Brain, Behavior and Evolution

The 44th Annual Meeting of the J.B. Johnston Club for Evolutionary Neuroscience and the 36th Annual Karger Workshop in Evolutionary Neuroscience

Loyola University, Chicago, USA, 3–4 October, 2024

Abstracts

Abstract Reviewer
Andrew N. Iwaniuk, University of Lethbridge, Lethbridge, Canada

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Conflict of Interest Statement

The abstracts included in this supplement were reviewed and selected by the JBJC Program Committee (Christopher Heesy, Andrew Iwaniuk, Muhammad Spocter). The committee has no conflicts of interest in connection with the congress and the selection of abstracts.

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The 44th Annual Meeting of the J.B. Johnston Club for Evolutionary Neuroscience and the 36th Annual Karger Workshop in Evolutionary Neuroscience

The 2024 meetings of the J.B. Johnston Club for Evolutionary Neuroscience and Karger Workshop in Evolutionary Neuroscience will be held immediately before the annual meeting of the Society for Neuroscience on Thursday, October 3 (the Karger Workshop), and Friday, October 4 (the regular JBJC meeting). Both meetings will take place at Loyola University, Chicago, IL, USA.

This year's Karger Workshop in Evolutionary Neuroscience, made possible by the continuing support of Karger Publishers, is organized by Daniel Miller and Katherine Bryant. It is entitled "*Mapping neurobiological diversity*". The Workshop will explore recent developments in brain mapping techniques across vertebrate taxa as well as online resources and communities to support the next generation of evolutionary neuroscientists.

On the following day, the program for the annual JBJC meeting will consist of 16 talks submitted by JBJC members selected by the JBJC Program Committee (Andrew Iwaniuk, Christopher Heesy, Muhammad Spocter) plus a presentation by this year's invited Karger Speaker, Dr. Claudio Mello. Additional information and the final schedule of talks will be mailed to JBJC members before the meeting and posted on the JBJC web site (www.jbjclub.org).

2024 Karger Workshop in Evolutionary Neuroscience: Mapping neurobiological diversity

Speakers giving presentations at the 2024 Karger Workshop in Evolutionary Neuroscience are listed below. The final schedule of talks will be sent to the membership prior to the meeting and will be available at the virtual registration desk during the meeting.

- Alison Bell, University of Illinois Urbana-Champaign, Champaign-Urbana, IL, USA
- Anna Kukekova, University of Illinois Urbana-Champaign, Champaign-Urbana, IL, USA
- Erin Hecht, Harvard University, Cambridge, MA, USA
- Katherine Bryant, Aix-Marseille Université, Marseille, France
- Loreta Sutkus, University of Illinois Urbana-Champaign, Champaign-Urbana, IL, USA
- Claudio Mello, Oregon Health and Science University, Portland, OR, USA
- Dan Miller, University of Illinois Urbana-Champaign, Champaign-Urbana, IL, USA
- Archie Fobbs, National Museum of Health and Medicine, Silver Spring, MD, USA

2024 J.B. Johnston Club for Evolutionary Neuroscience Meeting Abstracts

Abstracts for talks scheduled for the 2024 annual meeting of the J.B. Johnston Club for Evolutionary Neuroscience are listed in alphabetical order by presenting author. The final schedule of talks will be sent to the membership prior to the meeting.

A new model for post-TBI neurodegeneration: the G.O.A.T.

Nicole L. Ackermans, Rana M Adam, Ayodele Oyadeyi

Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, USA

There is currently no effective treatment for Alzheimer's disease and its related dementias (ADRD). This gap in part caused by translation difficulties from experimental results in rodent models to human patients. There has been a call to increase model species variety, to better understand the complexities of dementia-triggering events, disease onset, and long-term development. Goats can naturally develop Alzheimer's disease with age. They also headbutt continuously, potentially self-inflicting chronic brain trauma. Thus, we carried out a pilot experiment to explore using goats as a long-term, large animal model for Alzheimer's disease and related dementias. In a six-month pilot experiment on three adult male goats, we used longitudinal studies of positron emission tomography (PET) to measure physiological symptom progression in vivo. In addition, continuously recorded behavior used machine learning to measure cognitive changes post-impact. A Y-maze test was used to evaluate cognitive decline associated memory loss and accelerometers measured impact force and frequency. Levels of ADRD-specific fluid biomarkers in blood plasma, cerebrospinal fluid (CSF), and saliva were collected monthly to complement neuroimaging. Finally, Immunohistochemistry and transcriptomics were applied in key regions to determine pathology severity and differential expression of ADRD-related genes. Our goats sustained approximately 50 head impacts/day. We successfully imaged in the goat brain for the first time with PET-MRI, where [^{18}F]-FDG was used as a marker for glucose metabolism. All goats showed more frontal-dominant FDG activity, and one displayed an additional asymmetrical activation. CSF $A\beta_{42}$ was significantly negatively correlated with accumulated head impacts in the expected trend direction for clinical AD diagnosis. Behavioral measures and remaining fluid biomarker levels were stable over the course of the experiment. This pilot study demonstrated the ability to measure the complex interaction of pathologies contributing to ADRD within a single model. We aim to establish goats as a large animal model for ADRD to improve translational relevance and focus on early treatment strategies.

