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A Further Evaluation of the Effect of Age on Striate Cortex of the Rhesus Monkey

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PETERS, A., N. J. NIGRO AND K. J. McNALLY. *A further evaluation of the effect of age on striate cortex of the rhesus monkey.* NEUROBIOL AGING **18**(1) 29–36, 1997.—The brains of 14 rhesus monkeys (*Macaca mulatta*) between 4 and 35 years old were examined to determine the effects of aging on the thickness, neuronal frequency, fine structure, surface area, and volume of striate cortex. The effects of aging were ascertained by comparing the striate cortex in the six monkeys between 4 and 12 years of age with that of the eight monkeys over 25 years of age. The brains of the monkeys were all fixed by vascular perfusion and except for one of the old monkeys, whose age was estimated, the exact ages of all of the monkeys are known. One micron thick sections of plastic embedded cortex from one hemisphere of each monkey were examined by light microscopy to determine the thickness of the striate cortex, and neuronal frequency was determined by counting the numbers of neurons displaying nuclei in 250 μm-wide strips passing through the thickness of the cortex. When young monkeys were compared with the old ones, no differences were found in either the thickness of the cortex or in the numbers of neuronal profiles beneath unit areas of cortical surface. This suggests that neurons are not lost with age, and when the cortices were examined by electron microscopy there was no indication that the cell bodies of neurons are degenerating, except possibly in layer I. Serial, 30 μm-thick, Nissl stained frozen sections from the other hemisphere of each monkey were used to determine both the surface area and the volume of the striate cortex. Overall, the surface area varied between 702 and 1480 mm^2, with a mean value of 956 mm^2, but there was no indication that the surface area decreased with age, and the same is true for the volume of striate cortex. The conclusion is that while there is a large variation in the amount of cortex occupied by area 17, there is no indication that its thickness, volume, or number of neurons is altered by age. *Copyright © 1996 Elsevier Science Inc.*

Macaque Area 17 Aging Neurons Cortical volume

THE first[1] reports that neurons are lost from the cerebral cortex during normal aging were based upon examination of various regions of the cerebral cortices of human subjects between 18 and 95 years of age (5,6). On the basis of cell counts, a progressive decrease of some 50% in the neuronal cell density with age was described in the precentral gyrus and superior temporal gyrus. The losses from the visual cortex and precentral gyrus were between 20 and 30%, but no significant loss of neurons was detected from the inferior temporal gyrus and postcentral gyrus. Other investigators (1,8,10,18,32) have also reported similar losses of neurons from the human cerebral cortex with age (7). But there is another group of generally more recent investigators (9,15–17,21,22,36) who report that any cortical neuronal loss from the normally aging human cerebral cortex is either not significant, or much less than had been reported earlier.

Consequently, it would appear that the common belief that there is extensive loss of neurons from the aging human cerebral cortex is at least questionable and as has been pointed out (13), studies on even the same region of the cortex by different investigators have produced conflicting results. One source of these conflicts is the difference in the methods used to determine neuronal density, because it is on the basis of comparing neuronal densities in young and old brains that conclusions about neuronal losses with age have been based. It is to be hoped that some of the problems inherent in making comparative neuronal counts can be solved by the use of the more modern stereological techniques such as the optical disector (41). However, in the case of human material, there are a number of other factors that can seriously affect the results obtained. For example, the time that has elapsed between death and the fixation of the tissue can produce differences in the shrinkage or swelling of the tissue, and in tissue that is not optimally preserved it can be difficult to differentiate between small neurons and neuroglial cells (12).

As with the human studies, the first accounts of the effects of aging on the cortex of the rhesus monkey also indicated that there is some loss of neurons with age. In the sensorimotor cortex in young and old monkeys (2–4) no age related decrease in the thickness of the sensorimotor cortex was found, and the mean packing density of neurons over the total cortical depth was not significantly different in the young and old monkeys. However, in area 3 a decrease in the packing density of neurons was noted at a cortical depth characterized by the presence of small granular neurons, presumably layer IV, leading to the conclusion that this represented an age related neuronal loss. Subsequently, the gyri bordering the principal sulcus in the frontal lobe were examined (4) and it was concluded that neurons are also lost from this part of cortex with age. From an analysis of the data it has been estimated that the recorded neuronal loss was as much as 20% (7).

