Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 2007;70:57–70
DOI: 10.1159/000101491

Received: March 29, 2006
Returned for revision: June 10, 2006
Accepted after second revision: October 2, 2006
Published online: April 4, 2007

The Limbic System in Mammalian Brain Evolution

R.L. Reep[a] B.L. Finlay[b] R.B. Darlington[b]

[a]Department of Physiological Sciences and McKnight Brain Institute, University of Florida, Gainesville, Fla.,
[b]Department of Psychology, Cornell University, Ithaca, N.Y., USA

Key Words
Nervous system · Evolution · Morphometry · Carnivora · Perrisodactyla · Artiodactyla · Xenarthra · Sirenia

Abstract
Previous accounts of mammalian brain allometry have relied largely on data from primates, insectivores and bats. Here we examine scaling of brain structures in carnivores, ungulates, xenarthrans and sirenians, taxa chosen to maximize potential olfactory and limbic system variability. The data were compared to known scaling of the same structures in bats, insectivores and primates. Fundamental patterns in brain scaling were similar across all taxa. Marine mammals with reduced olfactory bulbs also had reduced limbic systems overall, particularly in those structures receiving direct olfactory input. In all species, a limbic factor with olfactory and non-olfactory components was observed. Primates, insectivores, ungulate and marine mammals collectively demonstrate an inverse relationship between isocortex and limbic volumes, but terrestrial carnivores have high relative volumes of both, and bats low relative volumes of both. We discuss developmental processes that may provide the mechanistic bases for understanding these findings.

Introduction

At each step and branch of mammalian brain evolution, multiple and often competing demands must be satisfied. Developmental constraints and covariation among developmental processes, metabolic limitations, general principles in the construction and scaling of information-processing systems, and special adaptations to particular niches all influence the range of variation seen among the adult brains of mammalian species. In exploring the general features of scaling of the brain and its component regions, comparative neuroanatomists have had an excellent resource: neuroanatomical data on 131 species including 40 insectivores, 48 haplorhine and strepsirhine primates, and 43 bats assembled by Stephan and his associates [Stephan et al., 1981; Baron et al., 1996] that have been the basis for a number of thorough analyses [Gould, 1975; Finlay and Darlington, 1995; Barton and Harvey, 2000; Clark et al., 2001; deWinter and Oxnard, 2001; Finlay et al., 2001; Barton et al., 2003]. This corpus has the advantage of meticulous measurement, a wide variety of niches (from burrowing to flying, nocturnal and diurnal, omnivores and specialists) and a wide range of brain sizes, including the human brain. Two limitations of this dataset are its inclusion of only 3 of the 26 orders of mammals [Wilson and Reeder, 1993], and the lack of specimens with large brains in taxa other than

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/bbe

Dr. R.L. Reep
Department of Physiological Sciences
University of Florida, Box 100144 HSC
Gainesville, FL 32610 (USA)
Tel. +1 352 392 4700, ext 3859, Fax +1 352 392 5145, E-Mail reep@mbi.ufl.edu

primates. Hence, the applicability of conclusions from this data to the full range of mammalian brain evolution is unknown.

One apparently singular feature of primate brain organization (compared to insectivores and bats) revealed by the Stephan et al. database is the reduction of the olfactory system [Finlay and Darlington, 1995], concomitant with the reduction of additional distributed structures of the limbic system (i.e., entorhinal cortex, amygdala, hippocampus and septum). In addition, the relative size of the isocortex in primates is increased by delaying and extending the period of isocortical neurogenesis [Finlay and Darlington, 1995; Finlay et al., 1998, 2001]. Adaptation to diurnality is the explanation that has been offered for these two linked features of the primate brain: primates became visual specialists (increasing the demand for cortex), less dependent on olfaction, thus reducing the demand for the limbic structures [Jerison, 1973]. Troublesome for this argument is that no other data exist to support the claim that wholesale reduction of the olfactory and limbic system is typical of diurnal mammals. Further, central components of the limbic system, the hippocampus and amygdala, are not dominated by olfaction, but rather appear to specialize in coding affective qualities of stimuli and in particular types of learning. Finally, although the term 'limbic system' is quite commonly used as collective term for the interconnected structures in the telencephalon and diencephalon described by Papez [see Reep, 1984], its status as an embryological, phylogenetic or functional unit has never been clearly defined.

Perhaps the apparent push-pull relationship between isocortex and limbic system is not related to the requirements of diurnality, but rather to constraining features of brain embryology and metabolic cost. For example, the forebrain shows a segmental, 'prosomeric' structure defined by the domains of regulatory genes [Puelles and Rubenstein, 2003]. Because isocortical and limbic components dominate different forebrain prosomeres, extending the domain of the developmental regulatory genes of one prosomere might directly reduce the number of progenitor cells available in a neighboring one, resulting in a 'zero-sum' reciprocal interaction. Alternatively, if each of these prosomeric domains could vary independently, a metabolic constraint – the simple fact that brain tissue is expensive – might produce a looser reciprocity if taxa were able to effectively specialize in one type of neural organization or the other. Is a reciprocal relationship between the limbic system and isocortex seen elsewhere in the mammalian lineage such that constraining embryological features might be suggested? To better address this question, we have decided to examine the mammalian radiation more broadly, with particular attention to species whose behavioral niches suggest the likelihood of variations in the elaboration of the olfactory and limbic systems. Here we report new data obtained from 29 mammalian species, including 18 Carnivora, 5 Artiodactyla, 4 Xenarthra, 1 Perrisodactyla, and 1 Sirenia. These new data are useful for assessing trends in mammalian brain evolution, and for determining whether patterns described for the earlier dataset apply to mammals generally.

Methods and Materials

Areas and Volumes

Volume estimates were made using 29 brain specimens in the Comparative Mammalian Brain Collection at the University of Wisconsin (table 1). Between 1958 and 1984 these brains were fixed by perfusion, embedded in celloidin and sectioned at 25–40 μm in the coronal plane. Closely spaced series of sections were stained alternately for cell bodies using thionin or, for myelinated axons, using hematoxylin.

For each brain region to be assessed, we first determined its rostrocaudal extent and chose 8–10 sections through that range, evenly spaced where possible. On each of these sections we measured the area of the region on one side of the brain (left side for Stellar Sea Lion, right side for all others) by projecting the image of the section onto a calibrated digitizing tablet and outlining the boundary of the region. The same side of the brain was used for all regions. Each area was multiplied by the number of sections it represented (n), and the section thickness (t). The resulting partial volumes were summed to obtain the volume estimate of that region (Vr). This is formalized in the formula below, where i is the index number of the section sampled, and ranges from 1–10.

$$V_r = \Sigma\, n_i \cdot t \cdot A_i$$

The estimated volumes for each region were corrected for shrinkage as described below. The resulting half-brain regional volumes were multiplied by 2 to obtain whole-brain regional volumes.

Regional Boundaries

Regions and their boundaries were identified as follows. Where boundaries occur gradually (e.g., transition from olfactory to entorhinal cortex), the section located at the midway point of the transitional region was designated as the boundary.

Olfactory bulb – Includes glomeruli, olfactory tract, but not olfactory nerve fascicles visible around surface of the bulb. Includes anterior olfactory nuclei. Does not include lateral ventricle inside olfactory bulbs.

Olfactory cortex – Includes all layers of primary olfactory cortex. If rostral boundary was not distinct from the anterior olfactory nucleus, it was designated to be the point where the olfactory peduncle joined the frontal cortex. Caudally, at the transition to entorhinal cortex a lamina dissecans becomes visible and layer II becomes intermittent.

