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Patrick R. Hof

Mount Sinai School of Medicine

Rebecca Chanis

Mount Sinai School of Medicine

Lori Marino

Emory University

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Cortical Complexity in Cetacean Brains

Patrick R. Hof,¹ Rebecca Chanis,^{1,2} and Lori Marino³⁻⁵

¹ Department of Neuroscience, Mount Sinai School of Medicine, New York, New York

² Bronx High School of Sciences, Bronx, New York

³ Neuroscience and Behavioral Biology Program, Emory University, Atlanta, Georgia

⁴ Center for Behavioral Neuroscience, Emory University, Atlanta, Georgia

⁵ Living Links Center for the Advanced Study of Ape and Human Evolution, Yerkes National Primate Research Center, Atlanta, Georgia

KEYWORDS

Artiodactyls, cetaceans, cytoarchitecture, delphinids, neocortex, odontocete

ABSTRACT

Cetaceans (dolphins, whales, and porpoises) have a long, dramatically divergent evolutionary history compared with terrestrial mammals. Throughout their 55–60 million years of evolution, cetaceans acquired a compelling set of characteristics that include echolocation ability (in odontocetes), complex auditory and communicative capacities, and complex social organization. Moreover, although cetaceans have not shared a common ancestor with primates for over 90 million years, they possess a set of cognitive attributes that are strikingly convergent with those of many primates, including great apes and humans. In contrast, cetaceans have evolved a highly unusual combination of neurobiological features different from that of primates. As such, cetacean brains offer a critical opportunity to address questions about how complex behavior can be based on very different neuroanatomical and neurobiological evolutionary products. Cetacean brains and primate brains are arguably most meaningfully conceived as alternative evolutionary routes to neurobiological and cognitive complexity. In this article, we summarize data on brain size and hemisphere surface configuration in several cetacean species and present an overview of the cytoarchitectural complexity of the cerebral cortex of the bottlenose dolphin.

Morphological and molecular evidence shows that cetacean ancestry is closely tied to that of Ungulata (the order of hooved mammals) and specifically Artiodactyla (the suborder of even-toed ungulates) (Gingerich et al., 2001; Thewissen et al., 2001; Geisler and Uhen, 2003). Molecular evidence shows a sister-group relationship between extant cetaceans and the artiodactyl family Hippopotamidae (Nikaido et al., 1996; Shimamura et al., 1997; Gatesy, 1998; Milinkovitch et al., 1998), though an early divergence with hippopotamids at least 52 million years ago (Gingerich and Uhen, 1998). The first cetacean suborder, Archaeoceti, derived from near-shore Indo-Pakistani locales (Thewissen et al., 1996) and survived until the late Eocene around 37 million years ago (Barnes et al., 1985) when the modern suborders, Mysticeti (comprising 13 species of baleen and rorqual whales) and Odontoceti (comprising 67 species of toothed whales, dolphins, and porpoises) appeared in the early Oligocene (Barnes et al., 1985).

Modern cetacean brains are among the largest in both absolute size and in relation to body size of all mammals [expressed here as encephalization level or encephalization quotient, EQ (Jerison, 1973)]. This is particularly striking in light of the fact that early cetaceans possessed small brains and, even more importantly, low EQs averaging about 0.5 (Marino et al., 2004). The largest brain on earth today belongs to the sperm whale (*Physeter macrocephalus*), with an average adult brain size of 8,000 g (Marino, 2002a). Furthermore, almost all odontocetes (toothed whales, dolphins, and porpoises) possess above-average encephalization levels (some EQs just under 5) compared with other mammals. Numerous odontocete species possess encephalization levels second only to modern humans (EQ \approx 7) and significantly higher than any of the nonhuman anthropoid primates (highest EQ \approx 3.3) (Marino, 1998; Marino et al., 2004).

TABLE 1. Average brain weight, body weight, and EQ for 29 cetacean species

Species Common Name (Taxonomic Name)	Brain wt (g)	Body wt (g)	EQ
Bottlenose Dolphin (<i>Tursiops truncatus</i>)*	1824	209530	4.14
Common Dolphin (<i>Delphinus delphis</i>)*	815	60170	4.26
Risso's Dolphin (<i>Grampus griseus</i>)	2387	328000	4.01
Pacific White-sided Dolphin (<i>Lagenorhynchus obliquidens</i>)*	1148	91020	4.55
Atlantic White-sided Dolphin (<i>Lagenorhynchus acutus</i>)	1103	244667	2.25
Long-finned Pilot Whale (<i>Globicephala melas</i>)	2893	943200	2.39
Killer Whale (<i>Orcinus orca</i>)	5059	1955450	2.57
False Killer Whale (<i>Pseudorca crassidens</i>)	2534	350098	4.03
Spinner Dolphin (<i>Stenella longirostris</i>)	660	66200	3.24
Striped dolphin (<i>Stenella coeruleoalba</i>)	940	261099	2.94
Rough-toothed Dolphin (<i>Steno bredanensis</i>)*	1542	124857	4.95
Tucuxi Dolphin (<i>Sotalia fluviatilis</i>)*	688	42240	4.56
Harbor Porpoise (<i>Phocoena phocoena</i>)	540	51193	2.95
Dall's Porpoise (<i>Phocoenoides dalli</i>)	866	86830	3.54
Chinese River Dolphin (<i>Lipotes vexillifer</i>)	510	82000	2.17
Ganges River Dolphin (<i>Platanista gangetica</i>)	295	59360	1.55
Amazon River Dolphin (<i>Inia geoffrensis</i>)	634	92004	2.51
Franciscana (<i>Pontoporia blainvillei</i>)	221	34859	1.67
Pygmy Sperm Whale (<i>Kogia breviceps</i>)	1012	305000	1.78
Dwarf Sperm Whale (<i>Kogia simus</i>)	622	168500	1.63
Sperm Whale (<i>Physeter macrocephalus</i>)	8028	35833330	0.58
Cuvier's Beaked Whale (<i>Ziphius cavirostris</i>)	2004	2273000	0.92
Gervais' Beaked Whale (<i>Mesoplodon europaeus</i>)	2149	1465000	2.11
Blainville's beaked whale (<i>Mesoplodon densirostris</i>)	1425	770500	1.39
Beluga Whale (<i>Delphinapterus leucas</i>)	2083	636000	2.24
Narwhal (<i>Monodon monoceros</i>)	2997	1578330	1.76
Fin Whale (<i>Balaenoptera physalus</i>)	7085	38421500	0.49
Blue Whale (<i>Balaenoptera musculus</i>)	3636	50904000	0.21
Humpback Whale (<i>Megaptera novaeangliae</i>)	6411	39295000	0.44

Sources of data are Marino (1998) and Marino et al. (2004).

*Those species whose EQ (as well as brain-body residual) is statistically significantly higher than that of anthropoid primates and the other cetacean species.

EQs of mysticetes are all substantially below 1 (Marino, 2002a) because of nonlinearities in EQ for very large animals. However, the large absolute sizes, high degrees of cortical convolutedness, and highly derived morphology establish that mysticete brains have, in addition to odontocete brains, undergone substantial enlargement and elaboration during the course of their evolution (Oelschläger and Oelschläger, 2002). Table 1 displays average brain weight, average body weight, and EQ based on the formula derived by Jerison (1973) for 26 species of modern odontocetes and 3 mysticetes with sexes combined.

The cetacean telencephalon is arranged into three concentric tiers of limbic, paralimbic, and supralimbic tissue. The high degree of cortical gyrification and resulting expansive surface area of approximately 3,745 cm² is unsurpassed among mammals, including humans (Ridgway and Brownson, 1984). Cerebral enlargement in cetaceans occurs most exuberantly in the parietal and temporal regions. Whereas primate brains feature large frontal lobes, no homologous frontal lobe region in the cetacean brain has been identified, leading many investigators to substitute the term “orbital lobe” for “frontal lobe” when referring to these modest, ventrally oriented hemispheric regions (Morgane et al., 1980). From a functional standpoint, a few electrophysiological mapping studies of cetacean cortex places primary visual cortex on the vertex of the hemisphere in the lateral gyrus and the primary auditory cortex lateral and directly adjacent to it in the suprasylvian gyrus. Secondary auditory cortex lies lateral to the primary auditory field in the medial ectosylvian gyrus (Supin et al., 1978) and somatosensory and motor cortices lie immediately adjacent and rostral to the visual and auditory regions (Lende and Akdikmen, 1968; Lende and Welker, 1972; Ladygina et al., 1978). Thus, the large mass and unusual surface configuration of cetacean brains sets the context for an interesting array of characteristics at the cytoarchitectural level. In this article, we explore several unusual features of cetacean neocortical architecture.

