Histol Histopath (1992) 7: 17-22

Histology and Histopathology

Ageing of the human entorhinal cortex and subicular complex

L. Trillo and L.M. Gonzalo
Department of Anatomy, University of Navarra, Pamplona, Spain

Summary. Age-dependent changes in the entorhinal cortex (EC) and subicular complex (SC) were studied in 30 brains of patients who died between 14 and 86 years of age, without CNS impairment, as determined by macro- and microscopic examination. The brains were fixed in 10% formalin and embedded in paraffin. Three series of coronal EC and SC sections (7 μm) were stained by Nissl, PAS or hematoxylin-eosin. Using neuronal count and Kariometry, age-dependent modifications were studied in layers II, III and V of the lateral area of the EC; in the pyramidal layer of the subiculum (S), and in layer II of the presubiculum (PS). All EC layers studied presented a slight (11-20%) although significant reduction up to 35 years, but from 35 to 75 years the decrease was not significant. After 75 years the neuronal loss increased slightly. The nuclear area decreased up to the age of 40-45 years, (10-18%) and augmented from this age up to 75 years (10-14%). During the last period of life, the nuclear area did not change. From 30-60 years, pyramidal layer in the S showed a significant neuronal loss (30%), thereafter, neuronal reduction was less. At early years, the nuclear area decreased insignificantly (15%), and from 35 years up to the most advanced age studied, it increased significantly (13%).

In the PS, layer II manifested a cell loss throughout the lifespan (32.9%) and the changes in the nuclear area did not reach statistical significance due to the dispersions of its values. These results lead to the conclusion that the neuronal loss in EC is notably less than in S and PS and, in general, than in other centres. The sequence of neuronal loss is also different in the EC and in the S. While in EC the maximal loss occurs up to 35 years of age, in S the most pronounced loss begins precisely after this age.

Offprint requests to: Dr. L.M. Gonzalo, Facultad de Medicina, Apdo. 273, Pamplona, Navarra, Spain

Key words: Entorhinal cortex, Subicular complex, Ageing

Introduction

The temporal lobe, with the entorhinal cortex (EC), the subicular complex (SC) and the hippocampus, is a very important structure involved in the mnemonic function. The EC acts as an interface between multimodal association areas and the temporal circuit (Hjörth-Simonsen, 1971; Beckstead, 1978; Goldman-Rakic et al., 1984). In fact, the EC, in addition to the efferent fibres to different cortical areas (Kosel et al., 1982; Sorensen, 1985; Swanson and Köhler, 1986), also projects to the gyrus dentatus (GD) (Andersen et al., 1966; Steward, 1976; Ruth et al., 1982; Witter et al., 1989) which in turn (following the temporal circuit) projects to CA3, Ca1, S, PS, (Sorensen and Shipley, 1979; Tamamaki et al., 1987) and the PS, closing the circuit, to the EC (Shipley, 1975; Köhler, 1984). The alteration of any one of these links is followed by an impairment of such circuitry and, consequently, of the mnemonic process (Hyman et al., 1984, 1986; Milner, 1970; Zola-Morgan et al., 1986).

It is well known that with the ageing of the CNS, memory is one of the most severely affected functions. To investigate the time-course of the ageing changes in the EC and the SC is an interesting point to study, but in the available bibliography the knowledge of the CNS changes in senescence is fragmentary since often only the extreme ages are compared. On the other hand, there is no parallel investigation of neuronal loss and the vicarious capacity of the remaining neurons.

Materials and methods

In this study 30 brains were selected based on the following criteria: absence of neurological or

psychiatric impairments, absence on macro- and microscopic examination of non age-dependent brain alterations, the age of the patients (a similar number for each decade of life), and finally, the postmortem delay inferior to 16 hours (average 6 hours). In all the cases, the left hemisphere was analyzed. The brains were fixed in 10% buffered formalin; the blocks containing the EC and SC were processed for paraffin embedding; and the coronal sections were serially made at a thickness of 7 μm. Three series were obtained and stained with Nissl, PAS, or Haematoxylin-eosin methods. The variables chosen to study the ageing process of the EC and the SC were neuronal number and karyometry. This study was limited to layers II, III, and V of the lateral area of the EC (Figs. 1, 2). In the S, the pyramidal layer and in the PS, layer II. All the neurons with a prominent nucleolus, included in the width of the layers, were counted by means of a manual method. Employing a semiautomatic method, the area of the neuronal nuclei was measured. The significance of the values obtained in neuronal counting and karyometry was assessed by the Fisher test and one-way analysis of variance. Linear and polynomial regression functions were used to evaluate correlation between parameters and age.

