

## **Effect of chronic alcoholism on the human hippocampus**

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**Summary.** The effect of chronic alcoholism on the human hippocampus was studied in 21 patients, divided in 4 groups: Group A under 45 years, group B 46-59 years, group C 60-69, and Group D over 70 years; and compared with age-matched control patients who died without neurological complications. The gyrus dentatus and the ammonic fields CA1 through CA4 were analyzed by counting the number of neurons and the size of the nuclear area. Both parameters were evaluated statistically.

The most important findings were a high neuronal loss in alcoholics in the first age group. In addition, the hippocampal neurons failed to display a vicarious reaction, since the nuclei did not show any increase in size despite the intense neuronal loss.

Our results point out an early neuronal loss in the hippocampus of alcoholic patients higher than age-matched controls, as well as a lack of reaction to the neuronal insult.

**Key words:** Chronic alcoholism, Hippocampus, Ethanol, Morphometry

### **Introduction**

Chronic alcoholism has a deleterious effect upon all body organs, but the most important, at least with respect to patient mortality, are those of the digestive system; especially the liver. However, the alterations in the central nervous system are also an outstanding feature at both neuropsychological and neuropathological levels. Common alterations are a decrease in the anterograde memory (Wilkinson and Carlen, 1980; Franceschi et al., 1984; Parsons, 1987), poor capacity for concentration and abstraction (Muuronen et al., 1984; Carlen and Wilkinson, 1987), emotional and affective

instability (Berglund and Sonesson 1976), and, occasionally, even dementia (Tarter, 1980; Lishman, 1981).

These behavioural disorders have a neuropathological substrate, consistent mostly in: diffuse cerebral atrophy (Ron et al., 1982; Melgaard et al., 1984; Harper and Holloway, 1985; Cala, 1987) with a severe involvement of the frontal and temporal lobes (Acker et al., 1982; Graff-Radford et al., 1982; Torvik et al., 1982; Dano and Guyader, 1988), decrease in the glucose brain consumption (Schachs et al., 1987; Eckhardt et al., 1988), and decreased cholinergic and noradrenergic activity (Miers, 1978; Mann and Mair, 1980; Lishman, 1986). There are also changes in the permeability of the neuronal membrane (Goldstein, 1983; Thomas, 1985), to potassium channels (Carlen and Wilkinson, 1987; Niesen et al., 1988).

The reports mentioned above undoubtedly account for some of the changes that take place in the central nervous system in a rather acute way. The information concerning how intense the neuronal loss in the human hippocampus is, and how the remainder of the neuronal population reacts before the reduction in the number of neurons is the aim of our present study, as well as the importance of the age of the patient at the beginning and in the course of those alterations.

We chose the hippocampus because chronic alcoholics exhibit a clear deficit in anterograde memory and, on the other hand, the hippocampus has a close relationship with the mnemonic function (Scoville, 1954; Milner, 1959; Squire, 1982; Olton et al., 1983; Zola-Morgan et al., 1986).

### **Materials and methods**

Human hippocampus samples were collected from necropsies of 3 hospital centers: Clínica Universitaria, Pamplona, Hospital of Navarra and Hospital Primero de Octubre, Madrid. The cases were selected according to their clinical history, by the anatomopathological

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study of the liver (only the cases with cirrhosis, steatosis or ethilic hepatitis with fibrosis, were chosen) and a third by brain examination. Those brains with macro or microscopic lesions which were not in accordance with the age of the patient or his alcoholic condition were discarded. Two other factors that were considered in the selection of the cases were sex (all were men) and age.

Since one of the aims of this study was to determine the hippocampal alterations which appeared in alcoholics of different ages, we selected brains from patients who died at 4 periods of life: under 50 years (6 cases), between 50-59 (5 cases), 60-69 (6 cases), and 70-80 (4 cases). The control cases were 21 patients of the same age, whose cause of death was extracerebral and without brain alterations not attributable to their age. In all cases the necropsy was carried out within 10 hours of death, the average being 5 hours.

The brains were fixed in 10% buffered formalin. The central portion of the hippocampus was embedded in paraffin, serially sectioned at 7  $\mu$ m and stained with cresyl-violet, PAS, or hematoxylin-eosin. The variation in the number of neurons as well as the karyometry of the remaining neurons was investigated.

