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Comparative Neuropathology of Brain Aging in Primates

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Among primates only humans exhibit a significant degree of pathological changes in the brain associated with deficits in certain cognitive functions during aging. In nonhuman primates pathological alterations have a more variable expression among individuals and cognitive decline appears less consistently than in humans [1, 2]. Therefore, nonhuman primates offer a remarkable opportunity to study brain aging in absence of the cellular changes that cause some of the most devastating age-related neuropsychiatric illnesses commonly seen in humans. Ceboid and cercopithecine species including those most frequently used in laboratory research, such as squirrel monkeys (*Saimiri*), macaque monkeys (*Macaca*), and baboons (*Papio*) have a potential life span in captivity of over 25 years, with some individuals surviving beyond 30 years, and a maximal longevity of approximately more than 40 years for macaques [3]. Great apes (*Pongo*, *Gorilla*, and *Pan*) have life spans in captivity extending considerably beyond that of macaques: the maximum life span of chimpanzees is above 60 years and many captive great apes live into their sixth decade (as we write,

there are over 200 chimpanzees older than 35 years of age in the USA alone [2]). At such an advanced age, these animals present all of the characteristic somatic senescence-associated changes that are typically observed in elderly humans, as many suffer from illnesses commonly seen in old people. Few aspects of brain aging have been addressed in the great apes, partly because few great apes in the past reached very advanced age in captivity, and partly due to the difficulty of obtaining a sufficient number of brains from great apes of different ages. In New World and Old World monkeys, brain alterations include amyloid deposition in several brain structures and impairment of delayed non-matching-to-sample task, delayed recognition span task, spatial and object reversal learning [4–19].

Monkeys are the only practical laboratory animal model to be so closely related to humans, and as such, a wide range of data on the process of primate senescence can be easily obtained from aging populations [3, 13, 18, 20–22]. In addition, macaque monkeys have a large repertoire of behaviors that can be extensively investigated by neuropsychological testing batteries derived from tasks developed originally for humans [19]. Monkeys and great apes apparently do not suffer from age-related neuropsychiatric illnesses which occur only in human, such as Alzheimer's disease, and therefore provide a model in which the effects of aging can be studied independently from the confounding effects of a concomitant dementia [22]. It is likely that substantial widespread cortical and subcortical dysfunction occurs in aged nonhuman primates, in view of the wide range in behavioral deficits that have been recognized, even though the cellular substrates of such changes are not fully described [23, 24]. Clearly, other factors, including hormonal status, reproductive senescence, dominance history, social rank, and degree of parenting, all contribute to the severity of cognitive decline and longevity potential in these species [25–29]. However, the vast majority of morphological studies of brains of aged nonhuman primates indicate that very few changes take place during aging. Importantly, all current studies agree that no decrease in total neuron numbers in the cerebral cortex and in subcortical structures occurs in nonhuman primates. However, a breakdown of myelin integrity and variable thinning of layer I in the cerebral cortex have been reported, mostly from studies of macaque monkeys [1, 2, 13, 21, 30–43]. Recent data on amyloid deposition, possible occurrence of Alzheimer's disease-type neurofibrillary degeneration, variability in estimates of volumetric and region- and cell type-specific changes in neuronal numbers and morphology, ultrastructural changes, and subcellular pathology at the level of neurotransmitter systems and their receptors have provided valuable insight into etiopathogenetic mechanisms leading to pathological alterations that occur during aging in the brain of old nonhuman primates in the context of identified neuronal circuits.

Age-Associated Deposition of Amyloid in Nonhuman Primates

Amyloid deposition in the form of senile plaques and diffuse deposits is thought to be a crucial factor in the development of Alzheimer's disease. It is consistently seen in the brain of elderly humans, independently of cognitive decline, as it can be observed in high levels even in cases displaying only very mild cognitive impairment or presenting with a normal cognitive status [44]. The amyloid protein and its precursor are highly conserved in vertebrates [45–47], and amyloid deposits have been reported during aging in the brains of trout and woodpeckers [48, 49]. Amyloid deposition has been studied in detail in many mammalian species as well as in several transgenic mouse models of Alzheimer's disease [10, 11]. In monkeys, the amyloid precursor protein has the same cellular localization as in human, as well as in large diffuse deposits and in neurites surrounding senile plaques in aged animals [50]. Furthermore, the predicted sequence of the 695-residue peptide in macaque monkey is similar to that of the human [46]. Thus, the frequent occurrence of amyloid protein in the brain of aged prosimians, monkeys, and great apes represents an interesting model of the dynamics of amyloid deposition in Alzheimer's disease, and permits assessment of the potential toxicity of the amyloid β protein ($A\beta$) in nonhuman primates [10, 11, 46, 51–62]. Electron-microscopic analyses of senile plaques in old macaque monkeys have shown that they resemble closely those seen in Alzheimer's disease, with a central dense amyloid core (fig. 1), although they do not exhibit the characteristic twisted tubules in monkeys [63].

In macaque monkeys, senile plaques are found predominantly in the prefrontal and primary somatosensory cortices, whereas lower densities of deposits have been reported in the hippocampus, insula, cingulate, motor, auditory, visual, temporal and parietal cortices and amygdala [9]. This distribution differs considerably from that observed in Alzheimer's disease patients in whom these lesions predominate in the temporal limbic and parietal regions [64]. Amyloid plaque densities are not correlated to cognitive deficits in old animals, as in humans [44, 65, 66]. In fact, a consistent burden of amyloid is seen only in some animals older than 25 [66]. Furthermore, a certain degree of glial response appears to occur prior to the development of senile plaques in macaque monkeys [67, 68]. It has been shown that the earliest change associated with the formation of senile plaques is the development in presynaptic terminals and dendrites of electron-dense bodies and the accumulation of the amyloid precursor protein in astrocytes and microglial cells [69]. In old monkeys, amyloid deposits containing nonfibrillar $A\beta$ have been documented in neurites, dendrites and glial elements, whereas the $A\beta$ peptide is found in extracellular fibrils around mature plaques, suggesting that synaptic pathology precedes the deposition of amyloid precursor protein in neurites and glial cells.