Results

Entorhinal Cortex

The 3 layers (II, III and V) studied in the EC showed a similar trend with regard to neuronal loss as well as to karyometry (Figs, 3, 5, 7). During the first period, up to 35 years of age, the most intense neuronal loss took place. Thereafter, until 75 years, the loss decreased, and in the last period it increased again. The global neuronal loss was 20.2% for layer II ($p < 0.01$), 11% for layer III ($p < 0.01$) and 14% for layer V ($p < 0.01$). The evolution of the nuclear areas showed a triphasic course: the first phase, until 40 years, was characterized by a significant atrophy

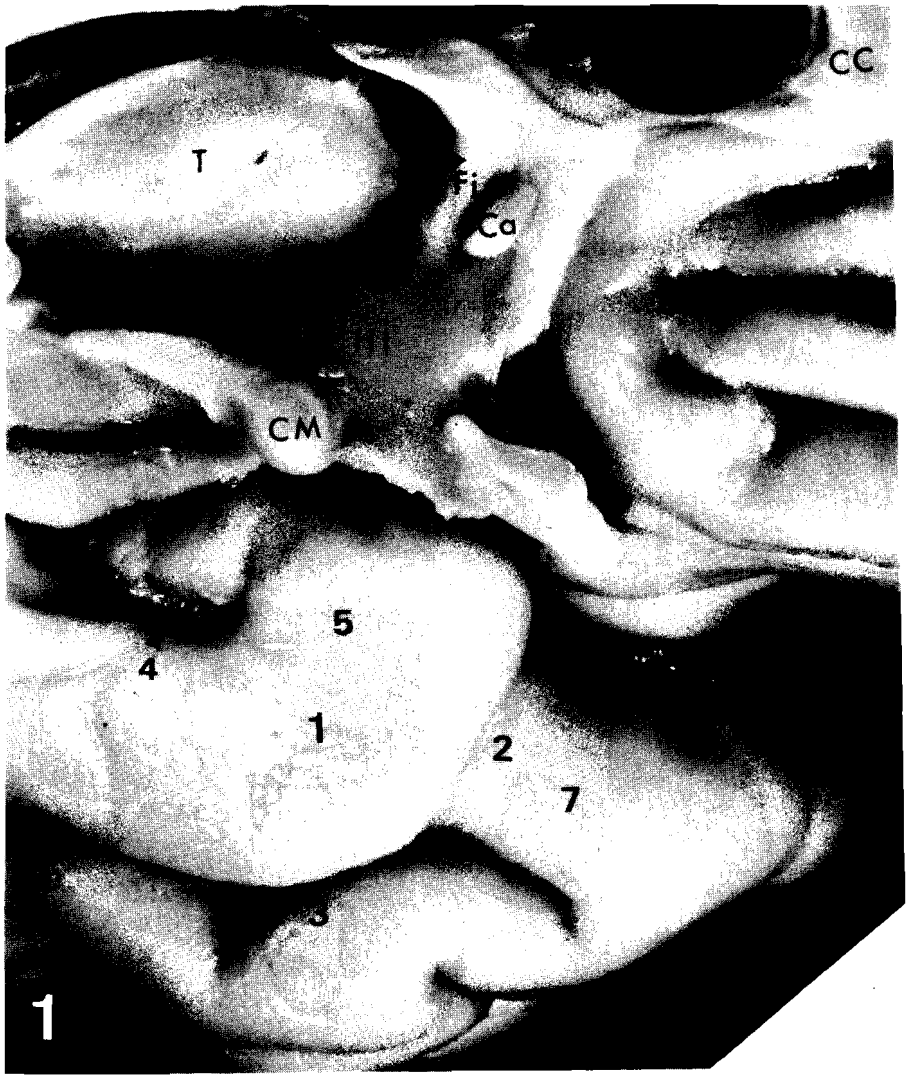

