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Lori Marino  
*Emory University*

Timothy L. Murphy  
*Michigan State University*

Lyad Gozal  
*Emory University*

John I. Johnson  
*Michigan State University*

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# Magnetic resonance imaging and three-dimensional reconstructions of the brain of a fetal common dolphin, *Delphinus delphis*

Lori Marino,<sup>1</sup> Timothy L. Murphy,<sup>2</sup> Lyad Gozal,<sup>1</sup> John I. Johnson<sup>2</sup>

<sup>1</sup> Emory University

<sup>2</sup> Michigan State University

## KEYWORDS

Neuroanatomy, development, cetacean, odontocete, MRI, Magnetic Resonance Imaging

## ABSTRACT

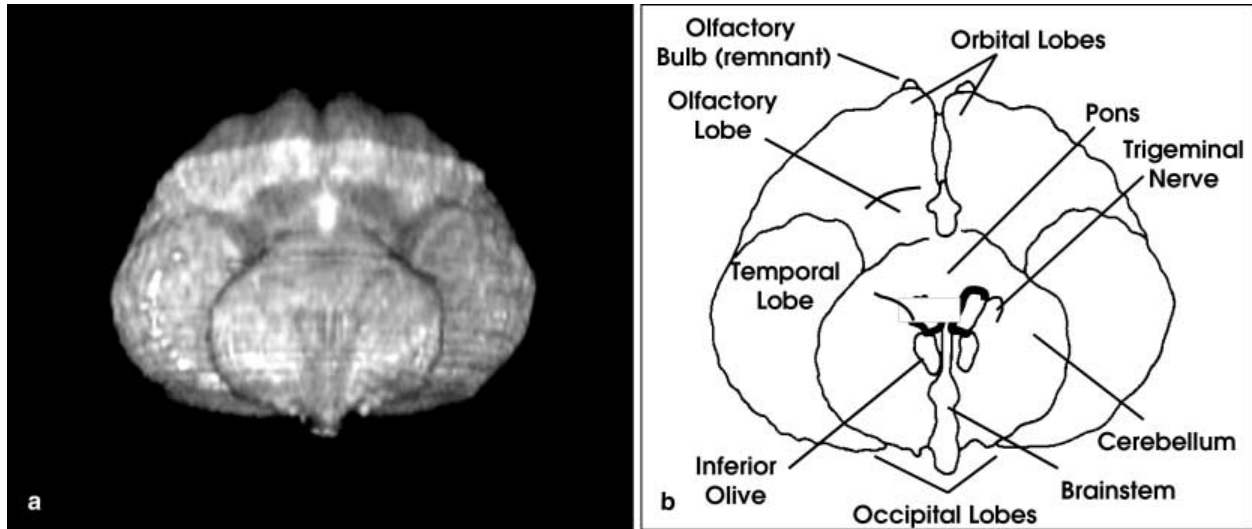
*To demonstrate the kinds of data that can be obtained non-destructively and non-invasively from preserved museum specimens using modern imaging technology the head region of a whole body fetal specimen of the common dolphin, *Delphinus delphis*, aged 8–9 months post-conception, was scanned using Magnetic Resonance Imaging (MRI). Series of scans were obtained in coronal, sagittal and horizontal planes. A digital three-dimensional reconstruction of the whole brain was prepared from the coronal series of scans. Sectional areas and three-dimensional volumes were obtained of the cerebral hemispheres and of the brainstem plus cerebellum. Neuroanatomical features identified in the scans include the major sulci of the cerebral hemispheres, well-differentiated regions of gray and white matter, the mesencephalic, pontine, and cervical flexures, the “foreshortened” appearance of the forebrain, and the large auditory inferior colliculi. These findings show that numerous features of the fetal common dolphin brain can be visualized and analyzed from MRI scans.*

## Introduction

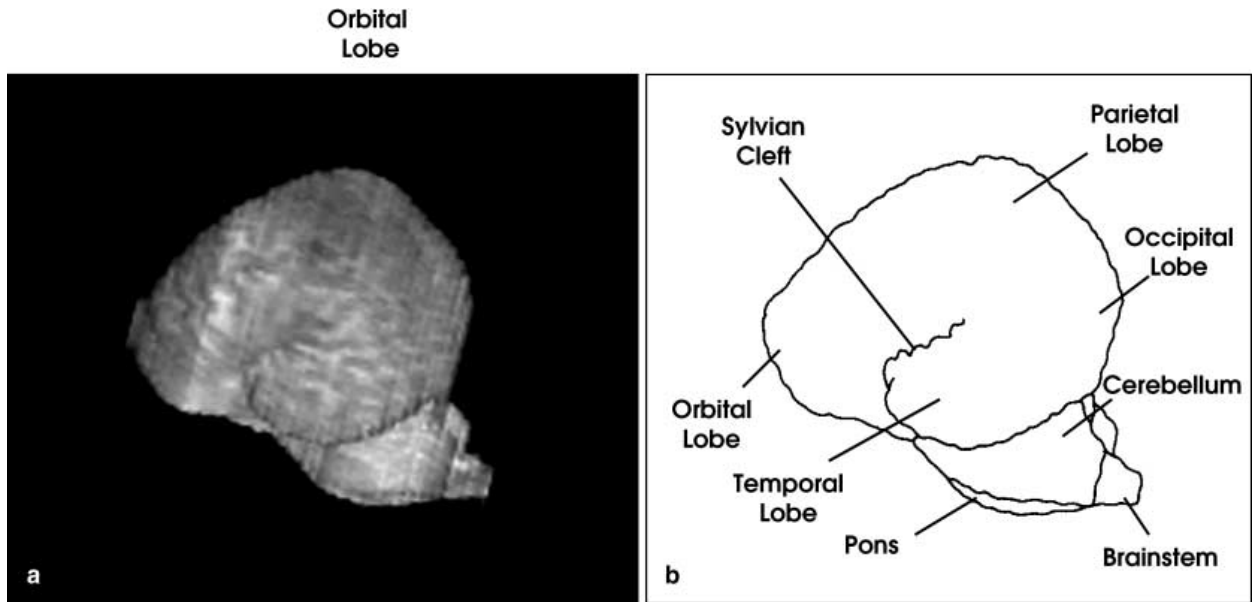
Cetaceans (whales, dolphins, and porpoises) have undergone a number of evolutionary modifications from their terrestrial ancestral state. One of the more dramatic transformations occurred within the suborder Odontoceti (toothed whales, dolphins, and porpoises) in brain size, neuroanatomical structure and organization. Compared with the brains of other mammalian orders, odontocete brain morphology is unusual in many respects. Researchers have 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, pp 105). Because of the fifty-five million year divergence between cetaceans and other mammals, odontocete brains are a blend of early mammalian features along with unique derived characteristics (Ridgway 1986, 1990; Glezer et al. 1988; Manger et al. 1998). There have been numerous published studies of adult odontocete brains. These studies have documented the differences between odontocete and other mammalian brains at the level of cortical cytoarchitecture and immunohistochemistry (Garey et al. 1985; Garey and Leuba 1986; Glezer and Morgane 1990; Glezer et al. 1990, 1992a, b; Hof et al. 1992; Hof et al. 1995; Glezer et al. 1998), cortical surface morphology

