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The Scaling of White Matter to Gray Matter in Cerebellum and Neocortex

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Mammals · White matter · Cerebellum · Neocortex · Scaling

Abstract
It is known that the white matter of neocortex increases disproportionately with brain size. However, relatively few measurements have been made of white matter/gray matter scaling in the cerebellum. We present data on the volumes of white and gray matter in both structures, taken from 45 species of mammals. We find a scaling exponent of 1.13 for cerebellum and 1.28 for neocortex. The 95% confidence intervals for our estimates of these two exponents do not overlap. This difference likely reflects differences in the connectivity and/or microstructure of white matter in the two regions.

Introduction

The neocortex and the cerebellum are the two most prominent laminar structures in the mammalian brain. They are alike in possessing an underlying layer of white matter, but they differ in the constituents which make up that white matter. In the neocortex, much of the white matter is thought to consist of axons projecting between neocortical regions. In contrast, in the cerebellum there are thought to be no cortico-cortical projections [Braitenberg et al., 1997]. Mossy fibers projecting from other parts of the brain probably make up a significant proportion of cerebellar white matter. Ramón y Cajal [1888] observed that they predominate in the granule layer, and the same is likely true in the white matter. Thus there is reason to suspect that white matter follows different scaling principles in the two structures.

Previous measurements of white matter/gray matter scaling in the neocortex have been in broad agreement that white matter increases disproportionately with brain size [Schlenska, 1974; Frahm et al., 1982; Rilling and Insel, 1999; Zhang and Sejnowski, 2000]. This has frequently been interpreted as a consequence of the high degree of interconnection in the neocortex [Frahm et al., 1982; Allman, 1999; Zhang and Sejnowski, 2000], though it has also been argued to be due to increases in axon diameter [Changizi, 2001].

There are far fewer published data on white and gray matter volume in the cerebellum. Sultan [2002], citing measurements in human and rat, suggested that the proportion of white matter in the cerebellum is almost constant [Andersen et al., 1992; Korbo et al., 1993].

To test this proposition, we have measured cerebellar white and gray matter volume in a larger set of mammals. For purposes of comparison, we also measured neocortical values in the same group.

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Table 1. White matter and gray matter volumes (cm^3) for neocortex and cerebellum for 45 mammals

Group	Species	Cer White	Cer Gray	Neo White	Neo Gray
Apes	*Hylobates lar*	2.93	12.8	21.9	45.1
	Pan troglodytes	8.63	36.1	104	163
Old World Monkeys	*Cercocebus torquatus*	1.96	4.47	40.4	47.9
	Cercopithecus nictitans	0.965	3.84	11.8	36.0
	Macaca mulatta	1.38	3.68	16.4	30.5
	Mandrillus sphinx	1.89	7.16	27.9	71.3
	Papio hamadryas	2.98	9.14	37.1	71.4
	Semnopithecus entellus	2.27	9.15	24.3	51.8
New World Monkeys	*Alouatta palliata*	1.05	3.32	11.6	17.4
	Aotus trivirgatus	0.174	0.753	1.49	4.32
	Ateles sp.	1.68	5.97	17.7	28.9
	Callicebus sp.	0.249	0.833	1.94	5.12
	Saimiri sciureus	0.393	1.49	3.70	10.4
Tarsier	*Tarsius syricta*	0.0473	0.247	0.151	1.47
Strepsirrhines	*Eulemur mongoz*	0.486	1.88	3.83	8.23
	Galago senegalensis	0.0634	0.354	0.195	1.20
	Lemur catta	0.409	2.27	2.12	8.96
	Microcebus urinus	0.0331	0.172	0.0976	0.591
	Nycticebus coucang	0.196	1.20	1.07	4.40
	Otolemur crassicaudatus	0.127	0.747	0.572	3.10
	Perodicticus potto	0.183	1.14	0.638	3.91
Artiodactyls	*Odocoileus virginianus*	4.16	11.1	24.4	52.0
	Tayassu tajacu	1.25	4.17	6.96	18.4
Marsupials	*Marmosa mitis*	0.0102	0.0871	0.0113	0.119
	Didelphis marsupialis	0.16	0.67	0.151	0.939
Xenarthra	*Myrmecophaga tridactyla*	3.19	9.02	8.58	19.9
	Tamandua tatradactyla	0.678	2.98	2.16	7.25
	Choloepus didactylis	0.682	2.30	2.63	7.91
Hyrax	*Procavia capensis*	0.31	1.12	1.33	4.21
Zebra	*Equus burchelli*	9.6	27.4	88.4	149
Rodents	*Applodontia rufa*	0.137	0.665	0.376	1.48
	Chinchilla laniger	0.173	0.792	0.269	1.16
	Myoxus glis	0.0481	0.245	0.0678	0.49
	Rattus norvegicus	0.0287	0.183	0.0534	0.529
	Sciurus carolinensis	0.184	0.904	0.44	2.48
Carnivores	*Ailurus fulgens*	1.10	3.97	6.25	13.8
	Canis latrans	1.34	4.17	15.5	30.4
	Crocuta crocuta	3.12	8.34	28.9	56.1
	Galictis vitatus	0.324	1.44	2.72	9.09
	Mustela putorius	0.122	0.626	0.569	2.14
	Mustela vison	0.217	0.859	1.21	4.75
	Nasua narica	0.794	2.94	4.80	12.1
	Potos flavus	1.07	4.32	6.16	13.2
	Vulpes zerda	0.271	1.21	1.53	5.73
	Zalophus californianus	13.6	67.1	88.7	213

