Abstracts

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The 30th Annual Meeting of the J.B. Johnston Club and the 22nd Annual Karger Workshop

The 2010 meeting of the J.B. Johnston Club and Karger Workshop will be held immediately before the annual meeting of the Society for Neuroscience on Thursday, November 11 (the Karger Workshop), and Friday, November 12 (the regular JBJC meeting), 2010. Both the Karger Workshop and the regular JBJC meeting will take place at the Horton Grand Hotel in San Diego, Calif., USA.

This year's Karger Workshop, made possible by the continuing support of Karger Press, was organized by Jon H. Kaas and is entitled 'Diversity in Cortical Organization'. The Karger Workshop will cover some of the recent advances in understandings of how cortex varies in structure and organization in mammals. On the following day, the program for the annual JBJC meeting will consist of fifteen talks submitted by JBJC members selected by the JBJC Program Committee (Ann Butler, Sabrina Burmeister, and Ken Catania) plus a presentation by this year's invited Karger Speaker, Dr. Suzana Herculano-Houzel. Additional information and the final schedule of talks will be mailed to JBJC members before the meeting, and will be posted on the JBJC web site (www.jbjclub.org).

2010 Karger Workshop: Diversity in Cortical Organization

Organized by: Jon H. Kaas (Vanderbilt University, Nashville, Tenn., USA)

Speakers giving presentations at the 2010 Karger Workshop are listed below. The final schedule of talks will be sent to the membership prior to the meeting and will be available at the registration desk during the meeting.

How Cortical Areas Vary in Number and Architecture

Jon H. Kaas, Vanderbilt University, Nashville, Tenn. (USA)

Building a Bigger Brain: New Views on Brain Scaling in Evolution

Suzana Herculano-Houzel, Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro (Brazil)

Variability in Neuron Densities across the Cortical Sheet in Primates

Christine Collins, Vanderbilt University, Nashville, Tenn. (USA)

Rats, All Rodents Are Not the Same

Leah Krubitzer, University of California at Davis, Davis, Calif. (USA)

Evolution of Cerebral Cortical Development

Zoltán Molnár, University of Oxford, Oxford (UK)

New Alternative Sources of Cortical Neurons and Related Structural Variation

Luis Puelles, University of Murcia & CIBER in Rare Diseases, Murcia (Spain)

2010 J.B. Johnston Club Meeting Abstracts

Abstracts for talks scheduled for the 2010 annual meeting of the J.B. Johnston Club are listed in alphabetical order by presenting author. The final schedule of talks will be sent to the membership prior to the meeting and will be available at the registration desk during the meeting. This year's Karger Invited Speaker will be Dr. Suzana Herculano-Houzel. The title of her talk will be 'Evidence against a Cortical Takeover in Mammalian Brain Evolution: Coordinated Scaling of Cerebellar and Cortical Numbers of Neurons'.

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Signal Diversification Drives the Evolution of Novel Patterns of Brain Organization