These studies (2–4) appear to be the only ones that have examined the effect of aging on the rhesus monkey cerebral cortex

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before we began our own investigations. In our studies, we have compared the cortices of young (4 to 12 years of age) monkeys with those of old monkeys that are generally over 25 years of age. We consider monkeys over 25 years of age to be old, because in an analysis of the longevity of rhesus monkeys housed at Yerkes Regional Research Primate Center we have found that only 25% of monkeys survive to 25 years of age (37). All of the brains that we have examined have been fixed by vascular perfusion. Consequently, they are well fixed and there is no problem with tissue preservation. In our studies of striate cortex (40) and of frontal cortex (29) we have used 1 μm-thick plastic sections passing through the entire depth of the cortex. In such sections it is easy to distinguish between neurons and neuroglial cells and so avoid one of the problems that may have confounded some of the earlier analyses of the effects of aging that used Nissl stained preparations. In these 1 μm-thick sections, counts were made of the numbers of neuronal profiles displaying nuclei in strips passing through the depth of the gray matter. Because the mean diameters of the profiles of the counting objects, the neuronal nuclei, do not change with age in either area 17 of the visual cortex (40) or in area 46 of the frontal cortex (29), these counts are a direct reflection of the numbers of neurons beneath a unit area of cortical surface. Consequently, a comparison of the counts obtained from sections through the cortices of young and old monkeys reveals if there is a loss of neurons with age, and this is irrespective of whether there is a decrease in the thickness of the cortex. We have concluded that there is no significant loss of neurons with age from either area 17 of visual cortex (40), including the population of deep Meynert cells (28), or from area 46 of frontal cortex (29).

In other studies of these same monkeys, area 4 of motor cortex has been examined using frozen sections, and again, there was no evidence of neuronal loss with age from either the total population or from the population of Betz cells (38).

In each case the cortices have been further examined by electron microscopy and except for some neurons in layer I, there has been no evidence that neuronal cell bodies are undergoing degeneration. In layer I some of the cell bodies of neurons in the older monkeys show varying degrees of vacuolation in the cytoplasm of their cell bodies.

To date, our studies have only addressed the question of whether there is a loss of neurons beneath a given area of cortical surface with age. We have now become concerned about whether there is a change with age in the amount of cortex devoted to a given function, because even if the numbers of neurons beneath a unit area of cortical surface remain unchanged, there would be a loss of neurons if there is a decrease in the cortical volume with age. To examine this problem we have carried out a further investigation of the effects of age on the striate cortex of the rhesus monkey using a larger number of monkeys than were included in our previous report (40). We have chosen to examine striate cortex because it has a very characteristic appearance in Nissl stained preparations. This makes it easy to determine the amount of surface that the primary visual cortex occupies in the occipital lobe, as well as the total volume of the striate cortex present in individual young and old monkeys. We have not attempted to determine the total numbers of neurons in the striate cortex of these monkeys, because to obtain accurate information would entail obtaining separate neuronal density values for each cortical layer. In striate cortex, in which there are four sublayers of layer IV, this would be extremely time consuming and would add little to the main point of this investigation, which is focussed upon the possible changes with age in the surface area and volume of striate cortex.

It should be pointed out that although there appears to be no loss of neurons with age from the primary visual cortex of primates, there are other effects. For example, it has been shown that with age there is a loss of myelinated fibers from the stripe of Gennari in the human cortex (20), and in a recent review (33) the point is made that with normal aging there are a number of changes in vision that cannot be solely explained by changes in the optics of the system, and must, therefore, be due to alterations in the retina or the central visual pathway. These include changes in visual acuity and contrast sensitivity, superthreshold contrast vision and contrast gain, temporal-frequency contrast sensitivity and resolution, spatial-temporal interactions, hyperacuity, binocular processing, and sensitivity to motion, but at present the bases for these age changes are not known. In Alzheimer s disease, of course, the effects of age on vision can be even more acute, perhaps due to the formation of senile plaques in area 17 and the formation of neurofibrillary tangles in pyramidal cells in the deep cortical layers (24).

METHOD

Animals

The brains of 14 rhesus monkeys (*Macaca mulatta*) between 4 and 35 years of age were used in the present study. As in our previous publications, the individual monkeys are identified by their AM code numbers, and the ages, sex, and body weights of the monkeys at death are given in Table 1. The ages are given to the nearest year, and except for one of the monkeys, AM 13, the exact date of birth of each animal is documented. AM 13 is estimated to be 35 years. Consequently, it is one of the oldest rhesus monkeys available for examination, because in an earlier study of the life span of the rhesus monkey (37) it was determined that only 6% of rhesus monkeys survive beyond 30 years of age.

