Neuronal Population of Area 4 During the Life Span of the Rhesus Monkey

JOHANNES TIGGES,[1] JAMES G. HERNDON AND ALAN PETERS

Yerkes Regional Primate Research Center and Department of Anatomy and Cell Biology
Emory University, Atlanta, GA 30322
and Department of Anatomy, Boston University, Boston, MA 02118

TIGGES, J., J. G. HERNDON AND A. PETERS. *Neuronal population of area 4 during the life span of the rhesus monkey.* NEUROBIOL AGING **11**(3) 201–208, 1990.—One right or left area 4 of each of 19 rhesus monkeys, ranging in age from 1 day to 35 years, was processed (frozen sectioned at 30 or 40 μm) for light microscopic analysis to assess age-related changes in the neuronal population. All neurons were examined regardless of their size. In addition, Betz cells were analyzed separately; to be regarded as Betz cells, pyramidal somata had to display a minimum height of 38 μm. A significant loss of approximately one-third was observed in the total number of neurons in *maturing* monkeys (<5.5 years). In contrast, in maturing rhesus monkeys significant increases with age were observed in the mean number of Betz cells, and in the means of Betz cell area, height, width, perimeter, and estimated volume. In *adult* monkeys (>4.5 years), no age-associated loss of neurons was observed. Also, no loss of Betz cells occurred, although the perimeter, area, and estimated volume of Betz cells decreased slightly, but significantly, with increasing age in adult monkeys. Lipofuscin granules were discernable in Betz cells beginning at the age of 5 years and their number increased with increasing age. In the older rhesus monkeys, the lipofuscin granules were so large and numerous that in some Betz cell somata they displaced the nucleus from its usual location in the center of the cell. No age-related change in thickness of area 4 was found.

Gerontology   Rhesus monkey   Development   Aging   Area 4   Cortical thickness   Neuronal number
Betz cells   Lipofuscin

DO the neurons in the aging human cerebral cortex die, shrink, or remain unchanged as a consequence of aging? The controversy regarding this question began when Brody (7) reported his results on brains aged from birth to 95 years. This question remains unsettled despite several reports on this topic (1, 3, 9, 12, 14–19, 23, 25, 36, 37). Coleman and Flood (8) critically reviewed the literature on this subject and concluded that there may be an age-related neuronal loss specific to certain cortical regions. The review points out, however, that uncontrolled differences in agonal state, postmortem delay, and other aspects of tissue handling as well as mental status, and nutritional and medical histories of patients may account for dissimilarities among the reported findings.

While most aging studies on the cerebral cortex focused on entire cerebral hemispheres or on populations of neurons throughout the depth of distinct cortical areas, others concentrated on the Betz cell, a very conspicuous neuron in layer 5 of area 4 (primary motor cortex) (33–35). In Golgi preparations of human brains aged 74 to 102 years, a sequence of age-related phenomena leading to cell death was observed. In the first stages of cell death, there was an irregular swelling of the cell body and principal dendritic shafts, and a progressive loss of dendritic spines. In later stages of decline, the dendrites died back and were reduced to stumps, after which the apical dendrite became fragmented and disappeared

before the disappearance of the soma. It was concluded that approximately 75% of Betz cells are lost from the human brain by the seventh decade of life, and that the Betz cells might be more vulnerable and show more age-related changes than the surrounding non-Betz pyramids. Unfortunately, those researchers did not define their Betz cells by size; this is important because there is a continuum of large to giant pyramids, the latter being generally designated as Betz cells. As in all studies of human material there is a nagging doubt as to whether these reported changes in neurons are real or are artifacts of suboptimal preservation of tissue caused by a postmortem delay in fixation. For example, Williams *et al.* (40) studied Golgi preparations of mouse brains and found that alterations in cell morphology, such as dendritic spine loss, varicosities of dendrites and truncation of dendrites can be induced by, and increased with, length of postmortem delay before fixation. The neuronal changes produced by delayed fixation can be obviated by using well-fixed tissue, but since this cannot be achieved in human brains, we have examined the motor cortex of rhesus monkeys. The motor cortex of this species is neuroanatomically closer to the corresponding area of the human brain than is that of any other widely available laboratory animal.

The present paper has two main goals. First, we examine the depth and the number of neurons of area 4 as a function of age of rhesus monkeys. Secondly, we focus on Betz cells to determine

[1]Requests for reprints should be addressed to Dr. Johannes Tigges, Yerkes Primate Center, Emory University, Atlanta, GA 30322.

