

Effects of Chronic Ethanol Treatment on Neocortex

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Neocortical inhibition and neuronal morphology were studied in rats following chronic ethanol treatment (CET). In terminal acute experiments, spontaneous neuronal discharges in pair-fed and naive rats were inhibited by epicortical stimulation, a procedure known to produce postsynaptic inhibition. Few units in CET rats were inhibited by such stimulation. Cortical recurrent inhibition, indicated by a surface-negative potential in response to antidromic stimulation of the cerebral peduncle, was little affected by a challenge dose of ethanol, compared with the response in pair-fed animals. Recurrent inhibition involves inhibitory interneurons. CET apparently made inhibitory interneurons and inhibitory postsynaptic receptors less responsive to ethanol. Apical dendritic spines on some portions of pyramidal neurons increased in number with CET. This could reflect a compensatory growth in neurons not damaged by CET. The overall observations are consistent with ethanol affecting one or more specific systems of cortical motor control as opposed to its presumed general disinhibitory effect.

THE EXPERIMENTS reported here are extensions of those on the effects of acutely administered ethanol described in the preceding paper.¹ Experiments were designed to evaluate neocortical effects of chronic ethanol treatment (CET) on 1) spontaneously firing neurons during epicortical stimulation, 2) an antidromically elicited cortical field potential, and 3) neuronal dendritic and spine morphology. Since experiments of this kind have not been reported until now, the results from an initial group of animals could guide direction for more extensive work.

There have been few reports on neuronal unit activity in primary projection areas of neocortex in animals given ethanol acutely. Ethanol ejected from micropipettes onto spontaneously discharging somatosensory cells in unanesthetized rats decreased firing rates in 23%, increased rates in 10%, and 65% were unaffected.² Similar experiments showed that of 13 cortical cells, 9 were unresponsive to ethanol and the rest were excited.³ Multiple unit activity in unanesthetized rabbits was especially responsive to intraperitoneal injections of ethanol.⁴ Ethanol reduced or abolished the suppression of input to particular cortical neurons during phases of the locomotor step cycle.⁵ The inhibitory effect of surface stimulation on neuronal firing

was potentiated by local injection of ethanol, and γ -aminobutyric acid inhibition was potentiated.^{6,7} No reports of neocortical neuronal activity after CET have been found, but other areas have been investigated. Studies on cortical neuronal morphology in cerebellum, cingulate gyrus, and hippocampus after CET have been reported.⁸⁻¹⁴ In a short report,¹⁵ CET in rats apparently caused neuronal degenerative changes and compensatory neuronal dendritic spine increases in neocortex. Also in rats, ethanol produced destructive effects on the cytoplasm of neocortical neurons.¹⁶

The results of the experiments reported here support our contention in the preceding paper that a primary effect of ethanol on neocortex is on neuronal inhibitory mechanisms. They are much less definitive in regards to ethanol effects upon neocortical neuronal morphology.

METHODS

In the various parts of this study, 23 male Sprague-Dawley rats (Charles River) were used. All were about 220 days of age at time of terminal acute experiments or death for histological study. Of the total, 12 were assigned to the CET (4 rats), pair-fed (4 rats) and chow-fed (4 rats) groups. The remaining 11 were obtained at about 205 days of age and kept in the animal colony on standard lab pellets and water, ad libitum, until used for acute experiments.

Electrophysiology

Unit Recordings. Terminal acute experiments were conducted on 10 rats anesthetized with ketamine and xylazine.¹ Four were naive controls and two each from CET, pair-fed, and control groups. An animal's head was fixed in a headholder and the mid-dorsal region of the neocortex was exposed under mineral oil. Oil pool and body temperatures were maintained by heating elements. For extracellular unit recordings, insulated tungsten microelectrodes (5–25 μ m exposed tips) were advanced vertically through the cortex with a hydraulic microdrive. Standard electrophysiological equipment included a window discriminator which counted the voltage signals generated by spike discharges in ten 50-msec time bins. Successive sweeps (usually 20–40), locked to a time signal, permitted bin accumulations of neuronal discharges during control (spontaneous firing) and cortical stimulation periods, and gave histograms displays with absolute bin values. Final recordings were not made until a unit's firing was stable for at least 10 min. Epicortical stimulation via bipolar silver ball electrodes spaced at 1.5 mm, and located within 2 mm of the microelectrode, was used to determine inhibitory and excitatory effects upon cell discharge.^{17,18} Epicortical stimulation was usually a single pulse of 0.1–0.2-msec duration, less than 1.0 mA at 0.5 Hz.