Five of the monkeys for which data are recorded had been included in our previous study of the effects of aging on striate cortex (40), but no data on the striate cortex of the other nine monkeys has been previously published. All of the monkeys used in this study were either housed at Yerkes Regional Primate Research Center, or at Boston University Laboratory Animal Science Center, and cared for in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 86:23) The monkeys are part of a population used in a program project to determine the effect of normal aging on the brain of the rhesus monkey.

TABLE 1

AGE, SEX, AND BODY WEIGHTS OF MONKEYS

Animal	Age	Sex	Body Weight (kg)
AM-58	4	F	5.4
AM-7	5	M	5.9
AM-10	6	M	4.6
AM-47	9	M	13.0
AM-20	12	F	5.6
AM-42	12	M	13.0
AM-19	25	F	5.3
AM-12	27	F	7.5
AM-15	27	F	6.2
AM-11	27	F	8.2
AM-27	28	M	10.1
AM-41	32	F	9.5
AM-23	32	F	5.5
AM-13	35	M	6.7

Fixation

As described previously (29), the monkeys were anesthetized and the brains perfused through the ascending aorta with warm fixing solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer or in 0.1 M phosphate buffer at pH 7.4. Immediately after perfusion the brains were removed and bisected. One-half of the brain was immersed in a cryoprotectant solution of 10% glycerol and 2% DMSO in 0.1 M phosphate buffer, and then into 20% glycerol and 2% DMSO in the same buffer (31) and subsequently used to prepare a Nissl stained set of serial frozen sections cut in the frontal plane at a thickness of 30 μm. The other half was immersed in a stronger solution of aldehydes containing 2% paraformaldehyde and 2.5% glutaraldehyde in the same strength buffer. These half brains were stored in this stronger solution for at least 1 week before blocks were removed from the opercular surface of area 17.

The tissue blocks removed from the opercular surface of striate cortex were taken from a region at least 3 mm caudal to and parallel with the lunate sulcus, in the region where central vision is represented. These tissue blocks were osmicated, dehydrated in an ascending ethanol series, stained en bloc with uranyl acetate and embedded in Araldite.

Thickness of Area 17

The thickness of area 17 was determined from the camera lucida drawings. The distance between the outside of the glial limiting membrane and the border between layers VIa and VIb was measured at the middle of each of the 250 μm-wide strips. Layer VIb was not included in the depth because it is often difficult to exactly determine how far its neurons extend into the white matter. The cortical thickness was not measured in one monkey (AM 23), because in the hemisphere that was to be used for plastic embedding a diverticulum of the posterior horn of the lateral ventricle had cavitated the white matter beneath the opercular portion of the striate cortex, and it was not clear how this had affected the grey matter above it.

Surface Area

Determinations of the surface area and the volume of area 17 were made as follows. For each hemisphere, the Nissl stained series of 30 μm thick sections was examined to ascertain the approximate extent of the striate cortex. A section from about the middle of the series was then selected as an arbitrary starting point and tracings made along the outer surface of sections through the occipital cortex that contained the primary visual cortex. The tracings were made at × 6.5 using an Aus Jena Dokumentor, and the extent of striate cortex marked. Striate cortex is readily discerned because of the distinctive lamination of layer IV (26,40). Every tenth 30 μm-thick section was drawn so that the effective distance between the drawings used in the integration was 300 μm. These tracings were then retraced into a computer using a computer-aided-drawing (CAD) program (AUTOCAD version 11 for the Macintosh computer), with a photosensitive tablet and a light pen. This allowed determination of the lengths of the tracings of area 17 as it appeared in each section. By multiplying the lengths of the surface tracings by the distance separating a plane passing through the middles of the sections traced, i.e., 300 μm, the amount of surface of the cortex devoted to area 17 was estimated. For each monkey, the estimations of surface area in the present study were based on tracings taken from between 30 and 55 sections.

