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Effect of chronic alcoholism on neuronal nuclear size and neuronal population in the mammillary body and the anterior thalamic complex of man

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Summary. The effect of chronic alcoholism on neuronal nuclear size and neuronal population of two memory-related diencephalic centres, the mammillary body and the anterior thalamic complex, has been examined in 24 chronic male alcoholics and 22 age-matched male controls. Cases were subdivided into three age groups (30-44 years, 45-59 years and 60-75 years). The results showed a significant reduction in both neuronal numbers and nuclear size in alcoholics compared to controls. Differences were especially high in the youngest alcoholics. The intensity of liver damage (steatosis vs. cirrhosis) did not have any significant effect. Moreover, an age-related decrease of neuronal number and karyometry was seen in controls but not in alcoholics. Our results suggest that chronic alcoholism accelerates the rate of neuronal loss in the mammillary body and anterior thalamic complex to a degree equivalent to aging. Likewise, chronic alcoholism impairs the compensatory increase in neuronal nuclei area seen in normal aging in these same structures. Our findings show that medial diencephalic memory centres are damaged in chronic alcoholism, which may contribute to the clinical symptomatology of these persons.

Key words: Alcoholism, Human, Mammillary body, Thalamic complex, Neurones

Introduction

Alcohol consumption, especially in its addictive form of ethanol, has a toxic effect upon different organs, particularly on the brain. The documented effect of alcohol on memory function raises the question of what anatomical pathways are impaired, either transiently or permanently (depending largely on the duration of the exposure to alcohol). Alterations in cortical and subcortical centres have been correlated with cognitive and memory deficiencies (Lee et al., 1979; Carlen and Wilkinson, 1980; Eckardt and Martin, 1986; Parson, 1987a,b; Tarter and Alterman, 1984). As far as the neuropathology associated to alcohol consumption is concerned, two systems seem to be involved: the medial diencephalic regions (Markowitsch, 1988) and the medial temporal lobe that comprises the hippocampal formation including entorhinal cortex and related cortical fields (Amaral, 1987; Squire and Zola-Morgan, 1991). The link between hippocampal formation and memory function is well established (Squire, 1987; Squire and Zola-Morgan, 1991). Modern methods of magnetic resonance imaging can distinguish in vivo lesions characteristic of one or the other system (Squire et al., 1990). However, memory, as an unitary function might involve simultaneously both systems, as they are closely interconnected by anatomical pathways (Amaral, 1987).

The most conspicuous changes associated to chronic alcoholism are found in the mammillary bodies, particularly in patients with Wernicke-Korsakoff's syndrome (Victor et al., 1989). However, the issue of what alterations are due to ethanol or to nutritional deficiencies, either alone or summed up to the alcohol effect, it is still unclear (Phillips, 1987; Thompson et al., 1983). Moreover, cases have been documented in which the memory loss of Wernicke-Korsakoff syndrome is present in the absence of alcoholism (Becker et al., 1990). Therefore, in order to exclude nutritional factors, one of the conditions of our study was to exclude the cases with Wernicke-Korsakoff's syndrome. Another assumption of our study is that alcohol effects take place simultaneously to those due to aging, therefore it would be interesting to dissociate both effects by dividing the whole population of alcoholics and controls of the study into three age groups. In this way it would be easier to discriminate between aging and alcohol effects upon the mammillary body and anterior thalamic nuclei. Another interesting issue is the role of liver damage in the observed effects of alcohol in the brain. We took into consideration this factor regarding the somewhat contradictory reports on the effect of liver damage upon

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Table 1. Distribution of control cases according to age, cerebral weight and cause of death. Cases have been divided into three groups: young (30-44 years), middle aged (45-59 years) and old (60-75 years).