Peristimulus and poststimulus time histograms of neuronal spike discharges are not usually subjected to statistical treatment. We have followed the statistical procedure of Dörrscheidt¹⁹ where the spike distribution is assumed to have a Poisson pattern typical of a randomly occurring process. A "goodness of fit" test was applied to the control (spontaneous firing) spike distributions and in all instances the spike counts were found to be randomly distributed in the 10 bins after 20 or

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Received for publication October 14, 1985; revised manuscript received January 21, 1986; accepted March 6, 1986.

This work was supported by Grant 05979 from the National Institute on Alcohol Abuse and Alcoholism.

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more repetitions. Confidence intervals for the difference between stimulated bin entries and the control rate were found in tables for the distribution of a Poisson variable.^{20,21} This statistical treatment permits a description of each bin entry deviation from control in terms of a probability level. Each neuron discharge was then characterized as to whether and when spike activity changed during the 500 msec after epicortical stimulation. Illustrated in Fig. 1 are three categories of neuronal discharges following stimulation and the probability level of difference from control.

Antidromic Potential. In the preceding paper,¹ we described a field potential, the surface-negative (SN) wave, antidromically evoked by stimulation of the ipsilateral cerebral peduncle. The SN wave is a summation of inhibitory postsynaptic potentials (IPSPs) in deep-lying somas and proximal dendrites and represents a measure of cortical recurrent inhibition (RI). Acutely administered ethanol depressed SN wave amplitude.¹ The SN wave was now studied after challenge doses of ethanol in acute experiments following CET. Studies were done on two CET and two pair-fed rats 1 week after the conclusion of 20.5 weeks of CET. Preparation under ketamine and xylazine was as described in the preceding paper.¹

Morphology

Data were obtained from 13 rats, including the two CET and two pair-fed controls used in the SN wave study. The aim was to evaluate dendritic branching and spines of neocortical pyramidal cells. The rapid Golgi method of staining was used.²² Tissue blocks of about 50 mm³ were removed from the sensorimotor cortex after head perfusion with saline and buffered formalin. Coronal sections from celloidin-embedded tissue were cut at 125 μ m, placed on slides, and cover-slipped. Processed along with the experimental material were blocks of tissue from the naive controls. Slides were coded, until the study was completed, to cover the identity of the animals. Selected for study were pyramidal neurons, in layers lower II to V, with apical dendrites extending into at least layer II. Criteria for study were: cells had to show uniform staining of neuronal processes (except axons); there was absence of debris or precipitate on cell membranes and background; good contrast between cells and background was necessary; and sections had to be of uniform thickness. These criteria were difficult to meet and resulted in a small number of cells which could be studied in each animal. Two kinds of measurements were made from scaled drawings: basal and apical dendritic branches in 50- μ m concentric circles (from soma), and spine counts in 20- μ m sections on basal dendrites, the midportion of the main apical shaft, oblique branches, and terminal sections of dendrites about 100 μ m from the pial surface. Data were treated with the Mann-Whitney U-test.²³

CET

Twelve rats were received at 52–54 days of age, randomly assigned to one of the three groups (CET, pair-fed, or control), and maintained on ad libitum lab chow pellets and water in individual cages for 2 weeks. Four CET rats and four pair-fed controls were then placed on a liquid diet (Bio-Mix, Bio-Serv, Inc., Frenchtown, NJ). The CET rats were fed increasing amounts of ethanol over the first 10 days until the diet contained 35% ethanol-derived calories. After 16 weeks the ethanol and sucrose caloric equivalents were increased to 38% to offset partially the tendency of rats to reduce slightly their ethanol consumption as their body weights increase.¹⁰ The total CET was 20.5 weeks. During the last 10 days of CET, diet ethanol was reduced from 38% to 28% (5 days), and then to 19% (last 5 days), in order to prevent withdrawal symptoms, including possible seizures and death. Even in the absence of motor seizures, abnormalities in brain function, as might be shown by spiking in the electroencephalogram, can occur within several days following abrupt ethanol withdrawal. We therefore waited 10 days before the acute experiments during which the rats were on ad libitum lab chow. Two blood alcohol levels were determined at 5 and 9½ weeks, in the afternoon, using blood from the tail and an enzymatic method¹ (Sigma, Procedure No. 332-UV).