Using these same drawings the area occupied by the primary visual cortex within each section was also delineated. This was done by marking the border between area 17 and area 18, and then tracing the lower border of the primary visual cortex at the boundary between layer VI and the white matter. Using the AUTOCAD program the area of the primary visual cortex within each section was determined. Multiplying these areas by the distance separating the middles of the 30 to 55 sections used allowed the total volume of the primary visual cortex to be estimated with reasonable accuracy, because it has been shown that an estimate of volume based upon a total of even 10 sections or less through an object provides an error of less than 5% (14).

Neuronal Profile Counts

Neuronal profile counts were made in the manner given in detail in two previous publications (29,40). In brief, plastic embedded tissue blocks from the opercular surface of striate cortex were sectioned using a calibrated ultramicrotome to produce sections 1 μm thick, and oriented normal to the pial surface, as determined by the fact that the plane of sectioning passed parallel to the courses of the apical dendrites of the pyramidal cells. The sections were stained with toluidine blue and coverslipped. For each animal in which neuronal profile counts were made, two plastic blocks were sectioned, and from each block two individual sections, at least 10 μm apart were used to make the neuronal profile counts.

In such preparations it is easy to distinguish between the profiles of neurons and those of neuroglial cells, and using a 40× objective camera lucida drawings were made to indicate those profiles of neurons and neuroglial cells that displayed nuclei and were contained in a 250 μm wide strip of the 1 μm-thick section passing through the entire depth of the primary visual cortex. The boundaries between the neuronal layers of the visual cortex were then marked on the drawings and counts made of the numbers of neuronal profiles with nuclei in each layer in the 250 μm strip. For each animal the individual counts obtained from the four sections were then pooled to determine the average neuronal count (Table 3).

It was not possible to obtain neuronal profile counts for all of the monkeys in which the surface area and volume of the striate cortex were estimated, because in some cases the other hemisphere was not available for plastic embedding, due to the needs of other investigators on this project. Counts were made on only five of the young monkeys between 4 and 12 years of age, and five of the monkeys over 25 years of age (see Table 3). To ensure uniformity, all of the counts were done by one investigator (K.J.M.) and checked by another investigator (A.P.).

Electron Microscopy

Thin sections were taken from the same blocks that were used to make the neuronal profile counts. The thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100S electron microscope. The examination was carried out to ascertain what types of morphological changes are associated with aging, particularly changes that might suggest that neurons are dying or being lost with age.

RESULTS

Thickness of Striate Cortex

The thickness of area 17 as measured in sections of cortex taken from the opercular surface of the occipital lobe are given in Table 2. While the thickness of the cortex can vary between 1.26

TABLE 2

THICKNESS, SURFACE AREA, AND VOLUME OF STRIATE CORTEX

Animal	Age	Thickness (mm)	Surface Area (mm^2)	Volume (mm^3)	Gender
Young Monkeys					
AM-58	4	1.36	804	1523	F
AM-7	5	1.37	1014	2039	M
AM-10	6	1.62	746	1432	M
AM-47	9	1.63	1480	2503	M
AM-20	12	1.60	840	1876	F
AM-42	12	1.71	978	1773	M
Mean (±SD)		1.55(±13)	977(±243)	1858(±353)	
Old monkeys					
AM-19	25	1.26	943	1575	F
AM-12	27	1.48	938	1874	F
AM-15	27	1.48	1189	2440	F
AM-11	27	1.78	702	1190	F
AM-27	28	1.77	969	1967	M
AM-41	32	1.62	977	1625	F
AM-23	32		927	1578	F
AM-13	35	1.49	878	1704	M
Mean (±SD)		1.55(±17)	940(±124)	1744(±340)	
All monkeys					
Mean (±SD)		1.55(±15)	956(±186)	1792(±351)	

and 1.78 mm, the mean depth for the group of six young monkeys is 1.55 mm, and it is the same for the seven old monkeys. In Fig. 1 the mean values for the thickness of the cortex in individual animals is plotted against age, and as can be seen there is no correlation between cortical thickness and age. Thus, the conclusion is the same as the one we arrived at previously on the basis of our earlier study (40), namely that there is no indication that the striate cortex decreases in thickness with age.

Surface Area Occupied by Striate Cortex

The surface area of the entire striate cortex in each of the 14 monkeys examined is given in Table 2, and the values are plotted against age in Fig. 2. There is obviously a great diversity in the areal extent of the striate cortex among individual monkeys, because the measured surface areas vary from 702 to 1,480 mm^2. Thus, in some monkeys the striate cortex occupies twice the amount of surface than in others. For the 14 monkeys included in this study the mean value for the surface area of striate cortex is 956 (SD ± 186) mm^2. For the six young monkeys between 4 and 12 years of age the mean value is 977 (SD ± 243) mm^2 and for the eight monkeys over 25 years of age the mean is 940 (SD ± 124) mm^2.