(Jacobs et al. 1979; Morgane et al. 1980; Haug 1987), and noncortical structures and features (Tarpley and Ridgway 1994; Glezer et al. 1995). In contrast, there have been relatively few published studies of cetacean prenatal neuroanatomy. These studies have been restricted to a few odontocete species such as the sperm whale, *Physeter macrocephalus* (Oelschlager and Kemp 1998) and harbour porpoise, *Phocoena phocoena* (Oelschlager and Buhl 1985; Buhl and Oelschlager 1988). To date, no published studies exist on the prenatal neuroanatomy of the common dolphin, *Delphinus delphis*.

**Fig. 1** Three-dimensional reconstruction (a) and labeled schematic illustration (b) of the whole fetal common dolphin brain from a ventral orientation



**Fig. 2** Three-dimensional reconstruction (a) and labeled schematic illustration (b) of the whole fetal common dolphin brain from a sagittal orientation



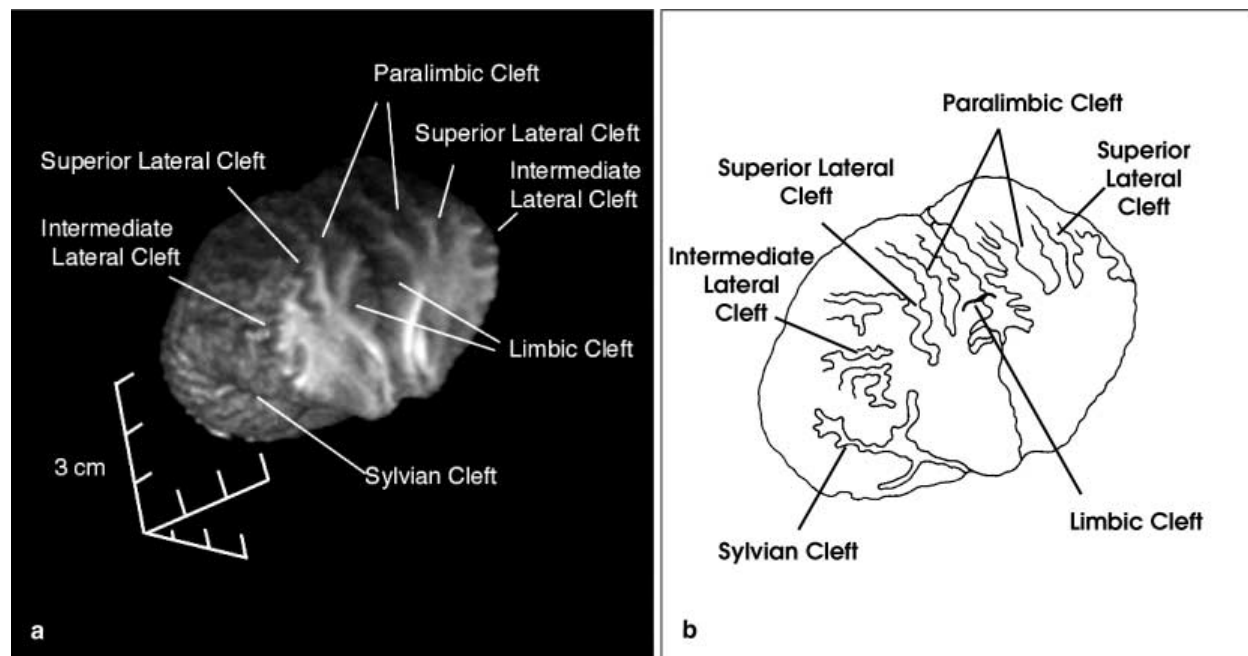
The relative dearth of neuroanatomical studies on prenatal cetaceans is due to both the relative rarity of such specimens and the difficulties involved in accessing and preparing the brain for conventional histological analysis. Thus, it would be extremely beneficial to take advantage of those specimens that already exist in museum and stranding facility collections. Neuroimaging, and Magnetic Resonance Imaging (MRI) in particular, provides a nondestructive and versatile way to visualize, measure, and reconstruct postmortem fetal cetacean brains in situ. In this paper we demonstrate the use of MRI for visualizing, measuring, and reconstructing the postmortem brain of a fetal common dolphin that had been fluid-preserved in a museum collection for 19 years.

## Materials and methods

### Specimen

The specimen is a male fetal common dolphin (*Delphinus delphis*) from the Marine Mammal Collection at the Smithsonian Institution. The specimen was found freshly dead in utero on Curritock Beach in North Carolina, where its mother stranded. Upon removal from the mother, the specimen was pressure perfused with 10% buffered formalin and placed in a container of 70% alcohol with brain intact. The body length is 53 cm, which is consistent with an estimated prenatal age of 8–9 months (Ferrero and Walker 1995; Perrin et al. 1977). Full gestation requires 11 months when the neonate is approximately 82 cm in length (Ferrero and Walker 1995).

