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Effects of chronic alcoholism on the amygdaloid complex. A study in human and rats

I. Alvarez, L.M. Gonzalo and J. Llor
Department of Anatomy, School of Medicine, University of Navarra, Spain

Summary. The effect of chronic alcoholism on the amygdaloid complex was studied in 16 humans and 10 rats. Eighteen patients whose death was due to extraneural causes were selected as controls with 3 rats.

The alcoholic cases, in addition to the data collected in their clinical history, showed, microscopically confirmed, liver cirrhosis or steatosis. The alcoholics and controls were divided into 4 groups: 35-44 years old (4 cases), 45-54 (5 cases), 55-64 (5 cases) over 65 (2 alcoholics and 4 controls). The alcoholic ingestion in the rats (Wistar, 10 weeks old) was 3 ml at a concentration of 30% in water solution administered by esophagic intubation, for 48 (5 rats) or 58 weeks (5 rats).

To judge the state of the amygdaloid nuclei, a neuronal count and caryometry were carried out. The numerical data obtained in this study were analyzed statistically. The results in humans have paralleled those obtained in rats and the behaviour of the different nuclei of the amygdala was uniform and can be summarized as follows: 1) ethanol provoked a prominent and early loss of neurons, and 2) the remainder of non-affected neurons did not react in order to compensate for this neuronal loss.

Key words: Chronic alcoholism, Amygdaloid complex, Ethanol and amygdala

Introduction

Chronic alcoholism has a deletereous effect upon the central nervous system (CNS). Neuropsychological examination reveals diminished anterograde memory function (Wilkinson and Carlen, 1980; Franceschi, et al., 1984; Parson, 1987), decrease in the concentration and abstraction capacity (Carlen and Wilkinson, 1987), emotional and affective alterations (Berglund and Ingrar, 1976), and in some cases, dementia (Tarter, 1980; Carlen et al., 1981; Lishman, 1981; Torvik et al., 1982). These clinical symptoms have an anatomic-pathological correlate: diffuse cerebral atrophy (Ron et al., 1982; Melgaard et al., 1984; Cala, 1987) with a more intense participation of the frontal and temporal lobes (Riley and Walker, 1978; Acker et al., 1982; Dano and Le Guyader, 1988), decreased cholinergic (Myers, 1978 and Lishman 1986) and noradrenergic activity (McEntee and Mair, 1980), etc. There are also changes in the neuronal membrane permeability (Goldstein, 1983; Thomas, 1985; Branchey et al., 1988) in potassium canals (Carlen and Wilkinson, 1987; Niesen et al., 1988), etc. These investigations provide information about the general changes that the CNS experiences under ethanol action. Papers, however, which study the gradual changes that appear in the different cerebral centres under the influence of ethanol are sparse. Likewise, investigations are necessary which study, comparatively, the ethanol effect in humans and in other animal species in which it is easier to make uniform all the factors that, besides alcohol, can influence the CNS.

Offprint requests to: L.M. Gonzalo, P.O. Box 273, Pamplona, Spain

Materials and methods

Humans

Sixteen brains of chronic alcoholics and 18 control brains, taken from normal patients with the following age distribution: 35-44 years, 4 cases; 45-54, 5 cases; 55-64 years, 5 cases; over 65 years, 4 control and 2 alcoholics, constitute the human material. In all cases death was due to extraneural causes and during their lifetime they were free of any diseases which could have affected the CNS. These were selected from among a larger group of brains, many of which did not fulfill the required conditions. For purposes of this study, cases were classified as alcoholics based upon hepatic alterations: cirrhosis, steatosis, etc.

Rats

Ten rats (Wistar) receiving alcohol were divided into 2 groups of 5 animals each, according to the duration of alcoholization: 48 or 58 weeks. The control group consisted of 5 rats. All these rats were males, 2 months of age when the experiment began. The control cases were sacrificed 53 weeks after the initiation of the experiment. Alcoholization was accomplished by means of esophageal canulation. During the first week, animals received 1 ml ethanol (15% solution) per day, 6 days/week; during the second, 2 ml ethanol/day (15%), 6 days/week; in the third week, 2 ml ethanol/day (30%), 6 days/week, and thereafter, 3 ml ethanol/day, 6 days/week. The animals were sacrificed under deep anesthesia with ether. The brains of both species, humans and rats, were fixed in buffered formalin (10%) for a period of 10 days. Then the rat specimens and, in the human cases, the bolck containing the amygdala, were embedded in paraffin, coronally and serially sectioned (7 mμ), and the slides divided in 3 series stained with cresyl Violet, PAS or hematoxilin-eosin.

Neuronal count

In rats, the neurons of 10 sections, regularly distributed from one end to the other of the corresponding nucleus, were counted. Only neurons with a prominent nucleolus were taken into account. For humans we utilized 20 sections per nucleus, and the neurons counted in each section were those included in a quadrat of 0.1024 mm^2, situated in the central part of the investigated nucleus.

Caryometry

The nuclear area was measured by a manual method: the contours of the nuclei (100 per each amygdaloid nucleus) were drawn (× 3,800) and then outlined with a digitizer coupled to a Hewlet-Packard computer that provided the area, the mean, the standard error, etc.

Statistical analysis

The numerical values obtained in the neuronal count and caryometry were statistically analyzed to determine their significance. ANOVA-1, Scheffé and Fisher probes and a Student test were performed to assess the differences between the alcoholic and control populations. ANOVA-2 was applied to judge the influence of alcohol and age in the morphometric results. P values $\leqslant 0.05$ were considered significant.

Nuclei investigated

In humans: basolateral (BL), lateral with its 2 portions: dorsolateral (Ld1) and ventromedial (Lvm), and the basomedial, pars profunda, (BM). In rats: Basolateral with its 2 areas: anterior (BLa) and posterior (BLp), and lateral with pars anterior (La) and posterior (Lp) (Figs. 1, 2).

Results

Rats

The results obtained in the investigated nuclei were very similar, therefore the description given for one can be applied to another with little variation.