Table 1. Estimated volumes (mm^3) of brain components in 29 selected species, with University of Wisconsin specimen numbers indicated

	Medulla	Cerebellum	Mesencephalon	Diencephalon[1]	Striatum[1]	Septum	Amygdala[2]	Paleocortex[2]	Hippocampus	Schizocortex	Isocortex
Order Carnivora											
Family Canidae											
Coyote 62-301 *(Canis latrans)*	3,392.64	6,244.80	1,818.72	3,256.32	1,847.04	144.48	849.12	2,272.32	2,158.56	1,603.68	49,435.68
Red fox 63-392 *(Vulpes vulpes)*	1,841.76	4,112.64	1,315.68	1,966.08	1,002.72	108.00	395.52	960.48	1,288.32	594.72	24,216.48
Fennec 63-388 *(Fennecus zerda)*	957.12	1,860.00	632.64	895.68	510.72	51.84	213.12	510.24	485.76	335.04	8,302.08
Polar bear 62-256 *(Ursus maritimus)*	20,285.28	61,999.68	7,129.92	14,129.28	9,889.92	617.28	1,947.36	7,370.40	5,588.64	2,806.08	259,556.64
Family Procyonidae											
Crab-eating raccoon 68-312 *(Procyon cancrivorus)*	1,596.96	4,155.84	765.60	1,942.08	1,039.68	107.04	291.84	812.16	1,025.76	565.92	24,555.36
Coatimundi 58-360 *(Nasua nasua)*	1,088.16	3,335.04	615.36	1,395.36	1,239.84	48.96	295.2	836.16	494.40	281.28	14,564.16
Olingo 62-113 *(Bassaricyon gabbii)*	683.52	1,795.20	496.32	1,016.64	811.20	57.12	191.52	575.52	579.36	512.64	90,30.72
Family Mustelidae											
Least weasel 63-10 *(Mustela nivalis)*	149.76	298.56	92.16	148.32	74.88	9.60	17.28	73.44	168	42.24	994.08
Badger 63-127 *(Taxidea taxus)*	1,912.80	4,135.20	1,037.28	2,561.28	2,120.64	118.56	422.4	1,641.12	1,447.68	865.92	26,457.12
Striped skunk 57-226 *(Mephitis mephitis)*	462.24	1,201.92	206.40	445.92	349.44	28.32	116.64	456.96	312.00	180.48	4,053.60
Family Hyaenidae											
Spotted hyena 64-352 *(Crocuta crocuta)*	7,089.12	14,094.72	3,419.52	6,756.48	5,801.28	336.00	1,471.2	3,902.88	3,080.64	2,154.24	92,500.80
Family Felidae											
Mountain lion 60-206 *(Felis concolor)*	5,176.32	12,413.28	2,940.00	4,935.36	3,000.00	259.20	855.36	2,014.08	1,798.56	1,082.88	70,327.68
Leopard 63-261 *(Panthera pardus)*	7,482.24	16,028.16	4,802.40	6,180.48	3,809.28	235.68	1,355.04	2,855.04	3,121.44	2,155.20	85,872.96
African lion 62-79 *(Panthera leo)*	11,861.76	32,487.36	6,199.20	8,932.32	3,633.60	422.40	1,578.72	3,082.56	4,496.16	1,324.8	157,070.40
Family Otariidae											
Northern fur seal 61-512 *(Callorhinus ursinus)*	10,992.00	40,414.56	4,153.44	12,811.68	6,391.20	391.68	905.28	2,115.84	1,953.12	1,680.00	186,232.80
California sea lion 62-294 *(Zalophus californianus)*	13,046.04	73,013.64	5,225.22	12,072.48	9,367.26	501.06	1,253.28	2,638.44	2,328.48	2,367.96	263,153.10
Stellar sea lion 61-513 *(Eumetopias jubatus)*	20,610.04	93,486.80	5,423.00	14,465.00	9,214.04	310.20	1,760	3,355.00	3,530.12	2,509.76	359,900.20
Family Phocidae											
Harbor seal 61-515 *(Phoca vitulina)*	9,209.82	33,550.68	3,720.12	10,289.78	6,096.96	348.58	932.06	1,838.02	2,113.52	2,545.04	169,256.18
Order Artiodactyla											
Family Tayassuidae											
Collared peccary 63-445 *(Tayassu tajacu)*	2,386.08	6,330.24	1,769.28	2,726.88	2,506.08	169.92	544.32	1,881.60	2,667.84	1,023.84	28,429.44
Family Camelidae											
Llama 65-139 *(Lama glama)*	8,955.36	22,925.76	6,617.76	7,921.92	7,429.44	310.56	1,191.36	2,737.92	3,524.16	1,693.44	99,180.00
Camel 60-227 *(Camelus dromedarius)*	26,781.12	70,519.68	14,904.00	19,111.68	21,388.32	768.96	3,966.24	7,468.80	8,577.12	3,760.32	375,959.52

Table 1 (continued)

	Medulla	Cerebellum	Mesencephalon	Diencephalon[1]	Striatum[1]	Septum	Amygdala[2]	Paleocortex[2]	Hippocampus	Schizocortex	Isocortex
Family Cervidae											
White-tailed deer 67-81 *(Odocoileus virginianus)*	6,521.28	14,463.84	5,060.16	7,130.40	3,750.72	406.08	1,048.8	3,132.48	3,259.68	2,596.80	68,266.08
Family Bovidae											
Zebu 64-322 *(Bos taurus indicus)*	17,187.84	41,459.04	12,054.72	20,231.04	17,609.28	444.96	3,838.08	9,524.64	7,708.32	5,050.08	362,993.76
Order Perissodactyla											
Family Equidae											
Zebra 61-820 *(Equus burchelli)*	18,975.36	47,924.64	12,012.00	16,176.48	15,858.72	541.44	2,971.2	7,879.68	10,334.88	3,980.16	294,262.56
Order Xenarthra											
Family Myrmecophagidae											
Giant anteater 67-29 *(Myrmecophaga tridactyla)*	3,816.96	8,314.08	1,813.92	3,568.80	2,063.52	163.20	425.28	2,377.92	2,684.64	915.84	19,503.36
Collared anteater 61-93 *(Tamandua tetradactyla)*	1,287.84	3,420.48	720.00	1,439.52	1,505.76	94.56	216.48	1,189.92	1,070.40	425.28	8,075.52
Family Megalonychidae											
Two-toed sloth 61-98 *(Choloepus didactylis)*	1,009.92	2,654.40	693.12	1,569.60	1,802.88	84.00	241.44	1,374.24	1,138.08	354.72	8,702.40
Family Dasypodidae											
Nine-banded armadillo 60-465 *(Dasypus novemcinctus)*	883.68	1,880.64	432.00	628.32	491.04	89.28	126.24	949.44	927.84	303.36	1,718.88
Order Sirenia											
Family Trichechidae											
Florida manatee 84-49 *(Trichechus manatus)*	13,635.54	44,297.28	5,456.70	1,2847.68	9,130.86	194.40	1,101.6	2,403.54	3,634.2	1,459.08	188,861.22

Species are arranged according to the taxonomic scheme of Wilson and Reeder [1993]. Brain component categories are identical to those of Stephan et al. [1981] except as noted.

[1] Diencephalon includes globus pallidus in the previous work of Stephan et al. [1981]. Here, globus pallidus is included with striatum.

[2] Amygdala volumes are reported separately and are also included in Paleocortex, to facilitate comparison with the dataset of Stephan et al. [1981].

Olfactory tubercle – Includes all layers of olfactory tubercle.

Septum – Includes the fornix when its fibers were intermingled with the septal nuclei; caudal to this point the fornix was considered part of the hippocampus. Does not include diagonal band, entopeduncular nucleus.

Amygdala – Rostral boundary at first appearance of nucleus of the lateral olfactory tract; caudal boundary at amygdalo-hippocampal area.

Isocortex – Includes gray and white matter, claustrum. Lateral ventricles measured separately.

Schizocortex – Includes entorhinal, parahippocampal, presubicular, parasubicular and subicular cortices.

Hippocampus – Includes the dentate gyrus, cornu Ammonis, alveus, hippocampal commissure, fornix-fimbria caudal to septum.

Basal ganglia – Includes caudate, putamen, nucleus accumbens, and globus pallidus. Includes internal capsule, whether dispersed as in rodents or coalesced as in carnivores.

Diencephalon – Rostral boundary located where the 3rd ventricle and anterior commissure are present together. The cerebral peduncle in the thalamic region was not included in volumetric estimates of diencephalon. Optic nerve and pituitary not included. Does not include 3rd ventricle.

Midbrain – Cerebral peduncle included.

Pons – Includes middle cerebellar peduncle until it joins the cerebellar cortex. Does not include 4th ventricle.

Medulla – Rostral boundary designated as that point where cranial nerve VII is first visible; caudal boundary denoted by disappearance of inferior olivary cells near the base of the pyramids, or disappearance of the cuneate-gracile nuclei dorsally. Does not include 4th ventricle.