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Social communication behavior is hypothesized to be a key determinant in the evolution of larger and more complex brains. Comparative studies have revealed correlations between variation in communication behavior and the size of specific brain regions. However, direct evidence that the diversification of communication signals can drive the evolution of novel patterns of brain organization is lacking. Here, we show that two subfamilies of mormyrid electric fish exhibit a dramatic difference in the structure of a sensory pathway devoted to communication behavior. In the subfamily Mormyrinae, the midbrain exterolateral nucleus (EL) is subdivided into anatomically distinct anterior and posterior regions that serve different functions in processing electric communication signals. In the subfamily Petrocephalinae, the EL is approximately four times smaller and lacks distinct anterior and posterior regions. This anatomical difference is linked to the functional organization of peripheral sensory receptors: in the Mormyrinae, receptors are distributed throughout the head as well as on the dorsal and ventral portions of the trunk; in the Petrocephalinae, receptors are limited to three clusters on the head termed rosettes. The most basal genus of the Mormyrinae, *Myomyrus*, exhibits an intermediate pattern of receptor organization, with a single head rosette and a relatively low density of receptors throughout the head and dorsal/ventral portions of the trunk. However, the EL of *Myomyrus* spp. is relatively small and undifferentiated, similar to the petrocephaline EL. The most likely reconstruction from these data is that the petrocephaline receptor rosettes and small, undifferentiated EL represent the ancestral mormyrid conditions. Within the Petrocephalinae, two distinct lineages have independently evolved the mormyrine pattern of receptor organization: in one of these species, *Petrocephalus microphthalmus*, the EL is relatively large and divided into anterior and posterior regions, as in the Mormyrinae; however, in the other species, *P.* sp. 2, the EL is small and undifferentiated, as in other Petrocephalinae. Therefore, changes in receptor organization have occurred without changes in brain organization in two different lineages (*Myomyrus* spp. and *P.* sp. 2). We hypothesize that the independent evolution of widely distributed receptors occurred to facilitate the spatial analysis of communication signals. This also would have allowed better temporal analysis of signal waveforms, potentially driving the diversification of waveforms as species recognition signals, as well as the enlargement and specialization of central brain regions for performing the necessary signal analysis. In support of our hypothesis, we found that the Mormyrinae exhibit dramatically higher levels of signal waveform divergence among species than do the Petrocephalinae. To directly test our hypothesis, we performed playback experiments in the field, using a dishabituation paradigm to assess behavioral discrimination of signal waveforms. The two species of Mormyrinae tested discriminated waveform variants. Of the four species of Petrocephalinae tested, only *P. microphthalmus*, the species with Mormyrinae-like brain anatomy, showed evidence of discrimination. Our results provide the first direct evidence that selection on social communication behavior can drive increases in the size and complexity of sensory pathways, and that these changes result in an improved perceptual ability to discriminate signal variation among closely-related species.

Binaural Sites in the Ascending Auditory Circuit of a Teleost Fish, *Opsanus tau*

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In general, binaural comparisons of interaural time of arrival differences (ITD) and/or interaural level differences (ILD) are common components of auditory circuits among vertebrates that localize sound sources. Although paired auditory receptors are present in teleost fishes, binaural processes were believed to be unimportant due to the speed of sound in water and the acoustic transparency of the tissues in water. In contrast, there are behavioral and anatomical data that support binaural processing in fishes. Sand [1974] provided evidence that the non-parallel saccules in cod have hair cell orientation patterns and response characteristics consistent with auditory endorgans that encode the axis of particle motion. Later, Horner et al. [1980)] identified binaural processing sites in the medulla and midbrain of cod. We have been investigating auditory processing in a teleost species for whom sound source localization may involve binaural processing: the oyster toadfish *(Opsanus tau)*. The behavioral repertoire of toadfish includes responses to conspecific sounds, which indicate that they are listening, but also, locating a vocalizing male is critical for successful reproduction. As in cod, toadfish saccules are not parallel, and binaural comparisons could be beneficial for sound source localization.

We have conducted a series of anatomical and physiological studies to evaluate binaural processing in the ascending auditory circuit of the toadfish. Primary auditory afferents from the saccule project ipsilaterally to multiple sites along the dorsal region of the descending octaval nucleus (dDON) [Edds-Walton et al., 1999]. Neurobiotin injected at auditory sites in DON traveled retrogradely to cells of the contralateral DON, confirming a commissural auditory tract [Edds-Walton, 1998]. Anterograde transport of neurobiotin revealed projections to the dorsal division of the secondary octaval population (SOdor) and to the auditory midbrain (nucleus centralis, NC). Retrograde transport of neurobiotin injected at characterized auditory sites in NC confirmed that a subset of dDON cells and SOdor cells project to the auditory midbrain. Physiological studies showed that the directional responses of primary auditory afferents are retained by cells in the DON and that a subset of cells exhibit directional 'sharpening' by neural computations in DON and NC. The data clearly indicate that calculating the direction of the sound source is an important task for the auditory system of this teleost, as in other vertebrates [Edds-Walton and Fay, 2005]. Recently, we demonstrated that binaural cells result from the convergence of excitatory and inhibitory inputs to cells in both DON and NC. Therefore, there are

potential sites in fishes for bilateral comparisons that may function similarly to binaural sites in the ascending auditory pathway of other vertebrates (e.g., nucleus laminaris in birds or nuclei of the superior olivary complex in mammals). Along with auditory scene analysis, binaural comparisons and sound source localization may be ancient functions of the vertebrate auditory system.

