Feature Article
Are Neurons Lost from the Primate Cerebral Cortex during Normal Aging?

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The concept that cortical neurons are lost with age and that this is the basis for cognitive decline is so embedded in our culture that when someone elderly is a little forgetful it is often said that 'He/she is losing his/her neurons'. The prime difficulty in determining if neurons are lost from the cerebral hemispheres during normal aging is that it is not possible to count neurons in the brain of the same individual at two different points in time. Consequently, the question of whether neurons are lost with age can only be answered by comparing the brains of older individuals with those of younger individuals, who have been raised in a different environment with a better diet and better health care. What effect this may have on neuronal numbers is not clear. For example, Haug (1985) points out that the increase in height due to secular acceleration, that is from generation to generation, amounts to ~1 mm per year, and data generated by Haug (1985) indicates that as body weight increases, so does brain weight; consequently, 'brain weight of one generation is proportionally higher than that of the preceding generation as measured in youth'. On the other hand, Pakkenberg and Gundersen (1997) conclude that sex and age are the main determinants of the overall number of neurons in the neocortex, and that body size does not affect neuron number. Such secular trends need to be considered when comparing the brains of young and old humans to determine if there is a neuron loss with age. Yet when comparisons are made in humans, as well as in laboratory animals that have a much shorter life span in a more controlled environment, we conclude that on the basis of the existing data there is no strong evidence to support the concept that significant numbers of neurons are lost from the cerebral cortex during normal aging. Instead, it appears that cortical neurons are largely preserved.

The idea that there is a significant loss of neurons during normal aging of the human cortex was put forward by Brody (1955, 1970), who examined the effects of aging of the cortices of subjects between 18 and 95 years of age. He concluded on the basis of cell counts that there is a progressive reduction in neuronal density with age, amounting to 50% in the superior frontal and superior temporal gyri, and between 20 and 30% in the precentral gyrus and visual cortex, although he detected no significant loss of neurons from the inferior temporal gyrus and the postcentral gyrus. This result received a great deal of publicity, and stimulated a number of other studies carried out in the 1970s and 1980s in which similar decreases in neuronal density with age were reported (e.g. Colon, 1972; Shefer, 1973; Devaney and Johnson, 1980; Henderson *et al.*, 1980; Anderson *et al.*, 1983). During that period, the only investigator who seemed to disagree with the conclusion that there is a significant loss of neurons with age was Cragg (1975), who found no differences in neuronal density in the frontal and temporal cortices with age. But there was no serious consideration given to the possibility that there is not a significant loss of neurons until the studies of Haug and his colleagues (Haug, 1984, 1985; Haug *et al.*, 1984) and of Terry *et al.* (1987) were published.

Haug and his colleagues carried out morphometric analyses on >120 human brains in a study lasting for 8 years (Haug, 1985) and measured neurons with a computerized digitizer to determine neuronal populations and densities. They concluded that the previously reported neuronal losses with age were probably due to the fact that during the processing of tissue for microscopic examination, the brains of younger individuals shrink as much as 15% more that those of older individuals. Consequently in cell counts made on tissue sections, the sections from younger brains give higher neuronal densities than those from older brains. After he made corrections for this difference in the amount of shrinkage, Haug (1985) concluded that some areas of the cortex, notably Brodmann areas 6 and 11 in the frontal lobe and area 17 in the occipital lobe, actually show an increase in neuronal density with age, with area 7 in the parietal lobe and area 20 in the inferior temporal gyrus showing similar tendencies. Haug concluded that these increases in neuronal density were the result of an age-related decrease in the volume of these areas, so that overall there is no change in the number of neurons within the entire human cerebral cortex with age. Haug determined that the mean number of neurons in the human cerebral cortex is 1.39×10^{10}, with a range of between 1.0 and 2.0×10^{10} neurons, indicating an inter-individual variation of up to 100% in cortical neuron numbers.