Basolateral nucleus, pars anterior (BLa)

Neuronal count

There is a couspicous loss of neurons in the group of rats with 58 weeks (G.A.) of alcohol ingesta in comparison with the controls ($p < 0.01$). The group with with 48 weeks (G.B.) showed a lesser decrease ($p < 0.05$), therefore, betwen both groups A and B there was a significant difference ($p < 0.05$) (Figs. 3-6).

Caryometry

A reduction ($p<0.05$) of the nuclear size appeared in group A. On the contrary, in group B there was a considerable increase ($p<0.01$). The cytologic study, however, showed that these nuclei were not hypertrophic but hydropic (Figs. 4, 7).

Basolateral nucleus, pars posterior (BLp)

Neuronal count

This portion of the BL nucleus asppeared to suffer a greater loss of neurons than the pars anterior (Fig. 8). The difference between groups C and A was very significant, but the difference between A and B just attained statistical significance.

Caryometry

In this subnucleus we observed the same phenomenon as that in the BLa: the nuclei in group A were smaller ($p<0.05$) than the controls, but in B were notably larger. Theri appearance, however, was hydropic (Fig. 9).

Lateral nucleus, pars anterior (La)

Neuronal count

In this subnucleus, the neuronal loss was lesser than that seen in the Bla or BLp, but statistically significant ($p<0.05$) (Fig. 10).

Caryometry

In group A the nuclear area was smaller than in group C ($P<0.05$), and in group B notably larger (Fig. 11).

Lateral nucleus, pars posterior (Lp)

Neuronal count

Although the difference between both alcoholic groups was not significant, it was very significant with respect to grupo C ($p<0.01$, $F = 88.9$) (Fig. 12).

Caryometry

The reduction of the nuclear area in group A was significant and its increase in group B very significant (Fig. 13).

Summarizing the results obtained in rats, we can conclude that all the amygdaloid nuclei investigated exhibited similar behaviour: there is a considerable neuronal loss in both alcoholic groups, a slightly higher one in group A, a nuclear atrophy in group A and a hydropic degeneration in the nuclei of group B.

Results in humans

Comparing the evolution of the neuronal population in alcoholics and controls, we found a clear difference between them: while in controls there was a uniform neuronal loss corresponding to age, in the alcoholics there was a notable neuronal loss at much younger age and the later decrease is, in all, significant. With respect to nuclear size, in alcoholic groups, the nuclei were smaller than in controls.

Basolateral nucleus (BL)

Neuronal count

There was a very significant difference between alcoholics and controls in the younger patients (group A) and this difference diminished with the progression of age. However, a difference was still noted in the last group (D) (Figs. 14-16).

Caryometry

There was a very significant difference between controls and alcoholics, that increased with the progression of age because the nuclear area tended to augment in the controls and to diminish in the alcoholics (Fig. 17).

Lateral nucleus, pars dorsolateralis (Ld1)

Neuronal count

In controls the neuronal density decreased uniformly throughout life (Fig. 18) but in alcoholics there was already a considerable neuronal reduction at an early age (Fig. 19).

Fig. 1. Microphotograph of a coronal section through the amygdala of the rat brain. The different nuclei of the amygdaloid complex appear schematized in Fig. 2a.

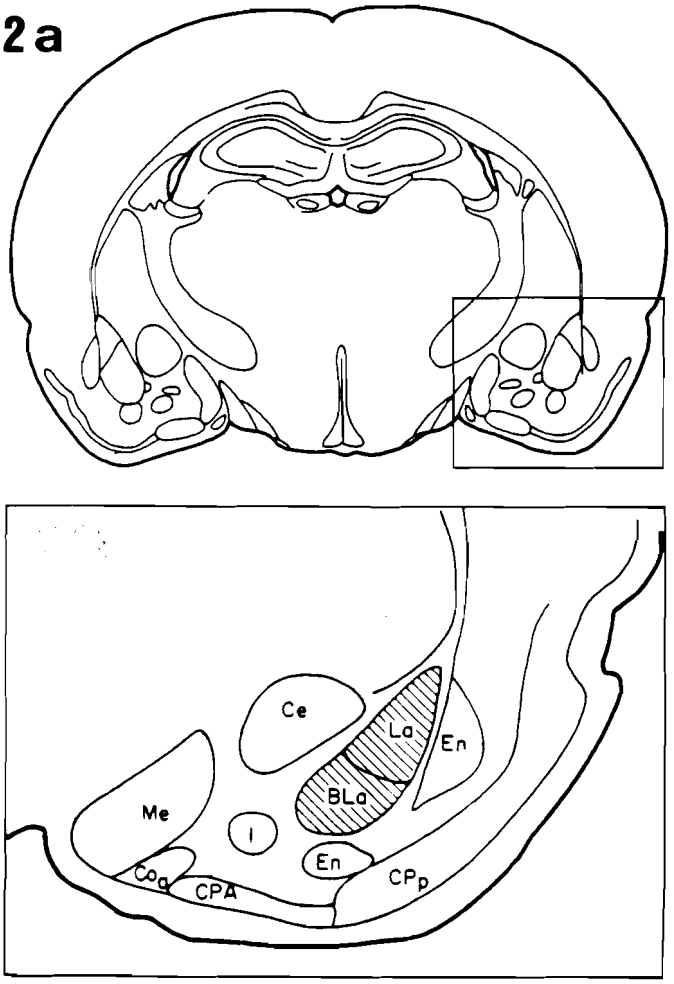

Fig. 2a. Scheme of the rat amygdala (pars anterior). BLa: basolateral nucleus, pars anterior; Ce: central nucleus; GPA: periamygdalar cortex; CPp: posterior piriform cortex; I: intercalar nucleus.

