# Neuronal Loss in Hippocampus Induced by Prolonged Ethanol Consumption in Rats

Abstract. *Quantitative neurohistological techniques were used to examine the hippocampal complex of laboratory rats maintained on ethanol-containing or control diets for 5 months followed by a 2-month alcohol-free period. Chronic ethanol consumption resulted in a significant loss of hippocampal pyramidal and dentate gyrus granule cells. This study provides direct evidence that long-term ethanol consumption, in the absence of malnutrition, produces neuronal loss in the central nervous system.*

A variety of neuropathological (*1*, *2*) and associated neuropsychological (*3*) alterations have been observed in chronic alcoholics. This brain damage and the resulting impairment in learning and memory have traditionally been attributed to malnutrition, especially thiamine deficiency (*4*) rather than to the direct neurotoxic effects of ethanol. Nevertheless, both neuropathological (*5*) and neuropsychological (*6*) deficits have been observed in long-term alcoholic patients with no history of malnutrition.

In the past few years, a number of nutritionally controlled studies in mice and rats have reported that long-term ethanol exposure (3 to 7 months) results in a residual impairment in the acquisition of a variety of behavioral tasks (*7*). Although the nature of the neuropathological alterations responsible for the ethanol-induced behavioral deficits remains to be completely specified, we recently observed that 4 months of ethanol consumption by mature mice resulted in a 50 to 60 percent loss of dendritic spines on hippocampal pyramidal cells and dentate gyrus granule cells as determined by quantitative analysis of Golgi-impregnated materials (*8*). The Golgi method is not useful for determining the total number of neurons in a tissue sample. Thus, more severe forms of neuropathology resulting in ultimate neuronal loss could not be evaluated in our previous study. We now report, using other methods, that 5 months of ethanol consumption in rats results in a significant loss of hippocampal pyramidal and dentate gyrus granule cells despite good nutrition.

Thirty male Long-Evans hooded rats (Charles River) were used. Rats were individually housed in standard stainless steel cages located in a temperature-controlled room with a 12 hour light-dark cycle throughout the experiment. When the rats were 75 days old, they were weighed and divided into three equal, weight-matched groups. One group (group E) received unrestricted access to an ethanol-containing liquid diet. A second group (group S) was fed an identical diet except that sucrose was substituted isocalorically for ethanol. Group S rats were given each day the amount of diet consumed by individual group E rats on the previous day (pair-feeding). A third group (group LC) received unrestricted access to standard pelleted laboratory food (Lab Chow, Ralston Purina) and tap water throughout the experiment. The liquid diets contained 35 to 39 percent of the total caloric content as ethanol- or sucrose-derived calories. The ethanol-containing diet was 8.1 to 9.4 percent ethanol (by volume). The percentage of total calories provided by ethanol or sucrose was increased from 35 to 39 percent in steps of 1 percent each month. The composition, nutritional adequacy, and procedures for administering the liquid diets have been described (*7–9*). After consumption of their respective liquid diets for 5 months, groups E and S were given unlimited access to lab chow and water for an additional 2 months before they were killed (*10*).

Two months after the liquid diets were discontinued and all groups changed to lab chow, the rats were intracardially perfused with 10 percent neutral buffered Formalin. Brains were removed and coded to allow subsequent analysis without knowledge of group designation. The brains were hemisected by a midline sagittal cut, and one-half of each brain was embedded in Paraplast (Sherwood Medical). Sagittal sections were cut at 4-$\mu$m thickness (*11*) beginning at the midline. Every tenth section was saved and mounted for subsequent staining with cresyl violet or hematoxylin and eosin. Anatomically matched sections from each brain, approximately 1500 $\mu$m from the midline (*12*), were used for histological analysis of the hippocampus and dentate gyrus. Quantitative determination of the total number of pyramidal cells per section was made by counting at a magnification of ×675 each soma containing a clearly defined nucleolus throughout the entire stratum pyramidale from CA1 through CA4 (*13*). In addition, separate counts were made for the number of pyramidal cells in regions CA1 and CA2 to CA4. The total number of granule cells for each section was determined by counting the number of cells in unit areas and multiplying by the total area of the granule cell layer (*14*). After all quantitative and qualitative data were collected for each of the 30 brains, the code was broken and the data were assigned to the appropriate group for analysis. Ten brains were quantitatively and qualitatively analyzed for each of the three groups.

