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Experimental Gerontology 38 (2003) 95–99

Experimental Gerontology

www.elsevier.com/locate/expgero

Aging and the human neocortex

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Received 14 May 2002; accepted 20 May 2002

Abstract

Neurostereology has been applied to quantitative anatomical study of the human brain. Such studies have included the total neocortical number of neurons and glial cells, the estimated size distribution of neocortical neurons, the total myelinated fiber length in the brain white matter, the total number of synapses in the neocortex, and the effect of normal aging on these structural elements. The difference in total number of neurons was found to be less than 10% over the age range from 20 to 90 years, while the glial cell number in six elderly individuals, mean age 89.2 years, showed an average number of 36 billion glial cells, which was not statistically significantly different from the 39 billion glial cells in the neocortex of six young individuals with a mean age of 26.2 years. The total myelinated fiber length varied from 150,000 to 180,000 km in young individuals and showed a large reduction as a function of age. The total number of synapses in the human neocortex is approximately 0.15×10^{15} (0.15 quadrillion). Although the effect of aging is seen in all estimated structural elements, the effect of sex is actually higher. The functional relevance of these differences in neuron numbers in both age and gender is not known.

Keywords: Fibre length; Glial cells; Neocortical volume; Stereology; Synapses; Total numbers

1. Introduction

The first estimate of total nerve cell numbers obtained by the disector counting method took place in the mid-80s to estimate the total number of nerve cells in the mediodorsal thalamic nucleus of schizophrenics and controls (Pakkenberg, 1990). Since then, the collection of data regarding quantitative studies of both the normal brain and brains from individuals suffering from some of the major diseases of the central nervous system have increased our knowledge about the quantitative structure of the human brain. Normal aging of the human neocortex is subject to obvious interest, especially the differentiation between normal aging and disease processes, since many of the brain changes that occur in old age such as appearance of senile plaques, neurofibrillary tangles, granulovacuolar degeneration, Hirano bodies and congophilic angiopathy are also shared with Alzheimer's disease (AD). One of the first stereological approaches to differentiate between aging and disease showed normal individuals to lose neurons in two regions of the hippocampus, subiculum and the hilus as a function of age, while patients with AD lost more neurons in these two regions than normal aging individuals (West and Gundersen, 1990; West et al., 1994; Simic et al., 1997). In CA1, a region that does not ordinarily show any age-related cell loss, the Alzheimer patients have lost most of their neurons at time of death. This confirms the impression that AD is not merely an advanced aging process, but a separate disease entity.

We report quantitative data concerning the primary structures of the normal human brain: the total neocortical number of neurons and glial cells, the estimated size distribution of neocortical neurons, total myelinated fiber length in the brain white matter, and the total number of synapses in the neocortex. In addition, some of the changes that take place as a function of age are also reported.

2. Estimations of the total number of neocortical neurons

From a large brain repository collected in accordance with Danish laws on autopsied human tissue, the total number of neocortical neurons was estimated in 62 males

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0531-5565/03/$ - see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0531-5565(02)00151-1

(average age 52 years; range 19–87 years) and 32 females (average age 64 years; range 18–93 years) (Pakkenberg and Gundersen, 1997). No attempt was made to match the two sexes according to age, since only a few young women met with the inclusion criteria, and only a few elderly men survived to a very old age without significant physical or mental disabilities. The data were, however, corrected for these differences before they were compared using regression techniques, insensitive to such range differences.

