Anatomy and Three-Dimensional Reconstructions of the Brain of the White Whale (*Delphinapterus leucas*) From Magnetic Resonance Images

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ABSTRACT

Magnetic resonance imaging offers a means of observing the internal structure of the brain where traditional procedures of embedding, sectioning, staining, mounting, and microscopic examination of thousands of sections are not practical. Furthermore, internal structures can be analyzed in their precise quantitative spatial interrelationships, which is difficult to accomplish after the spatial distortions often accompanying histological processing. For these reasons, magnetic resonance imaging makes specimens that were traditionally difficult to analyze, more accessible. In the present study, images of the brain of a white whale (Beluga) *Delphinapterus leucas* were scanned in the coronal plane at 119 antero-posterior levels. From these scans, a computer-generated three-dimensional model was constructed using the programs VoxelView and VoxelMath (Vital Images, Inc.). This model, wherein details of internal and external morphology are represented in three-dimensional space, was then resectioned in orthogonal planes to produce corresponding series of "virtual" sections in the horizontal and sagittal planes. Sections in all three planes display the sizes and positions of such structures as the corpus callosum, internal capsule, cerebral peduncles, cerebral ventricles, certain thalamic nuclear groups, caudate nucleus, ventral striatum, pontine nuclei, cerebellar cortex and white matter, and all cerebral cortical sulci and gyri. Anat Rec 262:429–439, 2001. © 2001 Wiley-Liss, Inc.

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Odontocetes (toothed whales, dolphins, and porpoises) have undergone a number of evolutionary modifications from their terrestrial ancestral state. Among these changes was a major increase in relative brain size. Several modern odontocete species possess encephalization levels second only to modern humans when brain-body allometry is taken into account (Ridgway and Brownson, 1984; Marino, 1998). An arguably equally dramatic transformation of odontocetes occurred in the anatomical structure and organization of their brains. Compared with many other mammalian brains, odontocete brain morphology is unusual in many respects. Researchers have
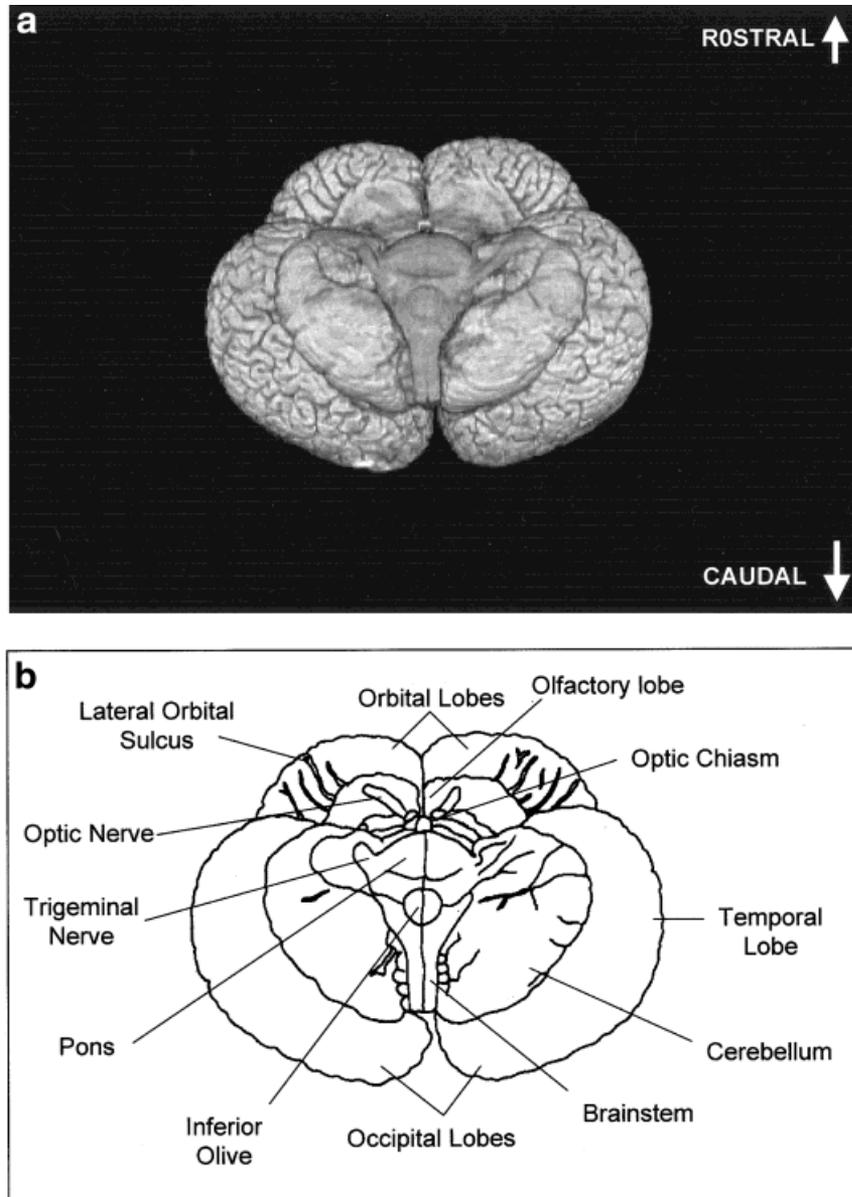
Fig. 1. Ventral surface of a three-dimensional digital reconstruction of the whole brain and labeled schematic illustration of the same image.

stated that "…the lobular formations in the dolphin brain are organized in a pattern fundamentally different from that seen in the brains of primates or carnivores" (Morgane et al., 1980). Because of the fifty-five to sixty million year divergence between cetaceans and other mammals, odontocete brains represent a blend of early mammalian features along with unique derived characteristics (Ridgway, 1986, 1990; Glezer et al., 1988; Manger et al., 1998). The differences between odontocete and other mammalian brains of similar size are present at the level of cortical cytoarchitecture and immunohistochemistry (Garey et al., 1985; Garey and Leuba, 1986; Glezer and Morgane, 1990; Hof et al., 1992, 1995; Glezer et al., 1990, 1992a,b, 1993, 1998), cortical surface morphology (Jacobs et al., 1979; Morgane et al., 1980; Haug, 1987), noncortical structures

and features (Tarpley and Ridgway, 1994; Glezer et al., 1995a,b), and ontogenesis (Oelschlager and Buhl, 1985; Buhl and Oelschlager, 1988; Oelschlager and Kemp, 1998).