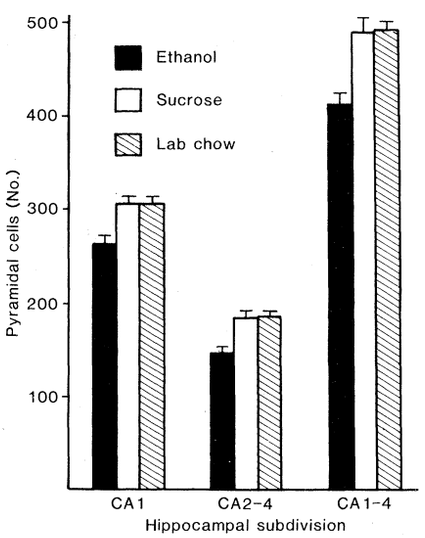


Fig. 1. The mean (+ S.E.) of dorsal hippocampal pyramidal cells per 4-$\mu$m sagittal section from ethanol-treated and control rats. The values for each group represent the mean of counts from ten rats. The counts for each CA subdivision within each group were taken from the same set of ten rats.

Group means for each CA subdivision were compared by one-factor analyses of variance (ANOVA). In each case, group E had significantly fewer pyramidal cells than groups S and LC (Fig. 1): for CA1 ($F = 6.8$; d.f. = 2, 27; $P < .005$); for CA2 to CA4 ($F = 8.83$; d.f. = 2, 27; $P < .005$); and for CA1 to CA4 ($F = 10.2$; d.f. = 2, 27; $P < .001$). Group E rats sustained an approximate 16 percent loss of hippocampal pyramidal cells as a result of 5 months of ethanol exposure. The total number of granule cells per section, reflected by both the reduced area and density of the granule cell layer, was significantly reduced by approximately 20 percent in group E relative to controls (Table 1) ($F = 7.4$, d.f. = 2,27, $P < .005$).

Qualitative changes in hippocampal morphology reflected the quantitative changes described above (Fig. 2). Both the CA1 pyramidal cell layer (Fig. 2, A and C) and the dentate gyrus granule cell layer (Fig. 2, B and D) were thinner in ethanol-exposed rats. Additionally, the width of the dentate molecular layer decreased substantially (Fig. 2, B and D), and that of the hippocampal stratum oriens decreased slightly (Fig. 2, A and C). These qualitative observations suggest


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Table 1. Effect of long-term ethanol consumption on the morphology of the granule cell layer of the dentate gyrus. The values represent the means ± S.E. These measurements are from the same histological materials as in Fig. 1.

| Treatment group | Area of granule cell layer ($mm^2$) | Granule cells per square millimeter | Granule cells per section |
|---|---|---|---|
| Ethanol | 0.285 ± .017 | 5664 ± 158 | 1595 ± 65* |
| Sucrose | 0.334 ± .024 | 5987 ± 320 | 1955 ± 98 |
| Lab chow | 0.341 ± .02 | 5945 ± 220 | 1995 ± 76 |

*$P < .005$.

that, in addition to the loss of neurons, the dendritic field of remaining dentate granule and CA1 pyramidal cells may be attenuated by ethanol exposure. These results are consistent with an earlier report from our laboratory (*8*).

The results make it clear that 5 months of ethanol intake results in significant neuronal loss in the rat hippocampus and dentate gyrus under nutritionally controlled conditions. Our previous observations (*8*) of a significant reduction in the number of dendritic spines on Golgi-impregnated hippocampal pyramidal and dentate gyrus granule cells in ethanol-treated mice may represent, then, an early phase of a progressive degenerative reaction ultimately ending in neuronal death. These results suggest that previous postmortem reports of histological and histochemical alterations of the hippocampal complex in chronically alcoholic patients (*15*) may have been associated with long-term ethanol abuse rather than some other coexisting condition.