TABLE 1

MEAN THICKNESS OF CORTICAL AREA 4 AND MEAN COUNTS OF NEURONS IN A 125 μm WIDE CORTICAL TRAVERSE IN 40 μm (R-MONKEYS) OR 30 μm (AM-MONKEYS) THICK SECTIONS

Monkey	Sex	Age	Mean Area 4 Thickness μm (SD)	Mean Number of Neurons (SD)	Mean Number of Neurons Beneath 1 mm²
R-607	F	1 d	3,150 (107)	366 (29)	73,000
R-618	M	1 m	3,380 (107)	374 (18)	74,800
R-624	F	5 m	3,700 (110)	327 (10)	65,400
R-628	M	1 y	3,640 (49)	322 (15)	64,400
R-733	M	3 y	3,250 (91)	309 (14)	61,800
R-732	F	4 y	3,450 (78)	303 (26)	60,600
R-706	M	5 y	3,190 (104)	241 (14)	48,200
R-712	F	9 y	3,380 (107)	238 (22)	47,600
R-711	M	15 y	3,060 (123)	219 (29)	43,600
R-622	M	19 y	3,250 (29)	246 (11)	49,200
R-614	M	20 y	3,120 (162)	252 (15)	50,400
R-608	F	20 y*	3,480 (114)	241 (23)	48,200
R-611	F	30 y*	3,090 (72)	241 (18)	48,200
R-623	M	34 y*	3,450 (98)	253 (29)	50,600
AM-7	M	5 y	3,150 (221)	— —	—
AM-10	M	6 y	3,050 (195)	— —	—
AM-12	F	26 y	3,060 (91)	— —	—
AM-15	F	27 y	3,410 (19)	— —	—
AM-13	M	35 y*	3,250 (114)	— —	—

SD = Standard deviation; d = day; m = month; y = year; *estimated.

whether their number and size change with age. For this purpose we collected 19 well-fixed brains of monkeys ranging from 1 day to 35 years of age. Area 4 was cut in the coronal plane and the sections stained for the light microscopic analysis of neuronal somata.

This study is part of a concerted effort among several investigators to evaluate the rhesus monkey as an animal model for human aging.

METHOD

Animals

Nineteen rhesus monkeys (*Macaca mulatta*) ranging in age from 1 day to 35 years (Tables 1 and 2) were used. This age range encompasses the entire life span of this species (38). Exact birth dates were available for 15 monkeys. The remaining 4 monkeys were obtained at a relatively early age; consequently, the margin of error for estimates of age was small. For example, the three oldest monkeys in this study (AM-13, R-611, R-623) came into captivity at an estimated age of 2 years. All monkeys were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23).

Two of the 19 monkeys had been subjected to ionizing radiation. At an estimated age of 2 years, R-611 was exposed to 1021 rads of fractionated cobalt radiation in 1958; R-623 was exposed to 554 rads of fractionated gamma radiation in 1954, also at the estimated age of 2 years. Parenthetically, it is interesting to note that exposure to radiation had no apparent effect on the parameters under study.

Fixation and Tissue Preparation

Before perfusion, each monkey was tranquilized with ketamine

TABLE 2

MEAN COUNTS AND MEASUREMENTS OF BETZ CELLS IN A 5 mm LONG STRIP OF 40 μm (R-MONKEYS) OR 30 μm (AM-MONKEYS) THICK SECTIONS

Monkey	Sex	Age	Betz Cell Mean Number	Mean Axes h × w μm (SD)	Mean Perimeter μm (SD)	Mean Projection Area μm² (SD)	Mean Volume × 1,000 μm³ (SD)
R-607	F	1 d	2.1	39 × 25 (2 × 3)	113 (6)	837 (108)	19 (4)
R-618	M	1 m	2.0	39 × 29 (1 × 3)	116 (4)	955 (75)	25 (4)
R-624	F	5 m	5.4	42 × 31 (3 × 4)	124 (9)	1085 (175)	31 (8)
R-628	M	1 y	4.5	41 × 27 (3 × 4)	118 (8)	902 (127)	21 (6)
R-733	M	3 y	6.7	48 × 32 (7 × 5)	143 (16)	1418 (332)	45 (16)
R-732	F	4 y	6.8	51 × 32 (7 × 5)	146 (18)	1430 (341)	43 (16)
R-706	M	5 y	11.9	50 × 38 (7 × 5)	153 (19)	1672 (399)	60 (21)
R-712	F	9 y	7.1	48 × 33 (7 × 5)	145 (16)	1448 (330)	46 (16)
R-711	M	15 y	8.0	53 × 36 (9 × 7)	158 (24)	1738 (494)	62 (25)
R-622	M	19 y	8.0	50 × 39 (8 × 6)	154 (22)	1698 (460)	61 (25)
R-614	M	20 y	6.6	49 × 36 (8 × 6)	147 (20)	1506 (416)	49 (21)
R-608	F	20 y*	6.5	51 × 30 (10 × 6)	142 (21)	1301 (325)	37 (19)
R-611	F	30 y*	7.1	47 × 33 (6 × 5)	141 (17)	1384 (321)	46 (22)
R-623	M	34 y*	6.4	48 × 32 (8 × 6)	140 (21)	1350 (408)	41 (19)
AM-7	M	5 y	6.1	48 × 40 (5 × 5)	151 (13)	1638 (274)	60 (15)
AM-10	M	6 y	9.4	46 × 37 (6 × 6)	144 (17)	1483 (373)	51 (20)
AM-12	F	26 y	7.2	44 × 35 (5 × 6)	138 (15)	1372 (314)	46 (17)
AM-15	F	27 y	8.7	45 × 34 (6 × 5)	140 (16)	1379 (332)	45 (17)
AM-13	M	35 y*	7.3	46 × 35 (8 × 7)	140 (23)	1432 (499)	49 (27)