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Neural crest–derived phenotypes in neurodevelopmental disorders are conserved from zebrafish to humans

Christieann Aprea, Amalia J. Napoli, Kiele Morgan, Sophia Cadolino, Jinah Kwak, Benjamin Martin, Howard I. Sirotkin, Lonnie P. Wollmuth

Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY, USA

The neural crest (NC) is a marvel of vertebrate evolution. It is a collection of highly migratory multipotent stem cells, unique to vertebrates, that gives rise to a vast array of both neural and non-neural progenies including the three branches of the autonomic nervous system (sympathetic, parasympathetic, and enteric), as well as chondrocytes, melanocytes, sensory ganglia, Schwann cells, smooth muscle, and more. Neurodevelopmental disorders (NDD) such as autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), and schizophrenia are commonly referred to as disorders of the brain, though many phenotypes associated with these disorders arise from outside the central nervous system with symptoms including gastrointestinal issues, sensory dysregulation, autonomic nervous system dysfunction and craniofacial abnormalities. Though a seemingly disparate suite of clinical manifestations, many of the phenotypes observed in NDDs develop in NC-derived lineages, suggesting that NC atypical development contributes to NDD pathogenesis. Yet, NC remains a virtually unexplored avenue of NDD disease etiology. To study the involvement of NC in NDDs, we have used zebrafish. The genetic and cellular mechanisms of NC development are highly conserved among all vertebrates, including zebrafish, a model that is highly advantageous in studying early development and NDDs. Here, we have used a mutant zebrafish line lacking all N-Methyl-D-Aspartate receptors (NMDAR). NMDARs are glutamate-gated ion channels that play a fundamental role in synaptic transmission, neuronal development, and nervous system function. NMDARs have a long evolutionary history, appearing in nearly all metazoan life suggesting fundamental organismal roles. Their role in NC development, however, is virtually unexplored. Notably, many missense and nonsense mutations in the genes (*GRIN*) encoding NMDAR subunits are highly associated with neurodevelopmental disorders such as ASD, ADHD and schizophrenia. NC derivatives in zebrafish develop from three distinct subpopulations - cranial, vagal, and trunk - based on their anteroposterior axis of origin prior to migration and differentiation. We have observed that zebrafish lacking NMDARs ($grin1^{-/-}$) exhibit excess pigmentation (cranial and trunk regions), craniofacial abnormalities (cranial region), and abnormal enteric nervous system development (vagal region), all of which are fully NC-derived, as well as abnormal cardiac function (vagal region), to which the NC makes major contributions. Abnormalities in all three subpopulations suggest that dysregulation is occurring at a more fundamental time point in NC stem cells, perhaps prior to specification of distinct lineages. We further analyzed this by performing PCNA (a marker of proliferation) and Sox10 (a NC transcription factor) IHC on $grin^{-/-}$ fish at 24 hours post fertilization and observed hyperproliferation in Sox10 expressing cells. Capitalizing on the power of zebrafish as a model, we have explored the connection between seemingly disparate abnormalities in non-CNS tissues and NDD pathogenesis. Furthermore, we have described a role for NMDARs in the regulation of NC development that is conserved between teleost fish and humans and thus linked NMDAR perturbation to NDD phenotypes associated with tissues outside the CNS.

Evaluation of male and female performance and the role of cerebellar lesions and local aromatase inhibition in postural and cognitive tasks in the zebra finch (*Taeniopygia castanotis*)

Lainy B. Day[a,b]*, Mathew Thibodeaux*[a]

[a]Department of Biology, University of Mississippi, Oxford, MS, USA; [b]Neuroscience Minor, University of Mississippi, Oxford, MS, USA

Most studies involving sex differences in songbirds are devoted to song learning and development. However, songbirds, such as zebra finches, are excellent models for studying general roles of steroids on behavior post-brain injury because they have high degrees of adult neuroplasticity and neurosteroidogenesis. The role of estradiol (E_2) in recovery of function after neural insults in mammals has been well-studied for decades. Only recently has the role of E_2 and aromatase in neuroprotection been examined in songbirds, and our lab is one of the few to study the role of E_2 and aromatase in behavioral recovery and the only to do so in the context of cerebellar lesions. Using novel spatial and postural task developed in our lab, we previously demonstrated cerebellar (CB) lesions plus aromatase inhibition slow learning of a spatial task while adding exogenous E_2 ameliorates this deficit in male zebra finches. However, postural deficits post-CB lesion are not increased by aromatase inhibition, nor improved by exogenous E_2. Only males have been studied in both tasks. Other labs have shown that female zebra finches upregulate local aromatase more than males at the lesion site post-CB lesion. It has been suggested that this sex difference in local upregulation by females is meant to compensate for a greater degree of systemic testosterone substrate for aromatization in males than females. If true, local inhibition of aromatase post-CB lesion could alter behavioral outcomes more for females than males without sex differences in birds with CB lesion only. The current set of experiments examines sex differences in spatial cognition and postural control post-CB lesion with or without aromatase inhibition. Given a lack of ecological relevant sex differences in male and female zebra finches related to postural tasks or spatial mapping, we did not expect sex differences in sham-lesioned birds. Our results did not suggest aromatase inhibition impacts males more than females, but did suggest sex-specific influences of CB lesions on spatial and postural tasks and support our previous results showing that aromatase inhibition alters outcomes in cognitive performance post-CB lesion, but does not increase a CB-dependent postural deficit in zebra finches. Our study suggests unexpected sex differences in spatial strategies and the impact of aromatase post-CB lesion on sexes in spatial and postural tasks and a novel role of the CB in the use of use of vicarious-trail-and-error to acquire spatial information.

