ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

The neocortex of cetaceans: cytoarchitecture and comparison with other aquatic and terrestrial species

Camilla Butti,[1] Mary Ann Raghanti,[2] Chet C. Sherwood,[3] and Patrick R. Hof[1]

[1]Department of Neuroscience, Mount Sinai School of Medicine, New York, New York. [2]Department of Anthropology and School of Biomedical Sciences, Kent State University, Kent, Ohio. [3]Department of Anthropology, The George Washington University, Washington, DC

Address for correspondence: Camilla Butti, Department of Neuroscience, Mount Sinai School of Medicine, Box 1065, One Gustave L. Levy Place, New York, NY 10029. camilla.butti@mssm.edu

The evolutionary process of readaptation to the aquatic environment was accompanied by extreme anatomical and physiological changes in the brain. This review discusses cortical specializations in the three major lineages of marine mammals in comparison to related terrestrial and semiaquatic species. Different groups of marine mammals adopted a wide range of strategies to cope with the challenges of aquatic living. Cetaceans and hippopotamids possess a completely agranular neocortex in contrast to phocids and sirenians; vertical modules are observed in deep layers V and VI in manatees, cetaceans, phocids, and hippopotamids, but in different cortical areas; and clustering in layer II appears in the insular cortex of hippopotamids, phocids, and cetaceans. Finally, von Economo neurons are present in cetaceans, hippopotamids, sirenians, and some phocids, with specific, yet different, cortical distributions. The interpretation of the evolutionary and functional significance of such specializations, and their relationships with the degrees of adaptation to the aquatic environment and phylogeny, remain difficult to trace, at least until comprehensive data, including representative species from all of the major mammalian families, become available.

Keywords: cetaceans; neocortex; evolution

What are marine mammals?

Marine mammals are a highly diverse group of species that resulted from the reinvasion of the aquatic environment by terrestrial species. Marine mammals are fully or partially dependent on the aquatic environment for survival and include cetaceans (whales, dolphins, and porpoises), sirenians (manatees and dugongs), and some carnivores (sea otters, polar bears, and the pinnipeds, seals, sea lions, and walruses). The degree of adaptation to the aquatic environment is extremely variable, with cetaceans and the unrelated sirenians being the only two extant groups fully dependent on an aquatic lifestyle.

Phylogenetic position, degree of adaptation to the aquatic environment, and lifestyles all resulted in major differences in morphological and physiological adaptations, including those of the brain. Cetaceans evolved extremely large and convoluted brains that set them apart from most other mammals; sirenians represent a unique departure from the usual organization of the brain in large mammals, having a lissencephalic brain with only few prominent fissures; and marine carnivores possess a brain that is comparable, at least in external morphology and proportions, to that of large terrestrial carnivores (Fig. 1).

Origin and evolution of cetaceans, sirenians, and pinnipeds

According to the fossil record, cetaceans diverged from terrestrial mammals approximately 52 million years ago (mya).[1] Early cetaceans, the Archeocetes, were a group of semiaquatic nonecholocating and nonfilter-feeding animals inhabiting marine and fresh waters that arose from terrestrial mammals such as anthracotheres, raoellids, and mesonychids.[2] In the early Eocene between 45 and 53 mya, Archeocetes diversified into Pakicetidae, Ambulocetidae, and Remingtonocetidae, all inferred to be
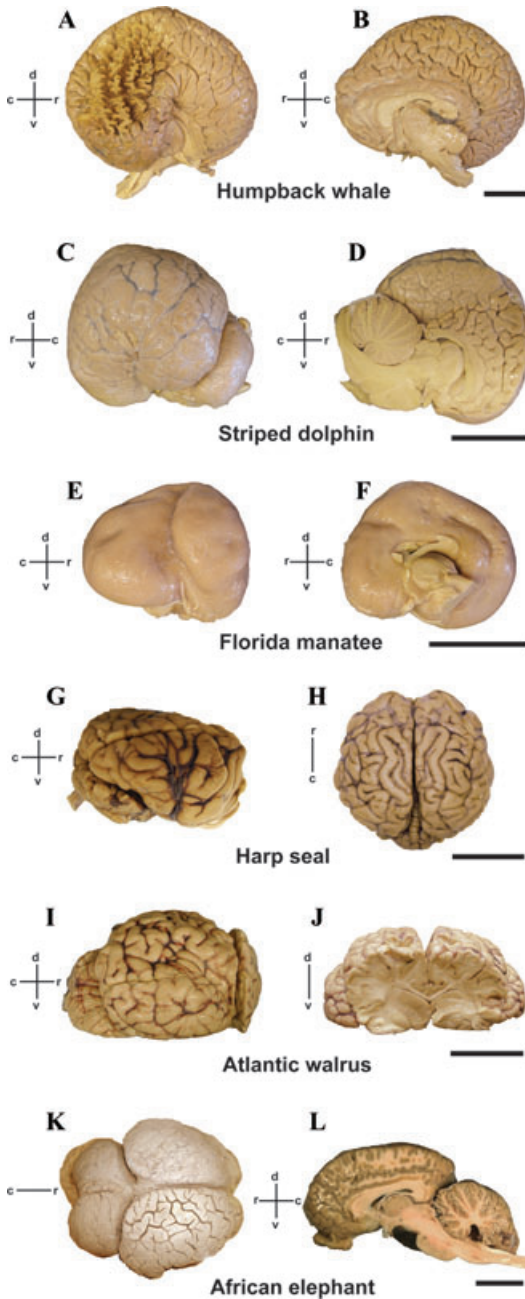
Figure 1. Macroscopic views of the brains of the species discussed in this review. Lateral (A, C, E, G, I), midline (B, D, F, L), dorsal (H, K), and coronal (J) views. c, caudal; d, dorsal; r, rostral; v, ventral. Scale bars = 5 cm.

semiaquatic mammals that could inhabit either land or sea.[3] In the middle Eocene, a more derived group of mammals arose, the Protocetidae, that possessed a lifestyle probably similar to modern pinnipeds and

lived in water but depended on a terrestrial lifestyle for reproduction. In the late Eocene, 38–40 mya, the Basilosauridae appeared, a group of fully aquatic mammals with morphological features and feeding and hearing capacities comparable to early odontocetes and mysticetes.[4] The early Oligocene, about 35 mya, is marked by the appearance of the Neoceti,[5] the clade of modern whales including their stem taxa. The oldest baleen-bearing mysticetes date back to the mid-Oligocene, about 28–29 mya, a period of great diversification of Mysticeti.[6] The oldest odontocetes are from the early Oligocene, about 32 mya.[7] Sperm whales (*Physeter macrocephalus* and *Kogia* spp.) are considered the most basal extant odontocetes.[8] However, only the early Pliocene, 2.5–5 mya, witnessed the divergence of the main crown cetacean genera.[4,5]