3. Cavalieri's principle

The hemispheres were fixed in 0.1 M sodium phosphate buffered formaldehyde for at least 5 months. The meninges were removed, and the cerebellum and brain stem detached at the level of the third cranial nerve. Right or left hemispheres were chosen at random, and the results from one hemisphere multiplied by two to provide results for both hemispheres. The five cortical regions, i.e. frontal, temporal, parietal, occipital, and archicortex, were delineated on the pial surface (Braendgaard et al., 1990). The archicortex, or the limbic lobe, comprises the uncus, hippocampus, parahippocampal gyrus, gyrus cinguli, subcallosal area, and amygdala. The hemispheres were embedded in 6% agar, sliced coronally at 4–7 mm intervals and the volume of neocortex, the different cortical regions, the white matter, the central gray structures and the ventricular system were estimated by the Cavalieri method using a transparent counting grid directly on the slices where an average of 25–35 slices were counted per brain. The total and regional neocortical surface areas were estimated by counting intersection points between the boundary and test lines (Gundersen, 1985).

The estimator of pial surface area, as opposed to the other stereological techniques, was not strictly unbiased because the brains were sliced in parallel, frontal slices. The actual bias in the estimates of pial surface area was, however, too small to be detected (Oster et al., 1993).

If a total of approximately 500 points per brain are counted on all neocortical sectional areas, the mean coefficient of error (CE = SEM/mean) on the estimates of total volume is approximately 3% (Gundersen et al., 1988; Regeur, 1999; Gundersen et al., 1999).

From every second slice, starting randomly, transcortical wedges were sampled uniformly from each neocortical region. Each wedge was cut into 2-mm-wide bars providing 25–50 bars per region. These were subsampled uniformly providing 6–10 bars per region. Each bar was embedded in Kultzer Technovit 7100® from which one 35–μm-thick section was cut and stained with a modified Wolbach Giemsa stain.

4. Counting procedure

The disector is a probe which samples isolated particles with a uniform probability in a three-dimensional space, irrespective of their size, shape or orientation in the tissue (Sterio, 1984). The concept 'disector' is an imaginary rectangular box, the *z*-axis (the height of the disector) is employed by using 40 μm-thick sections in which the plane of focus is moved up or down (Gundersen et al., 1988) and the *x*- and *y*-axis are defined by a square (the counting frame) superimposed on the magnified image of the tissue on the computer screen. The approximate number of particles counted in a disector, $\sum Q^-$, the height, *h*, of the disector, and the area of the counting frame, *a*(frame), are parameters defined by the investigator. Knowing the height of the disector and the area of the counting frame, the volume of the disector, *v*(dis), is given as

$$v(\text{dis}) = h \cdot a(\text{frame})$$

The total number of particles *N*(part) in a specimen or reference space of a given volume *V*(ref) is thus

$$N(\text{part}) = \left[\sum Q(\text{part})^- / \sum v(\text{dis})\right] \cdot V(\text{ref})$$

The optical disector counting equipment consists of a BH-2 Olympus microscope with a high numerical aperture (NA = 1.4) 100 × oil immersion objective, a motorised stage, and an electronic microcator with digital readout for measuring movements in the *Z*-direction with 0.5 μm-precision. Using the CAST-GRID® PC-program (Olympus, Denmark), optical disectors are superimposed onto a colour monitor. Each change in tissue volume between the estimates of the total volume and the numerical density estimate was quantified. In the study of the normal human neocortex a subset of approximately 220 uniformly selected disectors from the human neocortex was sufficient for the estimation of the total number of neurons, counting an average of 340 neurons per brain neocortex. The coefficient of error in the estimates of neuron number was 7%.

The number of neocortical neurons in females was 19.3 billion, and in males 22.8 billion, a difference of 16%. The difference in the total number of neocortical nerve cells over the observed range of 70 years (range 20–90 years) was 9.5%, providing an average 'loss' of neurons of about 85,000 per day, or approximately 1 per second. The relationship to age was the same for both sexes. The total number of neocortical neurons in this material varied more than a factor of 2 with a range of 118% (from 14.7 to 32.0 billion neurons) (Pakkenberg and Gundersen, 1997).