Exploring companion animals as models of Alzheimer's disease and aging in an evolutionary context

Alexandra de Sousa[a], *Brier Rigby Dames*[b,c], *Eli Morrow*[a], *Christine Charvet*[a]

[a]Department of Anatomy, Physiology and Pharmacology, Auburn University College of Veterinary Medicine, Auburn, AL, USA; [b]Department of Computer Science, University of Bath, Bath, UK; [c]Department of Psychology, University of Bath, Bath, UK

As companion animals, cats and dogs mirror humans in aspects of aging and could be valuable models for studying brain health conditions. Cats and dogs exhibit naturally occurring neuropathological signatures of Alzheimer's disease including evidence of plaques and tangles, as well as other aspects of brain atrophy and cognitive decline with age. In contrast, naturally occurring Aβ plaques and Tau tangles have been challenging to detect in rodents, suggesting they may be absent. Multiple factors could influence the similarities between humans and companion animals. These include shared factors such as a domesticated environment and a longer lifespan, which could potentially affect any mammalian species under these conditions. Cats and dogs live significantly longer in captivity, with known maximum lifespans of up to 37 years for cats and 27 years for dogs. Insights from evolution and development could better relate aging neuropathology in companion animals to humans.

Given that the potential for brain atrophy is widespread in mammals, do companion animals offer particularly interesting models? Rodents and non-human primates (NHPs) might not live long enough for some age-related diseases to manifest. To compare aging across species, we have been using a growing dataset used in Translating Time (translatingtime.org) that currently covers 1386 time points (n>11,000 observations) in 1117 species. A study investigating a subset of 494 time points (n=1,167 observations) in humans, rats, and mice found that mice at age 2 years correspond to humans at age 70, which is when risk for Alzheimer's disease increases substantially. Yet mice rarely live beyond 18 months, so they may not clearly show plaques, tangles, and brain atrophy because their lifespans do not extend into the stage when the pathologies would appear. Even our closest living relatives, the chimpanzees, might be limited as models of aging because they rarely live beyond their 40s, which, after time point calibration, equates to humans in their 50s. Like humans, companion animals have relatively extended lifespans when compared to closely related species. Companion animals reach later occurring time points during which they exhibit brain pathologies, atrophy, and cognitive dysfunction. To discover potentially unique features of aging in companion animals, we are comparing domesticated cats to other felid species, housed primarily in zoos. Preliminary results suggest differences in the timing and duration of events in the development and aging of domesticated versus big cats. We will discuss how the Translating Time dataset presents an opportunity to evaluate factors related to the appearance of AD-related pathologies in companion animals.

Detailing the occurrence and distribution of Aβ plaques and tau pathology provides context for examining the evolution of these features in cats and dogs. The classical condensed core plaques, such as those observed in Alzheimer's disease, are rare in mammals, but their presence has been documented in some mammalian species. For example, they have been observed in dogs and their caniform relatives (e.g., polar bears, sea lions), while only diffuse Aβ plaques have been reported in cats and other feliform species. Tau pathological features, such as hyperphosphorylated tau (pTau) and neurofibrillary tangles (NFTs), have been found in older cats and dogs as well as other mammals. There are also species of primates, cetaceans, and carnivores that show evidence of hyperphosphorylated tau, and many show non-fibrillary tangles. Differences in Alzheimer's neuropathological signatures across mammalian clades may be linked to genetic variation related to Alzheimer's pathology in humans. For example, species differ in the prevalence of the APOE4 isoform associated with Alzheimer's risk, and this could relate to differences in Alzheimer's pathology reported in mammals. By refining our variables and analysis, we aim to gain insights into estimating the ancestral states of these features and their timing during the lifespan and stimulate discussion about their evolution.

Thirsty for company: decoding social choices in wild mice

Ebru Demir, Ekin Ture, Duda Kvitsiani

Department of Biomedical Sciences, Southern Illinois University, Carbondale, IL, USA

Few decisions are as crucial as selecting the right time and partner for mating while meeting metabolic needs. These choices are essential for individual fitness and the evolution of diversity. Despite their importance, our understanding of the cognitive processes behind these decisions is limited. By exploring the mental frameworks and neural processes that guide these choices, we can reveal the complex systems underpinning social cognition, perception, and valuation, thereby enhancing our understanding of how organisms adapt and evolve.

In this presentation, we introduce a behavioral framework for a comprehensive analysis of social behaviors in mice within their Umwelt. This innovative approach elucidates the fundamental neurobiological principles of social cognition, valuation, and subjective experiences.

Our behavioral task, inspired by psychophysics, game theory and field ecology, evaluates social preferences when choosing between social and non-social rewards. In this setup, a thirsty mouse navigates between a water dispenser and a rotating carousel with several chambers. Each chamber may contain a mouse of the same strain, a different strain, a mouse lacking gender-specific pheromones, or be empty. As the carousel rotates and pauses, it aligns one chamber directly in the mouse's path, facilitating interaction with a potential social partner. The mouse must weigh the time spent engaging with the social cue against its need to drink water, balancing its social interests with physiological needs. This arrangement allows us to gauge the significance mice assign to social interactions relative to a basic need, termed 'water currency,' by observing how they prioritize social engagement over water access. This method enables us to quantify the subjective experiences of individual mice.

Furthermore, our 'Reward Trade-off Task' offers a quantifiable, standardized method to assess social value, adjustable by altering the water quantity. Moving forward, our plans include probing the neurobiological bases of how social values are formed, as well as exploring how phenotypic traits influence individual mice's social preferences, through the integration of neurophysiological data, optogenetic techniques and theoretical modelling.

