Evolution of Cytoarchitectural Landscapes in the Mammalian Isocortex: Sirenians (*Trichechus manatus*) in Comparison With Other Mammals

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ABSTRACT

The isocortex of several primates and rodents shows a systematic increase in the number of neurons per unit of cortical surface area from its rostrolateral to caudomedial border. The steepness of the gradient in neuronal number and density is positively correlated with cortical volume. The relative duration of neurogenesis along the same rostrocaudal gradient predicts a substantial fraction of this variation in neuron number and laminar position, which is produced principally from layers II–IV neurons. However, virtually all of our quantitative knowledge about total and laminar variation in cortical neuron numbers and neurogenesis comes from rodents and primates, leaving whole taxonomic groups and many intermediate-sized brains unexplored. Thus, the ubiquity in mammals of the covariation of longer cortical neurogenesis and increased cortical neuron number deriving from cortical layers II–IV is undetermined. To begin to address this gap, we examined the isocortex of the manatee using the optical disector method in sectioned tissue, and also assembled partial data from published reports of the domestic cat brain. The manatee isocortex has relatively fewer neurons per total volume, and fewer II–IV neurons than primates with equivalently sized brains. The gradient in number of neurons from the rostral to the caudal pole is intermediate between primates and rodents, and, like those species, is observed only in the upper cortical layers. The cat isocortex (*Felis domesticus*) shows a similar structure. Key species for further tests of the origin, ubiquity, and significance of this organizational feature are discussed. J. Comp. Neurol. 000:000–000, 2015.

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INDEXING TERMS: manatee; cortical lamination; neurogenesis; cortical column

In primate brains (e.g., baboons, macaques, capuchins) a pronounced variation in neuron numbers per unit of cortical surface area (neurons/unit column) aligns roughly with the original rostral-to-caudal axis of the embryonic isocortex, with as much as three times more neurons/unit column in posterior visual cortex than in prefrontal cortex (Collins et al., 2010; Cahalane et al., 2012). In smaller primate brains (e.g., owl monkey, tamarin) and large rodent brains (i.e., paca, agouti), a reduced version of this gradient is evident (Charvet et al., 2015). In all the brains described, more neurons/unit column are produced by more neurons in layers IV–II. In the small rodent brains (e.g., hamster and mouse), no gradient in neurons/unit column is in evidence, although a significant, but slight, cross-cortex reversal in the number of neurons in the upper versus the lower cortical layers can be seen (Charvet et al., 2015).

Correspondingly, in neural development, a gradient in duration of neurogenesis (or related aspects of neural maturation) has been reported in all mammals studied closely for this feature, shorter at variously situated rostral points and longest at the caudal pole (e.g., mouse: Suter et al., 2007; Caviness et al., 2009; rat: Bayer and Altman, 1987, 1991; possum: Sanderson and Weller,

1990; ferret: McSherry et al., 1984, Kroenke et al., 2009; macaque: Rakic, 1974, 2002; Smart et al., 2002; human: Miller et al., 2014). Species with longer neuro-development (e.g., macaque, human) produce greater relative discrepancies in neurons/unit column between the rostral-to-caudal pole than species with shorter development (Workman et al., 2013; Charvet and Finlay, 2014). One systematic deviation from this pattern can be seen in the timing of maturation in primary sensory areas (Kroenke et al., 2009) and reduced neuron loss in the same locations (Finlay and Slattery, 1983), which is echoed in increased neurons/unit column in the mature primary sensory areas superimposed upon the overall rostral–caudal gradient (Cahalane et al., 2012). These two effects of course do not exhaust all possible developmental sources of variation in neuron numbers and organization. The rostral-to-caudal gradient in neurogenesis duration can be viewed as the primary scaffold on which further variations may be situated. Using available information on progenitor numbers, cell cycle parameters, duration of neurogenesis and neuron death, and adult cortical numbers and disposition, we have recently produced an empirically constrained model, producing across-species and within-cortex differences in total cortical neuron numbers, cortical area, and laminar distribution of neurons within cortical regions as arising from a common suite of mechanisms operating over variable durations (Cahalane et al., 2014).

The collection of species represented in the studies described does not begin to exhaust or even explore the taxonomic divisions of mammals, however, and so the idea that this model might be mammalian-general must remain provisional until more species are represented. Finding any basic description of the scaling of the fundamental cortical scaffold would be an immense benefit to understanding its variations. Although studies of niche adaptations (e.g., nocturnal/diurnal: Kaskan et al., 2005; Krubitzer et al., 2011), species-specific sensorimotor adaptations (e.g. whiskers and star noses, hands and hooves: Van der Loos and Welker, 1985; Catania and Kaas, 1995; Catania, 2004; Nudo and Masterton, 1990) or learned variation in cortex (e.g. ocular dominance organization, musical expertise: Abraham and Bear, 1996, Herholz and Zatorre, 2012) are numerous, only a minority of these studies, like those cited, have attempted to place such specializations in the range of whole cortex variation. Valuable wholesale characterizations of basic cortical organization across taxonomic groups have been offered (Valverde, 1990; Kaas, 2011, Krubitzer and Seelke, 2012). These characterizations must necessarily undergo continual revision, as sampling the entire range of mammalian variation is

such an immense task. For example, the claim of a relative lack of differentiation of the cetacean cortex (Morgane et al., 1985) underwent major revision as more species and methods of describing cytoarchitecture became available (Hof et al., 2005). The evo–devo approach allows predictable scaling to be dissociated from species-specific adaptations, an intrinsically difficult problem as taxonomic groups are typically associated with particular brain size ranges and rates of development (Darlington et al., 1999; Workman et al., 2013).