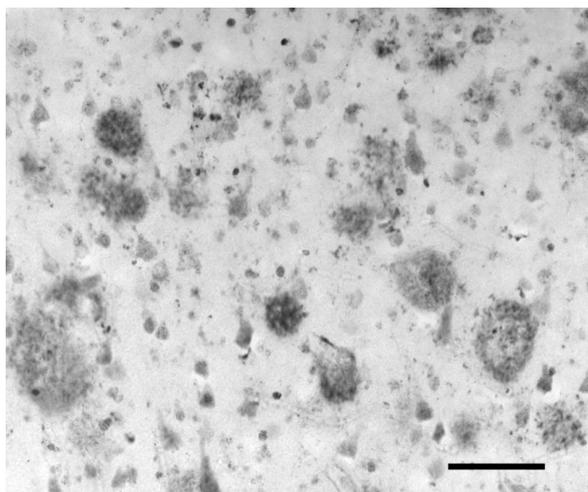


Fig. 1. Amyloid deposition in the temporal cortex of a 32-year-old rhesus monkey (*M. mulatta*). Classic senile plaques with well-visible amyloid cores as well as smaller deposits are numerous. Materials were immunoreacted using an antibody against A β 1–40. Scale bar = 100 μ m.

There is indeed evidence that amyloid protein elicits neuronal toxicity in old macaques. In fact, experiments in which A β 40 peptide was injected into the neocortex of long-tailed macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*) resulted in the presence in old animals of tau- and ubiquitin-immunoreactive neuronal somata and neuritic materials around A β depositions [57]. Similarly, injections in the neocortex of young and old macaque monkeys of fibrillar A β at concentrations equivalent to those found in senile plaques revealed age-dependent toxicity of A β [56], with severe neuronal loss, gliosis and formation of hyperphosphorylated tau-containing tangle-like lesions. These changes were not present in young animals and were not as severe in aged New World marmoset monkeys (*Callithrix jacchus*) compared to macaques. Injections of homogenates of human brains with Alzheimer's disease in the brain of marmosets could induce plaque formation independently of age, as amyloid accumulation is seen in this species only in animals older than 11 years [62]. Also, such lesions were not observed in rats suggesting that A β toxicity is likely to be order- and species-specific [56].

Amyloid deposits are also commonly seen in the small nocturnal Malagasy primate *Microcebus murinus* (grey mouse lemur), an interesting species for aging research that lives up to about 13 years in captivity [61]. This strepsirrhine primate belongs to the family Cheirogaleidae which includes at least 7 species

of mouse lemurs (*Microcebus*), the dwarf lemurs (*Cheirogaleus* and *Allocebus*), and the fork-marked lemur (*Phaner*). The A β peptide sequence in mouse lemurs is similar to human A β 40 [58]. In mouse lemurs older than 7 years, histological studies have revealed the presence of A β protein aggregates in the cerebral cortex, the meninges and the cerebral vasculature (amyloid angiopathy) of neuritic plaque-like deposits composed of degenerated neurites surrounding an amyloid deposit, and of neurofibrillary changes such as bundles of argyrophilic filaments in pyramidal neurons [51, 52, 70, 71]. The temporal and parietal cortex is especially affected by A β deposition, a distribution resembling that seen in humans but differs from that in macaque monkeys. In animals older than 7 years, A β protein deposits are observed as small cloudy deposits and as dense core of A β surrounded by a halo of amyloid fibrils [71]. Diffuse amyloid deposits can also be found in young adults suggesting the presence of changes in the morphology of the deposits as a function of age in *Microcebus*.

With respect to species-specific differences in amyloid deposition, it is worth noting that in the brain of the squirrel monkey (*Saimiri sciureus*), a New World monkey, widespread cortical angiopathy is the predominant type of amyloid lesion. In this species, vascular amyloid has been reported throughout the neocortex, the amygdala and the septal nuclei, but not in subcortical structures, and the density of affected vessels appears to be correlated to the amyloid load in the surrounding cortical tissue [10, 72]. This species difference indicates that whereas the macaques and mouse lemurs represent interesting models of cerebral amyloidosis, squirrel monkeys may be a better model of brain amyloid angiopathy, even though capillary changes and angiopathy of brain microvasculature have been reported in old macaques as well as in aged marmosets [73–75]. Also, vascular A β deposits in macaque monkeys are stained strongly by antibodies against A β 42(43), whereas both A β 40 and 42(43) are observed in senile plaques, unlike in humans. Furthermore, it appears that in monkeys the ratio of A β 40 to A β 42(43) is higher than in humans [76, 77], indicating that some differences exist in amyloid processing and deposition during aging between monkeys and humans. In spite of these differences, several molecular constituents of amyloid deposits in humans are also present in nonhuman primates. Apolipoprotein E, α_1 -antichymotrypsin and complement factors C1q and C3c are encountered in amyloid deposits in macaques [46, 78–80], suggesting an overall similarity in the molecular events leading to senile plaque formation in monkeys and humans. In this context, it would be informative to obtain data on age-associated brain amyloid deposition in great apes. Unfortunately, aside of a few incidental reports describing lesions in appearance similar to those seen in humans [53–55], no formal quantitative analysis has yet been performed in great apes due to the scarcity of brain specimens from old individuals.

Neurofibrillary Changes in Old Primates

Fibrillary neuronal and neuritic changes have been known to occur in old monkeys, although they are much rarer than in old human brains or in Alzheimer's disease cases, in the form of 10-nm helical filaments with a periodicity of 50 nm, and 13-nm granular, parallel filaments [63]. Neurofibrillary tangles have been reported in the brains of cognitively impaired monkeys [81]. More recently filamentous tau pathology, morphologically and biochemically comparable to the fibrillary lesions that are characteristics of a variety of human neurodegenerative disorders [for a review, see 82], has been described in a few aged baboons (*Papio hamadryas cynocephalus*), blue guenons (*Cercopithecus mona*), a brown lemur (*Eulemur fulvus*) and in an albino rhesus monkey [83–87a]. These lesions, that could be labeled by a panel of antibodies to hyperphosphorylated tau proteins as in human, occur in neurons, astrocytes and oligodendrocytes preferentially in the hippocampus and follow, at least in the affected baboons, an age-dependent progression in their severity [83–85]. The fact that these lesions were found not only in nerve cells but in glial cells as well, extend the nonhuman primate spectrum of age-related pathologies to a larger group of human degenerative diseases that differ from Alzheimer's disease by their profile of tau proteins and characteristic lesion distributions, as tau-containing glial lesions are observed most frequently in disorders like progressive supranuclear palsy and corticobasal degeneration [82]. The reason why such lesions develop in only some individuals in a restricted number of phylogenetically related species (they have been thus far documented only in a few macaques and baboons, but not in great apes) remains to be elucidated.