The classification and phylogenetic position of cetaceans within Eutheria has been debated given the divergent conclusions drawn by different authors.[4] Recent data provide both molecular[9] and morphological[10,11] evidence for the inclusion of cetaceans within the Artiodactyla (even-toed ungulates) and for a sister-taxon relationship between cetaceans and hippopotamids,[10,12] thereby creating a new clade, Cetancodonta.[13] The currently accepted classification groups are Cetacea (dolphins, whales, and porpoises) and Artiodactyla in the unranked taxon Cetartiodactyla. According to this classification, the Order Cetacea includes the two suborders, Mysticeti (baleen whales, with 14 species in 4 families) and Odontoceti (toothed whales, with 74 species in 10 families).[14]

Sirenians probably originated from large early herbivores, an ancestry that they share with elephants and hyraxes.[15] They also most likely adapted to aquatic life at approximately the same time as cetaceans, 50–60 mya during the Eocene.[16] The existence of the oldest sirenians, *Prorastomus* and *Protosiren,* is known from Eocene fossil records of the West Indies, Pakistan, North Africa, and Europe.[17] Sirenians are the closest phylogenetic relatives to elephants,[18] and the order includes only two extant families: Dugongidae (dugongs) and Trichechidae (manatees).

Pinnipeds (and otters) originated from ursids,[19] mustelids,[20] and possibly an unresolved ancestor, as supported by recent evidence.[21] The oldest pinnipeds, *Elianarctos* and *Pteronarctos*, are from the Oligocene, 25–27 mya, and Miocene, 19–15

mya, respectively, which supports a later adaptation to the aquatic environment than sirenians and cetaceans.[22]

The brain of fully aquatic marine mammals

Structure and function of the neocortex of cetaceans

Fossil evidence shows that modifications in cranial morphology occurred during cetacean evolution and that the process of telescoping and migration of the narial apertures onto the dorsal apex of the skull[23] led to the modern cetacean skull anatomy. The morphology of the cetacean brain reflects these anatomical changes in cranial morphology, with structural modifications such as foreshortening along the beak-fluke axis and lateral widening.[24] Modern cetaceans possess the largest brains in absolute size and relative to body sizes, and their structural complexity is increasingly recognized to be related to sociality and cognition rather than to adaptation to the aquatic environment.[25]

One of the most fascinating characteristics of the brain of cetaceans is the size and the extreme folding of the neocortex[26–28] (Fig. 1A–D). The limbic lobe is extensive and includes well-developed cingulate, insular, and parahippocampal cortices.[26,27,29] In contrast to the elaboration of the neocortex, the paleocortex (rhinencephalon) and archicortex (hippocampal formation) are very reduced, although the hippocampal formation contains all its subregions (dentate gyrus, hippocampus proper, and subiculum), while the entorhinal cortex is large.[26,29–31] This suggests that, in cetaceans, the pathways involved in learning, memory, and spatial navigation likely are organized very differently than in terrestrial mammals. The cetacean cortex is agranular owing to the lack or underdevelopment of layer IV.[26,29,31–33] The general layering pattern is characterized by a thick layer I that is far more cellular than in most terrestrial species, a densely populated layer II that contains extraverted neurons with dendrites extending into layer I, a wide pyramidal layer III, a layer V containing very large and clustering pyramidal neurons, and a multiform layer VI[26,34–36] (Figs. 2A–C and 3A, B). Specific cortical patterns are observed in the auditory and visual cortices where striking columns of neurons in layers V and VI (Fig. 3A and B) are proposed to be associated to specific thalamic afferents.[26]

The lack or underdevelopment of layer IV, given its major role as input for thalamocortical afferents, has been related to a possible different strategy in cortical wiring in cetaceans.[26,37] The remarkable development of the cetacean neocortex results in a complex pattern of gyrification characterized by a prominent and almost vertical sylvian fissure (technically a pseudosylvian fissure) that is surrounded concentrically toward the vertex of the hemisphere by the ectosylvian, suprasylvian, lateral, and entolateral sulci, respectively[26,28,29,38] (Fig. 4).

A few physiological mapping studies provide evidence about the functional organization of the neocortex of cetaceans. The caudal cortical domain situated between the ectosylvian and the suprasylvian sulci, the ectosylvian gyrus, corresponds to the secondary auditory field; almost the entire rostrocaudal extent of the cortex positioned between the suprasylvian and entolateral sulci, the suprasylvian gyrus, forms a belt along the vertex of the hemisphere that corresponds to the primary auditory field;[39–44] the cortex located between the lateral and the entolateral sulci, at the vertex of the hemisphere, in the lateral gyrus, corresponds to the primary visual field.[37,40,44] On the rostroventral extent of the cortex originates the cruciate sulcus that extends rostrocaudally, delineating the boundaries between the primary motor and primary somatosensory fields[40,45] (Fig. 5). The remainder of the lateral surface of the hemisphere is likely occupied by "association cortices" connecting the auditory, somatosensory, and motor fields.[28]

Studies of the neocortical distribution and morphology of neurons expressing neurochemical markers such as calcium-binding proteins show that the molecular organization of the neocortex of cetaceans is similar to that of ungulates but sets them apart from other groups, reflecting phylogenetic relationships among evolutionary distinct mammalian branches.[35,46–48] Moreover, the percent of GABAergic neurons in the visual cortex of cetaceans is comparable to that in terrestrial mammals such as the cat and macaque monkey.[49]