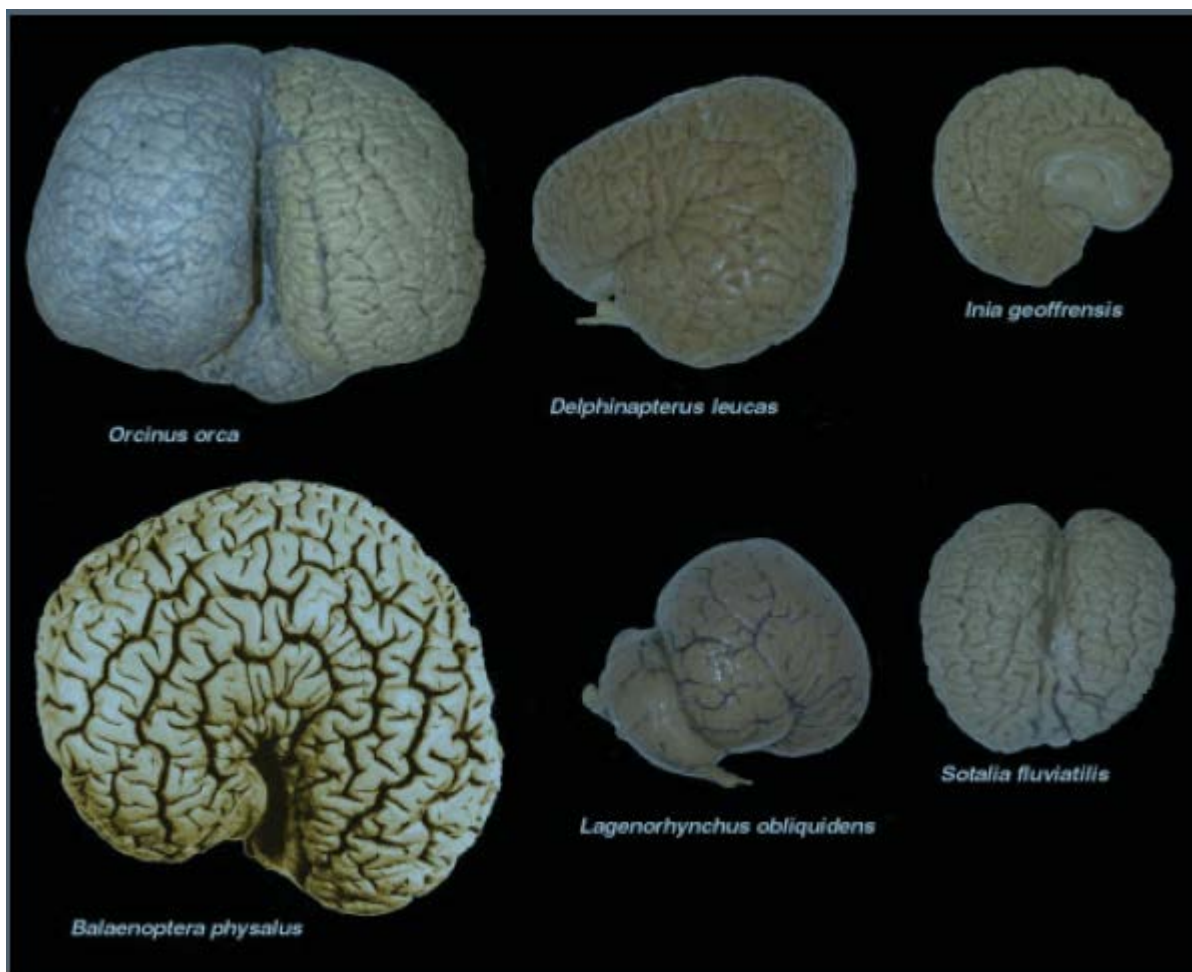
MATERIALS AND METHODS

The brains of three adult male bottlenose dolphins (*Tursiops truncatus*) were analyzed in detail in this study. These specimens were perfused by gravity with 40 l of Windle's fluid in situ using a cannula inserted into the descending aorta in animals that had been euthanized for medical reasons. The brains were then extracted and post-fixed in 8% formalin for 3 months (Jacobs et al., 1971, 1979). The brains were then dehydrated in graded alcohol solutions, embedded in celloidin, and cut serially at 35 µm on a modified large specimen microtome (Mico Instruments, Cambridge, MA). Each brain was cut in one of three planes (coronal, sagittal, horizontal) relative to the beak-fluke axis of the animal. Two 1:5 series of adjacent sections throughout these brains were stained for myelin with the Loyez-Weigert method or for Nissl substance with the Bielchowsky-Plien cresyl violet method (Bertrand, 1930). The sections were mounted on large glass slides and coverslipped in clarite for examination.

Additional adult specimens from *Tursiops*, a beluga whale (*Delphinapterus leucas*), a long-finned pilot whale (*Globicephala melas*), and a Cuvier's beaked whale (*Ziphius cavirostris*) were used for comparison across a few species. These specimens were obtained from stranded animals within a few hours of death and were fixed by immersion in neutral formalin for several months. Local samples of the regions corresponding to the primary visual and primary auditory cortex [i.e., from the mid-posterior portion of the lateral gyrus and the mid-posterior region of the supra-sylvian gyrus, respectively (Sokolov et al., 1972; Supin et al., 1978; Morgane et al., 1988)] were obtained from these cases, cryoprotected in graded sucrose solution, and cut on a cryostat (Reichert Jung, Vienna, Austria) at 60 µm and series of sections were then stained with cresyl violet.

All histological preparations were examined on a Zeiss Axiophot 2 photomicroscope with 5X, 10X, and 20X Fluor and Apochromat objectives (Zeiss, Oberkochen, Germany). Photomicrographs were acquired using a 10X PlanApochromat lens and an Optronics Microfire digital camera (Optronics, Goleta, CA). Photomontages were digitally assembled with Virtual Slice software (MicroBrightField, Williston, VT) and processed with Adobe Photoshop CS 8.0. The nomenclature of gyri and sulci follows that proposed by Morgane et al. (1980).

Fig. 1. Views of brains from different cetacean species. Clockwise from lower left, lateral view of the left hemisphere of the fin whale brain (*Balaenoptera physalus*), posterior superior view of the killer whale brain (*Orcinus orca*), lateral view of the left hemisphere of the beluga whale brain (*Delphinapterus leucas*), mid-sagittal view of the brain of an Amazon river dolphin (*Inia geoffrensis*), frontal superior view of the brain of a tucuxi (*Sotalia fluviatilis*), and lateral view of the right hemisphere of an Atlantic white-sided dolphin (*Lagenorhynchus obliquidens*). The brains are not represented to scale. See Table 1 for details on size.