Astrocyte diversity across mammals: a comparative analysis on distribution and single cell morphology

Caterina Ciani[a], *Giulio Pistorio*[a], *Marika Mearelli*[a], *Simona Giancola*[a], *Luca Mio*[a], *Laura Pinfildi*[b], *Simone Cauzzo*[c], *Ester Bruno*[c], *Fabio Anzà*[d], *Julio Hechavarria*[e], *Jean-Marie Graic*[f], *Chiara Magliaro*[c], *Carmen Falcone*[a]

[a]Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy; [b]Department of Woman, Child and General and Specialist surgery, University of Campania "Luigi Vanvitelli" Naples, Italy; [c]Centro di ricerca "E. Piaggio", University of Pisa, Pisa, Italy; [d]University of Trieste, Trieste, Italy; [e]Institute for Cell Biology and Neuroscience, Goethe University, Frankfurt am Main, Germany; [f]Comparative Biomedicine and Food Science, University of Padua, Legnaro, Italy

Over the recent years, there has been an increasing need for a better understanding of the identity and the roles of astrocytes and their involvement in the cognitive abilities. Indeed, they play a crucial role in brain functions, they show primate-specific features, and they are relevant in several diseases. Investigations on astrocytes across evolution have rarely been performed. However, a comprehensive comparison of astrocytes in a diverse range of mammals is pivotal for understanding the morphology across species and the related modifications in gene expression and functions. We have recently described primate-specific features of two subtypes of astrocytes, such as the Interlaminar astrocytes (ILAs) and the Varicose Projection astrocytes (VP-As), which can be of particular interest in the context of astrocyte evolution. Our project will expand from previous data and aims to investigate astrocyte diversity across mammals, by characterizing the distribution and the single-cell morphology of different subpopulations of astrocytes within different cortical layers across mammals. In details, we analyzed samples from prefrontal cortex of: Primates (chimpanzee, human), Carnivora (tiger), Artiodactila (cattle), Rodentia (mouse) and Chiroptera (Seba's short-tailed bat). We immuno-stained these samples with various astrocyte markers (*i.e.*, GFAP, ALDH1L1, S100β) in order to compare: (1) the distribution of different astrocyte subpopulations across species and layers of the cerebral cortex, and (2) the single-cell astrocyte morphology reconstructed with an algorithm-driven segmentation and Image- J plugin Neurotracer analyzer, and (3) the single-cell transcriptome of astrocytes by performing 10X Visium spatial transcriptomics analysis. Our previous results showed an increase of ILA morphological complexity and density in primates. With this project, we will unlock unprecedented details of the distribution, the single-cell morphological complexity and single-cell transcriptome of different astrocyte's subtypes across different layers and different mammals, with a special focus on primates and, in particular, humans. Data obtained from this research have the potential to lead to new fascinating hypotheses on the role of astrocytes in primate neuroanatomical, behavioural and cognitive complexity.

Topographic organization of the retino-tecto-diencephalo-telencephalic pathway in the Japanese eel: a feature common with mammals

Hanako Hagio[a,b], *Kana Watanabe*[a], *Naoyuki Yamamoto*[a]

[a]Laboratory of Fish Biology, Department of Animal Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8601, Japan; [b]Institute for Advanced Research, Nagoya University, Nagoya, 464-8601, Japan

Two ascending visual pathways to the telencephalon (pallium/cerebral cortex) are present in most vertebrates. In mammals, one of the two visual pathways is called the geniculate system that sends retinal inputs to the primary visual cortex via the lateral geniculate nucleus in the diencephalon. The other pathway is called the extrageniculate system, in which retinal information reaches the higher order visual cortex through the superior colliculus in the mesencephalon and then the lateral posterior nucleus/pulvinar complex in the diencephalon. Similarly, birds, reptiles, amphibians and cartilaginous fishes possess two pathways. Among actinopterygians (ray-finned fishes), two visual pathways have been observed in sturgeon, goldfish, and carp, while in squirrelfish and yellowfin goby, a geniculate-like pathway appears to be missing and only an extrageniculate-like pathway has been found. We revealed a topographic organization of the retino-tecto-diencephalic visual relay nuclei pathway in yellowfin goby, as in mammals. However, the topographic organization is unclear in other fishes. It also remains to be determined if the diencephalic visual relay nucleus projects to the telencephalon in a topographic manner.

We identified an extrageniculate-like pathway and a remnant of geniculate-like pathway of Japanese eel, as reported in JBJC annual meeting last year. Afterward, we found that both pathways are topographically organized. In the latter pathway, the diencephalic visual nucleus includes two regions. One of the regions receives visual information only from the dorsal visual field and the other from both dorsal and ventral visual fields. Our results suggest that spatial localization of objects in the visual field is important for eels, as in mammals. Especially, information in the dorsal visual field is probably of critical importance for eels to survive, because two regions of the diencephalic visual nucleus are involved in the visual processing of that field in the remnant of geniculate-like pathway.

The 20% solution for brain energetics

Christopher P. Heesy

Department of Anatomy, Colleges of Graduate Studies & Veterinary Medicine, Midwestern University, Glendale, AZ, USA

The brain is commonly believed to require 20% of an individual's daily energy budget; this figure has been cited as an energetic constraint in multiple hypotheses for relative brain size evolution not just in primates, but also multiple vertebrate taxa. This figure is based on estimates of the oxidative component of the resting energy expenditure of a 1350g brain in a 65kg human male. Intraspecific variation in energetic requirements of other high metabolic rate organs, such as the heart, kidney, and liver as well as the interspecific variation in these organs in primates or other taxa remains unaccounted for. I address this gap using comparative data on body, brain, heart, kidney, and liver masses collected from the same individuals as well as mass- and organ-specific metabolic rates to allometrically model the proportional and total organ-specific resting energy requirements in non-human primates and other mammals. Most mammal brains, including primates, require modest proportions of scaled resting energy expenditures, especially compared to the liver, which can be twice as 'expensive' as the brain. Comparisons with published data on daily total energy expenditure in primates demonstrates that brains require a small fraction of the total energy budget regardless of daily activity, while the energy required by other organs can vary widely depending on daily activity. For example, on a day the liver processes excess calories, the liver will require more energy, while the brain will require the roughly identical amount of energy as any other day.