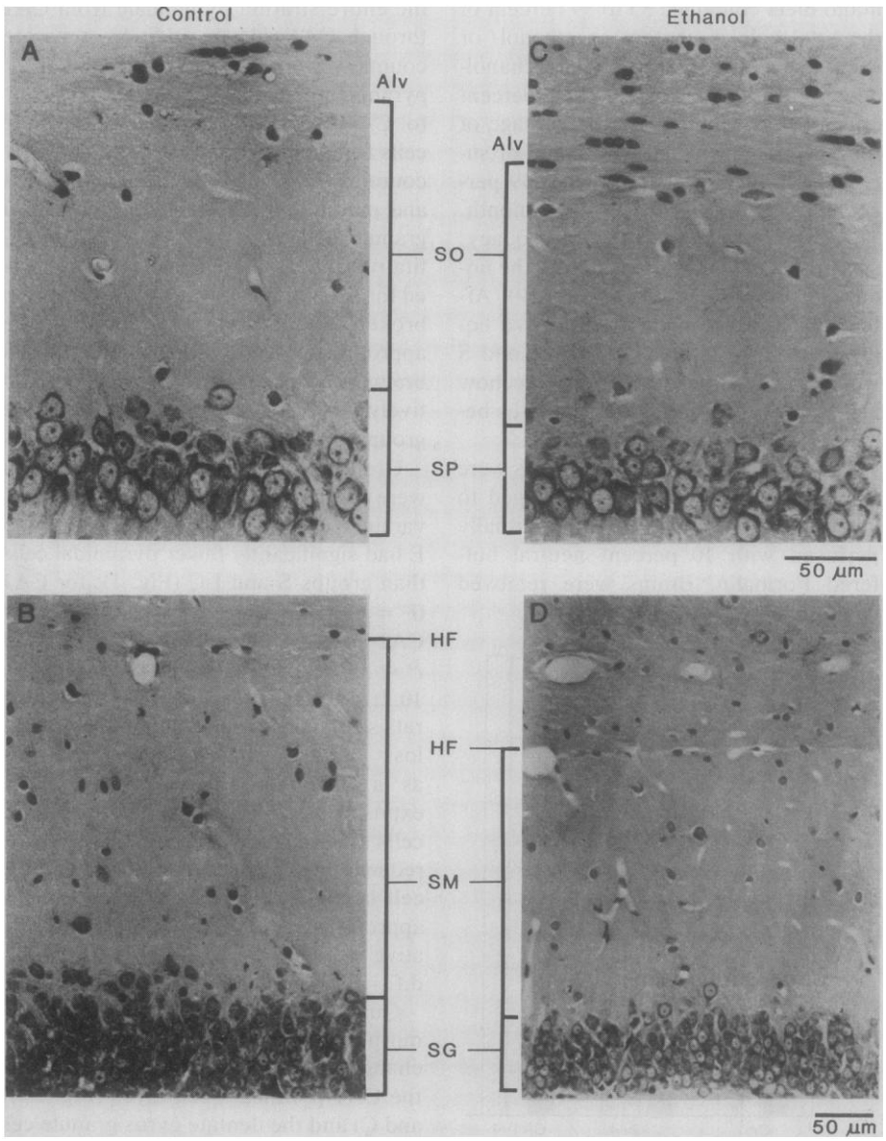


Fig. 2. Photomicrographs of anatomically matched sagittal sections through the rat dorsal hippocampus (A and C) and dentate gyrus (B and D) illustrating qualitative morphological alterations observed after 5 months of exposure to ethanol. Sections C and D are from an ethanol-treated rat and sections A and B are from a pair-fed sucrose control rat. Abbreviations: *SG*, stratum granulosum; *SM*, stratum moleculare; *HF*, hippocampal fissure; *SP*, stratum pyramidale; *SO*, stratum oriens; *Alv*, alveus. Upper calibration bar corresponds to (A) and (C) and lower calibration bar corresponds to (B) and (D). Sections were cut at 4 μm and stained with cresyl violet.