In this context it is worth noting that fibrillary lesions that resemble the neurofibrillary tangles of Alzheimer's disease have been described in the brain of old grey mouse lemurs [51, 59]. Tau proteins from this species have been shown to undergo age-related modifications in respect to their isoform composition [88, 89]. Antibodies directed against both normal and abnormally phosphorylated tau proteins show tau-positive aggregates in thick granules in nearly all animals independently of their age. This particular form of tau-containing pathologic profile seems to be specific of lemurs and has not been described in humans [89]. Numerous tau protein aggregates were always found in lemurs with numerous amyloid deposits, but were not consistently observed in animals devoid of amyloid [70]. In sharp contrast to humans, neocortical areas were frequently affected even in relatively young mouse lemurs, whereas the subiculum and entorhinal cortex were involved only occasionally in animals older than 8 years [59]. These data point out that in certain species the tau-related pathology of the cytoskeleton can exhibit specific patterns of regional and cellular distribution that does not necessarily match the situation seen in human. It is, however,

interesting that these lesions appear to have coextensive patterns in lemurs and therefore could represent an interesting model to study the relationships between the formation and distribution of tau and amyloid lesions in an animal whose biology and behavior are well documented and that can be subjected to extensive cognitive testing [for a recent review, see 61].

Subcellular Pathology in the Aging Macaque Monkey Brain

The density of profiles displaying a nucleus or the total number of neurons remains unchanged in the neocortex, and the morphology of neurons is not altered in old monkeys as demonstrated by studies using traditional as well as stereologic methods [34, 36, 40, 43]. The neurons in area 46 of the prefrontal cortex accumulate moderate amounts of lipofuscin in old macaques [34], although more severe changes in the morphology of the terminal dendrites of these neurons occur during aging with evidence of degeneration particularly affecting layer I, in which many dendritic branches exhibit changes in their cytoplasm such as loss of organelles and accumulation of membranous materials [34]. The authors also reported the existence of a thinning of layer I in this region and stereologic counts of the numbers of synapses revealed a consistent reduction in the old monkeys of 30–60% of the numerical densities of synapses per unit volume [21], in parallel to a decrease in the number of postsynaptic dendrites and spines in layer I. These data suggest that aging involves substantial damage of terminal dendritic arbors of neocortical neurons [21]. Data from our population of old macaque and patas monkeys confirm these observations and reveal a significant degree of alterations in the apical and basal dendritic trees of neurons in the prefrontal cortex (fig. 2) [1, 90]. Quantitative analyses of morphological changes at the level of dendritic segments revealed that a certain degree of impoverishment in the complexity of the dendrites occurs with aging and that the most consistently observed change is a decrease in the spine densities at all levels in the dendritic tree. Such changes imply that a reduction in synaptic function is likely to occur during aging in monkeys. Moreover, these dendritic and synaptic changes are accompanied by a degeneration of myelinated axons in the deep layers of the neocortex and in the white matter, which correlates in old animals with deficits in visual and spatial recognition tasks [34, 39]. Together, these ultrastructural changes indicate that age-related cognitive deficits in old monkeys do not result from a loss of neurons, but rather evolve from subtle cellular changes that lead to the localized disruption of certain cortical and corticosubcortical circuits.

There is consistent age-related axonal pathology in aged monkeys. Myelinated fibers undergo several types of alterations. In some fibers, dense

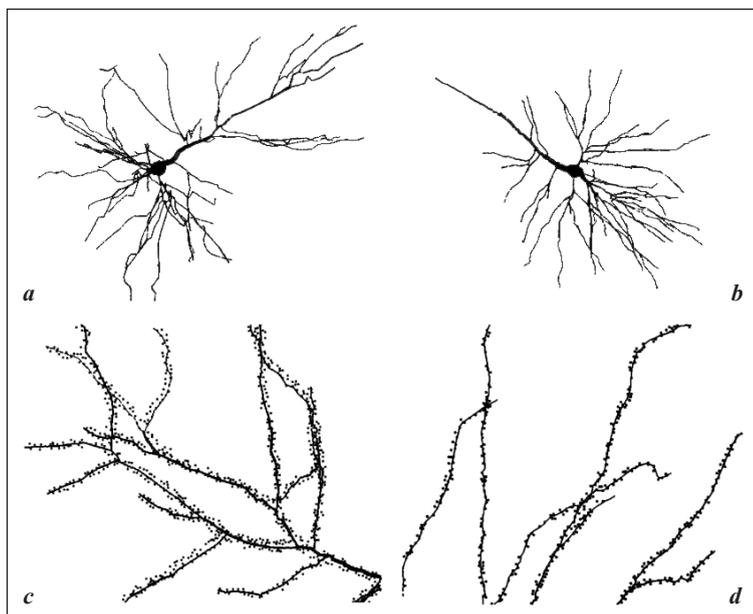


Fig. 2. Three-dimensional reconstruction of Lucifer Yellow-filled, retrogradely labeled neurons furnishing identified projections from the superior temporal cortex to prefrontal area 46 in a 9-year-old (*a*) and a 24-year-old (*b*) long-tailed macaque monkey (*M. fascicularis*). Note the decreased complexity and impoverishment in the dendritic arborization patterns in the old animal. Spine densities are also decreased in the old animal (*d*) compared to the young one (*c*). Spines were mapped at the same time the neurons were reconstructed using a three-dimensional tracing software [90].