Terry *et al.* (1987) examined 51 brains from individuals ranging in age from 24 to 100 years who had been carefully screened to determine that they were clinically and neuropathologically normal. They examined sections from the midfrontal, superior temporal and inferior parietal areas of the brain using an image analysis system. They noted significant age-related decrements in brain weight, cortical thickness in the midfrontal and superior temporal areas, and a shrinkage of large neurons in all of the areas examined, with a consequent increase in the numbers of small neurons. But they concluded that with age neuronal density is unchanged. Since they recorded a decrease in brain weight and a thinning of the cortex by 15% in the midfrontal region and 9% in the superior temporal gyrus, Terry *et al.* (1987) decided that there probably is some neuronal loss with age, but of much less a magnitude than had previously been supposed, and suggested that in some of the earlier studies reporting extensive neuronal loss, brains of some Alzheimer's patients may have been included in the material being studied.

In a series of other studies of the effects of aging, specific areas of the human cortex have been examined, most notably the visual cortex. Thus, Leuba and Kraftsik (1994a) found that although there is great variability in both the surface area of the adult primary visual cortex, which in the right occipital lobe ranges between 15 and 40 cm^2, and in the total numbers of neurons, there is no statistically significant neuronal loss during

the normal aging of the visual cortex. This is in marked contrast to the situation in the brains of Alzheimer's patients in which Leuba and Kraftsik (1994b) estimated the total number of neurons in areas 17 and 18 of the visual cortex to be decreased by as much as 30%.

Essentially, all of the above studies used profile counts with mathematical corrections for assumed profile shape to calculate the density of neurons, but in 1984 Gundersen (Sterio, 1984) introduced a new, so-called unbiased method, the disector, which when used in combination with an estimate of the volume of the brain structures under analysis could be used to estimate the total number of neurons (see West, 1993a; Coggeshall and Lekan, 1996). Numerous modifications of the original disector method, such as the optical disector and fractionator, were also developed by Gundersen and colleagues and have been used to obtain estimates of total neuron number in a given structure, independent of neuron density (Gundersen *et al.*, 1988a,b). Using this new approach, Braendgaard *et al.* (1990) calculated the total numbers of neurons in the right hemispheres of five normal 80-year-old men to be 1.37×10^{10}, with an inter-individual coefficient of variation of 12%. In a similar way, Pakkenberg and Gundersen (1997) examined a total of 94 normal brains comprising 62 male brains (age range 19–87 years) and 32 female brains (age range 18–93 years). They concluded that there is an overall loss of neurons from the cerebral cortex with age, and that it amounts to ~9.5%. Like Terry *et al.* (1987), Pakkenberg and Gundersen (1997) find no change in neuronal packing density with age, but in contrast to the result obtained by Terry *et al.* (1987), they indicate that there is no significant change in cortical thickness, although there is a diminution in the total volume of grey matter. This leads to their conclusion that there is an overall loss of cortical neurons with age.

However, contrary to this conclusion that there is a loss of grey matter, and a consequent loss of neurons during normal aging, there are data like those of Double *et al.* (1996), who have determined on the basis of point counting on sectioned material that there is no significant loss of grey matter with age. Double *et al.* (1996) did find a small decline in brain volume with age, but attributed that to a loss of white matter. In contrast, this group found a significant loss of grey matter in the brains of patients with Alzheimer's disease (AD), amounting to between 10 and 15% of the total cortical volume, with the greatest loss being from the temporal cortex.

One possible explanation for the discrepancies in reports of neuron loss with age may be that such losses are regionally specific and limited, as suggested by Coleman and Flood (1987). In this view, neurons could be lost from one architectonic area but not from the area next to it, or they may be lost from a specific cortical layer. The possibility of such a localized loss is difficult to dismiss, and the entorhinal cortex and the hippocampus to which it projects are prime candidates for age-related neuronal losses that could contribute to age-related cognitive decline. In this regard recent detailed studies of these areas are informative.

Gómez-Isla *et al.* (1996) have determined the neuronal densities and total number of neurons in the entorhinal cortex. They applied the modern principles of stereology to entorhinal cortices from a highly selected group of both normal individuals, 60–89 years of age, and individuals aged between 85 and 95 years with early-stage AD. Their conclusion is that in normal individuals there is no age-related change in the total numbers of neurons, the volume or the number of neurons in each lamina of the entorhinal cortex. In contrast, compared with the control group, the Alzheimer's group showed an average loss of 48% in the total number of neurons and the volume of the entorhinal cortex was reduced by ~40%.