This study examines neuron numbers across the isocortex of manatees (*Trichechus manatus*), a species that provides a maximal contrast from the species examined so far on multiple dimensions. Manatees belong to the order Siriena, which includes dugongs, and the superorder Afrotheria, which includes hyraxes and elephants. Manatees are distantly related to primates and rodents (Bininda-Emonds et al., 2007). Although large-brained compared with most rodents studied, manatees' "encephalization" (the ratio or population deviation of brain to body mass) is low (O'Shea and Reep, 1990). Although diurnal, they are not visual specialists like primates. In a further effort to gather any available data from additional taxonomic groups, particularly from brains of intermediate size between the rodents and primates gathered to date, we re-present data gathered by Beaulieu and Colonnier, 1989 on cortical organization in cats reorganized on the rostral-to-caudal dimension of interest

MATERIALS AND METHODS

Two adult manatee brains were used in this study. These specimens were obtained from a fresh carcass salvage program (Reep et al., 1989; Marshall and Reep, 1995). No manatees were killed for this study. One brain was sectioned coronally and the other brain was sectioned sagittally on a freezing microtome as described previously (Reep et al., 1989; Marshall and Reep, 1995). The sections were stained with cresyl violet. Specimen ID and histological information are listed in Table 1. Brain sections were examined with a Leitz Diaplan microscope and a Neurolucida imaging system with a mechanical stage, and estimates of neuron numbers were made with a lucivid (Microbrightfield, Colchester, VT). Coronal and sagittal sections were selected to cover the rostral–caudal axis and medial–lateral axes of the manatee isocortex.

In each selected coronal or sagittal section, we measured the length of the cortical surface. We then selected nine equidistant sites along the cortical surface area of each selected section. We then overlaid a

grid on the magnified image. The axes of a grid were aligned orthogonal to the isocortical surface, and counting boxes (41 µm × 41 µm) were placed at 100-µm intervals between the border between layer I and layer II and the border between layer VI and the underlying white matter (Fig. 1). In total, we selected 36 "cortical columns" for the sagittally sectioned hemisphere and 27 "cortical columns" for the coronally sectioned hemisphere.

As in prior studies, we used the optical disector method to estimate neuron numbers contained in each box's volume (Williams and Rakic, 1988; Williams et al., 2003). A 5-µm-thick guard zone was applied, whereby cells that lay on the three exclusion planes (x, y, and z planes) were excluded. Measurements of layers were made orthogonal to the cortical surface area. We quantified layers II–IV, layers V–VI neurons, and layers II–VI neurons across the isocortex in a manner similar to that described by Charvet et al. (2015), to facilitate comparison in cortical neuron numbers among manatees, primates, and rodents. Layer IV as defined by an aggregation of small cells, is evident although small in the manatee. It is evident across many different cortical areas although it is elusive in the rostral regions of the isocortex (Fig. 2; Reep et al., 1989; Marshall and Reep, 1995). We followed the work of Reep et al. (1989) and Marshall and Reep (1995) in defining cortical layers for the manatee.

We estimated neuron numbers under a unit of cortical surface area according to the following equation:

$$\text{Estimated neuron number} = \text{total neurons counted} \times 1/\text{asf} \times 1/\text{hsf}$$

where asf = B/D and hsf = H/T. B is the base area of the counting frame, D is the distance between counting boxes, H is the height of the optical disector, and T is the average tissue thickness after histological processing.

Correction for curvature of the isocortex

Although the manatee cortex is lissencephalic (Reep and O'Shea, 1990), its isocortex curves along its