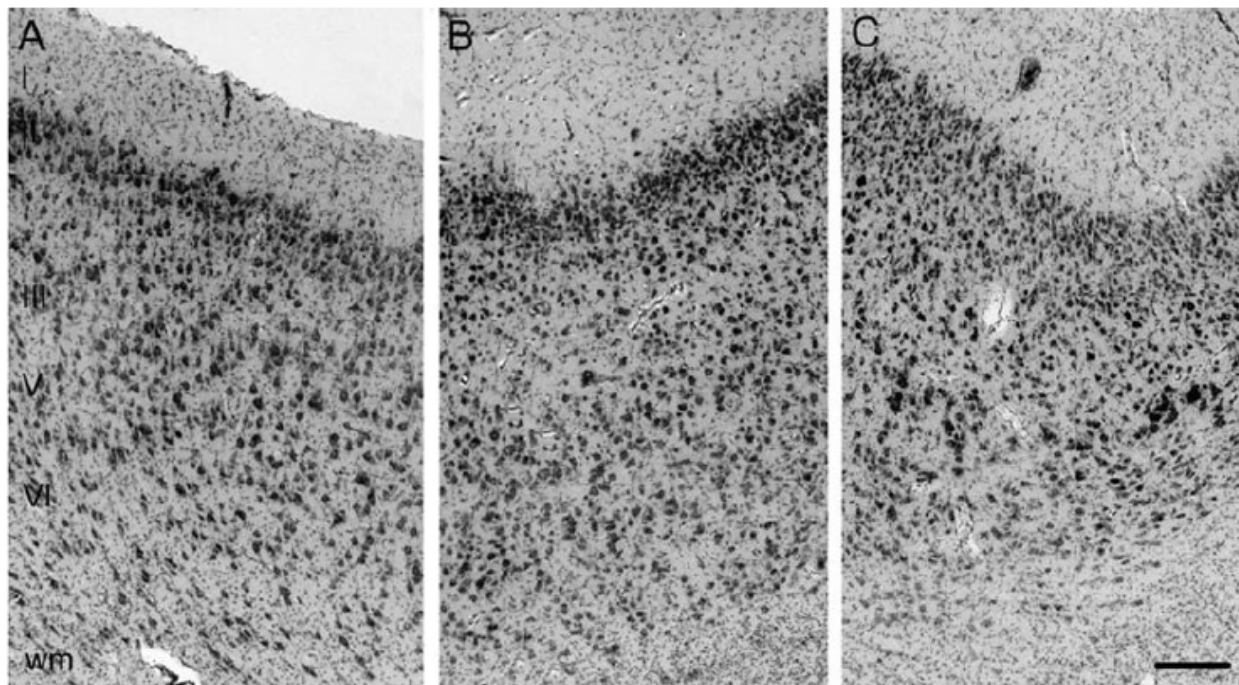


RESULTS

The histological organization of the cetacean cerebral cortex presents several unique features and appears complicated owing to the inordinate number of gyri and sulci that characterize the brains in these species (Fig. 1). In spite of a pervasive notion that neocortical structure is rather uniform throughout the cortical mantle with minor local variations, it is in fact quite complex, with a degree of regional parcellation at least comparable to that of large-brained terrestrial mammals such as anthropoid primates, carnivores, and ungulates. Short of a few studies, generally describing only restricted regions (Kojima, 1951;

Kesarev, 1969; Kesarev and Malofeeva, 1969; Jacobs et al., 1971, 1979; Kesarev et al., 1977; Morgane et al., 1982, 1988; Garey et al., 1985; Manger et al., 1998), the regional organization of the cerebral cortex in cetaceans remains poorly understood. Overall, the dolphin neocortex is thin and characterized by a general absence of granularity, a very prominent, thick layer I, which is far more cellular than in most terrestrial species, the presence of large, atypical neurons in the dense layer II, and very large pyramidal neurons frequently forming clusters at the border between layers III and V (layer IIIc/V). Layers III and VI vary considerably in thickness and cellular density across regions. Here we summarize some preliminary observations on the major neocortical domains in the bottlenose dolphin and a few comparisons with other species of the primary visual and auditory cortices. The archicortex, paleocortex, and cingulate cortex of dolphins have been described in detail by Jacobs et al. (1971, 1979) and by Morgane et al. (1982).

Fig. 2. Examples of cytoarchitecture in the frontal cortex of the bottlenose dolphin. A: Lateral orbital gyrus. B: Posterior level of the gyrus preureus. C: Cortex on the lateral bank of the cruciate sulcus, possibly corresponding to a motor field. Layers are indicated by Roman numerals. wm, white matter. Scale bar (on C) = 100 μ m.



Frontal Region

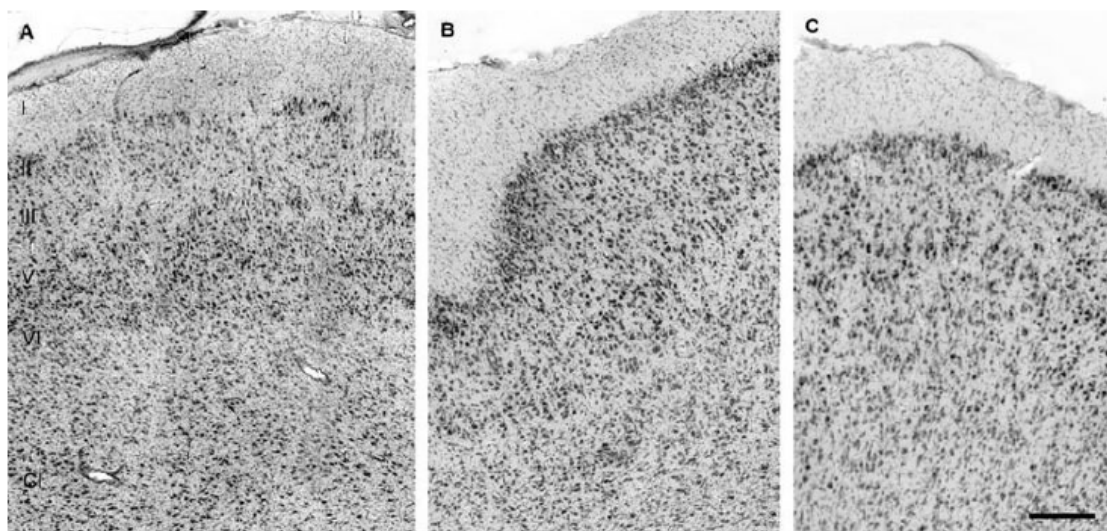
The anterior aspects of the lateral and medial orbital gyri are characterized by a well-defined laminar pattern with rather small pyramidal neurons in layers III and V (Fig. 2A). Layer II is very dense and thick. Posteriorly toward the olfactory lobe, the cortex becomes less differentiated with an increase in small neurons in the deep layers and considerable spacing of neurons in layer III, especially toward the junction with the ventral anterior insular cortex. At this level, layer II thins out and fragments into small islands. Medially, the cortex is thin on the gyrus preureus and adjacent gyri toward the pole of the frontal region, with fairly regularly distributed medium-sized pyramidal neurons in layer V. At more posterior levels, the cortex loses its lamination progressively and contains neurons of a smaller size (Fig. 2B), becoming a para-olfactory cortex in the subgenual region with no discernible laminar patterns. The frontopolar region, including the medial orbital gyrus and the cortex within most of the coronal gyrus, displays a cytoarchitecture comparable to the anterior orbital cortex. The dorsal and lateral aspects of the frontal

region include the anterior reaches of the paralimbic cleft (entolateral sulcus), cruciate sulcus, and the superior lateral sulcus. These regions exhibit considerable diversity of cytoarchitecture. However, a dominant theme is the presence of large to gigantic pyramidal cells in many of these fields, comparable to the description of a putative motor cortex in the sperm whale (Kojima, 1951). In the cortex of the cruciate sulcus, there is a gigantocellular region with very large layer V pyramidal cells occurring alone or in small groups of 2–3 cells, along with cell-dense layers II and III (Fig. 2C). The cortex along the paralimbic cleft shows very large layer V pyramidal cells that tend to form larger clusters than in the cruciate sulcus. It is possible that these giganto-pyramidal fields represent, on the basis of their layer V cell size and their topographic localization, the homologues of the primary, secondary, and supplementary motor cortices of other mammals. The anterior portion of the superior lateral sulcus is quite heterogeneous with regions showing similar characteristics as the large-celled field of the paralimbic and cruciate cortex, with a high degree of clustering, whereas other regions have slender and denser, elongated layer V pyramidal neurons.

