Neurobiological Bases of Age-Related Cognitive Decline in the Rhesus Monkey


Abstract. The rhesus monkey offers a useful model of normal human aging because when monkeys are tested on a battery of behavioral tasks that can also be used to evaluate cognition in humans, it is found that the monkeys undergo an age-related decline in several domains of cognitive function also observed in humans. In monkeys these changes begin at about 20 years of age. To determine what gives rise to this cognitive decline, we have examined several parameters in the brains of monkeys. Some parameters do not change with age. Examples of these are the numbers of neurons in the neocortex and hippocampal formation, and the numbers of synapses in the hippocampal formation. Changes in other parameters can be positively correlated with chronological age; examples of these are numbers of neurite plaques, a decrease in the numbers of neurons in the striatum, projecting pars compacta of the substantia nigra, and a decrease in the thickness of layer I in primary visual cortex. But the most interesting changes are those that correlate either with cognitive decline alone, or with both cognitive decline and chronological age. Among these are a breakdown in the integrity of myelin around axons, an overall reduction in the volume of white matter in the cerebral hemispheres, thinning of layer I in area 46 of prefrontal cortex, and decreases in the cell density in cortically projecting brain stem nuclei. To date then, our studies suggest that the cognitive declines evident in the rhesus monkey may be a consequence of changes in layer I and in the integrity of myelinated axons, rather than an age-related loss of cortical neurons or synapses, as has long been assumed.

Key Words: Aging; Behavior; Cerebral cortex; Hippocampal formation; Macaca mulatta; Myelin; Neuronal loss.

INTRODUCTION

This review provides new insight into age-related changes in the primate brain that account for cognitive decline. We have been motivated to present our results in this fashion because we believe that for the first time, we are gaining insight into the process of normal aging as it affects the rhesus monkey, which we consider to be an excellent model of normal aging in the human brain. Although this review is focused on our own results, it is not our intention to ignore or underrate the work of others. If we have omitted reference to the work of others, it is because the data are not directly pertinent to the main theme of this review. Please forgive any such omissions.

Investigation of the neurobiological basis of normal human aging has recently increased due to a series of behavioral studies showing that aging is accompanied by a decline in several aspects of cognitive function (1, 2). This increase has been fueled by a growing awareness that our population is shifting toward a proportionately greater number of older individuals. To date, however, the neurobiological factors underlying age-related cognitive decline remain largely unknown, mainly due to the obvious limitations imposed on studies of human subjects. Among these limitations is the low probability of obtaining behavioral, physiological, and morphological data from the same individual for long enough to allow meaningful correlation of the data. A second limitation is imposed by postmortem delays and the consequent problems of obtaining adequate preservation of brain tissue. A third is related to the lack of control over extraneous variables, such as widely varied or unknown health histories of the study population. And a fourth limitation is the ever-present potential for the inclusion of early, and hence undiagnosed, cases of Alzheimer's disease in a cohort of "normal" aged humans.

To circumvent such limitations it has been essential to develop suitable animal models of normal human aging, models which offer the opportunity to collect a wide range of data from the same subject within a short period of time. Animal models also permit the exploration of possible treatment modes and interventions to arrest or reverse age-related cognitive decline. A number of investigators have pointed to the monkey as a suitable model (3–5) for the following reasons: First, monkeys have rich and well-studied behavioral repertoires, as evidenced by their ability to perform difficult cognitive tasks adopted from human neuropsychological test batteries (6–8); Second, more than any other practical laboratory species, the monkey brain most closely resembles that of humans; Third, unlike those in humans, age changes in the monkey are not confounded by the potentially undetectable
presence of early stage Alzheimer’s disease. Sharing this view, several research groups, including our own, have pursued the study of the neurobiologic basis of aging in the monkey. In this article we will review recent behavioral and morphologic findings obtained from studies of aging monkeys. These findings point to a set of hypotheses that run counter to long-standing notions concerning the neurobiological basis of normal aging.

Before considering the behavioral and morphologic data, a brief description of the life span of the monkey is provided, as defining the criteria for considering a monkey to be elderly is essential for interpreting observations on age-related changes in the brain and behavior.

**LIFE SPAN OF THE RHESUS MONKEY**

It is only in the last 10 to 15 years that definitive data have been published on the life span of the rhesus monkey. Our data were obtained by examining the survival rates of 763 monkeys kept at two locations at Yerkes Regional Primate Research Center (9). The results indicated that only 50% of monkeys reached 16 years of age, and only about 25% reached 25 years of age. The longest life span observed in this population was a male monkey that died at 35 years of age. Since monkeys become sexually mature at 4 to 5 years of age, the maximal adult life span can be considered to be from 4 or 5 to about 35 years of age, while monkeys over 20 years old can be defined as aged animals, with those reaching over 30 years being “the oldest of the old.” These data also suggest that the ratio of monkey to human years of age is approximately 1 to 3.

A review of the literature indicates that our data are comparable to those from other primate centers. For example, the oldest rhesus monkey reported to be alive at the Wisconsin Regional Primate Research Center was 33 years of age (10). In another comprehensive long-term study of aging in rhesus monkeys, the three oldest subjects were sacrificed at an estimated age of 31 years because of growing infirmity (11), and from a survey of available data Bowden and Williams (12) estimated that the maximum life span of captive macaque monkeys was 37 years. Other data on longevity are available from the California Primate Research Center, and show that the probability of rhesus monkeys that are housed in separate enclosures reaching 14 years of age is 0.35 (13). As compared to the rhesus monkeys in captivity, rhesus monkeys kept under wild or semiwild conditions have even shorter lives. For example, in 1983 it was determined that of the 1161 free-ranging rhesus monkeys on the Island of Cayo Santiago, the oldest living animals were five 21-year-old, one 22-year-old, one 24-year-old, and one 26-year-old rhesus monkeys (14).

The conclusion from these data has to be that under optimal conditions of individual housing and with medical care, only 30 to 40% of rhesus monkeys attain an age of 20 years, no more than 25% survive to 25 years of age, and few animals survive to 30 years of age. It should also be noted that these figures do not include deaths at birth. Were these to be included the numbers would be even lower. In summary, the typical adult lifespan of the rhesus monkey may be considered to extend from 5 to 30 years of age.