oligodendrocytic materials accumulate and lead to splitting of the major dense line of the myelin sheaths [37]. Some sheaths display severe ballooning that ensues from splitting at intraperiod lines which secondarily are filled by fluid. These balloons in the myelin lamellae can be exceptionally large but usually have a diameter of about 10 μm [91]. Other fibers show doubling of their myelin sheaths with layers of compact myelin surrounding each other, while some sheaths contain too much myelin so that their axons are enclosed by sheaths that appear proportionally too large [37]. Although similar alterations of myelin sheaths are known to occur independently of aging [91], their consistent presence in aged macaque monkeys indicates an age-dependent deficit of oligodendrocytes in maintaining adequate myelin sheaths. Such changes are observed not only in the cerebral cortex but also in subcortical structures such as the inferior colliculus, cochlear and olivary nuclei, substantia nigra and the cerebellum [91, 92]. Interestingly, a recent study has also demonstrated severe age-associated

changes in the myelin sheaths of optic nerve axons with separation and ballooning of the myelin layers, a mild reduction in the number of axons, and an increase in the size of periaxonal septa and in the size of astrocytes [93]. Interestingly, even though there is no disruption in the morphology of the ascending bundles of axons in the primary visual cortex in old macaques, the myelin shows in these bundles a comparable and consistent pathology in absence of loss of visual fibers [92]. In addition, it has been reported that oligodendrocytes are commonly seen in groups or arranged in rows in old animals, whereas in young animals they occur singly [38]. Such clusters of oligodendrocytes may form an abnormal cellular network as they develop tight junctions among them [38]. In aged monkeys, the cell bodies of oligodendrocytes are also observed in close contact with brain microvessels, where they modify the limiting glial membrane normally formed by astrocytes. Finally, oligodendrocytes and other neuroglial cells accumulate abnormal inclusions or debris in their cytoplasm as do the pericytes [34]. These results point to the fact that glial cells may represent a crucial target of normal aging in the primate brain, even though their numbers do not change in old animals [34], and that these changes may be directly related to the alterations in brain metabolism that are known to occur in the aged primate brain [for review, see 1].

Neuron and Synapse Numbers in the Central Nervous System of Old Macaque Monkeys

Layer I of the prefrontal cortex displays a considerable loss of synapses during aging, although no obvious changes in the morphology of synapses have been observed, so that the relative proportions of symmetric and asymmetric synapses is preserved as is their distribution among axodendritic, abutting spines or dendritic shafts, and axosomatic subtypes. The cross-sectional area of the terminals and length of postsynaptic densities is also not affected by aging. This age-related synaptic pathology may be unique to the prefrontal cortex, because in other regions of the cerebral cortex, such as the dentate gyrus, the total numbers of axonal terminals making a synapse with dendritic spines or shafts does not vary, even though a minor decrease in the density of synapses on shafts is found if these synapses are considered alone [32, 33]. Thus, hippocampal circuits involved in episodic memory are likely to be morphologically preserved in old macaques. These data support the observation that there is no age-associated neuronal loss in layer II of the entorhinal cortex where the neurons of origin of the projection to the outer molecular layer of the dentate gyrus reside (fig. 3) [40]. Another stereologic study extended these data to all the layers of the entorhinal cortex and reached the same conclusion [94]. These authors also revealed that

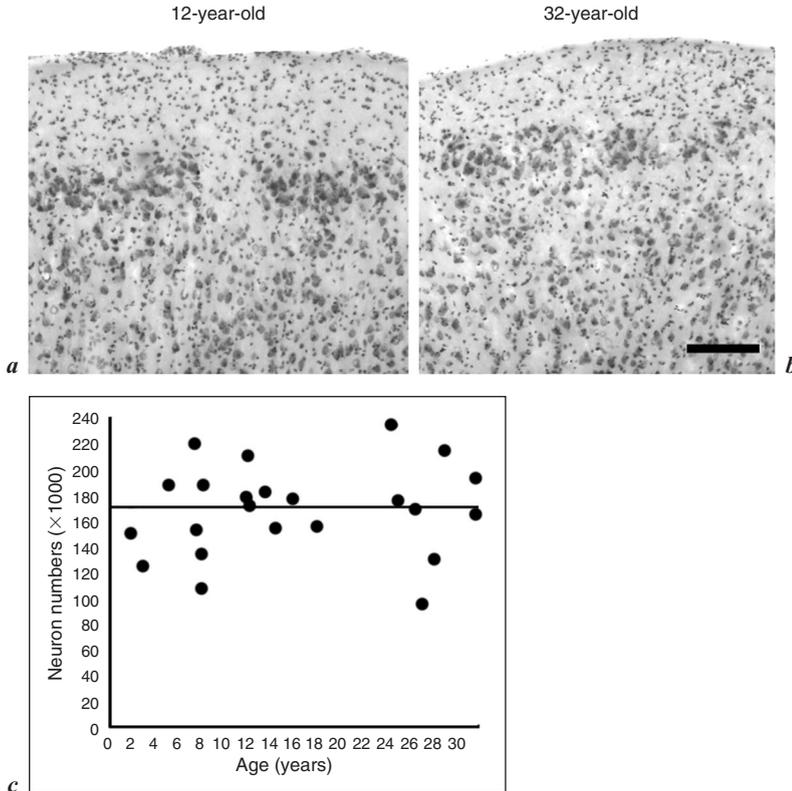


Fig. 3. *a* Layer II of the entorhinal cortex in a 12-year-old rhesus monkey. *b* The same area from a 32-year-old animal. No cell loss is observed in these neurons that provide the projection to the dentate gyrus in the old monkey. *c* Stereologic analysis of the total numbers of layer II neurons in a series of rhesus monkeys 2–32 years old is shown. There is considerable interindividual variability which was not related to the sex of the animals. Quantitative data reveal no age-related loss in neuron numbers. Scale bar = 100 μm .

there is no neuronal shrinkage associated with aging in macaque monkeys [94]. Contrasting with these observations, earlier analyses of the hippocampal formation in old macaques reported as much as a 50% decrease in the number of neurons in the CA1 field of the hippocampus proper, and smaller differences were found in the prefrontal cortex together with a reduced cortical thickness in these regions [95]. These data were unfortunately not obtained using rigorous stereologic methods and differ radically from a large number of recent and more robust quantitative analyses in primates that all demonstrate that neuronal loss is not a feature of normal aging [96]. Similarly, stereologic estimates of the numbers of neurons and neuronal volumes in the entorhinal cortex and the CA1