Insular Cortex

The insular lobe of the dolphin contains a variable number of transverse and vertical gyri (up to 16 in our specimens) that exhibit at least three major patterns of cytoarchitectural organization. The posterior and superior third of the insula and retroinsular region is characterized by a well-laminated type of cortex that is comparable to the immediately adjacent cortices in the lateral aspect of the sylvian cleft (Fig. 3A), with a thin, dense layer II, large pyramidal cells in layers III and V, but relatively small and nonclustering layer IIc/V pyramids. The middle third of the insular cortex shows a less differentiated cortical lamination than its posterior region, with a large field of vertically organized modules that span all cortical layers from the very thin layer II to the white matter (Fig. 3B). The neuronal density between these modules is very sparse. Anteriorly and ventrally, the insular cortex is characterized by the appearance in layer II of large clusters of small neurons (Manger et al., 1998) resembling the layer II islands traditionally seen in the mammalian entorhinal cortex (Fig. 3C). This pattern is especially distinguishable where the basal ganglia encroaches the lateral surface of the brain. The other layers are not as identifiable and contain moderately large pyramidal cells and layers V and VI mix with claustral neurons. The insular cortex extends rostrally along the basal ganglia and merges with the orbital cortex, at which level the layer II islands become smaller and less conspicuous as the cortex thins out ventrally.

Fig. 3. Cytoarchitecture of the bottlenose dolphin insula. **A:** Posterior superior region. **B:** Middle third of insula. **C:** Anterior inferior part of insula with typical layer II islands. Layers are indicated by Roman numerals. Cl, claustrum. Scale bar (on C) = 100 μ m.



Temporoparietal Region

The temporoparietal region of the cetacean brain constitutes the largest extension of neocortical surface. It is organized in several roughly parallel deep sulci around the somewhat verticalized sylvian cleft. Although there are many species-specific variations in the gyrification patterns, generally three major sulci can be recognized around the sylvian cleft, namely, the inferior lateral fissure (ectosylvian sulcus), the intermediate lateral fissure (suprasylvian sulcus), and the superior lateral fissure (ectolateral sulcus). These sulci define major gyri: the lateral gyrus superiorly, and moving laterally on the hemisphere, the ectolateral, suprasylvian, and ectosylvian gyri. The cortex in each of these gyri includes a rich diversity of cytoarchitectonic fields in either their superior (above and anterior to the sylvian cleft) or their inferior extent (below and posterior to the sylvian cleft). Such a pattern is reminiscent of the organization of the visual and auditory primary fields and their associated regions in carnivores, such as cats, in which the functional organization of these fields is well known. The localization of such fields or even their homology to the situation in other species is, however, uncertain in cetaceans, as only the primary auditory cortex has been located with any certainty in the suprasylvian gyrus and the primary visual cortex in the lateral gyrus. It is possible that as many as 10 different cytoarchitectural fields exist in the rostrocaudal extent of both the suprasylvian and the ectosylvian cortex.

A large expanse of cortex in the mid-posterior segment of the lateral gyrus corresponds to the primary and probably secondary visual cortex (Sokolov et al., 1972; Supin et al., 1978; Garey et al., 1985; Morgane et al., 1988). This region is characterized by the presence of midsized pyramidal neurons in layer V compared to adjacent fields in the paralimbic cleft and to a certain degree of granularity owing to the presence of small cells in layer III. Anterior and lateral to it lies the possible somatosensory cortex that contains small cell in all layers and small clusters of layer V pyramids of about 2–3 cells each (Fig. 4A). This comparatively small-celled region abuts the gigantocellular motor regions further rostrally in the frontal cortex. The cortex of the suprasylvian gyrus contains the primary auditory cortex. This region displays clear clusters of large pyramidal cells in layer V distributed with regularity. Of note, the clusters and the pyramidal cells of the auditory cortex are larger than those in the primary visual cortex and putative somatosensory cortex. Laterally in the superior portion of the ectosylvian gyrus, a thinner cortex is present with increased modularity identifiable by large aggregates of neurons below layer II and smaller cells (Fig. 4B). The modules are separated by wide gaps of white matter possibly corresponding to the projection of subcortical afferents to a region that may be related to auditory function. A similar pattern of modular organization is seen in the superior aspect of the cortex within the sylvian cleft. Ventrally toward the pole of the temporal lobe, the cortex exhibits thinner columns of cells with intercalated bundles and increased density of neurons in all layers. The temporopolar region shows medially a fragmented layer II with islands similar to those seen in the insula, and a less well-defined lamination overall, with relatively small layer V cells (Fig. 4C). Posteriorly, the inferior temporal cortex contains a progressively less differentiated cortex medially toward the entorhinal cortex, with layers III through VI having neurons of about the same size with no evidence of grouping in the deep layers. In contrast, the lateral aspect of the inferior temporal lobe (i.e., the inferior ectosylvian cortex) shows distinct clustering in layer V, although these pyramidal cells remain comparatively small in this region (Fig. 4D). Neurons become larger in the cortex of the inferior aspect of the sylvian cleft but the cortex is rather thin in these regions compared to frontal and parietal regions. These general patterns are seen along much of the rostrocaudal extent of the gyri, with local variations in cortical thickness and density of neurons that may identify a large number of individual cortical fields. This issue will, however, require a detailed and systematic study.

Posterior Polar Region

The posterior (occipital) cortex is carved by three major sulci, the paralimbic cleft that defines the posterior aspect dorsally of the lateral gyrus, the superior lateral fissure (ectolateral sulcus), and laterally

the intermediate lateral fissure (suprasylvian sulcus). Medially it contains the lingual lobule, bounded anteriorly by the calcarine sulcus. The cortex of the lateral gyrus is slightly thicker than on the adjacent gyri and contains very well-defined clusters of midsized pyramidal neurons in layer V and dense layers II and III. It is reminiscent of the organization seen in the primary visual cortex more rostrally in the lateral gyrus and may represent an accessory visual cortex. This pattern changes laterally along the suprasylvian gyrus, where the neurons in layer V appear smaller and less clustered and the granularity of layers II and III increases (Fig. 5A). Further laterally in the posterior ectosylvian cortex, a strong columnar pattern becomes apparent with vertical aggregates of neurons, 5–10 cells wide, spanning layers III through VI and separated by thin gaps that may represent ascending axonal bundles (Fig. 5B). This pattern is seen with relative regularity where the cortex is not cut tangentially by the plane of section, downward to the inferior tip of the posterior temporoparietal cortex. The medial and ventral aspect of the posterior polar cortex is relatively thin and shows a more regular arrangement of neurons in layer V, with no or low degree of clustering and slender neurons in the more ventral aspects of the lingual lobule. Dorsally toward the paralimbic cleft, larger neurons are present in layer V with an increased cellularity of layers II and III.

Fig. 4. Cytoarchitecture of the bottlenose dolphin temporoparietal operculum. **A:** Anterior suprasylvian cortex, possibly corresponding to the somatosensory region. **B:** Superior ectosylvian cortex. **C:** Lateral temporopolar region. **D:** Inferior ectosylvian cortex. Layers III and V are identified. Scale bar (on D) = 100 μm .

