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Mechanism of Action of Ethanol: Initial Central Nervous System Actions*

RICHARD A. DEITRICH,† THOMAS V. DUNWIDDIE, R. ADRON HARRIS, AND V. GENE ERWIN

University of Colorado School of Medicine, Department of Pharmacology, University of Colorado School of Pharmacy, and Alcohol Research Center, Denver, Colorado

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†To whom requests for reprints should be addressed: Department of Pharmacology, University of Colorado School of Medicine, 4200 E. 9th Ave., Denver, CO 80262.

I. Introduction

THIS review is concerned with the molecular mechanisms by which ethanol depresses central nervous system (CNS)‡ function. We do not consider other actions of ethanol such as preference, tolerance, dependence, or the initial activating effects of ethanol. The proposed association of activation with the rewarding properties of ethanol are also not included. The amount of material gathered in the past several years is impressive and the review is not meant to be encyclopedic. We have excluded much of the strictly behavioral work unless it bears on the molecular mechanisms by which ethanol acts.

Perhaps one of the more surprising developments in recent years has been the realization that ethanol has a specificity of action that was not predicted. We now realize that ethanol does not act exactly as do the gaseous anesthetics. Nor does it act exactly the same as the hypnotics, although it has much in common with both of these classes of agents. Ethanol has specificity for the type of neuron that is sensitive to its effects as well as specificity based on genetic makeup of the animal. Presumably this specificity is reflected in the cell by different components of the cell membrane or cellular milieu. What these are still eludes investigators. Better methods of analysis of membrane structures should eventually yield information that will allow investigators to pinpoint the action of ethanol, at least on some cells. Undoubtedly there will always be ethanol effects that must be attributed to bulk interaction of ethanol with lipid membranes. These interactions usually occur at very high ethanol levels and are, for the most part, not of concern when dealing with levels of ethanol compatible with life (generally <100 mM).

The relationship of the initial CNS-depressant effects to the eventual development of alcoholism in humans has yet to be clearly correlated. Conventional wisdom dictates that there must be a relationship, but the mechanism by which this occurs is anything but clear. What is clear is the enormous cost that intoxicated individuals extract from the society in which they exist both in human misery and economic cost. The eventual development of understanding of the process of CNS depression should make it possible to interfere with these processes in some way and thus prevent some of this cost.

‡Abbreviations: CNS, central nervous system; GABA, γ-aminobutyric acid; EPR, electron paramagnetic resonance; K_p, membrane/buffer partition coefficient; ACh, acetylcholine; AChR, acetylcholine receptor; i.p., intraperitoneal; i.v., intravenous; i.c.v., intracerebroventricular; LS, long-sleep mice; SS, short-sleep mice; HS, heterogenous stock mice; AT, alcohol-tolerant rats; ANT, alcohol-nontolerant rats; DS, diazepam-sensitive mice; DR, diazepam-resistant mice; LC, locus coeruleus; VTA, ventral tegmental area; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; MEPP, miniature end plate potential; LTP, long-term potentiation; mRNA, messenger ribonucleic acid; ATP, adenosine 5′-triphosphate; ATPase, adenosine 5′-triphosphatase; cGMP, cyclic guanosine 5′-monophosphate; cAMP, cyclic adenosine 5′-monophosphate; NMDA, N-methyl-D-aspartate; TRH, thyrotropin-releasing hormone; CCK, cholecystokinin; AHP, after-hyperpolarization; NA, nucleus accumbens; NT, neurotensin; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; TBPS, excitatory postsynaptic potential t-butylbicyclophosphothionate; Ca_i, intracellular calcium; I_A, A current; I_K, delayed rectifer; I_{Ca}, calcium current; Ca-g_K, calcium-activated potassium conductance.

II. Membrane Fluidity

A. Background

1. Historical. The development of organic chemistry in the 1800s led to attempts to reconcile chemical structures with biological action. Understanding the structure-activity relationship of anesthetic agents was a particular challenge because so many chemically diverse agents were observed to produce such a similar spectrum of intoxication and anesthesia. In the early 1900s, Meyer and Overton independently found that the anesthetic potency of many chemicals could be accurately predicted by their ability to partition from water to olive oil. This relationship between potency and lipid solubility was confirmed in countless studies carried out during the 20th century and led to general acceptance of the idea that anesthetics (including ethanol) act on hydrophobic portions of the neuron (400). The key question remains "Which hydrophobic site?" Proteins, lipids, sugars, and nucleic acids all represent biological macromolecules with hydrophobic regions. The leading contenders are the lipids that form the cell membranes and the leading hypothesis is that ethanol partitions into these mem-

branes, alters their physical properties, and thereby alters membrane function leading to intoxication and anesthesia. Before we discuss experiments that test this hypothesis, we should consider the thermodynamics of the interaction of the ethanol with macromolecules.

2. Thermodynamics of ethanol action. The search for the neurochemical basis of ethanol's actions has been hampered by the lack of pharmacological specificity of ethanol and the corresponding lack of pharmacological tools to assist us in studying this drug. For example, no high-potency analogs of ethanol are available; although the long-chain alcohols (octanol, decanol) are more potent than ethanol, aqueous concentrations of 0.1 to 0.3 mM are required for biological actions and the membrane concentrations are similar to those obtained with equieffective amounts of ethanol (210). In contrast, based on receptor-binding studies ligands for specific receptors are at least 1000 times more potent.

Because millimolar concentrations of ethanol are required for biological actions, the drug will not have a single site of action. This is because the change in free energy accompanying binding is small when dealing with sites of near-molar affinity. Thus, there are likely to be many sites (on proteins, lipids, carbohydrates, nucleotides, etc.) that have enough complementarity with the ethanol molecule to allow interaction with cellular macromolecules.