**Neuronal count.** We distinguished the 4 areas in the cornu ammonis: (CA1, CA2, CA3 and CA4) and gyrus dentatus (Figs. 1, 2). All neurons with a prominent nucleolus, contained in a quadrant of 10,000  $\mu$ m (with a similar location in all sections) were counted in the gyrus dentatus and the ammonic fields from CA4 to CA1; the operation was repeated in 10 different sections per case and area.

**Karyometry.** The nuclear area was measured manually. The outline of all the nuclei with a prominent nucleolus was plotted (X 3,800) with the aid of a camera lucida attached to a microscope. One hundred nuclei per ammonic field and gyrus dentatus were measured with a digitizing tablet coupled to a Hewlett-Packard computer. This operation provided the area of such nuclei. In the cornu ammonis only pyramidal neurons were measured.

#### Statistical analysis

The quantitative data obtained in the neuronal count and from Karyometry were analyzed statistically by means of the following tests:

ANOVA - 1, Schéffé, Fisher and Student tests were carried out to assess whether the differences between alcoholic and control populations were statistically significant.

ANOVA - 2, was applied to judge the influence of alcohol and age on the morphometric result. A p value equal to or less than 0.05 was considered significant.

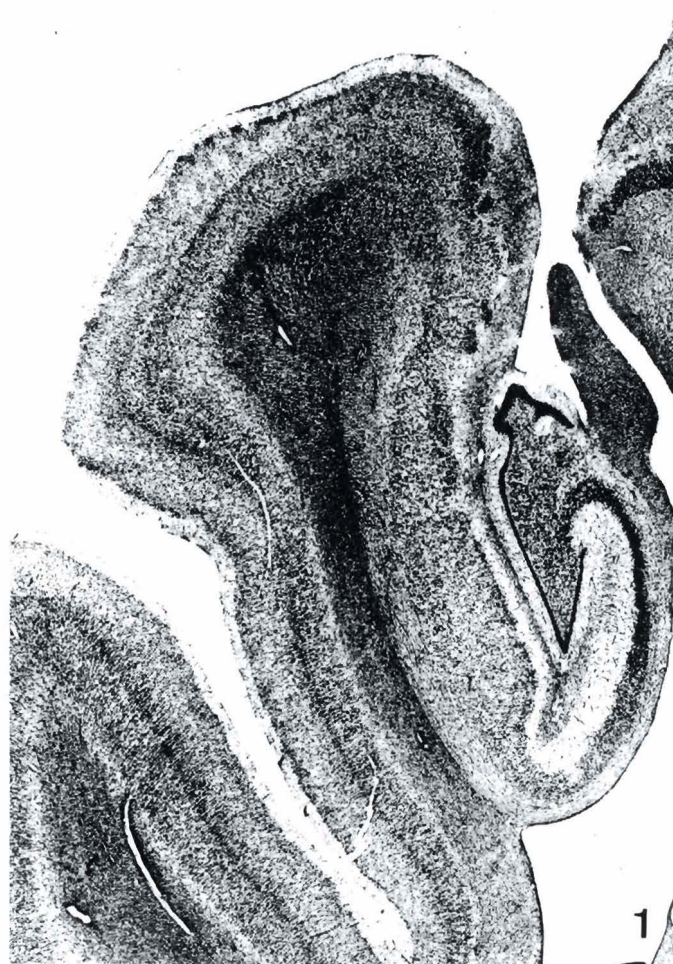
## Results

### Cornu Ammonis

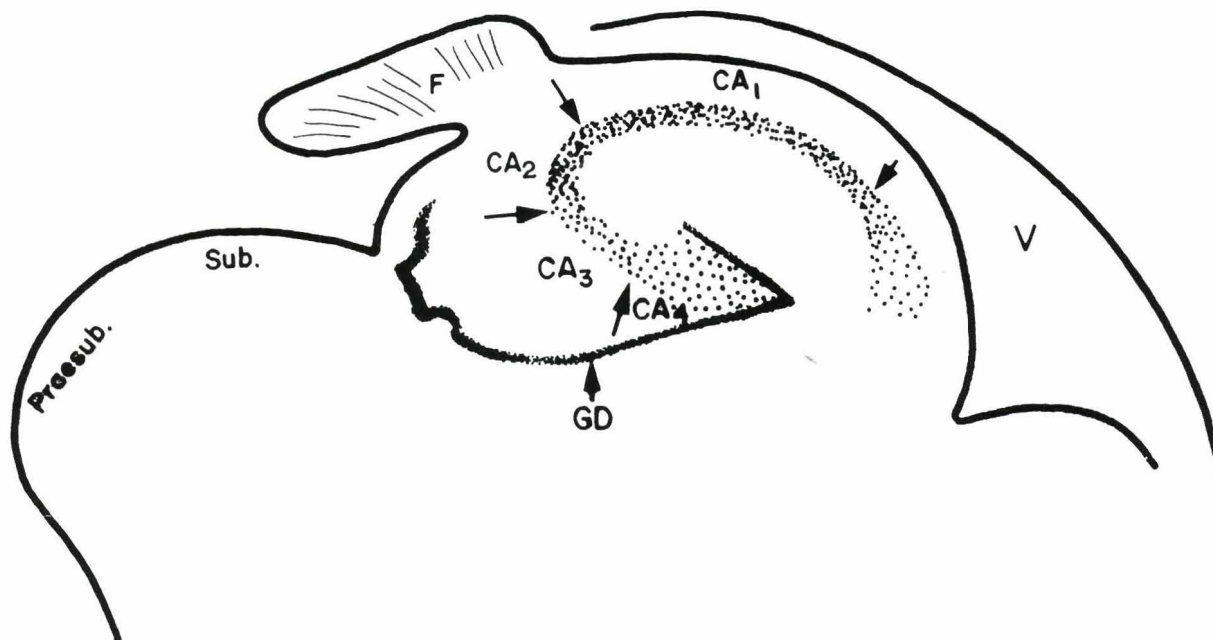
#### CA1

The number of neurons in field CA1 of the cornu ammonis was significantly reduced as compared to controls. Up to the age of 60 years the number of neurons in alcoholics was approximately one half of that of the controls. From 60 years onward, the difference between both groups decreased because in alcoholics, the number of neurons remained almost constant from the initial abrupt neuronal loss. On the other hand, the controls showed a more gradual decrease with aging (Fig. 3).

The evolution of the nuclear area was also different in controls and in alcoholics. In controls, there was a highly significant increase in the nuclear area from 55 years until the last period of life (Fig. 4a). In alcoholics the increase in the nuclear area was not significant (Fig. 4b).



**Fig. 1.** Microphotograph of a coronal section of the hippocampus and gyrus hippocampalis. The boundaries of the different fields of the cornu ammonis are represented in the line drawing of Fig. 2.



**Fig. 2.** Schematic representation of the hippocampus with the subdivisions of the cornu ammonis (CA1-CA4), GD = gyrus dentatus, F = fimbria, V = ventriculus lateralis.

**Table 1.** Neuronal counting. Differences between controls and alcoholics of group A (under 45 years)

	CA1	CA2	CA3	CA4	GD
Controls	18	13	17	11	85
Alcoholics	8 <sup>***</sup>	7 <sup>***</sup>	6 <sup>***</sup>	6 <sup>***</sup>	5 <sup>***</sup>
	55%	46%	64%	45%	35%

**Table 2.** Karyometry. Differences between both groups: controls and alcoholics of group A.

	CA1	CA2	CA3	CA4	GD
Controls	100	105	110	103	56
Alcoholics	83 <sup>*</sup>	78 <sup>**</sup>	84 <sup>**</sup>	90 <sup>*</sup>	45 <sup>**</sup>
	17%	25%	24%	13%	20%