CASE NUMBER	AGE	WEIGHT (gr)	CAUSE OF DEATH
Age group: 30-44 years			
1	32	1,150	Aids
2	32	1,570	Melanoma
3	36	unknown	Miocardiopathy
4	38	1,320	Pulmonary carcinoma
5	44	1,630	Pancreatic carcinoma
Age group: 45-59 years			
6	45	1,343	Miocardiopathy
7	46	1,200	Pulmonary carcinoma
8	50	1,440	Miocardial infarct
9	53	1,425	Leukemia
10	54	1,410	Pulmonary embolism
11	55	unknown	Pulmonary embolism
12	55	unknown	Pulmonary embolism
13	56	1,520	Tuberculosis
Age group: 60-75 years			
14	60	1,390	Pulmonary carcinoma
15	61	1,350	Pancreatic carcinoma
16	61	1,690	Pulmonary carcinoma
17	63	1,520	Pulmonary embolism
18	65	unknown	Pulmonary carcinoma
19	66	1,270	Prostatic carcinoma
20	67	1,485	Rectal carcinoma
21	68	1,360	Miocardial infarct
22	70	1,250	Esophagic carcinoma

Table 2. Distribution of alcoholic cases with the same criteria as in Table 1.

CASE NUMBER	AGE	WEIGHT (gr)	CAUSE OF DEATH
Age group: 30-44 years			
1	33	1,250	Drug overdose
2	38	1,300	Hepatopathy
3	41	1,600	Larynx carcinoma
4	44	1,380	Tuberculosis
5	44	1,640	Hepatopathy
Age group: 45-59 years			
6	49	1,300	Hepatopathy
7	49	1,750	Pancratic abscess
8	51	1,400	Hepatopathy
9	52	1,250	Cirrhosis
10	52	1,351	Cirrhosis
11	53	1,400	Hepatopathy
12	56	1,500	Hepatopathy
13	57	1,300	Hepatopathy
14	57	1,250	Cardiac insufficiency
15	58	1,250	Hepatopathy
Age group: 60-75 years			
16	60	1,440	Hepatopathy
17	62	1,450	Hepatopathy
18	63	1,100	Gastric carcinoma
19	64	1,300	Hepatopathy
20	65	1,400	Tongue carcinoma
21	67	1,500	Esophagic carcinoma
22	67	1,550	Pulmonary carcinoma
23	68	1,320	Hepatopathy
24	70	1,305	Kidney insufficiency

brain dysfunction (Lee et al., 1979; Arria et al., 1991; Harper and Kril, 1991). We have previously investigated some of the medial temporal lobe regions involved in changes provoked by alcohol, both in humans (Alvarez et al., 1989; Bengoechea and Gonzalo, 1990; Urbiola and Gonzalo, 1992; Ibáñez et al., 1995) and in rats (Bengoechea and Gonzalo, 1991; Ibañez et al., 1992). In the present work we aimed to investigate the effect of chronic alcoholism on medial diencephalic centres (the mammillary body and the anterior thalamic complex) in humans to disclose a specific effect of alcohol on these important classical relays on the subcortical output of the hippocampal formation (Amaral, 1987; Witter et al., 1989). Therefore, taking the precautions mentioned above, it seemed possible to disclose a specific effect of alcohol on the human mammillary body and the anterior thalamic complex.

Materials and methods

Necropsies from 24 male cases with clinical records of excessive and chronic alcohol drinking (an average of 120 ml of ethanol per day for several years) were collected (Table 1). Likewise, 22 male cases with no changes under neuropathological examination and no record of alcohol consumption, neurological psychiatric or liver damage were included as controls (Table 2). Age range was 30-75 years in both alcoholics and controls, and was subdivided into three age groups (Tables 1, 2); subgroup 1: 30-44 years (5 alcoholic cases and 5 controls); subgroup 2: 45-59 years (10 alcoholic cases and 8 controls); subgroup 3: 60-75 years (5 alcoholic cases and 9 controls). The alcoholic group was also divided into two groups according to the presence of steatosis (16 cases)