This assertion of pharmacological common sense can be evaluated more rigorously by calculation of free energy changes. The change in free energy (ΔG) is a measure of the strength of interaction between molecules. For ligand binding, this is expressed by $\Delta G = RT\ln K$, where R is the constant 1.99 cal/mol-°K, T is the temperature in °K, and K is the dissociation constant in moles/liter. The association constant of ethanol with a specific site of action has not been measured, but we can make three assumptions: (*a*) the largest reasonable dissociation constant would approximate that of the aqueous concentration of ethanol during deep anesthesia, about 100 mM; (*b*) another estimate would be the aqueous concentration during ataxia, about 10 mM; and (*c*) the lowest reasonable estimate would be the membrane concentration during ataxia, about 1 mM. (The use of membrane concentrations rather than aqueous concentrations is justified by the observation that behavioral potency of alcohols is best related to membrane concentrations.) These three estimates give ΔG values of −1.4, −2.7, and −4.1 kcal/mol, respectively.

The ΔG for partitioning of ethanol into lipid (dipalmitoylphosphatidyl choline) vesicles is about −0.9 kcal/mol, demonstrating that ethanol interacts rather weakly with this lipid (389). For comparison, a single hydrogen bond between an amide and a carbonyl group has a ΔG of about −1 kcal/mol (in a hydrophobic environment) (241). (Of course, weak interactions between small molecules and macromolecules can be of considerable importance as is shown by the binding of oxygen to hemoglobin which has a ΔG of −2 kcal/mol, yet is critical for vertebrate survival!) In contrast, binding of high-affinity ligands to neurotransmitter receptors give ΔG values of −10 to −12 kcal/mol (484). However, not all neurotransmitters bind with such high affinity to the active state of their receptors. For example, the affinity of γ-aminobutyric acid (GABA) for the receptor responsible for chloride channel opening is about 0.15 mM (54), which gives $\Delta G = -5.2$ kcal/mol. Thus, we see that the interaction of 10 mM (aqueous) ethanol with a protein in the lipid bilayer would require almost as large a change in free energy as the interaction of GABA with its receptor, whereas interaction of >100 mM ethanol with an a macromolecule in an aqueous environment would require only a weak interaction.

This is a thermodynamic explanation of why ethanol affects every biological process if a sufficiently large concentration is tested and why it has proven very difficult to identify neurochemical systems that are altered by low (10 mM) aqueous concentrations of ethanol. In the area of membrane fluidity, the challenge has been to identify lipid species or membrane types that are sensitive to low concentrations of ethanol and could serve as sites of action for ethanol.

3. Membrane physical properties. Mammalian cell membranes consist of a lipid bilayer containing a number of proteins. The lipids are also quite diverse, with many types of phospholipids, galactolipids, and neutral lipids represented. These lipids are not randomly distributed in the bilayers, but certain lipids are predominately localized in the outer leaflet of the bilayer (e.g., gangliosides) and others are mainly in the inner leaflet (e.g., phosphatidylserine). In addition, within each leaflet, certain lipids are clustered together to form domains (341). Many investigators have attempted to reduce the complexity of the biological membrane by using model membranes consisting of only one or two purified lipids.

The main physical properties studied in alcohol research are lipid order and viscosity. Order is a measure of the packing of the lipids or, more qualitatively, the volume occupied by each lipid molecule. Viscosity is the resistance to solvent drag or a measure of the freedom of lateral movement of the lipid molecules. The frequently used term "fluidity" does not have a precise definition but is usually taken to encompass both order and viscosity (186). Other membrane physical properties of potential importance are membrane surface charge and lipid-protein interactions, but little is known about effects of ethanol on these areas.

Thus far we have considered the membrane only as a lipid bilayer, but lipids can assume nonbilayer configurations which will coexist with the normal bilayer. The functions of these nonbilayer domains are not clear, but, as discussed below, ethanol can promote the formation of these phases.

B. Effects of Ethanol

1. In vitro. In 1977 Chin and Goldstein (67) demonstrated that brain membranes are disordered by in vitro exposure to concentrations of ethanol that could be attained in vivo. These investigators used electron paramagnetic resonance (EPR) probes inserted into synaptic membranes to study ethanol actions. During the next few years a number of studies (212) in which EPR and fluorescent probes were used demonstrated that ethanol concentrations in the range of 10 to 100 mM disordered brain membranes, but the effect of ethanol was small and the question of the functional importance of these changes in membrane properties arose immediately. To address this question, investigators asked whether differences in alcohol sensitivity in vivo are reflected in differences in membrane sensitivity in vitro. Membranes from mice with genetic differences in alcohol sensitivity [long-sleep (LS), short-sleep (SS), and individual heterogenous stock (HS) mice] and membranes from ethanol-tolerant (resulting from chronic consumption of ethanol) mice were tested, and sensitivity to the membrane-disordering action of ethanol correlated well with ethanol sensitivity in vivo (67, 162, 183). However, the decrease in ethanol sensitivity observed in vivo during early development and the increase in sensitivity that occurs during aging was not reflected in changes in membrane sensitivity to ethanol (197). Thus, some, but not all, alterations in ethanol sensitivity in vivo can be correlated with differences in membrane sensitivity.

One problem that arises is that different methods of measuring membrane order may lead to different conclusions regarding the actions of ethanol. For example, three probes which are located near the membrane surface, 5-doxylstearate (EPR), trimethylammonium-diphenylhexatriene, and 2-anthroyloxystearate (fluorescent), show that membranes from LS (ethanol sensitive) mice are more sensitive to ethanol than membranes from SS mice, but probes of the membrane core (diphenylhexatriene and 2-anthroyloxystearate-fluorescent) do not detect any difference between the lines (186). In addition, elevated levels of calcium in the assay buffer reduced the action of ethanol on LS membranes (192), the difference in ethanol sensitivity seen with 2-anthroyloxystearate and trimethylammonium-diphenylhexatriene. This suggests that the genetic difference in alcohol sensitivity is due to an alteration near the membrane surface rather than the membrane core and may involve calcium-binding sites. A molecule that could confer calcium-dependent alcohol insensitivity is G_{M-1} ganglioside. This galactolipid alters membrane surface properties, binds calcium, increases effects of ethanol on lipids (see below), and is the only membrane component known to be elevated in LS mice as compared to SS mice (17, 462).