Although there are a number of published descriptions of cetacean neuroanatomy (see Morgane et al., 1986; Ridgway, 1990; for reviews of this literature) there are only a handful of studies in which morphometric analyses were conducted in a systematic way permitting quantitative comparative analysis with other mammals (Jacobs et al., 1984; Johnson et al., 1984; Schwerdtfeger et al., 1984; Garey and Leuba, 1986; Johnson et al., 1994; Tarpley and Ridgway, 1994; Manger et al., 1998; Marino, 1998). Furthermore, with the exception of Morgane et al. (1980), Ridgway and Brownson (1984), Haug (1987), and Tarpley
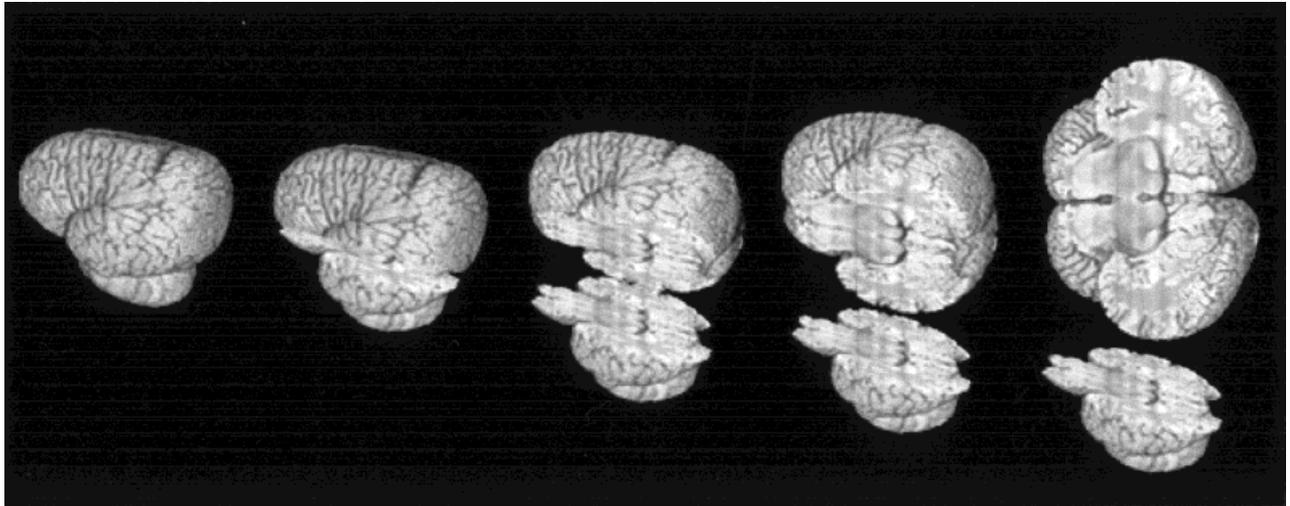
Fig. 2. Three-dimensional digital reconstructions of the whole brain and resectioning to produce "virtual" horizontal sections.
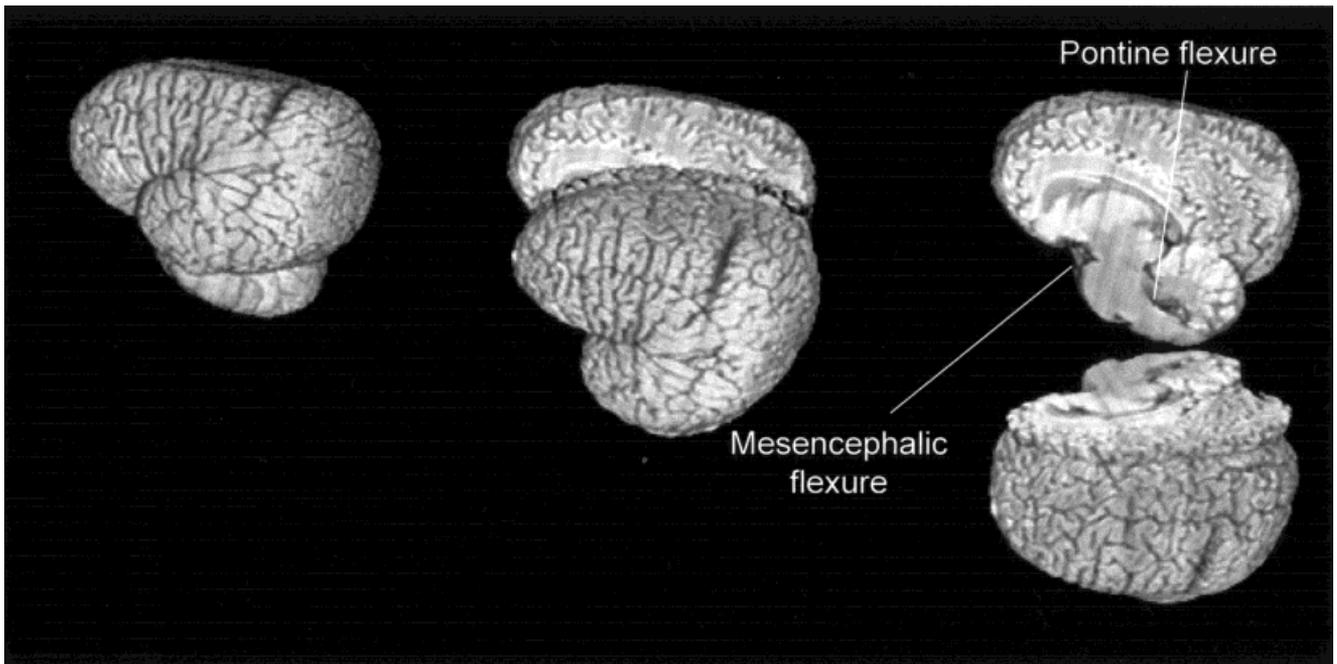

Fig. 3. Three-dimensional digital reconstructions of the whole brain and resectioning to produce "virtual" sagittal sections.

and Ridgway (1994) there are no systematic anatomical descriptions of whole cetacean brains and substructures at the qualitative level. There currently exists no comprehensive cetacean neuroanatomical atlas either in paper or electronic format on which to base studies of cetacean brain organization and function. This situation is mainly due to the time and practicality associated with the preparation of such large brain specimens. Magnetic resonance imaging (MRI) offers a means of observing the internal structure of the brain where traditional procedures of embedding, sectioning, staining, mounting, and microscopic examination of thousands of sections are not practical. Furthermore internal structures can be analyzed in

their precise spatial interrelationships, which is difficult to accomplish after the spatial distortions often accompanying histological processing. This study presents an anatomically-labeled three-dimensional atlas, created from MRI images, of the brain of one of the most behaviorally studied odontocetes, the white whale (*Delphinapterus leucas*).