TABLE 1.
Information on the Two Specimens Used in the Analyses

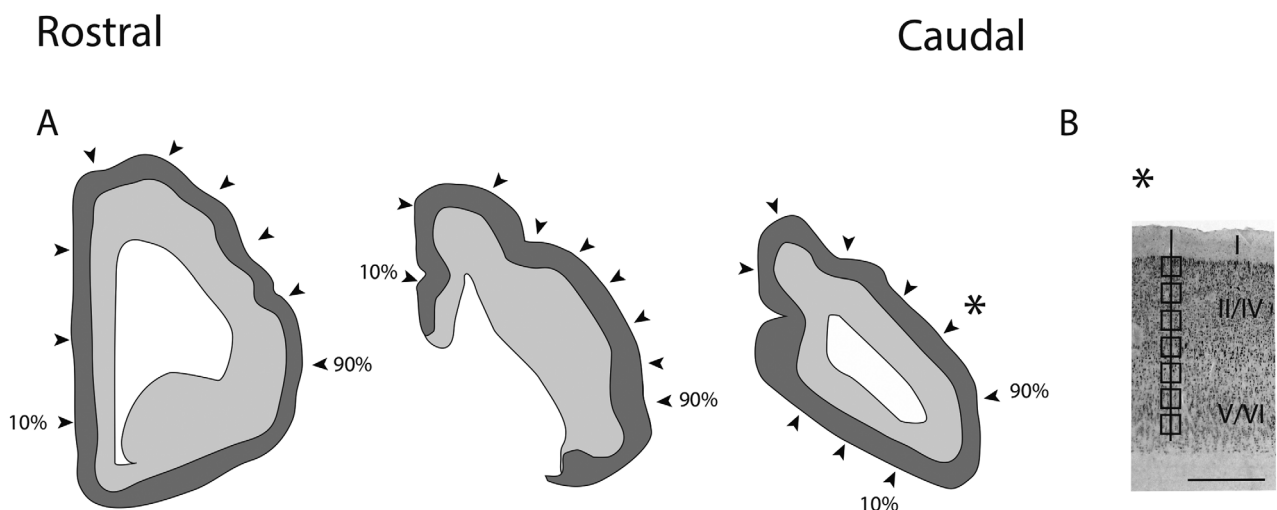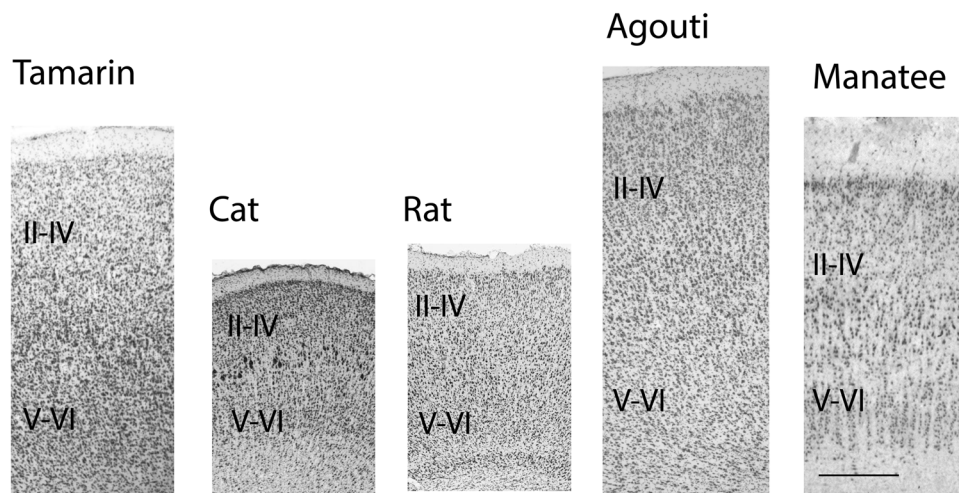|	Manatee	Manatee
ID	84-49	85-8
Brain weight (grams)	317 g	372 g
Body weight (kilograms)	485 kg	596 kg
Plane of section	Coronal	Sagittal
Section thickness	30 µm	30 µm
Guard zone	5 µm	5 µm
Sampled sites	27	36
Total number of layer II–VI neurons sampled	715	805

Figure 1. A: Illustration of a coronally sectioned manatee brain used for neuron number estimates. In coronal planes, we systematically sampled sites (i.e., every 10th percentile) along the medial to lateral axis of the isocortex. The most medially located region is designated as the 10th percentile and the most laterally selected region is designated as the 90th percentile. Arrowheads point to the approximate sites selected for neuron number estimates. We counted neuron numbers (layers II–VI neurons) under a unit of cortical surface area. B: Neuron number estimates were made through the depth of the cortex. Counting frames were selected at equidistant sites through the depth of the cortex. Sites were sampled orthogonal to the axis of the cortical surface. A star shows the approximate location of B. Scale bar = 1 mm in B.
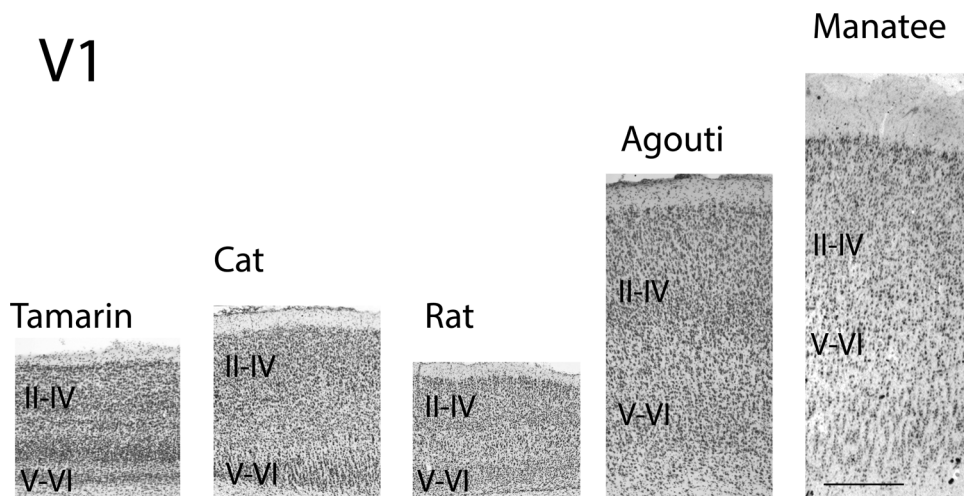
Frontal cortex