Gómez-Isla *et al.* (1997) have also studied the number of neurons present in the association cortex during normal aging and in AD. In this study 34 individuals with AD and 17 non-demented controls were assessed, using unbiased stereological counting techniques. They examined the cortex of the superior temporal sulcus, a high-order association cortex that receives projections from multiple association cortices, limbic areas and several subcortical structures. There was no loss of neurons in the control individuals, who were 50–90 years of age. A single individual whose age was in the early-20s was also studied, and this individual had the same number of neurons as all the other subjects. In sharp contrast, in the AD group an average 53% of neurons was lost. More recent data from this group looked at subsets of neurons, including SMI-32 immunoreactive neurons (large projection neurons), and found no change in the number of these neurons with increasing age in a smaller set of normal individuals (B.T. Hyman, unpublished results).

The hippocampal formation is the simplest of cortical areas and one that in AD shows severe loss of neurons, particularly in the CA1 and subicular subfields, where neurofibrillary tangles are prominent and diagnostic (e.g. Braak and Braak, 1991). Because of this neuron loss in AD and in other neuropathological conditions such as Ammon's horn ischemia (Zola-Morgan *et al.*, 1986), the hippocampal formation has been extensively examined for changes in normal aging. Like the early studies of the neocortex, those of the hippocampus all used profile counts in samples taken from limited areas of the hippocampus to determine neuronal densities. And, like the studies of the neocortex, these invariably led to reports of neuron loss (e.g. Ball, 1977). More recently unbiased stereological methods have been applied to the human hippocampus by West and colleagues, but again the results are somewhat inconsistent. In their first study, West and Gundersen (1990) reported that while there is neuron loss from the CA1 subfield, other subfields were preserved. However, in a subsequent study (West, 1993b) utilizing a larger sample it was reported that there is a loss of 31% of the neurons from the CA4 subfield in the hilus of the dentate gyrus and 52% from the subiculum, but no loss from CA1, CA2 or CA3. To further confound the issue Simic *et al.* (1997) report that in normal human brains there is a significant loss of neurons with age from CA1 (67%) and the subiculum (32%), but not from the hilus (CA4) or other subdivisions of the hippocampus. The reasons for the discrepancies between the three studies, all using the optical disector, are not obvious, but it suggests that in normal aging neuron loss from the hippocampus may be variable, or that there is a problem with the technique used in sampling.

In evaluating these results, and in particular the various discrepancies, it is important to consider the method of sampling used by Pakkenberg and Gundersen (1997) to determine the total number of neurons in the cerebral hemisphere. Because of the method of sampling, not all areas of the cerebral cortex are included in the estimate and this same approach led Regeur *et al.* (1994) to conclude that there is no global loss of neurons even from brains of patients with senile dementia of the Alzheimer's type. As pointed out by Mufson and Benzing (1994) in a commentary on this conclusion, this latter result is quite contrary to very solid data demonstrating that there is a

significant decrease in the numbers of neurons in the frontal, temporal and parietal cortex in individuals with AD (e.g. Terry and Davies, 1983; Terry and Hansen, 1988), as well as in the occipital cortex (Leuba and Kraftsik, 1994b), entorhinal cortex (Gómez-Isla *et al.*, 1997) and hippocampus (Braak and Braak, 1991). Thus, if neuronal loss in AD is selective in terms of both the layer and region of cortex affected (Hof and Morrison, 1994), it is possible that the method of sampling used by Regeur *et al.* (1994) leads to those areas of the cortex most affected by AD being under-represented in the neuronal counts. Regeur *et al.* (1994) also point out that the major limitation in their neuronal counts, obtained by using the optical disector, is the difficulty in distinguishing between neurons and neuroglial cells.

It is also of interest that in other publications, the Danish group of investigators give quite varying values for the total numbers of neurons in the human cerebral cortex. For example, Braendgaard *et al.* (1990) state that the total number of neurons in the right hemisphere of normal 80 year old men is 1.37×10^{10}, with an inter-individual variation of 12%, whereas Regeur *et al.* (1994) give a value of 1.81×10^{10} for the total number of neurons in the neocortex in their control group of non-demented females with a mean age of 82.6 years, and Pakkenberg and Gundersen (1997) give the mean total number of neurons in both hemispheres as 2.28×10^{10} in males and 1.93×10^{10} in females. This is not to diminish the importance of the investigations carried out by the Danish group and their role in generating the new stereological methods, but to merely point out that even the same group of investigators using the same unbiased counting technique can generate quite different absolute numbers, particularly when large heterogeneous structures such as the cerebral cortex are being treated as a single brain region.