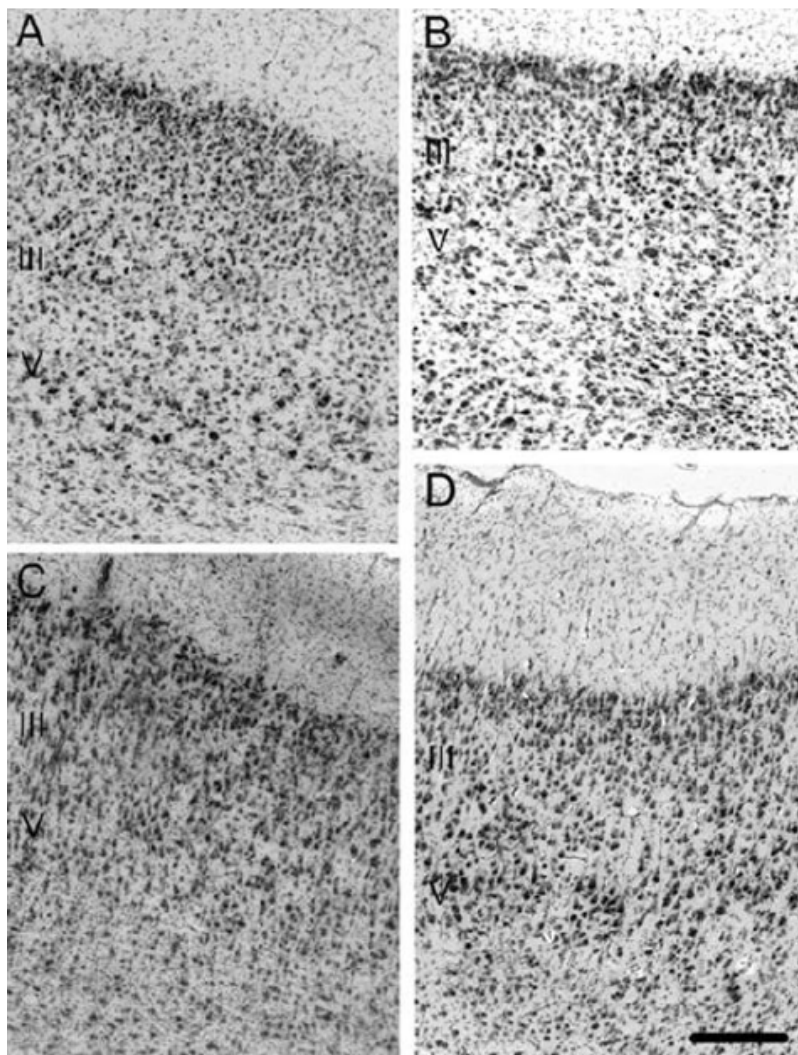
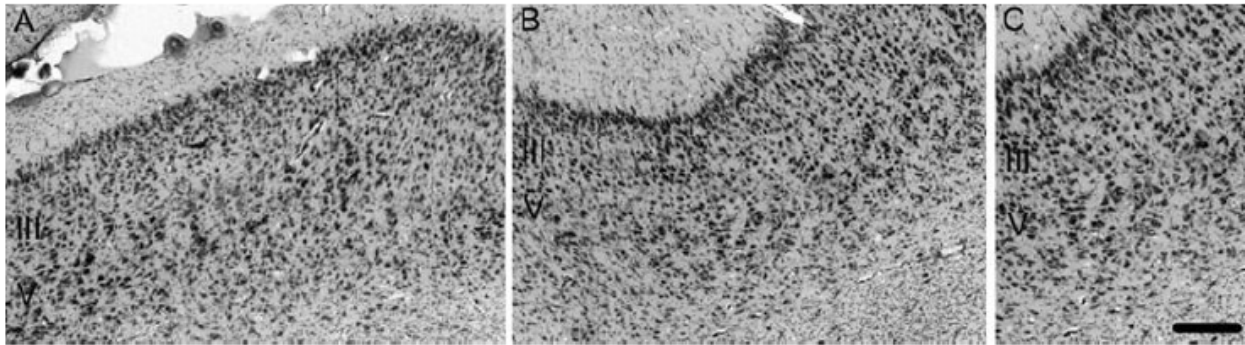


Fig. 5. Cytoarchitecture of the posterior polar region and oval lobule. **A:** Posterior suprasylvian cortex. **B:** Posterior ectosylvian cortex. **C:** Cortex at mid-level of the oval lobule. Layers III and V are identified. Scale bar (on C) = 100 μm .



Oval Lobule

The oval lobule is bounded ventrally by the limbic cleft, anteriorly by the superior marginal sulcus, dorsally by the paralimbic cleft, and posteriorly by the possible homologue of the calcarine sulcus. Its cortex is characterized by a well-demarcated layer II and a dense layer III containing small pyramidal cells. Layer V contains regularly spaced clusters of medium-sized pyramidal cells throughout most of the lobule (Fig. 5C). These neurons become smaller and less grouped posteriorly toward the junction with the lingual lobule. Dorsally, the pattern merges with the cortex within the paralimbic cleft, where the layer V pyramidal cells appear progressively larger and darker, and where the cellular density and thickness of layer II increase, rendering the layer II/III border difficult to assess.

Cingulate and Retrosplenial Cortex

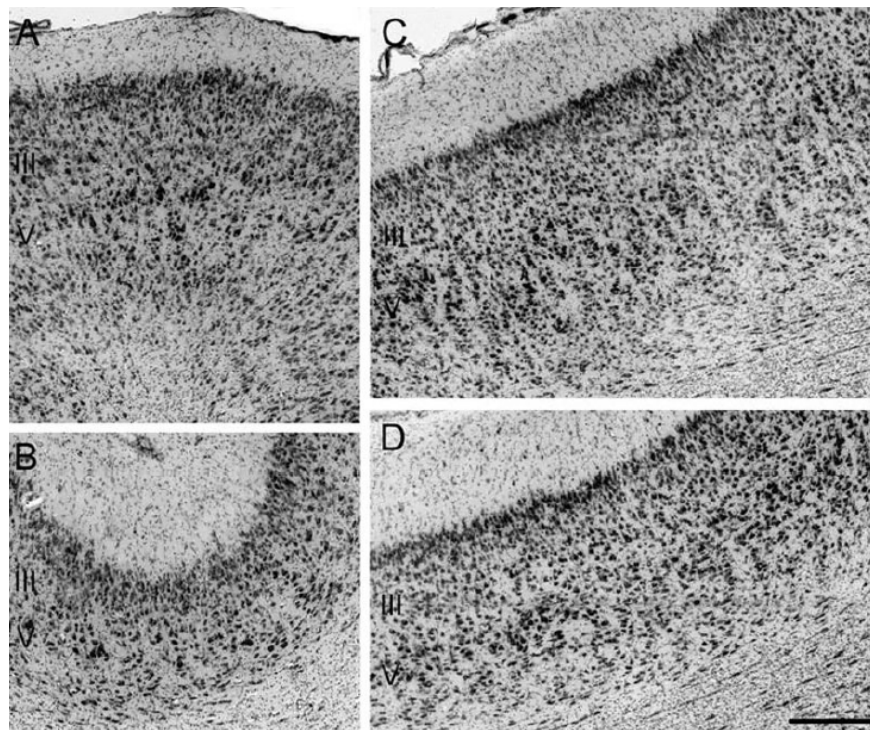
It is convenient to subdivide the limbic lobe into five distinct domains or lobules (Morgane et al., 1982): the parolfactory (subgenual) lobule; the supracallosal lobule that contains the cingulate cortex proper with its pregenual, anterior, and posterior divisions; the retrosplenial lobule with its anterior and posterior divisions; and the temporal region (parahippocampal lobule and hippocampal formation). The posterior aspect of the subgenual cortex lacks distinct layers. At the more anterior level of the root of the internal intercalate limbic sulcus, the cortex widens and the layers are clearly apparent with a patchy thin layer II and a small-celled layer V (Fig. 6A). The pregenual region is marked by the appearance of two distinct sulci (the internal and external intercalate limbic sulci) that course throughout the rostrocaudal extent of the supracallosal lobule. A limbic cleft limits the limbic lobe dorsally. As in other mammals, the supracallosal cortex of the limbic lobe abuts the callosal sulcus with the induseum griseum and the subicular remnant. The cortex then becomes clearly laminated, with thin layers and densely packed small cells. The cortex remains parvocellular around the full extent of the internal intercalate gyrus. In the external intercalate gyrus, layer V becomes progressively thicker with larger cells that tend to cluster and form modular elements spanning layers V and VI (Fig. 6B). This pattern is best appreciated in the anterior and posterior domains of the cingulate cortex. Dorsal to the external intercalate cortex, a transition (marginal) zone exists that shows increased cellularity of the deep layers anteriorly, with larger pyramidal cells than in the intercalate cortex, and posteriorly a clear modular pattern in layers V and VI until it abuts the retrosplenial cortex at the inferior marginal sulcus. Dorsally, within the limbic cleft, the marginal cortex borders the cortex of the paralimbic lobe, which is at these levels characterized by prominent, large pyramidal cells in layer V, forming clusters and what resembles the patterns observed in the frontal lobe, particularly in the cruciate sulcus.

The retrosplenial cortex occupies the region between the callosal sulcus and the limbic cleft. The cortical plate next to the subiculum shows a progressive differentiation of layers with a cytoarchitecture radically different from that seen in the supracallosal lobule. The cortex that faces the corpus callosum is characterized by a thin layer II and a relatively cell-poor layer III, but the neurons in that layer aggregate in somewhat regular groups of neurons defining a tiling pattern, which disappears medially toward the tip of the gyrus (Morgane et al., 1988). The cortex lining the variable retrosplenial intercalate sulcus presents a more usual clustering of rather small layer V pyramidal cells (Fig. 6C). Dorsally toward the limbic cleft, the marginal retrosplenial zone shows a thicker cortex, with a better-defined layer II and larger pyramidal cells in layer V (Fig. 6D). These features remain fairly constant throughout the anteroposterior extent of the retrosplenial cortex. Dorsally the marginal zone abuts the cortex of the oval and lingual lobules.