Fig. 1. Partial view of the medial surface of a human brain. Abbreviations: Ca, commissura anterior; CC, corpus callosum; CM, corpus mammillare; Fi, foramen interventriculare; Ht, hipothalamus; T. thalamus; 1, entorhinal cortex; 2, sulcus rhinalis; 3, sulcus collateralis; 4, fissura hippocampi; 5, gyrus ambiens; 6, gyrus semilunaris; 7, perirhinal cortex.

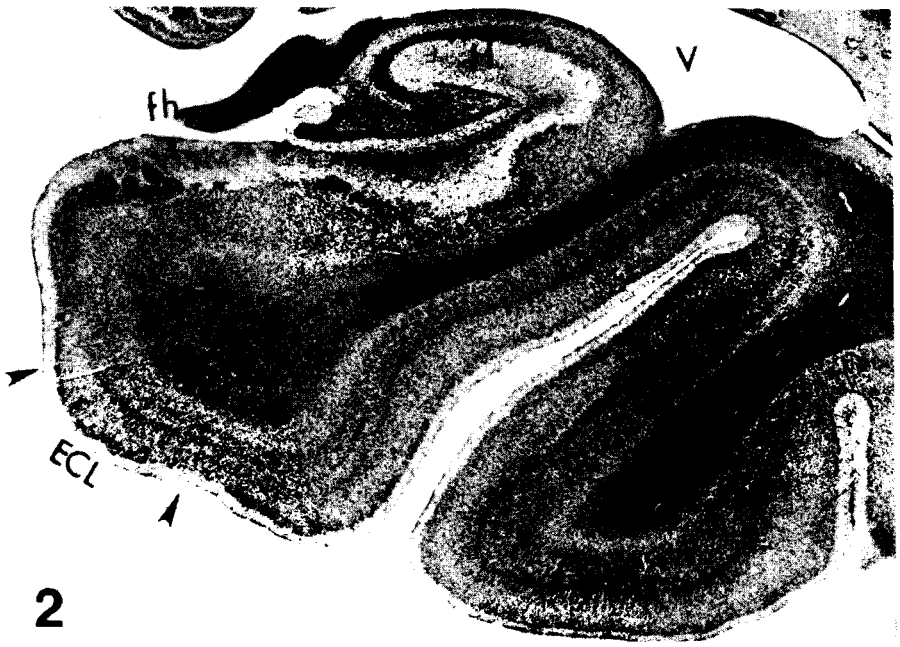

Fig. 2. Coronal section of the temporal lobe. Abbreviations: ECL, entorhinal cortex; fh, fimbria hippocampi; H, hippocampus, V, ventriculus lateralis.

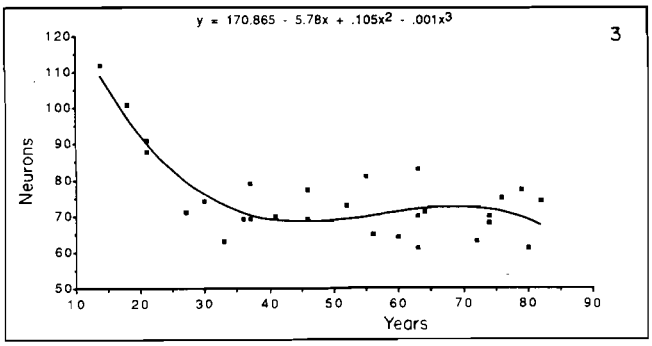

Fig. 3. Number of neurons in layer II of the EC according to age. The regression curve corresponds to the polynomial equation at the top of the figure.