**COGNITIVE FUNCTION**

Using a predominantly cross-sectional approach and a wide variety of behavioral tasks, data have accumulated from several laboratories to suggest that monkeys, like humans, undergo age-related decline in several domains of cognitive function. Deficits have been reported primarily in short-term memory and executive system function. With respect to memory function, deficits in aged monkeys have been repeatedly found on the delayed nonmatching to sample (DNMS) task (15–20). This is a benchmark visual recognition memory task that has been used by several laboratories to assess memory in monkeys with selective telencephalic and diencephalic lesions (8, 21–23). The DNMS task requires a monkey to identify a novel object from a previously presented familiar one, with increasing delays interpolated between the two presentation trials. With this trial unique paradigm, a different pair of objects is used for each trial. Though aged monkeys show impairment on the acquisition and performance of this task, they do not show the disproportionately reduced accuracy over delays that occur in monkeys with selective temporal lobe lesions. In fact, several studies have reported normal to near-normal performance by a subpopulation of aged monkeys (15–18, 20). Thus, like aged humans, old monkeys take longer to learn something new, but do not appear to forget this information more rapidly over lengthening delays.

An impairment in memory function has also been observed using the delayed response (DR) procedure (17, 24–27). In this paradigm the monkey is allowed to view the baiting of one of two test wells and, after a delay, is required to select the correct one of the two that are now covered with identical plaques. Increasing delays on the DR task, unlike that for the DNMS task, produce a disproportionately poor performance by aged monkeys. This suggests that aged monkeys may be more sensitive to tasks that make particular demands on spatial and temporal memory, and to tasks that are characterized by higher levels of stimulus interference.

The notion that memory for spatial position may be disproportionately affected in aged monkeys has gained support from a recent study which used the Delayed Recognition Span Task (DRST) (28). The DRST is a short-term memory task that was designed to investigate spatial recognition memory in monkeys following bilateral removal of the hippocampus. Monkeys with lesions of the hippocampal formation or entorhinal cortex show marked
impairment on this task (29). The task requires the subject to identify, trial-by-trial, a new stimulus among an increasing array of serially presented, familiar stimuli. The task is administered by using different classes of stimulus material, space, color, or pattern, in order to help determine whether recognition memory deficits are generalized or modality specific. This task has been used to assess recognition memory function in patients with Alzheimer’s disease (6, 7) as well as in patients with either Huntington’s disease or Korsakoff’s disease (30).

Intermediate aged monkeys (19 to 24 years of age), as a group, are impaired on the spatial version of the DRST but not on the color version (28). This finding is similar to the declines in performance on delayed recall seen in middle-aged humans. Aged monkeys (25 to 29 years of age), as a group, are impaired relative to young adults (5 to 7 years) under both conditions of the task, achieving about two-thirds of the span of that of young adults. This suggests not only that the performance of monkeys on the spatial version of the DRST may be functionally equivalent to the performance of humans on difficult recall tasks, but also that spatial function may be more sensitive to aging than other stimulus domains.

Executive function is the other cognitive domain in which aged monkeys and humans evidence impaired performance. While there is an impressive literature on age-related decline in memory, there are fewer studies aimed at the assessment of executive function. Executive function is the term applied to such abilities as cognitive flexibility, cognitive tracking, set maintenance, and divided attention. In large measure, these functions share the requirement of an inhibition of an interfering response, a capacity that has been attributed to the dorsolateral prefrontal cortex in the primate. In humans, a variety of studies have been reported showing age-related impairment on tasks of cognitive flexibility and cognitive tracking (2).

Only a few studies have investigated executive system function in aged monkeys, but of these, two have used pattern discrimination reversal learning paradigms (25, 31). Reversal learning involves responding to a change in reinforcement contingencies by first “unlearning” or breaking the initial stimulus-reinforcement bond, and then acquiring, or “shifting” to a new one. Previous findings on reversal in aged monkeys have been equivocal. Bartus et al (25) reported severe age-related impairment in reversal learning, but Rapp (31) reported no marked impairment in a group of intermediate and very old monkeys. In a recent study, we found that compared to young adult monkeys, aged monkeys as a group were impaired on spatial, but not on object reversal learning (32). Secondly, aged monkeys made more perseverative responses than young adults on both spatial and object reversal learning. However, it is important to note that the performance of some monkeys in the aged group often fell

<table>
<thead>
<tr>
<th>Age</th>
<th>DNMS acquisition (n)</th>
<th>DNMS delay (n)</th>
<th>DRST spatial (n)</th>
<th>DRST non-spatial (n)</th>
<th>Spatial reversals (n)</th>
<th>Object reversals (n)</th>
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<tr>
<td>20 to 24</td>
<td>50%</td>
<td>58%</td>
<td>75%</td>
<td>33%</td>
<td>55%</td>
<td>33%</td>
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<tr>
<td>25 to 29</td>
<td>80%</td>
<td>67%</td>
<td>100%</td>
<td>29%</td>
<td>75%</td>
<td>50%</td>
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<tr>
<td>30 to 35</td>
<td>100%</td>
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within the range of young adult monkeys. This variability has been reported in virtually all aging studies on non-human primates (15–18, 20, 33).

To demonstrate the range of performance in our population of aged monkeys, data are presented in Table 1, which shows six measures of cognitive function: (a) DNMS, acquisition errors; (b) DNMS, Delays; (c) DRST, spatial span; (d) DRST, color span; (e) Spatial reversal performance; and (f) Object reversal performance. A summary measure was derived by principal components analysis and each monkey was then ranked. Any monkey that was 2.5 standard deviations or more below the population mean for young adult monkeys was classified as severely impaired. Table 1 shows the prevalence of severe impairment on each of these six measures for monkeys 20 to 24, 25 to 29 and 30 years of age and over. The numbers in parentheses indicate the total number of animals in that cell.

It would appear from the data in Table 1 that the spatial form of the DRST task, a task weighted for spatial position and memory load, may be more sensitive to early changes in cognitive function than tests which tap either function alone (e.g. DNMS acquisition, DNMS delays, or Spatial Reversals). This pattern is consistent with evidence that performance on delayed response tasks that have both spatial and memory components is particularly sensitive to aging (17, 24, 26, 27). It is of further interest that by the beginning of the fourth decade monkeys show impairment in virtually all aspects of executive system and memory function, and evidence particular difficulty in learning the delayed matching to sample task. However, individual aged monkeys often perform within the range of young adults. Consequently, like some humans, some monkeys age successfully, while others show evidence of marked age-related decline.