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Cone Monochromacy in Sharks: Color-Blind Killers?

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Whales, dolphins and seals are all cone monochromats, i.e. they have only a single spectral class of cone photoreceptor in their retinas. Unless they are able to compare signals from their cones with those from the rod photoreceptors – which have a slightly different spectral sensitivity – they will be color-blind. Whilst it is not known whether aquatic mammals are able to do this, it begs the question: just how useful is color vision for predation underwater? We know that aquatic birds, reptiles (turtles) and many teleost fish have at least some form of color vision, but what about arguably the most efficient predators of all: the sharks? In this study, we used microspectrophotometry to measure the spectral absorbance characteristics of visual pigments in the cone photoreceptors of a number of shark species and found that, like whales, dolphins and seals, they have only a single spectral type. Moreover, the peak sensitivity (lambda max 532–561 nm depending on species) of those pigments is similar to that seen in the aquatic mammals. Is the prevalence of cone monochromacy in large marine predators an example of convergent evolution or does it simply reflect different ecophysiological 'bottlenecks' in the evolutionary history of these taxa? In addition to the ecological aspects of spectral sensitivity in sharks, the utility of color for survival in aquatic habitats is discussed.

How Do Birds Sense the Earth's Magnetic Field?

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Besides several other natural cues, the earth's magnetic field has shown to provide a major source of orientational information for migratory birds on their journeys between wintering and breeding grounds. However, the current state of knowledge on avian magnetoreception provides more questions than answers: how do birds sense the magnetic field? How do they derive orientational information from it? Finally, how is this information integrated and processed in the brain?

Unlike sensory modalities such as touch, taste, hearing or vision, whose peripheral organs and brain structures that process them are well described, magnetoreception has been an exception. A major source of controversy may evolve from the fact that magnetic fields freely pass through biological tissue. Consequently, a putative receptor does not require direct contact with the external world and therefore might be located anywhere within the organism. Moreover, a receptor tuned to this sense doesn't necessarily need to exist and the transduction process might occur as a set of biochemical reactions instead. Finally, only few materials of biological origin are affected by magnetic fields of naturally occurring strength.

Theoretically, birds could derive two fundamentally different kinds of information from the ambient magnetic field: (1) directional information, which could enable them to maintain a consistent heading, thus forming the basis for a 'magnetic compass'. This is supposed to be based on quantum-chemical processes in sensor molecules, putatively cryptochromes, associated with the visual system; (2) positional information, which a bird could use to assess its approximate geographical position. This could be mediated by iron-mineral-based structures in the upper beak of birds linked to the trigeminal system.

My talk aims to give an overview on the current state of knowledge, spanning from behavioral approaches to molecular biological, biophysical and neuroanatomical correlates, which, in recent years, have proven a powerful tool to elucidate major questions on avian magnetoreception.

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Ultrasound Detection by Clupeid Fishes

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One of the unique identifying features of clupeid fishes, including the herrings, sardines, and shads, is the presence of air-filled auditory bullae located in the skull. These auditory bullae are connected to the utricular macula and also the lateral line, which is restricted to the head. Blueback herring were discovered to respond to ultrasound when it was noticed that they avoided an echosounder. This sensitivity was then confirmed in the American shad *(Alosa sapidissima)* through behavioral, evoked potential and single unit recordings in the brain. A broad survey of clupeids suggests that ultrasound detection is limited to the subfamily Alosinae, which includes the shads and menhaden. Ultrasound detection has not been found in other subfamilies, including the sardines or herrings. The mechanism of ultrasound detection is not known.

High resolution CT scans of sardines and menhaden reveal that there are key differences in the location of pterotic bullae and in the elaboration of the lateral recess. In the menhaden, the pterotic bullae are positioned more laterally and also have an elaborate bony channel near adjacent to the lateral recess. The pterotic bullae are critical for ultrasound detection in that filling the bullae with saline reduces surface vibration of the bullae as well as eliminates evoked potentials in response to ultrasound. Furthermore, mechanical disruption of the area above the lateral recess eliminates evoked potential responses to ultrasound. These results lead to the suggestion that the lateral line is important in ultrasound detection in the Alosinae.