With advanced age, reductions occurred in the neocortical volume, the surface area, the white matter, the archicortex volume, and the brain weight, concomitantly with a large increase in the ventricular system. However, no changes were found in gray matter volume and the neocortical thickness. After accounting for sex and age, the neocortical neuron number was a dominating factor in

determining the size of other brain structures. Neuronal density was not a function of sex or age.

Other stereological studies have shown that alcoholics and patients with AD have a normal global total neuron number in the neocortex while patients dying with AIDS have up to 30% fewer neocortical neurons independent of their clinical state before death (Regeur et al., 1994; Oster et al., 1995).

5. Total neocortical glial cell number and aging

The total number of glial cells in the neocortex has been estimated preliminary in six old individuals, three males and three females, mean age 89.2 years, range 81–98 years, and showed an average number of approximately 36 billion glial cells, which was not statistically significantly different from the 39 billion glial cells in the neocortex of six young individuals, three males and three females, mean age 26 years, range 18–35 years. This suggests that the total number of glial cells in the neocortex is established already during young adulthood, and that increase in glial cell numbers apparently is not part of normal aging. The reduction by 11.7% in the total number of neocortical neurons was followed by a 13.9% reduction in the total number of oligodendroglial cells (D. Pelvig, unpublished data).

6. Macroscopic brain volumes in demented and non-demented individuals

Only a limited number of papers have estimated the volume of gross cerebral structures in senile dementia and AD compared to non-demented controls. The temporal and parietal regions are the two areas that most often show atrophy with concomitant ventricular enlargement. The results have, however, been somewhat conflicting in relation to the distribution of the atrophy. The volume reduction of the entire hemisphere has been found in AD patients using an image analyzing system (Miller et al., 1980) and using planimetry (de la Monte, 1989), with no change in the ratio between gray and white matter. In a study by Regeur (1999), the neocortical volumes, the cortical thickness, and the volumes of archicortex, the ventricular system, and the central gray matter and white matter were estimated using stereological methods on brains from 28 old females (mean age 81.8 years) with increasing degree of senile dementia and brains from 13 (mean age 82.7 years) non-demented controls. All the demented patients came from a psychogeriatric ward in Copenhagen, Denmark, and all had been evaluated prospectively with a psychometric dementia test and neurological examination once a year during their last years. The tests assessed a wide range of cognitive functions (intelligence, orientation, psychomotor performance, language, visuospatial abilities and various types of memory). Performances on the different psychological subtests were scored on a 7-point-scale, and a general score was obtained from 1 to 7 with a high score indicating severe dementia. The material was subdivided into four groups (group 0–3) according to severity of dementia.

Brains from the demented patients had decreased neocortical volume, and the thickness of the neocortex was significantly reduced in the demented patients, with the highest degree of reduction in the most demented patients and in the volumes of archicortex. Hubbard and Anderson (1981) found a similar global cerebral atrophy in demented patients below the age of 80, whereas patients over 80 showed selective atrophy of temporal cortex. As pointed out by Brun (1994) and Hyman and Gomez-Isla (1994), dementia of Alzheimer's type has often been called a global brain disorder, but regional focalization is evident. The finding of a significant reduction of the archicortex corroborates this point of view. Of the four neocortical regions investigated, all but the parietal region showed significant reduction in the demented patients. However, because of the high biological variation in the volume of the four neocortical regions and the evident problems in determining the different neocortical subdivisions (Flood, 1994), it was only concluded that the tendency towards reduction in volume was globally the same, even though only three out of four regions reached statistical significance.

In a previous paper we have reported normal global neocortical neuron number in severely demented females with AD (Regeur et al., 1994). This is not in conflict with the volumetric data, since the former also found reduced neocortical volume. The total number remained the same only because the neuron density, the number of neurons per volume, was significantly increased in the Alzheimer cases. As previously pointed out, large cortical atrophy only affects neocortical thickness in both AD and AIDS (Pakkenberg and Gundersen, 1997; Oster et al., 1993), while the surface area remains the same. This is in contrast to normal aging, where age affects the pial surface, but not the neocortical thickness (Pakkenberg and Gundersen, 1997). The mechanism involved in disease processes is thus much different from the one taking place during normal aging.