V1

Figure 2. Nissl-stained sections through the primary visual cortex and frontal cortex of a tamarin, agouti, and manatee. Labels refer to layers II–IV and layers V–VI combined. A well-defined granular layer is evident in the tamarin and to a lesser extent the agouti. A small granular layer is evident in the primary visual cortex of the manatee, although layer IV is less evident in the frontal cortex of the manatee. Scale bar = 0.5 mm.

rostrocaudal and mediolateral axes. Estimating neuron numbers under a unit of cortical surface area in sections may be compromised if the plane of section is not orthogonal to the cortical surface. Attempts to avoid regions of curvature eliminate large, essential regions of the rostral and caudal cortex, and judgments about curvature without reconstruction are likely to be in error. To correct for curvature, we used a previously reconstructed manatee brain from the comparative mammalian brain collection (http://brainmuseum.org/). We located the samples of interest on the recon-structed manatee brain sections and we estimated the length of the cortical column that was orthogonal to the cortical surface. First, we selected sites in which there was minimal variation in the curvature of the cortex when possible. We also estimated the length of the cortical column for the plane in which neuron number estimates were made. The correction factor was estimated by dividing the length of the cortical column that was orthogonal to the cortical surface area by the length of the cortical column that was used for neuron estimates.
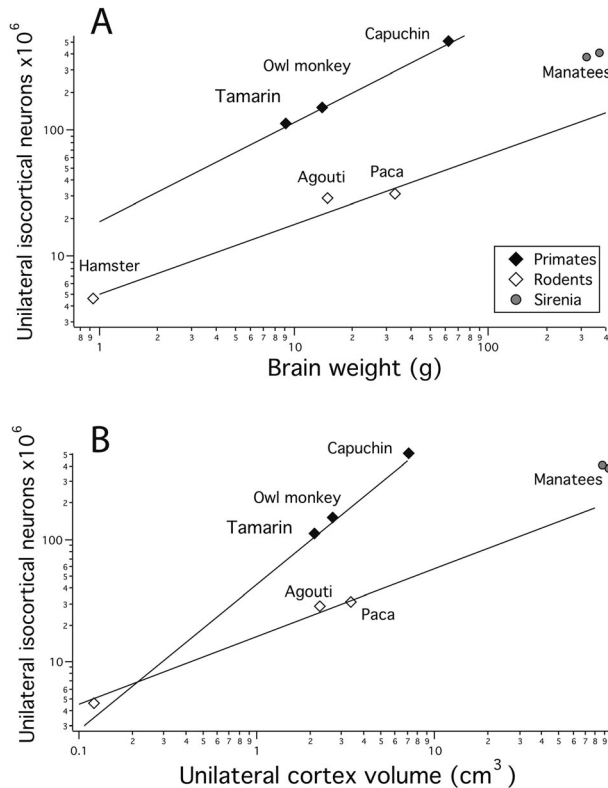
Figure 3. **A**: Total unilateral isocortical neuron numbers are plotted against brain weight in primates, rodents, and manatees. Isocortical neuron numbers of manatees and rodents are lower than would be predicted for by an equivalently sized primates brain. **B**: Total unilateral isocortical neuron numbers regressed against cortex volume in primates, rodents, and manatees paint a similar picture. That is, neuron numbers are fewer than would be predicted for by an equivalently sized primate brain. Natural-logged regressions were fit to primates and rodents separately. These regressions show that manatees resemble rodents more than primates in the scaling of neuron numbers versus brain weight or unilateral cortex volume.
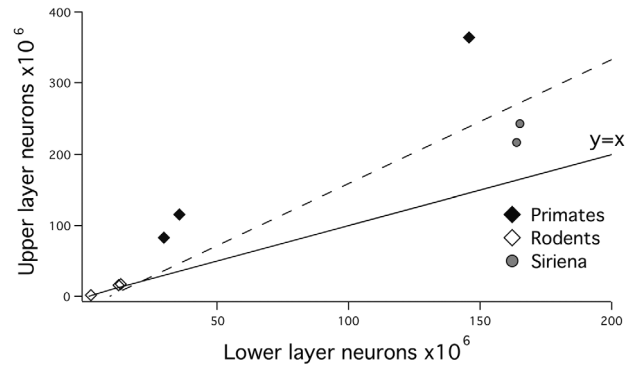

Figure 4. Upper layer neurons are plotted against lower layer neurons in primates, rodents, and manatees. Primates exhibit proportionally more upper layer neurons than manatees and rodents do. Across all three orders, upper layer neuron numbers expand disproportionately relative to lower layer neuron numbers.