A key question is how did ultrasound detection evolve? It is likely that the ability to detect ultrasound was due to selective pressure by echolocating odontocete cetaceans, which evolved much later than the clupeids. Why did it evolve in one subfamily, but not others? Most of the species in the Alosinae are anadromous fishes that spawn in fresh water. In southeastern Asia, these species currently live in the same river systems as river dolphins, which were one of the earliest odontocetes to evolve. Thus, it is hypothesized that predation by river dolphins may have been the key selective pressure in the evolution of ultrasound detection in the Alosinae.

Let's Sin: A New Model of the Zebrafish Forebrain

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The forebrain of zebrafish and other teleosts develops through a unique but poorly understood process of outward folding (eversion). As a result, pallial derivatives of zebrafish corresponding to the isocortex, hippocampus, and parts of the amygdaloid complex in mammals are still a matter of debate. In 2009, Nieuwenhuys tongue-in-cheek called those of us who do not pay enough attention to topological arguments 'new comparative neurological sinners'. That is, Nieuwenhuys critiqued the oversight of certain landmarks which, as he believes, point towards a simple eversion of the forebrain. His model defines the zebrafish forebrain as distinctly different from that of mammals. In particular, he assumes that a pallial division corresponding to the mammalian isocortex is missing in teleosts. Based on an evo-devo approach, this study introduces a new model of the teleostean forebrain and its organization and development. In contrast to traditional notions, my results show that the pallial organization of zebrafish is strikingly similar to those of mouse and man. I deciphered the eversion process in zebrafish studying histologically stained brain sections during different developmental stages (larval, juvenile, and adult) using a variety of molecular markers. For example, a histochemical stain for nicotine adenine dinucleotide phosphate diphorase (NADPHd) activity revealed a complex inward movement of the dorsal pallial division. Tracing this morphological shift, I topologically determined the dorsal pallial division homologous to the isocortex of mammals. I also redefined the pallial derivatives corresponding to the mammalian pallial amygdala, hippocampus, and piriform cortex in the adult brain of zebrafish. My model describes the zebrafish pallium as the result of what I call an outward-inward folding of a complex eversion process. These results have significant implications for both the interpretation of the evolution of the dorsal pallium in ray-finned fish and the applicability of zebrafish as a neurological model. The most immediate implication perhaps might be that we must part with Nieuwenhuys's simple pallial eversion and embrace the notion that already original sin brought us closer to the tree of knowledge.

Do Early Vertebrate Brain Subdivisions Develop in Similar or Different Ways?

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Early in vertebrate brain development, three units can be distinguished: the forebrain, the midbrain, and the hindbrain. Subsequently, the forebrain divides anteriorly into the secondary prosencephalon and posteriorly into the diencephalon. Each of these four primary segments begins as a single unit before subsequently dividing along transverse and longitudinal axes. The transverse segments are sometimes known as neuromeres, while the longitudinal areas become derivatives of the alar and basal plate. For the time period that begins when these four subdivisions are a single unit until each respective subdivision of the secondary prosencephalon, diencephalon, midbrain, and hindbrain undergoes internal parcellation, two questions are asked: First, do these four major segments undergo similar transformations? Second, do alar and basal plate areas share similar morphological features?

If these early brain parts follow similar rules of development, each unit should contain the same components: roof plate, alar plate, basal plate, and floor plate. With the exception of the hypothalamus, the remainder of the secondary prosencephalon is alar. Based on this feature alone, the secondary prosencephalon is distinguished from these other three areas. A portion of the alar midbrain, the optic tectum, is laminated. During this developmental time period, this character separates the midbrain from the diencephalon and hindbrain. Based on features such as cell proliferation, fiber tract location, and subsequent transformation into further subdivisions, the diencephalon and hindbrain differ in their formation. Taken together, these morphological characters of the secondary prosencephalon, diencephalon, midbrain, and hindbrain suggest that each develops differently. Alar and basal plate areas of these early brain divisions have some features in common such as fiber tract organization and certain patterns of longitudinal organization. However, neither of these features span major division boundaries as a continuum from hindbrain to the secondary prosencephalon in either the alar or basal plate, nor does the pattern of cell proliferation. Alar and basal plate derivatives of these early brain units seemingly form differently. If early brain transverse and longitudinal components undergo different morphological transformations and presumably follow different rules, what might the consequences be? One possibility is that evolution could act on individual parts early in development to increase potential brain variability among species.