In the past, quantitative studies of neocortical organization that used a variety of methodologies pointed to a high glial cell-to-neuron number ratio as a peculiar characteristic of cetaceans.[38,50–52] This subsequently gave rise to a controversial hypothesis about the functional significance of this ratio,[53] which was challenged on several grounds in
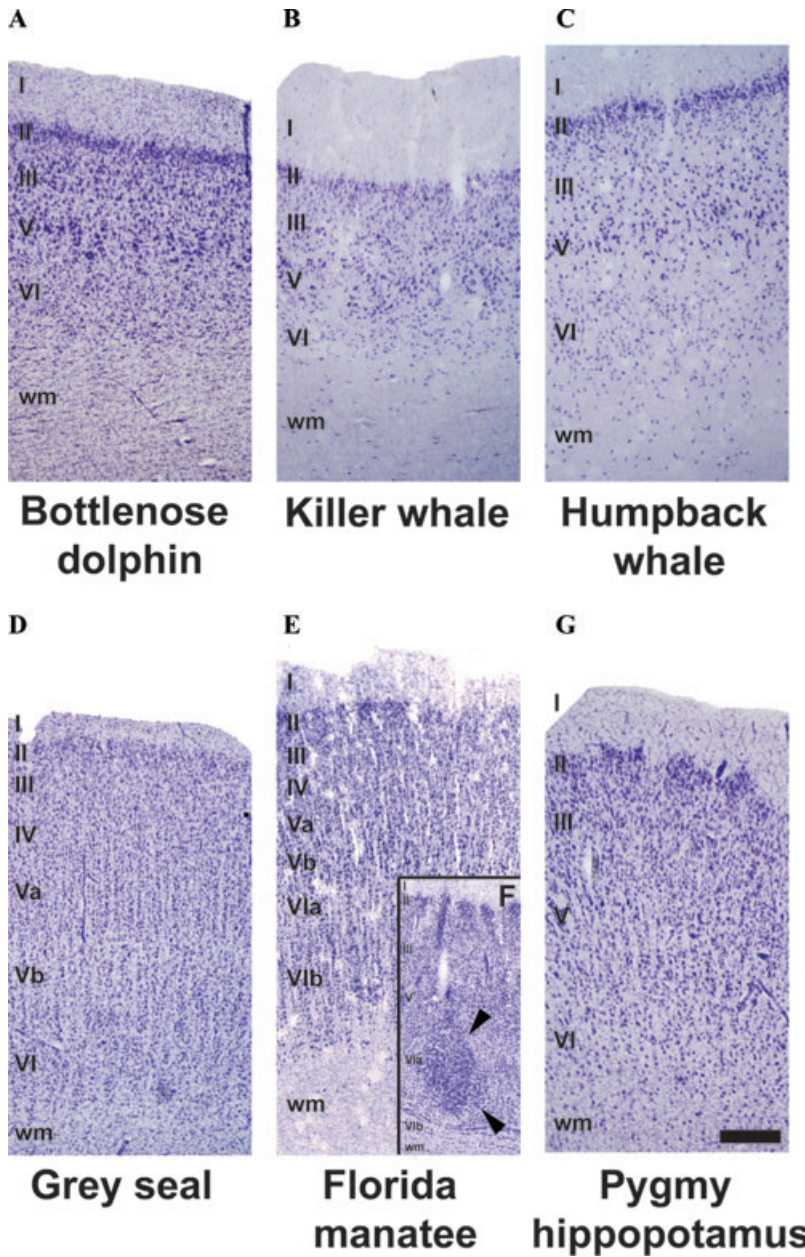
Figure 2. Examples of neocortical cytoarchitecture of somatosensory and insular cortices in some of the species discussed in this review. Somatosensory cortex (A–E and G); anterior insular cortex (F). Arrowheads (F) point to a neuronal cluster, or "Rindenkerne," in layer VI, a specialization unique to sirenians. Cortical layers are indicated by Roman numerals. Scale bar = 400 μm.

a recent review.[54] Marino *et al.* have stressed further the need for standardization of methods used in acquiring quantitative neuroanatomical data in comparative studies.[54]

Structure and function of the neocortex of sirenians

In contrast to the large size and the dramatic sulcation and gyrification of the cetacean brain, sirenians
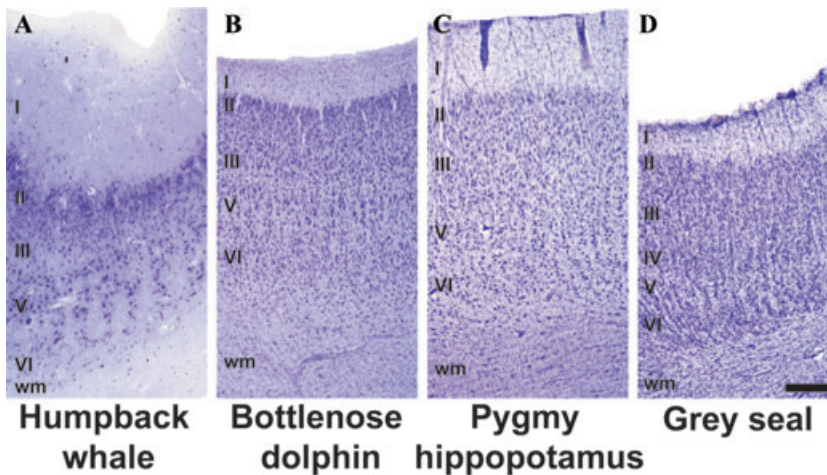
Figure 3. Examples of cytoarchitecture of the visual cortex in some of the species discussed in this review. Cortical layers are indicated by Roman numerals. Scale bar = 400 μm.

present an extreme and unusual case of lissencephaly with the presence of only a few pronounced fissures, which are accompanied by a relatively small size[24,55] (Fig. 1E and F). These differences are even more enhanced at a cytoarchitectural level, with the neocortex of sirenians being thicker and including both a well-organized internal granular layer IV[56–58]—unlike the situation in cetaceans—and columnar patterns of layers V and VI in most cortical areas[56,57] (Fig. 2E). In sirenians, seven cytoarchitectural areas, which represent 25% of the total neocortical surface area, are devoted to somatosensory functions,[56–58] and a possible overlapping of the entire primary auditory cortex with somatosensory function has been proposed in manatees.[58] The somatosensory nuclei of the thalamus and brainstem are much larger than those devoted to other functions,[59] which supports the primary role of the somatosensory perception of the environment for this species.[56,57,60–62] The formation of clusters of neurons in deep layer VI (Rindenkerne; Fig. 2F) is a unique specialization of sirenians that has been compared to the barrels of the somatosensory cortex of rodents, and has been suggested to be a functional representation of their unique tactile hairs.[58,62–64]

Comparison with large semiaquatic and terrestrial mammals

From a comparative viewpoint, the gross anatomy of the brains of both African and Indian elephants (*Loxodonta africana* and *Elephas maximus*, respec-

tively) is more comparable in size, level of sulcation and gyrification, and cytoarchitectural specialization to that of cetaceans rather than to that of its closest phylogenetic relatives, the sirenians[18] (Fig. 1E, F and K, L).

The few studies available on the gross anatomy and structure of the cerebral cortex of elephants[65] highlight a gyral complexity that is second only to cetaceans and includes an expanded neocortex with enlarged temporal, frontal, insular, parietal, cingulate, and hippocampal cortices, but with a poor development of the occipital cortex.[66] A recent study that examined the neuronal morphology of pyramidal neurons in the superficial layers of frontal and occipital cortices of the African elephant highlighted differences between this species, primates, and rodents, including dendritic length, branching patterns, and orientation.[67,68] The neocortex of the elephant, like that of cetaceans, contains superficial pyramidal neurons that possess bifurcating apical dendrites,[33,67,69,70] and has been suggested to have a high glia-to-neuron ratio.[71]