The fact that the genetic differences in alcohol sensitivity have only been detected at the membrane surface raises the question of where in the membrane is the most pronounced (and presumably most important) action of ethanol. Data from both EPR and fluorescent probes show that the disordering action of ethanol is most pronounced in the membrane core (lower methylene groups of the acyl chains), leading to the conclusion that the action of ethanol becomes stronger toward the interior of the bilayer (68). However, recent data from nuclear magnetic resonance studies support the intriguing alternative hypothesis that ethanol orders the membrane surface and disorders the membrane core (198). Thus, ethanol may have two distinct actions on the membrane and the EPR and fluorescent probe experiments measure an average of these two actions. Nuclear magnetic resonance may be particularly useful in studying these two sites of ethanol action because nuclear magnetic resonance does not require the use of an exogenous probe, but measures signals (resonances) from the lipids themselves (245, 246).

Thus far we have discussed effects of ethanol on membrane order, but viscosity is another membrane physical property often considered as a component of "fluidity." Studies of the motion of fluorescent probes by time-resolved techniques (186) and fluorescence recovery after photobleaching (457) indicate that ethanol does not usually alter the viscosity of neuronal membranes. Thus, it is more accurate to say that ethanol disorders membranes than to say that it fluidizes them. The implication of this distinction is that ethanol is unlikely to alter processes that require diffusion of proteins or other membrane components (e.g., interaction of protein subunits) but may alter processes that rely on lipid order. It is not clear which neuronal functions are clearly dependent on lipid order, but ion channels and membrane-bound enzymes are candidates.

Effects of ethanol on model membranes (lipid vesicles) have been studied by many groups. These studies support and extend results from brain membranes by showing that ethanol disorders the phospholipid vesicles and, as with brain, relatively large concentrations are required for small changes in membrane order. The effects of ethanol and other anesthetics on phosphatidlycholine vesicles are enhanced by addition of brain gangliosides to the vesicles. For example, vesicles composed of only dimyristylphosphatidylcholine require about 300 mM ethanol to reduce fluorescence polarization of diphenylhexatriene by 0.01, but dimyristylphosphatidylcholine vesicles with 10 mol % gangliosides (a concentration similar to synaptic membranes) require only about 50 mM ethanol to produce the same change in fluorescence polarization (188, 189). Conversely, the membrane-disordering action of ethanol (as measured by the EPR probes 12- and 5-doxylstearate) was attenuated by increasing the cholesterol content of mouse synaptic membranes (69). In addition, phosphatidylinositol or cardiolipin from liver of ethanol-fed rats reduces the disordering effect of ethanol on vesicles prepared from

microsomal phospholipids, but the molecular nature of these lipids has not been defined (113, 451).These results predict that certain membrane domains, e.g., ganglioside-rich, cholesterol-poor regions, would be particularly sensitive to the disordering actions of ethanol. This idea is supported by a study showing that the exofacial (outer) leaflet of the synaptic membrane, which contains all of the gangliosides, is more sensitive to ethanol than the inner leaflet (477). However, the role of cholesterol is less clear because genetic differences in synaptic membrane cholesterol concentration are not related to ethanol sensitivity (423). In addition, chronic ethanol treatment results in membrane tolerance regardless of whether membrane cholesterol is increased or decreased by the chronic treatment (183, 70, 272).

Membranes composed of a single phospholipid species display an abrupt phase transition or "melting" as the temperature is increased and as the lipid changes from a gel state to a liquid crystalline state. The temperature of the phase transition is initially decreased by ethanol but is increased by large concentrations of ethanol (389). The concentrations required to produce these actions are dependent on the type of phospholipid, particularly the acyl chain length. In the case of distearoylphosphatidylcholine (PC18:0), ethanol concentrations of approximately 100 to 500 mM decrease the phase transition temperature and concentrations of approximately 1500 to 2000 mM increase the transition temperature (389). The interpretation of these results is that ethanol selectively partitions into the liquid crystalline (fluid) phases and thereby decreases the phase transition temperature of the bilayer, but large concentrations of ethanol cause the bilayer to be converted to a nonbilayer, interdigitated, phase with a high transition temperature (389, 390). It is not known whether ethanol has similar actions on biological membranes.

2. Acute in vivo. A single injection of ethanol (100 mmol/kg, intragastrically) 18 hours before death renders brain synaptic membranes more sensitive to the in vitro disordering action of ethanol, as compared to membranes from rats treated with water. In vivo treatment with ethanol does not affect membrane order in the absence of in vitro ethanol (23). This sensitization to ethanol appears to be due to an increase in the partitioning of ethanol into the membrane (256). The mechanism has not been investigated; acute ethanol treatment decreases brain ganglioside levels (240) and may increase lipid peroxidation (1), but it is not clear that either of these changes would result in an increased sensitivity to ethanol. In fact, the opposite would be expected because LS mice have higher levels of gangliosides and are more sensitive to alcohol.

3. Measurement of ethanol partitioning. Effects of ethanol on the hydrophobic portions of membrane lipids or of proteins will depend directly on the concentration of ethanol in the membrane, which in turn depends on the membrane/buffer partition coefficient (K_p). Although these coefficients are of great importance, they are not easily determined experimentally. Three approaches have been used. The first is a direct measurement of the partitioning of radioactive ethanol into membranes (386). Although this technique works well with lipid-soluble compounds, the K_p of ethanol is low and the radioactivity found in the membrane is small compared to the amount in the aqueous phase resulting in poor signal to noise ratio. A second method is use of the depression of the transition temperature to calculate K_p (389). This technique works best with a pure lipid and has not been applied to biological membranes. The third approach is the use of deuterium nuclear magnetic resonance to measure the interaction of deuterated ethanol with membranes. This technique is based on the difference in resonance frequencies between bound and free ethanol (245, 246).

Before discussing the results of these techniques, it is important to note that the numerical value of K_p is markedly dependent on the units used. Rowe (389) pointed out that thermodynamic interpretations require use of molar units: (moles bound ethanol/moles lipid)/(moles free ethanol/moles water). However, some authors do not clearly specify the units used. Rowe (389) calculated the K_p of ethanol partitioning into dipalmatidylphosphatidylcholine as 3 to 7 (molar units). Kreishman et al. (245, 246) gave K_p values for ethanol partitioning into dipalmatidylphosphatidylcholine of 0.06 to 0.18 (depending on the concentration of ethanol) and stated that their data were "moles bound/moles free." The lipid concentration was 4 mg/ml (R. J. Hitzemann, personal communication) resulting in a molar K_p of 757. This value is more than 100 times larger than that calculated by Rowe (389) for the same lipid. Using radioactive ethanol, others (256, 386) reported K_p values for synaptic membranes of 0.8 to 1, but no units were given. Although these latter studies are not quantitatively interpretable, they make the points that ethanol may bind to two distinct sites on dipalmatidylphosphatidylcholine vesicles (245, 246) and that acute ethanol treatment increases K_p and chronic treatment decreases K_p (256, 385, 386).