Taken together, these findings suggest that aged monkeys show impairment in a wide range of cognitive function, but, like humans, the emergence of impairment appears related to the stage of aging. Thus, in the monkey, an impairment of visuospatial abilities and spatial recognition memory appears in intermediate stages of aging.
(19 to 24 years of age) and new rule learning is significantly compromised only toward the latter part of the life span (over 29 years). However, as with humans, there are marked individual differences in the presence and degree of cognitive impairment in aging. Indeed, as mentioned above, some individuals evidence only mild and limited impairments throughout their entire life span.

AMYLOID, SENILE PLAQUES AND NEUROFIBRILLARY TANGLES

Memory loss in humans with Alzheimer’s disease (AD) is frequently attributed to the formation of senile plaques containing amyloid, and to the presence of neurofibrillary tangles that presage neuronal death, and then leave their marks like tombstones. Although old monkeys may develop plaques (34), they do not develop neurofibrillary tangles, evidence cortical neuron loss, or show the magnitude of behavioral deficits indicative of AD (30–33). Consequently it appears that monkeys do not develop the age-related changes characteristic of AD in humans.

Despite the absence of AD, monkeys do show an accumulation of amyloid, which is in the form of senile β protein (Aβ), in plaques and in the walls of blood vessels. Aβ is a 40-42 amino acid peptide derived by proteolysis from the amyloid β protein precursor (AβPP) (35). The Aβ protein is found in soluble form in blood and cerebral spinal fluid (CSF), and conformational change is required for Aβ to aggregate and precipitate in plaques.

To determine if Aβ and amyloid-containing plaques are likely to be part of a causal cascade, Heilbroner and Kemper (34) examined 55 cytoarchitectural areas in the cerebral cortex of three monkeys over 27 years of age. They found that the frontal areas and the primary somatosensory cortex had a predilection for plaques. Lower densities of plaques were present in the amygdala and insula, and in the cingulate, limbic, temporal, occipital, and parietal association cortices. The lowest densities were in the hippocampus and the primary visual and auditory cortices. This pattern of plaque distribution, which is similar to that found by Struble et al (36) in another population of rhesus monkeys, is quite unlike that encountered in the brains of humans with AD, since in these brains plaques occur with the greatest frequency in the hippocampus, amygdala, entorhinal cortex, and temporal and parietal lobes (37). However, one monkey, 35 years old, examined by Heilbroner and Kemper (34), had a density of plaques in its motor and premotor areas that reached a level comparable with that found in Alzheimer diseased brains. But comparable numbers have not been found in any of the other aged monkeys examined subsequently.

Continuing our studies on plaques, more recently a larger population of young and aged rhesus monkey brains, on whom full behavioral testing had been conducted, have been examined using two different monoclonal antibodies to identify the presence of Aβ in plaques (Sloane et al, unpublished data). As suggested by the study of Heilbroner and Kemper (34), only a few aged monkeys show large numbers of plaques. Thus of 8 monkeys over 23 years of age, only two of them, aged 28 and 30 years old, had significant numbers of plaques, and these were most prominent in the frontal lobe. Two other monkeys, 25 and 30 years of age, had few plaques, and the other four monkeys, two 23, one 25, and one 28 years of age, had no plaques. When the density of plaques is compared with the animal’s behavioral performance, it is clear that there is no correlation with behavioral dysfunction, although there is a trend of increasing plaque burden with age (Sloane et al, unpublished data). This confirms previous reports (36, 38) of others that plaque density and distribution fail to account for cognitive deficits observed in aged monkeys (36, 38). This also suggests that amyloid accumulation (as detected by several Aβ antibodies) is not a major pathogenic mechanism in the normal aging of the rhesus monkey brain, at least for the first 25 to 29 years of life, and it seems likely that we can discount plaques as being the principal underlying cause of the cognitive impairment displayed by the majority of rhesus monkeys.

Another factor that may explain why Aβ in monkeys does not have the pathogenic effects predicted from Alzheimer’s studies is the kind of amyloid that occurs in monkeys. By using antibodies to the Aβ1-40 and the Aβ1-42 epitopes, Gearing et al (39) concluded that in aged monkeys the Aβ1-40 predominates. This is in contrast to AD in humans, in which the Aβ1-42 epitope predominates. This suggests that there is a species difference in the processing of the APP molecule in the monkey, and that the monkey does not accumulate amyloid in the same fashion as the human. Consequently, in the monkey model of normal aging, which is unconfounded by AD, neither neuritic plaques nor amyloid appear to play an important role in age-related cognitive decline, a conclusion supported by a recent study of amyloid plaques in normal aging humans (40).

However, it has been demonstrated in in vitro experiments that the aggregated Aβ is toxic to neurons, so that an elevated amount of Aβ could be neurotoxic in vivo. Furthermore, Aβ is able to activate microglia in vitro. Activated microglia produce cytokines, complement components, and nitric oxide. All three of these compounds are known to have deleterious effects on neurons, astrocytes, oligodendrocytes, and myelin and it may be that the Aβ deposits seen in the aging monkeys directly, or indirectly, participate in a cascade of events leading to the changes in myelin that will be described in subsequent sections of this article.
NEURONS IN THE CORTEX AND HIPPOCAMPAL FORMATION

In attempts to explain the reason for the cognitive decline that occurs during normal aging, a number of investigators have focused upon whether there is a loss of neurons from the cortex of primates, including humans, during the aging process. One of the first to investigate this question in the aging human brain was Brody (41), who counted cells through the thickness in the human cerebral cortex from birth to 95 years of age. He concluded that there was a progressive and significant neuronal loss from the precentral gyrus, superior temporal gyrus, and visual cortex, but not from the postcentral gyrus. Subsequently, Brody (42) went on to study the superior frontal gyrus and reported that there is a loss of as many as 48% of the neurons between the 5th and 9th decade of life. These reports were followed by a number of others, including those of Henderson et al (43) who reported a significant loss of neurons with age from the pre- and postcentral gyri as well as from superior temporal gyrus, inferior temporal gyrus, and gyrus rectus. In the same year, Devaney and Johnson (44) determined the numbers of neurons in the human visual cortex and concluded that from 20 to 87 years of age about 50% of the neurons are lost. The conclusion drawn from these reports was that neuronal loss from the human cerebral cortex with age is significant and might account for age-related cognitive impairments.