Morphometric Comparison of Telencephalic Areas in Selected Vertebrates in Relation to Function

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Numerous studies have already assessed the relative size of brains in different vertebrate groups. Many of those studies also measured and compared selected brain regions, mostly sensory input regions, to reveal sensory specializations and functional adaptations to specific habitats, ecology and behavior of the animal. The varying sizes of the telencephalon in different vertebrate species give rise to speculations whether size is related to higher cognitive functions, large sensory input areas and/or particular social behaviors. In mammals, there are detailed studies on the evolution of different areas within the telencephalon that give more information on the relative volume of limbic, olfactory, and cortical areas. Studies at this level are lacking in non-mammals and particularly in anamniotes. We examined brains of selected representatives of different vertebrate groups, especially elasmobranchs and ray finned fishes, by measuring different areas within the telencephalon in histological sections to get more detailed morphometric data on the evolution of the various parts of the telencephalon. In several species of elasmobranchs and many ray finned fishes information is already available on the relative size of the telencephalon and the olfactory bulb (OB). Generally, elasmobranchs have fairly large telencephali, but sizes vary, similar to teleosts, where also large differences exist in the size of the telencephalon. Surprisingly, however, it was never directly tested to what extend the size of the telencephalon correlates with the size of the OB. It is somehow generally assumed that a large telencephalon is related to higher integrative functions without considering the possibility that the size is correlated with an increase of olfactory related structures. Based on the proportion of the size of the OB to the rest of the telencephalon we found that there are great differences ranging from the squirrelfish with a huge telencephalon and a very small OB to sturgeons where the OB can be even bigger than the rest of the telencephalon. For example in *Anguilla*, the relative size of the telencephalon is in the range of cichlids, but this can be explained by an enlarged olfactory system and a reduced visual system. Surprisingly, *Polypterus* has one of the largest telencephali of all actinopterygians, but the OB/telencephalon ratio is in the range of *Anguilla*, *Salmo*, and *Esox*. In elasmobranchs, the telencephalon is on average larger than in actinopterygians, but the OB/telencephalon ratio is within a similar range. In summary, if the size of the telencephalon is taken in relation to the size of the OB, a more differentiated picture evolves of telencephalic evolution in vertebrates.

A Three-Dimensional MRI Brain Atlas of the Mozambique Tilapia *(Oreochromis mossambicus)*

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The African cichlid *Oreochromis mossambicus* has been used as a model system in a wide range of studies. The increasing number of genetic tools available for this species, together with the emerging interest in its use for neurobiology studies, increased the urgency in an accurate hodological mapping of the tilapia brain to complement the available histological data. The goal of our study was to elaborate a three-dimensional, high-resolution digital atlas using magnetic resonance imaging. Resulting images can be viewed and analyzed in all orientations (coronal, sagittal, and horizontal) and were manually labeled to reveal major brain nuclei in the olfactory bulb, telencephalon, diencephalon, optic tectum, and cerebellum. In this study, a 9.4 T Bruker BioSpec MR system (Bruker, Germany) was used to acquire T2-weighted 3D RARE images of 3 adult male tilapia brain (perfused with Paraformaldehyde + Dotarem® (4%)) immersed in Fluorinert (3M Co., Ltd.). The sequence timings were TE = 36 ms and TR = 350 ms. The voxel resolution of the 3D digital atlas was (50 × 50 × 50) μm^3. Image labeling and three-dimensional surface reconstructions were created using dedicated software (Amira, Mercury Computer Systems, USA). This high resolution tilapia brain atlas is expected to become a very useful tool for neuroscientists using this fish model and will certainly expand their use in future studies of the CNS.

