Cortical Surface Structure Analysis in Sharks using Magnetic Resonance Imaging (MRI)

K. E. Yopak[1], G. T. Balls[1], and L. R. Frank[1]

[1]Radiology, UCSD - Center for Scientific Computation in Imaging, La Jolla, CA, United States

Introduction Despite their basal place in vertebrate evolution, little quantitative data is available on shark (chondrichthyan) brain organization & its implications for nervous system adaptations [1,2], including our own. The cerebellum appeared at the onset of the chondrichthyan radiation, with extreme variations in cerebellar morphology during subsequent evolution, highlighting their role as key model systems. Physiological & behavioral research on the shark cerebellum has resulted in a lack of functional consensus; comparative data on cerebellar morphological patterns can inform the debate using representative cartilaginous fishes with disparate behavioral repertoires.

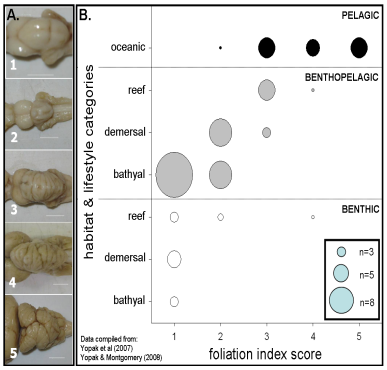
Visual Foliation Index Our recent work has demonstrated ecological correlations between cerebellar organization & habitat in sharks [1,3], even in phylogenetically diverse species [2]. A visual foliation index (1-5) of the cerebellar corpus (VFI) (Fig. 1a) was applied to approximately 60 species [1,2], showing extreme variation in corpus folding, with the highest foliation levels in agile predators that lived in 3D environments (Fig. 1b). However, visual indices are of limited quantitative utility for surface structure & can miss subtle but evolutionarily important species differences. Quantified foliation indices derived from histology have been documented for other vertebrate groups [4-5]. The histological cerebellar foliation index (CFI) used in avian studies [4] is based on the ratio of the length L_p of the surface (Purkinje cell layer) to the length L_e of the "envelope" of this layer, thus CFI = L_p/L_e. Although successful, such methods are highly manual & constrained to 2D. Further, derivation of 3D information for quantifying spatial complexity & shape variations is extremely difficult, & resolution (an advantage of histology) is typically lost during attempts of reconstruction. Here we use high resolution MR imaging and present preliminary quantification of foliation beyond the VFI & CFI by using the spherical harmonics decomposition (SHD) approach to shape analyses to rigorously quantify the variations in the shark cerebellum.

Imaging Anatomical MR scans were performed on a Bruker 7Tesla small animal scanner with gradient strengths of 46Gauss/cm. Brains were excised from the chondrocranium & formalin-fixed, then transferred for processing in saline + 0.01% sodium azide for a week followed by the addition of 5mM Prohance® for a further 7 days. MR images were T1-weighted, using a GRE gradient echo with no RF spoiling [6].

Shape Analysis Segmentation of the cerebellum was performed using ITK-SNAP (itksnap.com). Quantification of foliation was based upon the number of parameters necessary to accurately describe the cortical surface decomposed onto a spherical harmonic basis using the SHD. These methods have been used for characterization of global brain shape in humans [7-8], though their application to other vertebrate groups is novel. This parameterizes cerebellum shape in a rigorous fashion that allows comparison of cortical surfaces between shark species, as, unlike humans, neural morphology varies widely interspecifically. The SHD approach requires two steps: 1) an initial brain "inflation" step to map the surface uniquely onto a sphere, using Freesurfer (freesurfer.org), & 2) a Fourier decomposition on the sphere [7]. The result is a spectrum of coefficients from which the foliation index can be determined as the order of the SHD necessary to fit (in the least-squares sense) the brain shape, with higher degrees of foliation requiring more coefficients. The maximum degree L_{max} required to describe the shape (model selection) was determined using ANOVA [9], which results in an F-test comparison of the optimal model degree L_{max}. This L_{max} is then used as the foliation index, supplanting the original VFI [1].

Results An example of this methodology is shown in Fig. 2. Fig. 2a shows a validation method we have developed & Fig. 2b is the application to 3 sample shark species. The validation method uses the surface structure of a normal human brain (Fig. 2a, right), which represents the high end of the foliation scale, & successively smoothing it to produce synthetic brains of lower foliation (Fig. 2a, towards left). For each brain, SHD coefficients are calculated up to L_{max}=75 & their magnitude is plotted as a function of L & M. Larger coefficients at higher L values represent greater foliation, (Fig. 2a, towards left). Brighter pixels represent greater power. More foliated brains with high spatial frequency components (right) thus have brighter pixels at higher L than smoother brains (left). This well controlled synthetic dataset produces a continuum of foliations for the *same* brain that demonstrate the validity of the method & provides the rigorous determination of the L_{max} that best fits the data. The shark brains (Fig. 2b) show similar results, with larger coefficients at higher L values for the shark species (*Mustelus*) with the most foliated brain. These pilot results support that cerebellar complexity increases in more active species that occupy 3D environments [1,2].

Conclusions As basal vertebrates, the shark brain serves as an ideal model for our neural evolution. The combination of high-resolution MRI & rigorous 3D shape analysis methods [7] allows for a quantitative measure of cerebellar foliation based upon the complexity of the SHD required to accurately fit a given cortical surface. Our SHD analyses have redefined both the VFI & the avian CFI; we can now define CFI as the ratio of the surface areas of the segmented brain derived from the full SHD to the enveloping surface of the brain, computed from the low order (L_{max} =4) SHD fit. The next step is to apply these methods to a wider range of species [1,2], to fully confirm our previous qualitative scheme.



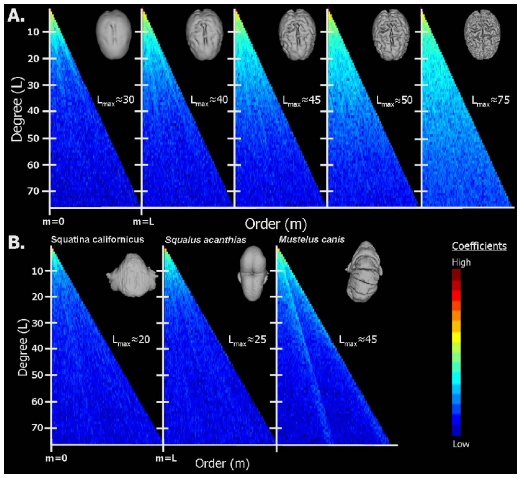


Fig 1. (**A**) VFI (1-5) in the brains of 5 representative shark species. (**B**) VFI from 60 shark species, graphed according to ecological grouping [1,2].

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