Although the lipid solubility of anesthetics was first evaluated using bulk solvents such as olive oil and octanol, partitioning into phospholipid bilayers is quite different from that measured with bulk phases (95). The measurement of partitioning of ethanol into different regions of biological membranes presents a number of technical problems which remain to be solved. As discussed below, partitioning of ethanol into membranes is likely to be important regardless of whether membrane disordering is a key action of ethanol. It is unfortunate that so little attention has been given to rigorous measurement of this process.

One example of a possible role of membrane partition-

ing in alcohol action is the observation that the LS/SS lines of mice differ markedly in sensitivity to ethanol and other compounds with low lipid solubility, but the genetic difference becomes less as the anesthetics become more lipid soluble (e.g., the lines do not differ significantly in sensitivity to halothane) (18, 283). Recently, Hitzemann (196) showed that phosphatidylcholine bilayers display a selectivity that could account for this sort of differential drug sensitivity. In brief, transition from the gel to liquid crystalline state increased the membrane-perturbing action of alcohols with low lipid solubility (e.g., ethanol and propanol) but did not increase the action of pentanol or hexanol. Thus, it is theoretically possible (but unproven) that a portion of the difference in drug sensitivity between the LS/SS lines could be due to differences in membrane physical properties resulting in differences in drug partitioning.

C. Importance in Alcohol Actions

1. Comparison with other membrane perturbants. The ability of *n*-alkanols and other compounds to disorder brain membranes is closely correlated with their potency as anesthetics (210, 273). However, this does not mean that these compounds are anesthetic because they disorder membranes. For example, they could act directly on a membrane protein and their effect on this protein as well as their membrane disordering action would depend on the concentration of the drug in the membrane and would thus be correlated but mechanistically unrelated.

One approach to evaluating causal effects of membrane disordering is to ask whether it is possible to disorder membranes without producing anesthesia. Indeed, elevation of temperature by 2 to 3°C (135, 219), administration of 2-[2-methoxyethoxy]-ethyl 8-[*cis*-2-*n*-octylecyclopropyl]-ooctanoate (42), centrophoxine (476), or an "active lipid" mixture (AL721) (12) all disorder brain membranes as much or more than anesthetic doses of ethanol yet do not produce behavioral effects such as ataxia or loss of righting. These and other data (136) argue that decreases in bulk lipid order cannot be responsible for anesthesia. This led to the "selective perturbation" (190) and "membrane domain" (477) hypotheses which propose membrane regions sensitive to low concentrations of ethanol but resistant to temperature and other nonanesthetic perturbants. Such domains have not been identified experimentally.

It should also be noted that ethanol is known to interact directly with proteins. For example, alcohol dehydrogenase has a low millimolar K_m for ethanol. Ethanol affects the function of purified (lipid free) proteins such as aequorin (328) and firefly luciferase (134), although concentrations of 100 to 300 mM are required to affect these proteins.

2. Influence of membrane order on protein function. One approach to the conundrum of whether the small changes in membrane order produced by ethanol could be responsible for its action on membrane function is to reconstitute purified membrane proteins with defined lipids with varying order parameters. This has been done with the nicotinic cholinergic [acetylcholine (ACh) receptor (AChR)] of *Torpedo.*

The AChR has three functions, binding of specific ligands, activation of a cation channel, and agonist-dependent desensitization-inhibiting channel function which increases receptor affinity. Reconstitution of the receptor with defined lipids demonstrates that the channel will display these three processes only if the membrane contains acidic lipids, cholesterol, and an optimal level of acyl chain ordering (130). Manipulation of lipid composition to decrease (or markedly increase) membrane order prevents ion flux and desensitization. However, rather large (at least to ethanol researchers) changes in order are required to abolish these functions. For example, the order parameter of the native membrane (measured with 5-doxylhexadecanoate) is 0.80 and asolectin mixtures with order parameters of 0.75 and 0.77 allow receptor function, but lipids (order parameters of 0.70 to 0.74) are required to abolish function (130, 226).

How do these changes in order parameter compare to actions of ethanol? Effects of ethanol on *Torpedo* membranes have not been measured with this probe, but use of a similar (and probably more sensitive) probe, 12-doxylstearate, demonstrated that approximately 1500 mM ethanol is required to decrease the order parameter by 0.06 (the change required to abolish AChR function) (305). Thus, it is unlikely that reasonable concentrations of ethanol would alter AChR function because they disorder membrane lipids. However, ethanol does alter the function of *Torpedo* AChR. Ethanol promotes receptor desensitization and increases the potency of carbamylcholine in stimulating ion flux (305). In both cases, the 50% effective concentration (EC_{50}) is approximately 300 mM. This may be a reasonable concentration for this species at the assay temperature of 4°C because, at 3°C, 333 mM ethanol is required to anesthetize frogs (305). Given that this is the opposite action predicted for membrane disordering (i.e., decreased desensitization, decreased channel function) and that the disordering produced by 300 mM ethanol is likely too small to alter channel function, it is reasonable to assume that ethanol acts either at the lipid-protein interface or directly on the AChR protein. The latter possibility was suggested by El-Fakahany et al. (112).

It is interesting to note that, in addition to the annular lipids required for AChR function, there is a nonannular site within the protein that must be occupied by cholesterol (or a suitable analog) for channel function (227). This site must represent a hydrophobic protein pocket within the membrane and the possibility that anesthetics could compete with cholesterol at this site has been suggested (226). Studies with AChR demonstrate the

power and elegance of using a purified, reconstituted protein in membranes to elucidate the role of lipids in neuronal function. One shortcoming of these studies is that activation-desensitization of the AChR is a rapid and complex event requiring a detailed kinetic analysis (53) and more complete analyses of flux rates and concentration dependence than have been carried out to date. It is possible that such analyses would reveal more subtle and compatible actions of ethanol and membrane order on AChR function.