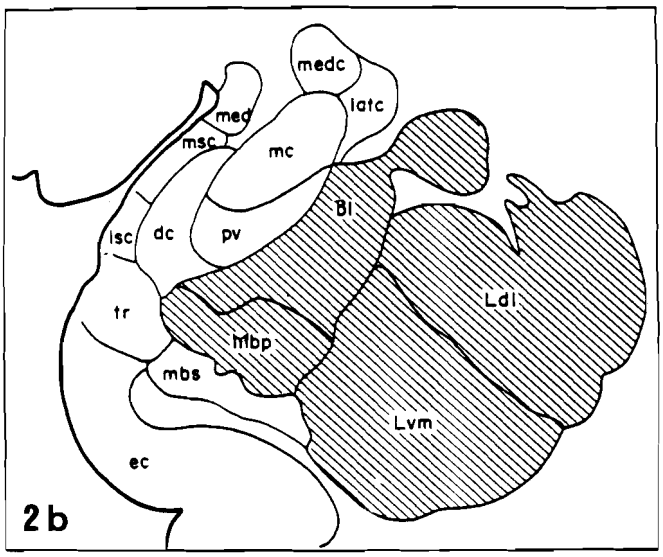

Fig. 2b. Scheme of the human amygdala. Bl: basolateral nucleus; Ldl: lateral nucleus (pars laterodorsalis); Lvm: lateral nucleus (pars ventromedialis); Mbp: basomedial nucleus (pars profunda).

Fig. 3. Microphotograph of the basolateral nucleus (pars anterior) of a control rat. Cresyl Violet, × 400

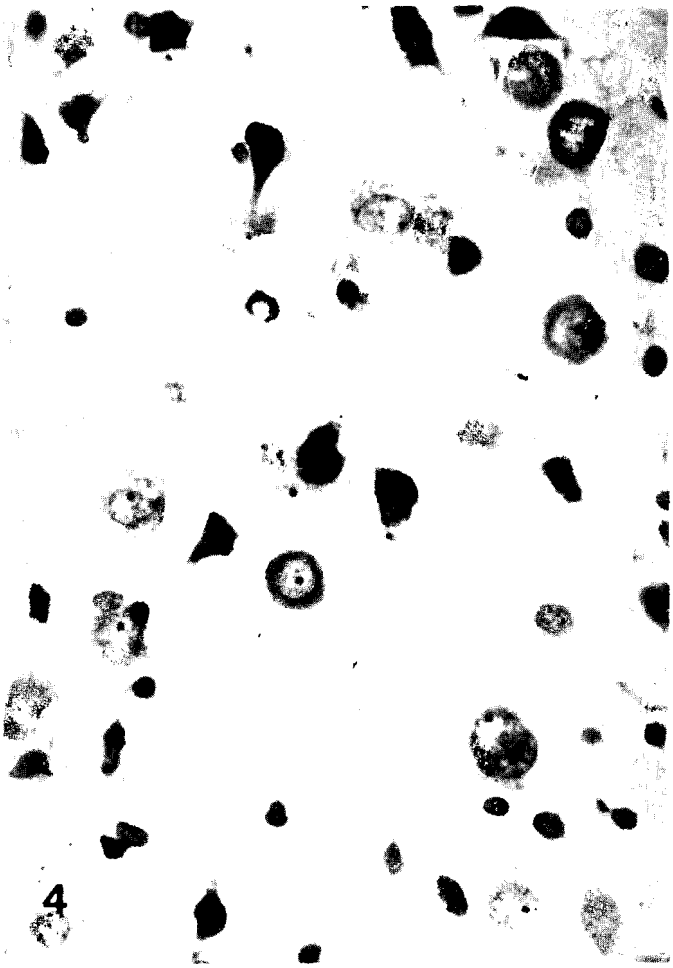

Fig. 4. Microphotograph of the basolateral nucleus (pars anterior) of a rat with 48 weeks of alcoholization. Some neurons show degenerative changes. Cresyl Violet, × 400

Caryometry

In group A, the nuclear size in controls was 41.7% greater than that in alcoholics and in group D, 50%. This difference shows that in alcoholics, in addition to the initial atrophy, the neurons did not develop a vicarious action, since the nuclear size did not augment as in the controls.

Lateral nucleus, pars ventromedialis (Lvm)

Neuronal count

The behaviour of this dorsomedial portion of the lateral nucleus was similar to that of the ventrolateral: in control patients there was an age-dependent neuronal density decrease (28.5% between groups A and D) while in alcoholics such a decrease was already very high in young patients as manifested by the difference between both A groups (38.8%) (Fig. 20).

Fig. 5. Microphotograph of the basolateral nucleus (pars anterior) of a rat with 48 weeks of alcoholization. The major part of the neurons are picnotic. The apparent neuronal density is due to tissue retraction. Cresyl Violet, × 400

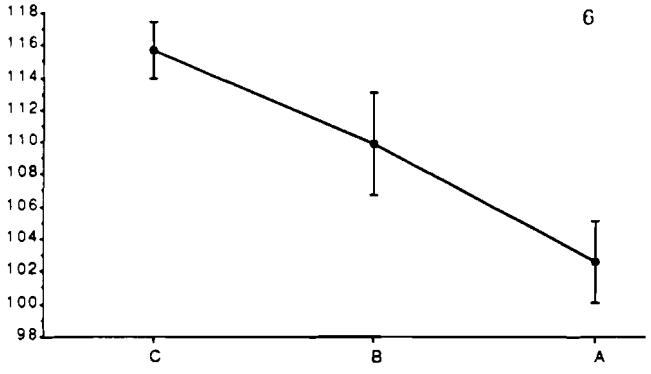

Fig. 6. Average neuronal count in the rat basolateral nucleus (pars anterior). C: control group; B: 48 weeks of alcoholization; A: 58 weeks of alcoholization.

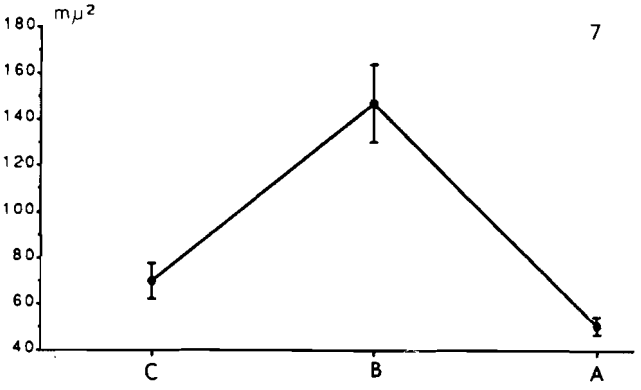

Fig. 7. Caryometry of the rat Bla nucleus.