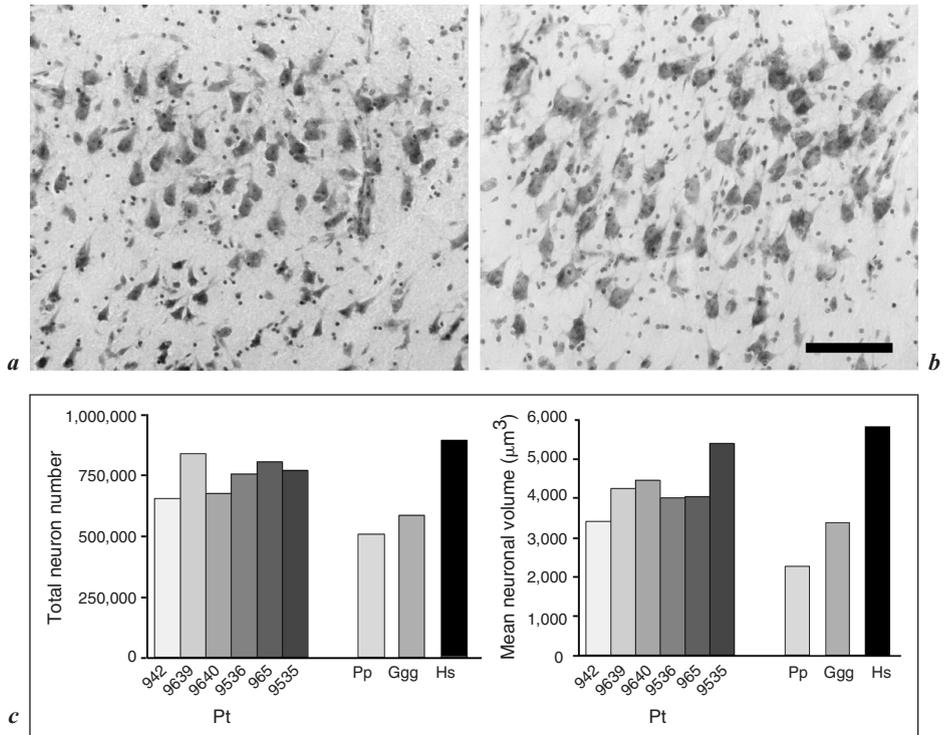


Fig. 4. Layer II of the entorhinal cortex in a young (*a*) and an old (*b*) chimpanzee is shown. The morphology and numbers of these neurons are well preserved in the old subject. The bottom histograms (*c*) show a stereologic study of total neuron numbers and mean neuronal volumes in layer II of the entorhinal cortex in 6 chimpanzees (Pt = *P. troglodytes*, 13–45 years old), a 37-year-old Borneo orangutan (Pp = *P. pygmaeus*), one 42-year-old gorilla (Ggg = *G. gorilla gorilla*), and 3 normal elderly humans (Hs; 75–82 years old). Neither the total number of neurons nor the neuronal volumes were affected by the aging process in chimpanzees. The chimpanzees are listed in the order of increasing age and the values from the 3 humans are pooled. Note that fewer and smaller neurons are present in the orangutan and the gorilla compared to the chimpanzees and humans. Scale bar = 100 μm.

field of great apes did not reveal any age-related differences [2, 97, 98] (fig. 4). These studies, which were performed in the context of the Great Ape Aging Project [2], involved several specimens from aged orangutans (*Pongo pygmaeus* and *Pongo abelii*), western lowland gorilla (*Gorilla gorilla gorilla*), and common chimpanzee (*Pan troglodytes*) that were compared to young specimens and to a series of brains obtained from elderly human controls. These analyses showed that in general the great apes have fewer neurons in the CA1 field and in layer II of the entorhinal cortex than normal humans, the orangutan and gorillas

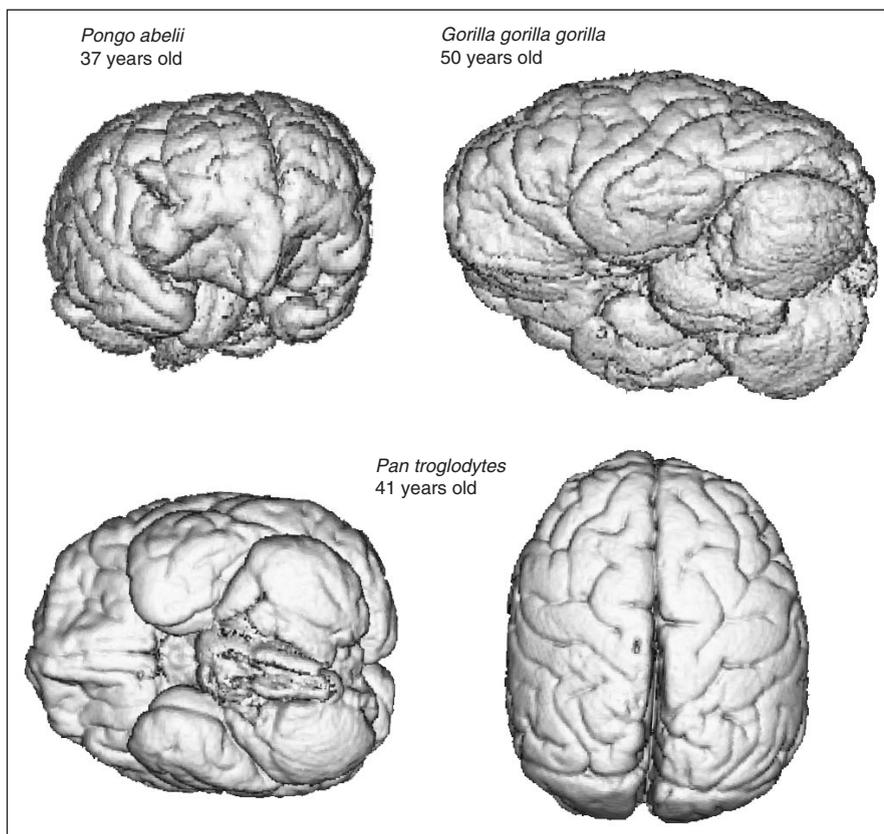


Fig. 5. Examples of volume rendition of postmortem MRI scans (1.5 T) of brain specimens obtained from an old Sumatran orangutan, an old western lowland gorilla and an old chimpanzee. Note that no gross brain atrophy is visible on these reconstructions.

displaying lower numbers than chimpanzees, and that old subjects from these species had neuronal numbers comparable to those in young individuals and that no neuronal shrinkage with aging was detected. We are currently analyzing regional volumes from postmortem magnetic resonance images that were acquired for archival purposes from the same brain specimens prior to histological processing, to assess whether age-related changes occur in the volume of certain brain areas or particular regions of the subcortical white matter (fig. 5). Preliminary observations of a limited number of samples do not reveal any substantial volumetric differences in old great apes [see also 98a].