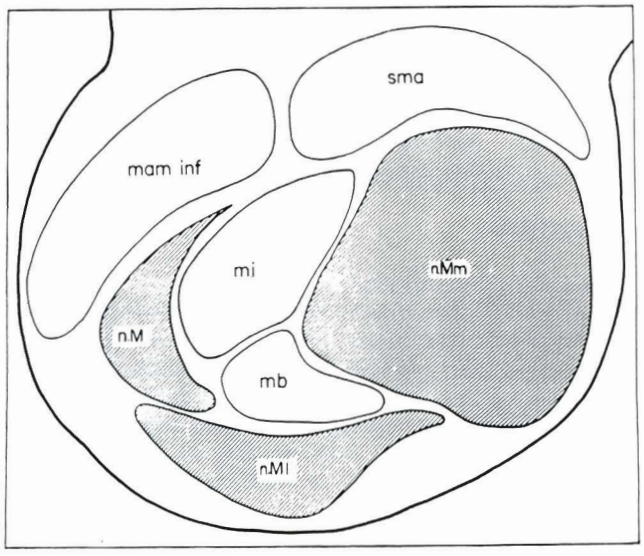

Fig. 1. Line drawing of the nuclei present in the human mammillary body. The areas studied are indicated by hatching.

Table 3. Average neuronal count in the mammillary body and the anterior thalamic complex in alcoholics and controls. Note the percentage of reduction found in alcoholics compared to controls and its statistical significance.

ZONE	ALCOHOLICS (means±SD)	CONTROLS (means±SD)	%	P
nMm	2480±100.8	3030±260.5	18	<0.001
nMl	3015±130.0	3765±350.4	20	<0.01
AD	2305±220.5	2755±230.5	16	<0.01
AV	3510±520.9	4695±510.2	25	<0.01
AM	3235±80.2	4300±480.0	25	<0.01

AD: anterodorsal nucleus of the thalamus; AM: anteromedial nucleus of the thalamus; AV: anteroventral nucleus of the thalamus; nMl: lateral mammillary nucleus; nMm: medial mammillary nuclus, medial portion.

Table 4. Average values for neuronal nuclear size in the mammillary body and the anterior thalamic complex in alcoholics and controls. As in Table 3, the percentage of reduction and statistical significance are indicated at the right-hand side of the table.

ZONE	ALCOHOLICS (means±SD)	CONTROLS (means±SD)	%	P
nMm	73.2±7.8	94.4±5.6	22	<0.01
nMl	79.5±8.4	110.7±17.6	28	<0.01
AD	68.7±4.7	87.4±5.5	21	<0.01
AV	86.1±13.7	116.5±7.1	26	<0.01
AM	79.2±10.2	109.7±13.4	28	<0.01

AD: anterodorsal nucleus of the thalamus; AM: anteromedial nucleus of the thalamus; AV: anteroventral nucleus of the thalamus; nMl: lateral mammillary nucleus; nMm: medial mammillary nuclus, medial portion.

or cirrhosis (8 cases) in the liver. The post-mortem delay ranged between 5 and 15 hours (average 7 hours) in both groups. The brains were fixed in 10% buffered formalin for three weeks. Blocks containing the whole extent of the mammillary body and the anterior thalamic complex were cut and embedded in paraffin, serially sectioned coronally at 7 µm and stained with cresyl violet or haematoxylin-eosin. A sample of the liver was cut in a cryostat at 15 µm and stained with scarlet red or haematoxylin-eosin. The neuronal population was determined by counting every neuronal soma present in sections spaced 70 µm, throughout the rostrocaudal axis of the nucleus considered. Separate counts were obtained for pars medialis and lateralis of the mammillary body (Fig. 1), and anteromedial, anterodorsal and anteroventral nuclei of the anterior thalamic complex (Fig. 2; Jones, 1989). Karyometry was assessed in the same sections. Fifty neuronal nuclei with a prominent nucleolus per section were measured with the image analysis system (1 pixel = 0.03 µm). Statistical analysis of the data was performed with the statistical package SPSS/PC+V.4.0. The tests employed were Kolmogorov-Smirnov, t-test or ANOVA of 1 or 2 factors for means comparison and lineal regression. Probability of 5% was considered significant.

Results

Results are summarized in Tables 4-6.