However, in contrast to these studies there is another set of studies that indicates that there is no extensive loss of cortical neurons from human cerebral cortex with age. Such reports include those of Cragg (45), and of Anderson et al (46), who used automatic counting methods. This same technique was also used by Terry et al (47), who concluded from examining the brains of 51 normal individuals 24 to 100 years of age in the midfrontal, superior temporal and inferior temporal areas, that the numbers of neurons, neuronal density and percentage of cell area are all unchanged. However, despite the preservation of total numbers of neurons, Terry et al (47) did find a decrease in the numbers of large neurons with age, coupled with an increase in the numbers of small neurons, suggesting that there is a shrinkage of large neurons with age, rather than a loss.

A similar conclusion has also been reached by Haug and his colleagues (48, 49, 50) on the basis of measurements made on 120 normal brains. They examined six cortical areas and concluded that the decrease in neuronal density with age, noted by others, is largely due to the fact that during the preparation of tissue for microscopic examination, cortex from young brains shrinks more than that from old brains, so that the neuronal density is apparently greater in young brains than in old ones. When shrinkage is taken into account, Haug and his colleagues conclude that no significant numbers of cortical neurons are lost during the normal aging process. Leuba and Garrey (51) and Leuba and Kraftsk (52) arrived at a similar conclusion in the studies of the effect of aging on the human primary visual cortex.

Studies of neuron number in the limbic system have also produced only equivocal evidence of neuronal loss. Thus many early investigations examined neuronal densities in the human hippocampus and reported loss in most of the subfields. For example, in the human hippocampus Ball (53), Anderson et al (46), Mann et al (54), Miller et al (55) and Dam (56) all found age-related declines in pyramidal cell density. According to Ball (53), there is a decline of 5.4% per decade for all the CA subfields and Dam (56) found a decline of 21% between a young group (21 to 56 years) and an old group (68 to 91 years). In contrast, Mann et al (54) found no loss of neurons in the various subdivision of Ammon's horn, except in CA4 where they saw a loss of 25% comparing a group under 65 and a group over 65 years of age. Recently West (57), using updated stereological techniques, reported that there was a loss of neurons only in the hilus of the dentate gyrus (CA4) and the subiculum, and not in CA1 where most of the investigators reported losses. Aside from the many technical problems that can contribute to such diverse results, an unaddressed problem with such studies of “normal” human aging is the potential presence of early cases of undiagnosed Alzheimer's disease since in this disease neurons are clearly lost. This problem is highlighted by a recent study of Gomez-Isla et al (58), in which exceedingly strict neuropsychological criteria were applied to separate “normal” aged cases from early Alzheimer cases. These investigators reported that even mild cognitive impairment was associated with neuron loss in entorhinal cortex, but that there was no age-related loss of neurons in the entorhinal cortex of “normal” aged adults compared with young adults.

What seems to emerge from these studies is that with better preservation of the material being examined, with increasingly sophisticated methods of neuron counting, and with more awareness of the need to ensure that brains being examined do not include ones with Alzheimer's disease, there is no strong evidence for an extensive loss of neurons from the human cerebral cortex or hippocampus during the normal aging process.

The studies on the effect of age on the cortical neurons of the human brain have been described in some detail because there have been few studies of the effect of age on the monkey cortex. Prior to our own studies, the only data were those generated by Brizdee and his colleagues (59–61). They examined the sensorimotor cortex of rhesus monkeys 4 to 6 years old and 18 to 20 years old and found no change in the thickness of the cortex with age. But they did find a significant decrease in the packing.
density of neurons, especially in the middle depths of the
cortex, leading them to conclude that there is a loss of
neurons with age. Subsequently, Brizzee et al (61) ex-
amined the gyri of the frontal cortex bordering the prin-
cipal sulcus, as well as the hippocampus. In the frontal
cortex they found the number of neurons per traverse
through the thickness of the cortex to be lower in the old
monkeys than in the young ones, and they obtained a
similar result in the CA1 zone of the hippocampus. Con-
sequently, these observations of decreased neuronal den-
sity are in accord with much of the early data from hu-
mans, before Haug and colleagues pointed out the pitfall
of differential shrinkage.

In contrast to the results obtained by Brizzee and his
colleagues, our studies of the effect of age on the cerebral
cortex and hippocampus of the rhesus monkey have led
us to the conclusion that, as in humans, there is no sig-
nificant loss of neurons with age. In our examination of
the primary visual cortex (62, 63) and of area 46 in the
prefrontal cortex (64) tissue was taken from monkeys that
had been behaviorally tested and fixed by perfusion, so
that the cortex could be examined both by light and elec-
tron microscopy. The cortices of young monkeys (4 to 6
years of age) were compared with those of old monkeys
(over 24 years of age), and the approach taken was to
count the numbers of profiles of neurons displaying nu-
clei in 250-μm-wide strips of 1-μm-thick plastic sections
passing through the depth of the cortex. To ensure that
the sections were not oriented obliquely, they were cut
to pass in a plane parallel to the lengths of the apical
dendrites of the pyramidal cells. Provided that the sizes
of the neuronal nuclei, which are the counting objects,
do not change, and they did not, the counts then reflect
the numbers of neurons beneath a unit area of cortical
surface. In both area 17 (62) and in prefrontal area 46
(64) there was no indication of a change with age in the
numbers of neuronal nuclear profiles through the depth
of the cortex or in the thickness of the cortex, and with
the exception of a few neurons in layer I, an electron
microscopic examination revealed no cytotological
changes in the cell bodies of the neurons, beyond a slight increase
in the amount of lipofuscin in the perikarya.