Shrinkage

In order to obtain accurate volume estimates, raw volume estimates must be corrected for shrinkage due to histological processing [Uylings et al., 1986]. For 6 of the 29 brains there were calibrated photographs of the brain from several angles, allowing

Table 2. Shrinkage estimates in six brains

Species	Linear shrinkage, %	Areal shrinkage, %	Volumetric shrinkage, %	Volumetric correction factor
Harbor seal	29.5	50.3	65.0	2.9
California sea lion	21.6	38.5	51.8	2.1
Stellar sea lion	23.2	41.0	54.7	2.2
Northern fur seal	25.8	44.9	59.1	2.4
Polar bear	25.6	44.6	58.8	2.4
Manatee	28.5	48.9	63.4	2.7
Mean values	25.7	44.8	59.0	2.4

Table 3. Precision of repeated measurements made on four brain regions

	Olfactory bulb Mountain lion	Amygdala Mountain lion	Pons Red fox	Isocortex Red fox
Mean area, mm^2	33.2	33.9	31.7	168.9
Std. Dev.	0.4	0.7	0.5	0.7
Coeff. Var.	0.012	0.020	0.016	0.004

Table 4. Variations in volume estimates derived from various numbers of sections of leopard brain

Isocortex # sections	5	6	8	11	22
Estimated volume	404.0	416.2	404.4	411.0	408.9
% error referenced to 22 sections	−1.2	+1.8	−1.1	+0.5	0
Thalamus # sections	3	4	6	8	16
Estimated volume	897.7	892.8	885.3	932.1	905.0
% error referenced to 16 sections	−0.8	+1.3	−2.2	+0.7	0
Medulla # sections	4	6	9	17	
Estimated volume	830.7	842.0	830.8	834.3	
% error referenced to 17 sections	−0.3	+0.9	−0.4	0	

for shrinkage estimates to be made. For this purpose, the maximum extent of the brain in the dorsoventral dimension was measured from the photograph and from the corresponding brain section. These values were compared to obtain an estimate of linear shrinkage. This was then converted to a volumetric shrinkage correction factor by taking the cubed value. Linear shrinkage estimates ranged from 21.6–29.5%, corresponding to areal shrinkage of 38.5–50.3%, and volumetric shrinkage of 51.8–65.0% (table 2). The associated correction factors were used to adjust raw volumetric estimates for these six taxa. The mean volumetric factor of 2.4 was used to adjust the raw values for volumes computed for the remaining 23 taxa for which accurate shrinkage estimates were not available. Because all brains were processed similarly (fixed by perfusion then embedded in celloidin), actual shrinkage in these 23 brains is likely to be in the same range as the 6 for which we obtained direct measurements.

Measurement Accuracy and Precision

To check the accuracy of the regional measurements, we compared the half-brain volume obtained by summing the component volumes to the half-brain volume obtained by direct measurement of the areas of 10 sections from each brain. These 10 sections spanned the entire region from the olfactory bulbs through the medulla. Lateral ventricle volumes were added as one component in the first case in order to match the second case, in which their volume is computed implicitly. The ratio of component-summed volume to 10-section volume averaged 96%, indicating that a small volume of each brain is not assessed. This likely includes areas in the basal telencephalon that were not included in septum, amygdala or basal ganglia, and the diencephalic portion of the cerebral peduncle.

In order to assess the precision of single areal measurements before using them to gather the present data, 10 repeated areal

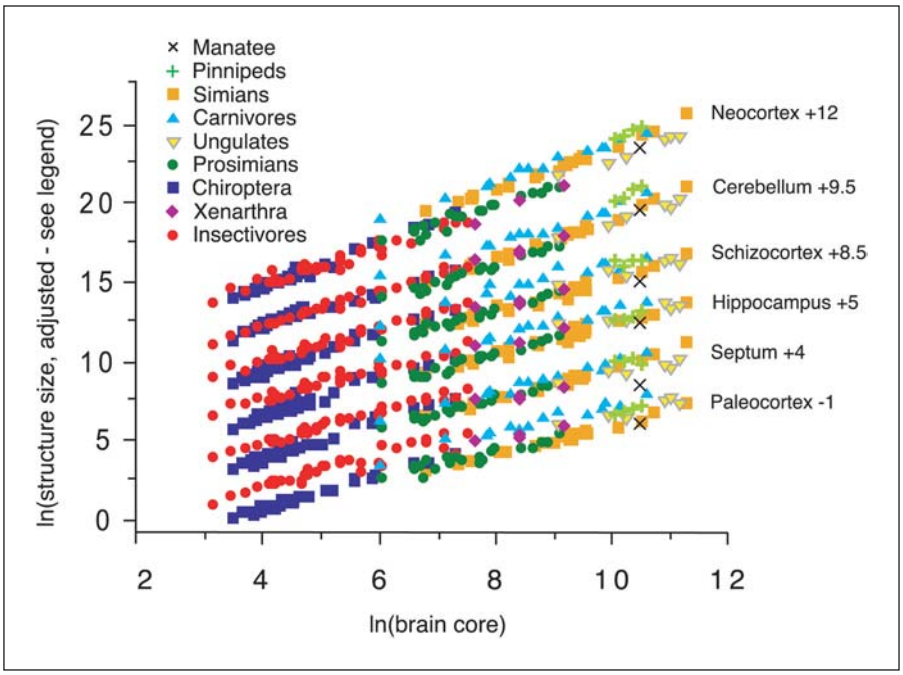

Fig. 1. The sizes of 6 non-core brain structures relative to brain core size, across 160 species in 9 taxonomic groups. The 6 structure sizes have been adjusted by the indicated arbitrary constants to separate the 6 scatterplots visually. Both scales are natural log scales. The regression slopes are: paleocortex 0.826, septum 0.847, hippocampus 0.912, schizocortex 0.963, cerebellum 1.150, isocortex 1.441.

measurements were made on four regions from two brains: olfactory bulb and amygdala in the mountain lion; pons and isocortex in the red fox. Coefficients of variation were all <0.02, indicating good measurement reliability (table 3).

In order to determine the optimum number of sections to use for volume estimates, we computed estimated volumes of three brain regions (frontal isocortex, thalamus, medulla) in the leopard specimen using variable numbers of sections, ranging from 3 to 22. As shown in table 4, for each of the three regions analyzed the errors fluctuated above and below the reference value determined from the largest number of sections sampled, and no error exceeded an absolute value of 2.2%. Errors involving 8 or more sections were no greater than 1.1% absolute value. Therefore, we used 8–10 sections per brain region in order to obtain volume estimates. This is consistent with the finding of Uylings et al. [1986] that using 5–7 sections per region is sufficient to obtain accurate volume estimates.

Half Brain and Whole Brain

The present data were collected from one side of the brain only, and were converted to whole brain regional volumes by multiplying all values by 2. This method assumes that regional volumes are comparable on the left and right sides. In order to test this assumption we obtained regional volume estimates on both sides of the white-tailed deer brain. Across regions, these estimates varied from 0.2–3.3%, with most differences less than 2%, suggesting that the use of one side is sufficient.

Statistical Analyses

We analyzed data from our new 29 species as well as from 131 species in the Stephan et al. dataset. A variety of techniques were employed to dissect the covariation of brain structure volumes, depending on the nature of the question asked. These are discussed briefly below, and in greater detail in the Results.

In order to determine whether the new dataset followed the overall pattern defined previously for the Stephan et al. dataset [Finlay and Darlington, 1995; Finlay et al., 2001], we used statistical procedures identical to the first analysis. In addition, we regressed the log-transformed sizes of various brain structures against the 'brain core' defined by Finlay et al. [2001], then analyzed the resulting slopes in order to differentiate correlations due to intrinsic linkages among brain regions from taxon-specific effects.