We reconstructed the distance of the sampled sites across the rostral to caudal and medial to lateral axes of the cortex. To that end, we located sampled sites on the reconstructed manatee cortex sections. We used the reconstructed manatee cortex to estimate the length of the cortical surface area across the rostral to caudal and medial to lateral axes (Fig. 1).

Statistical analysis

For cross-species comparisons, data on neurons under a unit of cortical surface area of three primate species (i.e., owl monkey, tamarin, capuchin) and three rodent species (i.e., hamster, agout, paca) were obtained from a previous publication (Charvet et al., 2015). Neuron estimates for these species were obtained with the optical disector method.

We used phylogeny generalized least squares regressions to correct for the fact that species are not independent data points. These statistics are highly similar to what we had done previously (Finlay et al., 2014). Briefly, we used phylogeny generalized least squares statistics (PGLS) to obtain phylogenetically controlled regressions in R (version 2.15.0; caper package). The phylogeny, which includes branch lengths, for the three rodent species (i.e., hamster, agouti, paca), three primate species (i.e., tamarin, owl monkey, capuchin), and manatee was taken from Bininda-Emonds et al. (2007). *Agouti paca* was used in place of *Cuniculus paca* because *Cuniculus paca* was not listed in the phylogeny of Bininda-Emonds et al. (2007). The two manatee specimens were averaged for cross-species comparisons. Regression parameters were found by maximum likelihood estimates.

We performed a natural-log regression of neuron numbers, upper and lower layer neurons versus the rostral-to-caudal axis of the cortical surface area in millimeters for each manatee to determine whether neuron numbers vary significantly across the rostral-to-caudal axis of the isocortex. Statistical analyses were performed in JMPPro11.

RESULTS
Cortical neuron numbers

The two manatee brains we measured yielded very similar estimates of 381–409 million neurons per unilateral cortex. In Figure 3, we compare the numbers of neurons in manatee cortex with the same estimations in rodents and primates we described previously (Charvet et al., 2015), in this case representing both with respect to whole brain volume. Manatees fall closer to

	Manatee 84-49				Manatee 85-8			
	Slope	Intercept	R^2	F ratio	Slope	Intercept	Adj R^2	F ratio
Total neurons	0.30*	10.00*	0.42	26.5*	0.27*	10.12*	0.22	8.36*
Upper layers	0.25*	9.61*	0.22	11.19*	0.31*	9.43*	0.14	5.31*
Lower layers	0.35*	8.88*	0.23	11.95*	0.26	9.23*	0.06	2.78

[1]Data are for total neurons (layers II–VI), upper layer neurons (layers II–IV), and lower layer neurons (layers V–VI) per mm^2 of cortical surface area regressed against the rostrocaudal axis of the cortex for each individual manatee.
*$P < 0.05$.

the slopes of cortical neuron numbers versus brain weight for rodents than for primates (Fig. 3A). Figure 3B compares neuron numbers in the cortex with respect to cortex volume, and we can see that with increasing cortex volume, the relative number of neurons decreases in rodents compared with primates, and the manatees close to the rodent line. Thus, both reduced allocation of tissue mass to cortex and reduced density of cortical neurons characterize the manatee brain, as they do in rodents, although no comparisons of allometry can be made without additional sirenian species.

Allometry of neurons by layer

Independent of the relative density of neurons in the cortex, the number of layer II–IV neurons relative to layer V–VI neurons increases regularly and disproportionately with total cortical neuron number, and manatees appear to conform to this trend (Fig. 4). A linear regression of layer II–IV neurons versus layer V–VI neurons demonstrates that layer II–IV neurons increase with a slope greater than 1 when regressed against layer V–VI neurons in these seven species ($y = 2.09x - 23.82$; adj $R^2 = 0.925$; $F = 75.05$; $P < 0.05$; $\lambda = 1$). That is, layer II–IV neurons increase with a positive allometry relative to layer V–VI neurons in this sample.

Rostrocaudal variation in neurons/unit column in the manatee

In the manatee cortex, neurons/unit column vary significantly across the rostral to caudal axis in both specimens (Table 2) with relatively fewer neurons in the rostral cortex compared with caudal regions of the cortex. Considering individual manatees, manatee 85-8 exhibits neurons per unit of cortical surface area that range between 52,600 neurons per mm^2 of cortical surface area in the most rostral regions and 88,700 neurons in the most caudal regions. That is, neurons vary by a factor of 1.68 between the four most rostrally (i.e., frontal cortex) and the four most caudally selected regions ($n = 4$; caudal pole; Reep et al., 1989; Marshall

and Reep, 1995), In the second manatee (84-49, coronally sectioned), neurons ranged between 74,500 most rostrally and 100,900 neurons per mm^2 caudally (see Fig. 1 for selected locations). That is, neurons vary by a factor of 1.35 between the nine most rostrally and nine most caudally selected cortical regions. Taken together, these findings demonstrate that neurons per unit of cortical surface area are higher in the caudal cortex relative to the rostral cortex in the manatee.

