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Golgi Analysis of Neuron Morphology in the Presumptive Somatosensory Cortex and Visual Cortex of the Florida Manatee *(Trichechus manatus latirostris)*

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Key Words

Manatee · Golgi · Neuron morphology · Pyramidal cells · Cerebral cortex · Somatosensory cortex · Visual cortex · Mammal · Afrotheria · Brain evolution

Abstract

The current study investigates neuron morphology in presumptive primary somatosensory (S1) and primary visual (V1) cortices of the Florida manatee *(Trichechus manatus latirostris)* as revealed by Golgi impregnation. Sirenians, including manatees, have an aquatic lifestyle, a large body size, and a relatively large lissencephalic brain. The present study examines neuron morphology in 3 cortical areas: in S1, dorsolateral cortex area 1 (DL1) and cluster cortex area 2 (CL2) and in V1, dorsolateral cortex area 4 (DL4). Neurons exhibited a variety of morphological types, with pyramidal neurons being the most common. The large variety of neuron types present in the manatee cortex was comparable to that seen in other eutherian mammals, except for rodents and primates, where pyramid-shaped neurons predominate. A comparison between pyramidal neurons in S1 and V1 indicated relatively greater dendritic branching in S1. Across all 3 areas, the dendritic arborization pattern of pyramidal neurons was also similar to that observed previously in the afrotherian rock hyrax, cetartiodactyls, opossums, and echidnas but did not resemble the widely bifurcated dendrites seen in the large-brained African elephant. Despite adaptations for an aquatic environment, manatees did not share specific neuron types such as tritufted and star-like neurons that have been found in cetaceans. Manatees exhibit an evolutionarily primitive pattern of cortical neuron morphology shared with most other mammals and do not appear to have neuronal specializations for an aquatic niche.

Introduction

The investigation of neuroanatomical variation among extant afrotherians and xenarthrans is of particular importance to understanding brain evolution because these taxa diverged near the stem of the placental mammal lineage [Murphy et al., 2001a, b, 2007; Archibald, 2003; Springer et al., 2004; Hallström et al., 2007; Nikolaev et al., 2007; Wible et al., 2007; Wildman et al., 2007; Prasad et al., 2008; Asher et al., 2009; Pyron, 2010; Meredith et al., 2011; Song et al., 2012; but see Kriegs et al., 2006] (fig. 1). Moreover, comparisons between members of these taxa and monotremes and marsupials can help to

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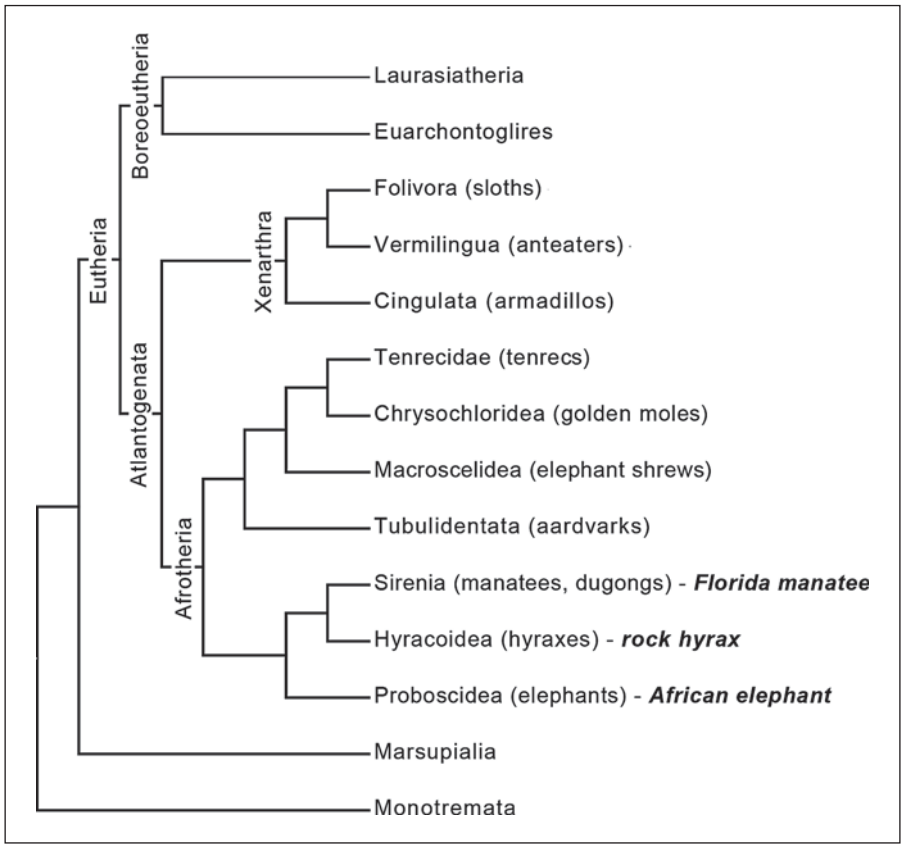

Fig. 1. Mammalian phylogenetic tree indicating the position of manatees within the order Sirenia. The current findings are discussed relative to other afrotherian species listed in bold italics (rock hyrax, African elephant). Adapted from Sherwood et al. [2009].

elucidate primitive and derived conditions in the eutherian clade. Examination of afrotherians and xenarthrans has shown that they retain many primitive neuroanatomical characteristics that were likely shared with the last eutherian common ancestor, including cellular scaling rules in the cerebral and cerebellar cortices, and aspects of the chemoarchitecture of the cerebral cortex, including particular interneuron subtypes at a relatively low density, as well as the morphology and laminar distribution of neurons containing nonphosphorylated neurofilament protein (NPNFP) [Sherwood et al., 2007, 2009; Neves et al., 2014; Reyes et al., 2015].