\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

### CA2, CA3, CA4

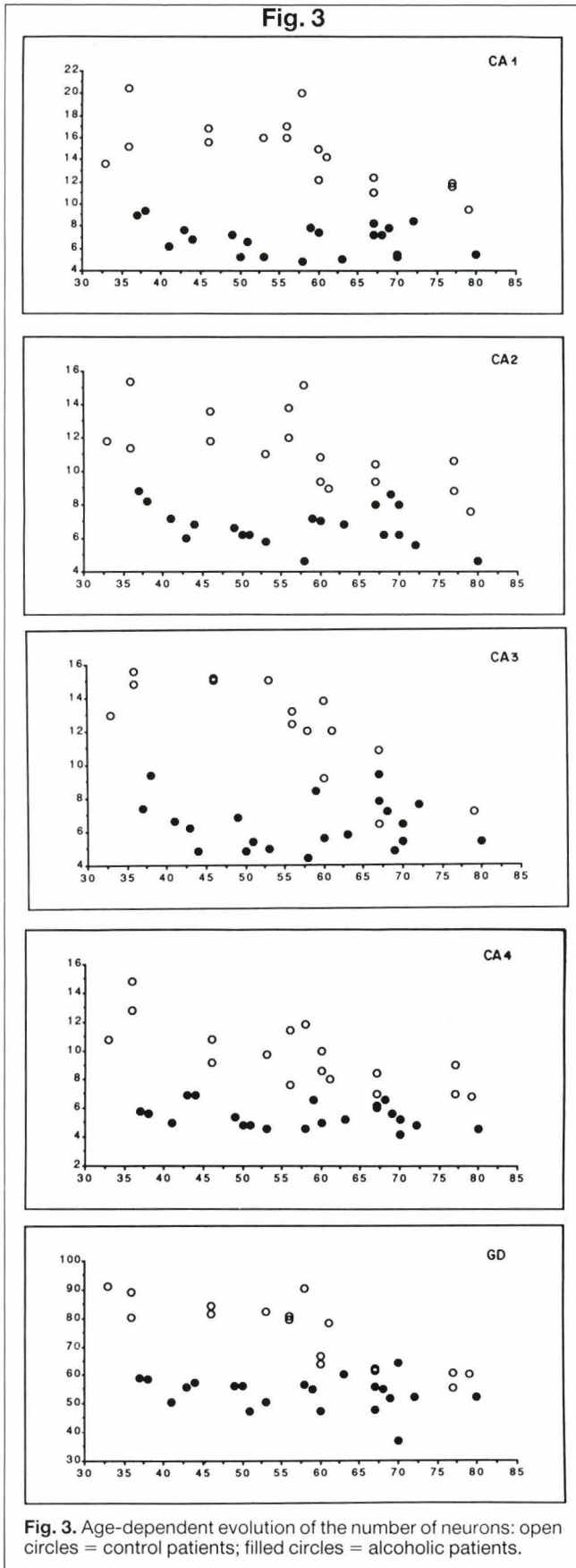
The results obtained in ammonic fields CA2, CA3 and CA4 were similar to those obtained in CA1. Neither, neuronal counting nor nuclear area differed from CA1. These results are quantitatively represented in Figs. 3-5 and in Tables 1 and 2.

### Gyrus Dentatus

In gyrus dentatus the value in neuronal number and nuclear area were clearly different in comparison to the cornu ammonis. In gyrus dentatus there was a highly significant difference in the number of neurons between

controls and alcoholics, less pronounced in the last period of life. The nuclear area showed highly significant increase from 55 years of age in controls, in alcoholics, this increase is absent (Figs. 3, 4, 6; Table 2). Moreover, changes in controls and alcoholics due to aging were similar to that in the cornu ammonis.

Thus, there are two features which differentiate alcoholics from controls: 1. An early and highly significant reduction in the neuronal number, and afterwards, a stabilization in cell loss. In controls the decrease in the number of neurons was uniform throughout the aging. 2. No significant changes in the nuclear size in spite of neuronal reduction. The controls, however, showed a significant increase in the last decades.



## Discussion

The most important finding of the present study is a profound neuronal loss in the hippocampus of young alcoholic patients, this could be as high as 50% of the estimated neuronal population. It seems that the remaining neurons were unable to compensate this loss.

Neuronal loss in several brain centres such as the granule cells of the cerebellum (Tavares and Paula-Barbosa, 1982), mammillary bodies (Lescaudron et al., 1984), pyramidal cells of the hippocampus (Mullen et al., 1984; Lescaudron and Verna, 1985), and the gyrus dentatus (Cadete-Leite et al., 1988), following chronic alcoholic consumption is described in experimental animals.