One problem in making these overall estimates may be due to the fact that, unlike the liver and kidney, the various areas of the cerebral cortex vary dramatically in the size and packing density of their component neurons. Thus, when assessing the primary visual cortex, for example, if counts were made only in the tightly packed laminae of layer 4, the estimate of the number of neurons would be higher than if counts were made in the less densely packed pyramidal cell layers, such as layer 5. Thus, if the sampling design is not appropriate, erroneous estimates can be generated regardless of which counting procedure is used. This problem has been recently emphasized by Popken and Farel (1997), who did a comparison of estimates of neuron number in dorsal root ganglia using both stereological (physical disector) and profile-counting methods. The mean values obtained from application of the physical disector and nucleolar counting were in good agreement, but when using the physical disector they found that, because of the heterogeneous distribution of the neurons in the ganglia, the recommended protocol for counting led to sampling errors of up to 50%. To improve the accuracy of the results Popken and Farel (1997) found it necessary to greatly increase the number of disector pairs examined. Thus a sample scheme has not only to satisfy the stereological rules to ensure an unbiased selection, but it must also take into account the particulars of the anatomical structure, with highly heterogeneous structures requiring more extensive sampling.

Another important factor in evaluating reports of neuronal loss in normal human aging is the suggestion by Terry *et al.* (1987) of the intrusion of early, undiagnosed cases of AD into a sample of cognitively and neurologically normal aged adults. In this regard, the studies of Gómez-Isla *et al.* (1996, 1997), using both formal cognitive assessments and unbiased stereological techniques, suggest that the neuronal loss that occurs in AD must begin well before the cognitive neurological changes that are diagnostic of AD can occur. And this neuronal loss could also begin before the pattern of neurofibrillary tangles and senile plaques that provides the neuropathological diagnosis for AD is firmly established. If this is so, the variably reported neuronal loss of 10% or less from some areas of the cortex may simply reflect the early stages of AD.

Perhaps the greatest problem in obtaining true estimates of any neuronal loss lies in the enormous variation in neuronal numbers between individuals. For example, as shown by Stensaas *et al.* (1974), Murphy (1985) and Leuba and Kraftsik (1994b), the size of area 17 in the human brain can vary by a factor of three times among individuals, and the same appears to be true of the numbers of neurons in one hemisphere, which Pakkenberg and Gundersen (1997) show can range from 1.47 to 3.20×10^{10}. Similar variations occur in the striate cortex of the monkey, because its surface area can differ by a factor of two when individual monkeys are compared (van Essen *et al.*, 1984; Purves and LaMantia, 1993; Peters *et al.*, 1997). Such large variations makes it virtually impossible to accurately determine if there is a significant loss of neurons from an individual brain, and raises doubt about the significance of a loss of up to 10%, when individual variations can be as much as 100%.

Evaluating the effects of aging on the brain of the rhesus monkey offers distinct advantages over studies that use human brains. Monkeys, only ~6% of which attain an age of 30 years (Tigges *et al.*, 1988), undergo an age-related decline in several domains of cognitive function, as do humans (e.g. Peters *et al.*, 1996), and a complete behavioral assessment can be made prior to death. The brain can then be well preserved without the imposition of post-mortem delay, and there is no indication that monkeys acquire AD, so that this does not confound studies aimed at determining the effects of normal aging. In addition, old monkeys being studied today have spent their entire lives in controlled husbandry environments, on standard diets and with relatively constant health care. Moreover, in a recent study Herndon *et al.* (1998) found that when they examined the weights of a sample of 399 rhesus monkeys obtained at necropsy, they could detect no decrease in brain weight with age, although the female brain is slightly smaller than that of the male. This study suggests that over the 30 plus years lifespan of the rhesus monkey secular effects do not affect the size of the brain. However, as with the human studies, the first accounts of the effect of aging on the cerebral hemispheres of monkeys also concluded that aging brings about a loss of neurons. Thus, Brizzee and his colleagues (Brizzee, 1973; Brizzee *et al.*, 1975, 1980) examined the sensorimotor cortex from rhesus monkeys 4–6 years of age and compared it with that of 18- to 20-year-old monkeys. They recorded no age-associated change in the thickness of the sensorimotor cortex, but found a significant decrease in the packing density of small granular neurons, presumably at the level of layer IV. They therefore concluded that there is neuronal loss from this cortex with age. Sub- sequently, the gyri bordering the principal sulcus in the frontal lobe were examined (Brizzee *et al.*, 1980) and it was also concluded that neurons are lost from this portion of the cortex with age. From their analysis of the data, Coleman and Flood (1987) calculated that the reported neuronal loss amounted to as much as 20%. The hippocampus was also examined and Brizzee *et al.* (1980) found the mean depth of the CA1 zone to be reduced in the aged monkeys, as well as the mean numbers of neurons counted in traverses through this portion of the hippocampus.