Identifying the distribution of peptide hormones regulating interrenal and gonadal steroids in the brains of diploid and polyploid treefrogs

Jordan Jackson, Christopher J Leary, Lainy B Day

Department of Biology, University of Mississippi, Oxford, MS, USA

Polyploidization can drive phenotypic change by altering gene expression patterns and/or by increasing gene mutation rates. How these effects potentially alter the endocrine physiology of extant polyploids is understudied. We are investigating the regulation of interrenal glucocorticoids and gonadal steroids in the gray tree frog complex, which consists of three tetraploid lineages collectively referred to as *Hyla versicolor* and its diploid ancestor, *H. chrysoscelis*. We hypothesize that polyploidization alters the expression levels of hormone receptors, which impacts the regulation of circulating hormone levels and hormone interactions, e.g., high glucocorticoids typically inhibit gonadal steroid production. Alternatively, polyploidization may promote mutations of duplicate genes coding for receptors. To examine these alternatives, RNA-seq will be used to compare expression levels and transcript sequences of receptors in brain regions regulating the hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal/interrenal (HPA/I) axes. The HPG axis is regulated by hypothalamic kisspeptin (KISS) that stimulate gonadotropin-releasing hormone (GnRH), which stimulates the pituitary to release luteinizing hormone (LH) and the production of gonadal steroids. The HPA/I axis is regulated by hypothalamic corticotropin releasing hormone (CRH), which stimulates pituitary release of adrenocorticotropic hormone (ACTH) to stimulate production of glucocorticoids. Negative feedback of the HPA/I axis involves glucocorticoids binding to glucocorticoid and mineralocorticoid receptors in the medial pallium, the hippocampal homolog. We are using immunohistochemistry to identify hypothalamic and pituitary peptide hormones to guide excision for RNA-sequencing and to characterize CNS distributions of these peptides. We used primary antibodies with avidin-biotin-complexing visualized by Novared or fluorescent secondaries. We performed immunohistochemistry for CRH, GnRH, and KISS (n=3/ species). CRH immunoreactive cells (CRH-ir) were located in preoptic and paraventricular nuclei as in other vertebrates. As in previous studies with *Xenopus lavius* and two *Rana* species we found CRH-ir in the bed nucleus of the stria terminalis, dorsal pallium, caudal nidopallium, preoptic area, median eminence, habenula, and parts of the ventral hypothalamus. Unlike previous reports for *Xenopus* and *Rana*, we found CRH-positive cells in the medial amygdala, dorsal hypothalamic nucleus, and epiphysis. Compared to *Xenopus* and *Rana*, we had minimal immunoreactivity in medial pallium, lateral pallium, caudal ventral hypothalamus, and striatum, and only sporadic CRH-ir in cerebellar Purkinje cells. Unlike *Xenopus*, but similar to Rana we found CRH-positive cells in the anterior pituitary, portal region, and optic chiasm. Similarly, we found that GnRH and KISS immunoreactivity did not overlap completely with distributions seen in other amphibians, a detailed analyses of these differences are ongoing. The basic pattern of CRH, GnRH, and KISS immunoreactivity is similar between *H. chrysoscelis* and *H. versicolor*. Diverse expression patterns within amphibians and across taxa suggest functional diversity in neuroendocrine networks.

A comparative neuroanatomic digitization project to provide worldwide access to the educational and research resources at the National Museum of Health and Medicine

John A. Morris[a], Daniel J. Miller[b], Chet C. Sherwood[c], Muhammad A. Spocter[d,e]

[a]West Newbury, MA, USA; [b]School of Integrative Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA; [c]Department of Anthropology and Center for the Advanced Study of Human Paleobiology, The George Washington University, Washington, DC, USA; [d]Department of Anatomy, Des Moines University, Des Moines, IA, USA; [e]Department of Biomedical Sciences, Iowa State University College of Veterinary Medicine, Ames, IA, USA

Studying the nervous system in a comparative context enables us to explore fundamental mechanisms governing the function of all nervous systems. Additionally, it offers valuable insights into the evolution of less commonly studied species. The National Museum of Health and Medicine houses an extensive collection of

materials for brain research, making it one of the world's most comprehensive neuroanatomical collections. This carefully curated collection has fueled numerous significant discoveries over decades. However, its accessibility is currently restricted to in-person research visitors.

Over 20 different repositories make up the Neuroanatomical Collections Division, including the immense Yakovlev-Haleem collection, consisting of ~1200 human brain specimens, ranging in age from preterm to 100+ years, with alternating Nissl and myelin whole-brain serial sections representing normal and a wide variety of neuropathology cases. In addition to the human series, similarly prepared comparative specimens (the Welker and Johnson collections) across 175+ species and 30 orders (such as primates, chiropterans, monotremes, carnivores, cetaceans) provide context of evolutionary changes in the cellular structure of neural systems.

A project is currently underway with the ultimate goal to increase access to these collections by acquiring cellular-level resolution scans (10X magnification) and creating an online database to access and analyze these images.