RESULTS

Animals

Average body weights of ethanol and pair-fed rats differed by only 1–7% during CET, and during the 12th to the 20th weeks of pair-fed rats weighed the same as the lab chow controls. Ethanol consumption was 7.8–11.1 g/kg/day, somewhat lower than that reported by others. Blood alcohol levels averaged 100 and 90 mg/100 ml for the two measurements. Liver weight to body weight ratios of the groups did not differ.

Electrophysiology

Unit Recordings. Fig. 1 illustrates how each neuron's discharge was classified following epicortical stimulation. A probability of 0.05 or better was accepted as showing a difference from the control, spontaneous rate. Complete recordings were obtained from 61 neurons of which 18 were from CET rats. Since the effect of epicortical stimulation on neurons among pair-fed, lab chow control, and naive control rats did not differ, the data are all included as those from "control." The proportions of cells placed in the various categories are shown in Fig. 2. Significantly fewer cells from CET rats were inhibited by epicortical stimulation within 500 msec and more cells were excited, compared with cells from control animals ($p = 0.049$). The effects of loss of inhibition and increased excitation in cells from CET rats are even more convincing ($p = 0.012$, Fig. 3) when the poststimulus analysis period is restricted to the first 150 msec (Fig. 3). During this time no cells from CET rats were inhibited (Fig. 3).

Antidromic Potential. When the SN wave was elicited by dual cerebral peduncle shocks, 25 msec apart, the response to the second shock was nearly totally blocked (from baseline) in a control animal (Fig. 4), but at the same intershock interval the second SN wave in a CET rat was little affected. Fig. 5 shows SN wave amplitudes as a function of dual shock intervals in a pair-fed and a CET rat and the change following challenge doses of ethanol to the latter. Throughout the range of shock intervals, the SN curve for the CET rat was shifted to the left indicating that the RI circuit was less responsive to the summative effects of a second cerebral peduncle shock than it was in a nonethanol-treated rat. Two challenge doses of ethanol to the CET rat brought the amplitude of the SN wave in response to the second shock (interval 25 msec) right to the point of nearly total block seen in the pair-fed animal's record. Ethanol had little effect on SN wave amplitude in response to the first shock.

Morphology

Although some 50 neurons were studied in 13 animals only 12 from CET and pair-fed animals met the rigorous criteria for selection and therefore the results can only be considered as suggestive of possible ethanol effects. No

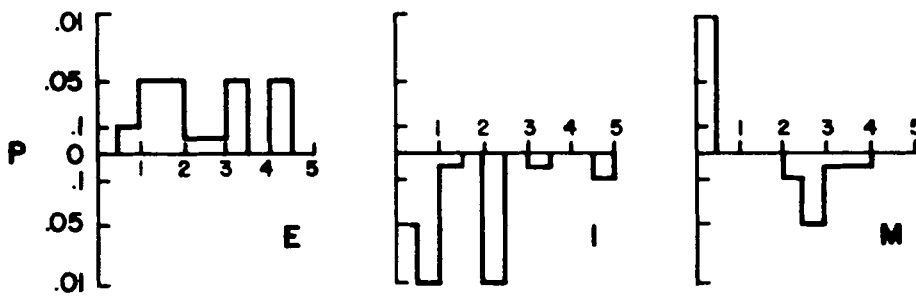


Fig. 1. Three main types of neuronal discharges during 500 msec following epicortical stimulation. Height of bars indicated on ordinate as probability of spike discharge being different from spontaneous period. Excitation above zero line, inhibition below. E, excitatory; I, inhibitory; M mixed. Time base: msec x 100.