**Fig. 3** Three-dimensional reconstruction (a) and labeled schematic illustration (b) of the fetal common dolphin brain, viewed from the right front of the brain, with the “orbital” lobes “dissected away” by a coronal section

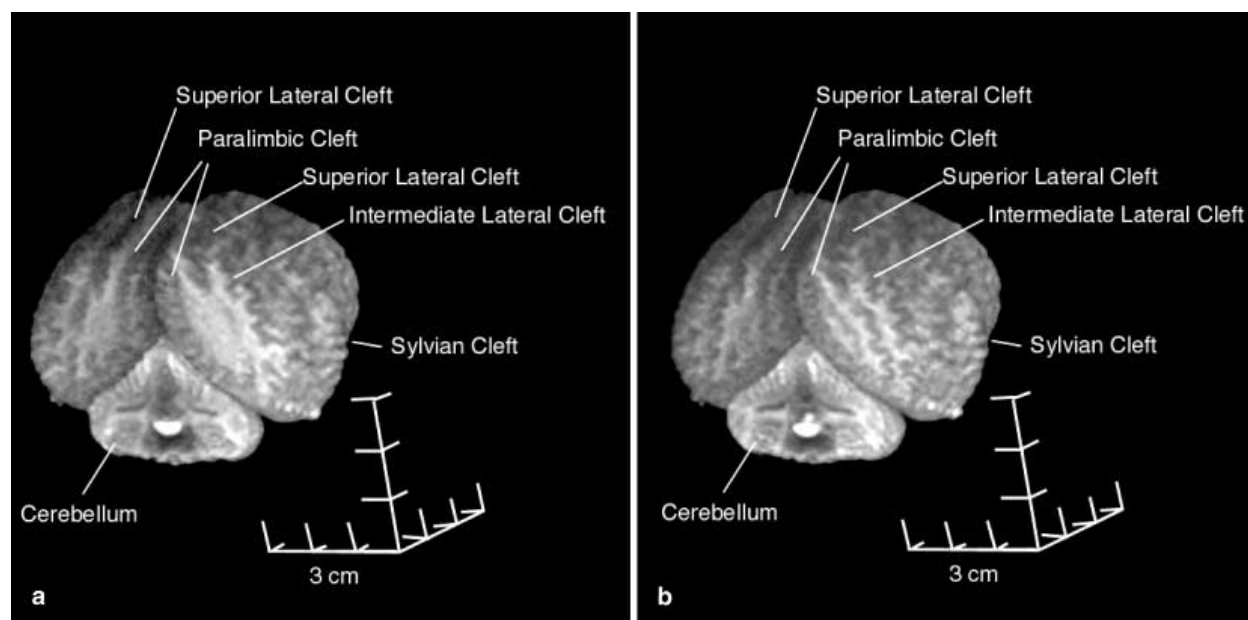


### MRI protocol

After 19 years in fluid preservative, magnetic resonance images of the entire brain in situ were acquired in the coronal, sagittal, and horizontal planes with a General Electric 1.5 Tesla scanner at the Dept. of Radiology at Walter Reed Army Medical Center. T1-weighted coronal images were obtained with the following protocol: pulse sequence=fast spin echo, slice thickness=1.5 mm, slice interval=1.5 mm,

TR=3,800 ms, TE=85 s, field of view=140 mm, matrix=256×256 pixels. T1-weighted sagittal images were acquired with two different spin echo pulse sequences. The first series was obtained with the following parameters: slice thickness=3 mm, slice interval=3 mm, TR=400 ms, TE=9 ms, field of view=120 mm, matrix=256×256 pixels. The second series was obtained with the same parameters as the first except TR=500 ms and TE=14 ms. T1-weighted horizontal scans were obtained with the following spin echo protocol: slice thickness=3 mm, slice interval=3 mm, TR=500 ms, TE=14 ms, field of view=120 mm, matrix=256×256 pixels. The specimen was scanned with the ventral side down in the human head coil. Approximate scan time for each of the series was 30 min.

**Fig. 4a, b** Two similar views of the posterior surface of the reconstructed brain of the fetal common dolphin



### **Volume measurements**

Whole brain, left and right cerebral hemispheres, and hindbrain (which included cerebellum) were measured with the image analysis software program Scion IMAGE for Windows (PC version of NIH IMAGE) using manually defined areas from successive slices that are integrated to arrive at a volume estimate.