Once we verified that intrinsic relationships among brain regions appeared to be producing the observed correlations, we performed a principal component analysis similar to that done previously on the Stephan et al. dataset [Finlay and Darlington, 1995], and investigated in some detail the second principal component. (Thus, although our figure 1 plots brain component sizes vs. brain core, the principal component analysis is identical to that performed in 1995, with the addition of our 29 new species) First, we computed the partial correlation between the second principal component and each of the various log-transformed structure sizes, partialing out the first principal component. Because this analysis revealed that the second principal component appears to be a 'limbic factor', we then compared the relative sizes of the olfactory bulbs for size-matched marine vs. non-marine mammals holding brain core constant. Upon discovering that the relative size of the olfactory bulb is markedly lower in marine vs. terrestrial taxa, we took a closer look at the pattern in which the remaining limbic brain regions scale when brain size is equalized across these taxa, and we used the method of independent contrasts [Price, 1997] to investigate the relationship between olfactory bulb size and that of isocortex. The phylogeny employed for this

analysis is based on Wilson and Reeder [1993], and is illustrated for the 29 new species in table 1.

In order to investigate the structure of the limbic factor as broadly as possible across our entire dataset, we performed a factor analysis on 112 species for which we had volumetric data on all the major components of the limbic system. This revealed a distinction between olfactory and non-olfactory limbic factors.

Results

Brain Data from New Orders

In this study we gathered data from a further sample of 29 species from 5 orders, including 18 Carnivora, 5 Artiodactyla, 4 Xenarthra, 1 Perrisodactyla, and 1 Sirenia (table 1). (Note: In the analyses and figures that follow, pinnipeds are analyzed separately from the terrestrial carnivores, and artiodactyls and perissodactyls are grouped as ungulates).

The carnivore and sirenian orders contain marine mammals in which the external olfactory system and olfactory bulbs appear greatly reduced, as is the case in cetaceans. Pinnipeds, although they are not exclusively aquatic, appear to have reduced olfactory systems [Welker et al., 2006] despite a lack of quantitative data until the present report; the manatee, a marine herbivore, is exclusively aquatic and exhibits greatly reduced olfactory system components [Reep et al., 1989], as does its sirenian relative the dugong. The artiodactyls include terrestrial herbivores that live in arid environments and utilize olfactory cues in a wide variety of social interactions [Hart, 1983; Deutsch, 1992]. The Xenarthra are olfactory and gustatory specialists [Eisenberg, 1981; Redford, 1985].

We asked a number of questions about this new collection of mammals. (1) Does the same factorial structure described for patterns of relative enlargement of brain parts in primates, bats and insectivores [Finlay and Darlington, 1995] also apply to these taxa? (2) If the peripheral components of the olfactory system are reduced in marine mammals, are the non-olfactory components of the limbic system also 'obligatorily' reduced; how do olfactory and limbic brain components covary overall? (3) Is the push-pull relationship of the relative size of isocortical and limbic structures observed in primates seen in other cases of olfactory/limbic system reduction or enlargement?

The Factorial Structure of the Brain

We defined the 'brain core' as the medulla, mesencephalon, diencephalon, and striatum [Finlay et al., 2001]. Figure 1 shows the log-transformed sizes of the other six measured brain structures (excluding the olfactory bulb) against the log-transformed size of the brain cores for all 160 species in our sample. Arbitrary constants were added to scores on the vertical axis to visually separate the six scatterplots and allow visual comparison with our earlier report [Finlay and Darlington, 1995]. The very high correlations visible in figure 1 reflect the very large percentage of variance explained by the first factor of a principal component analysis of the covariance matrix of the 11 structures. That percentage was 96.29 in the 1995 analysis and is essentially the same, at 96.47, in the new larger sample. Inclusion of the 29 new species increased total variance from 43.391 to 58.661. Total variance is not necessarily increased by new cases, but increased here because large animals such as polar bears and camels were added to a sample dominated by bats, insectivores, and small primates.

A second feature of the original analysis is also preserved – that different brain components increase with different slopes with respect to brain size or brain core, with isocortex increasing the most rapidly. Our previous analyses have related the slopes directly to the cross-mammalian conserved order and duration of neurogenesis in these structures [Clancy et al., 2000, 2001; Finlay et al., 2001]. It is also logically possible that the observed differences in slopes are caused by taxon-specific grade shifts [Rilling and Insel, 1998; Barton and Harvey, 2000; Barton, 2001]. We present here a new analysis to distinguish between these two hypotheses. The new analysis relies on three points. First, in simple regression we have

$$\text{Slope} = r(XY)*S(Y)/S(X),$$

where r denotes correlation, S denotes a standard deviation, and X and Y are the independent and dependent variables respectively. Second, when we consider the separate regressions predicting structure size from total brain size, the predictor variable X is the same in all the regressions, so S(X) is constant across all these regressions. Third, in the present dataset of 160 species, when olfactory bulb is excluded the correlations of log-transformed structure sizes with log-transformed brain size range only from 0.969 (for paleocortex) to 0.997 (for diencephalon). Thus we introduce only a small error by thinking of all these correlations as equal. These three points together imply that across the various brain structures, the slopes will be proportional to the values of S(Y), the standard deviations of the log-transformed brain structure sizes. Thus the original question about slopes can be translated into a question about standard deviations (SD's). This translation means the analysis is com-

pletely independent of the exact measure of total brain size used as X.

We next consider two measures of the variability of size for any particular structure. One is the mean SD within eight taxonomic groups (carnivores excluding pinnipeds, pinnipeds, artiodactyls and perissodactyls grouped as ungulates, prosimian primates, simian primates, Chiroptera, Xenarthra, and insectivores; manatees are not included because we cannot compute an SD for one species). For this within-taxon measure (WT), we compute SD within each taxon and then simply average the 8 values of SD. The second measure of variability is a between-taxon measure (BT), expressed as the SD of the within-taxon means. That is, compute the mean structure size within each taxon, then compute the SD of those 8 means.

According to a taxonomic grade-shift perspective, WT and BT are essentially independent qualities, and there should be no tendency for structures high on WT to be high on BT. However, if size of a given brain structure is tightly constrained by its relation to overall brain size, then r(WT, BT) should be high. In the present dataset, across the 10 structures, r(WT, BT) = 0.9711. Eight of the ten structures fall in exactly the same rank order on WT as on BT. From high to low, they are; isocortex, cerebellum, striatum, diencephalon, schizocortex, hippocampus, paleocortex, and septum. (Medulla and mesencephalon each miss only slightly in falling into this perfect rank order; mesencephalon is just below hippocampus on WT but above it on BT, and medulla is below hippocampus and schizocortex on BT but above them on WT). In fact, WT and BT are not merely positively related, but approximately proportional to each other; the BT/WT ratio varies only from 2.22 to 2.85 across the 10 structures. Thus, the present analysis supports the contention that the size of any brain structure is closely related to overall brain size, rather than merely appearing so due to taxon-specific grade shifts.

The earlier analysis of bats, insectivores and primates [Finlay and Darlington, 1995] concluded that among species matched on the first two principal components, the volume of a typical brain structure will have a range of about 2.55×. Repeating that calculation in the new larger sample produces a similar range of 2.54×. The total range of brain sizes is the same as before: 21,400× for the whole brain, and 142,000× for the isocortex. Thus our overall conclusion is unchanged: a ratio of 2.5 seems large in one sense but is trivial in comparison to the total range of overall brain sizes. (Incidentally, the volume range obtained using only the first principal component is 8.33×).

The Structure of the 'Limbic Factor' and Its Relationship to Olfaction

The percentages of total variance in brain component sizes explained by the first four principal components are 96.4702, 2.6076, 0.3617, and 0.2007. Even though the first of these values overwhelms the others, the second value is 7.21 times the third, and the second factor explains 74% of the variance not explained by the first factor. Thus the second factor is well worth studying. To study this second factor we used a smaller sample of 112 species. This reduced dataset included the 29 new species and 83 of the 131 species from the Stephan et al. dataset. For these 112 species separate volumetric data were available for the amygdala, which had been included in the paleocortex in our previous analyses. Thus in these 112 species we could use 12 non-overlapping brain structures, including the amygdala and the paleocortex without amygdala. We computed the correlations between the various log-transformed brain structure sizes and the second principal component, statistically holding constant the first principal component. The four largest positive correlations are all for limbic structures (olfactory bulb, 0.99; paleocortex, 0.82, schizocortex, 0.38; and hippocampus, 0.20), whereas the four largest negative correlations are all for nonlimbic structures. Thus the second factor is clearly a limbic factor. When the first factor is held constant, the second correlates +0.9940 with the log-transformed size of the olfactory bulb.