Are GnRH1 Cells Topographically Organized? Plasticity in the Songbird GnRH1 in Response to Photoperiod, Gonadal and Social Cues

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It is well established that the preoptic area (POA) plays a critical role in regulating reproductive physiology and behavior in vertebrate species. A number of studies that utilize lesion methods, immediate early gene expression, and/or a variety of histological techniques have suggested that the POA may be functionally topographically organized. The gonadotropin-releasing hormone 1 (GnRH1) cells are primarily distributed throughout the POA and are the key link between the brain and peripheral endocrine system. The primary aim of this presentation is to report on recent findings that suggest that the GnRH1 cells in the avian POA are topographically organized. Here we present evidence suggesting that (1) photoperiod, (2) social context, and (3) gonadal state independently regulate subregions of the POA GnRH1 population. In many avian species the GnRH1 system exhibits profound variation that is dependent on photoperiod experience. We found that particular subregions of GnRH1 cells within the medial-intermediate, medial-caudal, and lateral-intermediate POA exhibit greater variation in expression, suggesting localized anatomical effects of photoperiod. Specifically, breeding starlings have a greater number of GnRH1 mRNA expressing cells in all the aforementioned regions compared to non-breeding birds. We suggest that the annual change in photoperiod has widespread effects on the GnRH1 system, but specific regions are more sensitive to photic input. In order to investigate the effects of social environment on GnRH1, we conducted two experiments that examined (1) the number of GnRH1 cells and (2) the induction of the immediate early gene ZENK in GnRH1 cells from birds housed in different social conditions. In the first study, we paired male and female starlings or housed males alone for 2 weeks. We observed significantly more rostral and intermediate POA GnRH1 immunoreactive cells in male starlings housed with females compared to starlings housed alone. In the second study, we attempted to determine whether specific regions of GnRH1 cells are co-labeled with the immediate early gene ZENK after canaries were placed in dyads compared to those housed alone. Again, we observed a significantly greater proportion of GnRH1 cells expressing ZENK in male-female dyads compared to isolated birds. The greater proportion of double labeled ZENK-GnRH1 cells was observed in the rostral and intermediate POA. Finally, castration of starlings resulted in a significant increase in GnRH1 immunoreactive cells in the intermediate POA compared to intact starlings. Here, the increase in GnRH1 cells is presumably the result of decreased negative feedback from the lack of gonadal steroids. In light of findings collected from mammalian species that also show distinct variation in the GnRH1 system across the reproductive cycle, the data collectively suggest the possibility of an evolutionarily conserved mechanism by which external and internal stimuli can regulate a specific cell population that is critical for reproduction. We propose that different stimuli could regulate GnRH1 either directly through various unidentified neural circuitry or indirectly by the action of hormonal systems.

Beyond Language: Translational Implications of Seasonal Regression of the Avian Song Control System as a Model of Neuroprotection

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The study of songbird neurobiology and behavior has served as the premier animal model for human language for several decades. However, there are translational applications related to song control system neurobiology unrelated to speech and language. For example, hormonal sensitivity of the songbird brain makes it an ideal model to study the mechanisms of hormonally-mediated neuroprotection. In particular, seasonal regression of the song control system provides an excellent opportunity to better understand neuroprotection in vertebrates, including humans. In adult male Gambel's white-crowned sparrows, acute withdrawal of circulating testosterone (T) induces higher vocal center (HVC) volume to regress to nonbreeding size within 12 h of T withdrawal. The sudden collapse of the nucleus is initially due to a sharp increase in neuron density, which suggests a rapid degeneration of dendrites and/or axons. T probably protects HVC neurons directly, because infusion of T near HVC prevents the regression of HVC and significantly reduces the number and density of activated caspase-3 cells. T infusion near HVC also prevents regression of the efferent targets of HVC neurons, including RA and Area X volume, RA soma area and neuron density, which shows that T can act locally to have a neuroprotective effect and that the neuroprotective effects of T can act transsynaptically to prevent regression of efferent nuclei. Infusion of a cocktail of caspase inhibitors unilaterally near HVC via an osmotic pump and cannula prevents the regression of the ipsilateral HVC for at least 20 days after the transition to nonbreeding conditions. Though caspase inhibitors, like T, reduce cytosolic activation of caspase-3 after transition to nonbreeding conditions, the transsynaptic protection of RA and Area X are limited, which suggests that caspase inhibitors do not provide a full compliment of trophic support to degenerating brain areas. Last, data acquired from microarrays from the SoNG initiative have provided substantial insight into the changes in gene expression that mediate seasonal neurodegeneration of HVC and RA and generated hypotheses for future research. Taken together, these results demonstrate that the rapid and robust seasonal regression of the song control system provides an excellent opportunity for understanding what mechanisms underlie why a decrease in circulating levels of sex steroids leads to an increased likelihood of degeneration of hormone-sensitive brain circuits.