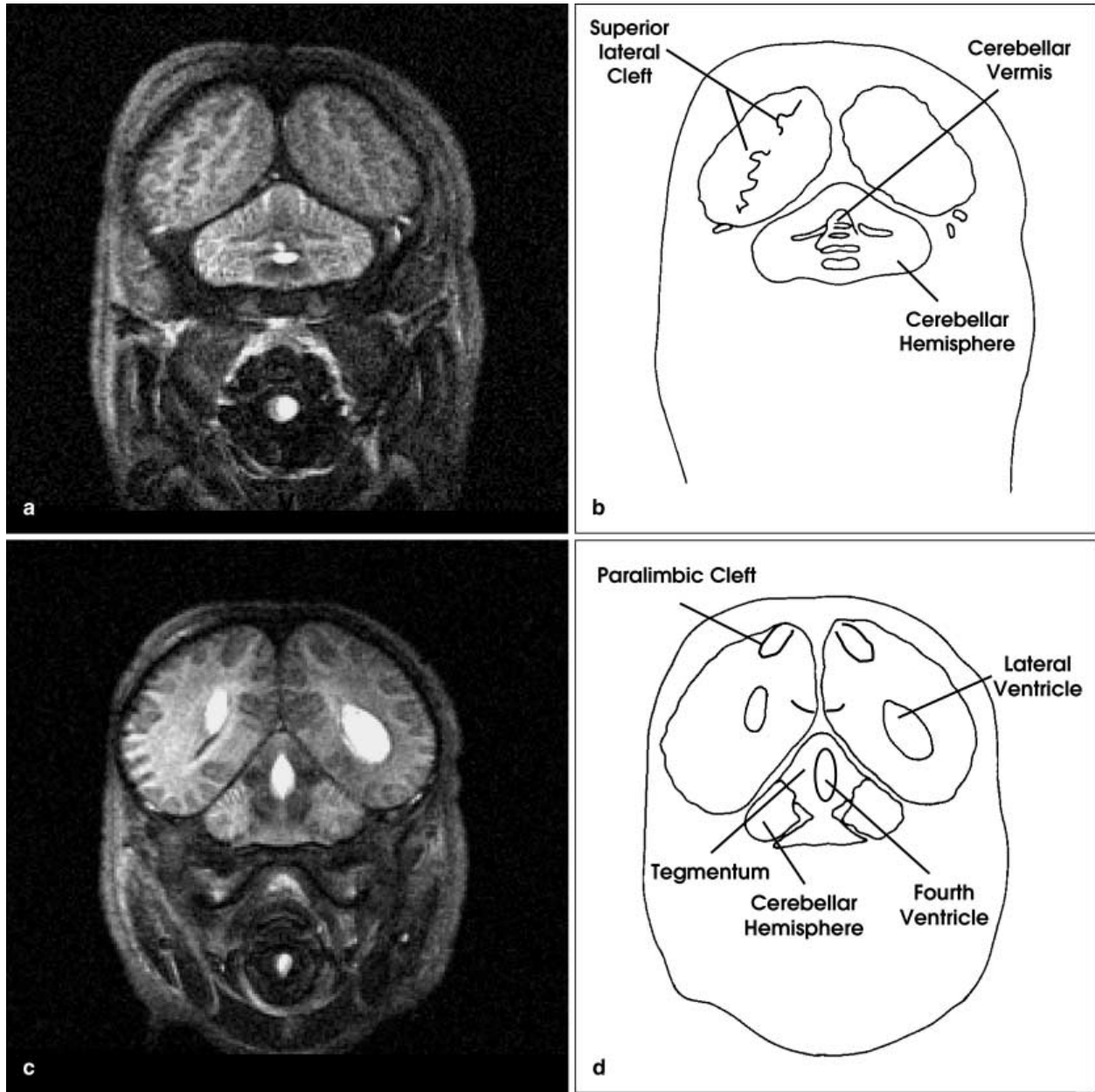
### **Three-dimensional reconstruction and reformatting**

Three-dimensional digital reconstruction images were created by Timothy L. Murphy, using VoxelView/E and VoxelMath programs (Vital Images) at the Laser Scanning Microscopy Laboratory at Michigan State University, Joanne Whallon, Director.

### **Anatomical labeling and nomenclature**

Identifiable neuroanatomical structures were labeled using the nomenclature from Morgane et al. (1980).

**Fig. 5** Posterior-to-anterior series of 1.5 mm thick coronal scans (**a, c, e, g, i, k**) of the fetal common dolphin brain and accompanying labeled schematic illustrations (**b, d, f, h, j, l**)



## Results

### *Qualitative anatomical descriptions*

Figures 1a, b and 2a, b display three-dimensional reconstructions of the specimen from a ventral and lateral viewpoint, respectively. Each MR image is accompanied by an anatomically labeled schematic drawing. These images show a number of major features of the specimen, including the almost vertically-oriented Sylvian cleft (Fig. 2a, b) and the large cerebellum (Figs. 1a, b, 2a, b). Figure 2a, b most clearly displays the “pronated boxing glove” shape typical of odontocete brains and noted by Morgane et al.

(1980) in reference to adult odontocete brains. Notable is the presence of structures that may be construed as residual olfactory bulbs (Fig. 1a, b). Despite the marked regression detectability of these olfactory structures at this relatively late fetal stage is surprising given that olfactory nerves and bulbs are lacking in adult odontocetes altogether (Breathnach 1960) with very few exceptions such as the sperm whale (Ries and Langworthy 1937; Kojima 1951). Therefore, these structures in the present specimen must be interpreted with caution. Secondary olfactory structures, such as the olfactory lobe, are present as expected (Morgane et. al., 1980).