Hippocampal Formation and Parahippocampal Cortex

Cetaceans have an extensive parahippocampal cortex, very large subicular complex, and a comparatively diminutive hippocampus proper and dentate gyrus (Fig. 7A). All fields traditionally described in other mammals exist with few variations in their architecture, except for the very small size of the dentate gyrus, whose granule cell layer is frequently reduced to a small smooth layer. The hippocampal fields are also somewhat small compared to the size of the subiculum. The entorhinal cortex can be divided into at least four lateral subregions in the dorsal lip of the parahippocampal sulcus, a medial field between the lateral field and the periamygdaloid cortex anteriorly and the pre-/parasubiculum posteriorly, and a posterior field. It has very clear layer II islands in the medial field and a well-defined lamina dissecans throughout (Fig. 7B) that makes its border with the inferior temporal neocortex unequivocal.

Fig. 6. Cytoarchitecture of the cingulate cortex and retrosplenial cortex. **A:** Anterior level of the anterior segment of the cingulate cortex through the internal intercalate sulcus. **B:** Mid-level of the anterior segment of the cingulate cortex through the external intercalate sulcus. **C:** Anterior level of the anterior segment of the retrosplenial cortex through the internal intercalate sulcus. **D:** Mid-level of the anterior segment of the retrosplenial cortex through the external intercalate sulcus. Layers III and V are identified. Scale bar (on D) = 100 μm .



Comparison of Areas V1 and A1 in Four Odontocete Species

In addition to a considerable variety of cytoarchitectural patterning in the cetacean brain, there exist differences in cortical organization among species for specific cortical regions. Here we provide a few examples based on the observation of the primary visual (area V1) and primary auditory (area A1) cortex in odontocete species, three delphinids, the bottlenose dolphin, the beluga whale, the long-finned pilot whale, and a ziphiid, Cuvier's beaked whale (Fig. 8). The interspecies differences concern mainly the size of layer V cells and the pattern of aggregation of neurons in layers V–VI. In both areas, the bottlenose dolphin and the beluga whale (Fig. 8A, B, E, and F) are characterized by fairly large neurons in layer V, while these neurons are fewer or smaller in the pilot whale (Fig. 8C and G) and the beaked whale (Fig. 8D and H). Also, these large pyramidal cells appear to be more clustered in the bottlenose dolphin than they are in the other species. The pilot whale exhibits very clear grouping of large numbers of cells in layer V and VI with relatively large cell-poor gaps in both areas, whereas such pattern is poorly defined or absent in the other species. The ziphiid species also shows a less prominent layer II (Fig. 8D and H). Whether this is a generalized phenomenon across the whole neocortex in the beaked whale cannot be established on our materials. That such differences exist in primary sensory regions across cetacean species suggests that larger variations in cortical organization are likely to occur in the association cortex and reflect taxon-specific functional specializations.

Fig. 7. Organization of the hippocampus and medial entorhinal cortex. **A:** The hippocampus proper (CA1 and CA3 fields) is relatively small and the dentate gyrus (DG) appears unfolded compared to the situation in other mammals. In contrast, there is a large subicular complex that extends over the adjacent gyrus at right. ProS, prosubiculum; SUB, subiculum; hf, hippocampal fissure. The medial-most region of the entorhinal shown here displays the typically large lamina dissecans (ld) and layer II islands (wm, white matter). Roman numerals on **B** indicate entorhinal cortex layers. The junction with the subicular complex (parasubiculum) occurs at the left side of the photomicrograph. Scale bar (on B) = 100 μm .

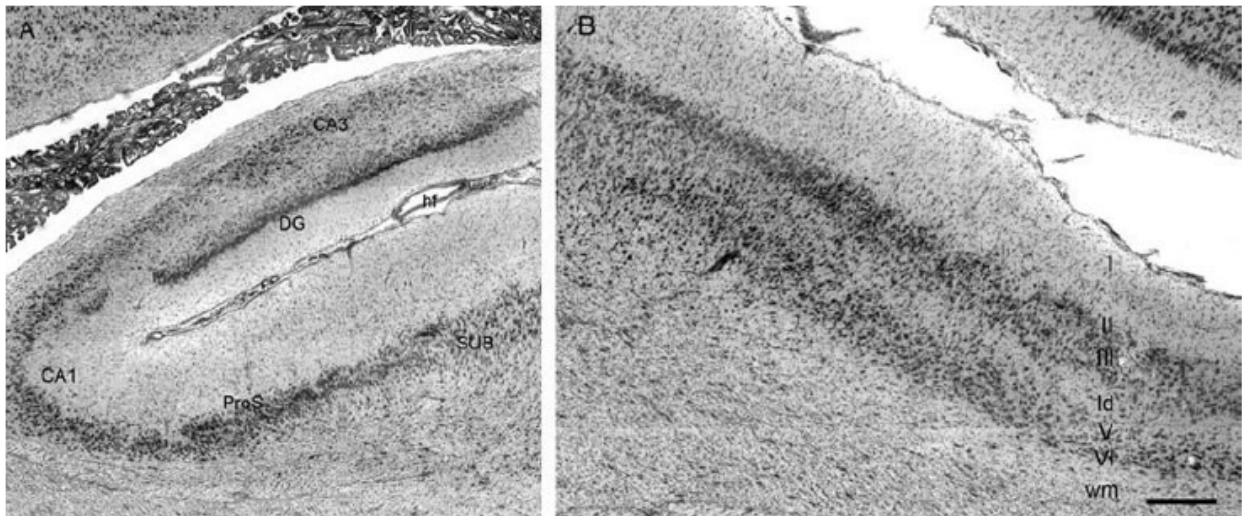
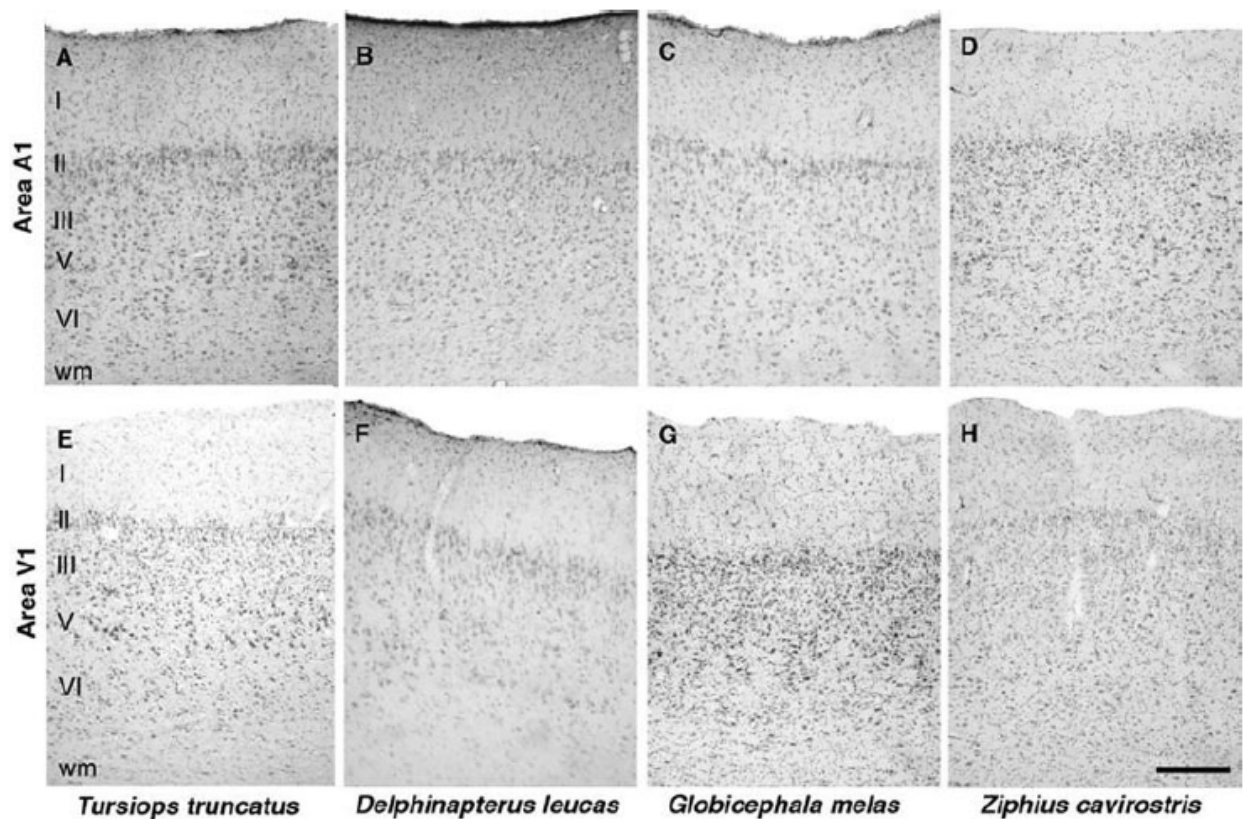