MATERIALS AND METHODS
Specimen

The specimen is the postmortem brain, fixed in 10% buffered formalin, of an adult female white whale (*Delphinapterus leucas*) who died of natural causes. The whale
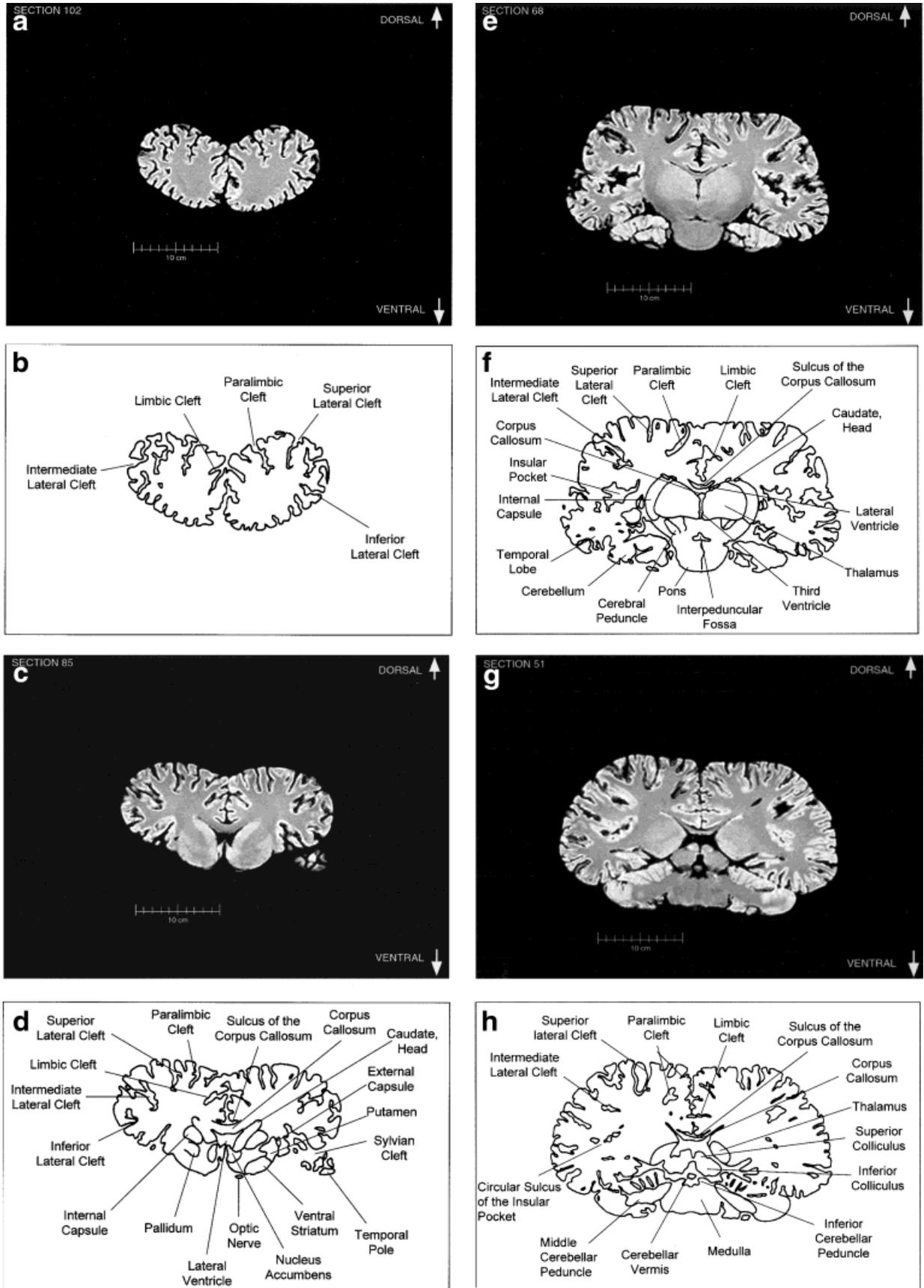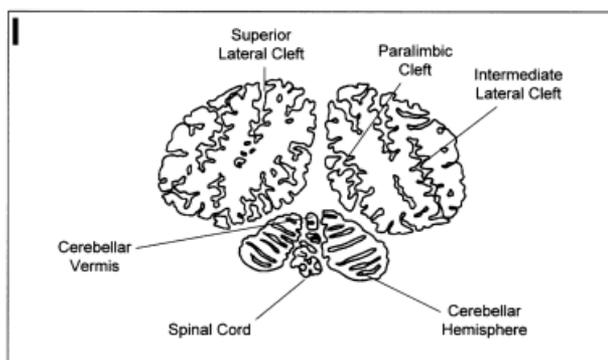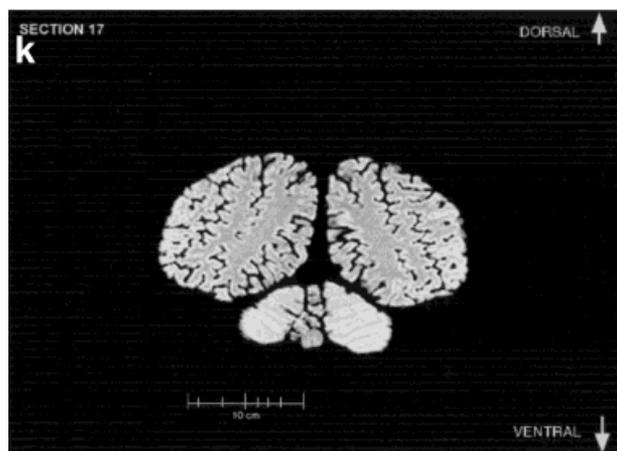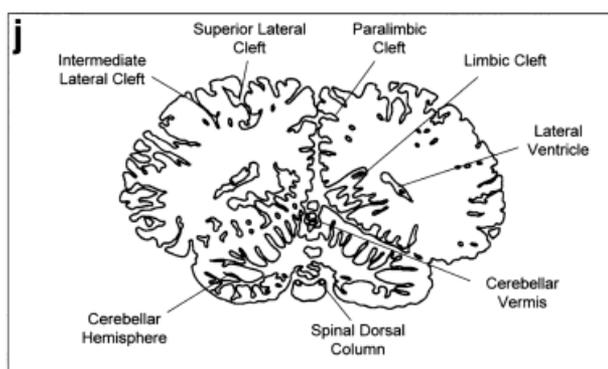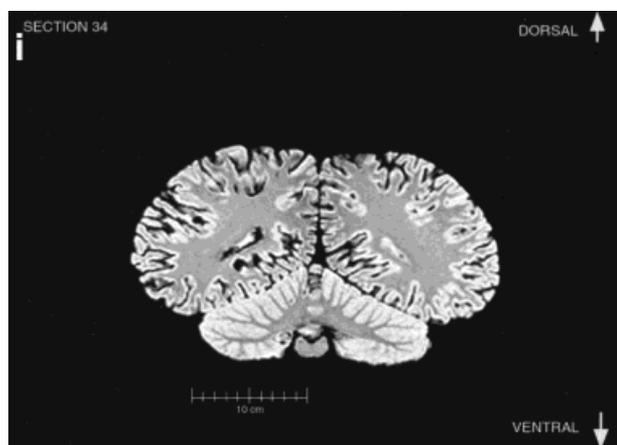
Figure 4.