Because the data generated by Terry et al (47) had
suggested that large neurons shrink with age, Peters and
Sethares (63) also examined a distinct population of large
neurons, the Meynert cells in layer VI of primary visual
cortex. There is no indication that Meynert cells are lost
with age and there is no change in the sizes of these
neurons, which surprisingly accumulate very little lipo-
fuscin as they age. A similar approach was used to ex-
amine the effects of aging on the large Betz cells and
other neurons in area 4 of the rhesus monkey (65). In
this case the cortices of monkeys ranging in age from 1
day old to 35 years were examined in frozen sections
passing through the depth of the motor cortex. No age-
related loss of the total numbers of neurons in strips
of sections passing through the depth of the cortex were
encountered. The large Betz cells in this cortex were as-
sessed separately, and although there was no indication
of a loss of Betz cells with age, their cell bodies did
decrease slightly in size and they began to accumulate
lipofuscin even in monkeys only 5 years of age. Lipo-
fuscin continued to accumulate with age and the Betz
cells in old monkeys had so much lipofuscin that the
nucleus was pushed to one side of the cell body. This is
in marked contrast to the effect of age on the large Mey-
nert cells in primary visual cortex, which, as pointed out,
accumulate only small amounts of lipofuscin (63). It was
also found that some Betz cells in older monkeys develop
perikaryal inclusion bodies that consist of a regular array
of three sets of equidistant, parallel sheets of filaments
(66). The origin and significance of the almost crystalline
inclusions are not yet known, but they might result from
an overproduction of neurofilamentous protein by the
Betz cells. Similar inclusions have not been encountered
in Meynert cells.

The conclusion from these studies of cortex is that
there is no significant loss of neurons beneath unit areas
of the neocortex as rhesus monkeys age, and no evidence
that cortical neurons are dying. Indeed, apart from an
accumulation of lipofuscin in some cells, the cytolology
of the neuronal cell bodies is not appreciably altered with
age. It is also worth noting that all the counts were made
in brains that were well fixed and carefully processed so
that the age-related differential shrinkage that can con-
found such studies (48, 49) was avoided.

Of course it can be argued that even if these recent
data are correct, there could still be an overall loss of
neurons if the volume of the cerebral cortex diminishes
with age—the reference volume problem addressed by
West, Oorschot, and others (57, 67, 68). In an attempt to
determine if there is a loss of cortical volume and hence
of total neuron number with age, Peters et al (unpublished
data) measured the surface area of the primary visual
cortex using sets of serial Nissl-stained frozen sections
taken through the occipital cortices of six rhesus monkeys
between 4 and 12 years of age and compared them with
those of eight monkeys over 25 years of age. In this pop-
ulation of monkeys it was calculated that the overall sur-
face area of area 17 varies from about 700 to 1,200 mm²,
with a mean value of 956 mm², a surprisingly large be-
tween-subject variability. Nevertheless, there was no in-
dication that the surface area or the volume of the pri-
mary visual cortex decreases with age. Interestingly,
there are similar large variations in the volume of human
primary visual cortex (e.g. 52). The large volume vari-
ations among individual monkeys and humans mean that
even if there are small losses in neurons with age, large
numbers of individuals would have to be examined be-
fore statistically significant values could be obtained.
Rosene et al (unpublished data) have also conducted a similar set of studies of the neuronal population in the hippocampal formation. In a series of 14 well-fixed monkey brains, the CA1 subfield and subiculum were examined throughout the longitudinal extent of the hippocampal formation. Neuronal counts revealed no age-related change in neuronal density within either the CA1 subfield or the subiculum. There was also no change in the thickness of the pyramidal cell layer, or in the sizes of the neurons. To address the issue of reference volume, two approaches were used. First the hippocampus was traced in its entirety and subdivided into individual subfields on a randomly chosen series of sections that systematically sampled the entire hippocampus. The Cavalieri estimator was used to calculate the volume of the hippocampus and this revealed that there was no age-related change in either the total volume of the hippocampus or in any of its subfields. Since there was also no change in neuronal density, this would indicate neurons are not lost from these subfields of the monkey hippocampal formation. Finally, because counts of neuronal density suffer from bias due to assumptions about the shape of the counting target and section thickness (57, 69), a subset of these cases was reanalyzed with the optical dissector and fractionator approach (67), which provides an estimate of the total number of neurons in a structure. The same result was obtained, namely, that there is no aggregated loss of neurons from the CA1 or subicular subfields of the hippocampal formation. This result is in accord with similar observations made on a smaller set of monkeys by Amaral (70), so that as in the neocortex, there is no evidence of age-related loss of neurons in the hippocampus.

However, the survival of neurons does not guarantee that they are functionally intact. A variety of functions, from membrane properties to neurotransmitter synthesis and neurotransmitter receptor composition could contribute to neuronal dysfunction, as could a reduction in oxidative metabolism (71). While little direct work has been carried out in primates, one study (68) has reported that homogenates of frontal and parietal lobe cortex showed an age-related reduction in the enzyme cytochrome oxidase. In addition, reductions in the levels of this enzyme have been reported to occur in Alzheimer's disease (72, 73). This enzyme is the final step in the mitochondrial oxidative metabolic pathway leading to the synthesis of ATP. Hence the reduction suggests that cerebral metabolism may be compromised. Rosene et al (unpublished data) have investigated this further with a combined study using Positron Emission Tomographic (PET) scanning and cytochrome oxidase histochemistry. The PET study demonstrated a statistically significant reduction in global cerebral blood flow in tranquilized monkeys. Cryostat sections from some of these monkeys were then processed for cytochrome oxidase (COX) histochemistry and for in situ hybridization with oligo probes to the mitochondrial-encoded COX subunit II and the nuclear-encoded COX subunit IV. Image analysis of the density of the reaction product over both cellular and molecular layers of the hippocampus as well as over the entire motor cortex, and the superior temporal gyrus of aged monkeys demonstrated that there was a statistically significant reduction in both COX reaction product and in the density of probe for subunit II, but not subunit IV. This suggests that the reduced blood flow detected in the PET study may be a response to reduced metabolic demand caused by reduced levels of COX activity in individual neurons of these areas, since other data demonstrates that neurons are not lost. While the causes and full implication of these findings remain unclear, they do raise the possibility that even though neuron number is preserved, cortical neuronal function may be compromised.

The conclusion from these studies is that in concert with the recent data from the human brain, recent observations on visual cortex, motor cortex, prefrontal cortex, and the hippocampal formation of the monkey strongly suggest that cortical neurons are not lost with age. Hence some other reason, such as reduced cytochrome oxidase activity, must be sought to explain the age-related deficits in cognitive functions that depend upon the cerebral cortex.