Fig. 5 e-h

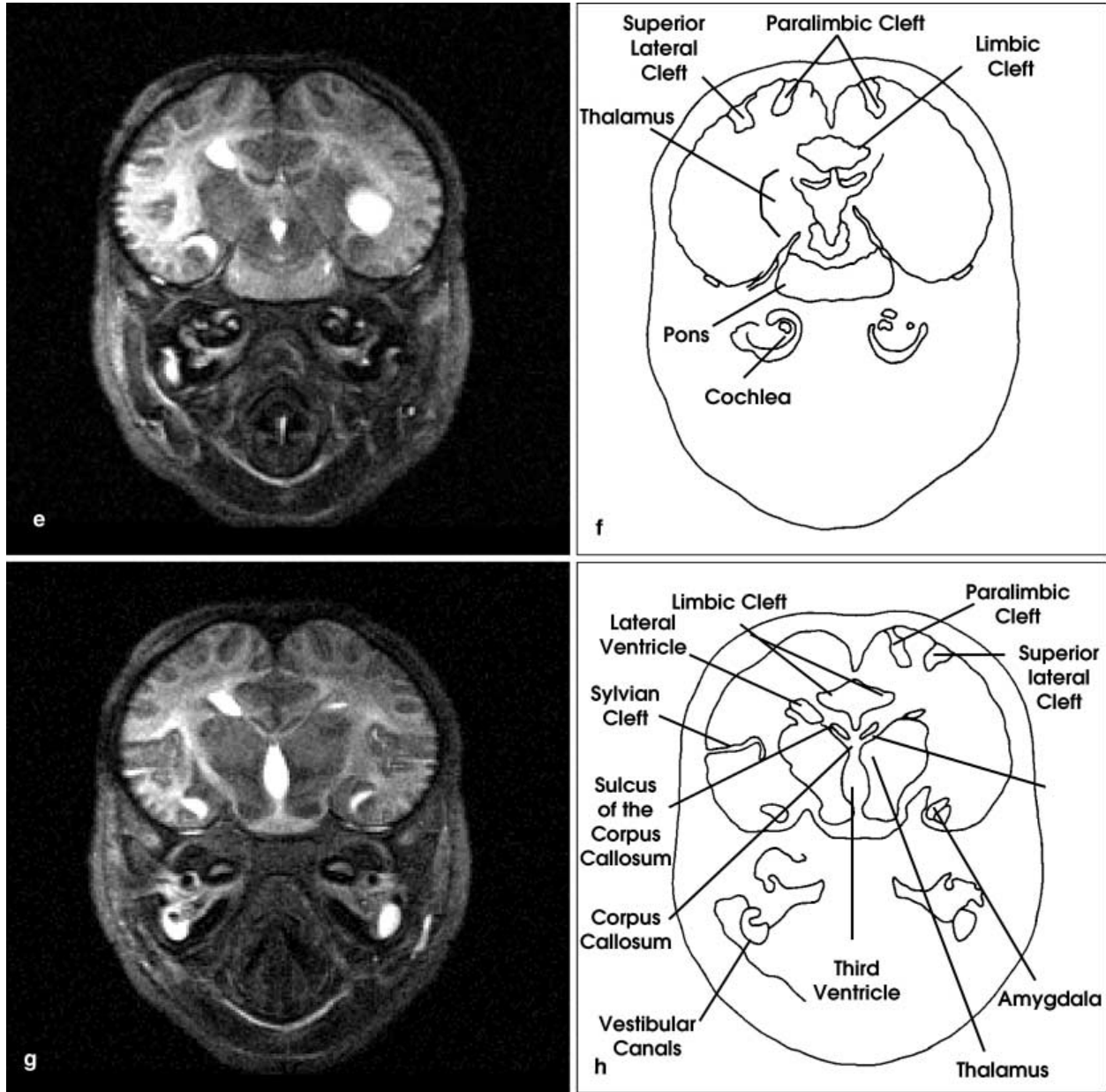


Fig. 5 i-l

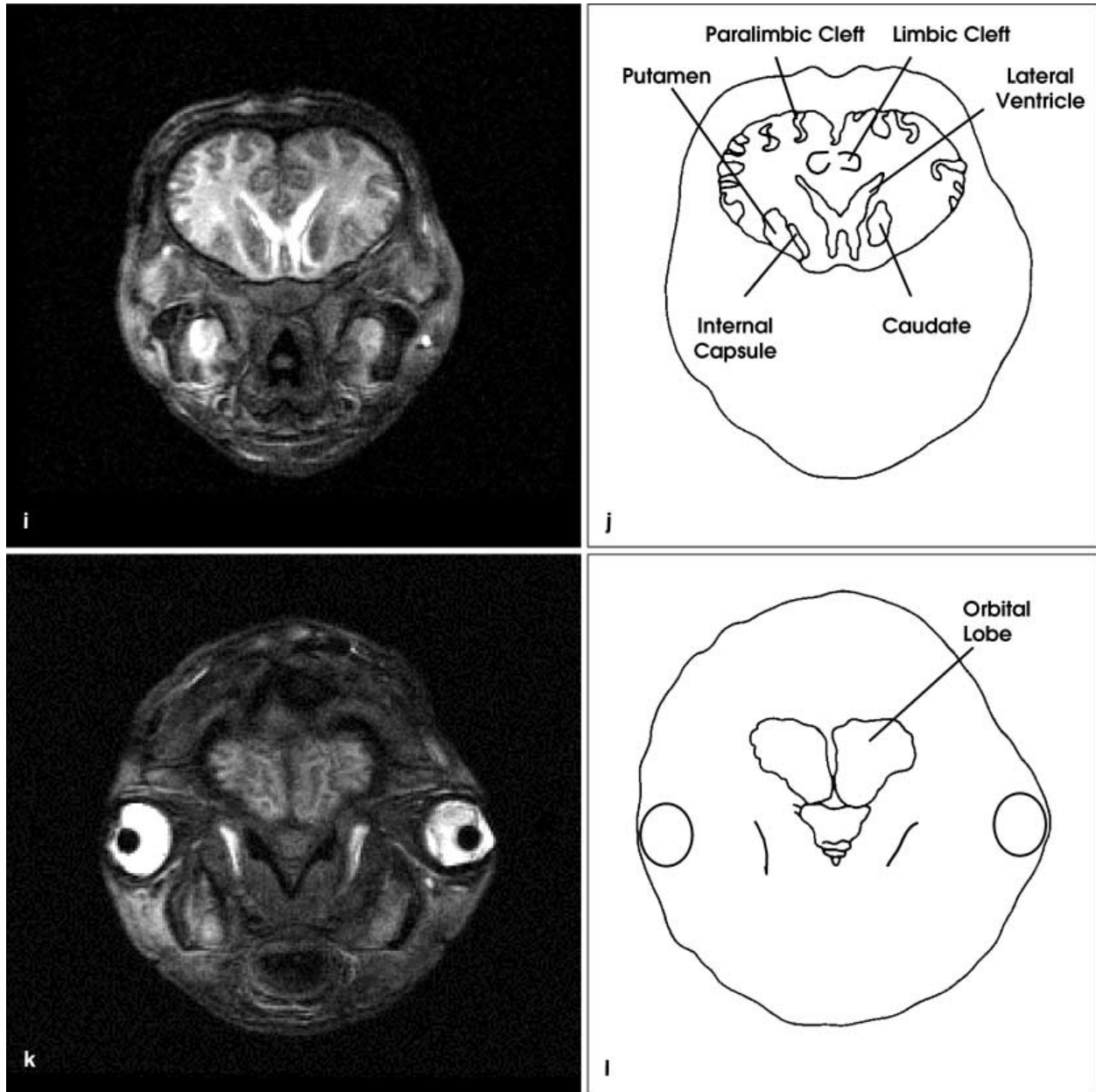


Figure 3a, b display a three-dimensional reconstruction of the brain, viewed from the right front of the brain, with the “orbital” lobes “dissected away” by a coronal section, to show the sulcal pattern on the surface and the depths of the sulci within the section. Major sulcal features are identified in Fig. 3b.