Though ethanol consumption is the most likely candidate for the neuronal loss, there are other possible candidates that should not be excluded: a) Vitamin deficit, which is due to incomplete diet usually associated with chronic alcoholism. Vitamin deficiencies are responsible for the neuropathological alterations in Wernicke-Korsakoff syndrome (Lambic, 1985). For this reason, all the patients diagnosed with Wernicke-Korsakoff syndrome were excluded from analysis. b) Age-dependent (Sass, 1982; Van Eden and Uylings, 1985; Uylings, et al. 1986) and tissue processing-dependent histological variabilities (Haug, 1980, 1986; Grace and Llinás, 1985; Scheff, 1987).

In our experience, the most determinant factor is the individual shrinkage, which is, in turn, dependent on pre- and post-mortem variables. Our experimental series obviates the technical and aging variability by using similar groups of age and the same tissue processing for alcoholics and controls.

The optimal method to avoid the problem of individual shrinkage is to make a total cell count, which is only feasible in small nuclei and with sharp boundaries. The human hippocampus does not meet such requirements. Therefore, total cell counts are practically impossible. In addition, the numerical estimation of the cell population (Mouritzen, 1979) is based on extrapolation of samples throughout the hippocampus. We tried to decrease the individual variability by counting all neurons in each section, and by taking each section roughly at the same rostrocaudal level. The dispersion of the values for the cell count and neuron size were very limited, indicating the validity of our results.

The prominent neuronal loss found in young alcoholics is less dramatic when compared to elderly controls. In fact, from the seventh decade onward, the differences between alcoholics and controls were not significant. We previously reported an important neuronal loss in the hippocampus of elderly persons, a feature not observed in the young ones (Baztán and Gonzalo, 1988). This led us to conclude that the neuronal loss observed in chronic ethanol abuse may be of the same magnitude as seen during aging. It remains an open question whether the neuronal population that disappears after chronic alcohol abuse will be the same or