SD = Standard deviation; d = day; m = month; y = year; *estimated.

and then given a lethal dose of Nembutal for anesthesia and 5,000 IU heparin for anticoagulation. The monkeys with R-prefixed codes were perfused through the ascending aorta with 500 ml saline followed by 600 ml of 4% paraformaldehyde and 0.1% glutaraldehyde. The fixative was then washed out with 500 ml 5% sucrose in phosphate buffer. (The washout was carried out because most of the R-monkeys were perfused for other projects which required this procedure, but area 4 was made available for the present study.) Immediately thereafter, a block of tissue from area 4 of the right hemisphere (Fig. 1, upper panel) was removed and kept for 1 month for further hardening in the same fixative used for the perfusion. The block of area 4 was then cryoprotected with 20% glycerol and 2% dimethylsulfoxide in buffer, before being rapidly frozen in −75°C isopentane and affixed to the freezing stage of the microtome (32). The tissue was cut in the coronal plane and as perpendicularly to the cortical surface as possible at a thickness of 40 μm in a cryostat set at approximately −40°C. The sections were collected in phosphate buffer; every fifth section was mounted on gelatin-coated slides and stained with cresyl violet acetate to reveal the neuronal somata.

The monkeys with AM-prefixed codes were also perfused through the ascending aorta. The blood was washed out with 400 ml saline containing 6% dextran and 1% $NaNO_2$, after which the brain was fixed by perfusion with 4 l of a warm (37°C) mixture of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4. The brains were immediately removed from the skull, bisected, and immersed in a stronger solution of aldehydes containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 7.4. Either the right or left hemisphere from each of these brains was cut in the coronal plane at 30 μm, and every tenth section, including area 4, was stained with thionin to show the neuronal somata. In addition, slabs of the other area 4 of the AM-brains were embedded in Araldite. Semithin sections were cut from this material and stained with toluidine blue to better visualize the presence of lipofuscin granules in Betz cells.

Following perfusion, the brains of all monkeys still fitted snugly in the cranial cavity. This suggests that no shrinkage of the brains occurred during the perfusion process, or that the shrinkage was so small that it could not be perceived during removal of the brains from the crania.

Counts, Measurements, and Calculations

From each brain, a series of 10 sections separated by intervals of 400 μm (R-monkeys) or 300 μm (AM-monkeys) was used for counts and measurements in the dorsal portion of area 4. To determine whether there is a change in the population of neurons with age, we counted, using an oil immersion objective, each neuron displaying a nucleolus in one 125 μm wide traverse from pia to the white matter in each of the 10 sections (Fig. 1, middle panel). All neurons were counted, regardless of their size. The counts were made with the aid of a square ocular reticule measuring 125 μm on the side, beginning at the pial surface and extending to the white matter. The border of area 4 with the subjacent white matter was not abrupt; instead, there was a gradual decrease of neuronal concentration in lower layer 6 and a concomitant increase in white matter. Counting was terminated in the white matter when the number of neurons within the square reticule fell below three. The mean number of neurons per traverse was then multiplied by 200 to arrive at an estimate of the mean number of neurons beneath 1 mm^2 (125 μm × 40 μm × 200 = 1 mm^2) of cortical surface (Table 1).