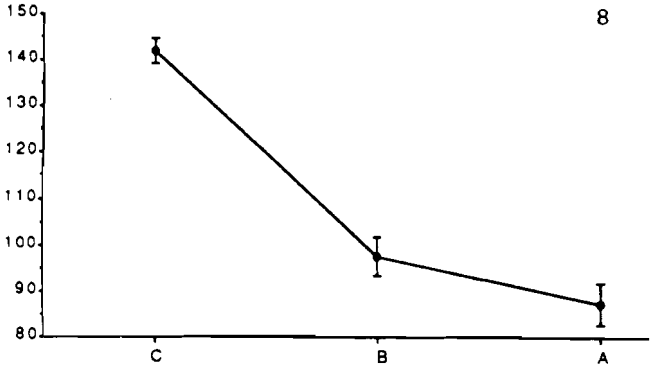

Fig. 8. Neuronal count in the rat Blp nucleus.

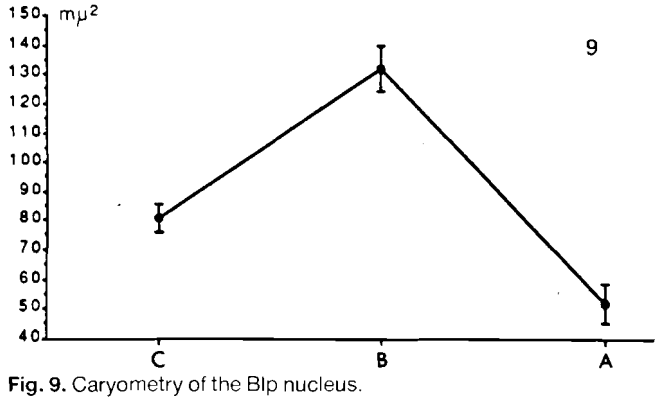

Fig. 9. Caryometry of the Blp nucleus.

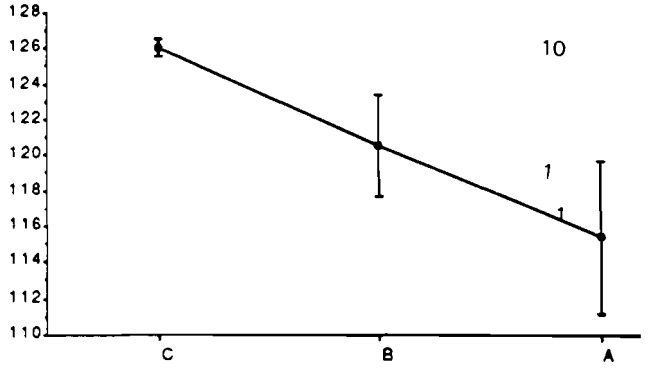

Fig. 10. Neuronal count in the rat La nucleus.

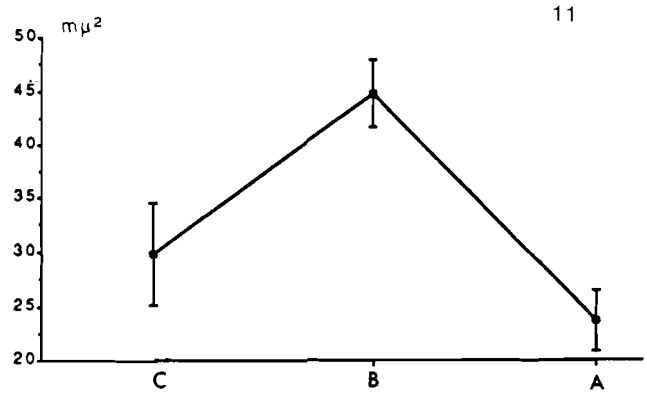

Fig. 11. Caryometry of the La nucleus.

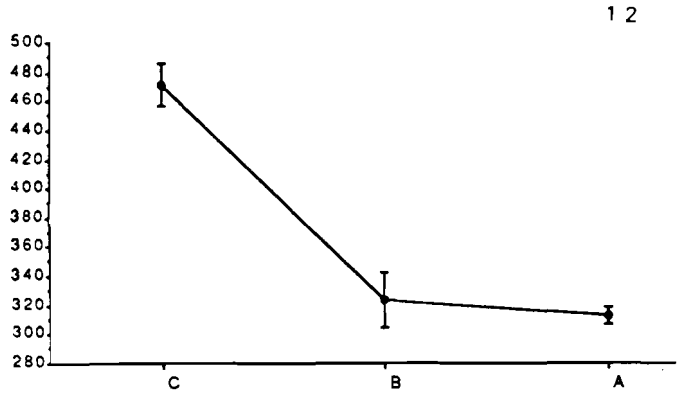

Fig. 12. Neuronal count in rat Lp nucleus.

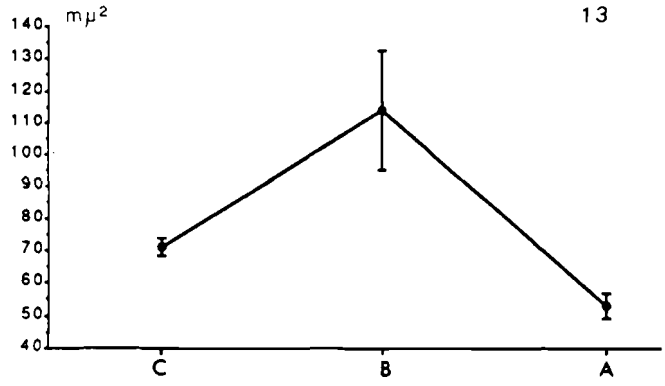

Fig. 13. Caryometry of the rat Lp nucleus.

Caryometry

Fig. 21 demonstrates very clearly the different size of the nuclei in controls and in alcoholics. In controls, the area tended to increase with age while in alcoholics it did not. In both, alcoholics and non-alcoholics of group A, the difference was 36.7%, while in groups D 49.1% (Fig. 21).