3. Conclusion. The membrane fluidity hypothesis has yet to be disproven, but this is faint praise. There are many impressive correlations between drug actions and membrane fluidity changes, but two alternative hypotheses could also explain these results. First, ethanol (and other anesthetics) may act on specific protein sites, but these sites are within the membrane; thus, the drug action is proportional to the membrane concentration, which is in turn proportional to the change in membrane fluidity. Second, the action of ethanol may be at another site (e.g., membrane calcium binding) and acyl chain fluidity will faithfully, but weakly, mirror this action. Although ethanol disorders membrane lipids, it is not clear that this action is responsible for any other actions of ethanol.

III. Effects of Ethanol on Neuronal Electrical Activity

A. Background

The effects of ethanol on the nervous system have been characterized in a number of ways utilizing biochemical, behavioral, and electrophysiological approaches. In one sense the electrophysiological approach can serve as a "bridge" between biochemical studies, on the one hand, and behavioral studies, on the other. If ethanol affects the function of a specific membrane protein (e.g., a neurotransmitter receptor or ion channel), then it is often possible to demonstrate, using electrophysiological techniques, the ways in which this action affects cellular activity. In the case of an ion channel, the rate of firing of a cell, the resting membrane potential, or other such cellular parameters could be affected by ethanol, and the mechanism by which ethanol acts can be inferred from the changes that are observed. In the case of a transmitter receptor, a biochemical effect (e.g., modulation of agonist binding) will often translate into an altered physiological response. If such effects can be demonstrated in brain regions with known function, physiological studies can sometimes link biochemical actions of ethanol to changes in behavior.

Even in cases in which the mechanism of action remains unknown, functional studies can often point to brain regions or cell types that appear to be particularly sensitive to the actions of ethanol and can then become the focus of more extensive studies aimed at determining cellular mechanisms.

B. Effects of Ethanol on Single-Unit Activity

A number of studies have characterized the effects of ethanol on the firing rates or patterns of activity of single cells. This type of approach has been used for decades to study the effects of a variety of drugs on neuronal activity. Single-unit studies are useful in identifying which brain regions are sensitive to the effects of ethanol and in determining the doses or concentration ranges in which such effects occur. These studies have not proved very informative concerning the cellular mechanisms of ethanol action but this may be, at least partially, because of the wide diversity of responses to ethanol. However, it must be borne in mind that responses to ethanol can depend on the preparation used, the brain regions tested, whether ethanol is administered systemically or through local application, whether or not recordings are made from identified populations of cells, and whether other drugs (e.g., general anesthetics) are also present.

1. Cerebellum. The cerebellum has been extensively studied for the effects of ethanol on single-unit activity. In particular, the activity of Purkinje cells, which are the major cerebellar output neurons, has been characterized in a number of different preparations. These studies have not yielded an entirely consistent view of the way in which ethanol affects neuronal activity, but, in many cases in which differences have arisen in the results of different laboratories, further studies have been able to resolve some of the discrepancies and to identify which factors in the experimental procedure are responsible.

Results of early studies suggested that the effects of ethanol on Purkinje cells were complex and could involve both depressant as well as excitatory actions (see refs. 212 and 344 for reviews). More recently, Sorensen et al. (428) demonstrated that ethanol [1–4 g/kg administered intraperitoneally (i.p.)] produced initial increases in Purkinje cell activity in urethane-anesthetized rats that were maximal at about 12 min following ethanol administration. This initial excitatory component could be blocked by 6-hydroxydopamine lesions, which suggested that the initial increase in rate was a secondary effect that resulted from inhibition of the activity of the locus coeruleus (LC) (see below).

Other studies have yielded somewhat variable results. Sinclair et al. (419) reported that intravenous (i.v.) injections of ethanol produced increases in Purkinje cell activity in urethane-anesthetized rats when the ethanol was infused over a 10-min period, but such changes were not observed when the ethanol was injected over 22 min (418). The disparities in the results seem to reflect the differences between a bolus i.p. injection of drug (428), as opposed to a slow or fast iv. injection, which suggest that the rapidity of onset of ethanol administration determines the response. In addition, virtually all of an i.p. injection of ethanol passes through the liver before it reaches the systemic circulation. With an i.v. injection, most of the ethanol escapes liver metabolism in the first

circulation time and only after several circulations will it all be exposed to liver metabolism. This might suggest a role for acetaldehyde in responses to i.p. ethanol.

In another study in which an unanesthetized paralyzed preparation was used (71), it was observed that i.p. injection of low doses of ethanol tends to excite, and high doses tend to inhibit spontaneous activity of Purkinje cells. In general, it was found that the inhibitory responses tended to promote more regular firing, whereas excitatory responses usually led to more bursting and occasionally to cessation of cell firing.

a. EFFECTS ON CEREBELLAR FIRING PATTERNS. Even in the absence of changes in the spontaneous firing rate of Purkinje cells, alterations in the pattern of activity have been noted. A commonly reported change is the increase in the regularity of discharge that occurs following ethanol administration (133, 419). In addition, synaptic responses may be diminished. For example, the duration of inhibition following local stimulation of the cerebellar cortex is reduced (418). The frequency of climbing fiber bursts are either markedly reduced (419) or enhanced (e.g., 384), depending on the anesthetics that were used. However, because these latter effects do not appear to be due to a cerebellar site of ethanol action, they will be discussed in greater detail in the next section dealing with actions on inferior olivary neurons.

b. STUDIES ON ISOLATED PREPARATIONS. In addition to the preceding studies, a number of studies have focussed on ethanol actions on Purkinje cells in in vitro systems. In vitro systems have the advantage that the cerebellum is deprived of the majority of its afferents, and thus the effects that are observed are more likely to reflect direct actions on the Purkinje cells. In addition, because ethanol is applied either through bath superfusion or from a pipette, the concentration can be controlled more directly than in vivo, and the tissue will not be exposed to metabolites that result from extracerebral metabolism.