The many functional and anatomical similarities that exist in the organization of primary motor and primary visual cortices between human and macaque

monkeys have prompted several studies of age-related changes in these systems in old macaque monkeys. Analyses of the primary motor cortex reported no age-related loss in the number of Betz cells and in the number of axonal contacts on their somata even though a marginal shrinkage in their cellular volume was observed [31, 99]. Tigges et al. [31] noted in this context that during the adult development of the macaque monkey, the number of neurons in the primary motor cortex decreases, but that the number of Betz cells increases during the maturation phase and remains subsequently stable for the entire life span of these animals. Therefore, a loss of upper motoneurons and/or of other components of the primary motor circuits is not the cause of the age-associated decrease in motility observed in aged macaques. Rather, distal causes such as bone involution and joint disorders are likely to be responsible for the reduced motility of these animals during aging as proposed by DeRousseau [100].

Visual abilities decline during normal aging in primates. Deficits in visual acuity, spatial contrast and temporal frequency contrast sensitivity and resolution, motion detection, and binocular processing have been documented in humans and in nonhuman primates [101, 102]. With the exception of a recent analysis of the optic nerve that demonstrated considerable age-related myelin pathology [93], anatomical and physiological studies of aged macaque monkeys have generally shown few alterations in the visual pathways, at least at the level of the retina and lateral geniculate nucleus, suggesting that visual deficits in aging may be related to cortical alterations [101, 102]. Important physiological data from old macaques have clearly demonstrated the existence of degradation in orientation and direction selectivity in the primary visual cortex, accompanied by increased spontaneous activity and responsiveness, possibly due to an age-related deficit in intracortical inhibition [103]. Such findings point to a pathology of intracortical GABAergic systems and careful quantitative analyses of these inhibitory circuits at the ultrastructural level will be needed to specify the anatomical substrate of these deficits. Retinal ganglion neurons and lateral geniculate nucleus parvocellular and magnocellular neurons do not show an age-related decline in their numbers or size [104, 105], indicating that aging has no clear effect on the visual system of functionally and histochemically identified compartments. Furthermore, the density of the cytochrome oxidase-rich blobs in the primary visual cortex remains unaltered in old animals [41], and the area, volume and thickness of the primary visual cortex are similar in young and old macaque monkeys [35, 43] (fig. 6).

As reported in the prefrontal cortex, vacuolar changes and degenerating myelin sheaths occur in the primary visual cortex, further stressing the fact that age-related deficits in visual function may affect cortical integrity at the ultrastructural level and involve preferentially certain neuronal subpopulations [30, 37, 38, 43, 91]. Neurons forming highly specific connections between the

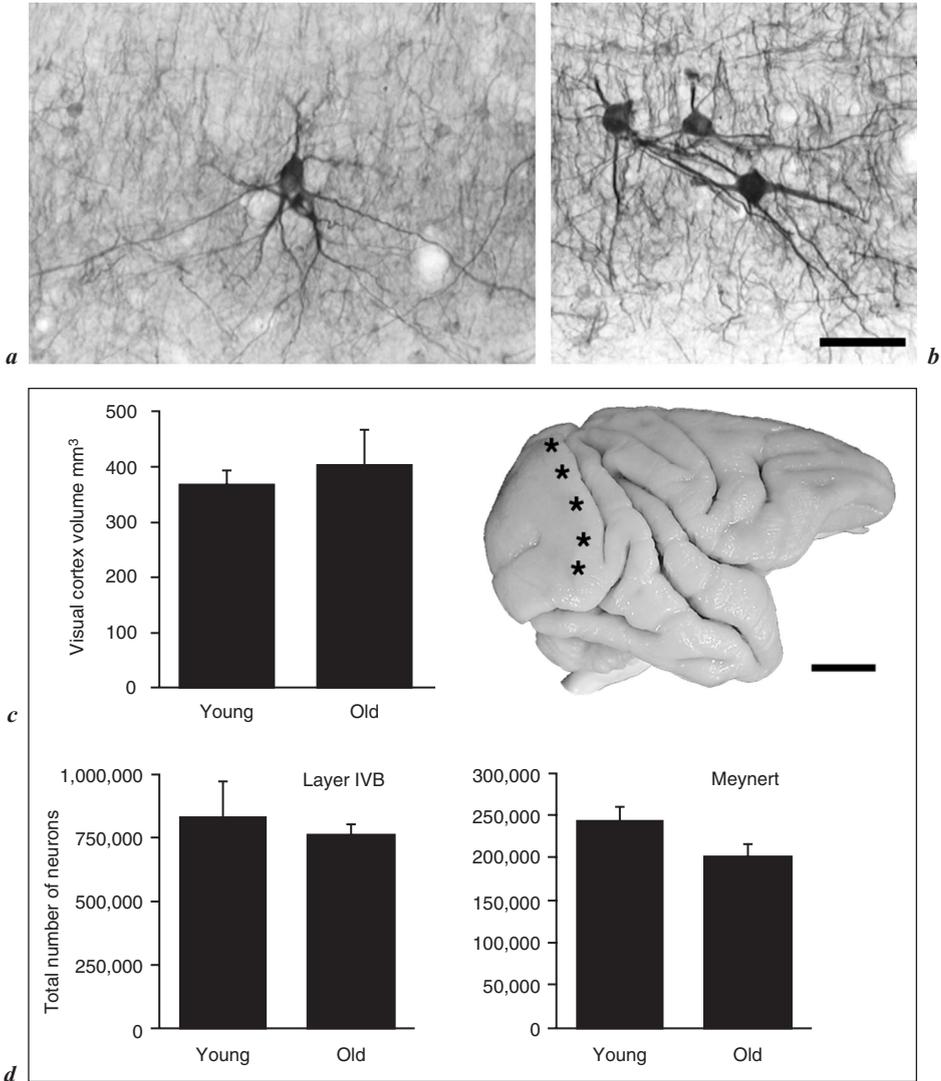


Fig. 6. Integrity of the primary visual cortex during aging in rhesus monkeys. Specific populations of projection neurons, the large layer IVB and the Meynert cells are not affected by aging and exhibit no age-related morphological alterations. Clusters of Meynert cells in layer VI of a young (*a*) and an old (*b*) animal are shown. Materials were stained with an antibody against nonphosphorylated neurofilament protein. No changes in the total volume of the primary visual cortex occur in old animals (*c*; the anterior margin of the primary visual cortex is indicated by asterisks on the lateral view of the cerebral hemisphere of an old animal). As demonstrated by stereologic analyses there is no age-related loss of these neurons in old animals (*d*). Quantitative data are shown as means \pm SD [43]. Scale bars = 100 μ m (*b*) and 1 cm (*c*).