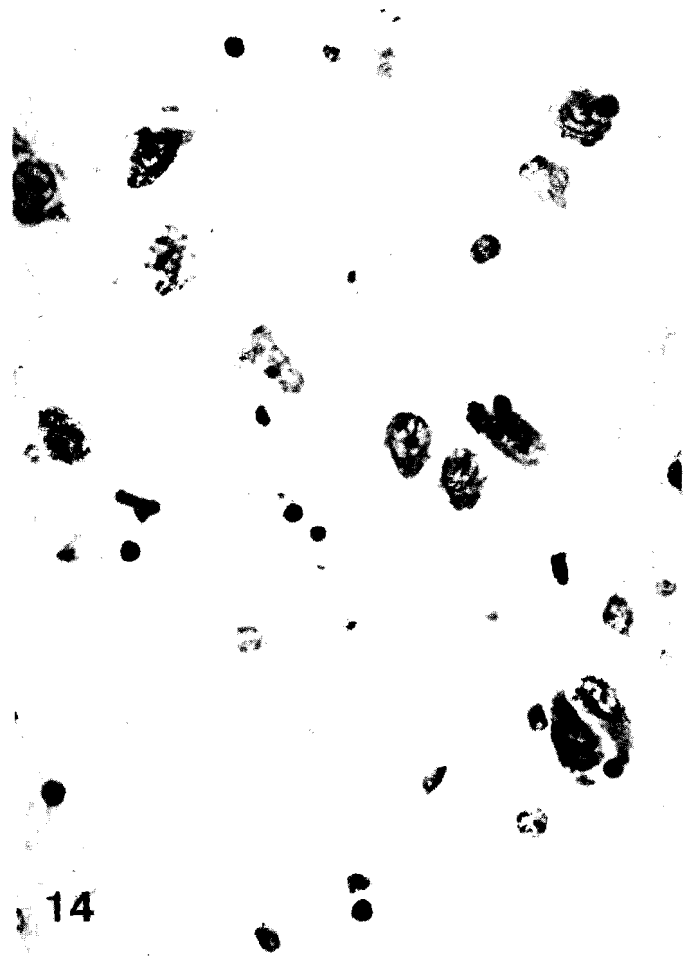

Fig. 14. Microphotograph of the basolateral nucleus of a control patient 44 years old. Cresyl Violet, × 400

Basomedial nucleus, pars profunda (BMp)

Neuronal count

The neuronal loss in the controls (groups A and D) was 25.5%, while in alcoholics the difference between groups A and D was insignificant, but the difference between controls and alcoholic patients in group A was 56.2% (Fig. 22).

Caryometry

The nuclear size difference between controls and alcoholics was noticeable: in alcoholics the nuclear area was half that of the controls.

Discussion

The realiability of the results that have been described are based upon strict selection criteria of the human cases and a proper selection of the methods employed to judge the neuronal state.

The control brains came from patients whose death

Fig. 15. Microphotograph of the basolateral nucleus of an alcoholic, 42 years old. Cresyl Violet, × 400

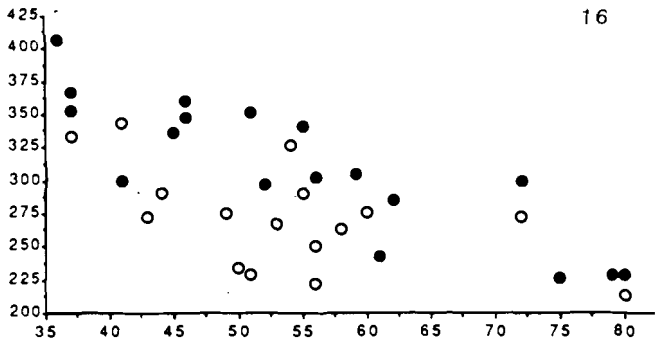

Fig. 16. Neuronal count in human BI nucleus. Closed circles: control patients; open circles: alcoholiccs. In abscissas, age of patients.

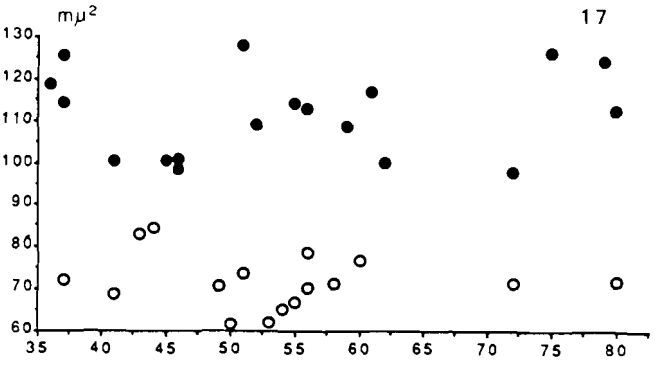

Fig. 17. Caryometry of the human BI nucleus. Closed circles: control patients; open circles: alcoholics. Abscissas, age of patients.

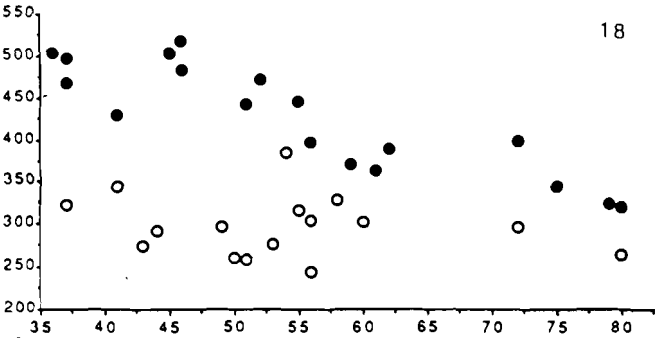

Fig. 18. Neuronal count in human lateral nucleus (pars dorsolateralis).

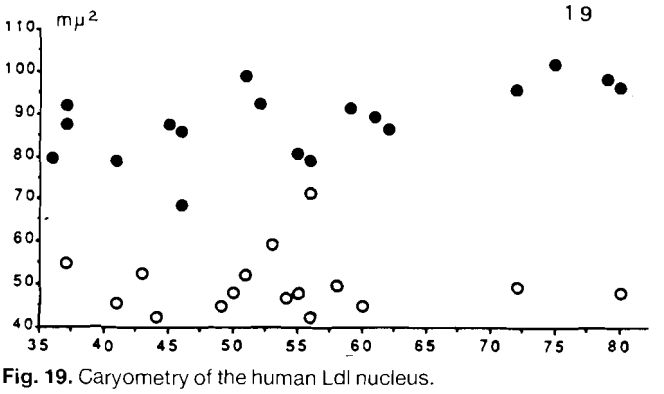

Fig. 19. Caryometry of the human LdI nucleus.

was due to extraneural causes and, in their clinical history, had not suffered diseases that could have influenced the structure of the CNS.