As displayed in Fig. 2, with increasing age there is no significant diminution in the surface area occupied by the striate cortex.

Volume of Striate Cortex

Values for the total volume of the striate cortex in the individual monkeys are given in Table 2. There is a wide variation in the volume of striate cortex among individual monkeys. The volume of striate cortex varies between 1,190 mm^3 for AM 11 to 2,503 mm^3 for AM 47, and as might be expected these monkeys are the same two that showed the greatest difference in the surface area occupied by striate cortex. The mean value for the volume of striate cortex is 1792 (SD ± 351)mm^3. For the six young monkeys 4 to 12 years of age the mean value is 1,858 (SD ± 353)mm^3 and for the group of monkeys over 25 years of age it is 1,744 (SD ± 340)mm^3. The volumes of the individual striate cortices are plotted against age in Fig. 3, and it is apparent that there is no significant decrease in the volume of the grey matter of striate cortex with age.

Neuronal Profile Counts

The numbers of neuronal profiles displaying nuclei in 250 μm-wide strips passing through the entire depth of the striate cortex in 1 μm-thick sections is given in Table 3. Values are given for the numbers of neurons within each of six laminae of the cortex, as well as the total for the cortical depth. The values given for each monkey are the means of counts from four separate strips. These mean counts are also shown plotted against age in Fig. 4.

There can be great differences in the neuronal profile counts obtained from different monkeys, and in this respect monkey AM 47, a 9-year-old monkey, stands out from the rest. This monkey had many more neurons than the other young monkeys and this is largely due to the high density of neurons in layer II/III. These differences could be due to the experimental design, because it is obvious that for each monkey neuronal profile counts were only taken in one portion of cortex, and no attempt was made to ensure that these neuronal profile counts are representative of the striate cortex as a whole. However, the total mean value for the number of neuronal profiles within the 250 μm strips passing through the entire depth of the cortex for the group of young monkeys, 4 to 12 years of age, is not significantly different from that of the group of five monkeys over 25 years old ($p > 0.2$), and there are no significant differences between the numbers of neurons in the individual laminae.

Electron Microscopic Examination

As illustrated in our earlier study of striate cortex (40), there is very little difference in the appearance of the cell bodies of neurons when the cortices from young and old monkeys are compared, other than the neurons in the old monkeys have more lipofuscin. Except for some neurons within layer I, no neuronal cell bodies have been encountered in the old animals that can be considered to show overt signs of degeneration. However, as pointed out earlier (40), large membrane bound vacuoles that can be as large as 10–15 μm have been occasionally encountered in the cortices of old monkeys, especially in the deeper layers. Obviously, such vacuoles are of the dimensions of neuronal cell bodies and could conceivably be produced by the dissolution of neurons, but no images that might be interpreted as supporting that such a process occurs have been encountered.

DISCUSSION

The results of this study of striate cortex using a larger population of rhesus monkeys are similar to those generated in our earlier examination of the numbers of neurons and the thickness of the cortex in young and old monkeys (40). As before, there is no evidence of a reduction in the thickness of the striate cortex with age, and no reduction in the numbers of profiles of neurons displaying nuclei within strips of sections passing through the depth of the cortex. Because there is no alteration in the mean sizes of the nuclei of the neurons with age in striate cortex (40), it is concluded that there is no change in the numbers of neurons beneath a given area of the surface. A similar conclusion was reached in our study

TABLE 3

NUMBER OF NEURONAL PROFILES WITH NUCLEI IN 250 μm WIDE STRIPS OF 1 μm PLASTIC SECTIONS

	Age	Layer II/III	Layer IVa	Layer IVb	Layer IVc	Layer V	Layer VI	Total
Young monkeys								
AM-10	6	183	40	36	130	50	85	522
AM-58	4	141	36	40	109	37	53	416
AM-47	9	247	40	43	114	46	84	574
AM-42	12	172	31	67	126	45	74	515
AM-20	12	178	38	55	124	51	75	520
Mean:		184	37	48	120	46	74	509 (SD ± 51)
Old monkeys								
AM-12	27	173	32	33	113	36	66	453
AM-13	35	196	29	42	109	44	70	485
AM-41	32	216	32	32	127	38	56	499
AM-15	27	202	32	55	114	42	74	519
AM-19	25	193	33	42	99	50	70	485
Mean:		196	31	41	112	42	69	491 (SD ± 22)

