in 29 species of birds of prey belonging to four avian orders. Our results show that even in closely related avian groups brains exhibit dramatically different neuronal organization. Unlike hawks, eagles and the seriema, owls and falcons have a neuron-rich telencephalon, but only owls have increased the size of their telencephalon, and thus the overall brain size. The increase in telencephalon size is specifically due to the increase in the size of the visual wulst, a structure involved in the processing of binocular visual input. Moreover, neuronal enrichment in the owl telencephalon, and especially in wulst, leads to the accumulation of high absolute numbers of neurons comparable to those seen in songbirds, parrots and primates. The neurons residing in wulst and known to be largely engaged in the detection of binocular stimuli, comprise on average half of all telencephalic neurons in owls. In contrast, raptors with limited binocular overlap have only 10-15% of telencephalic neurons contained within the wulst. Analysis of numbers of neurons in the tectum supports the conclusion that originally nocturnal owls underwent elaboration of the thalamofugal visual pathway for the enhancement of binocular summation, whereas diurnal raptors evolved high numbers of neurons in the tectofugal pathway for high-resolution daylight vision. Owls, similarly to primates and felids, independently evolved binocularity and neural architecture consisting of a pallial area with an expanded neuron-rich input layer specialized in processing binocular stimuli.

**P12**

Dionigi F., Marhounová L., Polonyiová A., Zhang Y., Kocourek M., Sommer G., Kverková K., Kotrschal A., Musilová Z. & Němec P.

Department of Zoology, Faculty of Science, Charles University, Prague, Czech Republic

**Extremely high numbers of brain neurons in weakly electric fish**

The ability to sense weak electric fields is widespread and likely ancestral in fish. This capacity has been lost in teleost fishes and afterwards independently reacquired in several teleost lineages. Some of them evolved passive electroreception, while others can produce weak electric fields and use them to orient in their environment and communicate with conspecifics. Here we used the isotropic fractionator to compare the numbers of neurons and their distributions across major brain parts in basal ray-finned fishes, teleosts without the ability to sense electric fields and those that secondarily evolved passive and active electroreception. We show that passively electroreceptive basal ray-finned fishes have rather modest numbers of brain neurons and there are no consistent differences between the brains of teleosts with and without passive electroreception. An enlarged cerebellum coupled with increased neuron density has evolved at least two times independently in weakly electric teleost fishes. In extreme cases, their cerebellum and telencephalon contain more than 95% and less than 0.5 % of brain neurons, respectively. Mormyrids are highly encephalized and feature the highest known cerebellar neuron densities, resulting in brain neuron counts equal to or greater than some large mammals (including monkeys) with much bigger brains. These findings strongly suggest that active electroreception is very computationally demanding.

**P13**

Yicheng Zhang, Alexandra Polonyiová, Kristina Kverková, Pavel Němec

Charles University, Prague, Czech Republic

**Cellular scaling rules for amphibian brains**

Relative brain size was traditonally used as the go-to proxy for cognitive abilities in comparative studies. In recent years, the focus has shifted towards better estimates of brain processing capacity, which is determined largerly by the number of neurons, inter-neuronal distances and the number of synapses. With the advent of the isotropic fractionator, it has become possible to reliably quantify numbers of neurons in whole brains with high throughput. Using this method, we analyzed the numbers of neurons and other cells in six major brain parts of 74 amphibian species representing all three amphibian orders - frogs (Anura), newts and salamanders (Caudata) and caecilians (Gymnophiona). The brain sizes varied starkly among the analyzed species, ranging from 8 mg to 312 mg. Number of brain neurons varied from 493,000 to 10,466,000. It was observed that newts and salamanders have significantly lower neuronal densities than frogs. These neuron numbers and densities will be compared with those of other vertebrates.

**P14**

Rahul Avaroth Bhaskaran[1], Pavel Osten[2], Pavel Němec[1]

1 Charles University, Faculty of Science, Department of Zoology, Prague; 2 Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY 11724, United States

**Quantitative whole-brain mapping of neuronal and glial cell type distributions in birds and reptiles**

The number of neurons in the entire brain is currently the best available proxy of brain information processing capacity. Using the isotropic fractionator we have estimated neuronal populations in more than 200 species representing diverse vertebrates. However, this technique is based on tissue homogenization and therefore does not allow the analysis of local brain microcircuits subserving behaviors. The next frontier is therefore quantitative mapping of specific cell type distributions across the brain. We have optimized a novel organic-based U-clear protocol for imaging whole-brain and standardized an analysis pipeline for quantitative mapping of neuronal cell type distributions in 3D brain data sets.