In the case of alcoholics, we only selected those patients whose alcoholism was confirmed by hepatic alterations: cirrhosis, steatosis, etc. On the other hand alcoholics with diseases such as AIDS, which could alter the CNS, have been excluded. In both, controls and alcoholics, in addition to the above mentioned causes of exclusion, a macro and microscopical examination of the brain and its arteries was carried out to eliminate those cases which showed alterations that did not correspond to their age.

Since differences have been described between men and women in the brain reaction to ethanol (Cala, 1984; Skullerud, 1985) only male brains have been collected.

In addition to ethanol, other factors could influence the human brain, such as genetics, diet, emotional stress, etc. and these may vary considerably from person to person. To know in what measure those factors act, it is necessary to use an experimental model in which all these factors are identical, so that the differences observed

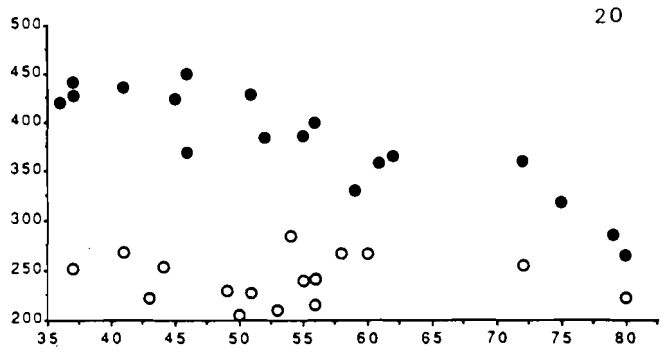

Fig. 20. Neuronal count in human lateral nucleus (pars ventromedialis).

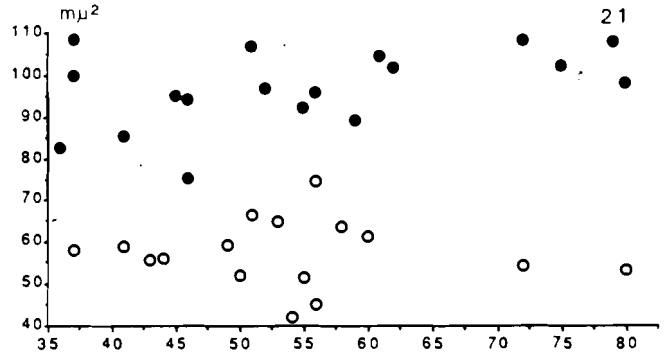

Fig. 21. Caryometry of the human Lvm nucleus.

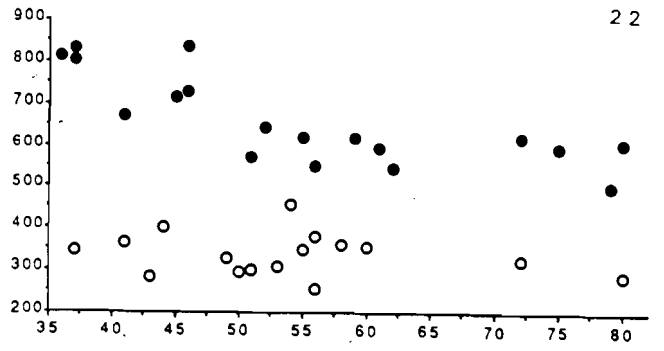

Fig. 22. Neuronal count in human basomedial nucleus (pars profunda).

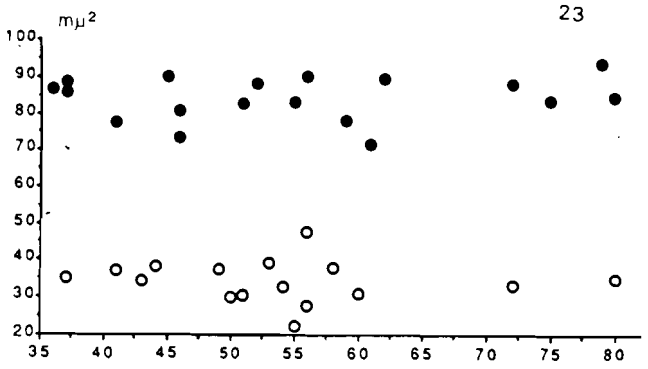

Fig. 23. Caryometry of the human BMp nucleus.

between controls and alcoholics can be attributed exclusively to the effects of ethanol. This is the reason why we have utilized rats of the same race and strain, and with the same diet and habitat.

Among the different procedures for the animal alcoholization: inhalation of ethanol vapour (Roach et al., 1973), ethanolization of the drinking water (Richter, 1976), liquid diet with ethanol (Lieber and de Carli, 1982), espohageal intubation (Majcrowicz, 1975), etc., we have chosen the last method. The animals tolerated the procedure very well, and their ingestion was normal (the weight of both control and alcoholic rats, at the end of the experiment, was similar).

Another basis for the reliability of the results is the accuracy of the methods employed for neuronal counting and caryometry. For neuronal counting, the most efficient method is a total cell count, but this is only possible when the studied structures are small. In our case, the neurons of 10 or 20 sections (for rats and humans respectively) of all the amygdaloid investigated nuclei, uniformly distributed from frontal to caudal end, have been counted. In the case of humans, since the extension of the nuclei in each section is large, only the neurons located in a quadrat of 0.1029 mm^2 were counted. In all cases only the neurons with a prominent nucleolus were taken into account.