We were particularly interested in relative olfactory bulb size in the five marine mammals in the new sample – four pinnipeds and a manatee – as well as in Xenarthra, this taxon chosen for its apparent behavioral dependence on chemosensation. We wished to verify the amount of olfactory variation in these animals and to explore the relationship between olfactory lobe variation and the sizes of additional neural structures, as a way of dissecting out the olfactory and non-olfactory components of the limbic system. Figure 2 shows the relative sizes of the olfactory bulb, on a natural log scale, with brain core controlled statistically, for these five taxa: the closest phylogenetic comparisons are pinnepeds to terrestrial carnivores, the manatee to ungulates, and the Xenarthra to both other groups. The results illustrated in figure 2 indicate that olfactory bulbs in marine mammals (pinnipeds and sirenians) are only a small fraction of the size of bulbs in terrestrial mammals with equal-sized brain cores.

Investigating further nervous system variation using the same statistical methods and the same 29 species used in figure 2, we estimated the size of each limbic structure for each of the 5 studied taxa, for a hypothetical taxon

member with brain core of 20,000 mm^3 – essentially the brain core size of a white-tailed deer. The results appear in figure 3, whose vertical axis is not on a log scale but is rather in mm^3. The top left panel of figure 3 repeats the information of figure 2 in this different format. The marine mammals (pinnipeds and sirenians) have markedly reduced olfactory (paleo) cortex, schizocortex (pre-, para-, and subicular cortices) and hippocampus; the septum and amygdala are not much altered. Notice that the Xenarthra, with their unusually large olfactory bulbs, have the reciprocal pattern. Extending this analysis to all available species, we subtracted the portion explained by brain core size from the sizes of all limbic structures (in this case defined as those structures labeled heavily with LAMP [Levitt, 1984; Levitt et al., 1997], plus the olfactory bulb) and performed a factor analysis with Oblimin rotation on the correlations of remaining portions of these structures in the 112 species for which we had amygdala data. In this restricted dataset we found two factors that correlated 0.290 with each other. The factor loadings (values from the factor pattern matrix) are depicted in figure 4. One factor loads highly on the olfactory bulb, paleocortex, schizocortex, and hippocampus, whereas the other loads highly on the amygdala and septum. We refer to these as the olfactory limbic factor and the non-olfactory limbic factor, respectively.

Inverse Relationship of Isocortical and Limbic Components across Taxa

In this analysis, we examine the relationship between the isocortex and limbic system (defined as olfactory bulb, olfactory cortex, schizocortex, hippocampus, and septum) while statistically holding constant the brain 'core' consisting of the medulla, midbrain, diencephalon and striatum (fig. 5). All possible relationships of the relative sizes of limbic system and cortex are in evidence, although the inverse pairings of high limbic/low isocortex and low limbic/high isocortex dominate the graph. Xenarthra (anteaters, sloths and an armadillo) and insectivores have relatively enlarged limbic systems and reduced isocortices compared to the brain core. Simians, pinnipeds and the manatee have relatively large isocortices and reduced limbic systems. However, terrestrial carnivores claim both large limbic systems and cortices, whereas some bats exhibit reductions in both isocortex and limbic system relative to the brain core, compared to other mammals. The notion of an obligatory inverse relationship of these two brain components is ruled out both by the patterns shown by carnivores and bats, and by the fact, visible in figure 5, that within taxa there is no consistent inverse relationship

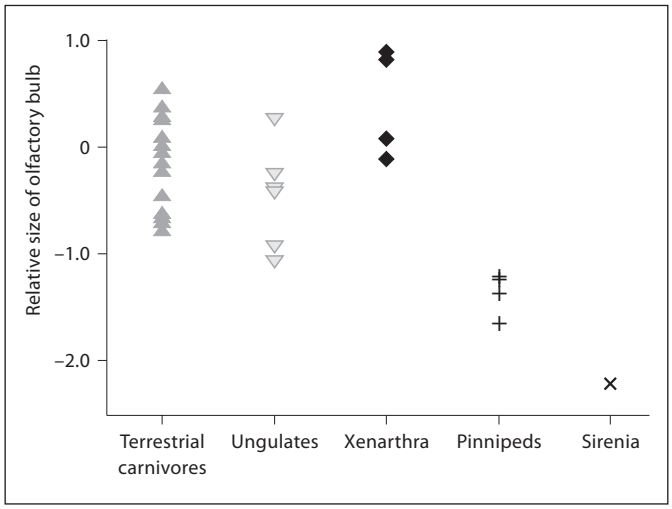

Fig. 2. Log-transformed sizes of the olfactory bulb in 29 species in 5 taxonomic groups, adjusted for differences in brain core size (each unit on the vertical scale corresponds to a factor of e or 2.72).

between isocortex and limbic system size. However, the clarity of the overall inverse relationship suggests that a more general constraint, such as reduction of metabolic cost, might apply. Analysis using the method of independent contrasts provides further evidence for the existence of a general inverse relationship (fig. 6). With brain core held constant, there was a significant negative relationship between the isocortex and limbic system (partial r = –0.353, t = –3.717, d.f. = 97, p = 0.000168). Similarly, there was a significant negative correlation between isocortex and olfactory bulb residuals (fig. 7) (partial r = –0.308, t = –3.18, d.f. = 97, p = 0.000977). All within-order correlations are negative, suggesting that the overall negative correlation represents biological variation that is independent of taxonomic effects.

Discussion

Causes and Effects of Olfactory Bulb Reduction in Marine Mammals

Unlike reduction of the olfactory bulb in primates, whose regression is hypothesized to happen secondarily to greater dependence on the visual modality [Jerison, 1973], the reason for reduction of olfactory bulbs in marine mammals is more direct because marine mammals have developed no way of employing olfaction underwater. There is no compelling behavioral reason to couple