Sirenians, which include manatees and dugongs, are unusual members of Afrotheria because they have adapted to an entirely aquatic lifestyle [O'Shea and Reep, 1990] and have a highly unusual, lissencephalic brain for their relatively large body size [Edinger, 1933, 1939; Reep et al., 1989; Reep and O'Shea, 1990] (fig. 1). A recent study of the chemoarchitecture of the presumptive somatosensory cortex (hereafter S1) in Florida manatees indicated that the neuron density and NPNFP-immunoreactive pyramidal neuron proportions were similar to those seen in other afrotherians and xenarthrans [Reyes et al., 2015]. More specific traits such as the predominance of calbindin- and calretinin-immunoreactive interneurons in S1 and the laminar distribution of NPNFP-immunoreactive neurons have also been shown to be common across a diverse range mammals including monotremes and marsupials and likely represent highly conserved neural features [Glezer et al., 1998; Hof et al., 1999].

Examination of the neuron morphology of the manatee may provide key information to trace the evolution of cortical organization in eutherian mammals and may also allow us to investigate whether specific features of neuronal morphology tend to evolve in a convergent manner in the context of aquatic adaptations. A previous study of neuron morphology in the cerebellum from the manatee and a sample of other mammals based on Golgi impregnations indicated that the types and overall morphology of cerebellar neurons were generally similar across species, although each species had a distinctive combination of soma size and dendritic traits. In cerebellar neurons, dendritic volume, dendritic tortuosity, and total dendritic length (TDL) were most distinctive,

while the dendritic segment count (DSC) was not predictive of species differences [Jacobs et al., 2015]. Neuron morphology in the cerebral cortex also exhibits variation across mammals. Primates and rodents tend to have a predominance of pyramidal neurons with a single, vertically oriented apical dendrite, with only a few atypical dendritic arrangements or pyramidal neuron types [Peters and Kaiserman-Abramof, 1970; Miller, 1981; Connor et al., 1982; Feldman, 1984; Galforé and Ferrer, 1987; Petit et al., 1988; DeFelipe and Fariñas, 1992; Elston and Rosa, 1997, 1998; Jacobs et al., 1997, 2001; Elston et al., 1998, 1999, 2001, 2005a, b, 2006, 2011; Hof et al., 2000; Elston, 2001; Anderson et al., 2009; Bianchi et al., 2013]. Interneuron morphology and laminar distribution, however, are much more consistent across mammals [Feldman and Peters, 1978; Ferrer and Perera, 1988; Hassiotis and Ashwell, 2003; Hassiotis et al., 2003; Ballesteros-Yañez et al., 2005; Bianchi et al., 2011; Jacobs et al., 2011].

The present study investigated the morphology of pyramidal and nonpyramidal neurons in S1 and presumptive primary visual (hereafter V1) cortices of the Florida manatee *(Trichechus manatus latirostris)* as revealed by Golgi impregnation. Manatees have vibrissae on the entire surface of their body, including facial vibrissae that are involved in oripulation and are adapted for feeding [Reep et al., 2001, 2002; Sarko and Reep, 2007]. Because manatees rely greatly on tactile sensations instead of vision [Sarko and Reep, 2007], we expected neurons from somatosensory areas to exhibit a greater size and dendritic complexity than those in visual areas. Placing these results within the context of other closely related mammals or those with similar aquatic adaptations may indicate the evolutionary dynamics that are associated with cortical neuron organization in manatees.

Materials and Methods

Sample Preparation

The brain of one adult female Florida manatee was obtained from the Lowry Park Zoo in Tampa Bay, Fla., USA. The manatee was wild and was euthanized after having been struck by a boat. Following euthanasia, a pathology examination was performed by the Florida Fish and Wildlife Conservation Commission Marine Mammal Pathobiology Laboratory in St. Petersburg, Fla., USA. The brain was gravity perfused with paraformaldehyde (2%) within 24 h of death. All procedures were carried out with the approval of University of Florida Institutional Animal Care and Use Committee (protocol No. C233).

Three separate blocks of tissue were obtained from the manatee brain, each containing a different region: dorsolateral cortex 1 (DL1) and cluster cortex 2 (CL2) from S1, and dorsolateral cortex 4 (DL4) from V1. DL1 was selected because it is the S1 region with the most pronounced granular layer IV, whereas CL2 was selected because it is one of the regions with Rindenkerne, or clusters of neurons in layer VI [Reep et al., 1989; Loerzel and Reep, 1991; Marshall and Reep, 1995]. DL4 was selected because it is a representative region of V1 with a visible granular layer IV [Marshall and Reep, 1995]. The tissue blocks had a mass of 1.60 g for CL2, 1.63 g for DL1, and 0.83 g for DL4. CL2 and DL1 have been identified in previous research as part of S1 using a combination of cytochrome oxidase staining [Sarko and Reep, 2007] and cytoarchitecture [Reep et al., 1989; Loerzel and Reep, 1991; Marshall and Reep, 1995]. DL4 has been identified as part of V1 based only on cytoarchitecture, particularly its well-developed layer IV and its close proximity to the caudal pole [Marshall and Reep, 1995]. These areas are considered presumptive sensory areas since they have not been investigated with electrophysiological or tracing studies.