Although the present and previous (*8*) results demonstrate that prolonged ethanol consumption can have neuropathological consequences despite good nutrition, the mechanism or mechanisms by which it does so remain unclear. Ethanol (or its metabolite, acetaldehyde) could be directly neurotoxic, or it could exert its effect by inhibiting neuronal protein synthesis (*16*) or by altering cerebral blood flow, resulting in chronic ischemia (*17*). Further research will be necessary before the mechanisms of action of the neuropathologic consequences of chronic ethanol exposure can be better specified.

The present results, considered together with other reports of morphological alterations of the hippocampal complex associated with prolonged ethanol exposure in mice (*8*) and nonhuman primate (*18*), suggest the possibility that the residual learning and memory deficits induced by long-term ethanol consumption in animals (*7*) and humans (*3*, *6*) may be related in part to hippocampal damage (*19*). Based on neuropathological studies of alcoholic Korsakoff patients, Victor *et al.* (*2*) have suggested that damage to the dorsomedial thalamus is correlated with impaired memory, whereas Brion (*20*) has placed more importance on the hippocampus and mammillary bodies. Future research in which both quantitative behavioral and neurohistological data are collected in the same set of animals after prolonged ethanol consumption under nutritionally controlled conditions should help clarify the relationship between the behavioral and regional neuropathological consequences of alcohol abuse.

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9. Briefly, the ethanol diet was prepared by mixing the proper proportion of a stock ethanol solution (63.3 percent by volume) with Nutrament (Mead Johnson) to result in a diet with 35 to 39 percent ethanol-derived calories. The sucrose control diet was prepared similarly, except that an isocaloric stock solution (87 percent weight to volume) was substituted for the stock ethanol solution. Both diets were additionally fortified with Vitamin Diet Fortification Mixture (0.3 g/dl) and Salt Mixture XIV (0.5 g/dl) (both from ICN Nutritional Biochemicals). Both the ethanol- and sucrose-containing liquid diets contained 1.3 kcal/ml.
10. The daily consumption of ethanol averaged over the 5-month period of exposure [mean ± standard error (S.E.)] for group E was 13.2 ± .33 g per kilogram of body weight. This is comparable to the level of ethanol consumption that results in residual behavioral deficits in rats (*7*). There were no statistically significant differences in body weight among the three groups at any point during the experiment. The body weights (mean ± S.E.) at the beginning of liquid diet administration were 245.0 ± 4.9 g for group E, 242.0 ± 4.1 g for group S, and 242.0 ± 5.6 g for group LC. The body weights at the end of the 5-month experimental diet period were 523.0 ± 22.5 g for group E, 517 ± 16.8 g for group S, and 497.0 ± 10.1 g for group LC.
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13. Cytoarchitectonic regions of the hippocampal formation are defined according to the criteria and nomenclature of R. Lorente de No [*J. Psychol. Neurol. (Leipzig)* **46**, 133 (1934)].
14. The number of granule cells per square millimeter was determined by calculating the average number of granule cells contained within a grid area of 0.0025 mm² at a magnification of ×1500. Six samples of granule cell density were taken in each section, three samples from each of the dorsal and ventral blades of the dentate gyrus granule cell layer. The total area (square millimeters) of the granule cell layer was determined by projecting each section onto paper by use of a projecting microscope, tracing the boundary of the granule cell layer and measuring the area with a compensating polar planimeter (Keuffel and Esser).
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19. The similarity of the neuropathological (*1*) and neuropsychological alterations associated with aging and chronic alcoholism have led to the hypothesis that prolonged alcohol ingestion may accelerate pathological processes associated with biological aging [M. J. Blusewicz, R. E. Dustman, T. Schenkenberg, E. C. Beck, *J. Nerv. Ment. Dis.* **165**, 348 (1977)]. That aging and long-term ethanol exposure result in markedly similar pathological alterations in the hippocampal complex of rats is consistent with such a hypothesis. For example, both aging and chronic ethanol ingestion result in a loss of dendritic spines on dentate gyrus granule cells [(*8*); W. Bondareff and Y. Geinisman, *Am. J. Anat.* **145**, 129 (1976)] and a loss of hippocampal pyramidal neurons [K. R. Brizzee and J. M. Ordy, *Mech. Ageing Dev.* **9**, 143 (179); this study].
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# Do Corals Lie About Their Age? Some Demographic Consequences of Partial Mortality, Fission, and Fusion


