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# Manatee Cerebral Cortex: Cytoarchitecture of the Caudal Region in *Trichechus manatus latirostris*

## Key Words

Cerebral cortex  
Brain  
Cytoarchitecture  
Manatee  
Sirenia

## Abstract

In several brains of the Florida manatee, *Trichechus manatus latirostris*, the architecture of caudal regions of cerebral cortex was examined in order to complete a map of cortical areas in the brain of this unique herbivore. Through observation of sections stained for Nissl substance, myelinated axons, acetylcholinesterase and cytochrome oxidase, we have identified 11 new cortical areas based on qualitative cytoarchitectural appearance and measurements of laminar thicknesses, for a total of 24 such cortical areas in manatee cerebral cortex. Some areas exhibit poorly differentiated laminae while in others there are 6 clearly demarcated layers, often with sublaminar organization. Some previously identified areas were found to extend into the region caudal to the vertically oriented lateral fissure. As in other mammalian brains, cortical areas in manatees are organized in concentric rings of allocortex, mesocortex, and isocortex. Putative functional roles have been assigned to most of the identified areas based on location, architecture, behavioral and anatomical considerations, and extrapolation from other taxa in which functional mapping has been done.

## Introduction

Neuroanatomical studies of mammalian cerebral cortex have been made in a large number of terrestrial taxa. However, of the three orders of marine mammals (Cetacea, Sirenia, and Pinnipedia), only cetacean cerebral cortex has been studied in any great detail [Ferrer and Perera, 1988; Glezer et al., 1988, 1989; Morgane et al., 1988; Garey et al., 1989; Glezer and Morgane, 1990; Revishchin and Garey, 1990, 1991; Duffield et al., 1992].

The order Sirenia consists of two families, Trichechidae and Dugongidae [Nowak and Paradiso, 1983]. The Tri-

chechidae are comprised of three extant species, *Trichechus manatus* (West Indian Manatee), *T. inunguis* (Amazonian Manatee), and *T. senegalensis* (West African Manatee). The West Indian Manatee is comprised of two subspecies, *T. m. latirostris* (the Florida Manatee) and *T. m. manatus* (the Antilles Manatee) [Domning, 1986]. The Dugongidae are represented by the single extant species *Dugong dugon* (Dugong) and the recently extinct *Hydrodamalis gigas* (Steller's Sea Cow).

Reports of sirenian brain anatomy are few and mostly on gross aspects only. The first detailed study of the internal structure of sirenian cerebral cortex examined the cytoar-

chitecture of a dugong brain [Dexler, 1913]. Nissl and myelin stains of dugong cerebral cortex revealed a well-laminated cytoarchitecture with a granular layer IV in some areas, as well as unique cellular aggregations (Rinddenkerne) in layer VI of some fields.

A second study of the internal organization of sirenian cerebral cortex [Reep et al., 1989] examined the cytoarchitecture of the frontal region of the Florida manatee (*T. m. latirostris*). We found the Florida manatee cerebral cortex to be well laminated, and some areas also exhibited Rinddenkerne as first reported by Dexler [1913] in dugongs.

The objective of the present study was to examine and document the cytoarchitecture of all areas caudal to those reported by us previously. This has resulted in a complete cytoarchitectural map of the cerebral cortex of the Florida manatee. Based on the locations and internal anatomy of these cortical areas, we have also suggested a scheme for functional localization.

## Materials and Methods

Four brains of the Florida manatee, *Trichechus manatus latirostris*, were obtained through the statewide manatee carcass salvage program administered by the Florida Department of Natural Resources and collected under U.S. Fish and Wildlife Permit PRT-684532. Heads were severed and perfused in situ by cannulation of the carotid arteries. First, 10–15 liters of 0.9% phosphate-buffered saline were used to flush remaining blood. Secondly, 10–15 liters of 4% phosphate-buffered paraformaldehyde were perfused as a fixative. The brains were extracted from fresh, postmortem (less than 12 h) animals.

All 4 brains were embedded in celloidin and cut serially at 30  $\mu$ m. Brains M-387, M-84-14, and M-395 were cut transversely, horizontally, and sagittally, respectively. Alternating sections of each brain were stained for Nissl substance with thionin, or for myelin by using a modified hematoxylin procedure (Heidenhain). Brain TM-8702 was stained for the presence of acetylcholinesterase [Hardy et al., 1976] and cytochrome oxidase [Wong-Riley, 1979] in alternating sections.

Cytoarchitectural delineations are based upon Nissl and myelin stained sections and also sections reacted for acetylcholinesterase (AChE) and cytochrome oxidase. Features such as laminar distribution, laminar widths, qualitative cell packing density, sizes of cell bodies, and laminar or sub-laminar specific patterns of AChE and cytochrome oxidase staining are used to describe the cytoarchitecture.