Golgi Staining and Neuron Morphology

Neuron morphology in the two regions of S1 (CL2 and DL1) and one region of V1 (DL4) (fig. 2) was explored with a modified rapid Golgi stain [Scheibel and Scheibel, 1978] on 120-μm-thick sections prepared as described elsewhere [Jacobs et al., 2001, 2011]. Neurons from all regions were quantified along x-, y-, and z-coordinates using an Olympus BH-2 microscope under a Planachromat ×60 oil objective interfaced with a Neurolucida software system (MBF Bioscience, Williston, Vt., USA). The system utilized a MicroFire Digital CCD 2-megapixel camera (Optronics, Goleta, Calif., USA) with 1,920 × 1,200 resolution that was mounted on the microscope. First, the soma was traced at the widest point on the 2-dimensional plane to provide an estimate of its cross-sectional area. Dendrites were then traced somatofugally in their entirety, accounting for dendritic diameter and quantifying all visible spines, regardless of the spine length or type. Dendritic processes were not followed into adjacent sections; as such, broken tips and unclear terminations were identified as incomplete endings. Neurons in all cortical layers were traced, with a maximum depth of 3,500 μm from the pial surface; however, the majority of neurons were from layers III and V.

Neurons were selected for tracing based on previously established criteria [e.g. Roitman et al., 2002; Anderson et al., 2009; Jacobs et al., 2011, 2015; Lu et al., 2013]. Traced neurons contained an isolated, darkly stained soma near the center of the 120-μm section and had as fully impregnated, unobscured, and complete dendritic arbors as possible. A variety of neuron types were traced across the three regions (table 1). It has been previously established that nomenclature for different neuron types is inconsistent and can therefore be problematic [Sanides and Sanides, 1972; Ferrer and Perera, 1988; Masland, 2004; Manger et al., 2008; Jacobs et al., 2011; DeFelipe et al., 2013]. Consequently, the existing nomenclature is used as much as possible in the current paper. Neurons were classified based only on the appearance of their dendritic morphology since we were not able to fully trace axons.

Although all well impregnated neurons were traced to illustrate the variety of neuron types, only typical pyramidal neurons were quantitatively compared because they were the most numerous. As in previous studies [e.g. Jacobs et al., 2001, 2011, 2015; Bianchi et al., 2011; Butti et al., 2014, 2015], typical pyramidal neurons were similar to those described in the primate and rodent cerebral cortex, with a triangular soma and a singular apical dendrite extend-

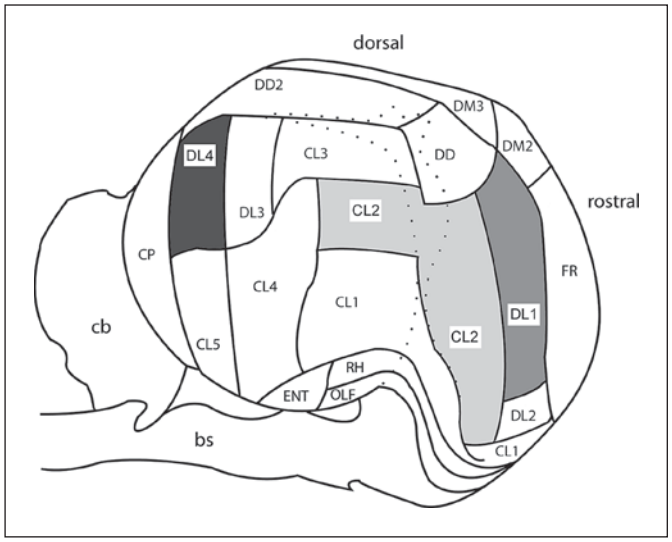

Fig. 2. Right lateral view of a manatee brain. The areas examined in the present study are shown in gray: dorsolateral somatosensory cortex (DL1, medium gray), cluster somatosensory cortex (CL2, light gray), and visual cortex (DL4, dark gray). Also shown are the frontal polar cortex (FR), the dorsolateral cortex (DL2, DL3), the dorsomedial cortex (DM2, DM3), the dorsal cortex (DD, DD2), cluster cortical areas (CL1, CL3, CL4, CL5), the rhinal cortex (RH), the olfactory cortex (OLF), the entorhinal cortex (ENT), the caudal polar cortex (CP), the cerebellum (cb), and the brainstem (bs). Areas adapted from Reep et al. [1989] and Marshall and Reep [1995].

Table 1. Number of Golgi-impregnated neuron types traced for each area

Neuron type	Neurons, n			
	somatosensory cortex		visual cortex	total
	CL2	DL1	DL4	
Aspiny	1	1	3	5
Aspiny bipolar	1	1	0	2
Aspiny bitufted	0	1	0	1
Bipolar	0	1	0	1
Extraverted	4	2	4	10
Fusiform	0	2	0	2
Horizontal	1	0	0	1
Inverted	1	0	0	1
Magnopyramidal	0	1	1	2
Pyramidal	10	10	10	30

ing from the apex of the soma toward the pial surface, and a skirt of basilar dendrites extending outward from the base of the soma [Feldman, 1984; DeFelipe and Fariñas, 1992]. Measurements for pyramidal neuronal morphology included: (i) cell soma area (μm^2), (ii) TDL (μm), (iii) DSC, (iv) mean segment length (MSL, μm), (v) dendritic spine number (DSN), and (vi) dendritic spine density (DSD, spines/μm) and were adapted from previous studies [Bianchi et al., 2011; Jacobs et al., 2011]. Nonparametric tests were employed to compare measurements because the data were not normally distributed. A Kruskal-Wallis test was performed to confirm consistent soma depths across the three regions and Wilcoxon's rank-sum tests were used to compare the neuronal morphology measurements between regions, with a Bonferroni correction to control the familywise error for multiple comparisons. All statistical analyses were performed using R [R Core Team, 2013]. Qualitative descriptions were also given for pyramidal neurons along with other neuronal subtypes identified in the manatee cortex.