Except for layer 1 and the border between layers 1 and 2, the distinction of neurons from glial cells presented little difficulty.
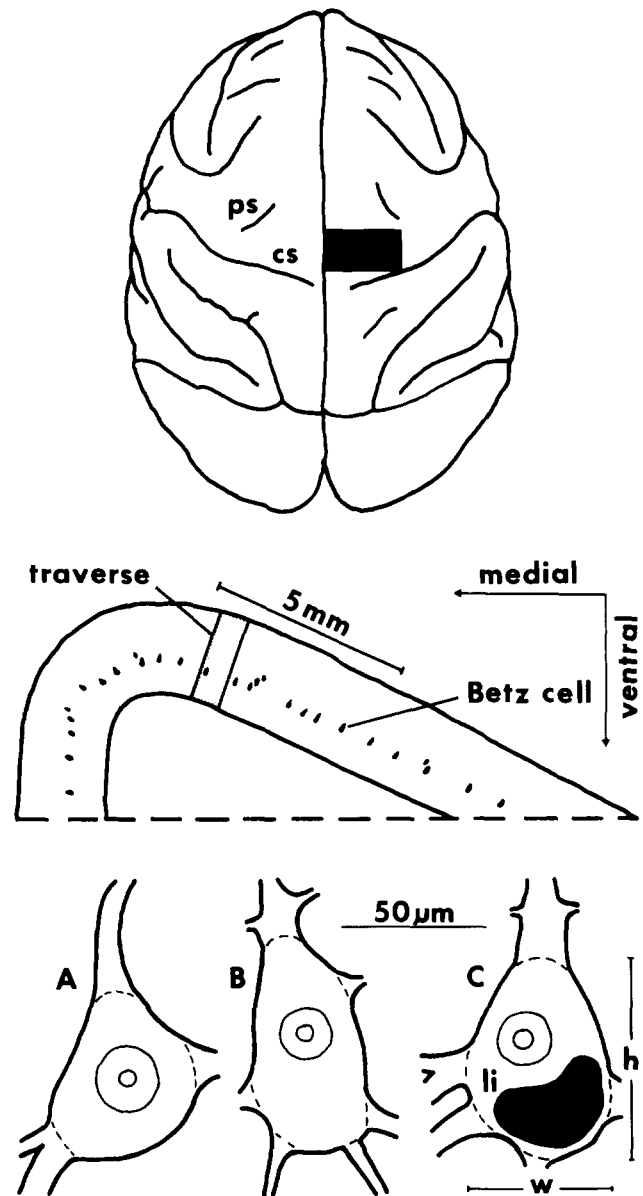

FIG. 1. Upper panel: drawing of dorsal view of an adult rhesus monkey brain. The black rectangle between the precentral sulcus (ps) and the central sulcus (cs) indicates the portion of area 4 used in this study. Middle panel: schematic drawing of a coronal section through area 4. Betz cells examined in this study were located in a 5 mm long strip on the dorsal surface of area 4, as indicated. Cell counts through the depth of area 4 were made in a 125 μm wide transverse at the location indicated. Lower panel: drawing of the cell bodies of three typical Betz cells (A–C). At its base cell C contains a moderately large accumulation of lipofuscin (li). For drawings of the perimeters of Betz cell somata with the camera lucida, the dendrites were ignored, as indicated by dashed lines. h, height or long axis; w, width or short axis.

Almost invariably the neurons displayed the initial portion of their apical dendrite and often the origin of one or more basal dendrites could be discerned. The rather lightly stained neuronal nucleus contained one distinctly dark nucleolus.

To determine whether cortical thickness changes as a function of age we measured the cortical thickness from the pia to the white matter at each traverse with a calibrated ocular micrometer. Again,

TABLE 3

SLOPES OF REGRESSION LINES AND p-VALUES

				1-Tailed Tests	
	Measurement Units	Maturing R-Monkeys (n = 7)	Adult AM- and R-Monkeys (n = 13)	Adult (n = 5) AM-Monkeys	Adult (n = 8) R-Monkeys
Area 4 thickness	μm	−41.58 (±43.29; ns)	4.08 (±4.22; ns)	—	—
Neurons per traverse	number	−19.43 (±4.14; p<0.01)	—	—	—
Betz cells	number	1.5 (±0.31; p<0.01)	−0.06 (±0.04; ns)	—	—
Height	μm	2.46 (±0.30; p<0.01)	−0.08 (±0.07; ns)	—	—
Width	μm	1.73 (±0.49; p<0.01)	−0.14 (±0.07; ns)	—	—
Perimeter	μm	7.78 (±0.77; p<0.01)	−0.38 (±0.14; p<0.05)	−0.32 (±0.13; p<0.05)	−0.43 (±0.22; p<0.05)
Area	μm²	149.66 (±20.47; p<0.01)	−8.04 (±3.31; p<0.05)	−6.17 (±2.98; ns)	−9.94 (±5.89; ns)
Volume	μm³	6,916 (±1,142; p<0.01)	−421 (±186; p<0.05)	−332 (±164; ns)	−519 (±340; ns)

Slopes are in units per year. Numbers in parentheses are standard errors of the slopes followed by significance levels (p). p-Values are derived from t-tests and indicate probability of a nonzero slope.
— = not calculated; ns = not significant.

the border between the grey and white matter was considered to be where the number of neurons with nucleoli within the 125 × 125 μm area of the reticule fell below three.