Unless otherwise specified, descriptions of features such as laminar widths, and laminar or sub-laminar specific cell patterns are in reference to area FR as a standard [Reep et al., 1989]. Area FR is the largest cortical area in a manatee brain and is easily distinguishable from all other cortical areas. It is used as a reference so that results are expressed consistently with those of Reep et al. [1989]. Features of Rinddenkerne are described in reference to areas CL<sub>1</sub> and CL<sub>2</sub> [Reep et al., 1989]. Abbreviations of all structures are defined in table 1.

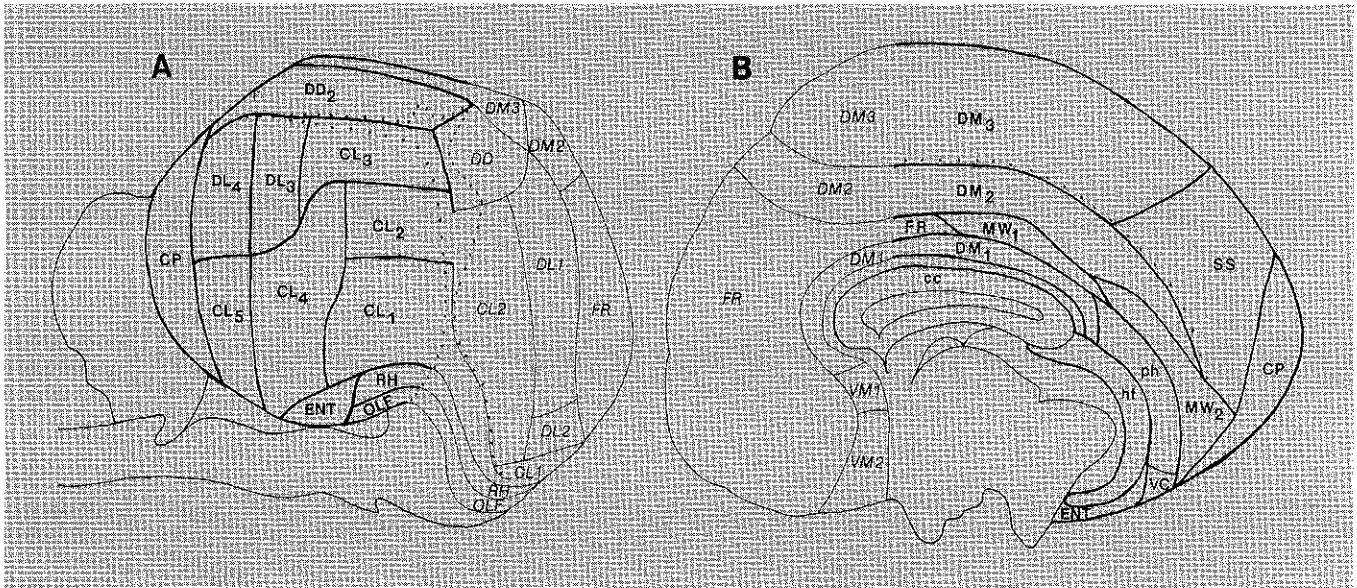
Sections were viewed under a Olympus model BH-2 microscope. Boundaries and tracings of cortical areas and laminae were made with the aid of a Bausch and Lomb microprojector. Boundaries between laminae were determined by changes in cell density, cell size and cell

## Abbreviations

|                 |                                 |
|-----------------|---------------------------------|
| cc              | Corpus callosum                 |
| CL <sub>1</sub> | Cluster cortex area one         |
| CL <sub>2</sub> | Cluster cortex area two         |
| CL <sub>3</sub> | Cluster cortex area three       |
| CL <sub>4</sub> | Cluster cortex area four        |
| CL <sub>5</sub> | Cluster cortex area five        |
| CP              | Caudal pole cortex              |
| DD              | Dorsal cortical area one        |
| DD <sub>2</sub> | Dorsal cortical area two        |
| DL <sub>1</sub> | Dorsolateral area one           |
| DL <sub>2</sub> | Dorsolateral area two           |
| DL <sub>3</sub> | Dorsolateral area three         |
| DL <sub>4</sub> | Dorsolateral area four          |
| DM <sub>1</sub> | Dorsomedial cortex area one     |
| DM <sub>2</sub> | Dorsomedial cortex area two     |
| DM <sub>3</sub> | Dorsal medial cortex area three |
| ENT             | Entorhinal cortex               |
| FR              | Frontal cortex                  |
| hf              | Hippocampal formation           |
| MW <sub>1</sub> | Medial wall cortex area one     |
| MW <sub>2</sub> | Medial wall cortex area two     |
| OLF             | Olfactory cortex                |
| ph              | Parahippocampal cortex          |
| PR              | Perirhinal cortex               |
| RH              | Rhinal cortex (RH1 and RH2)     |
| SS              | Splenic sulcal cortex           |
| TT              | Taenia tecta                    |
| VC              | Ventral caudal cortex           |
| VM <sub>1</sub> | Ventromedial cortex area one    |
| VM <sub>2</sub> | Ventromedial cortex area two    |