Results

Qualitative Descriptions of Neuronal Morphologies

Both S1 and V1 of the manatee contained a variety of aspiny and spiny neuron types (table 1). In addition to typical pyramidal neurons, the observed spiny neurons included atypical pyramidal types such as horizontal, inverted, and magnopyramidal neurons, as well as other nonprincipal neuron types such as extraverted and bipolar neurons. Aspiny neurons included multipolar and bitufted morphological types. Photomicrographs of neurons in S1 and V1 are shown in fig. 3.

Horizontal and Inverted Pyramidal

Horizontal (n = 1) and inverted (n = 1) pyramidal neurons in S1 were similar to pyramidal neurons in terms of their dendritic morphology and soma shape. The main difference between these three neuron types was their orientations. Whereas pyramidal neurons had an apical dendrite that extended toward the pial surface and basilar dendrites that extended radially and downward toward the underlying white matter (fig. 3a, b, d, g, h, 4 C–H, 5 B–F, 6 A–I), inverted pyramidal neurons were situated in the reverse position (fig. 4 B), and horizontal pyramidal neurons had a parallel or nearly parallel orientation relative to the pial surface (fig. 4 A).

Fig. 3. Photomicrographs of Golgi-stained neurons in the presumptive visual cortex (dorsolateral cortex area 4; DL4) and the presumptive somatosensory cortex (DL1 and cluster cortex area 2; CL2). Shown here are a pyramidal neuron from the dorsolateral primary visual cortex (DL4) (**a**), pyramidal neurons from DL1 (**b**, **d**, **g**), an extraverted neuron CL2 (**c**), an aspiny neuron from DL1 (**e**), a dendrite indicating the spine density at a higher magnification in CL2 (**f**), and a pyramidal neuron in CL2 (**h**). Scale bars = 100 (**a–e**, **g**) and 50 μm (**h**).

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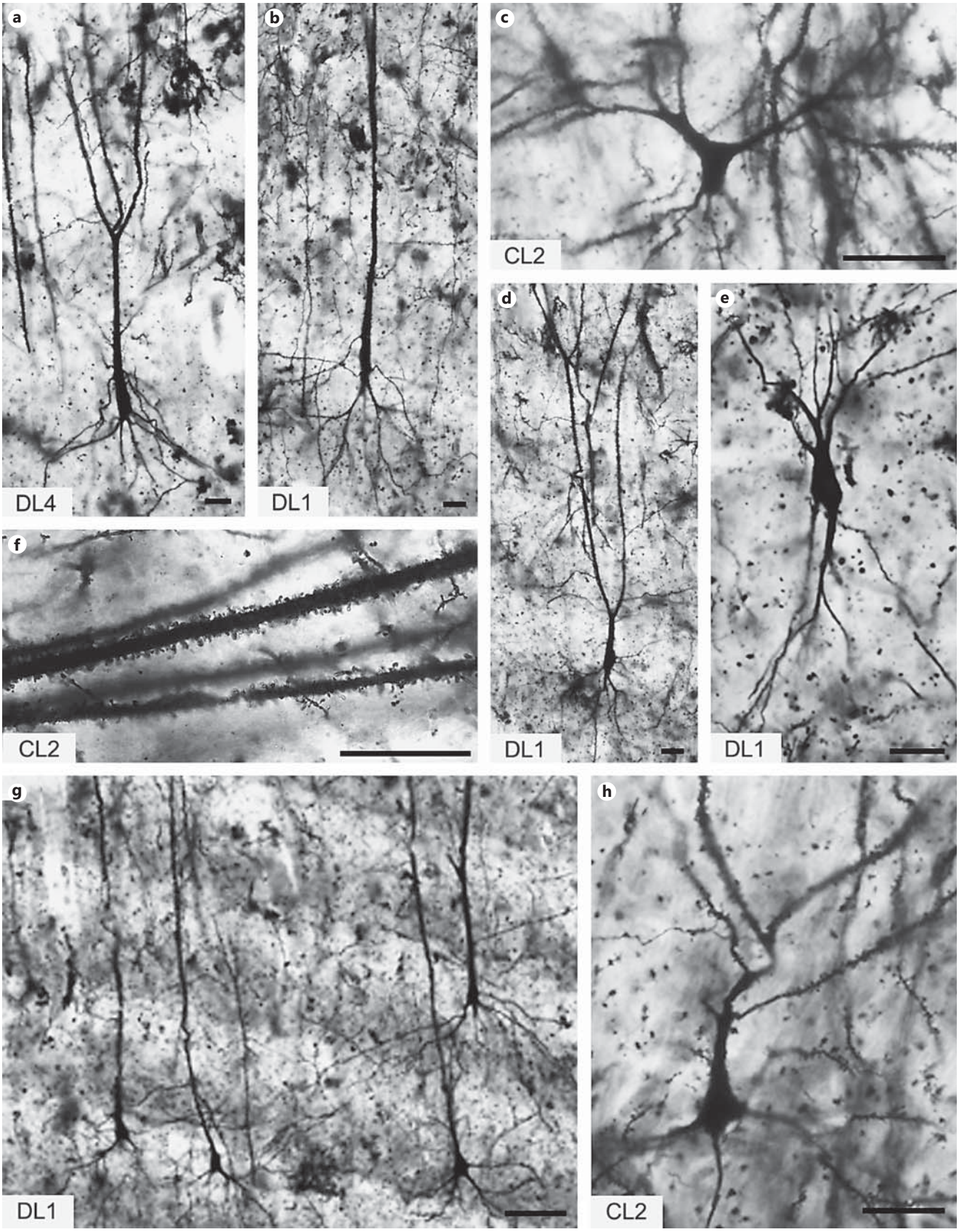