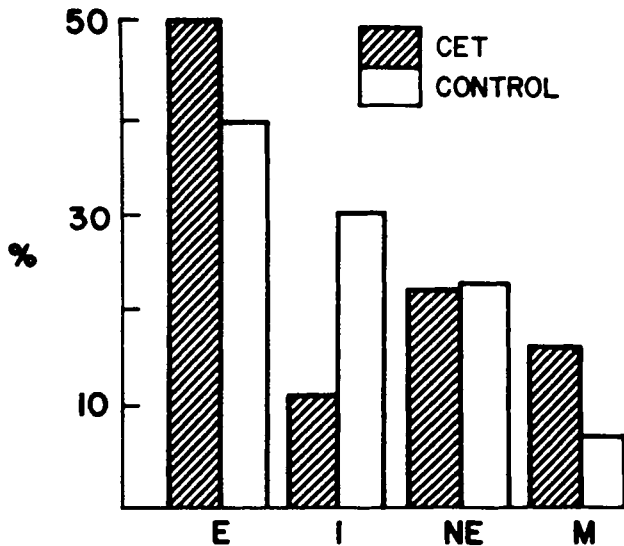


Fig. 2. Percentages of cells from CET and control animals classed as excited (E), inhibited (I), no effect (NE), or mixed (M) during 500 msec after epicortical stimulation. Differences between CET and control for E and I: $p = 0.0486$ (significance test, binomial distribution).

changes between basal and apical dendritic branching at different distances from the somas were found. Similarly, no differences were found in numbers of dendritic spines on basal and oblique dendrites. However, the mean of ranks for spines on main apical shafts (7.10 CET, 5.08 pair-fed) and terminal dendrites (7.67 CET, 4.00 pair-fed) was significantly greater for cells from CET rats (0.05 and 0.001 levels, respectively).

DISCUSSION

A main conclusion of this study was that CET resulted in a marked change in neocortical inhibitory functions. It has been found in several studies on cats that a single

electrical stimulus applied to the cortical surface inhibited spontaneous firing of cortical neurons below the surface electrodes.^{17, 18, 24, 25} The basis for the effect are IPSPs produced by the stimulation, presumably involving interneurons. Direct application of ethanol, to a neuron whose discharge was slowed by cortical stimulation, decreased the discharge further and this inhibition was thought to be γ -aminobutyric acid-mediated.^{6, 7} Presynaptic inhibition in amphibian spinal cord, involving γ -aminobutyric acid, was potentiated by the addition of ethanol to the perfusate.²⁶ At low doses of ethanol in acute rat preparations, there was a mixture of excitation and inhibition on spontaneous firing of hippocampal neurons and as doses increased inhibition of discharge prevailed.²⁷ In our experiments CET resulted in failure of epicortical stimulation to produce strong neuronal inhibition. The possible role of γ -aminobutyric acid-mediated inhibition in explaining our observations is unclear. Walker et al.²⁸ also suggested that CET has an effect upon neuronal inhibition. The cellular mechanism for block of inhibitory synapses is unknown, but Durand and Carlen²⁹ theorized that ethanol exposure could lead to a chronic impairment of Ca^{2+} -mediated potassium conductance. This could reduce or block the generation of IPSPs. Acute effects of ethanol on cortex are likely different from the chronic ones. Until shown otherwise, it is also best to assume that biphasicity of effects may be found under both experimental conditions.

A caution is in order. The results of our unit studies are clear in showing a loss of cortical inhibition by epicortical stimulation after CET, but there is no way of proving that the sample of cells obtained from CET rats came from the same population as those sampled in control animals. There is thus a possibility that "different" cells were being

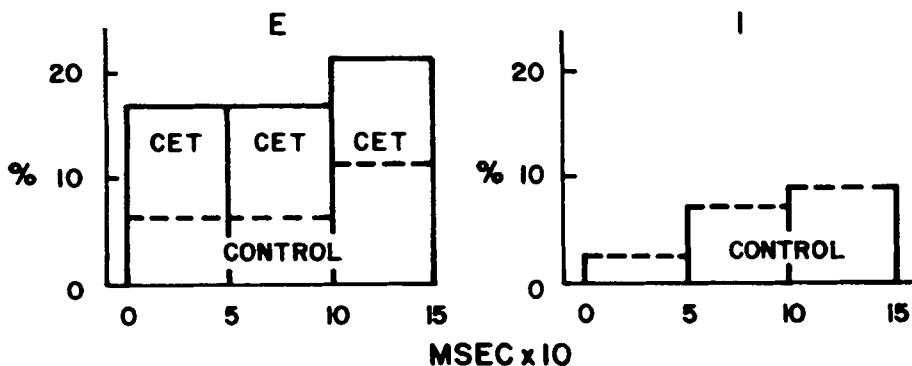


Fig. 3. Percentages of cells from CET and control animals excited (E) or inhibited (I) during first 150 msec after epicortical stimulation. No cells from CET animals were inhibited within 150 msec. Differences between CET and control: $p = 0.0122$ (significance test, binomial distribution).