Variation between specimens 84-49 and 85-8 with regard to neuron counts may be related to shrinkage differences. For brain 84-49, linear shrinkage was calculated to be 28.5%, corresponding to volumetric shrinkage of 63.4% (Reep et al., 2007). Brain 85-8 has linear shrinkage of 31.1%, corresponding to volumetric shrinkage of 67.3%. The greater shrinkage seen in specimen 85-8 may have contributed to variation in neuron density between the two specimens.

Rostrocaudal variation in upper and lower layer neurons/unit column in the manatee

Upper layer (i.e., layers II–IV) and lower layer neurons per unit of cortical surface area generally increase across the rostral to caudal axis of the isocortex (Fig. 5, Table 2). Some of the variation in total neurons per unit/column is accounted for by variation in upper layer neurons. This is evident from the observation that layer II–IV neurons per unit of cortical surface area vary by a factor of 1.45–1.49, ranging between 30,500 to 42,300 neurons per mm^2 of cortical surface area in rostral regions to 45,600 to 61,600 neurons in the caudal regions in both manatees (Fig. 5). Layers V–VI vary by a factor of 1.22–1.95 between the most rostrally and most caudally selected regions, ranging between 22,100–32,300 and 43,000–39,300 neurons per mm^2 of cortical surface area in both specimens.

Cross-species statistical comparison in cortical neuron numbers

Finally, we investigated whether the disparity in neurons per unit of cortical surface area increases as a
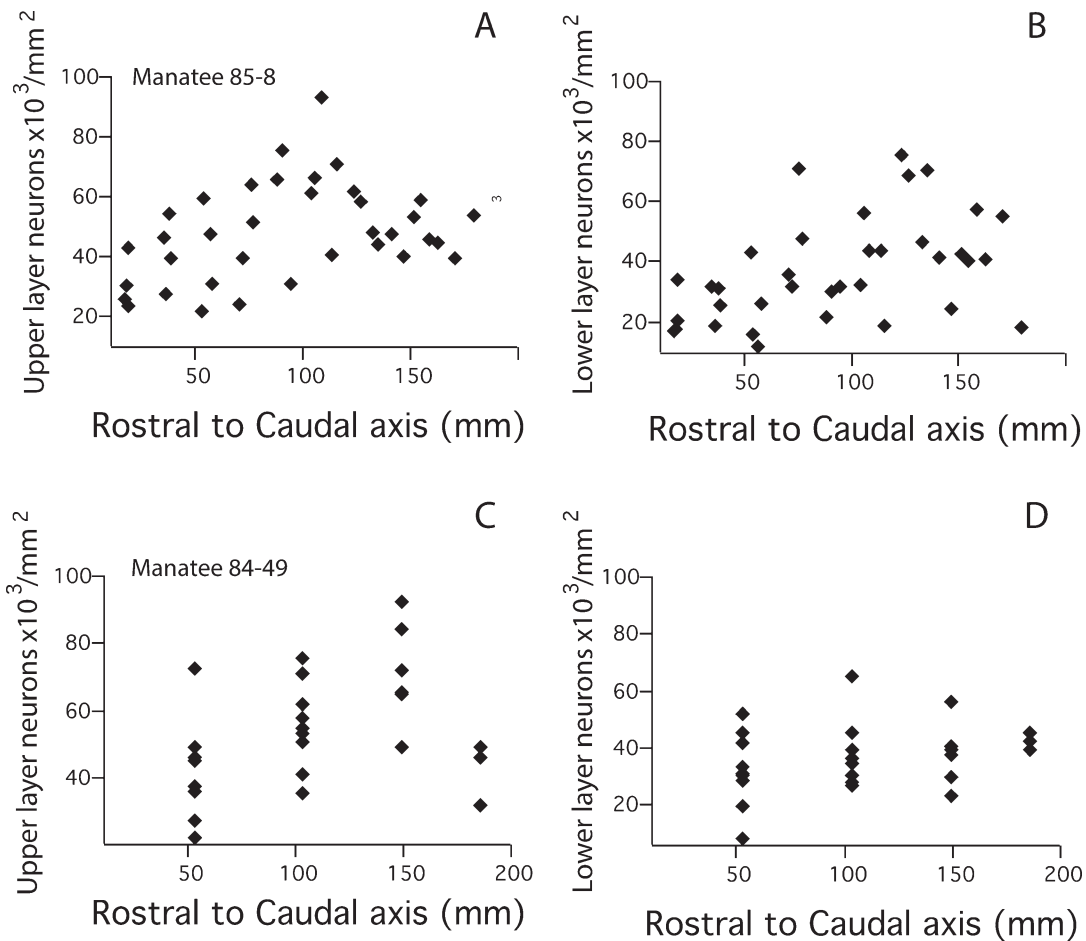
Figure 5. Layer II–IV and V–VI neurons per mm^2 of cortical surface area are plotted against the rostrocaudal axes of the cortex of each manatee. **A,B**: Manatee 85-8. **C,D**: Manatee 84-49.