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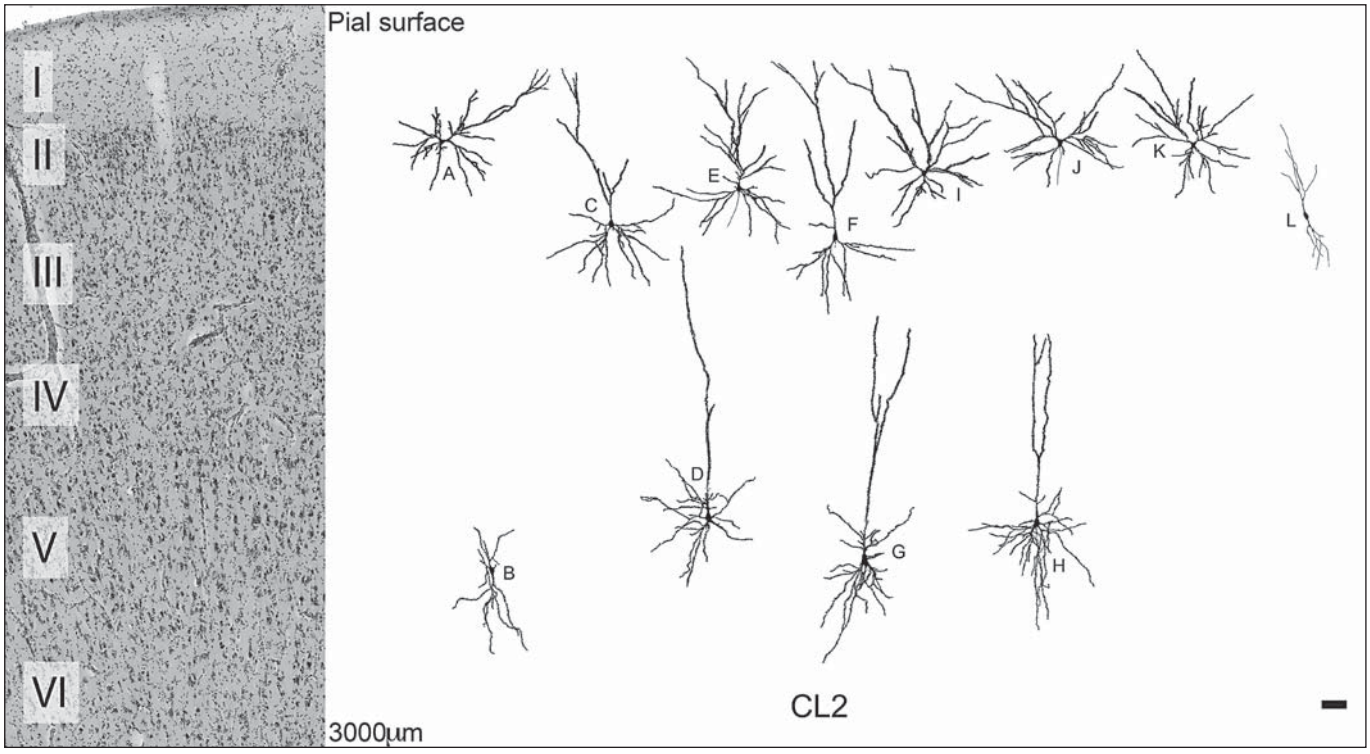

Fig. 4. Neurolucida tracings of spiny and aspiny neurons found in somatosensory area CL2 presented to indicate their location relative to the cortical layers: horizontal neuron (A), inverted neuron (B), pyramidal neurons (C–H), extraverted neurons (I–K), and aspiny neurons (L). Scale bar = 100 μm.

Magnopyramidal

Magnopyramidal neurons appeared similar in both regions (n = 2). These very large neurons were similar to pyramidal neurons and had a single apical dendrite extending toward the pial surface and many spiny basilar dendrites that spread radially from the soma. However, the single apical dendrite was very thick and extended further than those of pyramidal neurons and did not branch as it reached the pial surface (fig. 5 J, fig. 6 O).

Extraverted

Extraverted neurons in both regions (n = 10) resembled those originally identified in the hedgehog, the elephant shrew, and the big brown bat [Sanides and Sanides, 1974]. These neurons were classified as extraverted neurons based on the appearance of their dendritic morphology and how well this appearance matched previous images and descriptions [Sanides and Sanides, 1972, 1974] (specifically that the extent of the apical dendritic array, often containing 2 apical dendrites, exceeds that of the basilar dendritic array). Extraverted neurons in S1 had more extensive apical dendritic branching than those in V1 (fig. 3c, 4 I–K, U, 6 J–L).