For caryometry, similar precautions have been born in mind, but only 100 nuclei were measured in each subdivision of the amygdala. Caryometry is a morphometric method employed to evaluate the functional situation of cells. Casperson and Holgren (1934) utilized it for hepatic cells, Tonutti et al. (1954) for cells of the adrenal cortex, Kracht (1952) for thyroid cells, Pérez de Obanos and Gonzalo (1986) for luteal cells, and in the case of CNS, numerous authors have chosen this procedure (Hertl, 1952; Enestron, 1975; Roozendaal et al., 1987; etc.). There are also other morphological methods available, which can ascertain if there has been an increase or decrease in neuronal activity, e.g., quantification of dendritic ramifications or counting the number of synapses (West et al., 1982; Ferrer et al., 1986, 1987; Galofré et al., 1987). These procedures have, however, a limitation, i.e., that the small number of neurons investigated is incompatible with the number required for statistical analysis. The information acquired by neuronal counting or caryometry is interesting, but when both methods are simultaneously employed the interpretative value of experimental results is potentiated. The neuronal counting method provides information about the evolution of the cell population, and the caryometry indicates the reaction of the remaining cell population.

The results obtained in controls and alcoholics, either humans or rats, were uniform in all cases and similar in the different investigated nuclei. The parallelism of results obtained in both species indicates that those variable factors, whose action could influence throughout the lifetime the CNS of humans, have little importance in comparison with age and ethanol.

Two facts can be emphasized in our results: 1) in

alcoholics there is a pronounced and early neuronal loss, and 2) the absence of a compensatory reaction in the remainder of the neurons. Both facts contrast sharply with what occurs in the controls. These experience a uniform neuronal loss throughout life (Navarro, 1988), whereas young alcoholics have already lost more neurons than the 80-years-old control patients. The toxic action of ethanol upon neurons is also manifested by the lack of a vicarious response to compensate for the neuronal loss. In the control cases, on the contrary, the neuronal loss provokes a hypertrophy of the nuclei.

Are these effects due exclusively to the direct action of ethanol on the neurons, or also to the metabolic alterations caused by chronic alcoholism? It is known that ethanol produces metabolic alterations in the biological membranes (Goldstein, 1983; Thomas, 1985; Branchey et al., 1988) that induces a hyperpolarization, which possibly increases the conductance of potassium (Carlen and Wilkinson, 1987; Niesen et al., 1988), and alters the enzymatic systems bound to the membrane, e.g., the adenilato-cyclase (Saffey et al., 1988), the CAMP (Myers, 1978). However, other alterations seem to be caused indirectly, protein (Ogata et al., 1972; Branchey et al., 1988) and thiamine deficits as in the Wernicke syndrome (Duchen and Jacobs, 1984); on the other hand, the hepatic alterations can produce per se an encephalopathy (Adams and Foley, 1953; Victor and Adams, 1965; Zamora et al., 1973; Cavanagh, 1974; Duchen and Jacobs, 1984). It is, therefore, difficult to know to what extent the neuronal alterations correspond to a direct or to an indirect action of ethanol. However, the rats, whose liver was only slightly altered, and whose general state was satisfactory, showed neuronal alterations similar to that of humans. These findings favour the hypothesis that the most important effect of ethanol upon neurons is via a direct action.

References

Acker W.E.J., Majumdar, S.K. Shaw G.K. and Thomson A.D. (1982). The relationship between brain and liver damage in chronic alcoholics patients. J. Neurol. Neurosurg. Psychiatry 45, 984-987.

Adams R.D. and Foley J.M. (1953). The neurological disorder associated with liver disease. Research Publications Association for Research in Nervous and Mental Disease 32, 198-237.

Berglund M. and Ingvar D.H. (1976). Cerebral blood flow and its regional distribution in alcoholism and in Korsakoff's psychosis. J. Stud. Alcohol. 37, 586-597.

Branchey M.H., Branchey L.B., Korsten M.A., Richman E.I. Wilson J.S. and Lieber C.S. (1988). Alterations in synaptic membrane molecular order associated with alcoholization and protein deficciency in rats. Psychopharmacology 95, 43-48.

Cala L.A. (1987). Is CT scan a valid indicator of brain atrophy in alcoholism? Acta Med. Scand. Suppl. 717, 27-32.

Cala L.A., Jones B., Wiley B. and Mastaglia F.L. (1980). A computarized axial tomography (CAT) study of alcohol induced cerebral atrophy in conjunction with other correlates. Acta Psych. Scand. 62. (Suppl. 286), 31-40.

Carlen P.L., Lee M.A., Jacob M. and Livshits O. (1981). Parkinson provoked by alcoholism. Ann. Neurol. 9, 84-86.

Carlen P.L. and Wilkinson A. (1987). Reversibility of alcohol-related brain damage: clinical and experimental observations. Acta Med. Scand. Suppl. 717, 19-26.

Casperson T. and Holgren H.J. (1934). Varationen der Kerngrösse während der verschiedenen Phasen der Leberarbeit. Anat. Anz. 79, 53.

Cavanagh J.B. (1974). Liver bypass and the glia. Research Publications Association for Research in Nervous and Mental Disease 53, 14-35.

Courville C.B. (1955). The effects of alcohol on the Nervous System of man. Los Angeles. San Lucas Press.

Dano P. and Le Guyader J. (1988). Atrophie cerebrale et alcoholisme chronique. Rev. Neurol. (Paris) 144, 202-208.

Duchen L.W. and Jacobs J.M. (1984). Nutrional deficiencies and metabolic disorders. In: Greenfield's Neuropathology. 4th Edition. Adams J.H., Corsellis JAN., Duchen L.W. (Eds.). Edward Arnold. pp 573-626.

Eckardt M.J., Campbell G.A., Marietta C.A., Majchrowicz E. and Weight F.F. (1988). Acute ethanol administration selectively alters localized cerebral glucose metabolism. Brain Res. 444, 53-58.

Enestrom S. (1967). Nucleus supraopticus: a morphological and experimental study in the rat. Acta Path. Microbiol. Scand. Suppl. 186, 1-99.

Ferrer I., Fabregues I., Rairiz J. and Galofre E. (1986). Decreased number of dendritic spines on cortical pyramidal neurons in human chronic alcoholism. Neurosci. Lett. 69, 115-119.

Ferrer I. and Galofre E. (1987). Dendritic spine anomalies in fetal alcoholic syndrome. Neuropediatrics. 18, 161-163.

Ferrer I., Galofre E., López-Tejero D. and Llobera M. (1988). Morphological recovery of hippocampal pyramidal neurons in the adult rat exposed in utero to ethanol. Toxicology 48, 191-197.