Materials and Methods

We analyzed 45 mammalian species from 8 orders. These included 21 Primates, 10 Carnivores, 5 Rodents, 3 Xenarthra, 2 Artiodactyls, 2 Marsupials, as well as a Perissodactyl and a Hyrax. All brains were prepared at the Laboratory of Neurophysiology at the University of Wisconsin Madison and kept in the Comparative Mammalian Brain collection there. All were embedded in celloidin, sectioned exhaustively, and stained with thionin. For more details see for example Campos and Welker [1976].

For each brain we took a systematic random sample of 40 or more slices. We scanned these on a standard office flatbed scanner (Epson Expression 800) at 800 dpi. We then roughly aligned the resulting images to make the measurements easier.

We used a combination of semi-automatic and manual image segmentation tools in the Amira software package to segment the images. We calculated the coefficient of error (CE) of our measurements using the method of Gundersen et al. [1999]. For the measurements presented here the largest CE was 0.019.

Scaling coefficients were calculated by log transforming volume data and then calculating the slope of the major axis of log white matter on log gray matter. We established 95% confidence intervals for these slopes using the method of Jolicoeur [1968].

Tissue shrinkage resulting from celloidin embedding has two effects on volume measurements of the type we are making. First, the overall size of the brain may decrease substantially. Second, white matter and gray matter may shrink differently.

To correct for the effect of shrinkage on the overall size of the brains, we used pictures which were taken of the brains before sectioning. These were done from standard views at standard distances and always included a ruler. By comparing various measurements on these pictures with our scanned images, we were able to make estimates of slice dimensions before celloidin embedding.

This technique makes a gross correction for overall shrinkage, but still leaves a second problem. Gray and white matter shrink differently in celloidin. Note that the same is true of paraffin embedding techniques [Kretschmann et al., 1982].

Because we are interested in measuring a scaling exponent, differential shrinkage would not be a problem if it was consistent in different brains. That is, if the amount of shrinkage in gray and white matter differed by a fixed proportion, this would change the intercept but not the slope of the regression line of the log transformed data. If, however, the ratio of shrinkage in the two tissues varied systematically with brain size, then this would introduce a bias into estimates of the scaling exponent.

To address this problem, we examined two celloidin shrinkage studies from the University of Wisconsin brain collection. In these studies, one hemisphere of a brain had been cut frozen and the other embedded in celloidin. Neither study included a cerebellum. Both brains were in the 10–15 cm^3 range. In the beaver brain we found that neocortical gray matter shrank to 39% of its original volume, and white matter to 38%. In the capybara brain, gray matter shrank 34%, and white matter 41%. Earlier examination of a larger number of such studies did not reveal any clear or simple dependence of shrinkage on other factors [W. Welker pers comm.]