**P15**

Patrik Stehlík, Martin Kocourek, Yicheng Zhang, Seweryn Olkowicz, Lucie Marhounová, Kristina Kverková, Pavel Němec

Charles University, Faculty of Science, Department of Zoology, Prague

**Neuron numbers in hornbills, woodpeckers, and coraciiform birds**

Recent comparative studies have shown that bird brains, although small, have higher neuronal densities than brains of mammals. Especially large parrots and corvids compete with or even outnumber primates by the number of telencephalic neurons. However, the processing capacity of the avian brain appears to differ significantly between various phylogenetic lineages. Basal groups such as galliform birds and pigeons have much lower absolute numbers of neurons and lower neuronal densities than songbirds and parrots. However, these data do not allow us to reconstruct the evolution of the number of neurons in the brains of birds. Using the isotropic fractionator, we determined the number of neurons and non-neuronal cells in specific brain regions in 19 species of hornbills, woodpeckers, and coraciiform birds. The brains of hornbills and woodpeckers have numbers of neurons comparable to those of songbirds and parrots and have significantly more neurons than the equivalently sized brains of pigeons and galliform birds. Furthermore, hornbills have significantly higher numbers of non-neuronal cells than any other avian group so far. A more detailed analysis of the telencephalon in representative species showed that most telencephalic neurons (40–57 %) are allocated in the nidopallium, which is believed to be involved in numerous higher cognitive functions. The relative size and percentage of neurons in the hyperpallium are probably species-specific and rather reflect the ecology of a given species or taxon.

**P16**

Fabien Knoll[1], Soichiro Kawabe[2], Akinobu Watanabe[3]

1 ARAID-FCPTD, Teruel, Spain; 2 Fukui Prefectural University, Fukui, Japan; 3 New York Institute of Technology, Old Westbury, NY, USA

**A proxy for brain-to-endocranial cavity index in non-neornithean dinosaurs and other extinct archosaurs**

Whereas the brain fills most of the cranial cavity in birds, it can occupy a minor portion of it in crocodiles. In non-neornithean dinosaurs and other extinct archosaurs, the lack of data regarding the volume of the brain compared with that of the cranial cavity constitutes a serious shortcoming in assessing the degree of encephalization of these animals and, consequently, in inferring their cognitive capacities. However, empirical data suggest that the degree of endocranial doming is inversely related to the volume of the intracranial non-encephalic components across extant archosaurs. We build upon this to develop an equation relating endocast doming to brain-to-endocranial cavity (BEC) index in non-neornithean dinosaurs and other extinct archosaurs. We rely on measurements of endocast doming and BEC in extant relatives of non-neornithean dinosaurs, namely the crurotarsan *Crocodylus niloticus*, the paleognath *Struthio camelus* and the fowls *Phasianus colchicus* and *Gallus gallus*. Applying the equation to endocasts from major clades of dinosaurs, we found that the BEC varies from a little below 0.6 in thyreophorans and ceratopsians to near or over 0.7 in some theropods, sauropods and pachycephalosaurians, with ornithopods falling between these two extremes. We, therefore, warn against the use of a catch-all value, like 0.5, and instead encourage fine-tuning of the BEC in any given case.

## Functional studies

**(i) Contributed talks**

Ronen Segev

Life Sciences Department, Ben Gurion University of the Negev

**Finding the homolog of the hippocampus in the teleost brain: evidence from physiology of representation of space in the goldfish**

We set to find a functional homolog for the hippocampal formation in the telencephalon of teleost by looking for a representation of space in the activity of single cells in the lateral and central pallium. For this purpose, we recorded the activity of single neurons in the goldfish telencephalon while it was freely navigating in a water tank. We found cells that represent space and dynamics. Specifically, we found cells that represent border, speed, and head directions in the lateral pallium. In addition, cells with firing patterns which gradually increase or decrease with the position of the fish along different axes of space were recorded in the central telencephalon. While some of the cells were tuned along the major cartesian axes: horizontal and vertical, others were tuned to position along an axis from salient feature of the environment. This type of axial code for space representation in the brains of fish is unique among the space encoding cells in vertebrates and provides insights into spatial cognition in this lineage. Furthermore, it provides evidence for possible functional homologs of the hippocampal formation in teleost.