Neuronal counts (Fig. 3)

The neuronal count in the mammillary body of control cases presented a progressive decrease with age; it reached a 12% ($p<0.05$) reduction between groups 1 (the youngest) and 3 (the oldest). In contrast, the alcoholic group did not show differences among the subgroups of age. The nuclei of the anterior thalamic complex showed a neuronal reduction between subgroups 1 and 3 that ranged between 16% (anteromedial nucleus) and 25% (anterodorsal and anteroventral nuclei). Alcoholics presented a significant reduction in the number of neurons compared to age-matching controls in the two centres examined. The highest reduction was found in subgroup 1 (30-44 years), reaching up to 20% ($p<0.01$) in each division of the mammillary body. The same trend was observed in the anterior thalamic complex, where differences reached 28% in the anteroventral nucleus, 24% in

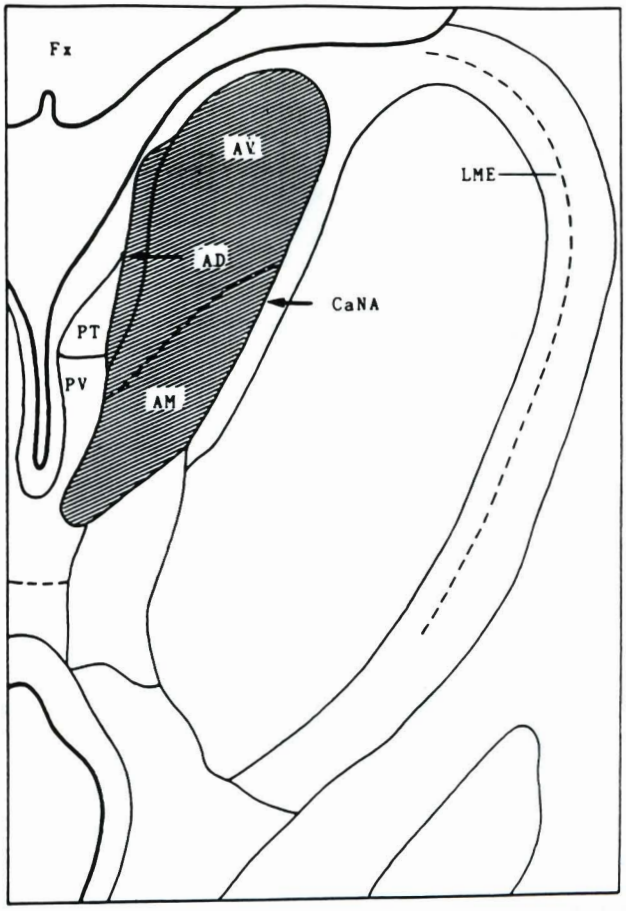

Fig. 2. Anterior thalamic complex and its nuclei in man. As in Fig. 1, the studied areas are hatched.

Table 5. Comparison of cirrhosis and steatosis alcoholic cases expressed in neuronal count and neuronal nuclear size. No significant differences could be demonstrated.

ZONE	NEURONAL COUNT			KARYOMETRY		
	Cirrhotic (mean±SD)	Steatotic (mean±SD)	Significance	Cirrhotic (mean±SD)	Steatotic (mean±SD)	Significance
nMm	2570.5±300.5	2290.0±15.3	N.S.	72.1±7.5	75.6±8.3	N.S.
nMl	3100.5±210.3	2840.0±13.2	N.S.	79.4±6.7	79.7±5.4	N.S.
AD	2390.0±100.5	2240.5±13.7	N.S.	68.9±5.5	68.5±7.2	N.S.
AV	3470.0±250.1	35.80.5±17.4	N.S.	85.4±13.7	87.4±12.8	N.S.
AM	3280.0±180.4	3140.0±19.2	N.S.	78.6±6.4	80.5±13.2	N.S.