We do not believe that shrinkage ratios in these brains vary systematically with brain size. However, as this factor could bias our result, we have considered what effect it might have on the estimated slopes. The values above come from neocortex, but they also probably give a general indication for the possible range of shrinkage in the cerebellum. We took two hypothetical data points from around the minimum and maximum values for our data set. We then 'shrank' the white and gray matter to either 30% or 40%, to systematically bias the result with size. We found that this could bias the resulting slope by up to 0.09.

Results

Total brain size in our sample varies from just under 1 to over 450 cm^3. We present our volume measurements for neocortex and cerebellum in table 1. We find that white matter/gray matter scaling differs significantly in cerebellum vs. neocortex. As can be seen in figure 1, a large neocortex has proportionally more white matter than does a large cerebellum.

This can also be seen in the slopes of the major axis regression lines. Table 2 gives these slopes for white matter and gray matter in cerebellum and neocortex. It includes values for the whole sample, and also for various

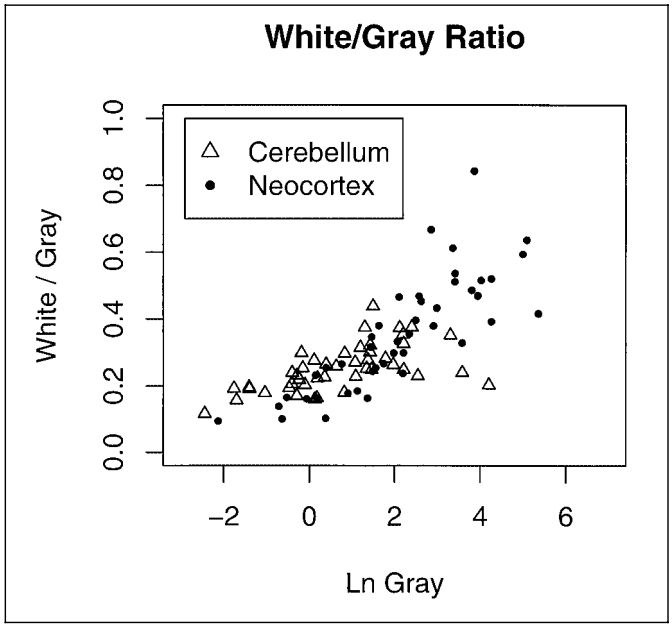

Fig. 1. The ratio of white matter to gray matter, plotted against ln gray matter for both neocortex and cerebellum.

Table 2. Major axis regression slopes and 95% confidence intervals for the whole sample of mammals, and for various subgroups of it

	Slope	95% CI	Sample size
Cerebellum			
All mammals	1.13	1.08–1.18	45
Primates	1.11	1.03–1.20	21
Carnivores	1.04	0.92–1.17	10
All non-primates	1.14	1.08–1.19	24
Neocortex			
All mammals	1.28	1.24–1.33	45
Primates	1.33	1.23–1.43	21
Carnivores	1.17	1.05–1.30	10
All non-primates	1.26	1.21–1.31	24