Franceschi M., Truci G., Comi G., Lozza L., Marclosely P. and Glardi G. (1984). Cognitive deficits and their relationship to other neurological complications in chronic alcoholic patients. J. Neurol. Neurosurg. Psychiatry 47, 1134-1137.

Galofre E., Ferrer I., Fabregues I. and López-Tejero D. (1987). Effects of prenatal ethanol exposure on dendritic spines of layer V pyramidal neurons in the somatosensory cortex of the rat. J. Neurol. Sci. 81, 185-195.

Goldstein D.B. (1983). The pharmacology of alcohol. Oxford University Press. New York.

Hertl M. (1952). Brunzstzeitige Kernschwellung in Tuber Cinereum der weissen Maus. Morph. Sb. 92, 15.

Kracht J. (1953). Wechsel Beziehungen zwischen Nebennieren-rinde und Schildrüse im Tierexperiment. Verh. Deut. Ges. Path. 36, 202-213.

Lieber C.S. and De Carli L.M. (1982). The feeding of alcohol in liquid diets: two decades of applications and 1982 update. Alcoholism Clin. Exp. Res. 6, 523-531.

Lishman W.A. (1981). Cerebral disorder in alcoholism. Syndromes of impairment. Brain 104, 1-20.

Lishman W.A. (1986). Alcoholic dementia: a hypothesis. Lancet 1, 1184-1185.

Lynch M.J.G. (1960). Brain lesions in chronic alcoholism. AMA. Arch. Pathol. 69, 342-353.

Majchrowicz E. (1975). Induction of physical dependence upon ethanol and the associated behavioral changes in rats. Psychopharm. 43, 245-254.

McEntee W.J. and Mair R.G. (1978). Memory impairment in Korsakoff's psychosis: a correlation with brain noradrenergic activity. Science 202, 905-907.

Meldgaard B., Andersen K., Ahlgren P., Danielsen U.T. and Sorensen H. (1984). Peripheral neuropathy, cerebral atrophy and intellectual impairment in chronic alcoholics. Acta Neurol. Scand. 70, 336-344.

Myers R.D. (1978). Psychopharmacology of alcohol. Ann. Rev. Pharmacol. Toxicol. 18, 125-144.

Navarro C. (1988). Proceso de senescencia del complejo basolateral de la amígdala y de la corteza periamigdalina. Tesis doctoral. Univ. Navarra.

Niesen C.E., Baskys A. and Carlen P.L. (1988). Reversed ethanol effects on potassium conductance in aged hippocampal dentate granule neurons. Brain Res. 445, 137-141.

Ogata H., Ogata F., Mendalson J.H. and Mello N.K. (1972). A comparison of techniques to induce alcohol dependence and tolerance in the mouse. J. Pharmacol. Exp. Ther. 180, 216-230.

Parsons D.A. (1987). Intellectual impairment in alcoholics: persistent issues. Acta Med. Scand. Suppl. 717, 33-45.

Pérez de Obanos M^{a}.L. and Gonzalo L.M^{a}. (1985). Cambios en la estructura ovárica y en la fertilidad de la rata tras diversas neurectomías ováricas. Rev. Esp. Fisiol. 41, 235-242.

Richter C.P. (1976). A study of the effect of moderate dose of alcohol on the growth and behaviour of the rat. J. Exp. Zool. 44, 397-418.

Riley J.N. and Walker D.W. (1978). Morphological alterations in hippocampus after long - term alcohol consumption in mice. Science 201, 646-648.

Roach M.K., Kahn M.M., Coffman R., Pennington W. and Dawson D.L. (1973). Brain (Na-K) - activated adenosine triphosphatase activity and neurotransmitter uptake in alcohol - dependent rats. Brain Res. 63, 323-329.

Ron M.A., Acker W., Shaw G.K. and Lishman W.A. (1982). Computarized tomography of the brain in chronic alcoholism: a survey and follow -up- study. Brain 105, 492-514.

Roozendaal B., Van Gool W.A., Swabb D.F., Hoogendijk J.E. and Mirmiram M. (1987). Changes in vasopresin cells of the rat suprachiasmatic nucleus with aging. Brain Res. 409, 259-264.

Sachs H., Russell G.A.G., Christman, D.R. and Cook B. (1987). Alteration of regional cerebral glucose metabolic rate in non - Korsakoff chronic alcoholism. Arch. Neurol. 44, 1242-1251.

Saffey K., Gillman M.A. and Cantrill R.C. (1988). Chronic in vivo ethanol administration alters the sensitivity of adenylate cyclase coupling in homogenates of rat brain. Neurosci. Lett. 84, 317-322.

Skullerud T. (1985). Variations in the size of the human brain. Acta Neurol. Scand. 7, 61-70.

Tarter R.E. (1980). Brain damage in chronic alcoholics: a review of the psychological evidence. In: Addiction and brain damage. Richter D. (Ed). Crom Helm. London. pp 267-297.

Thomas P.K. (1985). Alcohol and disease: central nervous system. Acta Med. Scand. Suppl. 703, 251-264.

Tonutti E., Bahner F. and Muschke E. (1954). Die Veränderungen der Nebennierenride der Maus nach Hypophysektomie und nach ACTH - Behandlung. Endocrinologie 31 265-276.

Torvik A., Lindboe C.F. and Rodge S. (1982). Brain lesions in alcoholics. A neuropathological study with clinical correlations. J. Neurol. Sci. 56, 233-248.

Victor M. and Adams R.D. (1985). The alcoholic dementias. In: Handbook of Clinical Neurology. Vol. 2 (46). Neurobehavioral disorders. Fredericks JAM (Ed).Elsevier Science Publishers BV.

West J.R., Lind M.D., Demuth R.M., Parker E.S., Alkana R.L. and Cassell M. (1982). Lesion induced sprouting in the rat dentate gyrus is inhibited by repeated ethanol administration. Science. 218, 808-810.

Zamora A.J., Cavanagh J.B. and Kyu M.H. (1973). Ultrastructural response of the astrocytes to portocaval anastomosis in the rat. J. Neurol. Sci. 18, 25-45.

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