Fig. 8. Comparative cytoarchitecture of the primary auditory cortex (A–D) and primary visual cortex (E–H) in *Tursiops truncatus* (bottlenose dolphin; A and E), *Delphinapterus leucas* (beluga whale; B and F), *Globicephala melas* (long-finned pilot whale; C and G), and *Ziphius cavirostris* (Cuvier's beaked whale; D and H). Note the differences in cellular composition of the two primary regions across these species. Layers are indicated by Roman numerals. wm, white matter. Scale bar (on H) = 100 μ m.



DISCUSSION

For several decades, the common view has been that cetacean cortex is homogeneous and fairly non-differentiated in character. This view, exemplified by Kesarev (1971, 1975), has engendered much hypothesizing about where the structural (and functional) complexity of cetacean brains lies if not in highly modified and differentiated cortical units as is the case in primates (Glezer et al., 1988). This view of cetacean neocortex as a rather indistinct and also sparse structure had implications for views of cetacean cognitive complexity and intelligence. On the one hand, the apparent uniformity of the neocortex implied a low level of behavioral complexity in cetaceans (Gaskin, 1982; Aronson and Tobach, 1988). On the other hand, the homogeneous nature posed a perplexing inconsistency to those that accepted the accruing evidence for considerable cognitive and behavioral complexity in cetaceans (Glezer et al., 1988; Marino, 2002b).

The present results, however, show that the cytoarchitectural patterns in cetaceans, at least based on the bottlenose dolphin, are far more varied and complex than generally thought. Although regions of less distinct lamination do exist, we identified numerous features of various neocortical regions that exemplify cytoarchitectural differentiation and diversity. Despite the modest gross morphological appearance of the frontal lobes, this region is distinctly laminated and comprises several cortical fields, as in the other lobes. The cytoarchitecture in the frontal lobe of cetaceans is indeed different from that of primates but it

displays its own unique pattern of differentiation. Also, highly distinct, vertically oriented modules are apparent in the insular cortex and posterior polar region, as well as in other areas, and such columnar or patchy modules vary considerably in length and width depending on the region considered. The patchiness or columnarity of these patterns may reflect the region-specific distribution of corticocortical or thalamocortical afferents and may be exaggerated in respect to other mammalian species due to the peculiar laminar organization of the neocortical mantle in cetaceans that lacks layer IV and exhibits a very thick layer I. As such, there appears to be a rich diversity of cytoarchitectural fields in most of the lobes, and the peculiar cortical cytoarchitecture in cetaceans may underlie different functional strategies for cortical processing compared to other mammals. Indeed, cetaceans, and especially odontocetes, have a highly developed auditory system and echolocation capabilities whose distribution and representation at the neocortical level are still poorly understood (Ridgway, 2000). Also, many aspects of cortical and subcortical connectivity are likely to differ in cetaceans from terrestrial species as exemplified by their unique patterns of sleep/wakefulness and hemispheric sleep regulation (Manger et al., 2003).

It is worth noting that the neocortex of cetaceans shares several cyto- and chemoarchitectural features with that of large terrestrial artiodactyls, consistent with the phylogenetic propinquity of these species (Nikaido et al., 1996; Shimamura et al., 1997; Gatesy, 1998; Gingerich and Uhen, 1998; Milinkovitch et al., 1998; Hof et al., 1999, 2000; Gingerich et al., 2001; Thewissen et al., 2001; Geisler and Uhen, 2003). From an evolutionary and developmental standpoint, in comparison to other mammals, both cetaceans and artiodactyls are born with early physical maturity, a key survival factor in the aquatic milieu and, for artiodactyls, to escape predation. The many specializations of the cetacean brain may also be related to the retention in adult forms of juvenile ancestral features, a phenomenon known as pedomorphosis. In cetaceans, possible pedomorphic features are the retention in adult stages of the pontine, mesencephalic and cephalic flexures found only in embryos in other mammals, and the very large brain size at birth (Glezer et al., 1998). Also consistent with pedomorphosis is the fact that the neocortex of cetaceans (and of large artiodactyls) is dominated by interneurons containing calbindin and calretinin, the calcium-binding proteins that appear first during development in rodents, carnivores, and primates (Hof et al., 1999), which may reflect the persistence in adults of an apparently less differentiated cortex compared to other mammals. Our findings reveal nonetheless that there are potentially as many neocortical regions that can be identified by cytoarchitectural criteria in cetaceans as in other mammals such as primates and carnivores. Part of the explanation for why cetacean neocortex has historically been considered so much less interesting and well organized is because of limitations on the number of species studied, the number of regions sampled, and methodological constraints owing to the large size of most specimens.

The present evidence for considerable complexity in cetacean neocortex, particularly when coupled with the large size of these brains, is clearly consistent with the large body of evidence for behavioral and social complexity in cetaceans (for a review, see Marino, 2002b). Decades of work on learning, memory, and artificial language comprehension have shown that bottlenose dolphins are at least as capable as chimpanzees in these domains (for a review, see Herman, 2002). Bottlenose dolphins have also demonstrated rare abilities related to self-awareness, such as mirror self-recognition (Reiss and Marino, 2001) and self-monitoring (Smith et al., 2003). Furthermore, many cetaceans, such as bottlenose dolphins, sperm whales, and killer whales exhibit complex social patterns that include coalition formation, cooperation, cultural transmission (Connor et al., 1992; Rendell and Whitehead, 2001), and tool use (Krutzen et al., 2005). Therefore, it is expected that these abilities would be underwritten by a similar level of complexity in brain organization in cetaceans as they are in primates. What remains a compelling question for future study, however, is how such dissimilar neocortical cytoarchitectural motifs, such as that found in cetaceans and primates, result in convergent cognitive and behavioral characteristics, and why.