Only a few reports are available on the external morphology of the brain and cytoarchitecture of the pinniped neocortex, and they are mostly focused on a particular species and a restricted cortical domain. In terms of gross morphology, the brain of pinnipeds is comparable in shape, proportions, and cortical folding to that of large terrestrial carnivores[72] (Fig. 1G–J). However, minor species-specific differences among pinnipeds, as well as between
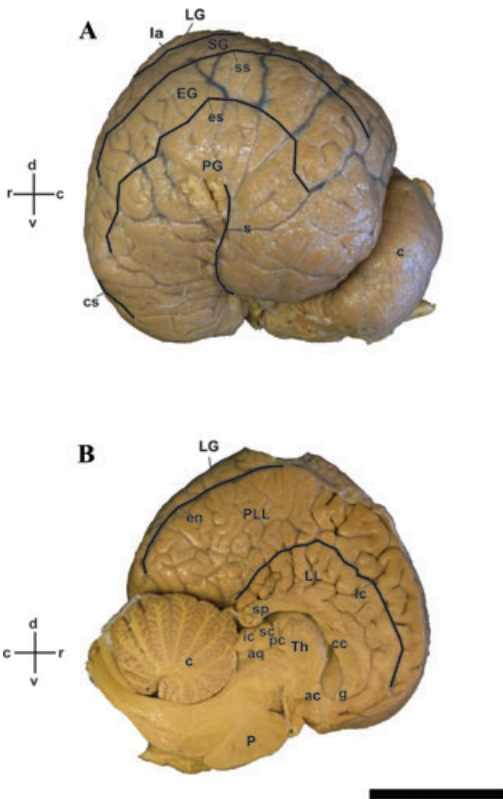
Figure 4. Lateral (A) and mediosagittal (B) aspects of the brain of the striped dolphin (*Stenella coeruleoalba*): ac, anterior commisure; aq, aqueduct; c, caudal; Cb, cerebellum; cc, corpus callosum; cs, central sulcus; d, dorsal; EG, ectosylvian gyrus; en, entolateral sulcus; es, ectosylvian sulcus; g, genu of the corpus callosum; IC, inferior colliculus; la, lateral sulcus; lc, limbic cleft; LG, lateral gyrus; LL, limbic lobe; P, pons; pc, posterior commissure; PG, perisylvian gyrus; PLL, paralimbic lobe; r, rostral; s, sylvian fissure; SC, superior colliculus; SG, suprasylvian gyrus; sp, splenium; ss, suprasylvian sulcus; Th, thalamus; v, ventral. Scale bar = 4 cm.

pinnipeds and terrestrial carnivores, have been reported in the shape, orientation, number, and extension of the cerebral fissures.[73] In his classical study of the carnivore brain, Fish summarized his observations as follows: " With regard to the ground plan of the fundamental fissures, and allowing for the difference in shape of the brains, that of the eared seals, *Callorhinus* and *Zalophus*, approximates in general more closely to that of the ursine carnivora than does *Phoca*. The latter, or earless seal, in some respects, appears aberrant." Fish also examined the gross morphology of the brain of a Caribbean monk seal (*Monachus tropicalis*) and reported this specimen as being in "a position intermediate between

the fur seal and *Phoca*, particularly in the frontal region which is somewhat foreshortened and broader than in *Callorhinus*." The same author also drew this conclusion from the analysis of the direction and extension of the sylvian fissure that, in both the Caribbean monk seal and the fur seal (*Callorhinus ursinus*), was described, similarly to cetaceans, to be almost vertical.[73] As a general conclusion, Fish reported an increase in number, size, and complexity of the fissural pattern going from Canidae, to Felidae, Ursidae, Otariidae (eared seals), and Phocidae (true seals), respectively.

From a functional standpoint, the motor cortex of the harbor seal (*Phoca vitulina*) lies anteroventrally to the cruciate sulcus, leaving only a small amount of cortex at the frontal pole of the brain, and presents histological characteristics consistent with descriptions of the motor cortex in carnivores.[72,74] This unusual rostral location of the motor cortex, also noted by Rioch,[72] is compared by the author to the rostral location of the motor cortex in a cetacean species, the harbor porpoise (*Phocoena phocoena*),[75] and is consistent with localizations in cetaceans.[26,76] Another functional study on the auditory and somatosensory cortices of the harbour seal revealed a limited regional extension of the cortex responding to auditory stimuli, compared to terrestrial carnivores, and mainly including, in this species, the ventrocaudal end of the sylvian gyrus.[77] Similarly, the somatosensory cortex of harbor and fur seals is located in the rostral part of the sylvian gyrus, rostrally to the sylvian fissure and ventrally to the suprasylvian sulcus.[77,78] (For details on the nomenclature of sulci and fissures, see Refs. 72 and 74.) The visual cortex is located on the parieto-occipital cortex in the Weddell seal (*Leptonychotes weddelli*), based on electrophysiological data[79] (Fig. 3D).

The cytoarchitectural organization of areas in the frontal cortex of the harbor seal was found to be comparable to that in the dog.[72] Moreover, at least in the grey seal (*Halichoerus grypus*), the deep layers V and VI show columnar patterns in several cortical regions (Fig. 2D; Butti *et al.*, unpublished observation) similar to manatees and cetaceans.

It is worth mentioning that the cytoarchitecture of the neocortex of the semiaquatic pygmy hippopotamus (*Hexaprotodon liberiensis*), a close relative of cetaceans, resembles that of cetaceans in many ways,
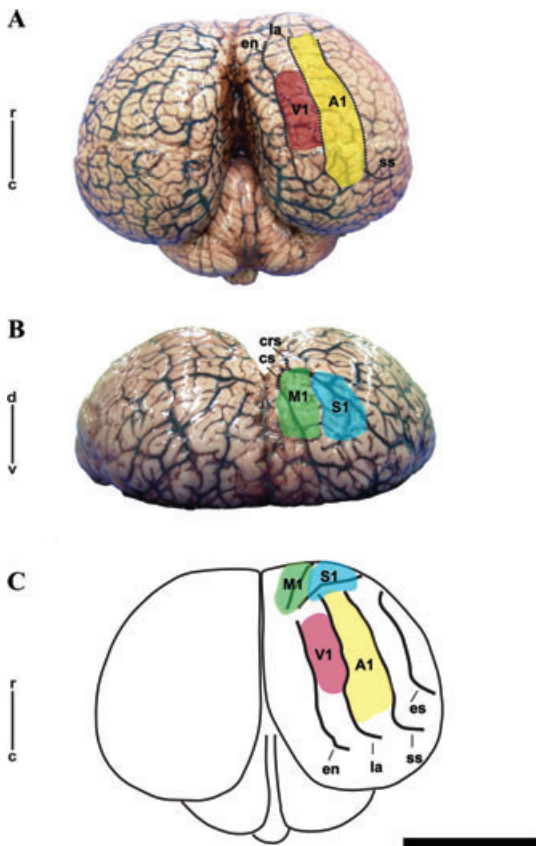
Figure 5. Dorsal (A) and rostral (B) aspects, and dorsal schematic view (C) of the brain of the bottlenose dolphin (*Tursiops truncatus*) showing the localization of primary cortical areas. A1, primary auditory cortex; c, caudal; cs, cruciate sulcus; crs, coronary sulcus; d, dorsal; en, entolateral sulcus; l, lateral; la, lateral sulcus; M1, primary motor cortex; r, rostral; S1, primary somatosensory cortex; v, ventral; V1, primary visual cortex. Scale bar = 5 cm.