This project would advance access to the NMHM brain collections from dozens of in-person visits to thousands of virtual visits, by posting high-quality scans on the internet and providing tools to measure and compare the specimens, thereby facilitating teaching and research efforts, and generating training data for automated computer analytic methods. Such interactive resources would enliven and streamline the training of medical students and researchers at all levels. Normal cases and rare pathologies across ages, as well as comparative animal examples, provide the raw materials needed to understand brain development, allowing insight into the neural architecture of underlying cell-type specialization and vulnerabilities in disease states. Professional training programs and teaching endeavors become possible year-round, supporting an array of interests such as digital pathology or Brain Awareness Week. The NMHM will continue to provide higher resolution scans on demand, but worldwide access using modest internet bandwidth enables anyone to take part in discovering shared similarities and differences across species, including human, bat, platypus, tiger, and dolphin brains among others, extending the utility of this resource to future generations.

Neural basis of auditory communication and social behavior in bats

Angeles Salles

Department of Biological Sciences, University of Illinois Chicago, Chicago, IL, USA

Bats are auditory specialists, processing acoustic signals to guide their behaviors, including prey tracking, navigation, and communication. Most bat species are very social and emit a wide array of communication calls, including food-claiming, aggressive, and appeasement calls. There is strong evidence that context plays a role in the processing of acoustic signals in humans and other animals. Yet, this process's circuits and mechanisms are still not fully understood. Bats emerge as outstanding mammalian models to explore the neural mechanisms underlying acoustic communication processing. In the lab, we work with two phylogenetically distant species of bats: *Carollia perspicillata* and *Rousettus aegyptiacus*. Though these are both frugivorous bats, these species have many differences. First, they have different echolocation mechanisms; *Carollia* bats are laryngeal echolocators (i.e. use their larynx to produce ultrasonic sonar signals), while *Rousettus* bats are lingual echolocators (i.e. use tongue clicks to produce ultrasonic sonar signals). Both species use their larynx to produce their communication signals, but their repertoire differs greatly in structure and spectral patterns. Furthermore, they have different social structures; while *Carollia* is a harem-forming bat with high-roost fidelity, Rousettus is promiscuous, and mating can occur with different males in a season. We are leveraging these differences to contrast and compare the underlying neural circuits for social communication and behavior across taxa. Here, I will provide an overview of our current work and our preliminary findings on the social behavior of these different bats and on the neural circuits used to process communication signals.

Cellular evolution of insect motor circuits: Muscles are variable and flexibly innervated by homologous motor neuron somata

Annika Sharma, Elizabeth S. Heckscher

Department of Biological Sciences, University of Chicago, Chicago, IL, USA

Motor circuits comprise muscles, motor neurons, and premotor networks. Changes to these components' number, location, connectivity, and activity produce a vast diversity of motor behaviors across animals. Yet, our understanding of which features of motor circuits are fundamental (or, evolutionarily constrained) is limited by: (i) in-depth knowledge in only a few distantly related model organisms, (ii) muscles and neurons being soft tissues that do not fossilize, (iii) technical challenges in tracing connections between the periphery and central nervous system (CNS), (iv) redundancy in motor circuits making ablation/inactivation studies infeasible, (v) intractability of single-cell resolution, and (vi) ambiguity in identifying homologous cells. We leverage the simple motor systems and vast evolutionary diversity of larval insects to overcome these challenges and investigate how motor circuits evolve at the cellular level. The unparalleled knowledge of motor circuits in larval *Drosophila melanogaster* of the Order Diptera (two-winged flies) serves as our reference for comparing several species of the same Order (~250MY). Adapting techniques developed in *D. melanogaster*, we examine muscle anatomy, motor neuron somata identities, and motor neuron innervation at the neuromuscular junction. We identify homologous cells based on anatomy: for muscles by relative position, orientation, and layering, and for motor neurons by relative position and the expression of marker transcription factors. We find two overarching patterns upon inferring the ancestral states and frequency of gains and losses of individual muscles: (i) muscles close to the body surface are more variable than internal muscles, and (ii) muscles innervated by a single motor neuron are more variable than those innervated by multiple motor neurons. Functional data from our lab indicates that the propulsive forces for locomotion are provided by the conserved muscles, suggesting that their conservation is

essential for locomotion and, therefore, survival. We are now investigating whether the variable muscles are responsible for species-specific motor adaptations in different ecological environments. The interspecies variability in musculature raises a fundamental question about how the nervous system adapts to altered muscles. We explore whether these changes are incorporated into motor circuits by producing new/different motor neurons or altered connectivity of existing motor neuron axons. In the species examined so far, the numbers and identities of motor neuron somata are conserved. However, axonal connectivity to muscles sometimes differs, even of motor neurons whose homologous muscle partners are present across species. Broadly, this shows that the constraint is in neuron number while flexibility is in connectivity. Beyond insights into the constraints on motor circuits, our technical approaches represent a novel way to analyze complex cellular data that is applicable to other tissues and organisms.

Associating gene evolution to brain evolution identifies biomedical targets

Jeroen B. Smaers[a], *Markus Riessland*[b,c], *Priya Aggarwal*[d], *Colista West*[b,c], *Joshua S. Rest*[d,e]

[a]Department of Anthropology, Stony Brook University, Stony Brook, NY, USA; [b]Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, NY, USA; [c]Center for Nervous System Disorders, Stony Brook University, Stony Brook, NY, USA; [d]Department of Ecology and Evolution, Stony Brook University, Stony Brook, NY, USA; [e]Laufer Center for Physical and Quantitative Biology, Stony Brook University, Stony Brook, NY, USA

Investigating developmental- and population scale genotype-to-phenotype associations is an essential tool for detecting genetic variants associated with human health and disease. Phylogenetic scale variation, accounting for macroevolutionary variation across a large and diverse set of species, has been underutilized for these purposes. A primary challenge is that fundamental questions on the molecular underpinnings of patterns of phenotypic evolution, such as convergence and evolutionary allometric shifts, have remained understudied.