Regeur (1999), reported that the ventricular volume increased with increasing degree of dementia, but did not differ statistically significantly in the demented group compared to the control group. Surface area did not change in the demented patients, and no significant reductions were found in the volumes of white matter or central gray structures in the demented patients compared to controls.

7. Estimation of cell size in neocortex

Most previous investigations on neuronal size in the neocortex have used conventional measuring designs (most often 2D area measurements), which cause

difficulties in the 3D interpretation of the results. In a stereological study of mean neuronal volume and absolute size distributions of the neocortical neurons in brains from controls and AD patients, the neocortex of eight patients with AD, mean age 81.1 years (68–94 years) was compared with nine non-demented controls, mean age 80.9 years (65–101 years). The brains came from Johns Hopkins University Hospital (JHUH) in Baltimore, Maryland, USA, the Netherlands Brain Bank (NBB), and from a large brain repository in Denmark. The rotator method was used to obtain an estimate of cell volumes providing absolute size distributions of the volume of both cell perikaryon and cell nuclei (Jensen and Gundersen, 1993). Group mean values and most statistical tests were performed on logarithmically transformed individual values. All uniformly sampled neurons were studied using the local stereological volume estimator, the vertical planar rotator. With this technique, estimates of individual neuronal nuclei and perikaryon volumes can be obtained in a vertical design without further assumptions about shape and orientation in the tissue. The individual relation between nuclear and perikaryon volume was analysed using ordinary parametric statistics.

The geometric mean volume of cell nuclei in neocortical neurons was 328 μm^3 (interindividual CV = 0.15) in the Alzheimer group compared to 277 μm^3 (interindividual CV = 0.17) in the controls, which was a statistically significant increase. The perikaryal volume was 1117 μm^3 in the Alzheimer group compared to 999 μm^3 in the controls, which was a non-significant difference. There was a highly significant correlation between the nuclear and perikaryon volumes in all individuals. The study confirmed that there is no simple correlation between neuronal numbers and size on one side and function and disability on the other (Bundgaard et al., 2001).

8. Nerve fibres and aging

Nerve fibres (myelinated fibres) from the human brain can be characterised stereologically by their length, diameter and volume (Tang and Nyengaard, 1997). A study shows that we have approximately 150,000–180,000 km myelinated nerve fibres in the white matter of the human brain at 20 years of age, and that we lose 40–50% of this fibre length as a function of age. Especially the thinner fibres are lost (L. Marner, unpublished data).

9. Synapses and aging

The total number of synapses has been reported by many to show a substantial loss as a function of age. Synapses have a diameter of 200–500 nm and can only be seen by electron microscopy. The primary problem in assessing the number of synapses in human brains is their lack of resistance to the decay starting shortly after death. In a study of big animals (dogs, pigs and cows), however, a period of up to 2 days after death did not seem to influence the number of paired pre- and postsynaptic dense specializations identified using a modified E-PTA stain. In five normal young males there was an average of about 0.15×10^{15} (0.15 quadrillion) synapses in the human brain cortex with just a moderate variation (CV = 0.17). On average, the neocortical neurons thus have about 7000 synapses each for intracortical reception and exchange of information. Data collection regarding the effect of aging on the total number of synapses is in progress.

10. Conclusion

The reported age-related changes in our population of normal individuals should serve as a first quantitative documentation of the events taking place in the elderly brain. When cognitive measures of performance are applied to elderly populations, they demonstrate a continuum of values with no clear delineation between those with and those without dementia. Many more studies are needed before it is possible to conclude which neuropathological changes are synonymous with normal aging and which must be characterized as consequences of disease. Neurostereology is a useful tool to collect such data and thereby increase our knowledge of the inevitable consequences of aging.

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