In these types of studies, ethanol has been reported to modify both the rate and pattern of spontaneous activity of Purkinje cells in cerebellar brain slices maintained in vitro (21, 147). Cells that were firing steadily were typically inhibited by superfusion with 10 to 300 mM ethanol. Other cells exhibited a different pattern of firing that consisted of intervals of high-frequency activity that were then followed by brief periods of electrical silence. Cells exhibiting this cyclic pattern of firing showed changes in rate and the duration of the firing part of the cycle (147). Similar results have been obtained in rat Purkinje cells in brain slices using intracellular recording techniques. Proctor and Dunwiddie (365) reported that perfusion with 80 mM ethanol had minimal effects on the resting membrane potential or membrane input impedance but induced significant changes in the pattern of spontaneous firing. Ethanol consistently modified the firing of cells that showed cycling behavior by shortening the period of repetitive spiking. Passing current through the intracellular recording electrode under control conditions to depolarize or hyperpolarize the cell did not reproduce the changes in the firing pattern produced by ethanol.

Ethanol has also been reported to increase the regularity of firing of Purkinje cells in cultures of cerebellar neurons (133), as has been reported in intact animals (e.g., 419). The rate of spontaneous firing either increased transiently or did not change. By contrast, the pattern of granule cell firing became more irregular, suggesting that the increased regularity of firing of the Purkinje cells is not a generalized effect on all neurons.

c. GENETIC STUDIES OF PURKINJE NEURON SENSITIVITY. One aspect of ethanol action on the Purkinje cell that has been extensively studied is the genetic determinants of sensitivity to the depressant effects of ethanol. As discussed previously, ethanol has a wide variety of actions on electrophysiological activity, and it has been difficult to determine which effects are behaviorally relevant. One approach to this problem is to use animals that have been genetically selected on the basis of sensitivity to ethanol using a specific behavioral response. It is then determined whether an electrophysiological response to ethanol is correlated with the behavior.

This approach has been used with the LS and SS lines of mice, which have been selected using the duration of the loss of the righting reflex following i.p. injection with ethanol as a measure of ethanol sensitivity. These two lines of mice differ markedly in the suppression of spontaneous Purkinje cell firing by locally applied ethanol (430). In these studies local pressure application of ethanol from a glass micropipette was used, so it is difficult to determine the actual concentration to which the cells are exposed; however, the amount that is ejected is linear with both pressure and time, so the total amount of ethanol that is applied is a function of pressure × time. When responsiveness was characterized as the dose (pressure × time) required to achieve a 50% inhibition of firing rate, it was found that the LS mice were approximately 30 times more sensitive than the SS to the effects of ethanol. The HS line of mice from which the two lines were selected, and which show an intermediate behavioral sensitivity to ethanol, also show an intermediate sensitivity to the effects of ethanol on Purkinje cell firing (429). This study also demonstrated that in the hippocampus there was no difference between the lines either in terms of the depressant effects of ethanol on pyramidal neuron spontaneous activity or in the ethanol-mediated depression of evoked potentials.

Subsequent studies of Purkinje neuron activity in brain slices made from SS, LS, and HS mice demonstrated multiple effects of ethanol on Purkinje cell spontaneous firing (21). Increases in firing rate were occasionally observed at the onset of superfusion but were

not dose dependent. The most consistent effect of ethanol was an inhibition of firing, and the electrophysiological sensitivity of the different lines paralleled the behavioral sensitivity. The concentrations of ethanol required to inhibit spontaneous activity by 50% were 76 mM for the LS, 187 mM for the HS, and 325 mM for the SS lines of mice. These actions did not depend on the presence of synaptic inputs, because they were maintained under conditions in which synaptic transmission was largely blocked. These studies demonstrate that ethanol must have some type of direct action on the Purkinje cell that is genetically determined and that is highly correlated with the behavioral sensitivity to ethanol.

Although these experiments demonstrate that ethanol must act directly on the Purkinje cell, it is unclear whether sensitivity is a factor that is under direct genetic control or whether environmental or developmental factors are involved. This issue has been addressed by studies on in oculo cerebellar grafts from SS and LS mice; in these studies, tissue is taken from fetal mice and transplanted into the anterior chamber of the eye of a host animal and allowed to mature in oculo (348). These studies have shown that electrophysiological sensitivity of Purkinje neurons to inhibition by ethanol is determined by the source of the donor material and not the identity of the host. Purkinje cells in grafts obtained from LS fetal tissue and grafted into LS hosts (LS × LS) showed a 50% inhibition of firing at an average concentration of 58 mM ethanol; LS grafts transplanted into SS hosts (LS × SS) were not significantly different (EC_{50} 43 mM ethanol). By comparison, SS × SS and SS × LS grafts both required much higher concentrations of ethanol to achieve 50% inhibition of spontaneous firing (421 mM and 432 mM, respectively) and showed no significant effect of the host on the sensitivity to ethanol. It is interesting to note that the electrophysiological sensitivity of Purkinje cells in grafts and cerebellar brain slices is comparable but that the relative sensitivity (4- to 8-fold difference between LS and SS) is much less than the 30-fold differences reported with local application of drug. These results imply that some of the effects that are observed with local application may rely on an interaction with extrinsic afferents to the cerebellum or that the response to locally applied ethanol may not be linear.

More recent studies have continued to provide strong evidence for a genetic correlation between the sensitivity of Purkinje cells to the depressant effects of local application of ethanol and behavioral sensitivity as measured by the duration of loss of the righting reflex. Studies in 8 inbred strains of mice showed a correlation of −0.997 between electrophysiological and behavioral measures of sensitivity (431). Similar studies have shown that Fischer 344 rats are more sensitive on both measures than Brown Norway rats (225), and a more extensive comparison of 6 inbred rat strains also showed a high degree of correlation between the sensitivity of Purkinje cells and behavioral sensitivity to ethanol ($r = -0.92$; 351).