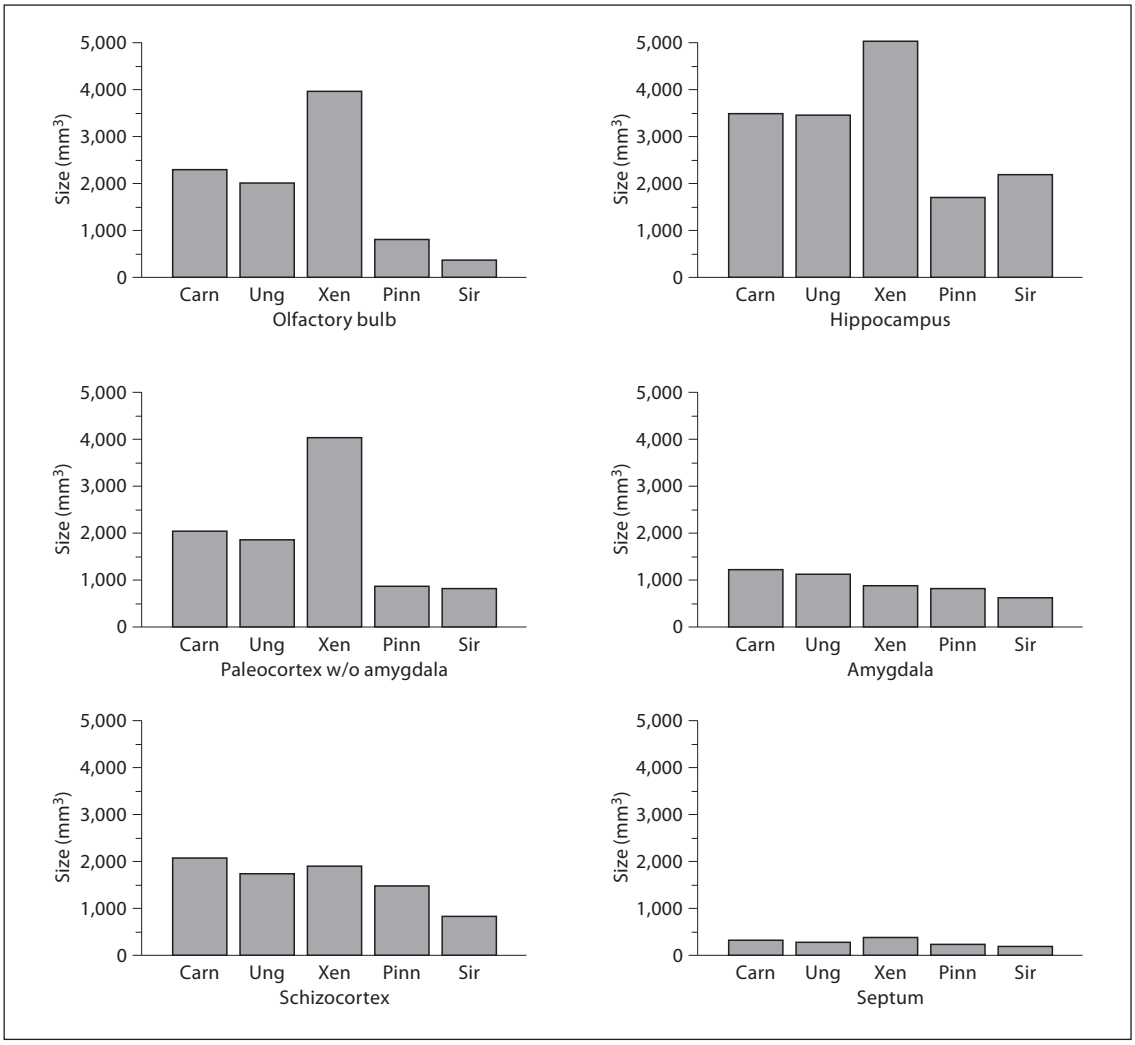

Fig. 3. The estimated sizes (in mm^3) of 6 limbic structures in 5 taxonomic groups for a hypothetical group member with the brain core size of a white-tail deer. Carn = terrestrial carnivores, Ung = artiodactyls and perissodactyls, Xen = Xenarthra, Pin = pinnipeds, Sir = sirenians (manatee).

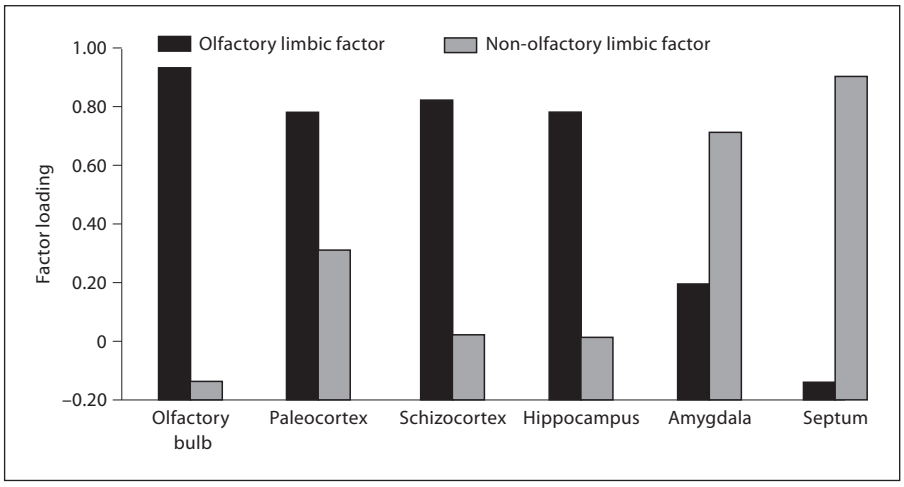

Fig. 4. Factor loadings of 6 limbic structures on two factors after the sizes of those structures have been adjusted for differences in brain core size.

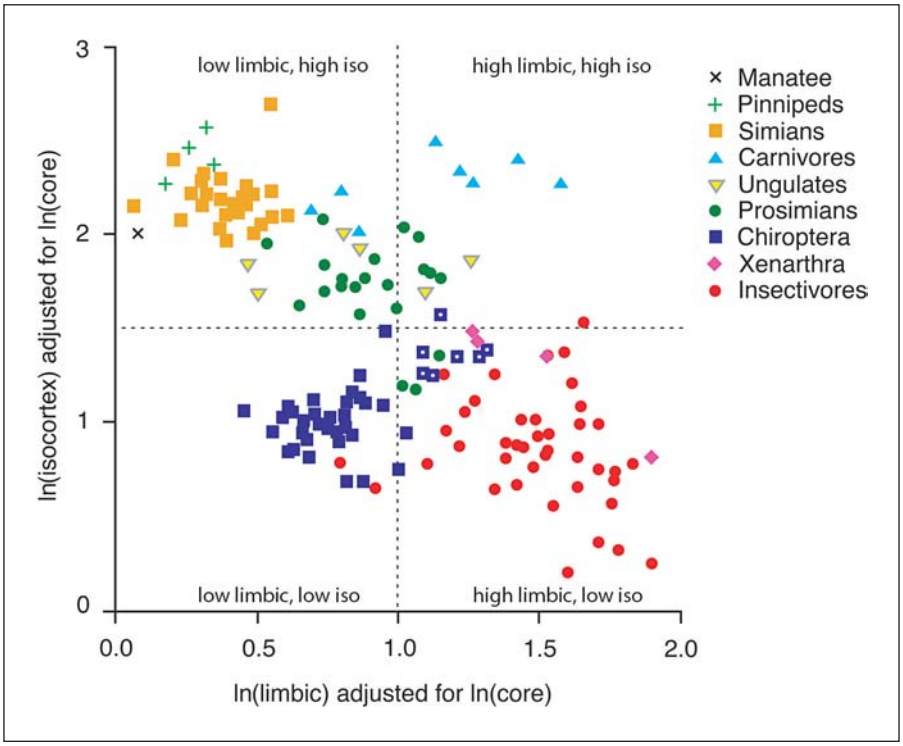

Fig. 5. Relationship between limbic and isocortical components of the telencephalon. Plotted are residual variance in the volume of limbic and isocortical components, referenced to brain core volume, for 160 species in 9 taxonomic groups. Overall there is a push-pull arrangement between the limbic and isocortical components, indicated by the preponderance of values in the upper left and lower right quadrants. However, some carnivores represent a balanced expansion of both components, and most chiropterans exhibit reduction of both components. Blue squares with white centers represent pteropids.

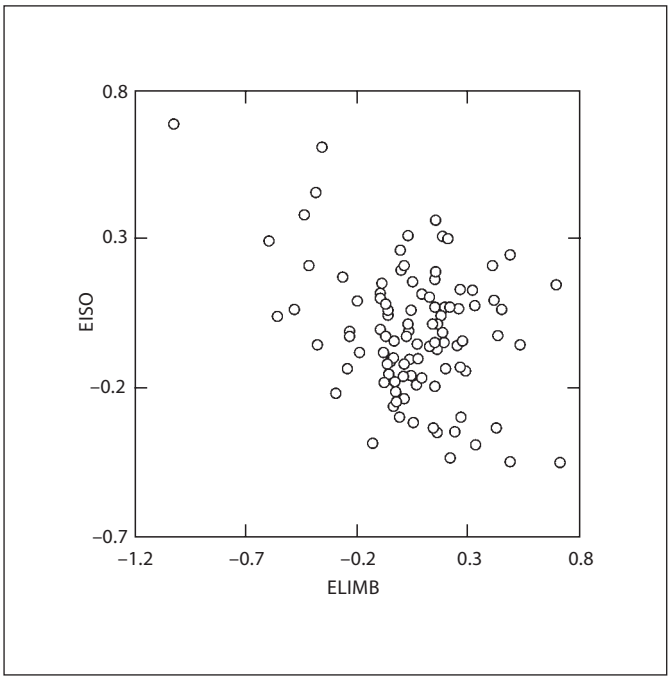

Fig. 6. The method of independent contrasts provides support for the general inverse relationship between limbic and isocortical components of the brain (partial r = –0.353, t = –3.717, d.f. = 97, p = 0.000168). The variables EISO and ELIMB represent the residuals for isocortex and limbic system, respectively.