had been involved in several behavioral studies including studies of its hearing (Awbrey et al., 1988). At death, the brain was extracted from the skull, weighed, and placed in neutral buffered formalin for 4 years before scanning. Fresh brain weight was 1,871 g. Fixed brain weight was 1,755 g at the time of scanning.

Magnetic Resonance Imaging

The brain was removed from the fluid and placed in a head coil ventral side down. Images of the entire brain were acquired in the coronal plane at 119 antero-posterior levels with a 1.5 T Siemens scanner (slice thickness = 1.3 mm, slice interval = 1.3 mm, isotropic 1 mm voxels, MP-RAGE sequence, field of view = 240 mm, matrix = 256 × 256). The scanning was done by Richard Buxton, PhD at the University of California, San Diego.

Three-Dimensional Reconstruction and Reformatting

Computer-generated three-dimensional reconstruction images were created by Timothy L. Murphy, using the software programs VoxelView and VoxelMath programs (Vital Images, Inc.) at the Laser Scanning Microscopy Laboratory at Michigan State University, Joanne Whallon, Director. The 3D rendered model, wherein details of internal and external morphology are represented in three-dimensional space, was then digitally resectioned in orthogonal planes to produce corresponding 0.9375 mm-thick "virtual" sections in the horizontal (163 "virtual" sections) and sagittal (236 "virtual" sections) planes.

Anatomical Labeling and Nomenclature

All identifiable anatomical structures of the white whale brain were labeled in the originally-acquired coronal plane images as well as in the images from the "virtual" sectioned brain in the sagittal and horizontal planes. The nomenclature used is from Morgane et al. (1980). As a guide to the identification of structures, the MRI scans and the sections from the three-dimensional reconstruction of the whale brain were compared with the few published illustrations (or images of real stained) sections through the white whale brain and photographs of the whole brain (Yablokov et al., 1964; Morgane et al., 1980). They were also compared with similar MRI scans and "virtual" sections and three-dimensional reconstructions from the scans of brains of bottlenose dolphins (*Tursiops truncatus*) (Morgane et al., 1980). All were compared with complete alternate series of sections from brains of bottlenose dolphins, stained, respectively, for cell bodies (Nissl method), and for myelinated fibers in the same three orthogonal planes (coronal, sagittal, and horizontal). These stained section series are from the Yakovlev-Haleem collection at the National Museum of Health and Medicine and the Welker collection at the University of Wisconsin-Madison.

RESULTS
Three-Dimensional Reconstruction

Three-dimensional reconstructions of the white whale's whole brain were produced from the original scans in the