Another way to modify the behavioral sensitivity to ethanol is to make animals tolerant to its effects by means of chronic exposure. Studies in LS and SS mice made tolerant to ethanol demonstrated that tolerant mice were less sensitive to the depressant effects of ethanol on Purkinje neuron activity. This was the case with both locally applied ethanol in situ and bath superfusion of ethanol in in vitro slices. In both cases, the relative differences in sensitivity between the two lines were maintained in the tolerant condition (345).

d. FETAL HUMAN PURKINJE NEURON SENSITIVITY. Perhaps some of the most provocative findings that have arisen from these in vitro studies stem from a recent study of human fetal tissue (cerebellar and neocortical) transplanted to the anterior chamber of the eye of rat hosts and then characterized for ethanol sensitivity (M. R. Palmer, B. J. Hoffer, L. Olson, and A.-C. Granholm, unpublished data). In such grafts, both cerebellar and cortical neurons were sensitive to the depressant effects of ethanol (concentrations generally between 1 and 100 mM were found to be effective), which would seem in keeping with the greater behavioral sensitivity to ethanol in humans as opposed to rodents. Moreover, it was found that, for the sensitivity to ethanol, there were two distinct populations of cells, one with an EC_{50} of approximately 5 mM for inhibition by ethanol and the other approximately 30 mM. Within any one graft, cells were of similar sensitivity, but different grafts showed distinct differences in sensitivity that depended on the source of the donor tissue. Grafts prepared from the same fetal tissue host showed comparable sensitivities to ethanol.

e. CONCLUSIONS. The genetic studies of Purkinje cell sensitivity have firmly established that factors controlling the electrophysiological sensitivity of Purkinje cells to the depressant effects of ethanol are genetically determined and can be expressed at the level of the Purkinje neuron itself. Nevertheless, there remain a number of unanswered questions concerning these responses. First, it is difficult to explain why there is such a high correlation between Purkinje cell sensitivity and behavioral sensitivity, when the cerebellum can be removed neonatally and the differential behavioral response (loss of righting reflex) is still maintained (347). What seems likely is that the cerebellum is not directly involved in the righting reflex but that the factors that control the sensitivity of the Purkinje neuron to ethanol also determine the sensitivity of cells in other parts of the brain that mediate in the righting response. Another issue is the relatively high concentrations of ethanol that are required to inhibit Purkinje cell firing (in the range of 75 to 432 mM to obtain a 50% inhibition). It is possible that other brain regions may show greater absolute sensitivity to ethanol than the cerebellum but that the same

basic factors regulate sensitivity in many different regions. For example, studies in the LC have shown that slowly firing cells are more sensitive to the effects of ethanol (411). Based on this observation, one might hypothesize that cells with relatively high rates of spontaneous activity such as the Purkinje cell would be minimally affected by concentrations of ethanol that would markedly affect the activity of more slowly firing cells. However, the same basic mechanism controls the ethanol response in both types of cells. Regardless of the factors that control neuronal sensitivity to ethanol, it is apparent that they are not uniformly expressed in brain, because regions such as the hippocampus demonstrate no differences between LS and SS mice not only in electrophysiological sensitivity (430) but in biochemical sensitivity to the effects of ethanol as well (5).

One issue that has not been resolved concerns the mechanism by which ethanol changes cell firing. A variety of conductances are affected by ethanol (see section IV), but intracellular studies have not identified the mechanism involved. If future electrophysiological studies can determine the specific ion channels that are affected in the Purkinje cells by ethanol, then molecular biological techniques may be able to pinpoint the specific ways in which these lines of mice differ and define the genetics of sensitivity to ethanol in humans as well.

2. Inferior olivary nucleus. As discussed in the previous section, there has been some controversy as to whether ethanol increases or decreases the rate of climbing fiber bursts observed in cerebellar Purkinje cells. Because these responses reflect the activity of inferior olivary neurons, several groups have looked directly at the actions of ethanol in this brain region and have, to some extent, resolved previous areas of disagreement concerning ethanol's actions.

Studies conducted in urethane-anesthetized rats have shown that both local and systemic (slow i.v. infusion) application of ethanol depresses both spontaneous and evoked activity of the inferior olive (177). On the other hand, Rogers et al., (384) found that the predominant effect of ethanol (i.p. injection) is a marked increase in activity in the inferior olive (although there were occasionally transient decreases in activity) in animals anesthetized with chloral hydrate or halothane or in paralyzed, respirated animals. However, under urethane anesthesia, only decreases in firing were observed, consistent with the observations of Harris and Sinclair (177). Nevertheless, the excitatory responses to ethanol are probably not direct; when animals have received lesions of 5-hydroxytryptamine-containing neurons, these responses are almost completely blocked (unpublished data cited in 32). This suggests that the inhibition seen with local application may be the direct effect of ethanol and that increases in activity are the indirect effect of changes in input from serotonergic neurons.

3. LC. There is also some disagreement concerning the effects of ethanol on the LC, although the majority of reports suggest that ethanol has primarily depressant effects in this brain region. In an early study in the paralyzed rat preparation, Pohorecky and Brick (357) reported that most cells were inhibited by i.p. injections of ethanol (2 g/kg), although 22% increased their firing, and 16% were unaffected. Similar effects of i.p. ethanol were also observed in chloral hydrate-anesthetized animals, although results from only 4 cells were reported (437). A more consistent action was reported in this same study for local application of ethanol to LC neurons, with nearly 100% of the cells showing inhibition.

On the other hand, LC neurons have been reported to show no changes in spontaneous rate with systemic ethanol up to 3 g/kg in chloral hydrate-, halothane-, or urethane-anesthetized rats (15). This study reported a marked depression in responses to sensory stimulation, a response that may be related to the observed facilitation of inhibitory responses elicited in the LC by antidromic stimulation. However, the lack of any effect on spontaneous activity is difficult to reconcile with previous reports, considering that the anesthetic and dose range for ethanol are comparable to those used previously (e.g., 437).

In in vitro studies of ethanol actions on LC neurons, primarily inhibitory effects have been reported. When slices are superfused with ethanol at concentrations of 1–60 mM, inhibition of cell firing is the most common response (411). It is noteworthy that the sensitivity of LC neurons from LS and SS mice differ in their sensitivity to ethanol, with the LS neurons about 2 times more sensitive to perfusion with ethanol in brain slices (EC_{50} values of about 25 mM and 50 mM; 410). Thus, the LC is another brain region in which sensitivity differences are observed between these lines of mice but at concentrations below those required to inhibit activity in the cerebellum. In addition, there were qualitative differences between the lines as well, in that SS mice (but not LS) occasionally showed excitations with lower concentrations of ethanol (410).

