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Dolphin insula reflects minicolumnar organization of mammalian isocortex

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Abstract

The brain of the bottlenose dolphin exhibits patterns of isocortical parcellation and cytoarchitecture distinct from those seen in primates, yet cell clusters in anterior insula are comparable in scale to module-like cell arrangements found throughout isocortex in other placental mammalian species with long divergent evolutionary histories. This similarity may be due to common ancestry, or to convergence as a result of selective constraints on organization of connections within such modules. Differences reflect alternate arrangements of minicolumns, an elemental cytoarchitectonic motif of isocortex defined by radially oriented pyramidal cell arrays. In contrast with larger modular structures incorporating them, minicolumns have been highly conserved in mammalian evolution. In this study a previously validated imaging method was employed to assess verticality, *D*, a parameter indicating radial bias of isocortex. Photomicrographs of coronal Nissl-stained sections of dolphin anterior insular cortex were compared with sections from human brains of putatively homologous areas as well as other isocortical areas differing in modular organization. Dolphin insula exhibited a high degree of verticality consistent with conserved minicolumnar organization. Our findings indicate that a basic structural motif of isocortex is synapomorphic in a species of marine mammal exhibiting unique phylogenetically derived isocortical characteristics.

Keywords

Cetaceans; Neocortex; Minicolumns; Pyramidal cells; *Tursiops truncatus*

The taxa encompassing the primate and cetacean orders diverged from a common line of placental mammals over 90 million years ago [1]. Some species of the suborder odontocetes—which includes dolphins, porpoises, and toothed whales—have acquired complex repertoires of problem solving, communication, and social behaviors that appear convergent with corresponding cognitive traits in primates [2]. Associated with this trend is an increase in absolute brain size and encephalization [3]. Larger brains in general exhibit more extensive parcellation with increased hierarchical organization of modular cortical subdivisions [4].

Odontocetes previously were thought to be exceptional in having a relatively simple, undifferentiated neocortical organization with few regional subdivisions [5,6]. More recent work has supplanted this view, demonstrating a high degree of cortical complexity in

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odontocetes with modules ranging from clusters of several pyramidal cells to arrays 20 or more cells wide evident in insula and other cortical areas [7,8,9]. This and comparably-scaled modular cyto- and chemo-architecture observed in other mammalian species are thought to reflect independent, convergent solutions to constraints of neuronal connection patterns and metabolism as brains increase in size phylogenetically [7], as well as species-specific adaptive requirements. These features are encompassed by columns characterized by physiological activity and thalamocortical projection fields as well as larger cytoarchitectonically-defined areas.

As observed in a number of mammalian species [10,11,12] such modular arrangements emerge during development from connections formed among reiterative arrays of microcircuits in the neocortical sheet. These fundamental cytoarchitectural building blocks are termed minicolumns and comprise radially-oriented cores of pyramidal cells with associated processes, interneurons, and peripheral neuropil [13]. Minicolumns are derived during development [14] from ontogenetic cell columns, post-mitotic neurons migrating from ventricular germinal zones outward into the cortical plate along common radial glial processes [15]. This process of gliophilic migration is exhibited among all mammalian species. The minicolumn is thus a conserved structural motif; this finding provides a basis for the exploration of independently derived ontogenetic mechanisms shaping modular features of neocortex.

We have previously used computer-assisted imaging methods [16] to model *directionality*, *D*, a parameter describing the degree of alignment of minicolumnar components along the radial axis, as well as other parameters of minicolumnar morphometry, establishing comparative values for radial organization among human and other mammalian species. We employed this method in the current study to determine *D* in an area of insular cortex of the odontocete *Tursiops truncatus* (bottlenose dolphin) exhibiting modular cell clusters (mean width of 255 μm) in layer II [7]. We obtained comparative data from putatively homologous or contiguous areas of insula as well as striate, temporal, somatosensory and dorsolateral cortex in humans, areas containing varied patterns of cell clustering.

The purpose of this study is to determine the extent to which verticality is comparable between bottlenose dolphins and humans and across human neocortical areas. As minicolumnar radial organization of isocortex, as determined by verticality, is synapomorphic among mammals, we seek to determine the contributions to radial organization of this conserved structural motif in comparison to superposed area- or species-specific modular arrangements of neurons.

Materials and Methods

Specimens

This study examines the vertical bias in Brodmann areas 4, 9, 17, 21, 22 and 40 from the brains of 21 humans (Table 1), and contrasts these data with measurements of insular cortex in Nissl-stained sections obtained from three bottlenose dolphins (*Tursiops truncatus*). One dolphin was known to be an adult female; data on the other two were unavailable. Brains were celloidin embedded, cut into 35 μm serial sections and Nissl stained (Figure 1). Adjacent slides, stained with the Loyez technique, were available to help identify brain parcellation. Regions of interest were identified according to both anatomical landmarks and cytoarchitectural criteria. Neuropathological examination of the brains and region of interest was normal. Photomicrographs were obtained using a Nikon E1000M microscope (40 $\times$ total magnification) and a DXM 1200. Obtained images had pixel dimension 0.85 μm . All specimens are part of the normative and comparative anatomy series located at the

Yakovlev-Haleem Collection, Armed Forces Institute of Pathology (AFIP), Washington, D.C.