Bipolar

A bipolar neuron was traced in S1 (n = 1). This neuron type had a fusiform soma and 2 long processes that extended vertically from either pole with sparse branching (fig. 5 A).

Aspiny

Aspiny neurons in S1 included multipolar (n = 5), bipolar (n = 2), and bitufted (n = 1) neurons. Multipolar neurons had a round soma, whereas bitufted neurons had a more fusiform soma shape. In V1, aspiny neurons were multipolar with larger round soma than those observed in S1 (fig. 3e, 4 L, 5 G, H, 6 M, N).

Analysis of Typical Pyramidal Neuron Morphology

Qualitatively, pyramidal neuron morphologies in S1 and V1 were similar, exhibiting a single apical dendrite that extended toward the pial surface, with many spiny basilar dendrites that spread out radially from the soma

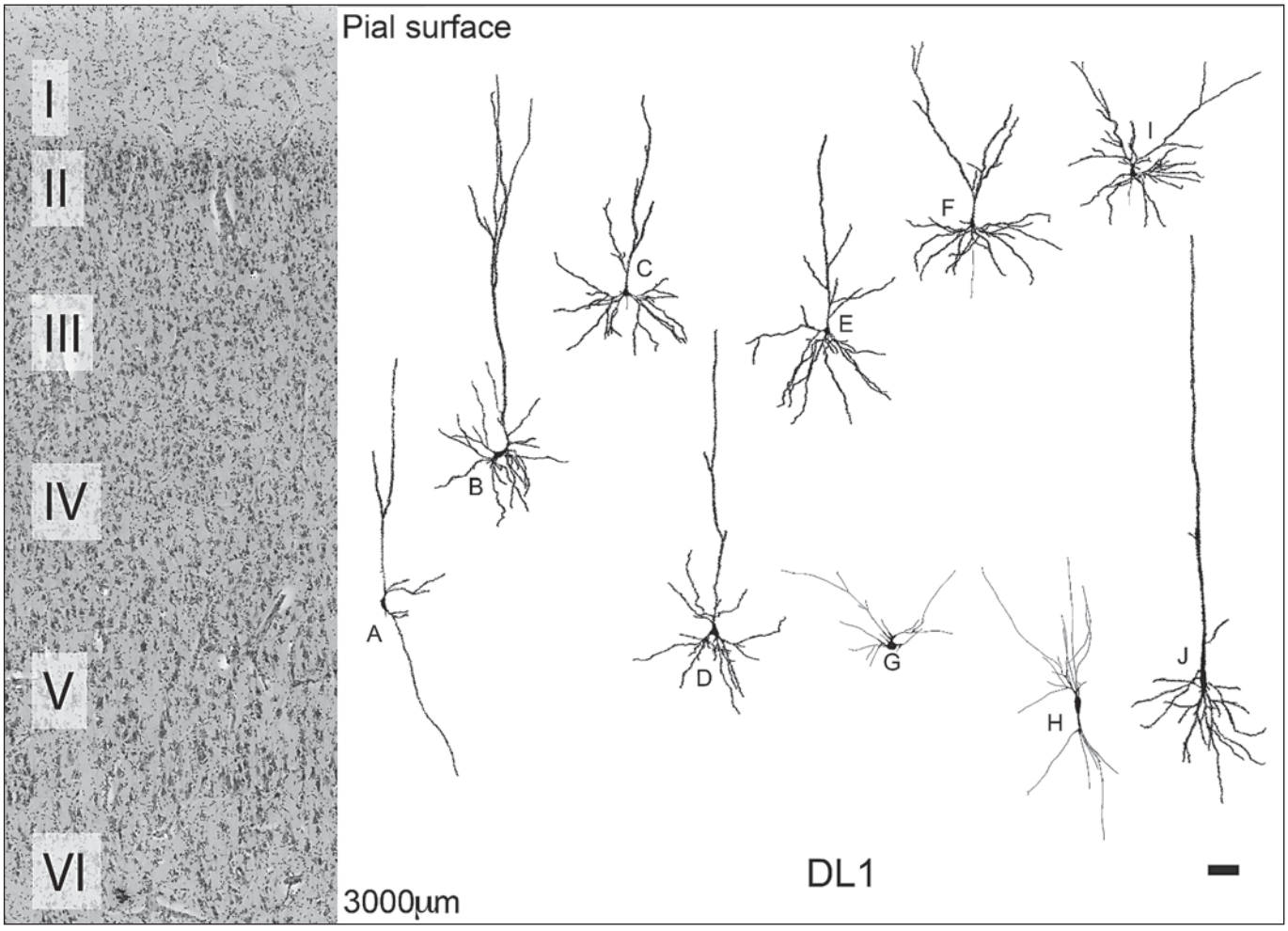

Fig. 5. Neurolucida tracings of spiny and aspiny neurons found in visual area DL1 presented to indicate their location relative to the cortical layers: bipolar neuron (A), pyramidal neurons (B–F), aspiny neurons (G, H), extraverted neurons (I), and magnopyramidal neuron (J). Scale bar = 100 μm.