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LITERATURE CITED

- Aronson LR, Tobach E. 1988. Conservative aspects of the dolphin cortex match its behavioral level. *Behav Brain Sci* 11:89–90.
- Barnes LG, Domning DP, Ray CE. 1985. Status of studies on fossil marine mammals. *Mar Mamm Sci* 1:15–53.
- Bertrand I. 1930. *Techniques histologiques de neuropathologie*. Paris: Masson.
- Connor RC, Smolker RA, Richards AF. 1992. Dolphin alliances and coalitions. In: Harcourt AH, deWaal FBM, editors. *Coalitions and alliances in humans and other animals*. Oxford: Oxford University Press. p 415–443.
- Garey LJ, Winkelman E, Brauer K. 1985. Golgi and Nissl studies of the visual cortex of the bottlenose dolphin. *J Comp Neurol* 240:305–321.
- Gaskin DE. 1982. *The ecology of whales and dolphins*. London: Heinemann
- Gatesy J. 1998. Molecular evidence for the phylogenetic affinities of cetacea. In: Thewissen JGM, editor. *The emergence of whales*. New York: Plenum Press. p 63–111.
- Geisler JH, Uhen MD. 2003. Morphological support for a close relationship between hippos and whales. *J Vert Paleontol* 23:991–996.
- Gingerich PD, Uhen MD. 1998. Likelihood estimation of the time of origin of cetacean and the time of divergence of cetacean and Artiodactyla. *Paleo-electronica* 2:1–47.
- Gingerich PD, ul Haq M, Zalmout IS, Khan IH, Sadiq M. 2001. Origin of whales from early artiodactyls: hands and feet of Eocene Protocetidae from Pakistan. *Science* 293:2239–2242.
- Glezer II, Jacobs MS, Morgane PJ. 1988. Implications of the “initial brain” concept for brain evolution in Cetacea. *Behav Brain Sci* 11:75–116.
- Herman LM. 2002. Exploring the cognitive world of the bottlenosed dolphin. In: Bekoff M, Allen C, Burghardt GM, editors. *The cognitive animal: empirical and theoretical perspectives on animal cognition*. Cambridge, MA: MIT Press. p 275-283.
- Hof PR, Glezer II, Conde´ F, Flagg RA, Rubin MB, Nimchinsky EA, Vogt Weisenhorn DM. 1999. Cellular distribution of the calcium-binding proteins parvalbumin, calbindin, and calretinin in the neocortex of mammals: phylogenetic and developmental patterns. *J Chem Neuroanat* 16:77–116.
- Hof PR, Glezer II, Nimchinsky EA, Erwin JM. 2000. Neurochemical and cellular specializations in the mammalian neocortex reflect phylogenetic relationships: evidence from primates, cetaceans, and artiodactyls. *Brain Behav Evol* 55:300–310.

- Jacobs MS, Morgane PJ, McFarland WL. 1971. The anatomy of the brain of the bottlenose dolphin (*Tursiops truncatus*). Rhinic lobe (rhinencephalon): I, the paleocortex. *J Comp Neurol* 141:205–272.
- Jacobs MS, McFarland WL, Morgane PJ. 1979. The anatomy of the brain of the bottlenose dolphin (*Tursiops truncatus*): rhinic lobe (rhinencephalon): the archicortex. *Brain Res Bull* 4(Suppl 1):1–108.
- Jerison HJ. 1973. *Evolution of the brain and intelligence*. New York: Academic Press.
- Kesarev VS. 1969. Structural organization of the limbic cortex in dolphins. *Arkh Anat Gistol Embriol* 56:28–35.
- Kesarev VS, Malofeeva LI. 1969. Structural organization of the dolphin motor cortex. *Arkh Anat Gistol Embriol* 59:48–55.
- Kesarev VS. 1971. The inferior brain of the dolphin. *Soviet Sci Rev* 2:52–58.
- Kesarev VS. 1975. Homologization of the cerebral neocortex in cetaceans. *Arkh Anat Gistol Embriol* 68:5–13.
- Kesarev VS, Malofeeva LI, Trykova OV. 1977. Structural organization of the cerebral neocortex in cetaceans. *Arkh Anat Gistol Embriol* 73:23–30.
- Kojima T. 1951. On the brain of the sperm whale (*Physeter catodon L.*). *Sci Rep Whales Res Inst Tokyo* 6:49–72.
- Krutzen M, Mann J, Heithaus MR, Connor RC, Bejdar L, Sherwin WB. 2005. Cultural transmission of tool use in bottlenose dolphins. *Proc Natl Acad Sci USA* 102:8939–3943.
- Ladygina TF, Mass AM, Supin AI. 1978. Multiple sensory projections in the dolphin cerebral cortex. *Zh Vyssh Nerv Deiat* 28:1047–1054.
- Lende RA, Akdikmen S. 1968. Motor field in cerebral cortex of the bottlenose dolphin. *J Neurosurg* 29:495–499.
- Lende RA, Welker WI. 1972. An unusual sensory area in the cerebral cortex of the bottlenose dolphin, *Tursiops truncatus*. *Brain Res* 45:555–560.
- Manger P, Sum M, Szymanski M, Ridgway S, Krubitzer L. 1998. Modular subdivisions of dolphin insular cortex: does evolutionary history repeat itself? *J Cogn Neurosci* 10:153–166.
- Manger PR, Ridgway SH, Siegel JM. 2003. The locus coeruleus complex of the bottlenose dolphin (*Tursiops truncatus*) revealed by tyrosine hydroxylase immunohistochemistry. *J Sleep Res* 12:149–155.
- Marino L. 1998. A comparison of encephalization between odontocete cetaceans and anthropoid primates. *Brain Behav Evol* 51:230–238.
- Marino L. 2002a. Brain size evolution. In: Perrin WF, Wursig B, Thewissen JGM, editors. *Encyclopedia of marine mammals*. San Diego, CA: Academic Press. p 158–162.

- Marino L. 2002b. Convergence in complex cognitive abilities in cetaceans and primates. *Brain Behav Evol* 59:21–32.
- Marino L, McShea D, Uhen MD. 2004. The origin and evolution of large brains in toothed whales. *Anat Rec* 281A:1247–1255.
- Milinkovitch MC, Berube M, Palsboll PJ. 1998. Cetaceans are highly derived artiodactyls. In: Thewissen JGM, editor. *The emergence of whales*. New York: Plenum Press. p 113–131.
- Morgane PJ, Jacobs MS, MacFarland WL. 1980. The anatomy of the brain of the bottlenose dolphin (*Tursiops truncatus*): surface configurations of the telencephalon of the bottlenose dolphin with comparative anatomical observations in four other cetacean species. *Brain Res Bull* 5(Suppl 3):1–107.
- Morgane PJ, McFarland WL, Jacobs MS. 1982. The limbic lobe of the dolphin brain: a quantitative cytoarchitectonic study. *J Hirnforsch* 23:465–552.
- Morgane PJ, Glezer II, Jacobs MS. 1988. The lateral gyrus (visual cortex) of the dolphin: an image analysis study. *J Comp Neurol* 273:3–25.
- Nikaido M, Rooney AP, Okada N. 1996. Phylogenetic relationships among cetartiodactyls based on insertions of short and long interspersed elements: hippopotamuses are the closest extant relatives of whales. *Proc Natl Acad Sci USA* 96:10261–10266.
- Oelschläger HA, Oelschläger JS. 2002. Brains. In: Perrin WF, Wursig B, Thewissen JGM, editors. *Encyclopedia of marine mammals*. San Diego, CA: Academic Press. p 133–158.
- Reiss D, Marino L. 2001. Mirror self recognition in the bottlenose dolphin: a case of cognitive convergence. *Proc Natl Acad Sci USA* 98:5937–5942.
- Rendell L, Whitehead H. 2001. Culture in whales and dolphins. *Behav Brain Sci* 24:309–382.
- Ridgway SH, Brownson RH. 1984. Relative brain sizes and cortical surface areas of odontocetes. *Acta Zool Fenn* 172:149–152.
- Ridgway SH. 2000. Auditory central nervous system of dolphins. In: Au WWL, Popper AN, Fay RR, editors. *Hearing by whales and dolphins*. New York: Springer. p 273–293.
- Shimamura M, Yasue H, Ohshima K, Abe H, Kato H, Kishiro T, Goto M, Munechika I, Okada N. 1997. Molecular evidence from retroposons that whales form a clade within even-toed ungulates. *Nature* 388:666–671.
- Smith JD, Shields WE, Washburn DA. 2003. The comparative psychology of uncertainty monitoring and metacognition. *Behav Brain Sci* 26:317–373.
- Sokolov VE, Ladygina TF, Supin AY. 1972. Localization of sensory zones in dolphin brain cortex. *Dokl Akad Nauk SSSR* 202:490–493.
- Supin AY, Mukhametov LM, Ladygina TF, Popov VV, Mass AM, Poliakova IG. 1978. Neurophysiologic characteristics of the cerebral cortex in dolphins. In: *Electrophysiological study of dolphin brain*. Moscow: Akademiya Nauka SSSR. p 29–85.

Thewissen JGM, Madar SI, Hussain ST. 1996. *Ambulocetus natans*, an Eocene cetacean (Mammalia) from Pakistan. *Cour Forsch Inst Senckenberg* 191:1–86.

Thewissen JGM, Williams EM, Roe LJ, Hussain ST. 2001. Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature* 413:277–281.