Andrea Messina[1], Davide Potrich[1], Anna Burato[1], Alessandra Gobbo[1], Valeria Anna Sovrano[1,2], Scott E. Fraser[3], Caroline H. Brennan[4], Giorgio Vallortigara[1]

1 CIMeC-University of Trento; 2 Dipsco-University of Trento; 3 Michelson Center for Convergent Bioscience - University of Southern California; 4 School of Biological and Behavioural Science - Queen Mary University of London

**Quantities in the brain: behavioural and neurobiological evidence in zebrafish**

The ability to deal with continuous and discrete quantity developed from an evolutionarily conserved system for approximating non-symbolic numerical magnitude. In fish, numerosity discrimination has been documented using spontaneous choice tests and operant conditioning procedures. However, little is knowns about the neural correlates of this ability. By combining a habituation/dishabituation behavioral paradigm with molecular biology assays, we have recently identified part of the neural network associated with quantity discrimination in adult zebrafish brain. Zebrafish were habituated to groups of 3 or 9 small red dots for four consecutive days. During this phase, the dots changed in density, position and size, while maintaining their numerousness and overall surface area. During dishabituation, zebrafish faced a change (i) in number (from 3 to 9 dots or vice versa, with the same overall surface), or (ii) in shape (3 or 9 red squares instead of dots), or (iii) in size. A control group was tested with the same stimuli as during the habituation. Thirty minutes after the dishabituation test, zebrafish brains were dissected to quantify the change in the expression levels of c-fos and egr-1 by quantitative polymerase chain reaction or probed with egr-1 in situ hybridization assays to identify the positional identity of neuronal correlates of changes in quantity (number, size) or shape. Results showed an involvement of the retina and optic tectum in the encoding of continuous magnitude. We also found a role of the habenula and the preglomerular complex, and of the caudal regions of the dorso-lateral and dorso-central pallium, in the encoding of discrete magnitude (e.g. change in numerosity). A response to shape discrimination was observed in the most rostral part of the dorso-central pallium. Results suggest an early involvement of thalamic and tectal areas for encoding of continuous quantity, and of more pallial (via thalamic nuclei) regions for discrete quantity.

**(ii) Contributed posters**

**P17**

Karina Santiago Gonzalez[1,2], Tim Boswell[2,3] and Tom V. Smulders[1,2]

1 Biosciences Institute, Newcastle University, Newcastle Upon Tyne, UK 2 Centre for Behaviour and Evolution, Newcastle University, Newcastle upon Tyne, UK; 3 School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK

**Hippocampal immediate early gene induction and HPA-axis activation in response to acute stress in chickens**

The mammalian and avian hippocampus are considered to be homologous structures. However, in contrast to mammals, the morphological organization of the avian hippocampal formation (HF) is not well characterized and despite extensive data addressing its role in spatial navigation and memory processing, little is known regarding its crucial role in the regulation of the neuroendocrine stress response.

Research in mammals indicates that electrical stimulation of the hippocampus decreases plasma corticosteroid levels, while lesions delay the termination of the stress response. Interestingly, a functional differentiation along the transversal and longitudinal axes of the mammalian hippocampus has been suggested: while the dorsal hippocampus appears to be more important in cognitive tasks, the ventral hippocampus participates in emotional processes.

This study aims to extend the understanding of the avian HPA axis functioning and to shed light on the role of the hippocampus in the regulation of stress in birds. We therefore evaluate the effect of an acute stressor on the activity patterns of the c-Fos immediate-early gene (IEG) expression in the chicken hippocampus, lateral Bed Nucleus of the Stria Terminalis, and paraventricular nucleus of the hypothalamus. Changes in plasma corticosterone (CORT) concentrations and pituitary gene expression levels of proopiomelanocortin (POMC), corticotropin-releasing hormone receptors (CRH-R1 and CRH-R2), and chicken vasotocin receptor two (VT2R) and four (VT4R) were also analysed.

Our study provides a better understanding of the neuroendocrine stress response in birds, and aims to provide valuable information about the subregional organization and functional specialization of the avian HF.

This project receives funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812777.

**P18**

Trinh Anh-Tuan, Ostenrath Anna Maria, Yaksi Emre

Kavli Institute for Systems Neuroscience and Centre for Neural Computation, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, Norway

**Neural circuit architecture and computations underlying sensory processing and learning in the zebrafish paleocortex**

Sensory information is first processed by various thalamic nuclei before reaching the pallium in amniotes. In teleost fish, the structure that is homologous to the amniote thalamus, the dorsal thalamic nuclei projects primarily to the subpallium. In contrast, the major sensory inputs from the teleost midbrain to pallium originate from the preglomerular nucleus (PG) which suggest that this pathway may perform analogous functions to thalamocortical computations in amniotes. We hypothesize that PG inputs would trigger sequential activation of teleost pallial circuits that are ancestral to mammalian cortico-limbic circuits. To test this, we first mapped the anatomical connections from PG to pallium using local tracer injections in the PG of juvenile (3-4 weeks old) zebrafish, and we have confirmed that PG projects ipsilaterally to the dorsal lateral (DL) and dorsal medial (DM) pallium. Next, we tested the function of these PG inputs, by micro-electrode stimulation of PG neurons while imaging calcium signals in the entire juvenile zebrafish brain explant. We observed that PG microstimulation leads to spatially restricted activity in DL, DM as well as in the anterior region of the dorsal telencephalon, which we termed DA. Notably, PG activation led to DM, DL and DA responses with different temporal dynamics, that outlasted the transient PG microstimulation. Moreover, direct activations of DL led to even longer lasting responses propagating across the pallium, suggesting strong recurrent connectivity. We are currently analyzing the temporal features of pallial ensembles upon PG, DL and DM microstimulations (*ex vivo*), as well as salient sensory stimulation (*in vivo*) to see if distinct temporal sequences of pallial neural responses can be triggered and if so, whether they can be facilitated by experience-dependent processes. Our results highlight an important role for PG inputs in the integration of sensory information by the zebrafish ancestral cortico-limbic circuits.