of the effects of age on area 46 in the frontal cortex of the rhesus monkey (29), and on area 4 of motor cortex (38). This conclusion is supported by the electron microscopic observations on these same monkeys. For neither in the visual, motor, nor frontal cortex have we encountered any neuronal cell bodies that could be interpreted as showing degenerative changes, except occasional ones in layer I. It might be parenthetically added that we are not sure what normally degenerating neurons within the mature cerebral cortex might look like. Some neurons in layer I of the aging monkey show a vacuolation of the perikaryal cytoplasm, similar to that occurring during the degeneration of neurons with age in the spiral ganglion (11), but such a vacuolation has not been seen in any of the neurons in deeper layers of the cortex. It might be supposed that an accumulation of lipofuscin could lead to the degeneration of cortical neurons, but few of the neurons within primary visual cortex show much lipofuscin, and even within area 4, in which the Betz cells accumulate vast amounts of lipofuscin with age (38), there is no indication that this results in their demise.

It might be added that although there are few changes in the neuronal cell bodies, the neuroglial cell population shows very obvious age changes. With age, all three types of neuroglial cells can accumulate inclusions within their cell bodies and the presence of these inclusions makes it very easy to distinguish the cortices of young monkeys from those of old ones (27).

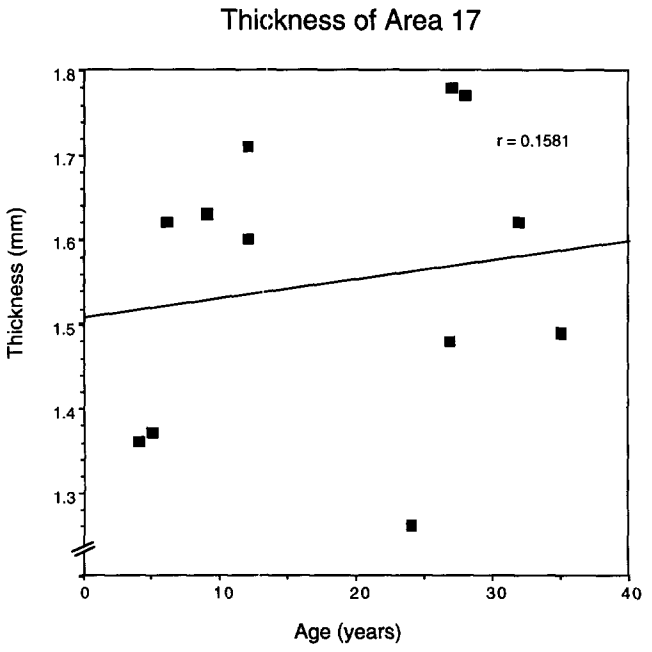

FIG. 1. The thickness of the striate cortex plotted against the ages of the monkeys.

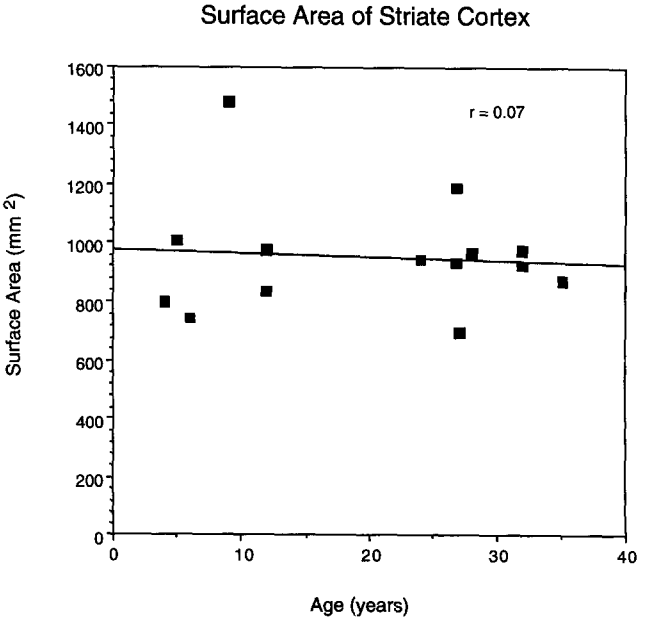

FIG. 2. The surface area of striate cortex plotted against the ages of the monkeys.