Figure 4a, b are similar views of the posterior surface of the reconstructed brain, viewed from the right posterior aspect. The coronal section revealing Fig. 4a is a few mm rostral to that of Fig. 4b. These figures show that the cetacean three-tiered pattern of major clefs (Morgane et. al., 1980) is already well-established by the mid-late fetal stage.



Figure 5a–l is a posterior-to-anterior selection of originally-acquired coronal scans, 1.5 mm thick, taken from the entire sequence of images, along with corresponding labeled illustrations. The MR images demonstrate that detectable differentiation of gray matter and white matter can be achieved with MRI in postmortem fetal cetacean brain specimens preserved for almost two decades.

**Fig. 6** A 3-mm-thick horizontal scan (a) and labeled schematic illustration (b) of the fetal common dolphin brain

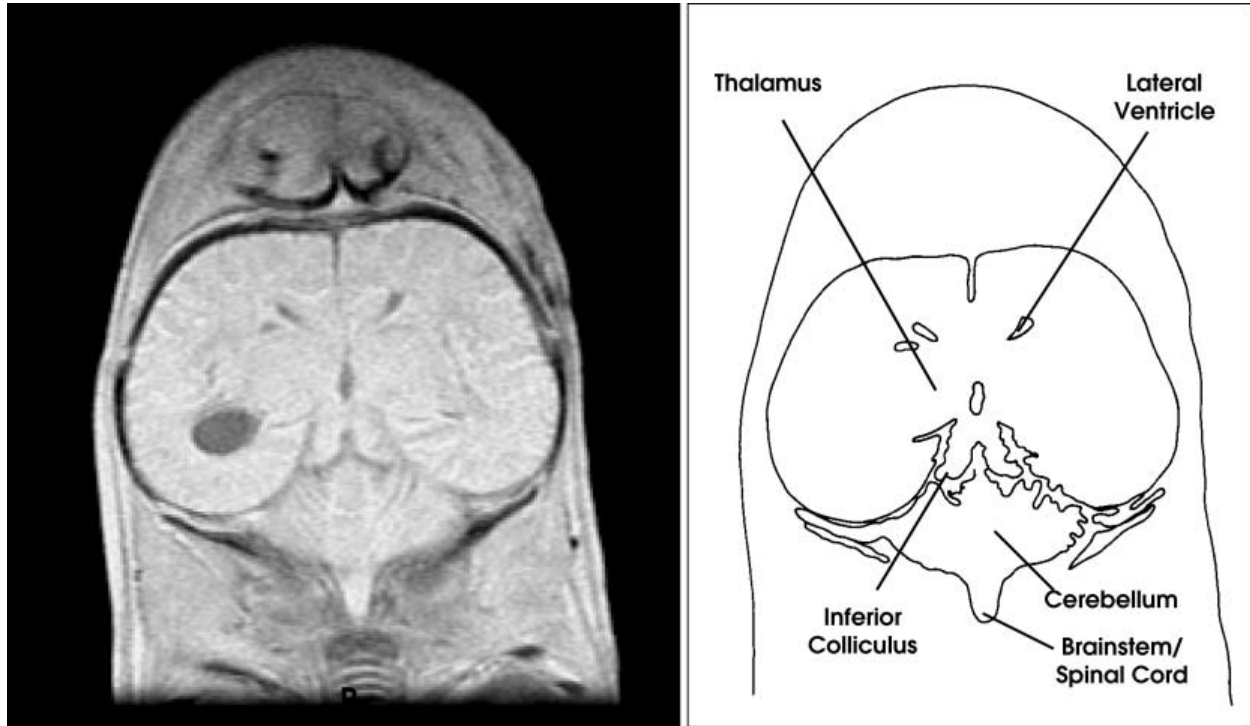
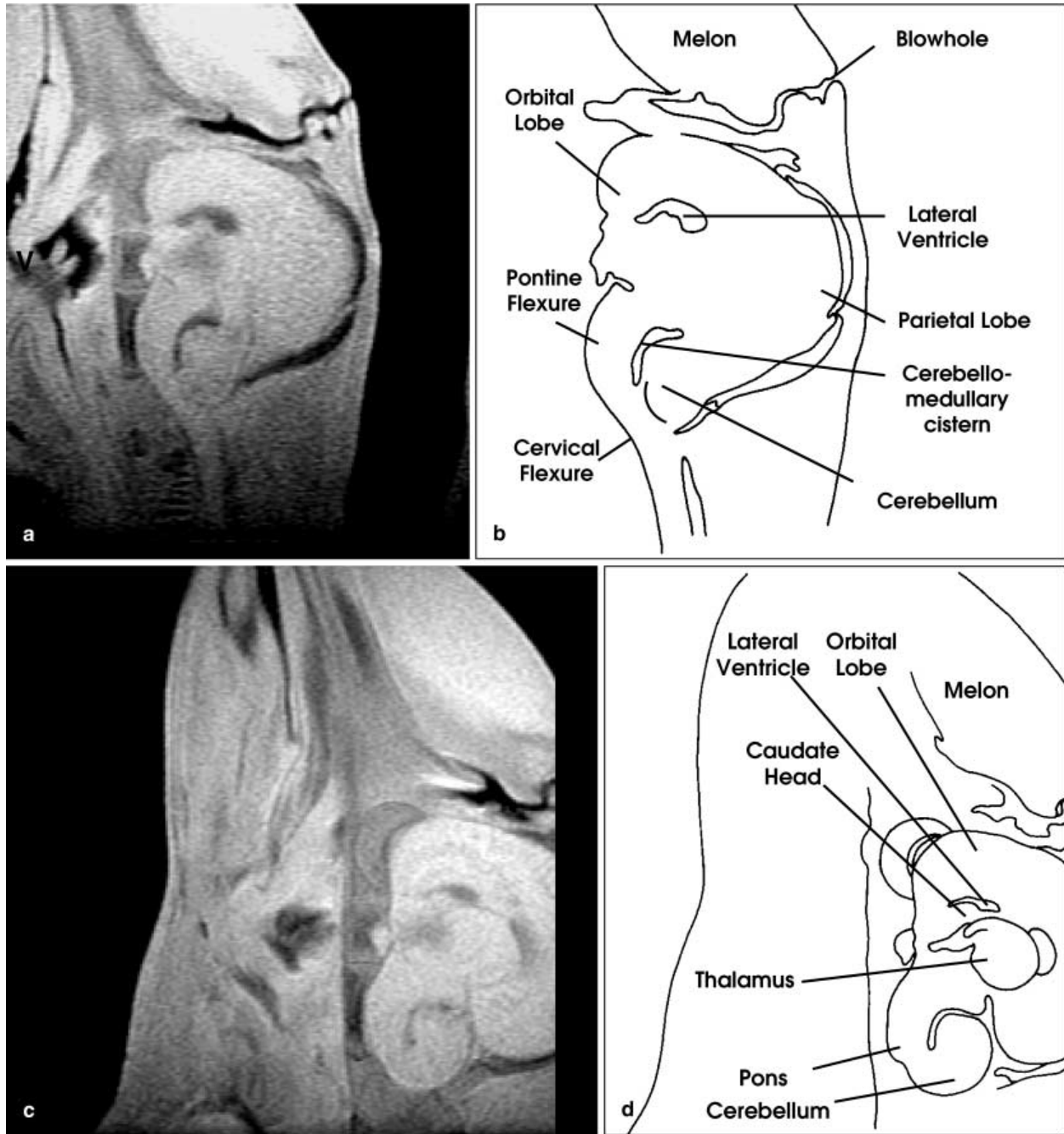