Fig. 4. Rostral-to-caudal sequence of originally-acquired 1.3 mm-thick coronal brain sections in 22 mm intervals and labeled schematic illustrations of each section.
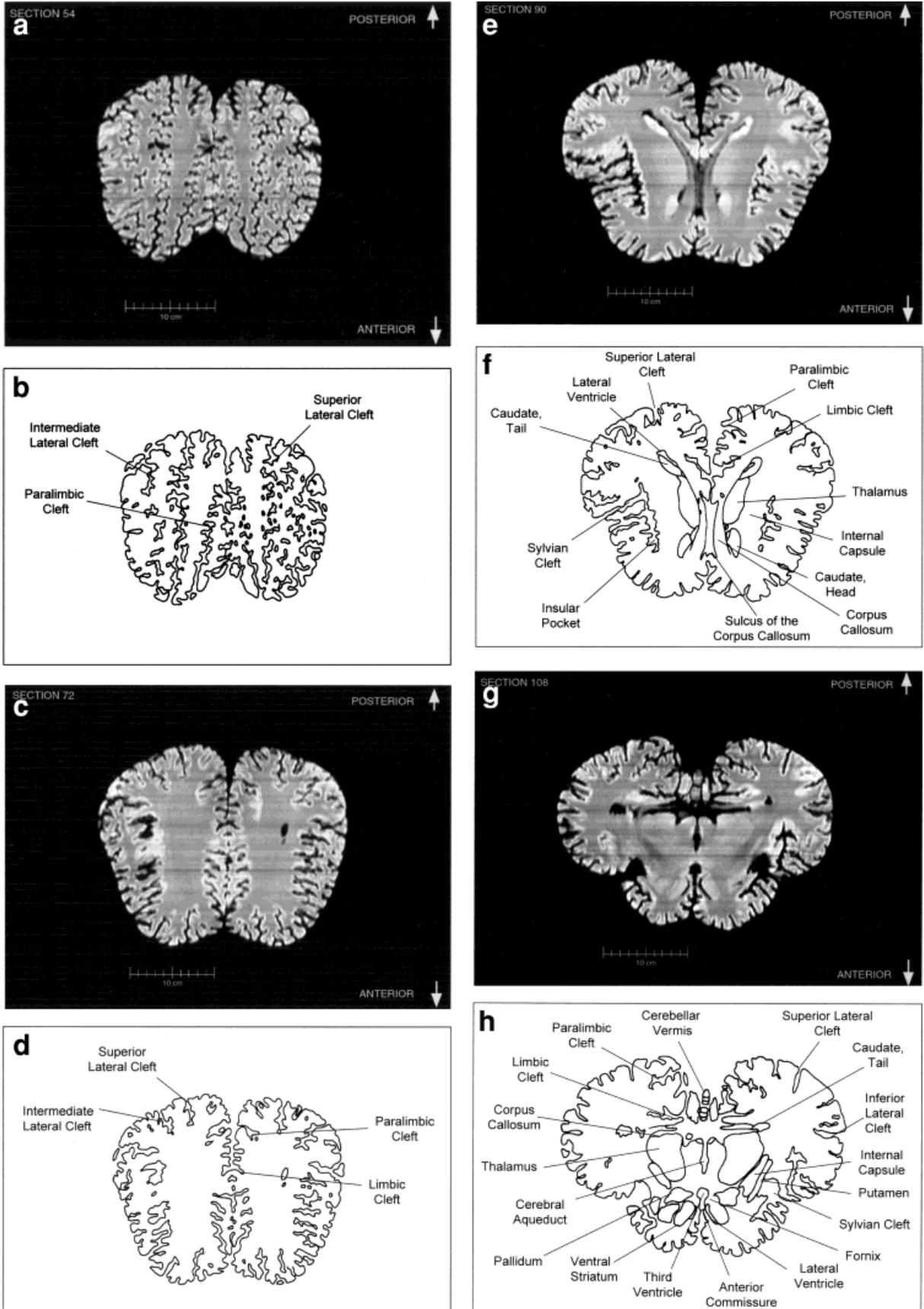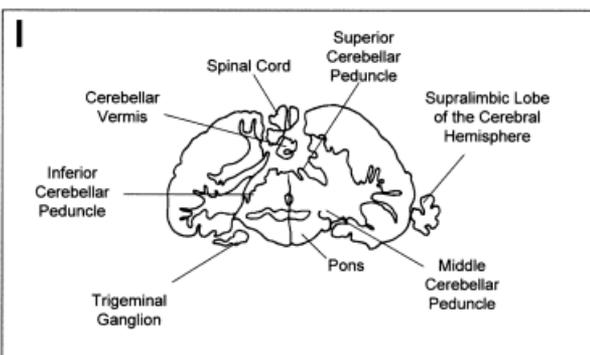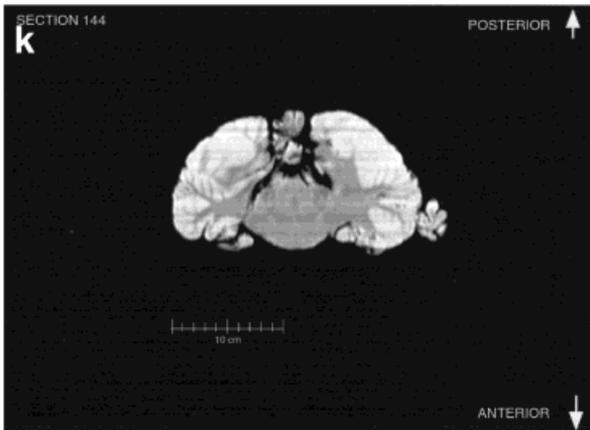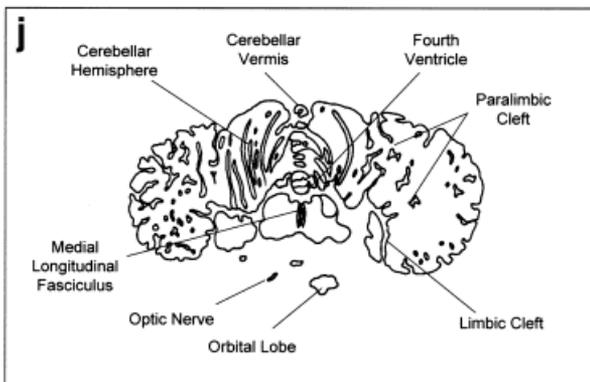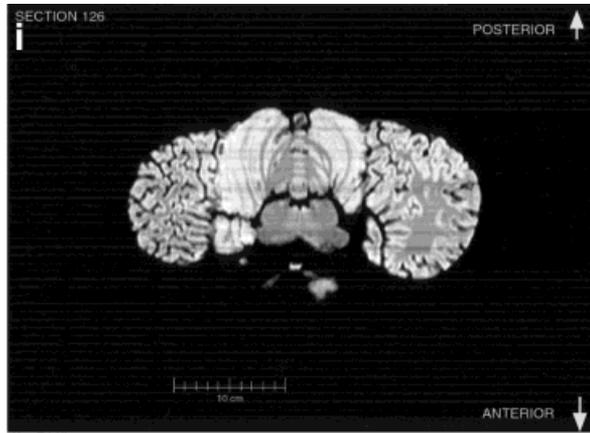
Figure 5.

coronal plane. Figure 1 displays an image (or view) of the posterior-ventral surface of a computer-generated three-dimensional reconstruction of the whole brain and a labeled illustration of the image. Figures 2 and 3 also display three-dimensional reconstructions of the whole brain and the brain digitally recut in the horizontal and sagittal planes, respectively. These three-dimensional reconstructions clearly display many noted characteristics of the cetacean brain that diverge from most other terrestrial mammalian brains (Morgane et al., 1980). The foreshortened orbital lobes are evident in Figure 3 as is the pronounced bitemporal width of the brain in Figures 1 and the last "virtual" cut in Figure 2. The labeled "virtual" cut in Figure 3 shows the mesencephalic and pontine flexures reminiscent of brainstem flexure patterns in the embryonic state of most terrestrial mammals. These flexures remain present in adult cetacean brains.

Anatomically-Labeled Two-Dimensional MRI Sections

Figure 4a–l displays a rostral-to-caudal sequence of originally-acquired 1.3 mm-thick coronal MRI brain sections at 22 mm intervals along with a labeled schematic illustration of each section. Figure 5 displays a dorsal-to-ventral sequence of reconstructed "virtual" horizontal sections (0.9375 mm thick) and a labeled schematic of each section. Figure 6 displays a lateral-to-medial sequence of reconstructed "virtual" sagittal sections, also 0.9375 mm thick, through the left hemisphere at 18.75 mm intervals (with the last two images 10.3 mm apart) and a labeled schematic for each section.