In each brain the most posterior section of the 10 sections used for counting was sufficiently anterior to the central sulcus so that the shape and size of Betz cells were not affected by the sulcus. All Betz cells containing a nucleolus were counted in a 5 mm long horizontal strip (parallel to the pial surface) in the dorsal portion of area 4 in each of these 10 sections (Fig. 1, middle panel). The dorsal portion was chosen because there are more Betz cells located here than in the portion of area 4 in the interhemispheric cleft (22). Also, this portion projects to the lumbar region of the spinal cord (26), which controls movements of the leg, hip, and thigh, and thus is comparable to the region of area 4 in the human brains analyzed by Scheibel et al. (35). In addition, the short (width) and long (height) axes of each Betz cell were measured with a calibrated ocular micrometer, and the perimeter of each Betz cell soma was outlined with the aid of a camera lucida at the magnification of × 1000 (Fig. 1, lower panel). The perimeters and the projection areas (within the perimeter) were measured from the camera lucida drawings by means of a computer-controlled graphics tablet (Houston Instruments Hipad® digitizer attached to a Zenith Z286 microcomputer and controlled by Jandel Sigma Scan® software). The values obtained were used to calculate the Betz cell volume according to a formula (V = PA²π/3DMAX; PA = projection area; DMAX = maximal diameter or height) for estimating pyramidal volumes (21).

The "giant" pyramidal cells in lamina 5 of area 4 are generally considered to be Betz cells. Since there is, however, a continuum of large to giant pyramids, there is no generally accepted lower limit of size to define Betz cells. This problem led Braak and Braak (2) to suggest that the presence of large numbers of lipofuscin granules can be used to distinguish Betz cells from other pyramids; since lipofuscin accumulates with age, this criterion may be appropriate for study of aged brains, but it cannot be used to identify Betz cells in maturing brains. Furthermore, even non-Betz cell pyramids accumulate lipofuscin, although to a lesser degree, and would therefore be indistinguishable from Betz cells on the basis of lipofuscin accumulation. Because of this difficulty we chose, after a general survey, to use the arbitrary size of 38 μm

in height as a criterion for regarding a neuron as a Betz cell.

In making the above measurements and counts, no attempt was made to carry out a blind analysis since accumulation of lipofuscin, which occurs with age in these monkeys, is so pronounced that a practiced observer can easily estimate the age of the monkey from which the sections were taken.

Statistical Analysis

For purposes of evaluating the statistical significance of any morphometric changes, maturing (R-monkeys <5.5 years, n = 7) and adult monkeys (AM- and R-monkeys >4.5 years, n = 13) were treated separately. Group boundaries were set so that 5-year-old monkeys, which are approaching asymptotal skeletal size (4), are regarded as being at the end point of maturation and the beginning of adulthood. Linear regression statistics were computed against age for each measurement. Dependent variables in these analyses were the means of the thickness of area 4, number of Betz cells per section, Betz cell projection area, height, width, perimeter, and volume. For the R-monkeys, the mean number of neurons per traverse was also included as a dependent variable.

For dependent variables displaying a statistically significant regression line slope ($p<0.05$) over age in adult monkeys, separate analyses were performed for the AM- and R-monkeys (Table 3).