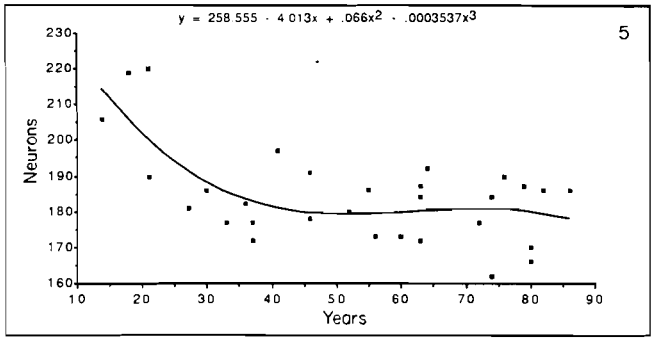

Fig. 5. Number of neurons in layer III of the EC.

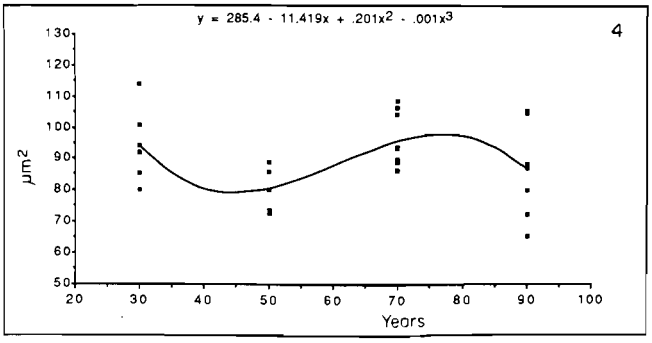

Fig. 4. Evolution of the nuclear area of layer II of the EC. The patients have been distributed in 4 groups (> 30, 30-50, 50-70 and 70-90 years of age).

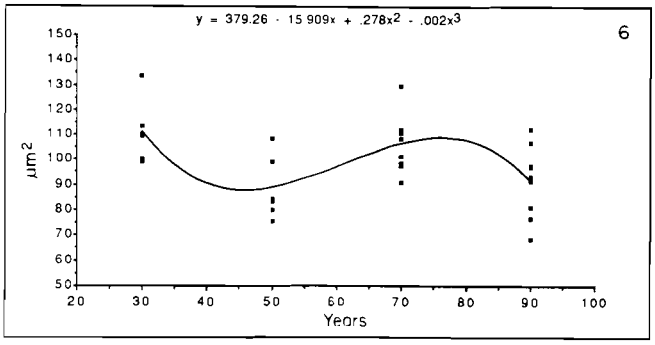

Fig. 6. Nuclear areas of the EC, layer III.

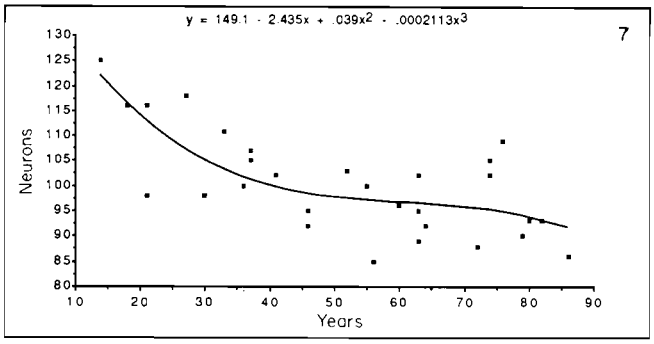

Fig. 7. Number of neurons in EC, layer V.

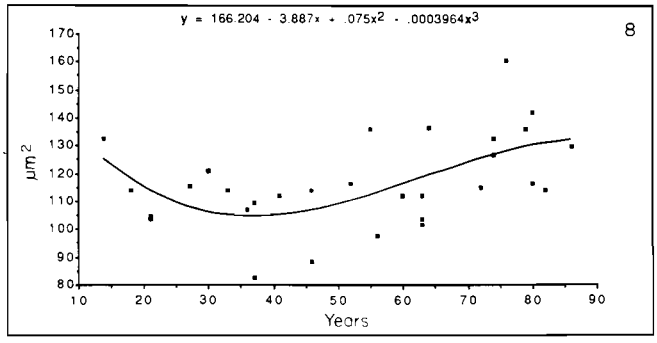

Fig. 8. Nuclear areas of the EC, layer V.