After the initial studies by Brizzee and his colleagues, no

further studies were done on the effects of aging on monkey cerebral cortex until 1989, when Vincent *et al.* (1989) compared the primary visual cortex of young rhesus monkeys (5–6 years of age) with that of old monkeys (25–35 years of age) and found no indication of a loss of neuronal numbers with age when the profiles of neurons displaying nuclei were counted in strips of semithick sections passing through the depth of the cortex. There was also no difference in the sizes of the neuronal nuclei, the counting objects, with age or in the thickness of the cortex. The specific population of large Meynert cells in this cortex was also examined (Peters and Sethares, 1993) and this population showed no indication of neuronal loss. In both studies the tissue used had been fixed by vascular perfusion to allow an electron microscopic examination to be made of the cortex, and except for a few cell bodies of some neurons in layer I, and degenerative changes in some dendrites, no indications of neuronal degeneration or death were encountered. Peters *et al.* (1997) repeated the evaluation of the effects of aging on cortical thickness and neuronal numbers, and again found no alterations with age. This study was carried out because it had been suggested that, although there may be no loss of cortical neurons beneath a unit area of cortical surface, there might nevertheless be a diminution in the surface area of the primary visual cortex with age. As with the primary visual cortex of the human brain, it was found that there is a wide variation in the surface area and volume, but there is no indication that either the surface area or the volume of the primary visual cortex of the monkey decreases with age. The primary visual cortex of the rhesus monkey was also examined by Kim *et al.* (1997), who employed an optical disector to count neurons in the cytochrome oxidase-positive blobs in layer II/III. In a comparison of young and old monkeys, they found no differences in the packing density of neurons within blobs. From these data and the results they obtained in previous quantitative studies on the effects of age on the retina and the lateral geniculate nucleus (Ahmad and Spear, 1993; Kim *et al.*, 1996) they conclude that the retinogeniculo-striate pathway of the rhesus monkey is relatively unaffected by aging.

Comparisons have also been made of the numbers of neurons beneath unit areas of cortical surface in area 4 of the motor cortex (Tigges *et al.*, 1990) and in area 46 of the prefrontal cortex (Peters *et al.*, 1994), and again, there is no indication of a loss of neurons with age.

Analyses of neuron numbers have also been carried out in the hippocampus of normal aging monkeys (Rosene, 1993). Initially the study concentrated on the CA1 and subicular subfields because of the prominent cell loss reported there in humans (West and Gundersen, 1990) and monkeys (Brizzee *et al.*, 1980). These studies, which used profile counts, showed no loss of neurons in either subfield (Rosene, 1993). More recently the sample size has been expanded and unbiased stereological methods have been applied. Again no loss of neurons or changes in volume of the CA1 or subicular subfields has been found (D.L. Rosene *et al.*, unpublished data). Recently Rosene (unpublished data) has looked at this same cohort using modern stereological techniques and examined the CA2, CA3 and CA4 subfields of the hippocampal formation and layers II, III and V–VI of the entorhinal cortex, and again found no loss of neurons with age. These data are in agreement with reports of no neuron loss with age from the hippocampus and entorhinal cortex by Amaral (1993) and by Gazzaley *et al.* (1997) for layer II of entorhinal cortex. Interestingly, precisely the same monkeys that Gazzaley *et al.* (1997) demonstrated to have no loss of neurons in layer II of entorhinal cortex did have a decrease in NMDA receptor immunoreactivity in the perforant path terminal zone of the dentate gyrus molecular layer (Gazzaley *et al.*, 1996), suggesting that a functionally relevant age-related biochemical change occurs in the entorhinal layer II to the dentate gyrus molecular layer projection without any loss of neurons.