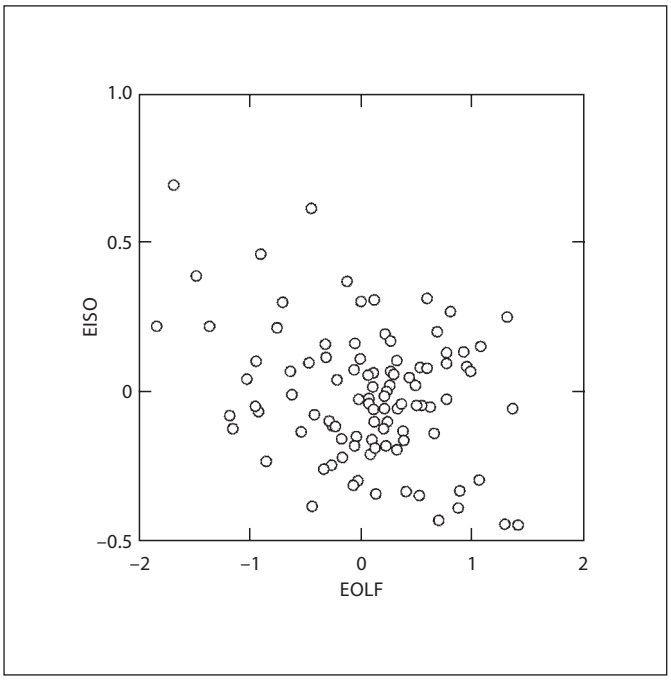

Fig. 7. There is a significant inverse relationship between relative size of isocortex and that of the olfactory bulb for 112 species examined (partial r = –0.308, t = –3.18, d.f. = 97, p = 0.000977). The variables EISO and EOLF represent the residuals for isocortex and olfactory bulb, respectively.

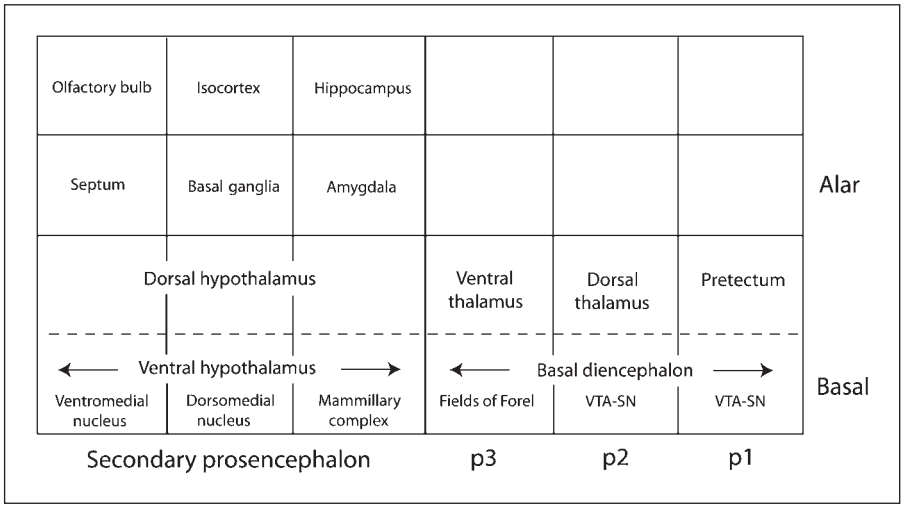

Fig. 8. Prosomeric model of the forebrain [redrawn after Puelles and Rubenstein, 2003]. The rostral pole of the brain is toward the left; dorsal toward the top. Dashed horizontal line represents the boundary between the alar and basal plates seen in early development.

olfactory reduction and neocortical expansion to privilege vision in marine mammals, as the visual capacities of pinnipeds are not obviously superior to terrestrial carnivores, nor the manatee to terrestrial ungulates although this has not been studied quantitatively. We can characterize the loss of the olfactory bulb in marine mammals more explicitly as the regression of an unused sensory system, such as the remnant visual system of burrowing mammals [Cooper et al., 1993] and ask in a similar fashion which other neural structures change in concert.

The olfactory (paleo) cortex, schizocortex (pre-, para-, and subicular cortices) and hippocampus are the most reduced in these animals but the septum and amygdala are not much altered. The cause of the reduction of the size of the olfactory and schizocortex could plausibly be attributed to the denervation caused by olfactory lobe reduction. The reduction of volume in the hippocampus, which gets only a minor olfactory projection compared to other sources of input, is suspiciously high for an explanation based on denervation.

Linkages of Evolution and Development in Brain Morphology

We offer one possible developmental source for the overall patterns of covariation we see in brain component structure. We hypothesize that the expansion and contraction of the domains of regulatory gene expression associated with prosomeres is a likely source of such structure (fig. 8). Particularly in the case of primates where the isocortex is enlarged and the limbic system reduced, the arrangement of neural components by prosomeres suggests this possibility. Within the alar portion of the secondary prosencephalon, its caudal component gives rise to hippocampus and amygdala; its middle component generates isocortex and basal ganglia; and its rostral component becomes the olfactory bulb, nucleus accumbens and the septum [Rubenstein et al., 1994; Puelles and Rubenstein, 2003]. Expansion of the alar domain of the middle portion could be the single genetic change accounting for cortex expansion/limbic system diminution in primates or the reverse pattern in Xenarthra. The approach of linking domains for expression of transcription factors with linked aspects of morphological change has been used successfully to understand the structure of cranial evolution in birds [Nemeschkal, 1999], and is a logical next step for describing morphological aspects of mammalian brain evolution.

Secondary epigenetic effects might further modify the original genetic changes. Olfactory nerve axons and arbors directly contribute their volume to terminal structures such as the olfactory cortex and the olfactory-recipient components of the amygdala, and also have direct trophic effects on postsynaptic cell survival and process elaboration in their target structures during development. Although this almost certainly accounts for significant reductions in the size of olfactory cortex and hippocampus and several of the more minor reductions in subicular cortex and amygdala, the near-50% loss of volume of the hippocampus in pinnipeds and the manatee seems very unlikely to be due to olfactory denervation alone, the reason we appeal to the segmental structure of the prosomeres.

Genomic and Functional Interactions in Brain Evolution

Evolution, when selecting on brain components, must be selecting on function, mediated strongly by the metabolic cost (or any other contingent cost) of the behavioral function. The developmental variation offered to selection – domains of expression of Hox genes, relative timing of receptor molecule generation and the like – are very rarely likely to be precisely isomorphic with these behavioral functions. The pattern of changes in brain components we see over phylogeny is likely the best compromise between particular behavioral demands and available developmental variation. The pattern of covariation in brain structures gives us evidence about what might constitute 'units of development.'

That there is a unit 'the limbic system' is a hypothesis that finds strong, although imperfect support in developmental, anatomical and physiological domains, despite its diverse behavioral functions [Reep, 1984; Squire, 1992; LeDoux, 2000]. Anatomically, a number of telencephalic structures, although spatially distributed, interconnect richly with each other including the olfactory bulbs and cortex, entorhinal, subicular and cingulate cortices, the hippocampus and the amygdala and septum, most marked with the LAMP factor described earlier. However, the notion that the limbic system is the 'reptilian brain' that has been overwritten by the mammalian cortex finds little current support, as the listed structures covary with respect to the rest of the brain between vertebrate taxa and within mammals. Functionally, the limbic system is often loosely equated with visceral, emotional and motivational systems. However, several of its components seem neither visceral nor emotional; for example, the analytical functions of the olfactory system, or the role of the hippocampus in spatial navigation and memory. It is clearly multifunctional: the olfactory system (in processing both social and environmental stimuli), the hippocampus (with respect to memory and spatial navigation) [Squire, 1992] and the general assignment of affect to cognition all depend on the limbic system [LeDoux, 2000].