The high level of convolution of the cortex is evident in almost all of the figures. The extreme depth and density of cortical sulci are particularly evident in Figure 6 (c–f). These images also display an orbital-to-occipital gradient of increased sulcation concordant with the increased elaboration of the occipital-parietal region over the orbital region. This occipital-parietal elaboration is evident in Figure 6 (g–j) in the striking triple-tiered arrangement of limbic, paralimbic, and supralimbic arcuate cortical lobules divided by the deep limbic and paralimbic clefts. This specific combination of occipital-parietal organization and elaboration is distinct from other mammals.

In contrast to the distinctive cortical features, the odontocete brain generally resembles other mammalian brains on a subcortical level. The volumetric proportions of various subcortical features, however, reveal even more of the distinctive adaptations and allometric rearrangements associated with odontocete evolution. As seen in Figure 1, the olfactory bulbs are absent. In contrast, auditory processing areas are enlarged (though visual structures are not necessarily reduced). These include the proportionately large inferior colliculus compared with the superior colliculus as seen most clearly in Figure 4 (g,h).

In keeping with behavioral and electrophysiological evidence for a high degree of hemispheric independence (Viamonte et al., 1968; Mukhametov et al., 1977; Mukhametov, 1984), the corpus callosum is small relative

Fig. 5. Dorsal-to-ventral sequence of reconstructed 0.9375-mm thick horizontal brain sections and labeled schematic illustrations of each section.
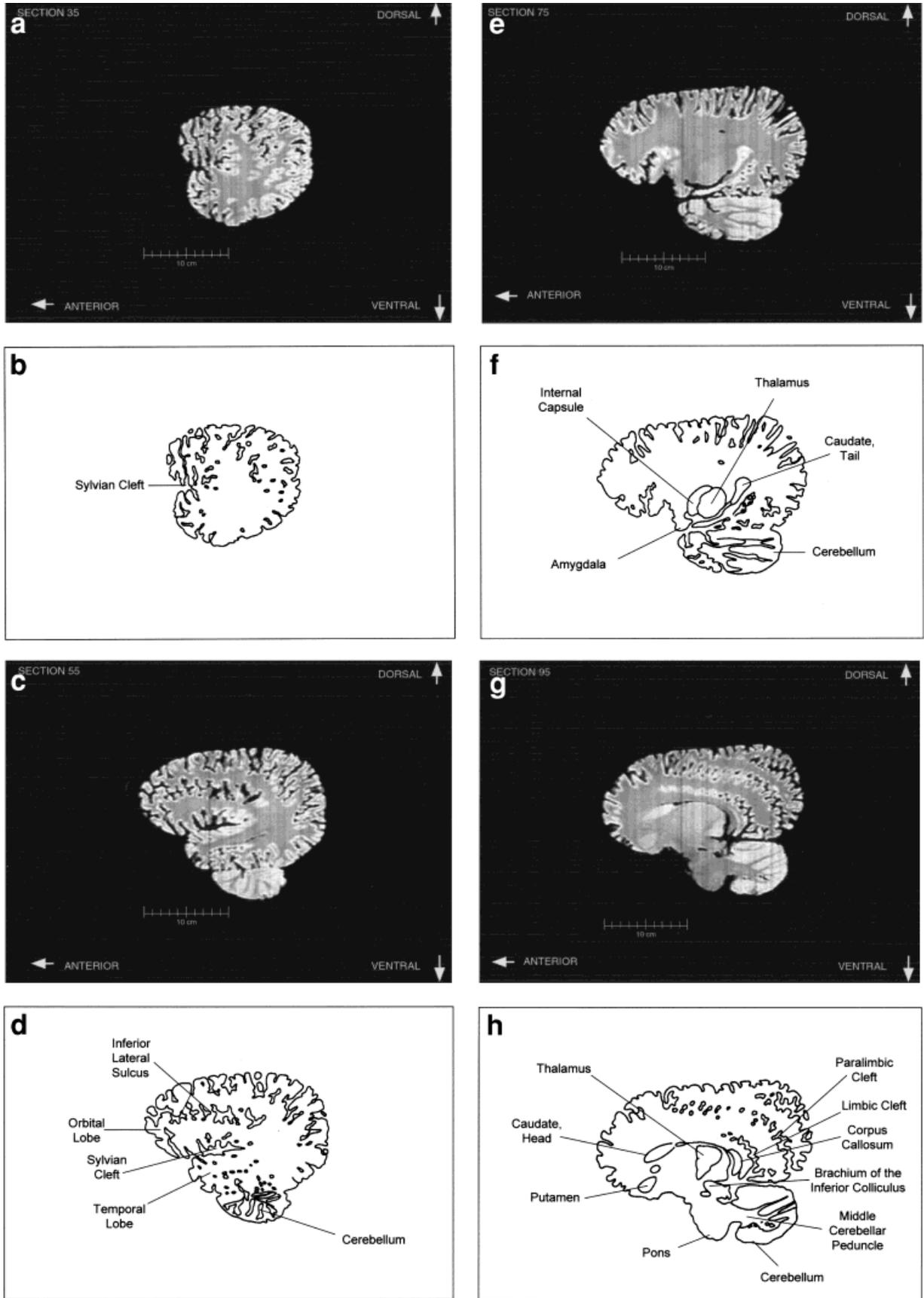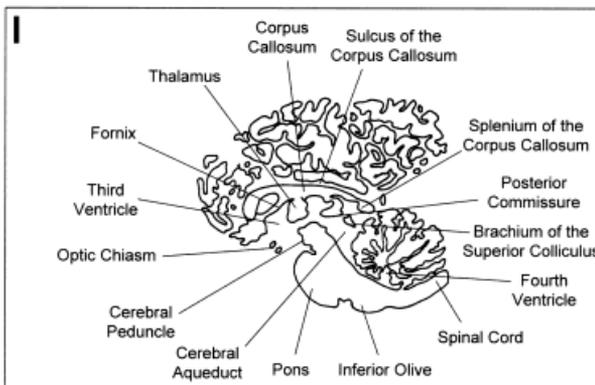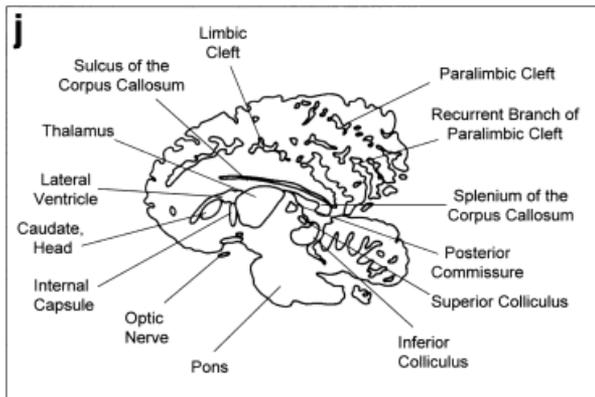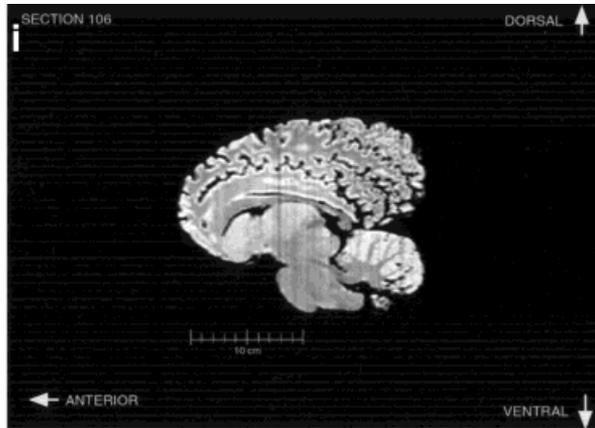
Figure 6.