Of course, studies of aging in laboratory animals are not limited to monkeys, and extensive studies have been conducted using the laboratory rat. While a complete review of this literature is beyond the scope of this article, a brief review of the effect of age on neuron numbers in rat hippocampus is instructive. As with the monkey, early studies of neuron numbers in rat hippocampus used profile counts and did not systematically sample the entire structure. The early studies concluded that there is a significant loss of neurons with age (e.g. Landfield *et al.*, 1981). However, in a recent study Rapp and Gallagher (1996) used the disector method to count neuron numbers of representative samples of the entire hippocampus of behaviorally tested rats, and they have reported that there is no age-related loss of neurons, even from those rats with the greatest age-related behavioral impairments. Hence, even in the rat there are indications that neurons in the forebrain are largely preserved with age.

Since it is possible to obtain well fixed material from monkeys, a number of electron microscopic evaluations have been made of the effects of age on cortical neurons in the primary visual cortex (Vincent *et al.*, 1989; Peters and Sethares, 1993) and prefrontal cortex (Peters *et al.*, 1994). In these studies, except in the relatively cell free layer I, no neuronal cell body profiles that could be construed as belonging to degenerating neurons have been encountered. Even the large Betz cells of the motor cortex, which can accumulate vast amounts of lipofuscin with age (Tigges *et al.*, 1990; Tigges, 1992), show no signs of degenerative changes. This is a particularly important point because, if there is a loss of cortical neurons with age, some degenerating profiles would be expected to be encountered, as well as neuroglial scars filling spaces that had previously been occupied by neurons.

In summary, as far as non-human primates are concerned, all of the recent studies on monkeys seem to agree that there is no evidence of significant loss of neurons from the cortex with age. In humans, the question of neuronal loss from the cortex is still not entirely resolved, and may never be resolved if neuronal losses due to AD begin before behavioral signs of cognitive decline or before neuropathological features allow a definitive diagnosis. But if there is any loss of cortical neurons during normal aging it is probably no more than 10%, and even this loss is probably limited to specific parts of the cortex. Consequently, total losses would be of a much smaller magnitude than had been supposed from earlier studies. The main problem in making determinations of neuronal loss is that there is a wide variation in the numbers of cortical neurons among individual normal brains. However, when there is a significant loss of neurons, as documented in AD (e.g. Leuba and Kraftsik, 1994a; Gómez-Isla *et al.*, 1996, 1997; Simic *et al.*, 1997), it is obvious: the pattern of loss is distinct and not a continuum of what occurs in normal aging, so that neuronal loss in AD is not simply an extension of a process that begins during normal aging.

Our conclusion is that little is likely to be gained by continuing studies aimed at linking an overall loss of cortical neurons to the cognitive deficits that arise during normal aging in humans or in non-human primates. Instead, efforts to reveal the underpinning of age-related deficits should be taken to a finer level of analysis, since it cannot be ruled out that there may be a dramatic loss from one of the specific subpopulations of cortical neurons. For

example, it has been estimated that nitric oxide synthase-containing neurons account for a small percentage of the total neuronal population, and a substantial loss from this subpopulation, or from subpopulations of inhibitory neurons such as the chandelier cells or double-bouquet cells, would not be detected in the existing stereological analyses. Yet substantial losses from such subpopulations of neurons could have important functional repercussions. The same could be true if substantial numbers of specific sets of dendritic spines are lost, as they are from layer 1 of area 46 in the aging monkey neocortex (A. Peters, unpublished data). On the other hand, molecular shifts in intact circuits, such as the decrease in NMDA receptors serving a crucial circuit, as has been described in the dentate gyrus of the monkey (Gazzaley *et al.*, 1996), could also impact specific functional systems during aging. This means that as far as aging non-human primates are concerned, whenever possible any morphological or biochemical analyses should be done on behaviorally characterized animals. Like humans, all monkeys do not age at the same rate (see e.g. Peters *et al.*, 1996), and the key to revealing those age-related neurobiological events that are potentially causally important is to relate them not only to age itself, but also to functional decline.

Notes

This work was supported by Program Project Grant 2PO1-AG00001 (A.P. and D.L.R), research grants AG 08487 (B.T.H.) and AG 06647 (J.H.M.) from the National Institute on Aging, and grant RR-00165 from the Division of Research Resources to Yerkes Regional Primate Research Center.

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