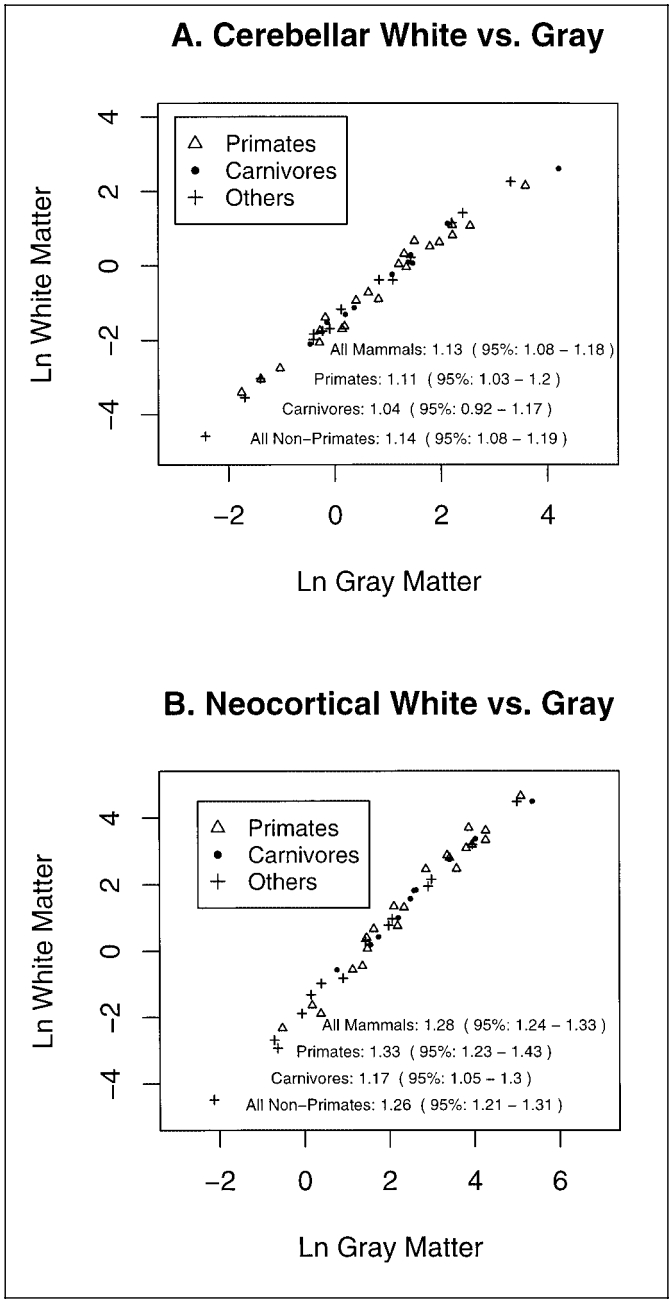

Fig. 2. A A plot of ln white matter vs. ln gray matter in the cerebellum, including various groups from our sample. Included are the major axis regression slopes, and the 95% confidence intervals for those slopes. B The same plot as A, but for neocortex.

subgroups. Figure 2 shows the separate groups plotted on logarithmic axes. Note that the 95% confidence interval for the cerebellum slope of the whole sample does not overlap with that for the neocortex slope. The cerebellum range is 1.08 to 1.18. The neocortex range is 1.24 to 1.33.

We also find that in the cerebellum, white matter scales with gray matter at a ratio significantly greater than 1:1. That is, a larger cerebellum tends to have proportionally more white matter than a small one. The general positive trend can be seen in figure 1, and is confirmed by the fact that the 95% confidence interval for the scaling exponent does not include zero.

Discussion

Our values for neocortex are consistent with other published data. Using data from H. Stephan s group, we calculated the neocortical major axis slope of 1.25 for a number of primates and insectivores [Frahm et al., 1982; Stephan et al., 1991]. If we calculate an exponent just for the 13 anthropoid primates in our sample, it is 1.16, close to Rilling and Insel's value of 1.12, which was calculated for 11 anthropoid species [Rilling and Insel, 1999].

The magnitude of the difference between cerebellum and neocortex is such that shrinkage bias with brain size seems an unlikely explanation. Based on our estimate above, if shrinkage was biased with brain size, it could affect the exponent at most by 0.09. For that to explain our data, neocortex and cerebellum must to have been affected in opposite directions – an unlikely eventuality. It seems similarly unlikely that shrinkage bias could explain our observation that the proportion of cerebellar white matter increases with brain size.

It has been suggested that the hyperscaling of neocortical white matter is due to increases in axon diameter with brain size [Changizi, 2001]. There are several recent studies on axon diameter in the corpus callosum. Those studies found that average axon diameter does scale up with brain size. Several studies found that the diameter of the largest fibers increases with brain size, but that other populations of fibers do not seem to vary [Schuz and Preissl, 1996; Olivares et al., 2001]. Another study found an increase in fiber diameter across all populations of callosal fibers [S. Wang, pers. comm.]. If such increases also exist in neocortical white matter outside the corpus callosum, they could explain the neocortical scaling exponent.

The comparative data on axon diameter in cerebellar white matter is even more sparse. Published ranges for the diameter of mossy fiber stem axons in the cat and the rat do not show increases related to brain size [Shinoda et al., 1992; Wu et al., 1999]. However this sample is too small to draw firm conclusions.