AD: anterodorsal nucleus of the thalamus; AM: anteromedial nucleus of the thalamus; AV: anteroventral nucleus of the thalamus; nMl: lateral mammillary nucleus; nMm: medial mammillary nuclus, medial portion; N.S.: no statistical significance.

the antero-dorsal nucleus and 21% in the antero-medial nucleus ($p<0.001$). Somewhat smaller differences between alcoholics and controls were found in subgroup 3 (60-75 years), although it was still statistically significant. Neuronal counts had no differences between cases with liver steatosis compared to those with liver cirrhosis.

Karyometry (Fig. 4)

Neuronal nuclear area presented differences among subgroups of age that did not reach statistical significance, neither in controls nor in alcoholics. However, nuclear size in alcoholics was clearly smaller compared to controls, although similar in the

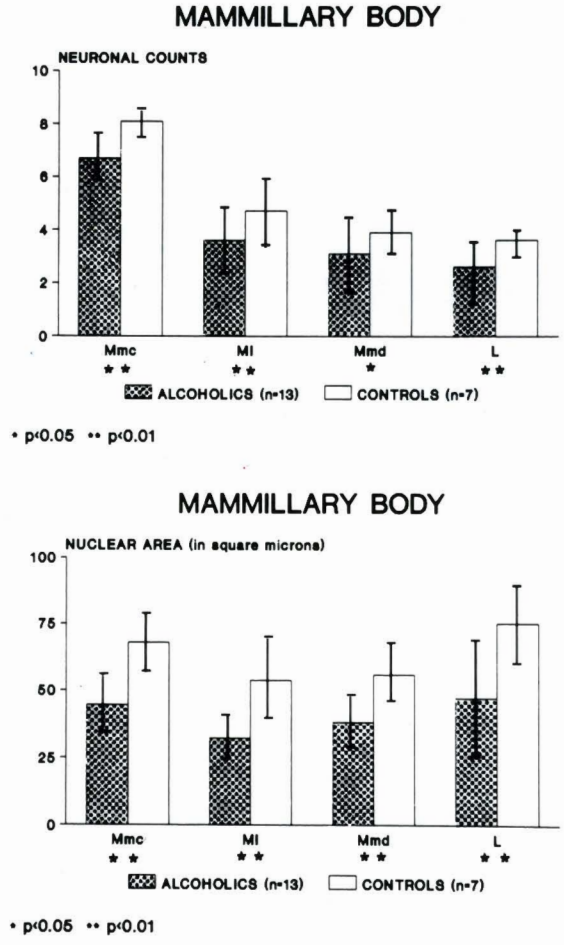

Fig. 3. Neuronal count (upper row) and neuronal nuclear area (lower row) in the mammillary body of alcoholic cases (stippled histograms) and in controls (open histograms). The reduction of both parameters in alcoholics is apparent.