type present. For example, generally the boundary between layers II and III was determined by an abrupt change from a cell dense, deeply stained region to a region of small pyramidal cells, lightly stained and relatively less dense than layer II. The layer III–IV boundary was determined by a change in cell type (small granular cells). In some areas layer IV exhibits distinctive horizontal striations. The transition between layers IV and V was generally an abrupt change in cell types, from small granular cells to large darkly stained pyramidal cells. Cell density was generally lower in layer V compared to layer IV. A thin cell sparse zone below layer V indicated the presence of layer Vb. This layer consistently contained smaller pyramidal cells relative to layer V. The boundary between layer Vb and VI was also abrupt. Cell density in layer VI was always higher than in layer Vb. Cells within layer VI were usually arranged in radial striations. An Olympus 35 mm camera attached to the Olympus BH-2 microscope was used for high contrast photomicrography for documentation purposes.

Seven additional manatee brains, housed at the Comparative Mammalian Brain Collection at the University of Wisconsin at Madison, were used for qualitative cytoarchitectural examination and compared with the more completely analyzed brains examined at the University of Florida.



**Fig. 1.** Surface map of manatee cerebral cortex and the location of cortical areas. **A** Lateral view, traced from brain 84-49. Dotted lines represent lateral and horizontal fissures. **B** Medial view, traced from brain M-354. Dotted line represents splenic sulcus. Thin solid lines in both **A** and **B** represent cortical area boundaries delineated by Reep et al. [1989]. Thick solid lines represent cortical area boundaries delineated in this study. Abbreviations in *italics* indicate cortical areas delineated by Reep et al. [1989]. Abbreviations in **bold** indicate cortical areas delineated in this study.

## Results

For purposes of the present report, the caudal region of the cerebral cortex is defined as the collection of those areas located caudal to the vertically oriented lateral fissure. Gross anatomical features specific to the caudal region include the appearance of three deep fissures. A horizontal fissure is located on the upper dorsolateral wall (fig. 1A). This fissure is continuous with the lateral fissure in most brains observed and continues caudally to the caudal pole. Another horizontal fissure is present on the medial wall and is similar to the splenic sulcus in other mammalian taxa (fig. 1B). In some manatee brains, this sulcus continues ventrally to the ventrocaudal region of the medial wall; in others, it is present as two separate sulci, one located horizontally in the dorsal portion of the medial wall and one located in the ventrocaudal area of the medial wall.

Cerebral cortex was examined and delineated into 11 new areas from the lateral fissure to the caudal pole (fig. 1-4). Cerebral cortex from the lateral fissure to the rostral pole was examined previously and delineated into 13 cortical areas [Reep et al., 1989]. Six of these areas have been found to extend caudally past the lateral fissure.

### *Lateral Cortical Areas*

The laterally situated cortical areas  $CL_1$  and  $CL_2$ , delineated by Reep et al. [1989], were found to extend caudal to the lateral fissure (fig. 1A). Six other cortical areas have been identified from the lateral fissure to the caudal pole on the lateral side of the brain (fig. 1A, 2, 3, 4A-G) and are described below.

**Dorsal Cortex** -  $DD_2$ . Area  $DD_2$  is located near the most dorsal extent of the brain and is bounded by area DD and the lateral fissure rostrally,  $DM_3$  medially, CP caudally, and areas  $CL_3$ ,  $DL_3$ ,  $DL_4$ , and the horizontal fissure ventrolaterally (fig. 1A, 2F-H, 3A, 4C-G).

Area  $DD_2$  is a subfield of area DD, previously defined by Reep et al. [1989]. It is characterized by a thick layer II, which is difficult to distinguish from layer III, a well-organized layer IV, a diminished layer V, and a thick layer VI (fig. 5C). Layer IV consists of small, almost granular cells, which are aligned in horizontal striations. The boundaries of layer V are variable and difficult to discriminate due to the thinness of the area and the small size of pyramidal cells (fig. 5C, 7A, B). A thin, cell-sparse sublayer Vb is present below layer V. Layer VI is identified by the presence of radially oriented groups of cells alternating with cell-sparse striations.