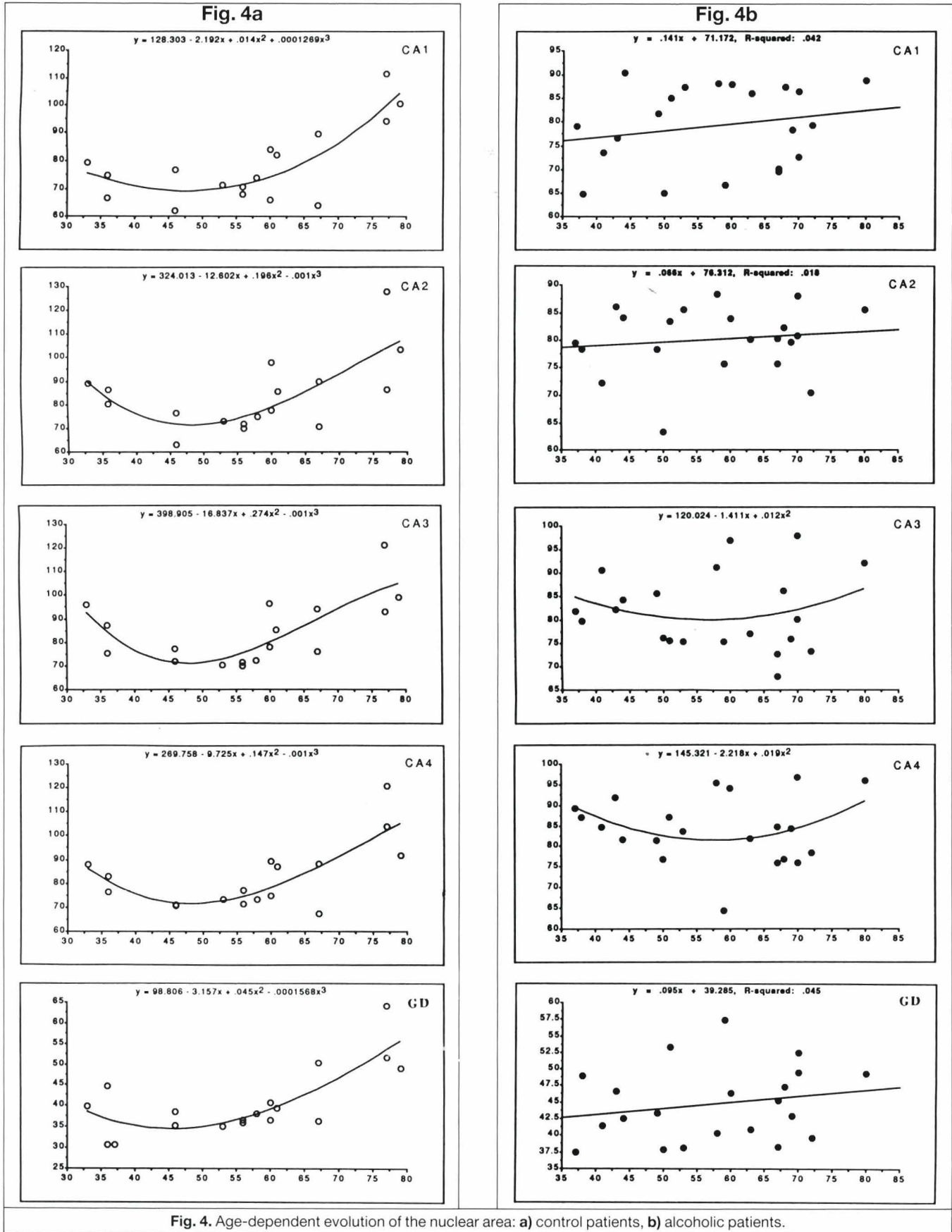


Fig. 4. Age-dependent evolution of the nuclear area: a) control patients, b) alcoholic patients.