higher. Alternatively, fresh tobacco smoke contains about 50 μM acetaldehyde at 1 atm between 20–37°C⁶⁻⁸ and chronic alcohol consumption increases the rise in blood acetaldehyde levels that follows the ingestion of ethanol.²⁴ Thus, the combination of drinking and smoking could result in acetaldehyde concentrations similar to those (50 or more μM) which produce metaplasia of tracheal epithelium when inhaled by experimental animals.^{7,8} The local acetaldehyde concentrations are expected to be even higher in those normally septic areas, such as the oropharynx,¹³ where the strongest synergism between alcohol and smoking on carcinogenesis has been found.¹⁻³ Even if the concentrations were smaller than those used in experimental animals, possible effects due to prolonged exposure to acetaldehyde in alcoholics who smoke should be considered as a possible factor in the synergism between alcohol and tobacco in the pathogenesis of upper respiratory cancer.

REFERENCES

- Rothman K, Keller A: The effect of joint exposure to alcohol and tobacco on risk of cancer of the mouth and pharynx. *J Chronic Dis* 25:711-716, 1972
- Wynder EL, Mushinski MH, Spivak JC: Tobacco and alcohol consumption in relation to the development of multiple primary cancers. *Cancer* 40:1872-1878, 1977
- Flanders WD, Rothman KJ: Interaction of alcohol and tobacco in laryngeal cancer. *Am J Epidemiol* 115:371-379, 1982
- Schmidt W, Popham RE: The role of drinking and smoking in mortality from cancer and other causes in male alcoholics. *Cancer* 47:1031-1041, 1981
- Herity B, Moriarty M, Daly L, Dunn J, Bourke GJ: The role of tobacco and alcohol in the aetiology of lung and larynx cancer. *Br J Cancer* 46:961-964, 1982
- Schmeltz I, Hoffmann D: Chemical studies on tobacco smoke. XXXVIII. The physicochemical nature of cigarette smoke, in Proceedings of the 3rd World Conference on Smoking and Health, DHEW Publ. (NIH) 76-1221, 1976
- Kruysse A, Feron VJ, Til HP: Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters. *Arch Environ Health* 30:449-452, 1975
- Appelman LM, Woutersen RA, Feron VJ: Toxicity of acetaldehyde in rats. I. Acute and subacute studies. *Toxicology* 23:293-307, 1982
- Obe G, Ristow H: Mutagenic, cancerogenic and teratogenic effects of alcohol. *Mutat Res* 65:229-259, 1979
- Jansson T: The frequency of sister chromatid exchanges in human lymphocytes treated with ethanol and acetaldehyde. *Hereditas* 97:301-303, 1982
- Stich HF, Rosin MP: Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human mucosa cells. *Int J Cancer* 31:305-308, 1983
- Baraona E, Julkunen R, Tannenbaum L, Lieber CS: Role of intestinal bacterial overgrowth in ethanol production and metabolism in rats. *Gastroenterology* 90:103-110, 1986
- Pikkarainen PH, Baraona E, Jauhonen P, Lieber CS: Contribution of oropharynx microflora and of lung microsomes to acetaldehyde in expired air after alcohol ingestion. *J Lab Clin Med* 97:631-636, 1981
- Jauhonen P, Baraona E, Miyakawa H, Lieber CS: Origin of breath acetaldehyde during ethanol oxidation. *J Lab Clin Med* 100:908-916, 1982
- Lesser M, Chang JC, Orłowski J, Kilburn KH, Orłowski M: Cathepsin B and prolyl endopeptidase activity in rat peritoneal and alveolar macrophages. Simulation of peritoneal macrophages by saline lavage. *J Lab Clin Med* 101:327-334, 1983
- Bernstein J, Martinez B, Escobales N, Santana G: The pulmonary ethanol metabolizing system (PET). *Res Commun Chem Pathol Pharmacol* 39:49-67, 1983
- Sorokin SP: The cells of the lung, in Nettsheim P, Hanna MG, Jr., Deatherage JW, Jr. (eds): Morphology of Experimental Respiratory Carcinogenesis. AEC Symposium Series 21. United States Department of Commerce, Springfield, VA, 1970, pp 3-41
- Azzopardi A, Thurlbeck WM: The histochemistry of the non-ciliated bronchiolar epithelial cells. *Am Rev Respir Dis* 99:516-525, 1969
- Cutlet T, Adnet JJ, Hopfner C: Etude histo-chimique de cellules alveolaires du poumon: les cellules a inclusions lipidiques complexes. *Histochemie* 15:21-37, 1968
- Devereux TR, Serabjit-Singh CJ, Slaughter SR, Wolf CR, Philpot RM, Fouts JR: Identification of cytochrome P-450 isoenzymes in non-ciliated bronchiolar epithelial (Clara) and alveolar type II cells isolated from rabbit lung. *Exp Lung Res* 2:221-230, 1981
- Devereux TR, Fouts JR: Xenobiotic metabolism by alveolar type II cells isolated from rabbit lung. *Biochem Pharmacol* 30:1231-1237, 1981
- Seitz HK, Garro AJ, Lieber CS: Enhanced pulmonary and intestinal activation of procarcinogens and mutagens after chronic ethanol consumption in the rat. *Eur J Clin Invest* 11:33-38, 1981
- Hook GER, Bend JR, Fouts JR: Mixed-function oxidases and the alveolar macrophage. *Biochem Pharmacol* 21:3267-3277, 1972
- Korsten MA, Matsuzaki S, Feinman L, Lieber CS: High blood acetaldehyde levels after ethanol administration: Differences between alcoholic and non-alcoholic subjects. *New Engl J Med* 292:386-389, 1975