including the absence of layer IV throughout the neocortex (Butti *et al.*, unpublished observations), a densely packed and clustered layer II in the insular cortex,[80] and in the putative somatosensory cortex, as well as the presence of vertical modules of neurons in layer VI of somatosensory and visual putative cortices (Figs. 2G and 3C; Butti *et al.*, unpublished observations). In our comparative study of the organization of the insular cortex, we observed a complete agranularity of the anterior sector of the insula in the Atlantic walrus (*Odobenus rosmarus rosmarus*),[80] which is similar to what has been observed in the cat[81] but contrasts with the situation in the dog, where a "dysgranular" cortex is present.[82]

Cortical specializations: von Economo neurons and laminar clusters

In most of the cetacean species studied, layer V of the anterior cingulate (ACC), anterior and frontoinsular (AI and FI), and frontopolar (FP) cortices contains von Economo neurons (VENs), [83] a population of projection neurons originally described in humans, great apes, and later elephants, which are suggested to play a role in interoception, sociality, and cognition.[26,80,84–91] Recent evidence shows a rapid increase in the number of VENs during the first eight months after birth in humans as well as an hemispheric asymmetry in their distribution in hominids, which is possibly related to asymmetries in the sympathetic and parasympathetic divisions of the autonomic nervous system.[89] VENs are larger than neighboring pyramidal neurons in cetaceans and hominoids,[84,88] send an axon out of the neocortex,[88] and possess a narrow and simplified dendritic tree, a morphology consistent with conveying information that is synthesized from within the space of a minicolumn.[92] Recent evidence suggests that VENs contain not only high levels of non-phosphorylated neurofilament protein (NFP), but also vasopressin 1a, dopamine D3, and serotonin 2b receptors; neuromedin B (NMB); gastrin-releasing peptide; DISC1 (disrupted in schizophrenia-1); activating-transcription factor 3 (ATF3); and interleukin 4 receptor α (IL-4Rα),[86,88,89] all of which are thought to be involved in social bonding, reward, punishment, digestion, and immune response.[93] Evidence also points to phylogenetic variation (and as such, possible biochemical specialization of VENs in select mammalian groups) in protein expression profile, with a higher proportion of VENs expressing ATF3, IL4Rα, and NMB in humans than in other hominoids.[93]

In view of the presence of VENs in phylogenetically divergent species that share large brains and complex social organization; their specific cortical distribution, morphology, biochemical profile; and selective disruption in neuropsychiatric disorders impairing cognitive and social functioning, these neurons may play a role in the integration of emotions, vocalization control, facial expression, social conduct, and regulation of autonomic visceral, olfactory, and gustatory functions. Furthermore, they may represent an anatomical substrate for the fast transmission of information along networks implicated in the emotional response to

external stimuli and generation of goal-directed behaviors in large mammals.[26,80,84,86,94–99] We have also observed VENs, with marked differences in distribution, in the neocortex of the pygmy hippopotamus, two marine mammals unrelated to cetaceans (the Florida manatee, *Trichechus manatus latirostris*, and the Atlantic walrus), and one perissodactyl, the common zebra (*Equus burchelli*),[80] as well as the domestic horse, *Equus caballus*, and the Eastern black rhinoceros, *Diceros bicornis michaeli* (Butti *et al.*, unpublished observations). Cetaceans, hominoids, and elephants share a selective distribution of high densities of VENs in specific frontal cortical regions (ACC, FI/AI, and FP/dlPFC in humans);[80,84,87,88,91] in contrast, the pygmy hippopotamus and common zebra have abundant VENs throughout the cortex,[80] and the Florida manatee is characterized by extremely rare and sparse VENs.[80] This suggests that the overall distribution of VENs in the pygmy hippopotamus represents a progressive reshaping of the projections provided by these neurons, possibly translating into a more specific function. Such specialization appears to have taken place during the evolution of the cetaceans from their common ancestor with hippopotamuses, where a broad distribution of VENs throughout the neocortex was refined to one preferentially involving the functions subserved by the ACC, FI, and FP (or dlPFC in hominids).[80] Similar mechanisms may have shaped other highly specialized pathways in primates, such as those supported in primates by Betz cells in the motor cortex and Meynert cells in the visual cortex, which are also characterized by sparsely distributed, large, clustering output neurons in functionally specific cortical and laminar domains.[100–102]

Another remarkable specialization of the neocortex of cetaceans is the clustering of neurons in layer II of the anterior insular cortex. Specifically, large clumps of neurons in layer II have been described in the anterior insular cortex of small odontocetes,[80,103,104] also extending to the temporal and occipital cortex in some mysticetes.[26] This modular organization was suggested[26] to be shaped by thalamocortical afferent and length of corticocortical projections, and represents a cost-effective strategy for efficient wiring in large brains.[26] Hof and Van der Gucht proposed that the peculiar patchiness of layer II in temporal and occipital regions could reflect a specific neocortical connectivity (and func-

tion) of these regions in balaenopterids that is not shared with other cetaceans.[26] Modules in layer II, comparable to those described in cetaceans, were also observed in the anterior insular cortex and in the putative somatosensory cortex of the pygmy hippopotamus and Atlantic walrus.[80] Particularly striking is the clustering observed in the anterior insular cortex of the manatee, where the presence of clusters in layer II is associated with the formation of large columns that extend to the deepest layers of the cortex.[80]

The importance of comprehensive sampling in comparative studies

The comparative data reviewed here demonstrate that the process of readaptation to the aquatic environment resulted in diverse brain morphology and cortical organization. This variation is manifest as the unique agranularity of the neocortex in cetaceans and artiodactyls and the similarities in external morphology of the brain and gyral pattern in phocids and terrestrial carnivores. However, similarities are evident across orders, including comparable gyral complexities, agranularity, columnar modularity of layer V and VI, clustering of layer II, and neuronal specializations such as VENs.

Interpreting the evolutionary significance of such traits is rendered difficult due to the lack of a clear pattern of appearance through the mammalian phylogeny, and is further complicated by the poor availability of many key species. Moreover, the absence of connectivity and functional data in species other than laboratory animals imposes critical limitations and stresses the need for comprehensive comparisons to avoid outright speculation. How the interpretation of the possible evolutionary significance of VENs has been changing as data on a larger number of species become available is a reflection of this situation. At first, the identification of VENs in homologous cortical regions of great apes and humans suggested that VENs could be related to cognitive and behavioral specializations unique to these species.[86,89] The later identification of VENs in cetaceans and elephants led to hypotheses relating VENs morphology to absolute brain size, other aspects of behavior, and the need for a fast conduction of information over great distances.[26] Most recently, the description of VENs in the brains of other large vertebrates[80] with different cortical distributions

than that described in hominoids, cetaceans, and elephants, suggests that VENs represent a common evolutionary trait among large mammals contributing to specialized neuronal networks in a taxon-specific manner, dependent upon their cortical distribution.

In this context, brain collections and zoological facilities constitute a unique source of critical information for the study of brain organization in a truly evolutionary context that goes beyond the handful of species commonly available in the laboratory. Such histological materials and anatomical repositories represent, in many cases, the only possibility of exploring brain organization of species otherwise impossible to study, to understand the evolutionary significance of neuroanatomical differences and similarities widely observed among mammalian lineages, and to add to the knowledge of poorly documented and endangered species.