### DENDRITES, AXONS, AND SYNAPSES

Even if age has no significant effect in reducing the numbers of neuronal cell bodies, it is possible that their dendrites and axons are affected. The literature dealing with age-related dendritic changes in primates has been reviewed by Coleman and Flood (74). As they point out, the few studies that have been carried out have used the Golgi method, which is not particularly reliable for quantitative studies. However, using this method, Copp and Uemura (75) examined the pyramidal cells in layers 3 and 4 of the superior frontal gyrus of monkeys 7 to 28 years of age, and concluded that until 20 years of age there is a continued growth and branching of the apical dendrites of the pyramidal cells, while their basal dendrites continue to grow, but do not add branches. However, in the older animals there seemed to be a loss of branches from both the basal and apical dendritic trees, as well as a reduction in the population of dendritic spines. Later Uemura (76) reported a loss of about 25% in the spine population. In their review Coleman and Flood (74) report that similar regressive changes in dendrites have been described in the aging human cortex. In our electron microscopic studies of the aging rhesus monkey cortex few changes have been found in the neuropil beyond degeneration of the tufts of the apical dendrites of pyramidal cells in layer I (Peters, unpublished data). Interestingly, in both primary visual cortex and in area 46 of prefrontal cortex, layer I becomes thinner with age, and electron microscopic examination shows that many
of the spiny dendrites in this layer lose their characteristic organelles and at the same time accumulate membranous inclusions. This suggests that the apical dendritic tufts of pyramidal cells may be undergoing degenerative changes, but a more complete study needs to be undertaken before a definite statement to this effect can be made. Moreover, in these same cortices from old monkeys the glial-limiting membrane formed by astrocytes often becomes very thick and is composed of numerous layers of astrocytic processes whose cytoplasm contains a profusion of filaments (77). After making a more careful analysis of the effect of age on layer I it was surprising to find that the thinning of layer I in area 17 seems to be purely an effect of age, and is not related to cognitive dysfunction. In contrast, in layer I of area 46 the amount of thinning correlates with the decline in cognitive function of individual monkeys. This may be especially significant in light of the fact that area 46 of prefrontal cortex is an association area that has been often implicated in cognitive function.

The literature on the effects of age on the synapses in cortices of primates appears to be equally modest. Using ethanolic phosphotungstic acid (E-PTA) to label synaptic junctions for electron microscopic examination, Uemura (76) found a loss of synapses in older monkeys, paralleling the loss of dendritic spines described above. A comparison of the numbers of synaptic junctions in the prefrontal cortices of young and old monkeys indicated a loss of 27% of synapses in the upper third of the cortex, of 21% in the middle third, and 18% in the lower third. Huttunen (78) reported a similar age-related loss of synapses from the human frontal cortex. In contrast, the three studies that our group has carried out on monkeys do not indicate that synapses are lost with age. Using electron microscopy, Tigges et al (79) examined the axon terminals synapsing with the perikarya of Betz cells in area 4, and found no change with age in their total numbers. This result agrees with that obtained by Zecevic et al (80) who found no change in the frequency of symmetric synapses in the neuropil of area 4 in rhesus monkeys between 3 and 20 years of age. In another study, Tigges et al (81) have examined the synapses in the outer third of the dentate gyrus of the rhesus monkey, where the cortical afferents from the entorhinal cortex terminate. Again, no changes were found in the total numbers of synapses with age. However, when synapses were subdivided into those involving spines and those involving dendritic shafts, it turned out that the shaft synapses (which account for only about 13% of the total number of synapses) showed a statistically significant loss (p < 0.04). However, in terms of the total number of synapses, this loss of shaft synapses would amount to only a 3% loss of synapses over the total 35-year life span of the rhesus monkey.

Because of the importance of synaptic loss, the study of the hippocampal formation was extended to the inner third of the molecular layer of the dentate gyrus, where mainly intrinsic association and subcortical afferents terminate (Tigges et al, unpublished data). Using electron micrographs and the dissector method of analysis, the number of synapses per unit volume was determined in young and old monkeys, and again, no age-related loss of synapses was found in the inner third of the molecular layer of the dentate gyrus.

In the context of potential alterations in the synaptic population with age, it is worth pointing out that Beal et al (82) detected no differences in the levels either of glutamate, the principal excitatory neurotransmitter in the cortex, or of GABA, the principal inhibitory neurotransmitter, an observation entirely congruent with the finding that synapse numbers are largely preserved during aging.

In contrast to our electron microscopic observations on well-fixed monkey brains, Masliah et al (83) used synaptophysin antibodies to label axon terminals in immersion-fixed human prefrontal cortex and reported that analysis of confocal microscope images indicated that there is an age-related loss in the numerical density of synapses. While this contrary observation may be a consequence of examining different anatomical locations, or using different methodologies, it could also reflect the presence of early Alzheimer's cases in the sample examined by Masliah et al (83). In any case it will be important to initiate additional studies of aged human and rhesus monkey material to resolve the question of whether there is a preservation or diminution of synapse number within various portions of the telencephalon.

SUBCORTICAL EXTRINSIC INPUTS TO CORTEX

The vast majority, probably over 95%, of the synapses in cerebral cortex are formed by the intrinsic neurons. For example, the most extensive extrinsic input to primary visual cortex in the monkey is from the dLGN, but this accounts for only about 5% of the input to layer 4 (84). In addition to such specific thalamic afferents, there are diffuse extrinsic inputs to cerebral cortex from a number of subcortical nuclei including the midline thalamus, the substantia nigra, ventral tegmental area, median raphe nuclei, locus coeruleus, and nucleus basalis, and it is these diffuse inputs that do seem to be affected by aging. Thus, using monkeys 2 to 18 years of age, Goldman-Rakic and Brown (85) have shown that with age there is a decrease in the endogenous concentrations of dopamine in some regions of the cortex, but the decrease is most evident in the prefrontal cortex, where it is over 50%. A reduction in the level of dopamine with age has also been demonstrated by Beal et al (82). Presumably most of this dopamine is derived from the terminals of neurons in the substantia nigra or the ventral tegmental area, and the preliminary studies on the monkey indicate that there is a significant loss of neurons with age from these nuclei.
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(86, 87). Beal et al (82) have also demonstrated a reduction in the level of choline acetyltransferase (ChAT) with age in monkeys. ChAT is the enzyme involved in the synthesis of acetylcholine and a reduction in the levels of this enzyme presumably reflects changes in the basal nucleus of Meynert, which is the main source of the cholinergic input to cerebral cortex. However, data on age-related changes in the cholinergic basal forebrain is still equivocal. For example, studies of the basal forebrain (88) indicate an age-related loss of acetylcholinesterase positive (and presumptively cholinergic) neurons throughout all of the magnocellular nuclei. Amaral and colleagues (70, 89) have also demonstrated a loss of ChAT-positive neurons in the medial septal nucleus. On the other hand, using antibody to the p75 nerve growth factor receptor, which is another marker of presumptive cholinergic neurons, Voytko et al (90) reported that there is no age-related loss of neurons in the nucleus basalis. Clearly, a comprehensive study of the cholinergic magnocellular nuclei using multiple cholinergic markers is required to resolve this issue.