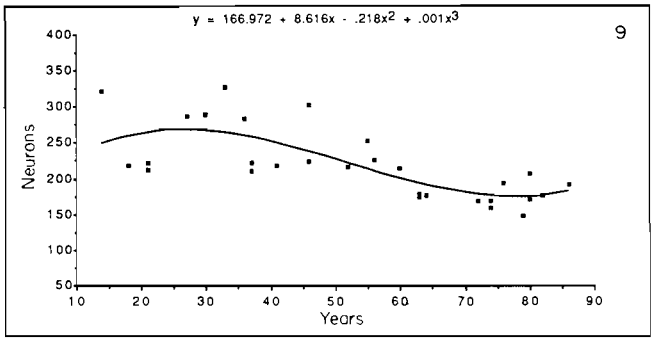

Fig. 9. Number of neurons in S pyramidal layer.

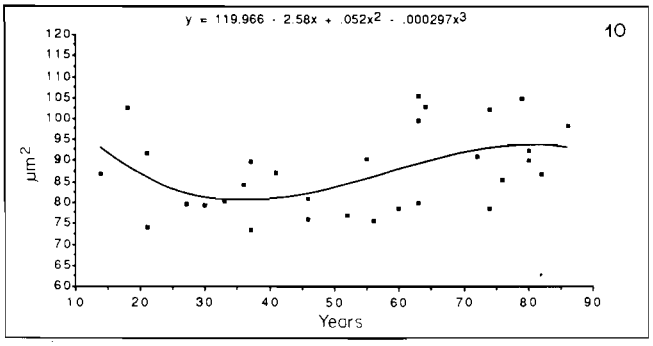

Fig. 10. Nuclear areas of the S pyramidal layer.

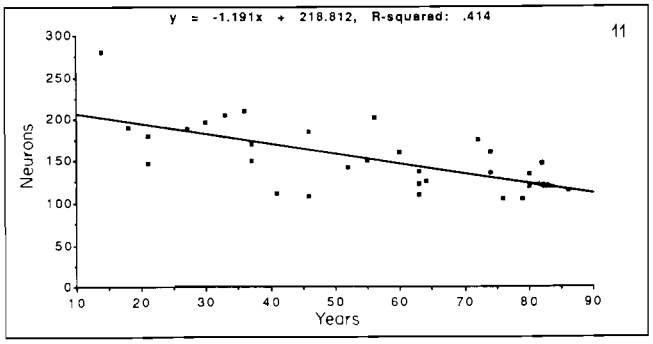

Fig. 11. Number of neurons in PS, layer II.

ranging between 10.4% and 18% ($p < 0.05$) according to the layer (Figs. 4, 6, 8); the second phase, to 75 years of age, showed an increase of the nuclear size, ranging between 10% and 14% ($p < 0.05$); and, finally, in the third phase, the nuclear size experienced a non-significant reduction in layers II and III. In layer V, however, the nuclear hypertrophy continued (12.3%, $p < 0.05$).

Subiculum

The neuronal number in the subiculum followed an evolution similar to that described for the EC. However, the neuronal reduction began later (about the age of 30) and lasted for a longer period till the age of 60. The global neuronal loss was of 32.1% ($p < 0.001$), (Fig. 9). The nuclear area decreased up to the age of 35 (5%) and, after that, increased (13%, $p < 0.05$) until the end of life (Fig. 10).

Presubiculum

The percentage of the neuronal loss in layer II of the PS was 32.9% ($p < 0.001$). Relatively uniform during the lifespan, the neuronal loss could be fitted to a regression line (Fig. 11). The nuclear size, on the other hand, was variable, therefore, differences between decades were not significant.