to the massive hemispheres, consistent with quantitative findings in other odontocete species and qualitative observations of the white whale brain (Tarpley and Ridgway, 1994). This is apparent in most of the figures but particularly in Figure 6 (g–l).

The cerebellum is large relative to the hemispheres. This is especially evident in Figure 4 (g–j), and Figure 5 (i,j), and also in the sagittal images. As shown in Figure 4 (e,f) the cerebral peduncles are high on the lateral surface of the caudal diencephalon and through the entire midbrain, rather than on the basal inferior or ventral surface as in most mammals. The basal surface is instead occupied by a large mass of gray matter that appears to be continuous with the ventral striatum and the dorsal and ventral pallidum of the forebrain reaching from these structures to the pontine nuclei caudally. The approximation of apparent basal ganglia and pons may be a consequence of the flexing of the diencephalon and the midbrain bringing the brain stem into contact with the basal forebrain, or it may represent a specialized development of the basal ganglia.

DISCUSSION

This study presents the first MRI-based, anatomically-labeled, three-dimensional atlas of the brain of the white (beluga) whale (*Delphinapterus leucas*). In addition, we have constructed three-dimensional models of the white whale brain and produced "virtual" horizontal and sagittal sections from these original images. These reconstructed images allow for the visualizing of a range of distinctive white whale brain features from various orientations by preserving the gross morphological and internal structure of the specimen. Because there are none of the distortions associated with histological processing, we have a more realistic view of the brain as it was in situ.

Many cortical features are easily identified from the original MRI scans and "virtual" images. These include the distinctive lobular formations, gyral and sulcal patterns, and general gradient of elaboration in the parietal, occipital, and temporal regions. Subcortical allometry, including that of both gray and white matter structures, is easily assessed as well. Our findings are consistent with what has been noted in the few existing histological studies of the odontocete brain. Moreover, because we are able to preserve the internal structure of the specimen, neuroanatomical studies of brains from MRI set the stage for much-needed accurate and reliable morphometric analyses of various brain structures in odontocetes. These studies are underway.

Evolutionary Considerations

There is a deep evolutionary divergence of the Order Cetacea (of which Odontoceti is a suborder) from other mammalian lines. Furthermore, cetacean evolution is characterized by distinctive environmental pressures associated with a fully aquatic existence versus a terrestrial lifestyle. These related attributes make the comparative study of structure-function relationships in cetacean brains, compared with those of other mammals, uniquely

Fig. 6. Lateral-to-medial sequence of reconstructed 0.9375 thick sagittal sections and labeled schematic illustrations of each section.

valuable for improving our understanding of the parameters of mammalian brain evolution.

The brain of the white whale as revealed in this study is characterized by similar morphological trends as those found in the bottlenose dolphin and other cetaceans (Morgane et al., 1980). Although there are differences among cetacean brains, these differences are relatively minor compared with the striking dissimilarities to brains of other mammals. The most obvious difference between cetacean brains and those of other mammals is in the gross morphological configuration of the whole structure and the lobules of the cerebral hemispheres. These are well-visualized in MRI scans. Evolution of overall brain shape in cetaceans may have been partly due to migration of the blowhole and telescoping of the skull, i.e., antorbital elongation and postorbital compression. This in turn may account for the distinctive construction of the midbrain, i.e., the corticopontine, corticobulbar and corticospinal fibers travel high on the lateral surface whereas the ventral surface is occupied by a large continuous mass of gray matter extending from the diencephalon rostrally to the pontine nuclei caudally. There may be distinctive organizational features of the basal ganglia that also contribute to this uniquely cetacean architecture.

There is also adequate evidence that many of the anatomical changes in the cetacean brain represent changes in function, e.g., loss of olfactory structures and enlargement of acoustic structures. Similar, convergent changes in function, along with their neuroanatomical correlates, are observed in several brains of unrelated clades, such as many bats and primates (Johnson et al., 1984, 1994). In general, the cetacean brain possesses some common mammalian features in combination with specialized and highly unusual features, the function of which we have barely begun to understand.

CONCLUSIONS

If we are to eventually understand the functional significance of this mosaic of typical mammalian and uniquely cetacean features, the structural organization of the cetacean brain must be further elucidated. This can be rapidly and effectively accomplished by MRI-based studies of neuroanatomy. In comparison, already-existing data from the more traditional methods of sectioning and staining are very time-intensive, expensive, and vulnerable to spatial distortion compared with the data acquired by MRI. Studies like the present one are crucial for establishing the structural basis of and templates for future functional studies using non-invasive neuroimaging techniques to investigate the neurobiological basis of cetacean cognition and behavior.