Beal et al (82) have also shown reductions with age in the levels of somatostatin, neuropeptide Y, norepinephrine, and serotonin in the occipital cortex and cerebral cortex of the monkey. Kemper (91) has examined neuron numbers in the raphe nuclei of the midbrain, which are the principal source of serotoninergic projections to the cerebral cortex, and has found that there is a clear age-related loss of neurons both in the dorsal raphe and in the median raphe nuclei. Moreover, preliminary comparisons of neuron numbers with behavioral measures have identified a number of strong correlations between loss of neurons and behavior. These include a statistical correlation between the age-related diminution of cell packing density in both the pars paragranilis of the ventral tegmental area and the median raphe nuclei with an overall score of decline in age-related cognitive function (both p < 0.01) and with the delayed nonmatching to sample test (both p < 0.05).

It is interesting to note that dopaminergic and cholinergic fibers provide a significant input to layer I (e.g. 92, 93), where we have observed a thinning of this layer as well as the presence of dystrophic dendrites. There is also a concomitant thickening of the glial limitans (77) that might indicate the formation of a scar in response to degeneration of the apical dendrites and the subcortical afferents. Vogt (94) has advanced the notion that because of its rich cholinergic innervation from the nucleus basalis, layer I may play a role in attention “setting” or gating. If this is true it would place layer I in a pivotal position to regulate information processing within the cerebral cortex. Indeed there is evidence that in one rare form of dementia, called progressive subcortical gliosis, the degenerative changes in the cerebral cortex are disproportionately localized to layer I (95–98).

Smiley and Goldman-Rakic (99) have examined the role of the dopamine-containing axons in the monkey prefrontal cortex and find that the labeled terminals form small symmetric synapses. However, only 39% of the vesicle-filled terminals form synaptic junctions. The termination of the cholinergic axons in the prefrontal cortex is somewhat similar, for Mrzljak et al (92) find that when the terminals form synaptic junctions they are symmetric ones. But over 50% of the cholinergic terminals do not form definitive synaptic junctions, even though they are apposed to spines and small dendrites. Mrzljak et al (92) conclude that acetylcholine has a dual modulatory effect in the cortex, acting both through conventional synapses or through nonsynaptic appositions. For both dopaminergic and cholinergic synapses, the main postsynaptic targets appear to be pyramidal neurons.

The conclusion from these studies is that there is evidence of significant age-related loss of neurons from the dopaminergic substantia nigra and ventral tegmental area, the serotonergic raphe nuclei, and probably the cholinergic basal forebrain, along with a reduction in their specific markers in the cerebral cortex. Although subcortical afferents to the cerebral cortex account for only a small fraction of the total number of synapses in the cerebral cortex, the loss of the cells of origin of this subset of synapses has shown strong correlations with behavioral deficits that are generally attributed to cortical function. These subcortical afferents have in common a prominent projection to layer I, a cortical layer that shows an age-related thinning, and it seems likely that the neuronal loss and the cortical thinning are causally related.

NEUROGLIAL CELLS

Although cortical neurons appear to show few changes with age in the rhesus monkey, this is not true of neuroglial cells because a striking change in cortex is that astrocytes and microglial cells in the aging monkey usually contain phagocytosed debris (see 64, 77, 100). The debris is so obvious that when histological preparations are examined these cells are much better indicators than the neurons that a cortical sample is from an old monkey (100). Interestingly, although the appearance of the phagocytosed material in each neuroglial cell type within cerebral cortex is very characteristic, its appearance gives no clue as to its source. However, the frequency of occurrence of debris inside glial cells is likely to be a good indicator of the extent of degeneration that has occurred within the cortex of a particular old monkey.

As far as we are aware, apart from the studies of Peters et al (100), there have been no systematic studies on the effects of aging on the numbers of neuroglial cells in monkey cortex. This study suggests that the total numbers of glial cells increases only slightly during aging, due to an increase in the number of oligodendroglia and microglial cells. Thus, in young monkeys (5 and 6 years
of age) microglial cells account for about 7.6% of the
neuroglial cell population, but in old monkeys (over 25
years of age), they account for 9.4% of the total popu-
lation. But because of the increase in the total numbers
of neuroglial cells, the population of microglia cells in-
creases by an average of 44% with age. Since the mi-
icroglia are the principal phagocytic cells of the CNS, this
increase raises the possibility that they may play a major
role in age-related changes in the cortex.

As far as we have been able to ascertain, the oligo-
dendroglial cells, which are responsible for the formation
of the myelin sheaths in the central nervous system, do
not undertake phagocytosis. Nevertheless their cell bod-
ies do accumulate inclusions with age and in areas 46
and 17, at least, their processes tend to become thicker
and develop bulbous swellings. These swellings also con-
tain inclusions that are similar in appearance to the ones
within the perikaryon (101). An intriguing question is
whether these changes in the oligodendrocytes are cau-
sally related to the degenerative changes in myelin that
occur with increasing frequency in the brains of old mon-
keys (e.g. 64).