Acknowledgments

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Gingerich, P.D. & M.D. Uhen. 1998. Likelihood estimation of the time of origin of cetacean and the time of divergence of Cetacea and Artiodactyla. *Paleo-Electronica* **2:** 1–47.

2. Fordyce, R. 2008. Cetacean evolution. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 201–207. Academic Press. San Diego, CA.

3. Thewissen, J.G., L.N. Cooper, M.T. Clementz, *et al.* 2007. Whales originated from aquatic artiodactyls in the Eocene epoch of India. *Nature* **450:** 1190–1194.

4. Uhen, M. 2010. The origin(s) of whales. *Annu. Rev. Earth Planet. Sci.* **38:** 189–219.

5. Fordyce, R.E. 2008. Neoceti. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 758–763: Academic Press. San Diego, CA.

6. Fitzgerald, E. 2006. A bizarre new toothed mysticete (Cetacea) from Australia and the early evolution of baleen whales. *Proc. R. Acad. Sci. B.* **273:** 2955–2963.

7. Fordyce, R.E. 2002. *Simocetus rayi* (Odontoceti: Simocetidae) (new species, new genus, new family), a bizarre new archaic Oligocene dolphin from the Eastern North Pacific. *Smithsonian Contrib. Paleobiol.* **93:** 185–222.

8. Fordyce, R.E. 2008. Cetacean fossil record. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 207–215. Academic Press. San Diego, CA.

9. Nikaido, M., A.P. Rooney & N. Okada. 1999. 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.

10. Boisserie, J.R., F. Lihoreau & M. Brunet. 2005. The position of Hippopotamidae within Cetartiodactyla. *Proc. Natl. Acad. Sci. USA* **102:** 1537–1541.

11. Thewissen, J.G., E.M. Williams, L.J. Roe & S.T. Hussain. 2001. Skeletons of terrestrial cetaceans and the relationship of whales to artiodactyls. *Nature* **413:** 277–281.

12. Geisler, J.H. & J.M. Theodor. 2009. Hippopotamus and whale phylogeny. *Nature* **458:** E1–4; discussion E5.

13. Arnason, U., A. Gullberg, S. Gretarsdottir, *et al.* 2000. The mitochondrial genome of the sperm whale and a new molecular reference for estimating eutherian divergence dates. *J. Mol. Evol.* **50:** 569–578.

14. Rice, D. Classification (overall). 2008. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 234–238. Academic Press. San Diego, CA.

15. Murphy, W.J. *et al*. 2001. Molecular phylogenetics and the origins of placental mammals. *Nature* **409:** 614–618.

16. Domning, D.P. 2000. The readaptation of Eocene sirenians to life in water. *Historical Biol.* **14:** 115–119.

17. Heyning, J.E. & G.M. Lento. 2006. The evolution of marine mammals. In *Marine Mammal Biology: An Evolutionary Approach*. A.R. Hoelzel, Ed.: 38–72. Blackwell Publishing. Malden, MA.

18. Kellogg, M.E. *et al.* 2007. Chromosome painting in the manatee supports Afrotheria and Paenungulata. *BMC Evol. Biol.* **7:** 6.

19. Vrana, P.B., M.C. Milinkovitch, J.R. Powell & W.C. Wheeler. 1994. Higher level relationships of the arctoid Carnivora based on sequence data and "total evidence". *Mol. Phylogenet. Evol.* **3:** 47–58.

20. Arnason, U. & B. Widegren. 1986. Pinniped phylogeny enlightened by molecular hybridizations using highly repetitive DNA. *Mol. Biol. Evol.* **3:** 356–365.

21. Lento, G.M., R.E. Hickson, G.K. Chambers & D. Penny. 1995. Use of spectral analysis to test hypotheses on the origin of pinnipeds. *Mol. Biol. Evol.* **12:** 28–52.

22. Berta, A. 2008. Pinniped evolution. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 861–868. Academic Press. San Diego, CA.

23. Klima, M. 1999. Development of the cetacean nasal skull. *Adv. Anat. Embryol. Cell. Biol.* **149:** 1–143.

24. Marino, L. 2008. Brain size evolution. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 149–152. Academic Press. San Diego, CA.

25. Marino, L. 2007. Cetacean brains: how aquatic are they? *Anat. Rec.* **290:** 694–700.

26. Hof, P.R. & E. Van der Gucht. 2007. Structure of the cerebral cortex of the humpback whale, *Megaptera novaeangliae* (Cetacea, Mysticeti, Balaenopteridae). *Anat. Rec.* **290:** 1–31.

27. Morgane, P.J., M.S. Jacobs & W.L. McFarland. 1980. The anatomy of the brain of the bottlenose dolphin (*Tursiops truncatus*). Surface configuration of the telencephalon of the bottlenose dolphin with comparative anatomical observations in four other cetaceans species. *Brain Res. Bull.* **5**(Suppl. 3): 1–108.

28. Oelschläger, H.A. & J. Oelschläger. 2008. Brain. In *Encyclopedia of Marine Mammals*. W.F. Perrin, B. Würsig & J.G.M. Thewissen, Eds.: 134–149. Academic Press. San Diego, CA.

29. Jacobs, M.S., W.L. McFarland & P.J. Morgane. 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.

30. Breathnach, A.S. & F. Goldby. 1954. The amygdaloid nuclei, hippocampus and other parts of the rhinencephalon in the porpoise (*Phocaena phocaena*). *J. Anat.* **88:** 267–291.

31. Hof, P.R., R. Chanis & L. Marino. 2005. Cortical complexity in cetacean brains. *Anat. Rec.* **287:** 1142–1152.

32. Furutani, R. 2008. Laminar and cytoarchitectonic features of the cerebral cortex in the Risso's dolphin (*Grampus griseus*), striped dolphin (*Stenella coeruleoalba*), and bottlenose dolphin (*Tursiops truncatus*). *J. Anat.* **213:** 241–248.

33. Garey, L.J., E. Winkelmann & K. Brauer. 1985. Golgi and Nissl studies of the visual cortex of the bottlenose dolphin. *J. Comp. Neurol.* **240:** 305–321.

34. Glezer, I.I. & P.J. Morgane. 1990. Ultrastructure of synapses and golgi analysis of neurons in neocortex of the lateral gyrus (visual cortex) of the dolphin and pilot whale. *Brain Res. Bull.* **24:** 401–427.

35. Hof, P.R. & C.C. Sherwood. 2005. Morphomolecular neuronal phenotypes in the neocortex reflect phylogenetic relationships among certain mammalian orders. *Anat. Rec.* **287:** 1153–1163.

36. Morgane, P.J., I.I. Glezer & M.S. Jacobs. 1988. Visual cortex of the dolphin: an image analysis study. *J. Comp. Neurol.* **273:** 3–25.