function of total isocortical neurons across the three orders (i.e, Siriena, primates, rodents), comparing the percentage variation in neuron numbers per mm^2 of cortical surface area between the rostral and caudal poles in each of the selected species (three primate species, three rodent species, a manatee). A phylogenetically controlled regression shows that total isocortical neuron number predicts the magnitude of disparity between the rostral and caudal pole across three mammalian orders. More specifically, the percentage variation in neurons per unit of cortical surface area between the rostral and caudal pole increases in species with greater isocortical neuron numbers ($y = 0.001x + 1.28$; adj R$^2 = 0.71$; F $= 15.81$; $P < 0.05$; $\lambda = 1$).

DISCUSSION

The new data compiled from the manatee show the increase in neuron number/unit column from rostral to caudal cortex. The density of neurons in the manatee cortex is more like the prior rodent sample than the primate sample. The allometry of the rostral-to-caudal increase in neuron numbers appears to vary between orders and depends on absolute neuron number in the cortex, not neuron density or resultant cortical volume, and is most marked in the upper cortical layers.

Rostral-to-caudal variation in neuron numbers in the cat

We give an overview of the variation in neurons per unit of cortical surface area with respect to the rostro-caudal axis across the cat isocortex to broaden our cross-species comparative analyses. Estimates of neurons under 1 mm^2 of cortical surface area for cats ($n = 6$) were obtained with the size-frequency distribution method (Beaulieu and Colonnier, 1989). Neurons per mm^2 of cortical surface area vary systematically across the rostral-to-caudal axis of the cat cortex (Fig. 6). In particular, rostrally selected regions
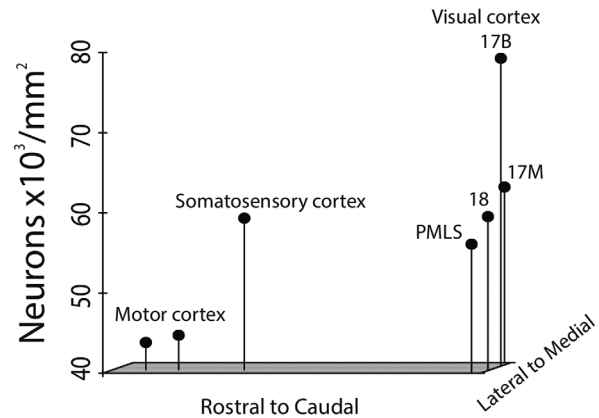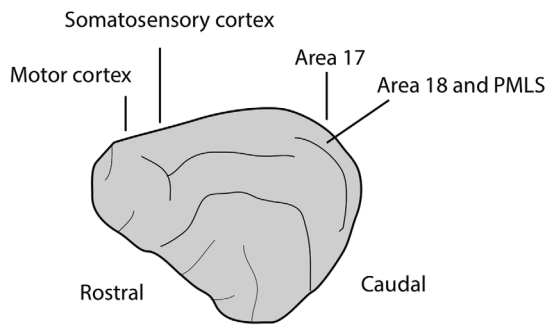
Cat cortical areas

Figure 6. Beaulieu and Colonnier (1989) estimated neuron numbers per mm^2 of cortical surface area in various cortical areas in the cat. The relative positions of neuron numbers per mm^2 of cortical surface area are represented across the rostral to caudal and medial to lateral axis of the cat cortex. Neuron numbers are highest in the caudomedial region (i.e., primary visual cortex) and lowest in rostral regions (i.e., motor cortex).

(i.e., motor cortex) exhibit approximately 40,000 neurons per mm^2 of cortical surface area whereas more caudally selected regions exhibit up to twice as many neurons, ranging between 60,000 and 80,000 neurons per mm^2 of cortical surface area. One noticeable feature is that the increase in neurons across the cat cortex favors the caudomedial pole. This is in contrast to what is observed in primates, in which variation in neuron numbers favors the entire caudal pole of the cortex (Collins et al., 2010; Cahalane et al., 2012). Both the new data compiled for manatees and cats demonstrate that the variation in neurons per unit of cortical surface area is intermediate between the rodents and primate samples, although they resemble rodents more than primates.

Methodological assessments and counterclaims

Our studies were designed to obtain neuron counts across the isocortex, identified for laminar position and corrected for section curvature and stereology, to relate evolutionary patterns of cytoarchitecture in the cortex within and between species to the developmental mechanisms that might produce them. Of particular interest is the gradient of neurogenesis duration and other maturational features across the dorsal cortex and its possible linkage to the rostral-to-caudal increase in neuron numbers/unit column, which is most amplified in the upper cortical layers (Cahalane et al, 2014). Cingulate, entorhinal, olfactory, and all other allocortex areas do not participate in either the developmental maturational gradient, or the gradient in neural numbers in adulthood (note the falloff in neuron number per

column occasionally observed in the extreme caudomedial pole; Fig. 5 and Charvet et al., 2015) when it has been unclear which class of cortex the region might be assigned to.