WHITE MATTER AND MYELIN

A point that is often overlooked is that in aging the
loss of white matter is obviously greater than the loss of
gray matter. For example Haug and Eggers (50) report
that while the volume of the entire cortex is reduced by
only 3% when brains of humans aged 20 to 40 years are
compared with those aged 75 to 85 years, the volume of
white matter is reduced by 11%. And after examining the
volumes of components of brains from nondemented per-
sons aged 69 to over 80 years of age, Anderson et al (46)
report that compared to the 69 to 74 age group, the brains
of persons over 80 years of age show a 2.6% decrease in
the volume of cortex and an 11.4% decrease in white
matter. These results have been confirmed by Albert (2)
who obtained axial MRI scans from 70 carefully screened
human subjects ranging in age from 30 to 80 years. She
found that although the volume of gray matter does not
decrease with age, there is a decrease in the amount of
white matter. This is accompanied by an increase in the
amount of space occupied by cerebrospinal fluid. Other
observations on changes in the white matter with age have
been reported by Kemper (37) who examined myelin-
stained sections of human brains in the Yakovlev collec-
tion. Kemper (37) found that the staining of the white
matter in the older normative brains is much paler than
in the younger ones, indicating a loss of myelin, and con-
cluded that this pallor is most common in the cortico-
cortical fibers of the centrum semiovale and the stratum
sagittale interna. Similarly, Lintil and Braak (102) have
reported that the myelin staining in the stripe of Gennari
in the human cortex is gradually reduced with age in
normal human brains ranging in age from 18 to 96 years
of age.

While these data from human studies are provocative,
the monkey offers the opportunity to determine how age-
related changes in white matter relate to cognitive dys-
function. As a first step we have begun a systematic study
of the MRIs of our behaviorally tested monkeys using
point counting on MRI scans to compare the brains of 8
young (5 to 14 years) and 8 elderly (20 to 32 years of age)
monkeys. As in humans there is a significant age-
related decline in the overall forebrain volume. Although
the volume of gray appears to decrease slightly, it is not
statistically significant. However, there is a much greater
and statistically significant decrease in the volume of
white matter, as well as a statistically significant increase
in the ventricular volume (Rosene et al, unpublished
data). A comparison of these data with the behavioral
performance of the individual monkeys reveals a signif-
icient correlation between the extent of the white matter
loss and overall cognitive dysfunction.

Although these volumetric studies indicate gross
changes in white matter, they cannot reveal whether white
matter is being lost because axons are degenerating, or
whether myelin is degenerating. However, in our electron
microscopic preparations it is obvious that in the cortex
the myelin of many sheaths is breaking down or losing
integrity, leaving the axons largely unscathed. Although
we have noted this myelin dystrophy in a number of pub-
cations (e.g. 62, 64, 77, 79, 81), the most striking find-
ing emerged in a study of the effect of aging on the
prefrontal cortex (64). It was found that when the old
monkeys are ranket in a sequence from the ones that
show the least to the ones that show the most extensive
myelin degeneration, there is a correlation between the
extent of the myelin breakdown and the performance def-
cits of the monkeys on recognition memory tasks. It is
important to emphasize that the correlation is not with
age, but with myelin degeneration. For example, one
31-year-old monkey performed better and had less myelin
degeneration than a 27-year-old monkey.

CONCLUSIONS

Our studies indicate that aging monkeys show the same
kinds of cognitive impairment displayed by normally ag-
ing humans, and that although the degree of impairment
increases with age, it is not a simple function of age. Like
humans, some monkeys show more impairment than oth-
ers at the same chronological age. The ones that show
the least impairment can be regarded as aging "success-
fully", and the goal of research into the effects of normal
aging is to determine what makes this successful aging
possible.

Some hints of how this might be achieved are emerg-
ing from our studies, since they reveal that there are dif-
f erent patterns of changes in the brain, relative to the
cognitive decline in our aged monkeys. While these are tentative conclusions that need to be confirmed in a larger sample of animals, they suggest that aging in the brain follows one of three different patterns. Some parameters such as neuronal and synapse numbers in cortex and hippocampus do not appear to change significantly with age, and while there are many obvious changes in neuroglial cells, it seems unlikely that they are directly responsible for the cognitive decline. These cells may be simply responding to the degenerative alterations in other components of the cortex, but on the other hand they may be initiating some of the pathogenic changes. In contrast, some changes appear closely correlated with both the degree of cognitive decline and chronological age, and yet others are correlated only with the degree of cognitive decline, or only with chronological age. Among the ones that can be correlated both with cognitive decline and chronological age are decreases in cell density in cortically projecting brainstem nuclei (the median raphe nucleus and the dopaminergic paragranular of the substantia nigra/ventral tegmental area). In contrast, decreases in thickness of layer I of area 46 of the frontal cortex, the breakdown in the integrity of myelin, and the reduction of overall forebrain white matter volume are significantly correlated only with cognitive decline. Other alterations, such as decreases in thickness of layer I of area 17, decreases in cell number of the striatally projecting pars compacta of the substantia nigra, and frequency of neuritic plaques are positively correlated with chronological age, but not with cognitive decline. All of these changes have significance, but the ones that are of particular interest are those changes that correlate with cognitive decline but not age, because these are the changes that must be addressed if “successful” aging is to be facilitated. Being able to prevent or ameliorate some of the changes of this type could provide the greatest social benefit.

On the basis of the existing data there seem to be three processes that correlate with cognitive declines. One is the thinning of layer I that is probably associated with a decrease in the cell density in brain stem nuclei, leading to a diminution in the extrinsic subcortical input to the cortex and to degeneration of the apical dendritic tufts of pyramidal cells. We know little about the significance of these changes at the present time. The second is the extent of myelin breakdown, and the third, which is likely to be the consequence of the myelin breakdown, is the volume reduction in white matter as measured by MRI scans. A breakdown of the integrity of the myelin sheaths of axons would bring about a reduction in the speed with which the affected fibers conduct impulses (e.g., 103, 104). Similar degenerative changes in myelin have long been known to occur in the peripheral nervous system, in which they lead to nerve conduction abnormalities and to slowing down of the conduction rate (e.g., 105). A change in the conduction rates of myelinated axons involved in cortical circuits would lead to a disruption or loss of coherence in the critical timing of synaptic events upon which neuronal circuits depend for their normal functioning, and could well bring about the changes in cognitive behavior displayed by aged primates. As far as we are aware, the role of myelin in the aging process has not been previously emphasized, but before its role in cognitive decline can be firmly established more work needs to be done to confirm the correlation between myelin breakdown and the behavior of aged monkeys. At the same time we must determine what brings about the disruption of the oligodendroglial/myelin system during the aging process. Then studies can be initiated to determine how this disruption can be retarded and “successful” aging facilitated.

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