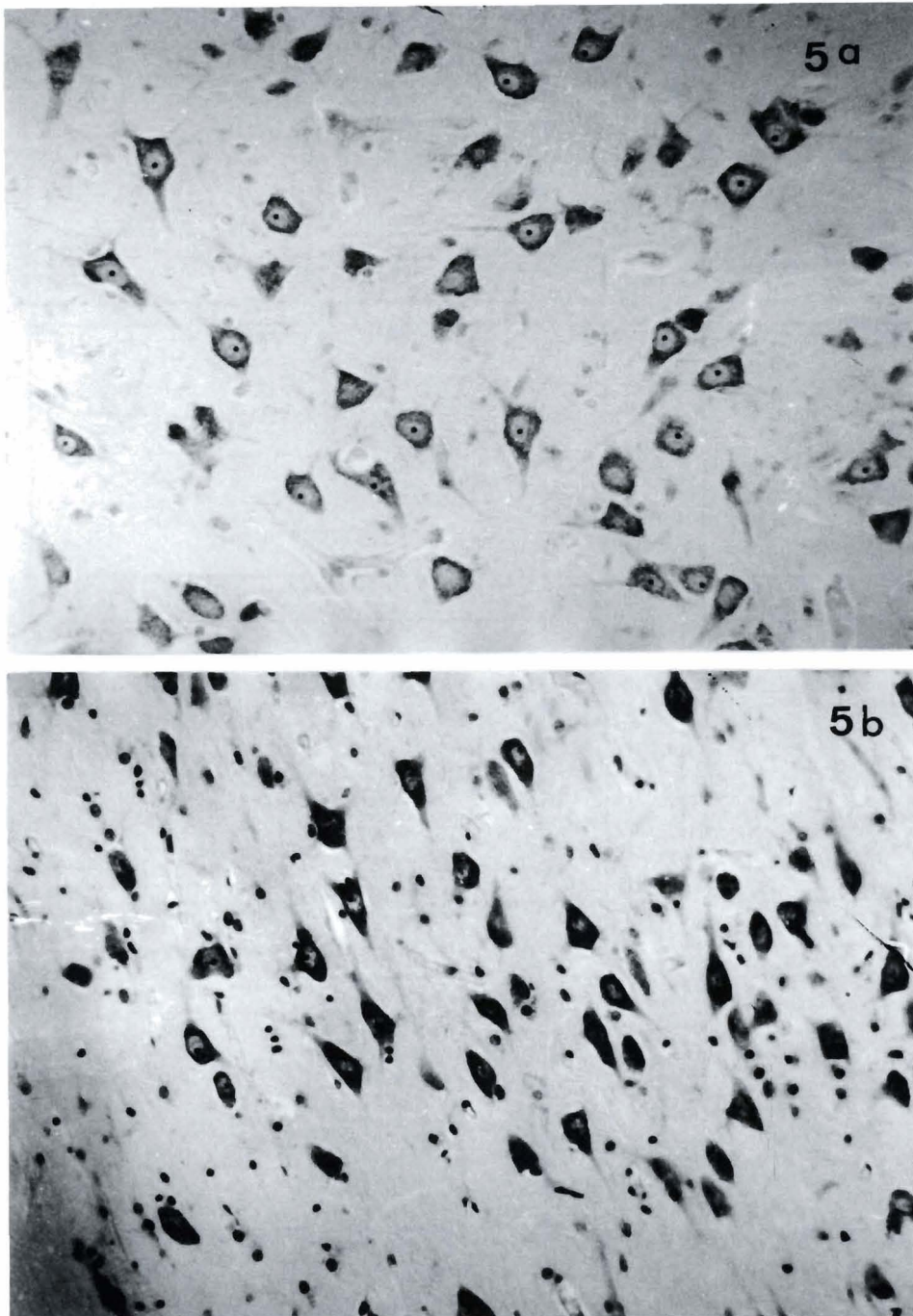


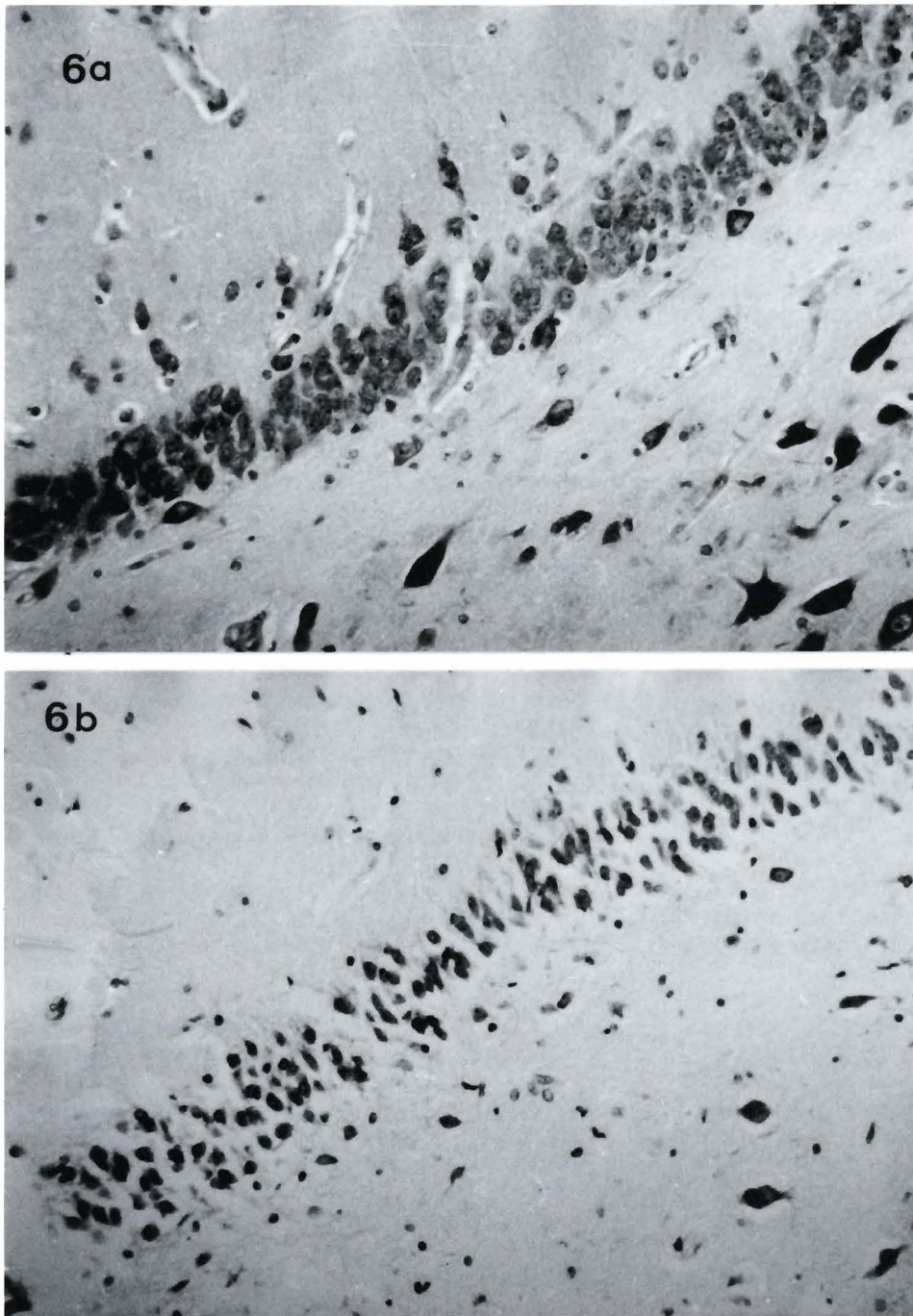
Fig. 5. Microphotograph of field CA4, a) control (35 years old), b) alcoholic (34 years old). Cresyl-violet,  $\times 160$