RESULTS

Qualitative Results

In neonatal rhesus monkeys, the neurons in area 4 are relatively small and densely packed, and appear almost uniformly round and darkly stained with cresyl violet acetate. As the monkeys mature, some pyramids grow larger than others and become more prominent. At the same time, the Nissl substance in these larger cells aggregates to form distinct blocks. In lamina 5, some of these enlarged pyramids grow to become the Betz cells, which occur either in solitary fashion or in groups. Although they are usually more or less aligned in a row in sections cut perpendicular to the cortical surface, Betz cells are not strictly confined to a specific
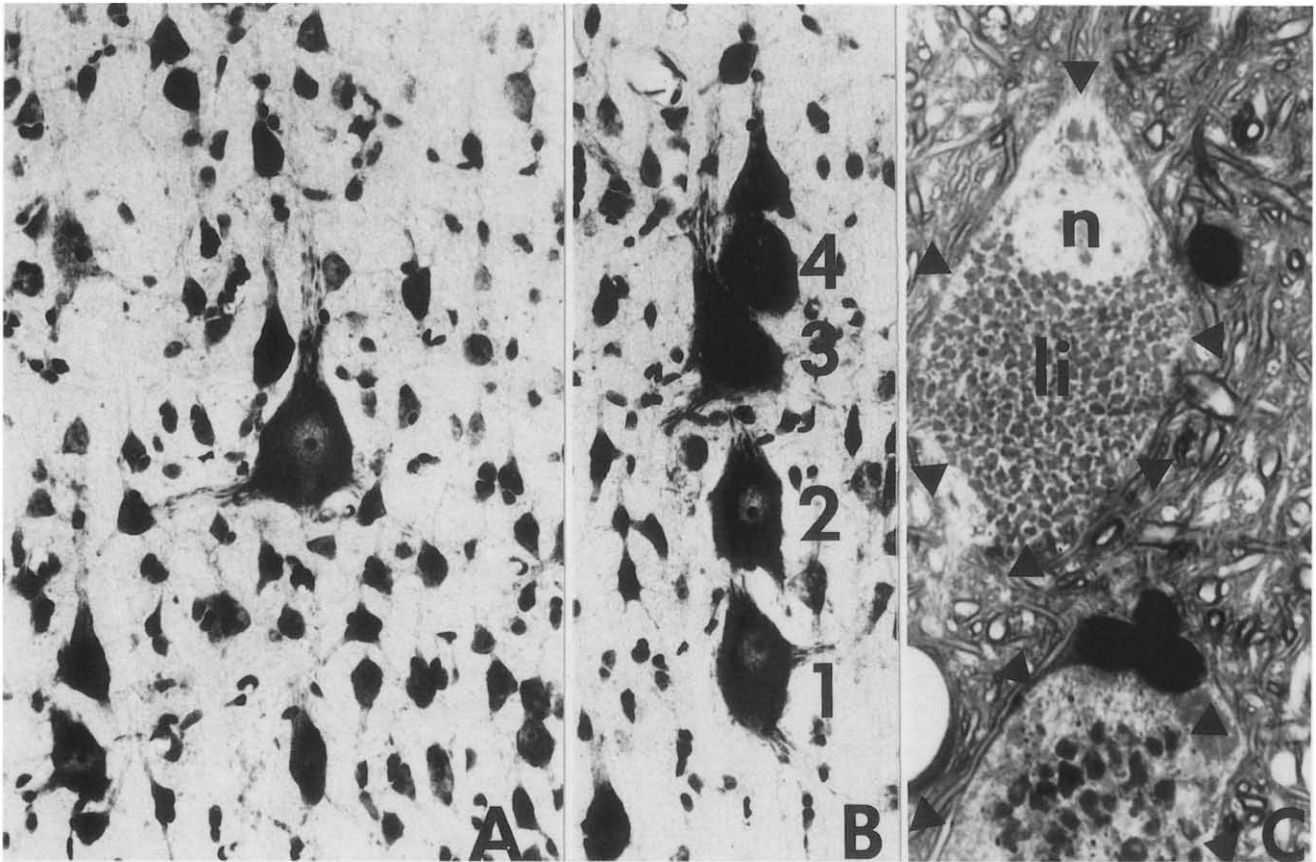
FIG. 2. Photographs of Betz cells. (A) Solitary Betz cell surrounded by large and small pyramidal cells. (B) Four (1–4) Betz cells are stacked on top of one another. Directly on top of Betz cell 4 is a pyramid that is too small to be considered as a Betz cell. (C) Betz cell (outlined by arrow heads) from a 35-year-old monkey (AM-13) is almost completely filled with lipofuscin (li) granules which have pushed the nucleus (n) to the apical pole of the cell. A portion of another Betz cell with fewer lipofuscin granules is seen at the bottom of this micrograph. A and B are from a 20-year-old monkey (R-614), cresyl violet acetate stain, × 250. (C) Semithin section stained with toluidine blue, × 1200.

sublayer of layer 5. Indeed, their depth beneath the pia varies. The variation in depth is particularly obvious when Betz cells occur in clusters and are stacked on top of one another (Fig. 2).