primary visual cortex and higher cortical areas are of particular interest in this context, such as the large layer of IVB neurons and the Meynert cells that provide direct projections to temporoparietal cortical regions involved in motion detection, ocular tracking, smooth pursuit movements, and saccade production [43], because the functions they are likely to subservise are those that present deficits in old subjects. Using an unbiased stereologic method, estimates of the total numbers of layer IVB cells and Meynert cells have been obtained separately in the calcarine cortex and in the opercular cortex in aged macaque monkeys [43]. A considerable degree of interindividual variability in neuron numbers and cortical volume was observed among both young and old animals and no differences in either Meynert cell or layer IVB cell numbers between the aged and young groups in either parts of the primary visual cortex were found (fig. 6) [43, 106]. Peters and Sethares [106] had also reported that Meynert cells do not shrink during aging and our study revealed no morphological alterations of these neurons (fig. 6) [43]. These data suggest that the visual deficits occurring in aged animals are not likely due to the loss of highly specific neocortical neuronal populations, but that more subtle alterations in the neurochemical characteristics or synaptic organization of the functional pathways subserving the different visual modalities are directly linked to impaired visual function (it is important to note that the aged animals included in these studies of visual function had no ocular pathology). Also, in the light of reported dendritic alterations in other cortical regions [1, 36, 90], it would be important to investigate the possible involution of dendritic arborizations as well as spine changes in Meynert cells in old monkeys.

Subcortical Systems Show Neuronal Alterations and Loss in Aged Macaque Monkeys

Unlike the cerebral cortex, alterations have been observed more consistently in the subcortical nuclei that contain the neurons of origin of the cholinergic and aminergic systems. Cholinergic deficits have long been implicated in the memory deficits of aging and are strongly correlated with the late stages of Alzheimer's disease. In old macaque monkeys, a study has documented a loss of about 40% of the cholinergic neurons in the caudalmost part of the nucleus of Meynert [8, 107]. Age-related decreases in packing densities and numbers of a population of small neurons and a group of serotonergic neurons, the nucleus raphe centralis superior, have been reported in old rhesus monkeys, with a loss reaching 50% [108]. A substantial loss of dopaminergic neurons in the substantia nigra pars compacta and the ventral tegmental area also occurs in old macaque monkeys accompanied by a certain degree of shrinkage of these neurons [109–111a].

No changes in the volume of these nuclei were found by these authors and the most severe neuronal loss in the substantia nigra involved small, presumably GABAergic neurons with an overall loss of about 25% of the substantia nigra pars compacta and 34% of the ventral tegmental area neurons in the aged macaques. Minor changes in the size of individual nigral and tegmental neurons were observed and many cells accumulated lipofuscin and Marinesco bodies, and displayed severe dendritic alterations during aging [109]. This study also documented neuropil alterations in the substantia nigra pars compacta in aged animals comparable to those observed in the cerebral cortex, with an increase in the number of astrocytic processes, and the presence of astrocyte-derived spheroids, oligodendrocytic inclusions and breakdown of myelin sheaths, lending further support to the notion that glial changes, and particularly ultrastructural alterations of the myelin, represent a signature of aging-related brain pathology.

Importantly, changes in all these subcortical systems furnishing divergent projections to the cerebral cortex are correlated with the severity of cognitive deficits in aged animals [8, 107, 109, 110]. These cellular alterations may influence the integrity of neurochemically specific afferent projections on neocortical function. In fact, the development of cognitive and memory impairment in aged monkeys are known to be linked to age-related morphological and pharmacological alterations of catecholaminergic systems [112, 113]. Importantly, pharmacological and physiological studies of aged macaques have demonstrated that the cortical dopaminergic mesocortical system is particularly affected during aging [112–115], a fact that is supported by the morphological evidence described above [109–111]. These data indicate that subcortical systems may be far more sensitive to the aging process than the cerebral cortex. Because they each provide a chemically specific type of afferent to their cortical projection domains, they represent interesting targets for the development of therapeutic strategies. Interestingly, a recent study demonstrated that delivery of human nerve growth factor in old macaque monkeys reverses age-related atrophy and loss of cholinergic neurons [116]. These data suggest that not only loss, but cellular atrophy also is an important contributing factor to the cognitive decline observed in old primates that can be potentially improved by gene transfer of neurotrophic molecules to specific neuronal systems in spite of their possibly higher vulnerability during aging.

Conclusions: Cognitive Deficits Related to Normal Aging in Old Primates Are due to Subcellular and Molecular Alterations

The fact that only minor morphological modifications in cortical circuits are evident in old monkeys and that major changes appear to be restricted to a

small number of specific systems, the cognitive deficits that are observed in most, but not all, aged subjects are likely to be more strongly correlated with subtle alterations affecting certain populations of cortical neurons that are not necessarily revealed at the level of light microscopy. Such alterations will therefore not be easily described even by refined morphometric and stereologic methods, or during general neuropathological evaluation of the brain, but require quantitative investigations at the ultrastructural level. The apparently severe age-related changes in monoaminergic and cholinergic systems that have been described in morphological and pharmacological investigations of old animals [113] indicate the existence of deficits in specific receptor molecules or specific sites on the dendritic and somatic compartments of the neurons receiving these subcortical projections.