Abstract. *Population dynamics of corals and other colonial animals are complicated by their modular construction and growth. Partial colony mortality, colony fission, and colony fusion distort any simple relationship between size and age among reef corals.*


Life expectancies of scleractinian corals and other colonial animals are poorly known, largely because their modular mode of growth presents difficulties not evident in the demography of more conventional, solitary organisms (*1–5*). The few longevity estimates available for corals are primarily based on extrapolation from data on short-term growth and the untested assumption that colony size is proportional to colony age (*5*, *6*). In this report, we show how relationships of coral size to age can be severely distorted by three modular processes (partial colony mortality, colony fission, and colony fusion) and point out some of the more important consequences of these phenomena to population studies of reef corals.

The numbers and sizes of five species of foliaceous scleractinian corals were recorded for 1 year at Rio Bueno, Jamaica. More than half of the 662 colonies censused were *Agaricia agaricites*. The

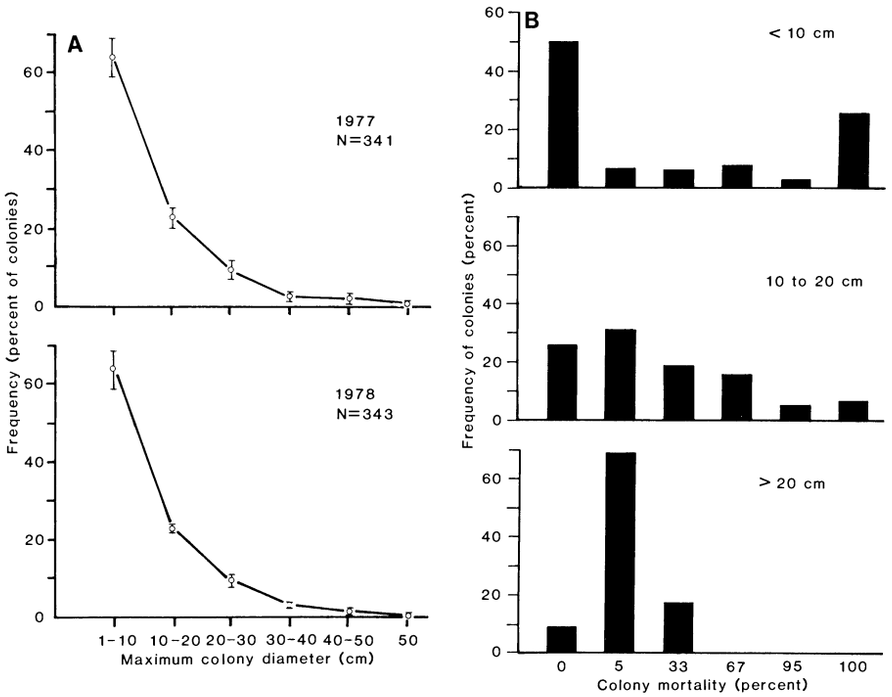


Fig. 1. (A) Size-frequency distributions of colonies of *Agaricia agaricites* on a vertical reef face just west of Rio Bueno Harbour, Jamaica, West Indies. Six quadrats (1 m²) were tied to the reef at −10 m, −15 m, and −20 m. Nails were fixed adjacent to corals for spatial reference. All corals larger than 1 cm in diameter were photographed and measured in situ in July 1977 and again in July 1978. Changes in living coral dimensions over the year were determined to an accuracy of about 1 cm. Error bars indicate 1 standard deviation from the mean frequency for corals from the three depths combined. (B) Frequency distribution of the percentage of colony mortality over 1 year for three size classes of *Agaricia agaricites*. Coral sizes are maximum colony diameters. The sample size for corals < 10 cm is 216, for corals 10 to 20 cm it is 78, and for corals > 20 cm it is 47.



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