Discussion

Counting all the neurons, the only method to overcome the biassing effect of individual tissue shrinkage (Haug, 1980, 1986; Sass, 1982), is made difficult by the large extent of the human EC and SC. Therefore, we have restricted our study to the lateral area of the EC (Tuñón, 1989; Amaral et al. 1987) and the territory of the SC. The lateral area is one of the territories of the EC with denser afferents from the neocortex (Kosel et al., 1982; Goldman et al., 1984; Sorensen, 1985; Swanson and Köhler, 1986; Amaral et al., 1987; Insausti et al., 1987). On the other hand, its layers II, III, and V were chosen for neuronal counting and karyometry because they form the origin

of projections to the GD, CA3 and to different cortical areas (Andersen et al., 1966; Hjörth-Simonsen, 1972; Steward, 1976; Ruth et al., 1982; Witter et al., 1989). By counting all the neurons with a prominent nucleolus, included in the width of the studied layers (and keeping in mind that the sections were of 7 μm) we believe that the counting error was reduced to a minimum. As far as karyometry is concerned, since the dispersion of values was relatively small, measuring 100 nuclei per layer and case, we have assured the reliability of these results. Among the results described above, the parallel course of the neuronal loss in layers II, III and V of the EC, and the fact that they were rather small (11% for layer III, 15% for layer V, and 20% for layer II) are worth noting. This slight reduction of neuronal number contrasts with that observed in S and PS (32%) or in hippocampus, (26%) (Baztán and Gonzalo-Sanz, 1988), corpus mammillare (mean 33%) (Navarro, 1988). In the rat, the neuronal loss in the EC was considerably higher than in humans (38%) (Trillo, 1989). Despite the consistent neuronal loss in S and PS, the increase of the nuclear size in these territories was small (11.1%), notably inferior to that observed in hippocampus (mean 35%, $p < 0.0001$) (Baztán and Gonzalo-Sanz, 1988), corpus mammillare (mean 25%, $p < 0.01$) (Panadero and Gonzalo, 1988) and amygdala (mean 35%, $p < 0.001$) (Navarro, 1988). These results favor the hypothesis that, in the mnemonic circuit, the S is the link most severely damaged in ageing. Another feature to be emphasized is the different chronology of the neuronal loss in the three territories studied: while in the EC the maximal loss occurs during the first 35 years, in the S the process is the opposite (until the age of 40 years the neuronal reduction is minimal and between 40 and 60 years, it reaches its peak). This trend of the S agrees with that of the mnemonic function which begins to be impaired at the same time of life. The triphasic evolution of the nuclear area, in correspondence with the evolution of neuronal loss, could be related to the phenomenon of neuronal redundance and plasticity. The second phase, that of nuclear hypertrophy, pointed out that the redundance was becoming exhausted. The third phase, characterized by a stabilization of the nuclear size or a slight decrease, indicates reduction or loss of the neuronal plasticity that, in the previous phase, tended to compensate the neuronal loss. It is more difficult to explain the significance of the first phase wherein there is a simultaneous neuronal loss and decrease of the karyometric index. Perhaps, neuronal redundance as well as the increasing number of skills that become automtized could explain this apparently paradoxical behaviour.

Acknowledgements. This work was supported by a grant from the ECHEBANO foundation. We thank Dr. T. Tuñón and Dr. J.L. Guijarro for their help in obtaining the brains employed in this study.

References

Amaral D.G., Insausti R. and Cowan W.M. (1987). The entorhinal cortex of the monkey. J. Cytoarchitectonic organization. J. Comp. Neurol. 264, 326-355.

Andersen P., Holmquist B. and Voorhoeve P.E. (1966). Entorhinal activation of dentate granule cells. Acta Physiol. Scand. 66, 448-460.

Baztán S. and Gonzalo-Sanz L.M. (1988). Modificaciones en hipocampo humano y de rata, debidos a la edad. Rev. Esp. Geriatr. Gerontol. 23, 221-230.

Beckstead R.M. (1978). Afferent connections of the entorhinal area in the rat as demonstrated by retrograde cell-labeling with horseradish peroxidase. Brain Res. 152, 249-264.

Goldman-Rakic P.S., Selemon L.D. and Schwartz M.L. (1984). Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience 12, 719-734.

Haug H. (1980). Die AbhängigKeit der Einbettungschrumpfung des Gehirngewebes vom Lebensalter. Verh. Anat. Ges. 74, 699-700.

Haug H. (1986). History of neuromorphometry. J. Neurosci, Methods. 18, 1-17.