In *summary*, results presented in this and the preceding paper,¹ showing that ethanol has definite effects upon neocortical processes of inhibition and excitation, should not be interpreted as establishing a basis for "disinhibition of the cortex under alcohol." The measures studied in these experiments show biphasicity of effects, tolerance, and differences under acute and chronic treatments. The cortical systems studied are involved in the complex organization and control of motor expression. Our results point to ethanol's effects upon more specific cortical functions than have been studied to date. For the human condition of acute ethanol effects upon motor expression, we view our data as suggesting that ethanol intake beyond a certain low level leads to a loss of one component of cortical organization and control, recurrent inhibition, rather than simply decreasing some kind of general functional inhibition. We also suggest that, at least in the initial stages of chronic alcohol intake, humans may reacquire some of the previously blocked neocortical mechanisms of motor control.

ACKNOWLEDGMENT

Grateful thanks are extended to David Tellner for his technical assistance.

REFERENCES

- Rutledge LT, Chi S-I, Sutter MA: Effects of acute ethanol administration on neocortical inhibition. *Alcohol Clin Exp Res* 10:506-511, 1986
- Lake N, Yarbrough GG, Phillis JW: Effects of ethanol on cerebral cortex neurons: Interactions with some putative transmitters. *J Pharm Pharmacol* 25:582-584, 1973
- Wayner MJ, Ono T, Nolley D: Effects of ethyl alcohol on cortical neurons. *Pharmacol Biochem Behav* 3:499-506, 1975
- Klemm WR, Mallari CG, Dreyfus LR, Fiske JC, Forney E, Mikeska JA: Ethanol-induced regional and dose-response differences in multiple-unit activity in rabbits. *Psychopharmacology* 49:235-244, 1976
- Chapin JK, Woodward DJ: Ethanol's effect on selective gating of somatic sensory inputs to single cortical neurons. *Pharmacol Biochem Behav* 18:489-493, 1983
- Nestoros JN: Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. *Science* 209:708-710, 1980
- Nestoros JN: Ethanol selectively potentiates GABA-mediated inhibition of single feline cortical neurons. *Life Sci* 26:519-523, 1980
- Kunz G, Englisch H-J, Wenzel J: Untersuchungen zur Spines-Verteilung an Pyramidenneuronen der CA1-region des Hippocampus der Ratte nach langzeitiger oraler Alkoholapplikation. *J Hirnforsch* 17:351-363, 1977
- Schulz E, Schönheit B: Quantitative Untersuchung über apikale Dendriten-Spines an cingulären Lamina-V-Pyramidenneuronen der Ratte nach oraler Alkohol-Applikation. *Z Mikrosk Anat Forsch* 91:453-459, 1977
- Walker DW, Barnes DE, Zornetzer SF, Hunter BE, Kubanis P: Neuronal loss in hippocampus induced by prolonged ethanol consumption in rats. *Science* 209:711-713, 1980
- Lee K, Dunwiddie T, Deitrich R, Lynch G, Hoffer B: Chronic ethanol consumption and hippocampal neuron dendritic spines: a morphometric and physiological analysis. *Exp Neurol* 71:541-549, 1981