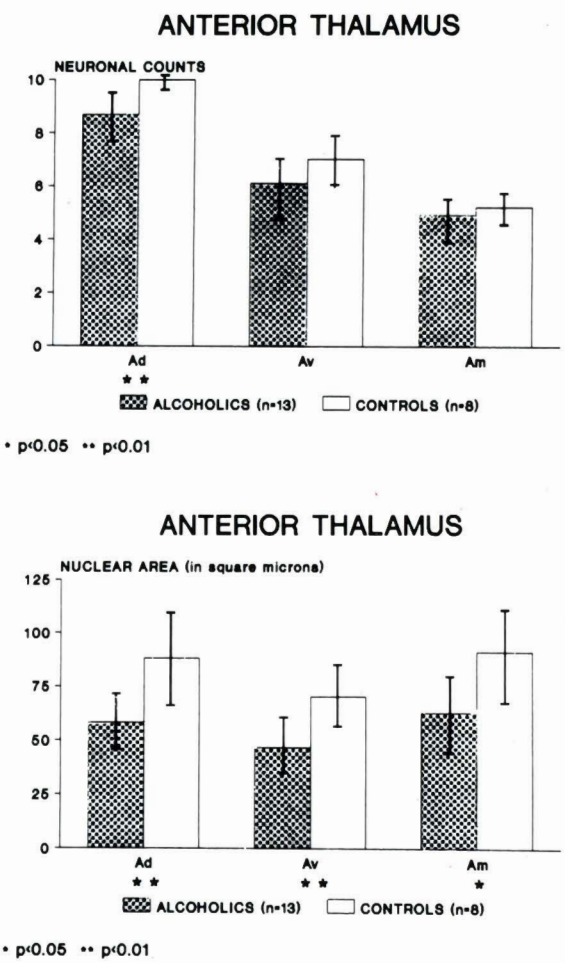

Fig. 4. Neuronal count (upper row) and neuronal area (lower row) in the anterior thalamic complex of alcoholic cases (stippled) and in controls (open bars). Note the reduction in alcoholics compared to controls.

mammillary body than in the anterior thalamic complex. The largest reduction was noted in the anteromedial thalamic nucleus (30%, $p<0.1$), while the medial division of the mammillary body had the smallest reduction (19%, $p<0.01$). The occurrence of liver steatosis or liver cirrhosis did not have any effect on neuronal nuclear size.

Discussion

There are two major findings in our study: the reduction in neuronal counts, specially in the first subgroup (30-44 years); and the reduction in neuronal nuclear size in the mammillary body and in the anterior thalamic complex of alcoholics compared to age-matched controls.

Methodological consideration

The methods used to quantify the changes produced in the brain by ethanol (neuronal counts and karyometry) provide accurate information about neuronal loss and functional capacity of the surviving neurons (Lescaudron and Verna, 1985; Cadete-Leite et al., 1988; Alvarez et al., 1989; Lacalle et al., 1991). Quantitative morphometric studies often use only one method to evaluate the effects of alcohol or aging on the brain (Lescaudron et al., 1984; Beracoechea et al., 1987; Roozendaal et al., 1987). The use of both methods offers more detailed information on the consequences of a noxious agent upon nervous centres.

The study of human material always needs to be interpreted with some caution because, in addition to the problem under examination (in this case abusive alcohol intake), many other undetectable individual factors such as genetic, dietetic, stress, etc, can potentially bias the results. We tried to minimize these factors in our selection of cases. By excluding Wernicke-Korsakoff cases, we ensured the elimination of potential alterations due to malnutrition associated with these diseases (Thompson, 1983; Phillips, 1987).

Another potential biasing factor is tissue shrinkage associated with age or cerebral hypoxia after death (interval between death and fixation). In order to overcome this, neuronal counts of all the cell bodies contained in every tenth section through the antero-posterior axis was performed. The use of samples of the neuronal population was avoided (Haugh, 1980; Sass, 1982; Man, 1987).

Liver damage effect

Our findings that hepatic lesion did not substantially influence neuronal counts or karyometry support the contention that liver steatosis or cirrhosis is not reflected in anatomical changes in the brain (Lee et al., 1979; Harper et al., 1988; Harper and Kril, 1991), although it does not exclude functional changes (Arria et al., 1991; Lister et al., 1991). The above considerations lead us to conclude that the alcoholic group in our study had a uniform damage in the brain due to alcohol (Schlesselman, 1982). Therefore, the main difference between alcoholics and controls was due to the abusive alcohol intake. In fact, one measure of the minor role played in our study by additional genetic or environmental factors is the confirmation of a direct ethanol damage to mammillary body and anterior thalamic complex in rats (Belzunegui, unpublished observations) where the same genetic strain and rearing conditions (except for the alcohol diet provided to the experimental group) were used.

Alcohol effect on the mammillary body and the anterior thalamic complex

Our results clearly indicate that alcoholic cases had fewer neurons in the centres here studied. Controls presented a reduction in the number of neurons, very likely associated with age. In contrast, the alcoholic group did not show such reduction with age. This indicates that the neuronal loss observed in young alcoholics was roughly equivalent to an effect of 30-40 years of aging. This difference happens in young individuals, easing off in older drinkers.