The Presumptive Isthmic Region in a Mouse as Defined by *fgf8* Expression

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The isthmus is recognized as a substantial part of the hindbrain of adult birds and reptiles, situated between the midbrain and the first rhombomere. However, most studies on mammals ignore its potential existence. Transient *fgf8* expression at the border separating the *Otx2* and *Gbx2* fields plays a crucial role as a secondary organizing center in avian and mammalian embryos. We hypothesized that *fgf8* might be a marker for the isthmic region in mammals. We therefore mapped the distribution of cells of the *fgf8* lineage in postnatal mice (n = 4) possessing an *fgf8*-cre-lacZ transgene. The neuron groups expressing *fgf8* in the mouse correspond well to the predictions based on avian studies; the trochlear, dorsal raphe, caudal linear, and parabigeminal nuclei are all intensely labelled with lacZ. Our study suggests that a large part of the substantia nigra (SN) and all of the A8 dopamine group are derived from the isthmus. The whole cerebellar vermis is also labeled in these preparations. This study indicates that many structures at the midbrain-hindbrain junction in mammals might be isthmic in nature.

Avian Cerebellum Specialization in Relation to Acrobatic Courtship Displays in Manakins (Pipridae)

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The courtship displays of male manakins (Aves: Pipridae) involve an array of acrobatic and postural elements. One might expect species with more elaborate displays to exhibit specializations in motor planning and coordination areas of the brain. Several studies have suggested a relationship between cerebellum (Cb) morphology and distinct motor-related functions for the anterior Cb (somatosensory, flying, hopping/walking), posterior Cb (vision, audition, flying, hopping/walking, vestibular), vestibulocerebellum (flying, hopping/walking, vestibular), and cerebellar nuclei (CBN). Therefore, we compared Cb morphology of four species of manakins collected in Panama: *Manacus vitellinus* (n = 3), *Pipra mentalis* (n = 4), *Chiroxiphia lanceolata* (n = 3), and *Lepidothrix coronata* (n = 3), representing a range of display complexities. *M. vitellinus* performs the most complex display, which consists of very rapid hops between vertical saplings. During each hop loud snaps are made by explosively lifting the wings behind the back. Once perched on the vertical sapling the male quickly assumes a posture in which his beard feathers are extended outward. *P. mentalis* males' displays consist of swooping flights in the lower canopy with non-vocal 'pop' noises upon landing. While perched on a branch, males also perform a 'moonwalk' display in which tiny, rapid hops give the illusion that the bird is sliding backwards along a branch. *C. lanceolata* males perform a cooperative display involving two males on a low horizontal display perch. One male jumps up and hovers while the other male slides under him, then jumps and hovers while the other slides beneath. *L. coronata* males perform the simplest display which consists of normal but quick flights between perches in the lek. Birds were caught by mist net, and brain tissue was prepared by first performing an intracardial perfusion with 0.1 M PBS and neutral buffered formalin (NBF), dissecting the brain, postfixing in NBF, and cryoprotecting in sucrose solution. Tissue was then placed in an 8% gelatin block, submerged in NBF and cryoprotected again, then frozen rapidly on dry ice. Brains were sectioned sagittally at 30 μm, placed on slides, and Nissl stained with cresyl violet. Every 9th section was used for volume measurements. We scored each species' display for overall complexity, taking into account acrobatic elements and the presence of sound production by the wings or coordination between two displaying males. We then tested for a correlation between display complexity score and area of the midsagittal Cb, the length of the Purkinje cell layer for anterior Cb, posterior Cb, vestibulocerebellum, and lastly the volume of combined CBN. Such relationships would suggest specializations related to the function of these Cb regions. Cb volume was corrected for the size of the bird by regressing Cb volume against the volume of the brain minus Cb, then using the residuals in subsequent analyses. There was no relationship between the size of the CBN and Cb volume. In order to correct for phylogenetic relatedness, independent contrasts for each variable were derived employing the most recent molecular phylogeny for Pipridae. Pearson-product moment correlations of these contrasts revealed a positive relationship between display complexity and CBN volume ($r = 0.968$; $p < 0.032$), but no other relationships were detected, suggesting that the amount of Cb inputs and outputs passing through the CBN may reflect the complexity of acrobatic courtship displays in manakins.