Considering this, we can see two possible interpretations of the difference between neocortical and cerebellar

gray/white scaling exponents. One possibility is that axon diameter scales similarly in neocortex and cerebellum. In this case differences in white matter scaling exponent would reflect differences in connectivity. The comparatively lower cerebellar value could result from the lack of long range white matter connections in cerebellar cortex [Sultan, 2002]. Such connections in neocortex might become more numerous as brains become larger, reflecting an increase in the absolute number of connections per neuron. The lower cerebellar value could also reflect numerical changes in the mossy fiber projection onto granule cells. It is possible that in larger brains, a single mossy fiber projects to significantly fewer granule cells.

Another possibility is that axon diameter in fact scales differently in the two structures, and this difference explains the differences in white matter scaling. If this were true, it might reflect different selective forces acting on conduction velocity in neocortical or cerebellar axons.

Distinguishing between these explanations will require new comparative data, particularly measurements of axon diameter and the proportions of different fiber types in cerebellar white matter.

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References

Allman, J. (1999) Evolving Brains. Scientific American Books, New York.

Andersen, B., L. Korbo, and B. Pakkenberg (1992) A quantitative study of the human cerebellum with unbiased stereological techniques. J. Comp. Neurol., *326:* 549–560.

Braitenberg, V., D. Heck, and F. Sultan (1997) The detection and generation of sequences as a key to cerebellar function: Experiments and theory. Behav. Brain Sci., *20:* 229–77.

Campos, G., and W. Welker (1976) Comparisons between brains of a large and a small hystricomorph rodent – capybara, *Hydrochoerus* and guinea-pig, *Cavia* – neocortical projection regions and measurements of brain subdivisions. Brain Behav. Evol., *13:* 243–266.

Changizi, M. (2001) Principles underlying mammalian neocortical scaling. Biol. Cyber., *84:* 207–215.

Frahm, H. D., H. Stephan, and M. Stephan (1982) Comparison of brain structure volumes in Insectivora and Primates. I. neocortex. J. Hirnforsch., *23:* 375–389.

Gundersen, H., E. Jensen, K. Kieu, and J. Nielsen (1999) The efficiency of systematic sampling in stereology reconsidered. J. Microsc., *193:* 199–211.

Jolicoeur, P. (1968) Interval estimation of the slope of the major axis of a bivariate normal distribution in the case of a small sample. Biometrics, *24:* 679–682.

Korbo, L., B. Andersen, O. Ladefoged, and A. Moller (1993) Total numbers of various cell types in rat cerebellar cortex estimated using an unbiased stereological method. Brain Res., *609:* 262–268.

Kretschmann, H. J., U. Tafesse, and A. Herrmann (1982) Different volume changes of cerebral cortex and white matter during histological preparation. Microsc. Acta, *86:* 13–24.

Olivares, R., J. Montiel, and F. Aboitiz (2001) Species differences and similarities in the fine structure of the mammalian corpus callosum. Brain Behav. Evol., *57:* 98–105.

Ramon y Cajal, S. (1888) Estructura de los centros nerviosos de las aves. Rev. Trimestr. Histol. *1:* 1–10.

Rilling, J. K., and T.R. Insel (1999) The primate neocortex in comparative perspective using magnetic resonance imaging. J. Hum. Evol. *37:* 191–223.

Schlenska, G. (1974) Volumen und Oberflächenmessungen an Gehirnen verschiedener Säugetiere im Vergleich zu einem errechneten Modell. J. Hirnforsch., *15:* 401–408.

Schuz, A., and H. Preissl (1996) Basic connectivity of the cerebral cortex and some considerations on the corpus callosum. Neurosci. Biobehav. Rev., *20:* 567–570.

Shinoda, Y., Y. Sugiuchi, T. Futami, and R. Izawa (1992) Axon collaterals of mossy fibers from the pontine nucleus in the cerebellar dentate nucleus. J. Neurophysiol. *67:* 547–560.

Stephan, H., G. Baron, and H. Frahm (1991) Insectivora. Springer-Verlag, New York.

Sultan, F. (2002) Brain evolution – analysis of mammalian brain architecture. Nature, *415:* 133–134.

Wu, H., I. Sugihara, and Y. Shinoda (1999) Projection patterns of single mossy fibers originating from the lateral reticular nucleus in the rat cerebellar cortex and nuclei. J. Comp. Neurol., *411:* 97–118.

Zhang, K., and T. Sejnowski (2000) A universal scaling law between gray matter and white matter of cerebral cortex. Proc. Nat. Acad. Sci. (USA) *97:* 5621–5626.