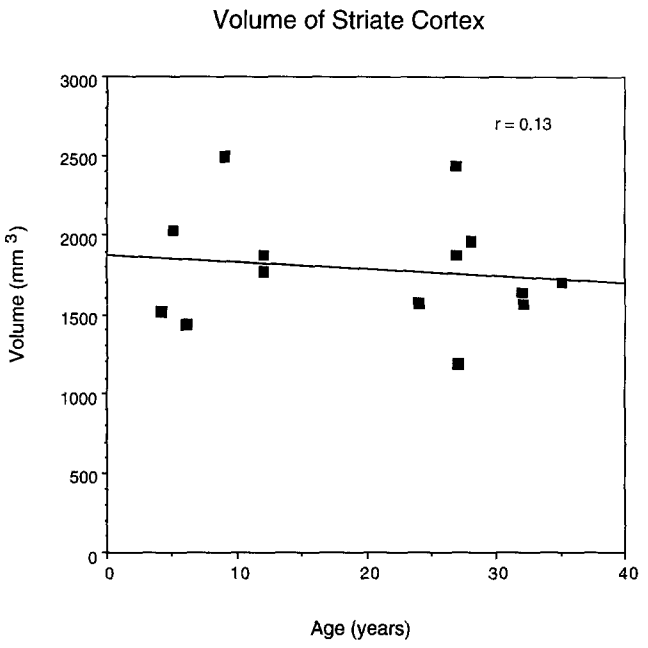

FIG. 3. The volume of the striate cortex plotted against the ages of the monkeys.

Even if there is no suggestion that striate cortex becomes thinner with age and that the numbers of neurons beneath a given area of cortical surface are not reduced, it might be proposed that neurons could still be lost if the total, or reference volume of striate cortex diminishes with age [see (41)]. Because the striate cortex is not becoming thinner with age, this would have to happen as a consequence of a contraction in its surface area. But there is no indication from our measurements that with age there is a diminution in either the surface area occupied by striate cortex, or in its volume. However, a confounding problem is that there is an enormous variation in the size of the striate cortex among individual monkeys. It could be argued that some of this is due to difference in brain size between male and female monkeys, and it should be noted that four of the sample of six young monkeys are male, while in the group of old monkeys six of the eight monkeys are female. However, it is evident from Table 2 that there is no direct correlation between gender and the volume of area 17. If the monkeys are ranked according to increasing volume of area 17, among the six young monkeys the two females rank second and forth, and that among the eight old monkeys, the two males rank fifth and seventh.

This large variation in the amount of cortex devoted to vision in primates has been often overlooked and yet it has been described in a number of publications. In one study (39) 31 hemispheres from the brains of 24 *Macaca fascicularis* were examined and values obtained for the area of striate cortex ranged between 690 to 1560 mm^2, with a mean value of 1195 mm^2. More recently, the striate cortex in neonatal and in young adult male rhesus monkeys (*M. mulatta*) has been examined (30), and in a sample of 11 young adult monkeys, which ranged from 6 to 12 years of age, the surface area of striate cortex was found to vary between 885 and 1,346 mm^2, with a mean value of 1,069 (SD ± 42) mm^2. This is similar to the range of size that we have measured (Table 2). In the study cited (30) the weights of the brains are also given, and although there is a tendency for the largest brains to have the most extensive striate cortices, there is not a rigid correlation between brain weight and the size of area 17. Interestingly, it was also found that there is a large variation in the number of cytochrome oxidase blobs beneath 1 mm^2 of cortical surface among individual monkeys, as well as in the total number of these blobs within the striate cortices of individual monkeys (30). This is again an indication that although the cortices of all monkeys may look similar histologically, there are large variations from monkey to monkey.

An interesting question that is raised by these variations in the volume on area 17 among monkeys is whether the monkeys with the largest cortical volumes have the greatest numbers of neurons throughout the visual pathway. The answer to this question is not known, but in a recent study (34) it has been shown that there is no significant correlation between the numbers of ganglion cells in the retinae and the numbers of neurons in the LGNs of individual monkeys. While the average ratio of retinal ganglion cells to LGN neurons is 1:1, for individual monkeys the ratio varies twofold, from 0.78:1 to 1.64:1.

