same family is also documented in humans (*13*).

The present study, involving the only mammalian true hermaphrodite known to function as a fertile female (*14*), is informative for several reasons: (i) it implies that excess H-Y antigen on the cell membrane is associated with supernumerary male-determining H-Y genes in the nucleus; (ii) it shows that the H-Y$^+$ cellular phenotype does not rule out the presence of functional ovarian tissue in a fertile "female" of the dog and, by inference, of other mammalian species including the human; (iii) in view of the simultaneous gestation of XX male and XX female pups, it argues against the involvement of any environmental (or in utero) factor in the etiology of the XX male condition; and (iv) it indicates that the XX male syndrome and XX true hermaphroditism represent varying degrees of a common masculinizing event associated with abnormal transmission of Y-chromosomal (H-Y) genes.

Given a common genetic basis for XX male syndrome and XX true hermaphroditism, it remains to be determined how one gonad differentiates as a testis and another as an ovotestis. Earlier (*15*) we suggested that disseminated H-Y antigen is bound by plasma membrane receptors in the developing mammalian gonad. In this context it will be interesting to learn whether H-Y antigen expression differs in the two types of gonad and, if it does, whether this difference is related to the presence of other cell membrane components—for example, those determined by the major histocompatibility complex (*16*).

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17. Suspensions were scored as coded samples by an observer who recorded the counts of rosettes and free sperm. The values (percentage shown) were derived from the formula: (number of rosettes/number of rosettes plus free sperm). Positive absorption, indicating that the absorbing cells were H-Y$^+$, is indicated in the mixed hemadsorption-hybrid antibody test as a decrease in the number of rosettes when compared to the number of rosettes counted for unabsorbed H-Y antiserum [see (*9*) for details of procedure].
18. This work was supported in part by grants from NIH (CA 08748, AI 11982, GM 20138, HD 10065, HD 00171), ACS (FRA 167), and the Rockefeller Foundation. We thank K. Krupen-Brown, V. Kafantaris, C. L. Goldberg, and M. L. Oehlert for technical assistance; J. R. Church and R. F. Buchanan for referral of cases. R. S. Kenney assisted with the histology, and V. Scher with the manuscript.

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Morphological Alterations in Hippocampus After Long-Term Alcohol Consumption in Mice

Abstract. *Golgi methods were used to examine the hippocampus of laboratory mice that received alcohol-containing or control diets for 4 months followed by a 2-month alcohol-free period. Long-term alcohol consumption resulted in a significant loss of dendritic spines on hippocampal pyramidal cells and dentate granule cells. This study provides evidence that long-term alcohol consumption, in the absence of malnutrition, produces morphological damage to the central nervous system.*

The long-term consumption of ethyl alcohol is associated with a variety of neuropathological changes in central nervous system morphology (*1–3*). While many of these alterations are thought to be due to the secondary effects of malnutrition (*2, 3*), evidence of brain damage has been found in human alcoholic patients with no history of malnutrition (*4*). In addition, well-nourished animals receiving alcohol-containing diets show impairments in the performance of a variety of behavioral tasks, including shuttle-box avoidance (*5, 6*), timing behavior (differential reinforcement of low rate responding) (*7*), and maze learning (*8*). The ethanol-induced impairment in the acquisition of these behavioral tasks is present even after an ethanol-free period of 10 weeks (*5, 6*). Although a number of factors probably contribute to these ethanol-induced behavioral deficits, we report here that, in a nutritionally controlled animal model, long-term alcohol consumption results in the loss of dendritic spines on neurons in the hippocampal formation.

Three groups of mature (90 days old) female mice (C57Bl/6J, Jackson Laboratories) were used. One group (group A) received an ethanol-containing liquid diet. A second group (group S) was pair-

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fed an identical diet, except that sucrose was substituted isocalorically for ethanol. A third group (group LC) received standard pelleted laboratory food (Lab Chow, Ralston Purina) and unrestricted access to water. The liquid diets contained 35 to 42 percent of the total caloric content as ethanol or sucrose-derived calories. The ethanol-containing liquid diet was 8.1 to 10.3 percent ethanol (by volume). The percentage of total calories provided by ethanol or sucrose was increased from 35 to 42 percent in steps of 2 percent per month. The composition, nutritional adequacy, and procedures for administering the experimental diet have been described (*9*). After 4 months of being fed on the experimental diets, all groups were maintained for 2 months on Lab Chow, with free access to water.