Figure 6a, b displays one of the originally-acquired 3-mm-thick horizontal scans and a labeled schematic version. Prominent in this image are the large inferior colliculi and the extensive lateral development of the cerebellum, both of which are characteristic of odontocete brains (Breathnach 1960; Ridgway 1990). The inferior colliculus appears to already have taken on adult proportions as it is clearly larger than the superior colliculus in size. In contrast, the inferior colliculus does not surpass the superior colliculus in size in very late fetal stages in the harbour porpoise (*Phocoena phocoena*) (Buhl and Oelschläger 1988).

Figure 7a–f displays examples of originally-acquired 3-mm-thick sagittal sections and corresponding labeled illustrations. These images clearly show some of the more distinctive features of the dolphin brain pertaining to the position of the brain in the cranial cavity with respect to the longitudinal body axis (beak/fluke axis). The pronounced pontine and cervical flexures typical of odontocete brains and the mesencephalic flexure, which largely gives the brain its “boxing glove” appearance, are evident in the images of Fig. 6. These flexures are found in the brains of other mammals, including humans, but only during the embryonic state. In the dolphin, these flexures are maintained in the adult brain. The dolphin forebrain is also foreshortened, giving the appearance that the cerebral hemispheres are rotated on the transverse axis 90 degrees from the beak-fluke axis. This appearance is also characteristic of the adult dolphin brain. Because of the distinctive orientation of the brain, the most anterior cortical lobe is called “orbital” instead of “frontal” (Morgane et al. 1980).

Fig. 7 Three-mm-thick sagittal sections (a, c, e) through the fetal common dolphin brain and accompanying labeled schematic illustrations (b, d, f)

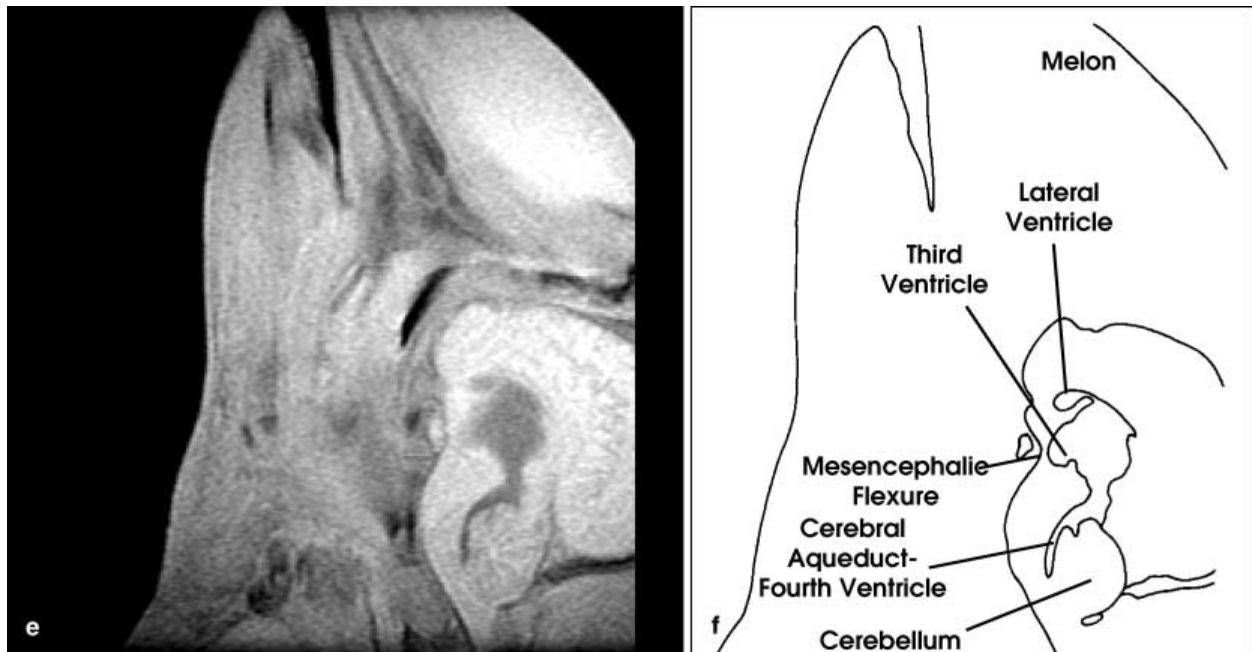


### Volumetric measurements

Volumetric measurements indicate that whole brain volume for the present specimen is 194.91 cc. There is no substantial difference in volume between the two cerebral hemispheres. The left hemisphere volume is 84.02 cc and the right hemisphere volume 83.37 cc. Hindbrain volume (which includes the cerebellum) is 27.51 cc. The hindbrain made up 14.1 percent of the whole brain volume. Ridgway and Brownson (1984) reported a brain weight of 476 g for a neonatal common dolphin. Because the specific gravity of brain tissue is close to one, weight units can be reasonably substituted for volume units. When this is