The soma of a Betz cell may either be plump and rounded or more triangular in appearance. Numerous Nissl bodies extend from the soma into the primary dendrites, making the origins of the dendrites visible. This is especially true of the prominent apical dendrite, which may arise either smoothly or abruptly from the soma and can often be followed for some distance on its way toward the pial surface. Some of the basal dendrites are also pronounced, but the smaller dendrites which may emanate from any point of the soma are less obvious. The nucleus is relatively small compared to the soma and contains a darkly staining nucleolus which measures between 5 and 7 μm in diameter.

From 5 years of age on, yellowish-brownish particles of lipofuscin become discernible in the somata of Betz cells in sections stained with thionin. In semithin plastic sections stained with toluidine blue and studied under high power, the lipofuscin particles appear as relatively large and irregularly formed bodies. With advancing age, the lipofuscin granules increase in number and aggregate to form large masses. In our oldest monkeys, 26 to 35 years of age, these masses sometimes occupy half or more of the Betz cell body and can force the nucleus from its central

position toward one side or pole of the cell (Fig. 2C). There is no preferential location of the lipofuscin in the soma.

Quantitative Results

The thickness of area 4 varies between 3050 and 3700 μm (Table 1), averaging approximately 3.3 mm, but does not change significantly with age (Table 3). In contrast, the number of neurons counted in a 125 μm wide traverse decreases steadily from 1 day to 5 years of age. This decrease of approximately one-third in the neuronal packing density in maturing monkeys is statistically significant (Table 3). The number of neurons stabilizes by early adulthood (5 years), however, and thereafter remains unchanged. During maturity, the average number of neurons beneath 1 mm^2 of surface remains at approximately 48,000 (Table 1).

Table 2 summarizes the counts, measurements, and calculations on Betz cells. A summary of linear regression analyses for maturing and adult monkeys is presented in Table 3 and the data can be interpreted as follows. In maturing (<5.5 years) monkeys, significant increases occur with age in the mean number of Betz cells per 5 mm strip of the section, as well as in the means of Betz cell projection area, length, width, perimeter, and estimated volume.

Although there is no change in numbers of Betz cells after 5 years of age, there is evidence of decreasing Betz cell size. Thus, Betz cell projection area, perimeter, and volume are all found to decrease significantly with increasing age. When the adult AM-monkeys (n = 5) and R-monkeys (n = 8) are analyzed separately (with 1-tailed tests), significant decreases with age remain only in Betz cell perimeter. Declines in volume and area are not statistically reliable in these subgroups. In adult monkeys, other measures (see Table 3) are not significantly correlated with age.

DISCUSSION

A recent study on the life span and survival rate of rhesus monkeys has shown that the median age at death is about 16 to 17 years (38). By the mid-twenties, approximately 25% of the population remain, while about 6% live beyond the age of 30 years. Thus, the present study includes a number of extremely rare specimens.

Our counts revealed a steady decrease in the number of neurons in area 4 of maturing monkeys. Since the thickness of area 4 does not significantly change as a function of age, the lower packing density indicates a real loss of neurons. Neuron losses from maturing brains have been previously reported. For example, O'Kusky and Colonnier (27) observed a loss of neurons from area 17 of infant macaques. Similarly, the number of neurons in human area 17 drops by 35 to 40% before it stabilizes between 2 and 4 months postnatally (23), and neuronal density in layer 3 of the middle frontal gyrus of human brain shows a very rapid decline during the first 6 months of life (20). It is noteworthy, however, that the cell loss in area 4 of the present material extended over a much longer time period (up to 5 years). In another part of the nervous system of the rhesus monkey, the optic nerve, axons may continue to be lost over a similarly long period. The loss of retinofugal axons begins before birth and continues to adulthood (29,30). Although regressive phenomena such as loss of perikarya, dendrites, axon collaterals and/or axon terminals are recognized as playing a major role in determining the form of the mature nervous system (10), the functional significance of cell loss during the first 5 years of the rhesus monkey is unknown at the present.

The present study confirms a previous report (5) that the thickness of area 4 in rhesus monkey remains unchanged during aging, but conflicts with the finding in that report of a significant decrease in neuronal density from early adulthood through 20 years of age. The present study reveals no overall neuronal loss in area 4 between the ages of 5 and 34 years. The discrepancy between the two studies may, in part, be a consequence of the fact that in the earlier study 4-year-old monkeys were used as the baseline, whereas the present results suggest that a stable adult neuron population is not attained until 5 years of age.