Hjörth-Simonsen A. (1971). Hippocampal efferents to the ipsilateral entorhinal area: An experimental study in the rat. J. Comp. Neurol. 142, 417-437.

Hjörth-Simonsen A. (1972). Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata. J. Comp. Neurol. 143, 219-231.

Hyman B.T., Van Hoesen G.W., Damasio A.R. and Barnes C.L. (1984). Alzheimer's disease: Cell specific pathology isolates the hippocampal formation. Science 225, 1168-1170.

Hyman B.T., Van Hoesen G.W., Kromer L.J. and Damasio A.R. (1986). Perforant pathway changes and memory impairment of Alzheimer's disease. Ann. Neurol. 20, 427-481.

Insausti R., Amaral D.G. and Cowan W.M. (1987). The entorhinal cortex of the monkey. II. Cortical afferents. J. Comp. Neurol. 264, 356-395.

Köhler C. (1984). Morphologic details of the projection from the presubiculum to the entorhinal area as shown with the novel PHA-L immunohistochemical tracing method in the rat. Neurosci. Lett. 45, 285-290.

Kosel K.C., Van Hoesen G.W. and Rosene D.L. (1982). Non hippocampal cortical projections from the entorhinal cortex in the rat and rhesus monkey. Brain Res. 244, 201-213.

Milner B. (1970). Memory and the medial temporal regions of the brain. In: Biology of memory. (Pribram K.H. and Broadbent D.E. (eds) Academic Press. New York. pp 29-50.

Navarro C. (1988). Proceso de senescencia del complejo basolateral de la amígdala y de la corteza periamigdalina. Estudio en humanos y ratas. Doctoral dissertation, Univ. of Navarra.

Panadero A. and Gonzalo L.M. (1988). Memoria y senescencia: cambios en cuerpo mamilar y núcleos talámicos anteriores debidos a la edad. Rev. Med. Univ. Navarra, 32, 191-200.

Ruth R.E., Collier T.J. and Routtenberg R. (1982). Topography between the entorhinal cortex and the dentate septotemporal axis in rats I. Medial and intermediate entorhinal projecting cells. J. Comp. Neurol. 209, 69-78.

Sass N.L. (1982). The age-dependent variation of the

embedding-shrinkage of neurobiological sections. Mikroskopie (Wien) 39, 278-281.

Shipley M.T. (1975). The topographical and laminar organization of the presubiculum's projection to the ipsi- and contralateral entorhinal cortex in the guinea pig. J. Comp. Neurol. 160, 127-146.

Sorensen K.E. and Shipley M.T. (1979). Projections from the subiculum to the deep layers of the ipsilateral presubicular and entorhinal cortices in the guinea pig. J. Comp. Neurol. 188, 313-334.

Sorensen K.E. (1985). Projections of the entorhinal area to the striatum, nucleus accumbens, and cerebral cortex in the guinea pig. J. Comp. Neurol. 238, 308-322.

Steward O. (1976). Topographic organization of the projections from the entorhinal area to the hippocampal formation of the rat. J. Comp. Neurol. 167, 285-314.

Swanson L.W. and Köhler C. (1986). Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. J. Neurosci. 6, 3010-3023.

Tamamaki N., Abe K. and Nojyo Y. (1987). Columnar organization in the subiculum formed by axons branches originating from single CA1 pyramidal neurons in the rat hippocampus. Brain Res. 412, 156-160.

Trillo L. (1989). Cambios de la corteza entorrinal y del complejo subicular en el proceso de senescencia: estudio morfométrico. Doctoral dissertation. Univ. of Navarra.

Tuñón T. (1989). Citoarquitectura de la corteza entorrinal humana. Doctoral dissertation. Univ. of Navara.

Witter M.P., Van Hoesen G.W. and Amaral D.G. (1989). Topographical organization of the entorhinal projections to the dentate gyrus of the monkey. J. Neurosci. 9, 216-228.

Zola-Morgan S., Squire L.R. and Amaral D.G. (1986). Human amnesia and the medial temporal region: Enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J. Neurosci. 6, 2950-2967.

Accepted June 1, 1991