- Tavares MA, Paula-Barbosa MM, Gray EG: Dendritic spine plasticity and chronic alcoholism in rats. *Neurosci Lett* 42:235-238, 1983
- McMullen PA, Saint-Cyr JA, Carlen PL: Morphological alterations in rat CA1 hippocampal pyramidal cell dendrites resulting from chronic ethanol consumption and withdrawal. *J Comp Neurol* 225:111-118, 1984
- Lescaudron L, Verna A: 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, 1985
- Popova EN: Changes of cortical neuron dendrites during experimental alcohol intoxication. *Biull Eksp Biol Med* 85:87-89, 1978
- Karwacka H: Ultrastructural and biochemical studies in brain and other organs in rats after chronic alcohol administration. *Exp Pathol* 18:127-135, 1980
- Krnjević K, Randić M, Straughan DW: An inhibitory process in the cerebral cortex. *J Physiol (Lond)* 184:16-48, 1966
- Anderson TE, Rutledge LT, Dyer RS: Altered neuronal excitability accompanying experimental prevention of supersensitivity in undercut cortex. *Brain Res* 95:147-153, 1975
- Dörscheidt GA: The statistical significance of the peristimulus time histogram (PSTH). *Brain Res* 220:397-401, 1981
- Crow EL, Gardner RS: Confidence intervals for the expectation of a Poisson variable. *Biometrika* 46:441-453, 1959
- Pearson ES, Hartley HO (eds): *Biometrical Tables for Statisticians*, vol I, 3rd ed. Cambridge, Cambridge University Press, 1966
- Scheibel ME, Scheibel AB: The methods of Golgi, in Robertson RT (ed): *Neuroanatomical Research Techniques*. New York, Academic Press, 1978, pp 89-114
- Krauth J: The interpretation of significance tests for independent and dependent samples. *J Neurosci Methods* 9:269-281, 1983
- Krnjević K, Randić M, Straughan DW: Nature of a cortical inhibitory process. *J Physiol (Lond)* 184:49-78, 1966
- McLennan H: Inhibitions of long duration in the cerebral cortex. A quantitative difference between excitatory amino acids. *Exp Brain Res* 10:417-426, 1970
- Davidoff RA: Alcohol and presynaptic inhibition in an isolated spinal cord preparation. *Arch Neurol* 28:60-63, 1973
- Grupp L: Biphasic action of ethanol on single units of the dorsal hippocampus and the relationship to the cortical EEG. *Psychopharmacology* 70:95-103, 1980
- Walker DW, Hunter BE, Wickliffe C, Abraham BA: Neuroanatomical and functional deficits subsequent to chronic ethanol administration in animals. *Alcohol Clin Exp Res* 5:267-282, 1981
- Durand D, Carlen PL: Decreased neuronal inhibition *in vitro* after long-term administration of ethanol. *Science* 224:1359-1361, 1984
- Kalant H, LeBlanc AE, Gibbins RJ, Wilson A: Accelerated development of tolerance during repeated cycles of ethanol exposure. *Psychopharmacology* 60:59-65, 1978
- Juntunen J, Widenius T, Reed TE, Ylikahri R, Matikainen E, Sarviharju M, Teräväinen H: Tolerance for the decrease in nerve conduction velocity and for the motor impairment produced by ethanol in mice: differential development during chronic ethanol consumption. *Neurobehav Toxicol Teratol* 6:245-248, 1984
- Winger JR, Tiffany TM, Bombardier C, Nicholls K, Woods SC: Ethanol tolerance in the rat is learned. *Science* 213:575-577, 1981