(fig. 3a, b, d, g, h, 4 C–H, 5 B–F, 6 A–I). Apical dendrites in DL4 formed a single thick branch before splitting into 2 or 3 smaller branches in a V-shaped pattern. Those closer to the pial surface displayed single thick apical dendrites that extended a short distance before splitting into 2 or more secondary main branches, whereas pyramidal neurons deeper in the cortex more closely resembled those in area DL4, with single apical dendrites with little or no branching.

Because there were no significant differences for any measure of pyramidal neuron morphology between CL2 and DL1, we pooled these regions and analyzed regional differences in pyramidal neuron morphology between S1 and V1. Descriptive statistics are shown in table 2. The DSC was significantly higher in S1 (W = 183.0, corrected p = 0.01), while there were no significant differences between regions in terms of soma area (W = 55.5, corrected p = 0.14), TDL (W = 152.5, corrected p = 0.49), MSL (W = 64.5, corrected p = 0.36), DSN (W = 143.5, corrected p > 0.99), and DSD (W = 124.0, corrected p > 0.99).

Discussion

The present study investigated the neuronal morphology of 2 cortical areas of S1 and 1 area of V1 in a Florida manatee *(T. manatus latirostris)*, providing qualitative descriptions of the different neuron types and a comparative analysis of S1 and V1 pyramidal neuron morphol-

Fig. 6. Neurolucida tracings of spiny and aspiny neurons found in visual area DL4 presented to indicate their location relative to the cortical layers: pyramidal neurons (A–I), extraverted neurons (J–L aspiny neurons (M, N), and magnopyramidal neuron (O). Scale bar = 100 μm.

ogy. Manatee neurons exhibited a variety of morphological types, with pyramidal neurons appearing as the most common. Although the sample is relatively limited in terms of both cytoarchitectural areas and the number of individuals, several morphological types that are comparable to those found in other mammalian species, including afrotherians, were identified [Bianchi et al., 2011; Jacobs et al., 2011, 2015; Butti et al., 2014, 2015]. A quantitative comparison between pyramidal neurons in S1 and V1 indicated a relatively greater DSC in S1 and might reflect an increased reliance on tactile sensation compared to vision in manatees.

Neuron Morphology in S1 and V1 as It Relates to Manatee Ecology

We found that one measure of dendritic branching (indicated by DSC) was increased in pyramidal neurons of S1 compared to V1. This might be related to feeding adaptations in the manatee. Manatees inhabit a shallow and turbid aquatic environment and thus rely heavily on tactile sensation instead of vision [Sarko and Reep, 2007]. Vibrissae are present over the entire surface of the manatee body and provide high-resolution tactile information about the aquatic environment [Reep et al., 2002]. Facial vibrissae are highly adapted for feeding [Sarko and Reep, 2007] and are efficient in their movements during grazing and browsing on aquatic vegetation [Marshall et al., 1998; Reep et al., 1998, 2001; Sarko et al., 2007]. The perioral vibrissae participate in oripulation and are active in both grasping and sensing vegetation [Reep et al., 2001], and they are highly innervated [Sarko and Reep, 2007].

Increased dendritic branching in pyramidal neurons has been identified in the occipital cortex of rats reared in complex, enriched environments compared to rats reared in more impoverished environments, including cages with individual housing [Diamond et al., 1964; Volkmar and Greenough, 1972; Greenough and Volkmar, 1973; Wallace et al., 1992] and visual deprivation [Holloway,

Table 2. Quantitative measures of pyramidal neuron dendrites in the manatee S1 (DL1 and CL2) and V1 (DL4) regions

	CL2 (n = 10)	DL1 (n = 10)	DL4 (n = 10)
Soma area, μm^2	468.28±149.26	349.66±105.26	605.66±254.97
TDL, μm	4,953.15±1,063.35	4,841.87±1,373.14	4,044.05±741.93
MSL, μm	88.71±16.09	90.72±17.46	106.97±29.24
DSC, n	56.40±9.35	54.10±14.40	39.73±10.40
DSN, n	4,873±688.75	4,775.80±1,296.92	4,174.45±1,680.38
DSD, spine n/μm	1.01±0.17	0.99±0.14	1.01±0.29

Values are presented as means ± SD.

Table 3. Quantitative measures of basilar dendrites of typical pyramidal neurons in the cortex of the Florida manatee, the African elephant, and the rock hyrax

	Florida manatee (somatosensory and visual cortex)[a]	African elephant (frontal and occipital cortex)[b]	Rock hyrax (frontal and occipital cortex)[c]
Soma area, μm^2	466.68±199.96	450.43±135.13	252.60±72.50
TDL, μm	2,751.98±860.27	3,922.16±605.09	1,711.29±699.31
MSL, μm	83.36±17.57	94.91±4.16	57.65±9.94
DSC, n	33.8±10.58	42.50±8.63	28.98±10.32
DSN, n	2,556.40±272.18	2,355.60±477.23	453.38±318.08
DSD, spine n/μm	1.01±0.30	0.60±0.04	0.25±0.11

Values are presented as means ± SD. African elephant values are from Jacobs et al. [2011]. Rock hyrax values are from Bianchi et al. [2011]. [a] n = 30. [b] n = 7. [c] n = 40.

1966], and this difference has been attributed to an increased environmental complexity. A reduction of visually guided feeding and an increase in reliance on tactile information may therefore account for a significantly higher DSC in S1 pyramidal neurons of the manatee.