In frozen sections from area 4 of two rhesus monkeys, Cragg (11) found between 16,100 and 20,700 neurons/mm^3. Based on brain weights (72 to 75 g) supplied by the author, we are led to believe that his material came from 3- to 4-year-old monkeys. [Brains of rhesus monkey weigh approximately 54 g at birth and reach adult weights of 83 g at 4 years of age (6).] Assuming an average cortical thickness of 3.3 mm (present report), this is equivalent to 60,700 neurons beneath 1 mm^2 of cortical surface, a number similar to the 60,600 to 61,800 neurons present in our 3- to 4-year-old monkeys (Table 1). This is far fewer than the number of neurons encountered by Rockel et al. (31). From the numbers provided by these authors (31), it can be calculated that there are 147,000 neurons present beneath 1 mm^2 of cortical surface of area 4. Even a linear shrinkage of 18% (28) of their paraffin embedded brain material cannot account for the large discrepancy between their findings and our average of 48,000 neurons beneath 1 mm^2 of surface of adult monkeys.

The number of pyramids fulfilling our height criterion of 38 μm for Betz cells roughly triples from birth to 3 years (Table 2). From then on and through 35 years of age the number of Betz cells does not change significantly. This finding is in sharp contrast to studies on human brains by the Scheibels (33–35) who reported a loss of approximately 75% of Betz cells by the seventh decade of life. At present the disparity between the results in the human and monkey brains cannot be explained, except to suggest that we may be confronted with a true species difference.

Betz cell size is only weakly related to age in adult monkeys. The decreases with age in Betz cell volume (421 μm^3/year), projection area (8 μm^2/year), and perimeter (0.38 μm/year) are of borderline statistical significance. The decreases are an order of magnitude smaller in absolute value than the highly reliable increases seen over the period from one day to 5.5 years of age, and the functional significance of the small decrease in size during adulthood seems questionable.

One interesting hypothesis regarding the function of Betz cells put forward by the Scheibels (33–35) is that the Betz cells fire briefly before antigravity-muscle activity but are silent during contraction. The ensuing contraction of these weight-bearing muscles is supposed to be caused by non-Betz pyramids. It was suggested by the Scheibels that this brief burst of Betz cell firing before contraction initiates a lysis or relaxation of muscles involved in maintaining antigravity postures. The age-related decline and ultimate disappearance of Betz cells from the human brain was invoked by these authors as a further mechanism, in addition to degenerative joint diseases and basal ganglionary changes, for slowing of lower limb and hip activities so often observed in aging people.

Aged monkeys, like aged human beings, also appear generally to be less active and more reluctant to move about than younger monkeys. Since we find no loss of Betz cells, however, the tentative and reluctant mobility of old macaques cannot be attributed to reduced numbers of these neurons. The small decrease in size and substantial increases in lipofuscin content of Betz cells may contribute to an impaired function of these cells and lead to a reduced or slowed voluntary movement, but it seems more likely that degenerative bone and joint disorders of hip and spine reported to occur in aged rhesus monkeys (13) account for decreased movement.

In a study specifically designed to examine changes in the lipofuscin content of neurons in the cerebral cortex of monkeys, the neuronal lipofuscin concentration in layer 5 of area 4 was found to increase progressively after the age of 4 years (6), approximately the age when we observed the first granules. In this context it is worth pointing out, that large quantities of lipofuscin can apparently be tolerated by Betz cells and that the presence of excessive lipofuscin does not necessarily lead to the demise of these giant neurons. There may nevertheless be subtle changes towards incapacitation undetectable in a morphological study. It has been suggested, for example, that accumulating lipofuscin either displaces or reduces the amount of the protein synthesizing endoplasmic reticulum (24).

The AM-monkeys used in the present study were included, with other monkeys, in a parallel study on area 17. The study resulted in essentially similar findings to the one reported here. Specifically, neither a loss of neurons nor an appreciable change in thickness of area 17 was found between 5 and 35 years of age (39). Both the present study and our study on area 17 (39) contrast with numerous reports of marked cell loss in human brains (see above). More recent studies on human material, however, are consistent with our findings in the monkey. For example, Braak and Braak (3) found the number and laminar distribution of pyramidal cells in area 11 to be almost unchanged throughout adulthood. Likewise, Leuba and Garey (23) also found a stable number of neurons in

area 17 of adult human brains. Finally, Haug and his collaborators (14–18) reported on a variety of cortical areas in a large number of human brains. After taking into consideration an array of technical factors (fixation, paraffin embedding, etc.), they concluded that there is no neuronal loss during the aging process. The preceding discussion has focused exclusively on the cerebral cortex of (human and nonhuman) primates; however, it should be briefly pointed out that stability of some cortical neuron populations has also been reported for aged rodents [for review see (8)]. Such findings, in concert with those presented here, suggest that death of neurons can no longer be considered an inevitable consequence

of advancing age in all neocortical regions of the mammalian brain.

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