Karyometry in controls did not change significantly with age. A report from our laboratory (Panadero and Gonzalo, 1988), in which nuclear size was studied in the same centres from adolescence to old individuals, showed an increase in nuclear size only from 75 years on, but not in younger cases, which is consistent with the results reported here. Alcoholics presented a much smaller nuclear size. This reduced nuclear size might indicate a functional impairment of the neurons still present in the brain. A reduction in nuclear size of surviving neurons in alcoholism has also been reported in other limbic centres, i.e. the hippocampus (Bengoechea and Gonzalo, 1990), the dentate gyrus, (Lind et al., 1988; Orona et al., 1988) as well as the neocortex (Ferrer et al., 1986).

Conclusion

Excessive alcohol intake has an effect on neuronal loss and reduction in nuclear size, indicative of a direct toxic effect on memory centres not associated to nutritional factors as in Wernicke-Korsakoff's syndrome. The damage inflicted to the medial diencephalon, in conjunction with the damage to the medial temporal lobe might explain the memory impairment associated with alcohol intake (Lister et al., 1991).

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References

Alvarez I., Gonzalo L.M. and Llor J. (1989). Effects of chronic alcoholism on the amygdaloid complex. A study in human and rats. Histol. Histopathol. 4, 183-192.

Amaral D.G. (1987). The anatomical organization of candidate brain regions. In: Handbook of phisiology. Sect 1. Vol V. part 2. Higher functions of the neurons system. Plum F. (ed). American Physiological Society. Bethesda, Maryland. pp 211-294.

Arria A.M., Tarter R.E., Kabene M.A., Laird S.B., Moss H. and Vanthiel D.H. (1991). The role of cirrhosis in memory functioning of alcoholics. Alcohol. Clin. Exp. Res. 15, 932-937.

Becker J.T., Furma J.M.R., Panisset M. and Smith C. (1990). Characteristics of the memory loss of a patient with Wernicke-Korsakoff's syndrome without alcoholism. Neuropsychologia 28, 171-179.

Bengoechea O. and Gonzalo L.M. (1990). Effect of chronic alcoholism on the human hippocampus. Histol. Histopathol. 5, 349-357.

Bengoechea O. and Gonzalo L.M. (1991). Effect of alcoholization on the rat hippocampus. Neurosci. Lett. 123, 112-114.

Beracoechea D., Lescaudron L., Verna A. and Jaffard R. (1987). Neuroanatomical effects of chronic ethanol consumption on dorsomedial and anterior thalamic nuclei and on substancia innominata in mice. Neurosci. Lett. 73, 81-84.

Cadete-Leite A., Tavares M.A. and Paula-Barbosa M.M. (1988). Alcohol withdrawal does not impede hippocampal granule cell progressive loss in chronic alcohol fed rats. Neurosci. Lett. 86, 45-50.

Carlen P.L. and Wilkinson D.A. (1980). Alcoholic brain damage and reversible deficits. Act. Psychiatr. Scan (Suppl) 286, 100-117.

Eckardt M.J. and Martin P.D. (1986). Clinical assessment of cognition in alcoholism. Alcohol. Clin. Exp. Res. 10, 123-127.

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.

Harper C. and Kril J. (1991). If you drink your brain will shrink. Neuropathological considerations. Alcohol Alcoholism Suppl. 1, 375-380.

Harper C., Kril J. and Daly J. (1988). Does a «moderate» alcohol intake damage the brain? J. Neurol. Neurosurg. Psychiat. 51, 909-913.

Haug H. (1980). Die aghèngigkeit der Einbettung-schrumpfung des Gehirngewebe vom Lebensalter. Verh. Anat. Gest. 74, 699-670.

Ibañez J., Herrero T., Insausti R., Belzunegui T. and Gonzalo L.M. (1992). Short-term ethanol intoxication in rat. Effect on the entorhinal cortex. Neurosci. Lett. 138, 199-201.

Ibañez, J., Herrero M.T., Insausti R., Belzunegui T., Tuñuñ T., García-Bregado F. and Gonzalo L.M. (1995). Chronic alcoholism decreases neuronal nuclear size in the human entorhinal cortes. Neurosci. Lett. 183, 71-74.