The brains from five animals from each group were used for histological analysis. After intravascular perfusion with 10 percent formalin, the brains were removed, coded to prevent experimenter bias, and hemisected by a midline sagittal cut. Sections from one half of each brain were stained with thionin while the other half of each brain was processed according to a modified Golgi-Kopsch technique (*10*). The Golgi-Kopsch material was analyzed quantitatively. Dendritic spines were counted on 30-μm segments of CA1 pyramidal cell basilar dendrites and on 30-μm segments of dentate granule cell dendrites (*11, 12*). In addition, the branching pattern of dentate granule cell dendrites was examined by the method of Sholl (*13*). Measurements were made on five neurons for each animal (*11*). The mean of five measurements for each animal was used to compute group means. Group means were compared with an analysis of variance (ANOVA) procedure and the data from the Sholl analysis were analyzed with a nonparametric procedure (*14*).

The average daily consumption of ethanol [mean ± standard error (S.E.M.)] for group A was 24.9 ± 1.3 g/kg. This is comparable to the amount of alcohol that results in mice showing behavioral deficits on a variety of behavioral tasks (*5–8*). Concentrations of ethanol in the blood were not determined in the present experiments. However, female C57BL/6J mice of the same age as those used in the present experiment, and maintained under identical conditions on the same ethanol-containing liquid diet, were found to have blood ethanol levels averaging approximately 100 to 150 mg/100 ml (*9*). There were no statistically significant differences in body weights at the time the animals were killed (mean ± S.E.M. for group A was 25.3 ± 0.8 g, 24.8 ± 0.9 g for group S, and 25.0 ± 1.4 g for group LC). All mice gained weight normally throughout the 4-month period of diet administration. There were no statistically significant differences in body weight among the three groups at any point in the experiment.

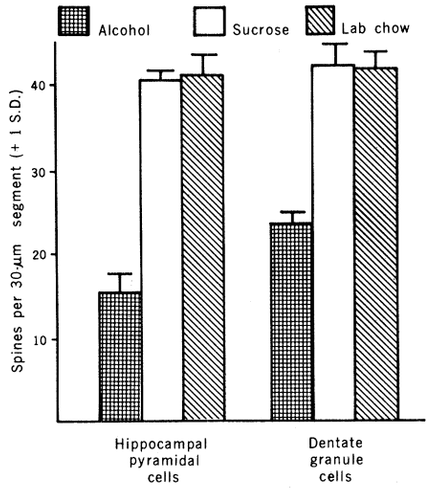

Fig. 1. The number of spines per 30-μm dendritic segment of hippocampal (CA1) pyramidal cells and dentate gyrus granule cells in alcohol-treated and control mice. The values for each group represent the mean of values from five mice. The value for each mouse was the mean of five measurements made on segments of each cell type. The alcohol-treated group received a nutritionally adequate ethanol-containing liquid diet. The sucrose control group received a sucrose-containing liquid diet (pair-fed to the alcohol group), and the Lab Chow (Ralston Purina) control group received pelleted laboratory food and water without restriction. All three groups received their respective diets for 4 months and then, for a 2-month period, the mice in all groups were given Lab Chow and water without restriction before they were killed.

No marked abnormalities or gross neuropathological changes were observed in Nissl-stained sections of the hippocampus. In contrast to the Nissl material, a number of abnormal neurons were observed in the Golgi material. These morphological alterations were reflected in the quantitative analysis of Golgi impregnated neurons. The number of spines on CA1 pyramidal cells is shown in Fig. 1. The mean (± standard deviation) number of spines per 30-μm segment of dendrite was 15.5 ± 2.3 for group A, 40.5 ± 1.4 for group S, and 40.8 ± 2.6 for group LC. The difference between group A and groups S and LC was statistically significant (ANOVA, $F = 34.2$; d.f. = 1, 12; $P < .01$, two-tailed). The number of spines on dentate granule cells is also shown in Fig. 1. The mean (± S.D.) number of spines per 30-μm segment of dendrite was 23.4 ± 1.4 for group A, 42.9 ± 2.5 for group S, and 42.5 ± 2.1 for group LC. The difference between group A and groups S and LC was statistically significant (ANOVA, $F = 44.6$; d.f. = 1, 12; $P < .01$, two-tailed). No statistically significant differences were found in the data from the Sholl analysis (data not shown).