Recent studies using isotropic fractionator in the elephant (Herculano-Houzel et al., 2014) and the pig, kudu, springbok, and giraffe (Artiodactyla; Kazu et al., 2014) have concluded that neuron numbers do not vary significantly across the isocortex in these mammals (Kazu et al., 2014). The assumption that NeuN, which is used to label neurons, would label the same populations of neurons equivalently across a broad range of mammals has not been validated. Moreover, the methodology of these studies prohibits any conclusion about the neuron number gradients of the isocortical area in question—the coronally amassed samples do not contain isocortex alone but contain major varying components of various allocortex areas (olfactory and subicular cortices as well as some basal forebrain cell groups), only omitting the hippocampus. The relative contribution of allocortex to cortex neuron numbers varies with coronal position. The collated coronal section counts are likely misaligned to variable degrees with the developmental rostrolateral-to-caudomedial gradient of interest depending on brain size. Laminar contributions cannot be distinguished, further diluting the signal from the superficial layers. Remarkably, even with these handicaps, the rostral-to-caudal gradient in neuron number can be seen for four of the six species, with the greatest densities of neurons in the posterior samples (Fig. 10 of Kazu et al., 2014), but the methodological limitations of these studies prohibit their use as positive or negative evidence.

The lissencephaly of manatees

The manatee cortex is peculiar in that it is large and lissencephalic (O'Shea and Reep, 1990; Sun and Hevner, 2014). This is unlike the cortices of many big-brained mammals, which are generally gyrencephalic (Van Essen, 1997; Martínez-Cerdeño et al., 2006; Kriegstein et al., 2006; Lewitus et al., 2013; Zilles et al., 2013). Our findings demonstrate that gyrencephaly coincides with the relative expansion of upper layer neurons relative to lower layer neurons (Fig. 4). That is, New World monkeys (e.g., capuchin, which are gyrencephalic) exhibit proportionately more upper layer neurons than the lissencephalic manatees (Fig. 4; Charvet et al., 2015).

In gyrencephaly, the outer cortical surface area is generally expanded relative to the inner cortical surface, which should accommodate relatively more upper layer neurons relative to lower layer neurons. The finding of a relative expansion of upper layer neurons in gyrencephalic primates relative to the lissencephalic manatee cortex supports the notion that gyrencephaly may coincide with an expansion of upper layer neurons relative to upper layer neurons (Fig. 4). Whether gyrencephaly coincides with an expansion of upper layer neurons across a broad range of mammalian species remains to be investigated.

The seemingly disparate hypotheses concerning the evolution of gyrencephaly may be intrinsically linked. That is, alterations in neurogenetic schedules coincide with evolutionary changes in the relative proportion of different progenitor cells (e.g., subventricular zone) and the relative expansion of late generated neurons (i.e., upper layer neurons; Finlay and Darlington, 1995; Cahalane et al., 2014; Charvet and Finlay, 2014). Heterochronic changes in neurogenesis duration might entail changes in progenitor cell populations in development as well as the disproportionate increase in upper layer neurons and concomitant changes in connectivity patterns and gyrencephaly in adulthood.

Evolutionary changes in gradients in neuron numbers

Our analysis suggests that the manatee and the cat follow the hypothesized mammalian pattern derived from rodents and primates, with higher neurons per unit of cortical surface area in caudal than rostral cortex (Fig. 5). The gradient of neuron number per unit of cortical surface area appears to be steeper in primates than manatees, but the limited sample of species and individuals prevents much speculation on the mechanistic source of the difference.

The "grade shift" in cortical numbers with respect to whole brain volume in manatees and rodents has at least two possible sources. Primates and carnivores, including several marine mammals, allocate relatively more neural mass to isocortex and less to multiple limbic and olfactory structures (Finlay and Darlington 1995; Reep et al., 2007) by delaying and extending cortical neurogenesis with respect to other mammalian taxa (Workman et al., 2013). Additionally and likely independently, the density of neurons in the primate cortex is higher than in rodents and other groups, and the allometry of change in density in primates with cortical volume is close to 1, while the density in larger rodent brains is reduced compared with smaller brains (Herculano-Houzel et al., 2011; Charvet et al., 2015).

Useful species for further quantification of the detail of isocortical neuron number topography may be relatively easy to locate, as large domesticated mammals (goats, sheep, pigs, cows, and horses) would provide a range of brain sizes, some quite large. These species provide a further benefit, as details of their gestation and relative rate of growth have been reasonably well studied. The alterations in rate and relative timing of neural development they exhibit should provide a further window into the theme and variations of the mammalian cortex.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors contributed substantially to the manuscript. Christine Charvet formulated ideas, gathered and analyzed data, wrote the paper, and prepared figures; Roger Reep prepared the histological material, provided expertise on manatee anatomy, and edited the paper; Barbara Finlay helped formulate ideas, organized the basic plan, wrote sections, and edited the paper.

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