These observations, concurrently with morphological studies of neuropil changes in the prefrontal cortex [13, 36], indicate that subcellular alterations may impact considerably the functional connectivity of cortical circuits in aging. This fact is further supported by a recent study of the cortical volume of prefrontal cortex area 46 that revealed no gross change in the volume of layer I and of the total cortex in macaque monkeys cognitively impaired on a delayed-response task, indicating that cognitive dysfunction is not necessarily related to gross alterations (fig. 7) [42]. A few important studies have revealed specific shifts in the expression of certain ionotropic glutamate receptor subunit proteins in aged monkeys compared to juvenile and adult monkeys that were interpreted as subserving a functional decline in the absence of overt neurodegeneration [117, 118]. In particular, NMDAR1 receptor levels detected by quantitative immunohistochemistry were shown to decrease specifically and consistently in the outer molecular layer of the dentate gyrus which corresponds to the zone of projection of the medial perforant path. These changes are specific as there are alterations in AMPA or kainate receptor subunits, and no morphological reflection of degeneration of the perforant path could be documented by this analysis [117]. Most remarkably, there is no neuronal loss in layer II of the entorhinal cortex in these aged animals, which are the neurons of origin of the projection to the dentate gyrus [40]. These data are consistent with the minor age-related changes in synapse counts reported in the dentate gyrus by Tigges et al. [32], and with reports of receptor binding assays in aged monkeys [115, 118]. Similarly, a considerable downregulation in the expression of the glutamate receptor subunits NMDAR1 and AMPA GluR2 was demonstrated in old rhesus and long-tailed macaques, and patas monkeys in corticocortical projection neurons identified by tract-tracing, with 20–40% fewer neurons expressing these subunit proteins in old animals [1, 119]. Interestingly, the level of downregulation was more severe in, but not limited to, neurons participating in long corticocortical pathways, which correspond to those suspected to be particularly prone to

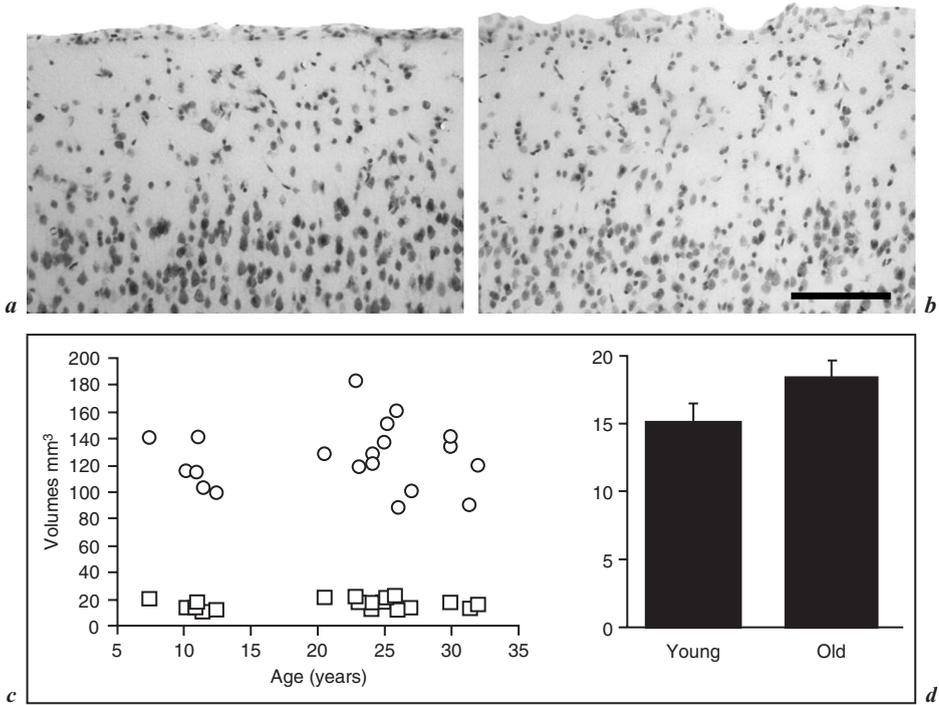


Fig. 7. Morphology and stereologic analysis of layer I in area 46 of the prefrontal cortex in old rhesus monkeys. Young (*a*) and old (*b*) animals show comparable thickness and morphology of layer I. Volumetric analyses show no correlation between volumes of layer I or total volume of area 46 and age (*c*; ○ = total volumes; □ = layer I volumes), or between young and old animals (*d*). Quantitative data are shown as means ± SD [42]. Scale bar = 100 μm.

neurofibrillary degeneration in Alzheimer's disease [96]. Furthermore, the three-dimensional reconstruction and modeling of these cortical projection neurons in *Macaca* and patas showed that they undergo a consistent impoverishment of the complexity of their distal dendritic arborizations and exhibit a severe decrease (20–30%) in spine densities (fig. 2).

Altogether, these findings suggest that the intradendritic distribution of a neurotransmitter receptor as well as the density, shape, and spatial arrangement of dendritic spines undergo age-related modifications in a circuit-specific manner and that these changes are anatomically positioned to influence the information processing capacities of systems critical for normal learning, memory, and behavior. Defining the functional significance of these alterations, however, will require a multidisciplinary approach, combining behavioral, morphological

and biochemical investigations of normal aging in a variety of primate species. It is in this context very important to keep in mind that many taxon-specific idiosyncrasies exist in terms of cellular specificity, not only at the morphological level but at the molecular and pathological levels as well. Such factors could be interpreted as phylogenetic trends that influence the susceptibility of a particular taxon to a given pathological condition, such as Alzheimer's disease. It has been proposed that the human brain has acquired unique characteristics during its recent evolution that render the species prone to neurodegenerative disorders [120–123]. The recently described spinal neurons that occur only in the anterior cingulate and anterior insular cortex of hominids, to the exclusion of all other mammalian species, are present in high densities only in humans, and are highly susceptible to degeneration during Alzheimer's disease; they may represent one such characteristic [122, 123]. Another example of primate-specific features that demonstrates a distinct evolutionary pattern within the order of primates is the considerable morphological differences that exist in the size of Betz and Meynert cells among primates [124, 125]. Considering carefully the evolutionary trajectories of such traits will be necessary to assess adequately their influence on the process of aging in the primate brain in a proper evolutionary context and further our knowledge of aging and age-related neuropathology in our own species as well as in all of the primates.

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