done, the brain weight reported in Ridgway and Brownson (1984) is almost 2.5 times the brain “weight” reported in the present study for a specimen of 8–9 months prenatal age. However, because actual ages of the specimens are unknown, this comparison might not be especially meaningful. Rapid prenatal brain growth and some shrinkage of tissue may account for any real discrepancy. The large relative size of the hindbrain is consistent with reports of a relatively large cerebellum in odontocetes that apparently develops its large size after the embryonic stage in dolphins (Ridgway 1990). Because much of the hindbrain volume includes the cerebellum, the fact that the entire hindbrain constitutes 14.1% of the whole brain volume suggests that the cerebellum in the present fetal specimen forms a slightly lower proportion of total brain volume than in adults, in which the cerebellum constitutes 15% of the entire brain volume (Marino et al. 2000).

Fig. 7 e–f



## Discussion

The study of prenatal cetacean brain development, and cetacean neuroanatomy in general, has been constrained by the lack of enough data to formulate a database on the normative range of morphometric values for whole brains and substructures in cetaceans. This situation can be resolved by using MRI to analyze the numerous postmortem specimens available in museums and similar facilities. The present study demonstrates that useful neuroanatomical and morphometric data can be obtained non-invasively and efficiently from postmortem prenatal cetacean specimens that have been in fixative for long periods of time. MRI images can be produced that allow anatomical labeling, volumetric measuring, and three-dimensional reconstruction of the brain. MRI also allows for the preservation of spatial aspects of internal structures and their relationship to one another to a degree that cannot be readily achieved through conventional histological techniques. This enables accurate three-dimensional reconstruction that then offers the flexibility to view any part of the brain from any angle and in any plane of sectioning while maintaining the intactness of the specimen.

Comparative analyses of prenatal developmental patterns in and among cetaceans and other mammals are extremely promising for elucidating the history of phylogenetic divergence among cetacean species

and between cetaceans and other mammals. The importance of including prenatal specimens in comparative evolutionary studies is achieving wide recognition: there has been a recent upsurge of contributions to the literature that point to the value of examining developmental patterns in studies of evolution. One of the questions that may benefit substantially from studies of cetacean prenatal brain development is that of the phylogenetic relationship among the various families and species of cetaceans. There are still unresolved issues in the literature about various aspects of the cetacean phylogenetic tree. Perhaps on an even more profound level, studies of cetacean brain development may provide valuable data that will assist efforts to reconstruct the evolutionary history of cetaceans since their divergence from terrestrial ancestors. This enterprise, recently dubbed “evo-devo”, requires the kinds of data presented in this paper and in the few other existing studies on cetacean prenatal brain development. MRI studies provide a way to more efficiently collect and analyze these data in the future.

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### **References**

- Breathnach AS (1960) The cetacean nervous system. *Biol Rev Camb Philos Soc* 35: 187–230
- Buhl EH, Oelschlager HA (1988) Morphogenesis of the brain in the harbour porpoise. *J Comp Neurol* 277: 109–125
- Ferrero RC, Walker WA (1995) Growth and reproduction of the common dolphin, *Delphinus delphis* Linnaeus, in the offshore waters of the North Pacific Ocean *Fish Bull* 93: 483–494
- 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, 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, 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, Leranath C (1990) Immunohistochemistry of neurotransmitters in visual cortex of several toothed whales: Light and electron microscopic study. In: Thomas JA, Kastelein RA (eds) *Sensory abilities of cetaceans: laboratory and field evidence*. Plenum Press, New York, pp 39–60
- Glezer II, Hof PR, Leranath 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 (eds) Marine mammal sensory systems. Plenum Press, New York, pp. 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, Morgane PJ (1995) Cytoarchitectonics and immunocytochemistry of the inferior colliculus of midbrain in cetaceans. *FASEB J* 9: A247–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 Neuroanat* 15: 203–237
- 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, 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: 1–108
- Kojima T (1951) On the brain of the sperm whale (*Physeter catadon* L.). *Sci Rep Whales Res Inst Tokyo* 6: 49–72
- 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
- Marino L, Rilling JK, Lin SK, Ridgway SH (2000) Relative volume of the cerebellum in dolphins and comparison with anthropoid primates. *Brain Behav Evol* 56: 204–211
- 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
- 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 (eds). *Functional morphology in vertebrates*. Fischer, New York, pp 695–698
- Oelschlager HA, Kemp B (1998) Ontogenesis of the sperm whale brain. *J Comp Neurol* 399: 210–228
- Perrin WF, Holts DB, Miller, RB (1977) Growth and reproduction of the eastern spinner dolphin, a geographic form of *Stenella longirostris* in the eastern tropical Pacific. *Fish Bull* 75: 725–750

- Ridgway SH (1986) The central nervous system of the bottlenose dolphin. In: Schusterman RJ, Thomas JA, Wood FG (eds) *Dolphin cognition and behavior: a comparative approach*. Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp 31–60
- Ridgway SH (1990) The central nervous system of the bottlenose dolphin. In: Leatherwood S, Reeves R (eds) *The bottlenose dolphin*. Academic Press, San Diego, pp 69–97
- Ridgway SH, Brownson RH (1984) Relative brain sizes and cortical surface areas of odontocetes. *Acta Zool Fisiol* 172: 149–152
- Ries FA, Langworthy OR (1937) A study of the surface structure of the brain of the whale (*Balaenoptera physalus* and *Physeter catodon*). *J Comp Neurol* 68: 1–47
- Tarpley RL, Ridgway SH (1994) Corpus callosum size in delphinid cetaceans. *Brain Behav Evol* 44: 156–165