Manatees in the Context of Other Afrotherians

A qualitative comparison of neuron morphology in the manatee S1 and V1 versus that from previously published studies in the frontal and occipital lobe of the other afrotherians, the rock hyrax *(Procavia capensis),* and the African elephant *(Loxodonta africana)* indicates that neuronal morphology in the manatee resembles that of the hyrax yet differs considerably from that of the elephant. Both the hyrax and the manatee exhibited considerably less variability in neuronal morphology than did the elephant. Furthermore, the apical dendrites of the pyramidal cells in the manatee tended to resemble the single, vertically oriented apical dendrites found in the rock hyrax, more so than the widely bifurcating ones that typified elephant pyramidal neurons. In addition, the atypical pyramidal neurons (e.g. flattened and multiapical) that are abundant in the elephant cortex were absent in the manatee. As in the elephant, both regions of the manatee cortex contained large magnopyramidal neurons. Magnopyramidal neurons in both species lacked the extensive branching observed in other neuron types; however, these magnopyramidal neurons in the manatee cortex were qualitatively different from the large magnopyramidal-taproot, or matriarch neurons, seen in the elephant frontal cortex. In the manatee, the magnopyramidal neurons appeared similar in both regions, with a single, nonbranching apical dendrite, while the matriarch neurons in the elephant often had bifurcation in their apical dendrites [Jacobs et al., 2011]. Manatees shared other neuronal subtypes with the rock hyrax and elephant, including extraverted, bipolar, aspiny bitufted, and aspiny multipolar neurons. These other neuronal subtypes were morphologically similar across species [Bianchi et al., 2011; Jacobs et al., 2011].

Direct quantitative cross-species comparisons are often challenging since there are currently data from only a limited number of individual specimens for each species and also a relatively limited sample of traced neurons. Because staining of dendritic spines is highly susceptible to preservation artifacts, comparisons of DSN and DSD are

not reliable for consideration across species. Table 3 presents mean values for measures of soma area, TDL, MSL, and DSC across cortical areas in the manatee, the rock hyrax, and the elephant. These values are smaller than in the elephant, and they are larger than in the rock hyrax (table 3) [Bianchi et al., 2011; Jacobs et al., 2011].

Overall, neuronal morphology in the manatee cortex is more similar to that seen in the rock hyrax, which suggests that elephant neurons are evolutionarily derived and have more diverse morphologies with a greater extent of dendritic branching. Similarities between the manatee and the elephant in terms of soma area and MSL, however, may be a result of scaling and could be linked to an increase in absolute brain size in these two species. An investigation of the relationship between these measurements and brain size across a wider range of afrotherians and other mammals is needed to better understand the relationship among soma size, dendritic morphology, and brain size.

Manatees in the Context of Other Mammals

There is considerable variation among mammals in terms of neuron morphology. Most mammalian taxa examined thus far, including monotremes, marsupials, cetartiodactyls, carnivores, and afrotherians, have a considerable variety of neuron types, with a high frequency of neurons classified as atypical pyramidal types such as inverted, horizontal, and magnopyramidal neurons, and nonprincipal types such as extraverted, fork, and fusiform neurons [Walsh and Ebner, 1970; Hassiotis et al., 2003, 2004; Ashwell et al., 2005; Sherwood et al., 2009; Jacobs et al., 2011, 2015; Butti et al., 2014, 2015; Reyes et al., 2015]. Manatees and other afrotherians also appear to lack spiny stellate neurons, a type commonly seen in rodents, primates, and carnivores [Jones, 1975; Simon and Woolsey, 1984; Tarczy-Hornoch et al., 1998; Smith and Populin, 2001].

The dendritic morphology of pyramidal neurons is also variable among mammals. Mammalian taxa, including monotremes, marsupials, cetartiodactyls, carnivores, and the African elephant, exhibit a range of bifurcation in apical dendrites [Walsh and Ebner, 1970; Hassiotis et al., 2003, 2004; Ashwell et al., 2005; Sherwood et al., 2009; Jacobs et al., 2011, 2015; Butti et al., 2014, 2015], with African elephants having more widely bifurcating dendrites even compared to these other taxa [Jacobs et al., 2011]. The rock hyrax and the manatee, however, tend to have a single, think apical dendrite extending from each pyramidal neuron [Bianchi et al., 2011], similar to what is observed in primates and rodents. This variation in pyramidal neuron morphology may be linked to the presence or absence of an identifiable layer IV in these species; the rock hyrax and the manatee both share the presence of a visible layer IV [Reep et al., 1989; Marshall and Reep, 1995; Reyes et al., 2014, 2015], while taxa with widely bifurcating dendrites, such as the African elephant, lack a visible layer IV in the regions that were examined [Jacobs et al., 2011].

Because manatees and cetaceans are both fully aquatic, a comparison between these taxa may suggest whether neuron morphology shows similarities in association with this unique environment. Recent work has demonstrated that cetaceans have neuron types not observed in their close relatives, the terrestrial artiodactyls. These neuron types included tritufted and star-like neurons with no clear apical dendrite [Butti et al., 2015], which are also absent or observed in relatively low numbers in the manatee. Instead, manatee cortical neuron types more closely resemble those in the rock hyrax due to phylogenetic affinities [Bianchi et al., 2011]. Thus, convergent evolution of cortical neuron types related to an aquatic habitat does not appear to occur between manatees and cetaceans. Our analysis of neuron morphology in the manatee contributes to reconstructing the ancestral state for mammals and eutherians and to assessing the evolution of neuron types across all mammals.

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