37. Revishchin, A.V. & L.J. Garey. 1989. Sources of thalamic afferent neurons, projecting into the suprasylvian gyrus of the dolphin cerebral cortex. *Neirofiziologia* **21:** 529–539.

38. Morgane, P.J., W.L. McFarland & M.S. Jacobs. 1982. The limbic lobe of the dolphin brain: a quantitative cytoarchitectonic study. *J. Hirnforsch.* **23:** 465–552.

39. Ladygina, T.F., A.M. Mass & A. Supin. 1978. Multiple sensory projections in the dolphin cerebral cortex. *Zh. Vyssh. Nerv. Deiat. Im. I.P. Pavlova* **28:** 1047–1053.

40. Ladygina, T.F. & A. Supin. 1977. Localization of the sensory projection areas in the cerebral cortex of the dolphin, *Tursiops truncatus*. *Zh. Evol. Biokhim. Fiziol.* **13:** 712–718.

41. Popov, V.V., T.F. Ladygina & A. Supin. 1986. Evoked potentials of the auditory cortex of the porpoise, *Phocoena phocoena*. *J. Comp. Physiol.* **158:** 705–711.

42. Popov, V.V. & A. Supin. 1976. Detemination of the hearing characteristics of the dolphin by the evoked potential method. *Fiziol. Zh. SSSR Im. I.M. Sechenova* **62:** 550–558.

43. Revishchin, A.V. & L.J. Garey. 1996. Mitochondrial distribution in visual and auditory cerebral cortex of the harbour porpoise. *Brain Behav. Evol.* **47:** 257–266.

44. Sokolov, V.E., T.F. Ladygina & A. Supin. 1972. Localization of sensory zones in the dolphin cerebral cortex. *Dokl. Akad. Nauk. SSSR* **202:** 490–493.

45. Kesarev, V.S. & L.I. Malofeeva. 1969. Structural organization of the motor zone of the cerebral cortex in dolphins. *Arkh. Anat. Gistol. Embriol.* **56:** 48–55.

46. Glezer, I.I., P.R. Hof, C. Leranth & P.J. Morgane. 1993. Calcium-binding protein-containing neuronal populations in mammalian visual cortex: a comparative study in whales, insectivores, bats, rodents, and primates. *Cereb. Cortex* **3:** 249–272.

47. Hof, P.R. *et al.* 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.

48. Sherwood, C.C. *et al.* 2009. Neocortical neuron types in Xenarthra and Afrotheria: implications for brain evolution in mammals. *Brain Struct. Funct.* **213:** 301–328.

49. Garey, L.J., J. Takacs, A.V. Revishchin & J. Hamori. 1989. Quantitative distribution of GABA-immunoreactive neurons in cetacean visual cortex is similar to that in land mammals. *Brain Res.* **485:** 278–284.

50. Garey, L.J. & G. Leuba. 1986. A quantitative study of neuronal and glial numerical density in the visual cortex of the bottlenose dolphin: evidence for a specialized subarea and changes with age. *J. Comp. Neurol.* **247:** 491–496.

51. Hawkins, A. & J. Olszewski. 1957. Glia/nerve cell index for cortex of the whale. *Science* **126:** 76–77.

52. Eriksen, N. & B. Pakkenberg. 2007. Total neocortical cell number in the mysticete brain. *Anat. Rec.* **290:** 83–95.

53. Manger, P.R. 2006. An examination of cetacean brain structure with a novel hypothesis correlating thermogenesis to the evolution of a big brain. *Biol. Rev.* **81:** 293–338.

54. Marino, L. *et al.* 2008. A claim in search of evidence: reply to Manger's thermogenesis hypothesis of cetacean brain structure. *Biol. Rev.* **83:** 417–440.

55. Reep, R.L. & T.J. O'Shea. 1990. Regional brain morphometry and lissencephaly in the Sirenia. *Brain Behav. Evol.* **35:** 185–194.

56. Marshall, C.D. & R.L. Reep. 1995. Manatee cerebral cortex: cytoarchitecture of the caudal region in *Trichechus manatus latirostris*. *Brain Behav. Evol.* **45:** 1–18.

57. Reep, R.L., J.I. Johnson, R.C. Switzer & W.I. Welker. 1989. Manatee cerebral cortex: cytoarchitecture of the frontal region in *Trichechus manatus latirostris*. *Brain Behav. Evol.* **34:** 365–386.

58. Sarko, D.K. & R.L. Reep. 2007. Somatosensory areas of manatee cerebral cortex: histochemical characterization and functional implications. *Brain Behav. Evol.* **69:** 20–36.

59. Sarko, D.K. *et al.* 2007. Somatosensory nuclei of the manatee brainstem and thalamus. *Anat. Rec.* **290:** 1138–1165.

60. Marshall, C.D., L.A. Clark & R.L. Reep. 1998. The muscular hydrostat of the Florida manatee (*Trichechus manatus latirostris*): a functional morphological model of perioral bristle use. *Mar. Mamm. Sci.* **14:** 290–303.

61. Marshall, C.D. *et al.* 1998. Prehensile use of perioral bristles during feeding and associated behaviors of the Florida manatee (*Trichechus manatus latirostris*). *Mar. Mamm. Sci.* **14:** 274–289.

62. Reep, R.L. *et al.* 2001. Microanatomy of facial vibrissae in the Florida manatee: the basis for specialized sensory function and oripulation. *Brain Behav. Evol.* **58:** 1–14.

63. Reep, R.L., C.D. Marshall & M.L. Stoll. 2002. Tactile hairs on the postcranial body in Florida manatees: a mammalian lateral line? *Brain Behav. Evol.* **59:** 141–154.

64. Sarko, D.K., F.L. Rice, & R.L. Reep. 2011. Mammalian tactile hair: divergence from a limited distribution. *Ann. N.Y. Acad. Sci.* **1225:** 90–100.

65. Cozzi, B., S. Spagnoli & L. Bruno. 2001. An overview of the central nervous system of the elephant through a critical appraisal of the literature published in the XIX and XX centuries. *Brain Res. Bull.* **54:** 219–227.

66. Shoshani, J., W.J. Kupsky & G.H. Marchant. 2006. Elephant brain. Part I: gross morphology, functions, comparative anatomy, and evolution. *Brain Res. Bull.* **70:** 124–157.

67. Jacobs, B. *et al.* 2011. Neuronal morphology in the African elephant (*Loxodonta africana*) neocortex. *Brain Struct. Funct.* **215:** 273–298 [Epub ahead of print].

68. Bianchi, S. *et al.* 2011. Neocortical neuron morphology in Afrotheria: comparing the rock hyrax with the African elephant. *Ann. N.Y. Acad. Sci.* **1225:** 37–46.

69. Ferrer, I. & M. Perera. 1988. Structure and nerve cell organisation in the cerebral cortex of the dolphin *Stenella coeruleoalba*: a Golgi study. With special attention to the primary auditory area. *Anat. Embryol.* **178:** 161–173.