Here, we reconstruct a detailed macroevolutionary history of gene-level traits (protein structure, gene structure and regulatory architecture) extracted from whole-genome information for 36 mammalian species and used this information to identify 'hit' genes based on their evolutionary association with a brain phenotype (lateral cerebellum, 'LC') that underpins cognition and is associated with aging and neurodegeneration. Results show significant and specific enrichment of hit genes in pathways fundamental to human neurodevelopment and neurodegeneration, primarily due to hit genes that show evolutionary changes in lineages that also demonstrate phenotypic evolutionary allometric shifts. Species that convergently expanded LC size (humans, dolphins, pinnipeds and elephants) demonstrate analogous pathway modifications. Among these hit genes, we identified two potential schizophrenia target genes and experimentally validated new insights into their disease-relevant role. These findings demonstrate direct links between molecular and phenotypic brain evolution and highlight their implications for biomedicine.

Exploring woodpecker brain physiology: new insights and conflicting observations

Daniel J Tobiansky[a,b]

[a]Department of Biology, St Mary's College of Maryland, MD, USA; [b]Program in Neuroscience, St Mary's College of Maryland, MD, USA

Woodpeckers routinely withstand high-velocity head impacts exceeding 400g during pecking, far surpassing human concussive thresholds. In contrast to previous work (Farah *et al.*, 2018), our findings reveal minimal evidence of tau pathology (typically present in repeated brain trauma) in woodpecker brains as assessed by Gallyas-Braak silver staining. Moreover, there is no significant difference in baseline neural degeneration in woodpeckers versus songbirds (see below) as assessed by Fluoro-Jade C staining. This unexpected resilience led us to investigate potential protective mechanisms. We discovered that woodpeckers possess an enlarged choroid plexus (up to 130× larger) compared to non-woodpecker Piciforme species (e.g., Toucans), suggesting altered cerebrospinal fluid dynamics or function. Additionally, transcriptomic analysis of woodpecker meninges and associated vasculature shows significant upregulation of genes involved in protein turnover (e.g., ubiquitin-mediated degradation pathways) and unique immunological processes (e.g., interleukin-17 signaling). These mechanisms may contribute to the woodpeckers' remarkable ability to withstand repeated impacts. Our ongoing research employs a comparative approach across multiple woodpecker species [Downy Woodpeckers (*Dryobates pubescens*), Red-bellied Woodpecker (*Melanerpes carolinus*), and Yellow-shafted Flicker (*Colaptes auratus*)] and ecologically- or size-matched songbird species [European Starlings (*Sturnus vulgaris*), Tufted Titmice (*Baeolophus bicolor*), White-breasted Nuthatches (*Sitta carolinensis*)]. We combine behavioral assessments, histological analyses, molecular techniques, and transcriptomics to further examine neuroinflammatory responses and neuroprotective physiological processes. By unraveling these mechanisms, we seek to provide new insights into impact resilience in these remarkable vertebrates.

Why does the left side of the brain control the right side of the body?

Martin Wessendorf, Daniel Wang

Department of Neuroscience, University of Minnesota, Minneapolis, MN, USA

In humans, the right side of the cerebral cortex controls the lower motoneurons innervating the left hand and left leg. Similarly, the right side of the cerebral cortex receives somatosensory information from the left side of the body; moreover, our right visual cortex encodes the left visual field. So how did we arrive at this seemingly odd state of affairs? A number of authors, beginning at least with Cajal, have speculated about the adaptive value of our contralaterally organized sensory and motor systems. However, no one appears to have examined the evolutionary history of these traits in detail. We have attempted to do so and conclude the following:

(1) The last common ancestor of the vertebrates had two camera-type eyes, the lenses of which rotated each retinal image 180 degrees (i.e. each image was left-right reversed and inverted). It also had an optic chiasm, which made movement inferred from comparing the two eyes to be in the same direction as movement projected on each retina.

(2) The last common ancestor of the vertebrates also had an optic tectum in which contralateral visual space was represented retinotopically. In addition, it had contralateral representations of other spatially organized senses (e.g., vibration and somatosensation) in registration with it, as well as a representation of contralateral motor space that was also in registration with the representation of visual space.

(3) In addition to the above, the last common ancestor of the tetrapods appears to have had a contralaterally organized rubrospinal tract (i.e. its cell somata in the brainstem lay contralateral to its axons in the spinal cord) and a contralaterally organized somatosensory system (i.e., its axon terminals lay contralateral to the stimuli that caused it to fire.)

(4) The last common ancestor of mammals appears to have had a contralaterally organized somatosensory system, a contralaterally organized visual system, a contralaterally organized optic tectum/superior colliculus that integrated visual, auditory, and somatosensory input with motor responses, and a contralaterally organized rubrospinal tract. We propose that our contralaterally organized mammalian cerebral cortex evolved in the context of a brain that was already extensively contralaterally organized.