Computerized Image Analysis

Computerized image analysis of minicolumnar structure in laminae II through VI (see below) was performed with algorithms described in the literature [16]. The feature extraction properties of the program correct for minicolumnar fragments, curvature of the tissue section, and have been adapted to 3D proportions (stereological modeling) [16,17]. The results have also been validated (i.e., minicolumnar width) against measurements of both physiological (intrinsic optical signaling) and anatomically (myelinated bundles) derived minicolumns [16].

Let I be an image of Nissl-stained cerebral cortex, with Fourier transform J . Pyramidal cell arrays give rise to a striped appearance, or texture, while the individual cell somata produce a blotchy texture. Superposition of these textures is reflected in J , where minicolumnar structure stands out as a strongly directional signal against an isotropic background [16]. The dominant orientation θ of the signal is found using the formula [18]

$$\theta = \frac{1}{2} \tan^{-1} \frac{\sum_k |J_k|^2 \sin 2\theta_k}{\sum_k |J_k|^2 \cos 2\theta_k},$$

where $|J_k|$ is the amplitude of component k of the discrete Fourier transform, and the sum is over all two-dimensional frequencies $\mathbf{f}_k = (f_k, g_k)$. The angle θ_k is not the phase of J_k , but the direction of the signal giving rise to the Fourier component at that frequency, i.e. $\tan \theta_k = g_k / f_k$. The directionality, or strength of the directed texture, is given by [18]

$$D^2 = \frac{\sum_k |J_k|^2 \cos 2(\theta_k - \theta)}{\sum_k |J_k|^2},$$

which is normalized to lie between zero and one.

In practice, we apply this technique using windowed Fourier transforms, where the window size (approximately $500 \mu\text{m} \times 500 \mu\text{m}$) is large compared to minicolumnar scales, but small enough that the columnar orientation does not change appreciably due to the curvature of the cortex. Within the window, nonzero weight is given only to cortical laminae II through VI. Directionality D of an image is the root mean square of D^2 values from overlapping windows covering the image. Since windowed Fourier transforms are used rather than scale-space techniques, D measures an amalgam of oriented textures at all scales simultaneously (Figure 2). In particular, two equally anisotropic textures oriented at right angles to each other would lead to $D = 0$. The method relies on the assumption, based on prior anatomical and physiological studies [10,11,12], that minicolumns are the most significant anisotropic structure in isocortex.

Statistical analysis employed univariate ANOVA with species as a fixed factor and repeated measures (multiple cortical areas) within humans. The dependent variable was D , untransformed, but q-q plots were used to verify the empirical distribution of observed values of D were not strongly non-normal.

Results

Mean D was 0.245 in *Tursiops truncatus* insular cortex, and ranged from 0.258 in area 17 to 0.342 in area 21 of human cortex (Table 2, Figure 3). The difference between species was significant ($F = 8.10$ with 1 numerator and 92 denominator degrees of freedom; $p = 0.0055$) as was the difference between cortical areas within *Homo sapiens* ($F = 8.97$ with 5 numerator and 92 denominator d.f.; $p < 0.0001$). Though statistically significant, the difference between dolphin insular D and human D averaged across the six areas of interest amounted to 0.071, less than the range of values observed in the human brain (0.084). Standard deviation in D within a cortical area, across individuals of the same species, was estimated 0.043 from the residuals of the ANOVA model.

Discussion

This study provides quantitative data indicating a significant degree of vertical bias in dolphin anterior insular cortex. Values for a verticality parameter, D , in this region were comparable to those obtained in sampled areas of human cortex. This similarity in vertical bias is notable as dolphin anterior insular cortex exhibits module-like clusters of neurons in layer II and other features differing from compared areas in humans [7,8]. The extent to which these distinguishing features account for differences in cytoarchitectonic organization ought to be reflected in the degree of difference in verticality between areas.

This vertical bias has been associated with a modular organization of single cell-wide columns extending radially in layers II through VI of isocortex and demonstrated throughout cortex in a wide range of mammalian brains [19]. Previous studies have demonstrated temporal contiguity in measures of vertical bias in human brains beginning with emergence of ontogenetic cell columns in the cortical plate and subsequently maintained throughout the lifespan [14,20]. Minicolumns in various mammalian species may therefore be appropriately viewed as true homologous structures, that is, having similarity due to shared common ancestry rather than adherence to an adjacency rule [21].

Manger and colleagues [7] described irregularly distributed layer II cell clusters, 125 μm to 450 μm in diameter, in area 1a of dolphin insular cortex. As areas of insular cortex are contiguous with auditory association cortex in various mammalian species, they posited that these cell clusters may subserve the processing of complex auditory stimuli associated with dolphin communication. They noted that similarly scaled modular units, either as cytoarchitectonic ensembles (e.g., barrel fields in layer IV of rodent cortex) or as chemically defined connectional fields (e.g., CO blobs in layers II, III, or VI of primate striate cortex) vary in size by perhaps a factor of two in brains differing in size by up to several orders of magnitude. From this perspective, the restricted range in size across highly divergent mammalian species of modules having diverse cytoarchitectonic or histochemical features is the consequence of convergence arising from universal functional constraints, rather than the product of a shared ancestral developmental mechanism.