Jones E.L. (1989). A new parcellation of the human thalamus on the basis of histochemical staining. Brain Res. Rev. 14, 1-34.

Lacalle S., Iraizoz J. and Gonzalo L.M. (1991). Differential changes in cell size and number in topographic subdivision of human basal nucleus in normal aging. Neuroscience 43, 445-456.

Lee K., Moller L., Hardt F., Haubek R. and Jensen E. (1979). Alcohol-induced brain damage and liver damage in young males. Lancet II, 759-761.

Lescaudron L. and Verna A. (1985). Effects of chronic ethanol consumption on pyramidal neurons of the mouse dorsal and ventral hippocampus: a quantitative histological analysis. Exp. Brain Res. 58. 362-367.

Lescaudron L., Beracochea D. and Verna A. (1984). Chronic ethanol consumption indices neuronal loss in mammillary bodies of the mouse: a quantitative analysis. Neurosc. Lett. 50, 151-155.

Lind M.D., Goodlett C.R. and West J.R. (1988). Time course and reversibility of ethanol's suppressive effect on axon sprouting in the dentate gyrus of the adult rat. Alcohol. Clin. Exp. Res. 12, 433-439.

Lister R.G., Gorenstein C., Risherflowers D., Weingartner H.J., Eckardt M.J. (1991). Dissociation of the acute effects of alcohol on implicit and explicit memory processes. Neuropsychologia 29, 1205-1212.

Mann D.M.A. (1987). Neuronal loss in old people: the effects of ageing or disease. Neurobiol. Aging 8, 550-551.

Markowitsch H.J. (1988). Diencephalic amnesia: a reorientation toward tracts? Brain Res. Rev. 13, 351-370.

Orona E., Hunter B.E. and Walker D.W. (1988). Ethanol exposure following unilateral entorhinal deafferentation alters synaptic reorganization in the dentate gyrus: a quantitative analysis of acetylcholinesterase histochemistry. Exp. Neurol. 101, 114-131.

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.

Parson O.A. (1987a). Neuropsycological deficitis in alcoholics: facts and fancies. Alcohol. Clin. Exp. Res. 1, 51-56.

Parson O.A. (1987b). Intellectual impairment in alcoholics: persistent issues. Acta Med. Scand. (Suppl) 717, 33-46.

Philips S.C. (1987). Can brain lesions occur in experimental animals by administration of ethanol or acetaldehide? Acta Med. Scand. (Suppl) 717, 67-72.

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

Sass N.L. (1982). The age-dependent variation of the embedding shrinkage of neurohistological sections. Makroskopie (Wien), 39, 143-175.

Schlesselman J.J. (1982). Case-control studies. Design, conduct, analysis. Oxford University Press.

Squire L.R. (1987). Memory and brain. Oxford University Press. Oxford.

Squire L.R. and Zola-Morgan S. (1991). The medial temporal lobe memory system. Science 253, 1380-1386.

Squire L.R., Amaral D.G. and Press G.A. (1990). Magnetic resonance imaging of the hippocampal formation and mammillary nuclei distinguish medial temporal lobe and diencephalic amnesia. J. Neurosci. 10, 3106-3117.

Tarter R.E. and Alterman A.T. (1984). Neuropsycological deficits in alcoholics: etiological considerations. J. Stud. Alcohol. 45, 1-9.

Thompson A.D., Ryle P.R. and Shaw P.K. (1983). Ethanol, thiamine and brain damage. Alcohol 18, 27-43.

Urbiola E. and Gonzalo L.M. (1992). Efectos del alcoholismo crónico sobre las áreas corticales de asociación 21 y 38 (Brodman). Patología 25, 19-28.

Victor M., Adams R.D. and Collins G.H. (1989). The Wernicke-Korsakoff syndrome and related neurological disorders due to alcoholism and malnutrition. 2d ed. F.A. Davis. Philadelphia.

Witter M.P., Groenewegen H.J., Lopez da Silva F.H. and Lohman A.H.M. (1989). Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Prog. Neurobiol. 33, 161-253.

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