The co-evolutionary history of striatum and neocortex size in mammals

Wiggenhauser, Nicolás G.[1] *and Smaers, Jeroen B.*[1, 2]

[1]Interdepartmental Doctoral Program in Anthropological Sciences, Stony Brook University, Stony Brook, NY, USA; [2]Department of Anthropology, Stony Brook University, Stony Brook, NY, USA

The cortico-striatal system (CSS) has evolved as an important neural network that enables reinforcement learning, optimal behavior regulation, decision-making, and habit formation, all essential cognitive adaptations for animals to survive in their ecological niches. Despite the fundamental role of the CSS in the emergence of behavioral complexity in mammals, no studies have explored its detailed macroevolutionary history across mammals. To investigate the evolution of the CSS, we analyzed which patterns of co-evolution between striatum and neocortex size explain the extant mammalian diversity (N = 547 species). Our objective is to characterize the pattern and timing of the evolutionary allometric relationship between striatum and neocortex size in mammals.

We have identified several grade shifts in the evolutionary allometry of the striatum relative to the neocortex in diverse clades. Multiple shifts on an increased reliance on the striatum were identified, such as in afrotherians, xenarthrans, eulipotyphlans, and to a lesser extent in select bat clades (Rhinolophidae, Pteropodinae, and Vespertilionidae). These shifts may indicate a selection towards more reinforcement learning (in which actions are selected based on the reward or punishment produced) to maximize the value of future action choices. Furthermore, other shifts exhibit a decreased reliance on the striatum, as evidenced in ferungulates and anthropoid primates, with an even stronger signal in pinnipeds, mustelids, canids, and the human genus. These patterns might relate to a stronger reliance on the neocortex to mediate unsupervised learning (geared towards the generation of statistical regularities of the perceived environment). This study provides a more comprehensive understanding of the changes in brain evolution that explain major behavioral adaptations and significant lifestyle changes along the mammalian phylogeny.

How can we unify brain nomenclature across vertebrates?

Kei Yamamoto

Paris-Saclay Institute of Neuroscience (NeuroPSI), Université Paris-Saclay / CNRS, 151 route de la Rotonde, 91400 Saclay, France

Nomenclature is a crucial aspect of anatomical studies, as we first need to name structures without ambiguities in order to efficiently work with and communicate about them. Anatomical atlases have long been major tools for propagating and validating the use of a unified nomenclature within a community of researchers working on a given species, and complete brain atlases are available for commonly used model species (e.g. mouse, pigeon, zebrafish).

Once a community of researchers has adopted a certain nomenclature, however, they - often dedicated to a single model species or even to a specific region of its brain - might be hesitant to adopt a revised nomenclature that takes into account updates concerning homology across different taxonomical lineages. As a result, using the same term for non-homologous structures across different species continues to cause errors when comparing species in neurosciences.

The most successful nomenclature modification was probably the one proposed by the "Avian Brain Nomenclature Forum", in which avian neurobiologists collectively deliberated on nomenclature modifications (Reiner et al. 2004 J Comp Neurol; Jarvis et al. 2005 Nat Rev Neurosci). The main motivation was to correct terms with the suffix "-striatum" for structures initially believed to be subpallial, but later found to be pallial. In this forum, some avian nomenclature was also unified with mammalian nomenclature to facilitate the communication with the mammalian community and to better reflect homology.

Conversely, as the mammalian nomenclature is the most popular, what happens when non-mammalian studies reveal misidentifications in the mammalian nomenclature itself? I envision three options: 1) Modify the nomenclature of mammals, 2) Accommodate the non-mammalian nomenclature to the accepted mammalian nomenclature, or 3) Create a third nomenclature that fits both taxa.

I will discuss this issue using the example of the discrepancy in the anatomical location of Otp-dependent neuroendocrine cell populations: the hypothalamus in mammals and the preoptic area in teleosts, which have been proposed to be renamed the optic recess region (ORR) as they are part of the eye field (Yamamoto et al. 2017 Dev Growth Diff). It has been almost a decade since this

misidentification of the mammalian "hypothalamus" was revealed, nonetheless, the new idea does not gain traction. This is at least partially due to the difficulty of modifying the well-accepted mammalian nomenclature.

I would like to provoke a discussion with this question: in order to use terminology that better reflects ontogeny throughout vertebrates, to what extent can we free ourselves from ancient nomenclature and a mammal-centric view?

"Spinal enlargements" in zebrafish

Naoyuki Yamamoto[a], *Ryo Takaokaa, Hanako Hagio*[a,b]

[a]Laboratory of Fish Biology, Department of Animal Sciences, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya, 464-8601, Japan; [b]Institute for Advanced Research, Nagoya University, Nagoya, 464-8601, Japan

In tetrapods there are cervical and lumbar enlargements in the spinal cord, which are readily appreciated with the naked eyes. These enlarged spinal levels innervate anterior and posterior limbs, respectively, with higher somatosensory acuity and finer muscles relative to the trunk. Spinal enlargements are not recognized in teleosts as observed macroscopically. However, the anterior and posterior limbs are homologous to the pectoral and pectoral fins, respectively, and spinal enlargements may perhaps be present in teleosts, which can be appreciated only by more detailed analyses. We therefore set out for histological analyses of zebrafish spinal cord to address this issue.

We prepared series of transverse sections and measured the areas of spinal cord and spinal grey matter. These analyses revealed increased areas of the spinal cord and grey matter at levels innervating fins.

The present study indicates that spinal enlargements are also present in teleosts, although they are detected only at microscopic level. Spinal enlargements may be more subtle in zebrafish, since sensory organs and muscular systems of fins are less elaborated than those of limbs in tetrapods. Another point that should be stressed is that spinal enlargements are not specific for paired fins/limbs, since enlargements occur at spinal levels innervating unpaired fins.