70. Hof, P.R. *et al.* 1992. The primary auditory cortex in cetacean and human brain: a comparative analysis of neurofilament protein-containing pyramidal neurons. *Neurosci. Lett.* **146:** 91–95.

71. Haug, H. 1987. Brain sizes, surfaces, and neuronal sizes of the cortex cerebri: a stereological investigation of man and his variability and a comparison with some mammals (primates, whales, marsupials, insectivores, and one elephant). *Am. J. Anat.* **180:** 126–142.

72. Rioch, D.M. 1937. A physiological and histological study of the frontal cortex of the seal (*Phoca vitulina*). *Biol. Bull. Woods Hole.* **73:** 591–602.

73. Fish, P.A. 1898. The brain of the fur seal, *Callorhinus ursinus*: with a comparative description of those of *Zalophus californianus*, *Phoca vitulina*, *Ursus americanus* and *Monachus tropicalis*. *J. Comp. Neurosci.* **8:** 57–91.

74. Langworthy, O.R., F.H. Hesser & L.C. Kolb. 1938. A physiological study of the cerebral cortex of the hair seal (*Phoca vitulina*). *J. Comp. Neurol.* **69:** 351–369.

75. Langworthy, O. 1932. A description of the central nervous system of the porpoise (*Tursiops truncatus*). *J. Comp. Neurol.* **350:** 337–356.

76. Kojima, T. 1951. On the brain of the sperm whale (*Physeter catodon, L.*). *Sci. Rep. Whales Res. Inst. Tokyo* **6:** 49–72.

77. Alderson, A.M., E. Diamantopoulos & C.B.B. Downman. 1960. Auditory cortex of the seal (*Phoca vitulina*). *J. Anat.* **94:** 506–511.

78. Ladygina, T.F.P., V.V. Supin & A.Ya. 1985. Topical organization of somatic projections in the fur seal cerebral cortex. *Neirofiziologia* **17:** 344–351.

79. Gruenau, S.P. & J.T. Shurley. 1976. Visual evoked response (VER) changes during maturation in the Weddell seal. *Dev. Psychobiol.* **9:** 477–493.

80. Butti, C. & P.R. Hof. 2010. The insular cortex: a comparative perspective. *Brain Struct. Funct.* **214:** 477–493.

81. Clasca, F., A. Llamas & F. Reinoso-Suarez. 1997. Insular cortex and neighboring fields in the cat: a redefinition based on cortical microarchitecture and connections with the thalamus. *J. Comp. Neurol.* **384:** 456–482.

82. Salazar, I. *et al.* 1988. The neocortex of the dog.I. A classical cytoarchitectonic map. *Anat. Histol. Embryol.* **17:** 169–187.

83. von Economo, C. 1926. Eine neue Art Spezialzellen des Lobus cinguli und Lobus insulae. *Zschr. Ges. Neurol. Psychiatr.* **100:** 706–712.

84. Butti, C. *et al.* 2009. Total number and volume of von Economo neurons in the cerebral cortex of cetaceans. *J. Comp. Neurol.* **515:** 243–259.

85. Allman, J., A. Hakeem & K. Watson. 2002. Two phylogenetic specializations in the human brain. *Neuroscientist* **8:** 335–346.

86. Allman, J.M., K.K. Watson, N.A. Tetreault & A.Y. Hakeem. 2005. Intuition and autism: a possible role for von Economo neurons. *Trends Cogn. Sci.* **9:** 367–373.

87. Nimchinsky, E.A. *et al.* 1999. A neuronal morphologic type unique to humans and great apes. *Proc. Natl. Acad. Sci. USA* **96:** 5268–5273.

88. Nimchinsky, E.A., B.A. Vogt, J.H. Morrison & P.R. Hof. 1995. Spindle neurons of the human anterior cingulate cortex. *J. Comp. Neurol.* **355:** 27–37.

89. Allman, J.M. *et al.* 2010. The von Economo neurons in frontoinsular and anterior cingulate cortex in great apes and humans. *Brain Struct. Funct.* **214:** 495–517.

90. Fajardo, C. *et al.* 2008. Von Economo neurons are present in the dorsolateral (dysgranular) prefrontal cortex of humans. *Neurosci. Lett.* **435:** 215–218.

91. Hakeem, A.Y. *et al.* 2008. Von Economo neurons in the elephant brain. *Anat. Rec.* **292:** 242–248.

92. Watson, K.K., T.K. Jones & J.M. Allman. 2006. Dendritic architecture of the von Economo neurons. *Neuroscience* **141:** 1107–1112.

93. Stimpson, C. *et al.* 2011. Biochemical specificity of von Economo neurons in hominoids. *Am. J. Hum. Biol.* **23:** 22–28.

94. Kaufman, J.A. *et al.* 2008. Selective reduction of von Economo neuron number in agenesis of the corpus callosum. *Acta Neuropathol.* **116:** 479–489.

95. Seeley, W.W. *et al.* 2007. Divergent social functioning in behavioral variant frontotemporal dementia and Alzheimer disease: reciprocal networks and neuronal evolution. *Alzh. Dis. Assoc. Disord.* **21:** S50–57.

96. Seeley, W.W. *et al.* 2006. Early frontotemporal dementia targets neurons unique to apes and humans. *Ann. Neurol.* **60:** 660–667.

97. Brune, M. *et al.* 2010. Von Economo neuron density in the anterior cingulate cortex is reduced in early onset schizophrenia. *Acta Neuropathol.* **119:** 771–778.

98. Santos, M. *et al.* 2011. Von Economo neurons in autism: a stereologic study of the frontoinsular cortex in children. *Brain Res.* [Epub ahead of print]. doi: 10.1016/j.brainres.2010.08.067

99. Allman, J.M. *et al.* 2011. The von Economo neurons in the frontoinsular and anterior cingulate cortex. *Ann. N.Y. Acad. Sci.* **1225:** 59–71.

100. Hof, P.R., E.A. Nimchinsky, W.G. Young & J.H. Morrison. 2000. Numbers of Meynert and layer IVB cells in area V1: a stereologic analysis in young and aged macaque monkeys. *J. Comp. Neurol.* **420:** 113–126.

101. Rivara, C.B., C.C. Sherwood, C. Bouras & P.R. Hof. 2003. Stereologic characterization and spatial distribution patterns of Betz cells in the human primary motor cortex. *Anat. Rec.* **270:** 137–151.

102. Sherwood, C.C. *et al.* 2003. Evolution of specialized pyramidal neurons in primate visual and motor cortex. *Brain Behav. Evol.* **61:** 28–44.

103. Jacobs, M.S., A.M. Galaburda, W.L. McFarland & P.J. Morgane. 1984. The insular formations of the dolphin brain: quantitative cytoarchitectonic studies of the insular component of the limbic lobe. *J. Comp. Neurol.* **225:** 396–432.

104. Manger, P. *et al.* 1998. Modular subdivisions of dolphin insular cortex: does evolutionary history repeat itself? *J. Cogn. Neurosci.* **10:** 153–166.