ACKNOWLEDGMENTS

LITERATURE CITED

Awbrey FT, Thomas JA, Kastelein RA.1988. Low-frequency underwater hearing sensitivity in belugas, *Delphinapterus leucas*. J Acoust Soc Am 84:2273–2275

Buhl EH, Oelschlager HA. 1988. Morphogenesis of the brain in the harbor porpoise. J Comp Neurol 277:109–125.

Garey LJ, Leuba G. 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.

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.

Glezer II, Hof PR, Leranth C, Morgane PJ. 1992a. Morphological and histological features of odontocete visual neocortex: immunocytochemical analysis of pyramidal and nonpyramidal populations of neurons. In: Thomas JA, Kastelein RA, Supin AY, editors. Marine mammal sensory systems. New York: Plenum Press. p 1–38.

Glezer II, Hof PR, Morgane PJ. 1992b. Calretinin-immunoreactive neurons in the primary visual cortex of dolphin and human brains. Brain Res 595:181–188.

Glezer II, Hof PR, Istomin VV, Morgane PJ. 1995a. Comparative immunocytochemistry of calcium-binding protein-positive neurons in visual and auditory systems of cetacean and primate brains. In: Kastelein RA, Thomas JA, Nachtigall PE, editors. Sensory systems of aquatic mammals. The Netherlands: De Spil Publishers. p 477–513.

Glezer II, Hof PR, Leranth C, Morgane PJ. 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.

Glezer II, Hof PR, Morgane PJ. 1995b. Cytoarchitectonics and immunocytochemistry of the inferior colliculus of midbrains in cetaceans. FASEB J 9:A247.

Glezer II, Hof PR, Morgane PJ. 1998. Comparative analysis of calcium-binding protein-immunoreactive neuronal populations in the auditory and visual systems of the bottlenose dolphin (*Tursiops truncatus*) and the macaque monkey (*Macaca fascicularis*). J Chem Neuro15:203–237.

Glezer II, Jacobs M, Morgane P. 1988. Implications of the 'initial brain' concept for brain evolution in Cetacea. Behav Brain Sci 11:75–116.

Glezer II, Morgane, PJ. 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.

Glezer II, Morgane PJ, Leranth C. 1990. Immunohistochemistry of neurotransmitters in visual cortex of several toothed whales: light and electron microscopic study. In: Thomas JA, Kastelein RA, editors. Sensory abilities of Cetaceans: laboratory and field evidence. New York: Plenum Press. p 39–60.

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, marsupialia, insectivores and one elephant). Am J Anat 180:126–142.

Hof PR, Glezer II, Archin N, Janssen WG, Morgane PJ, Morrison JH. 1992. The primary auditory cortex in cetacean and human brain: a comparative analysis of neurofilament protein-containing pyramidal neurons. Neurosci Lett 146:91–95.

Hof PR, Glezer II, Revishchin AV, Bouras C, Charnay Y, Morgane PJ. 1995. Distribution of dopaminergic fibers and neurons in visual and auditory cortices of the harbor porpoise and pilot whale. Brain Res Bull 36:275–284.

Jacobs MS, Galaburda AM, McFarland WL, Morgane PJ. 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.

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.):1–108.

Johnson JI, Kirsch JA, Switzer III RC. 1984. Brain traits through phylogeny: the evolution of neural characters. Brain Behav Evol 20:97–117.

Johnson JI, Kirsch JA, Reep RL, Switzer III RC. 1994. Phylogeny through brain traits: more characters for the analysis of mammalian evolution. Brain Behav Evol 43:319–347.

Manger P, Sum M, Szymanski M, Ridgway S, Krubitzer L. 1998. Modular subdivisions of dolphin insular cortex: does evolutionary history repeat itself? J Cognitive Neurosci 10: 153–166.

Marino L. 1998. A comparison of encephalization levels between adult Anthropoid Primates and Odontocetes (toothed whales). Brain Behav Evol 51:230–238.

Morgane P, Jacobs M, Galaburda A. 1986. Evolutionary morphology of the dolphin brain. In: Schusterman RJ, Thomas JA, Wood FG, editors. Dolphin cognition and behavior: a comparative approach. Hillsdale, NJ: Lawrence Erlbaum Associates. p 5–30.

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.):1–107.

Mukhametov LM. 1984. Sleep in marine mammals. Exp Brain Res 8:227–238.

Mukhametov LM, Supin AY, Polyakova IG. 1977. Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. Brain Res 134:581–584.

Oelschlager HA, Buhl, EH. 1985. Occurrence of an olfactory bulb in the early development of the harbor porpoise (*Phocoena phocoena L.*). In: Duncker HR, Fleischer G, editors. Functional morphology in vertebrates. New York: Fischer. p 695–698.

Oelschlager HA, Kemp B. 1998. Ontogenesis of the sperm whale brain. J Comp Neurol 399:210–228.

Ridgway SH. 1986. The central nervous system of the bottlenose dolphin. In: Schusterman RJ, Thomas JA, Wood FG, editors. Dolphin cognition and behavior: a comparative approach. Hillsdale, NJ: Lawrence Erlbaum Associates. p 31–60.

Ridgway SH. 1990. The central nervous system of the bottlenose dolphin. In: Leatherwood S, Reeves R, editors. The bottlenose dolphin. San Diego: Academic Press. p 69–97.

Ridgway SH, Brownson RH. 1984. Relative brain sizes and cortical surface areas in odontocetes. Acta Zool Fennica 172:149–152.

Schwerdtfeger WK, Oelschlager HA, Stephan H. 1984. Quantitative neuroanatomy of the brain of the La Plata dolphin, *Pontoporia blainvillei*. Anat Embryol 170:11–19.

Tarpley RL, Ridgway, SH. 1994. Corpus callosum size in delphinid cetaceans. Brain Behav Evol 44:156–165.

Viamonte M, Morgane PJ, Galliano RE, Nagel EL, McFarland WL. 1968. Angiography in the living dolphin and observations on blood supply to the brain. Am J Physiol 214:1225–1249.

Yablokov, AV, Bel'kovich BM, Tarasevich MN. 1964. The central nervous system and sense organs. In: Kleinenberg SE, Yablokov AV, Bel'kovich BM, editors. Beluga (*Delphinapterus leucas*) investigation of the species. USSR: Academy of Sciences of the USSR. p 169–183.