different to the cell population lost during aging. If it were the same, attempts should be made to determine which is the common mechanism responsible for neuronal death in aging and alcoholism, and the preservation of the remaining neurons.

The remaining neurons in hippocampus of alcoholics presented a limited capacity for compensatory changes, since the nuclear size did not increase. In contrast, in elderly controls this capacity was unaltered until the last decade (Baztán and Gonzalo, 1988). These two facts: neuronal loss and inability to compensate it, could

explain the severe psychic impairment shown by young alcoholics. Similar results have been reported in animals (West et al., 1982; Schachs et al., 1987; Trillo and Gonzalo, 1990).

The accuracy of investigation depends of the homogeneity of the materials used. Working with human material, there are factors such as genetic ones nutritional ones, stressors, etc., that are highly variable from one patient to another and make it impossible to compare. Other factors of sex, age, pathological state, could be easier to be uniformed. This discrepancy was



**Fig. 6.** Microphotograph of gyrus dentatus of the same cases that in the Fig. 5: **a)** control, **b)** alcoholic, Cresyl-violet,  $\times 160$

minimized in our human material by selecting male cases with chronic alcoholism, diagnosed not only by the clinical history but also by the necropsic findings. On the other hand, only the left hippocampus was used. Chronic alcoholics were considered only those with hepatic alterations of ethilic etiology, e.g. cirrhosis or steatosis (Lynch, 1960; Skullerud, 1985). The cases with special cerebral atrophy, i.e. cases with Wernicke-Korsakoff syndrome were excluded.

The homogeneity of the material was also impaired by the tissue shrinkage that is not only age-dependent (Sass,

1982; Van Eden and Uylings, 1985; Uylings et al., 1986) but also individual. In our experience the individual variations clearly surpass those of age. This tissue shrinkage is mainly due to fixation process, according to the histological methods employed (Haug, 1980, 1986).

To avoid this difficulty, there are three possibilities: to increase the number of cases, to utilize in all cases the same histological procedures and, to count all neurons found in a section. With the last procedure, the shrinkage in the coronal plaque is taken into consideration. These possibilities were strictly followed in our study. Since the

influence of shrinkage is age-dependent groups of alcoholics and controls of the same age were compared.

The neuronal counting gives a quantitative evaluation of the neuronal population of a nucleus or cortical area, which is much more precise than a global observation such as cerebral weight, amplitude of the sulci, etc. (Harper et al., 1985; Lishman et al., 1987). Neuronal counting has been frequently utilized as an objective method (Ebbesson and Tang, 1965; Kiss et al., 1983; Lescaudron and Verna, 1985; Mouritzen, 1979; Satorre et al., 1985; Cadete-Leite et al., 1988) to estimate neuronal loss. When this method is accompanied by karyometry we can evaluate cell vitality i.e. the vicarious capacity of the remaining neurons. The parallelism between cellular activity and nuclear size is well known (Kalimo, 1975; Roozendaal et al., 1987). Other morphometric methods such as the quantification of the dendritic arborization of the dendritic spines (Ferrer et al., 1986; Ferrer and Galofré, 1987; Galofré et al., 1987) are objective and inform us about the functional state of a neuron, but only allow the study of a small number of neurons, and therefore, are inadequate for the study of a large number of neurons in different neuronal centres in order to perform a statistical evaluation.

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