The linkage of limbic system components in the segmental structure of the forebrain might force some unsuspected pleiotropic effect on behavioral evolution, and might account for the fact that the limbic system acts statistically as a single structure despite the diversity of its behavioral capacities. To select on one component is, in part, to select on all. For example, consider the case of the high covariation of the olfactory bulb and the hippocampus (fig. 4). It would be interesting, for example, to determine if a reduction of the olfactory system in marine mammals requiring a secondary diminution of the hippocampus has any consequences for either the accuracy of their spatial navigation or the neural systems employed to execute spatial navigation. The isocortex and limbic system show evidence of both independence and linkage (fig. 5). Because all quadrants are filled in the high/low limbic/isocortex matrix, it is clear that there is no necessary embryonic linkage between the two. It is possible to have both a relatively large limbic system and isocortex, either or neither. However, the push-pull axis between isocortex and limbic system does dominate the data, with most species falling into the high limbic/low isocortex and low limbic/high isocortex categories. We might understand this as two fully independent components of the brain offered as possible loci of selection, each offering a range of pleiotropic effects. Both are expensive to maintain, and if an animal is able to successfully execute its functions emphasizing one mode or the other, it will be energetically beneficial to do so. 'Success' will be a complex calculation of the types of functions dependent on the limbic complex, the isocortex, and their dependence on size. Perhaps carnivores, under pressure for both good olfactory discrimination and good spatial navigation, might not sacrifice either brain component [Gittleman, 1991, 1995]. This solution could be metabolically affordable due to a very high quality diet. In contrast, aerial hawking microchiropteran bats might exhibit reductions in both components due to extreme selection pressure for light weight to aid flight dynamics. This factor seems also to drive a reduction in overall brain size in this group [Eisenberg and Wilson, 1978; Ratcliffe et al., 2006]. This hypothesis is supported by the fact that the relatively large-bodied and slow flying megachiropterans (pteropids), most of which do not use echolocation, lie along the main regression line in figure 5.

We can perhaps understand the evolution of the primate brain in a different light by focusing on the tradeoffs between two factors. The reduction in the primate limbic system has been attributed to a reduction in the olfactory system because of the primate dependence on vision. However, the reduction in the limbic system could be secondary to selection for expansion of the isocortical system for improvement in long term memory. Primates are large-brained and long-lived as a group compared to their ancestors and the isocortex is the critical structure for the storage of long term memories [Allman et al., 1998]. Short-term consolidation processes dependent on the hippocampus need not necessarily change – the information acquired in a single day might have no relationship to whether the lifespan is two years or twenty. So it is quite

possible to reverse the typical causal scenario given for the relative sizes of primate brain parts; perhaps the reduction of the olfactory system in primates and humans is the unfortunate but tolerable result of selection to increase the size of the isocortex by reassigning stem neurons from olfactory bulb and hippocampus to the isocortex. Our sense of smell could be the casualty of our requirements for longevity.

Acknowledgements

Jeremy Yost helped with the phylogenetic classification used for the independent contrasts analysis. We thank Gabe Trinity for help constructing the figures, and Nicholas Nicastro for his critical reading of the manuscript. Thanks to Jack Johnson at Michigan State University, Wally Welker at the University of Wisconsin, and Archie Fobbs at the National Museum of Health and Medicine for encouragement, hospitality, and access to specimens. Supported by NSF grant IBN-9120450 to R.L.R., NSF grant IBN-0138113 to B.L.F., and The Maxwell Fund. University of Florida College of Veterinary Medicine Journal Series No. 630.

References

Allman J, Rosin A, Kumar R, Hasenstaub A (1998) Parenting and survival in anthropoid primates: Caretakers live longer. Proc Natl Acad Sci USA 95:6866–6869.

Baron G, Stephan H, Frahm HD (1996) Comparative Neurobiology in Chiroptera (3 vols.). Basel: Birkhäuser Verlag.

Barton RA (2001) The coordinated structure of mosaic brain evolution. Behav Brain Sci 24: 281–282.

Barton RA, Harvey PH (2000) Mosaic evolution of brain structure in mammals. Nature 405: 1055–1058.

Barton RA, Aggleton JP, Grenyer R (2003) Evolutionary coherence of the mammalian amygdala. Proc R Soc Lond B 270:539–543.

Clancy B, Darlington RB, Finlay BL (2000) The course of human events: Predicting the timing of primate neural development. Devel Sci 3:57–66.

Clancy B, Darlington RB, Finlay BL (2001) Translating developmental time across mammalian species. Neurosci 105:7–17.

Clark DA, Mitra PP, Wang SS-H (2001) Scalable architecture in mammalian brains. Nature 411:189–193.

Cooper HM, Herbin M, Nevo E (1993) Visual system of a naturally microphthalmic mammal – the blind mole rat, *Spalax ehrenbergi*. J Comp Neurol 328:313–350.

de Winter W, Oxnard CE (2001) Evolutionary radiations and convergences in the structural organization of mammalian brains. Nature 409:710–714.

Deutsch JC (1992) Olfactory cues influence female choice in two lek-breeding antelopes. Nature 356:596–598.

Eisenberg JF (1981) The Mammalian Radiations. Chicago IL: The University of Chicago Press.

Eisenberg JF, Wilson DE (1978) Relative brain size and feeding strategies in the Chiroptera. Evolution 32:740–751.

Finlay BL, Darlington RB (1995) Linked regularities in the development and evolution of mammalian brains. Science 268:1578–1584.

Finlay BL, Hersman MN, Darlington RB (1998) Patterns of vertebrate neurogenesis and the paths of vertebrate evolution. Brain Behav Evol 52:232–242.

Finlay BL, Darlington RB, Nicastro N (2001) Developmental structure in brain evolution. Behav Brain Sci 24:263–308.

Gittleman JL (1991) Carnivore olfactory bulb size – allometry, phylogeny and ecology. J Zool Lond 225:253–272.

Gittleman JL (1995) Carnivore brain size, behavioural ecology and phylogeny. J Mammal 67: 3–36.

Gould SJ (1975) Allometry in primates, with emphasis on scaling and the evolution of the brain. Cont Primatol 5:244–292.

Hart BL (1983) Flehmen behavior and vomeronasal organ function. In: Chemical Signals in Vertebrates III (Silverstein RM, Muller-Schwarze D, eds), pp 87–103. New York: Plenum Press.

Jerison HJ (1973) Evolution of the Brain and Intelligence. New York: Academic Press.

LeDoux JE (2000) Emotion circuits in the brain. Ann Rev Neurosci 23:155–184.

Levitt P (1984) A monoclonal antibody to limbic system neurons. Science 223:299–301.

Levitt P, Barbe MF, Eagleson KL (1997) Patterning and specification of the cerebral cortex. Ann Rev Neurosci 20:1–24.

Nemeschkal HL (1999) Morphometric correlation patterns of adult birds (Fringillidae: passeriformes and columbiformes) mirror the expression of developmental control genes. Evolution 53:899–918.

Price T (1997) Correlated evolution and independent contrasts. Phil Trans R Soc Lond B 352:519–529.

Puelles L, Rubenstein LR (2003) Forebrain gene expression domains and the evolving prosomeric model. Trends Neurosci 26:469–476.

Ratcliffe JM, Fenton MB, Shettleworth SJ (2006) Behavioral flexibility positively correlated with relative brain volume in predatory bats. Brain Behav Evol 67:165–176.

Redford KH (1985) Feeding and food preference in captive and wild giant anteaters (Myrmecophaga-tridactyla). J Zool 205:559–572.

Reep RL (1984) The relationship between prefrontal and limbic cortex – a comparative anatomical review. Brain Behav Evol 25:5–80.

Reep RL, Johnson JI, Switzer RC, Welker WI (1989) Manatee cerebral cortex: cytoarchitecture of the frontal region in *Trichechus manatus latirostris*. Brain Behav Evol 34: 365–386.

Rilling JK, Insel TR (1998) Evolution of the cerebellum in primates: Differences in relative volume among monkeys, apes and humans. Brain Behav Evol 52:308–314.

Rubenstein JLR, Martinez S, Shimamura K, Puelles L (1994) The embryonic vertebrate forebrain: The prosomeric model. Science 266: 578–580.

Squire L (1992) Memory and the hippocampus – a synthesis from findings with rats, monkeys and humans. Psych Review 99: 195–231.

Stephan H, Frahm H, Baron G (1981) New and revised data on volumes of brain structures in insectivores and primates. Folia primatol 35:1–29.

Uylings HBM, van Eden CG, Hofman MA (1986) Morphometry of size/volume variables and comparison of their bivariate relations in the nervous system under different conditions. J Neurosci Meth 18:19–37.

Welker WI, Johnson JI, Noe A (2006) Comparative Mammalian brain collections. Worldwide web: http://brainmuseum.org/index.html

Wilson DE, Reeder DM (1993) Mammal Species of the World: a Taxonomic and Geographical Reference, 2nd Edition. Washington, D.C.: Smithsonian Institution Press.