Brain Regions Associated with Female Mate Preference Behavior in a Teleost

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In many taxa, females vary in their mate preference response towards males. This individual variation in mate preferences is one of the factors that have allowed poeciliid fishes (e.g. guppies, mollies and swordtails) to become a model system for studying the evolution of female mate choice. In swordtails, female preferences demonstrated in the laboratory reflect mating preferences in the wild. In one particular species of swordtails, *Xiphophorus nigrensis*, females prefer males that have a large body, court, and possess ultraviolet ornamentation. Here we present our findings on nuclei that may underlie female mate preference behavior in wild caught *X. nigrensis*. Females were subjected to one of several conditions in a non-contact dichotomous choice setup for 30 min. We recorded the association time and two behaviors exhibited during the trial to assign a preference score for a stimulus. Using in situ hybridization we localized and quantified the expression of a gene associated with mate preference *(neuroserpin)* in this species in ten nuclei. Utilizing the individual variation in female preferences in this species, we demonstrate that several forebrain nuclei show a significant difference between 'high' and 'low' preference score females and have a significant positive relationship between *neuroserpin* expression and preference score only in mate choice conditions. Further, there is a unique *neuroserpin* expression network between the brain regions examined that is seen only in mate choice conditions.

Exploring Adaptive Evolution in the Brains of Bathyal Skates (Family: Rajidae): Phylogenetic and Ecological Perspectives

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The deep sea is a vast and still largely unexplored habitat. As neural development in fishes has been shown to reflect morphological adaptations, particularly in extreme environments, quantitative analysis of deep-sea species is potentially highly informative. Members of the family Rajidae (skates) make up a highly diverse group, comprising approximately 40% of all batoids, with 30 genera across approximately 280 species. Despite their diversity, few studies have quantified interspecific brain size (encephalization) or the relative development of major brain areas (telencephalon, diencephalon, mesencephalon, cerebellum, medulla) and discrete sub-sections of these brain structures that receive direct sensory input (e.g. optic tectum and the dorsal and medial octavolateral nuclei) in rajids. The brains of eight species of deep-sea skate, *Bathyraja aleutica*, *B. parmifera*, *B. abyssicola*, *B. trachura*, *Raja binoculata*, *R. rhina*, *R. stellulata*, and *Rhinoraja interrupta*, ranging in primary habitat depth from ~5 m to as deep as 2,900 m, were assessed and compared to the brain organization of other batoids (n = 24) as well as a broad dataset of sharks and holocephalans (n = 84). Trends show both strong phylogenetic patterns as well as possible ecological adaptations. Brains of these eight skate species were scanned using magnetic resonance imaging (MRI), and major brain areas were digitally segmented using user-guided automatic segmentation in ITK-SNAP. Encephalization of the brain was assessed using species as independent data points (generalized least squares regression) and using phylogenetically independent contrasts. Results indicated that rajids have small brains relative to their body mass in comparison to other batoids, and indeed other chondrichthyans, even when free of phylogenetic constraints. Brain organization in members of Rajidae was assessed using a relative deviation analysis (θ), and connectivity to other chondrichthyans was determined using a multivariate hierarchical cluster analysis, based on Euclidean distance. Though morphologically and phylogenetically dissimilar, pilot data suggest similar patterns of brain organization between the deep-sea members of Rajidae and the deep-sea sharks and holocephalans, such as *Etmopterus baxteri*, *Centroselachus crepidater*, and *Harriotta raleighana*, which have the common characteristics of a relatively small telencephalon, a small, smooth cerebellum, and a large medulla, particularly the octavolateralis areas, which receive electroreceptive and lateral line input. A relatively small brain, with a poorly developed telencephalon and smooth cerebellar corpus, has been suggested as the ancestral condition of the cartilaginous fishes, though the dorsal and medial octavolateral nuclei seem disproportionately large in deep-sea chondrichthyans. We hypothesize that a combination of phylogenetic and ecological pressures is contributing to brain development in these species.

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