**P19**

Daniel Divín[1], Mercedes Goméz Samblas[1], Nithya Kuttiyarthu Veetil[1], Eleni Voukali[1], Zuzana Świderska[1], Tereza Krajzingrová[1], Martin Těšický[1], Daniel Elleder[2], Oldřich Bartoš[3] and Michal Vinkler[1]

1 Charles University, Faculty of Science, Department of Zoology, Czech Republic 2 Institute of Molecular Genetics, Czech Academy of Sciences, Czech Republic; 3 Military Health Institute, Military Medical Agency, Czech Republic

**Chromosomal rearrangements are responsible for altered neuroinflammatory regulation in parrots**

Parrots (Psittaciformes) are birds with advanced cognitive abilities outperforming mammals of similar size. In their evolution they came through massive chromosomal rearrangements, which caused several gene losses. Here we provide genomic evidence of cannabinoid receptor 2 (CNR2) gene loss shared in parrots. Our results based on interspecific comparison of immune response regulation in parrots (CNR2 loss) and passerine birds (functional CNR2) suggests susceptibility of parrots to neurinflammation. In budgerigar (Melopsittacus undulatus), we detected a significant upregulation of interleukin 1 beta (IL1B) expression in the brain after experimentally induced sterile peripheral inflammation. In contrast with the parrots, no such upregulation was detected in zebra finch (Taeniopygia guttata). Further we analysed effects of neural inflammation on neuropeptides expression in parrots (NPY, VIP, TAC1, PDYN, POMC). We propose that CNR2 loss, which acts as an immune regulator expressed mainly in immune cells including microglias in brain, might have contributed to parrot susceptibility to neurological disorders like depression-related behaviours. For this purpose parrots may serve as suitable models in future neurological studies.

**P20**

Eva Landová[12], Silvie Rádlová[12], Jakub Polák[1] , Kristýna Sedláčková[12], Anna Pidnebesna[2], David

Tomeček[2], Jaroslav Hlinka[2], Daniel Frynta[12]

1 Faculty of Sciences, Charles University, Viničná 7, 12844 Prague

2 National Institute of Mental Health, Topolová 748, 25067, Klecany

**Emotional response to spiders, fearful snakes, and disgusting animal stimuli: fMRI study**

Spiders are common objects of phobias. It is unclear, however, whether they evoke primarily fear or disgust in fearful subjects. We prepared an experiment to examine the neural aspects of emotional priming spider stimuli either by fear or disgust eliciting stimuli . We presented various emotional stimuli to 30 arachnophobics (Aph) and 32 healthy controls (HC) inside the magnetic resonance. There were three measuring sessions; first, we presented the block „neutral“, which included the stimulus categories tarantula spiders, beetles, leaves and daddy-long legs spiders. Second, we presented either the block „fear“ (tarantula, beetle, fear-eliciting snakes, lizards), followed by „disgust“ (tarantula, beetle, disgusting animal carcasses, sleeping animals), or vice versa. In the subsequent analysis, we found stronger activation of the higher-level visual processing areas (both the ventral and dorsal stream) when watching the tarantulas, fear-evoking, and disgusting stimuli, in both Aph and HC. When we analyzed the difference between the groups of respondents, we found that the Aph showed a higher activation of the ventral visual stream when watching the tarantulas. We found no substantial difference between the Aph and HC when watching the fear-evoking and disgusting stimuli. Moreover, we analyzed the effect of priming with the fear-eliciting and disgust- eliciting stimuli when watching the tarantula spiders. The poster shows the differences between the Aph and HC respondents and more detailed analyses of each of the priming condition. However, emotional priming either by fear eliciting snakes or disgust eliciting animals caused lower emotional activation when participants watched the spider stimuli in this specific contexts. These results are important for their potential of reduction of perceived fear and disgust and should be used in development of new cognitive behavioral therapy for arachnophobia.