There are also large differences in the size of the striate cortex among human brains, as determined from a computer analysis of the size of the primary visual cortex using coronal sections from 52 brains procured at autopsy (35). This study showed that there can be a threefold difference in the surface area of the primary visual cortex among individual brains, because the smallest surface area of striate cortex measured was 1,284 mm^2 and the largest 3,702 mm^2, with a mean value of 2,134 mm^2. This is a much wider range of variation than we and others (30) have encountered in the rhesus monkey brains. In another study, in which the primary goal was to examine the asymmetry between left and right hemispheres, 31 serially sectioned human brains that ranged in age from 33 weeks of gestation to 94 years of age were examined to determine the volume of striate cortex (25). If only the brains over 25 years of age are considered, the results of this study show that the volume of the striate cortex can vary from about 1549 mm^3 to 4157 mm^3. In another study, the volume of area 17 was measured in a number

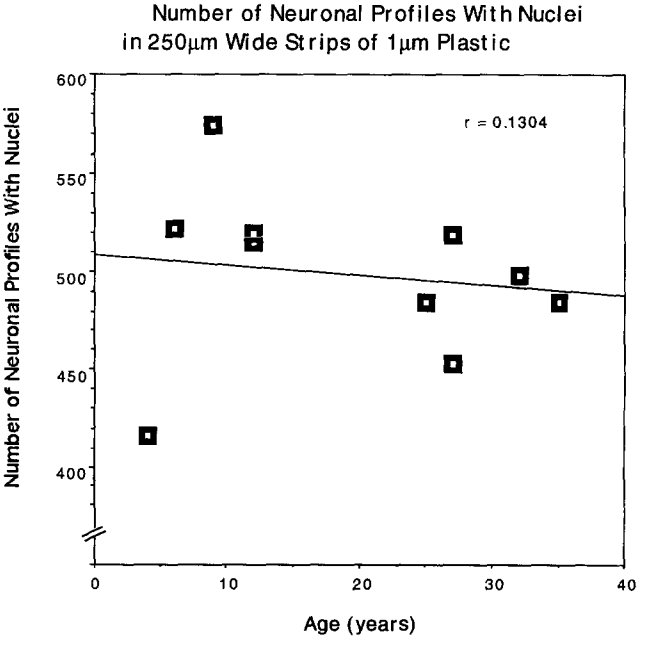

FIG. 4. The number of neuronal profiles with nuclei in 250 µm wide strips of 1 µm sections plotted against age.

of normal human brains aged between 66 and 93 years and found it to vary between 3,250 mm^3 to 6,380 mm^3, with a mean volume of 4,950 mm^3 (23). This, again, shows the wide range in the size of area 17, and interestingly, in this study the largest normal brain was from the oldest specimen, a female 93 years of age.

In this same study (23) a comparison was made between the normal brains and brains from patients with Alzheimer's disease (AD). Although no difference was found in the volume of area 17 between the two groups, the AD brains had an average of 33% fewer neurons beneath 1 mm^2 of cortical surface than the control brains. The point to make here is that while, as in the rhesus monkey, there is a wide variation among individual brains in the size and amount of cortex devoted to the primary visual function, when there is a neuronal loss it is detectable from the counts of neurons present beneath unit areas of cortical surface. This suggests that a shrinkage in an area or volume does not compensate for neuronal loss.

Based on the existing evidence, the only reasonable conclusion is that there is no significant loss of neurons from the striate cortex of the rhesus monkey as a consequence of the normal aging process. And, indeed, this same conclusion is also likely to apply to other areas of the neocortex. But in view of the large variation in volume among the cortices of individual monkeys, and of humans, there is probably little to gain by continuing studies that involve only counting profiles per unit area of sections or determining neuronal densities as a means to evaluate the possibility of neuronal loss from cortex during normal aging in primates. Whether similar large variations in the volumes of specific cortical areas occur in other species appears not to be known. But as far as the cortices of primates are concerned it would be more profitable to try to produce a marker that would display any neurons that are vulnerable to degeneration, such as the neurons that are prone to death and label with antibodies to nonphosphorylated epitopes of the medium and heavy molecular weight subunits of neurofilament protein in Alzheimer's brains (19). Such markers could be used to determine which parts of the brain are most succeptible to the aging process and would reveal whether cortical neurons are as prone, or less prone, to death than neurons in other parts of the central nervous system.

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