These authors assert that “[T]he ubiquity of modules and the apparent convergent evolution of module size indicate that there may be underlying homologous rules of cortical development that cause initial segregations, and that there is independent evolution of a restricted size of module, possibly due to selection for an optimal connection length.” [7, p 161] That is, they hypothesize that these modular arrangements are not the direct products of universal, conserved ontogenetic mechanisms, but may represent independent, convergent solutions to common metabolic, wiring, and information-processing constraints as brains “scale-up” [22]. They suggest that structural diversity may represent area- or species-specific variations on common organizational motifs. Alternatively, we propose as a more

parsimonious explanation that verticality of isocortical structure is determined as a first-order function by ontogenetic mechanisms of cell column formation synapomorphic among mammals.

We are arguing for a perspective complementary to that of Manger et al. [7], but focus on the conservation of parameters of minicolumns rather than cortical modules. Similarities of cortical modularity and verticality between *Homo sapiens* and *Tursiops truncatus* are likely the result of common ancestry, as well as independent adherence to selection for optimal placement in the sense of Cherniak [21]. Minicolumns are the product of conserved ontogenetic mechanisms (e.g. radial glia-guided neuronal migration). Accordingly, minicolumns serve as ‘initial segregations’ or elemental building blocks from which are assembled various higher-order modules such as the clusters in dolphin insular cortex described by Manger et al. [7].

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Figure 1.

Dolphin insular cortex (left) and Brodmann area 40 in human (right). The insular cortex of the dolphin is characterized by a cell dense layer II and columnar modules weaving their way through layers V and VI. Clustering of layer II cells is only minimally suggested in this photomicrograph. As in other brain parcellations, the dolphin cortex is agranular and exhibits a thickened layer I relative to human cortex. Cortical width is greater in humans. Brodmann area 40 contains a clearly demarcated layer IV, which is absent in the dolphin insular cortex. Micrographs are shown to scale. The scale bar measures 0.5 mm.

Figure 2.

Images with greater and lesser directionality (D). Top left: Ontogenetic cell columns in the fetal cerebral cortex at 28 weeks of gestation have a strikingly linear appearance. Bottom left: The previous image has been transformed into the frequency domain. The power spectrum, or magnitude of the Fourier transform, is shown as grayscale, with darker patches indicating more signal—image structure—at the corresponding frequency. Since the ontogenetic columns are oriented vertically in the image, most of the power is concentrated in a horizontal band; thus $D \approx 1$. Top right: In this low-magnification image of Brodmann area 4 from an adult human, there is a large amount of folding so that the local radial direction changes appreciably across the region. Consequently, the power spectrum (bottom right) is practically isotropic, so that $D \approx 0$.

Figure 3. Distribution of columnarity. Box plots summarize the human data, grouped by cortical area. Data for the insular cortices of three *Tursiops truncatus* specimens are plotted (x) on the far right.

Table 1

Metadata for the human tissue used for this study. PMI: interval between death and autopsy. PMI and cause of death are unavailable in many instances; however, none of the tissue was listed as a pathological specimen in the brain collection's inventory. The absence of any gross abnormalities was confirmed by one of us (MFC) by way of visual inspection of the slides.

	Sex	Age (y)	PMI (hr)	Cause of death
1.	male	0.2	5.25	Sudden infant death syndrome
2.	male	0.4	16.3	Aspiration of vomitus
3.	male	0.7	22	Asphyxia due to strangulation
4.	male	3.3	2.3	Lye ingestion
5.	female	4.5	22	Asphyxia due to strangulation
6.	male	6.5	< 14.3	Metastatic Wilms' tumor
7.	female	11	12	Metastatic embryonal carcinoma
8.	male	19	—	—
9.	male	20	—	—
10.	male	25	—	—
11.	male	25	—	—
12.	female	32	—	—
13.	male	44	—	—
14.	female	50	—	—
15.	male	50	—	—
16.	male	71	—	—
17.	male	72	—	—
18.	female	74	—	—
19.	male	86	3.7	Myocardial infarction
20.	female	94	< 13.5	Pulmonary embolus
21.	male	98	—	—

Table 2

Mean verticality index (D) and standard deviation by cortical area. N : number of micrographs actually analyzed. While there were 21 human cases in all, not every one provided for useful images of all six regions of interest.

Species	Region	N	D	
			Mean	Std. dev.
Dolphin	Ins	3	0.245	0.016
Human	area 4	21	0.320	0.039
	area 9	20	0.326	0.049
	area 17	19	0.263	0.039
	area 21	18	0.345	0.052
	area 22	19	0.318	0.065
	area 40	19	0.334	0.034
Human (all areas)		115	0.318	0.053