A number of morphological changes were observed in group A animals. Whereas there were no apparent differences in the morphology of neurons from groups S and LC animals, a wide variation was found in the hippocampal formation of group A animals. The most severe alterations were observed in region CA1. The basilar dendrites of CA1 pyramidal cells in group A animals often appeared severely attenuated. Although no systematic measurements were made, the apical shafts of CA1 pyramidal cells of group A animals appeared relatively well preserved, but the distal branching at the apical tip appeared somewhat attenuated. In contrast, dentate granule cells were well preserved in group A animals. Neither the Sholl analysis nor subjective inspection indicated any severe alteration in neuronal morphology other than the loss of dendritic spines. The only major changes in dendritic morphology observed in dentate granule cells and CA1 pyramidal cells were the loss of spines and attenuation of dendrites; "beading" of dendrites and swelling of cell bodies were not observed.

The present results suggest that long-term alcohol consumption results in the reduction of the number of dendritic spines on the dendrites of hippocampal pyramidal cells and granule cells of the dentate gyrus. As indicated by the Sholl analysis, these changes can occur without signs of severe alterations in general dendritic morphology. Although a number of functions have been suggested for dendritic spines (*15*), the functional consequences of spine loss are not known. Nor do the present results clarify the causal factors responsible for the reduction of dendritic spines. The reduction could be due to degenerative processes within the affected neurons (*16*) or to transynaptic degeneration due to the loss of afferents (*17*). In order to determine the degree of neural specificity of ethanol-induced morphological alterations of the brain, other brain regions must be examined (*18*) and related to ethanol-induced alterations in behavior (*5–8*).

It is possible that long-term ethanol exposure resulted in an alteration in neural tissue that rendered the dendritic elements less susceptible to impregnation by the Golgi stain. Such a phenomenon could result in the apparent loss of spines even though they were still present. Matthews and Powell (*19*) reported that neu-

rons of the pyriform cortex undergoing transynaptic degeneration as a result of deafferentation are more resistant than normal cells to impregnation with the Golgi method. These investigators considered such reduction in impregnation to be a result of the deafferentation and not an expression of unreliability of the Golgi method (*19*). An electron microscopic investigation of deafferented pyriform cortex indicated that dendritic spines may retain their integrity even though their presynaptic terminals are degenerated (*20*). It has been recently reported, however, that the reduction of dendritic spines on dentate granule cells of the aging rat, as determined with the Golgi method, is accompanied by a decrease in axo-dendritic synapses as determined by electron microscopy (*21*). In the present investigation only neurons that met rigid criteria (*11*) for adequate impregnation were used for quantitative analysis. Therefore, we consider it highly improbable that long-term ethanol exposure selectively reduced the susceptibility to impregnation of a portion of the dendritic spines in an otherwise fully impregnated neuron. It is possible, however, that dendritic spines with presynaptic terminal degeneration could have become less susceptible to impregnation, as previously suggested (*19*), because of some unspecified alteration in deafferented tissue. Future studies with electron microscopy should answer the question of whether the loss of spines observed in the present investigation is due to deafferentation or to spine degeneration. Regardless of whether the decreased number of spines observed in the present experiment is due to the actual absence of spines or to a lack of impregnation because of presynaptic terminal degeneration, the results demonstrate a persistent alteration in brain tissue induced by long-term exposure to ethanol.

Alterations in the hippocampal morphology have been reported in a number of other conditions. For example, in the rat, the number of spines on dentate granule cell dendrites is reduced with age (*21*). In addition, Golgi methods have been used to detect morphological alterations in the hippocampus in senile humans (*16*), temporal lobe epileptics (*22*), and mentally retarded infants (*23*).

The results of the present study may be relevant to the understanding of the effects of long-term alcohol consumption in humans. It is interesting that chronic alcohol consumption is associated with severe disturbances of mnemonic functions in some alcoholic patients (*12*). Disruption of mnemonic functions in humans is also associated with damage to the hippocampus (*24*). Although other brain areas must yet be examined, it seems reasonable to speculate that part of the disruption of mnemonic abilities in some alcoholic patients could be related to damage of the hippocampus even though such damage might not be detected with routine neurohistopathological methods.

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