As with the Purkinje neuron, the electrophysiological basis for ethanol inhibition of LC neuronal activity remains poorly understood. When the mechanism underlying this response has been better characterized, it may be possible to use more extensive genetic approaches to better characterize the relationships between these effects and the behavioral aspects of ethanol intoxication.

4. Ventral tegmental area (VTA) and substantia nigra. The VTA has been a subject of particular interest in ethanol research. Drugs of abuse commonly are reported to increase either the activity of VTA neurons themselves or to potentiate the actions of dopamine, the primary transmitter in the projections of the VTA to the mesolimbic and mesocortical areas (475).

In this context, it has been noted that i.v. administration of ethanol produces increases in spontaneous activ-

ity in the VTA and substantia in unanesthetized rats but not those anesthetized with chloral hydrate (152, 296). In paralyzed preparations, anesthetics by themselves increase activity in the nigral neurons, and the response to ethanol is blocked (296). VTA neurons were approximately 5 times more sensitive to this effect (EC_{50} 0.16 g/kg i.v.) than were dopamine-containing neurons in the substantia nigra pars compacta. In both cases, it was hypothesized that the increases in activity are the indirect consequence of ethanol suppressing the activity of GABAergic neurons that tonically inhibit the firing of dopamine-containing cells (152).

More recently, the effects of ethanol on the spontaneous activity of VTA neurons in brain slices have been characterized as well (39). In such slices, ethanol increases the spontaneous rate of firing, and preliminary results suggest that these effects are observed under conditions in which synaptic transmission is blocked. This observation, if correct, would suggest that the excitatory action of ethanol on these cells need not be indirect, as proposed by Gessa et al. (152). However, there are a number of differences in the activity of VTA neurons in brain slices and in the intact animal. It could well be that different mechanisms underlie the increases in firing rate under the two conditions.

Indirect support for an ethanol-mediated increase in VTA activity, comes from the observation that ethanol also increases the concentrations of extracellular dopamine in the nucleus accumbens (NA; a major projection area of the VTA) in freely moving rats, (97). These effects were more pronounced in the accumbens than in the striatum.

These reports of ethanol effect on dopaminergic systems clearly suggest a mechanism that could underlie ethanol self-administration. If mesolimbic and/or mesocortical dopamine systems mediate endogenous reward mechanisms, (475) then a drug such as ethanol that activates these cells would be likely to be rewarding. In the global view, effects in the VTA seem unlikely to relate to measures of behavioral sensitivity such as duration of loss of the righting reflex but might be linked to the propensity for alcohol self-administration. On this basis, rats that demonstrate preference for alcohol might be more sensitive to the stimulatory effects of ethanol on VTA activity. By analogy, people at risk for the development of alcoholism might be expected to have a greater activation of dopaminergic systems as well.

5. Raphe nuclei. In addition to studies of the effects of ethanol on norepinephrine and dopamine-containing neurons, there have also been effects reported on serotonergic neurons as well. In the dorsal raphe of intact unanesthetized, paralyzed animals, slowly firing units (which are probably the serotonergic neurons) generally responded to i.p. injection of ethanol with a slowing of unit activity broken by occasional periods of complete inhibition (72). Cells with faster basal rates were typically not as sensitive. Median raphe units showed increases in the regularity of firing and decreases in rate as well. Somewhat similar effects were observed in a brain slice preparation (73). Perfusion with 22 mM ethanol increased the firing rate of the majority of slowly firing cells. At 44 mM, however, more than half of the neurons were inhibited. At all higher concentrations the predominant response was inhibition of firing.

6. Hippocampus. Early studies of the responses of hippocampal neurons to ethanol reported generally depressant effects on spontaneous activity. Grupp and Perlanski (173) reported that systemic administration (0.5–3.9 g/kg) of ethanol produced a primarily depressant effect on the firing of hippocampal neurons in restrained unanesthetized rats. Although some cells showed increased firing rates at low concentrations, almost all were depressed at higher levels. The dose of 1 g/kg was the lowest dose that produced a statistically significant depression of firing rate, although individual neurons showed significant effects at lower doses. In this study the cells were not differentiated as being from the regio superior as opposed to the dentate gyrus. No attempt was made to differentiate between interneurons and pyramidal neurons. A more recent study of hippocampal CA1 and CA3 neurons reported that local application of ethanol increased the firing rate of more than 50% of the cells in halothane-anesthetized rats, whereas about one-third showed decreases (26). Parallel experiments on cortical neurons showed primarily depressant effects as well.

In another study of hippocampal neurons in mice, depressant effects of locally applied ethanol were consistently observed (430). The effects reported by Berger et al. (26) also differ from what has been reported in the dentate gyrus of halothane-anesthetized rats. Here, ethanol (2 g/kg, i.p.) produced a rapid decline in spontaneous firing rate (472). In the dentate, i.p. ethanol injections induced a period of near total inhibition of spontaneous activity that lasted about 15 min, which was followed by a much longer period of reduced activity.

In brain slice experiments, a high proportion of cells also respond with decreases in firing rate. Carlen et al. (49) reported that 83% of hippocampal cells were inhibited by ethanol application. Siggins et al. (416) also found that slowing was the most frequently observed response (50% of cells). A variety of factors could account for the primarily excitatory responses reported by Berger et al. (26) and the findings of other laboratories (type of anesthetic, method of drug application, etc.). However, none of these potential explanations is wholly satisfactory. Perhaps the most likely of the alternatives concerns the level of tonic inhibitory activity in the different preparations. Opiate drugs (which are thought to inhibit hippocampal interneurons produce excitatory effects on single-unit activity in intact animals but depress spontaneous unit activity in brain slices (107). The critical

difference between these situations appears to be the level of activity of interneurons. If ethanol also acts on interneurons, then the varying reports on ethanol action in the hippocampus might be at least partially explained.

7. Septal area. When one considers the effects of ethanol on hippocampal neurons in intact animals, it is also important to consider the indirect effects that might result from changes in regions that project to the hippocampus, such as the septal region. In general, ethanol inhibits the firing of cells in the medial septal/diagonal band region. This type of inhibition is dose dependent at doses between 0.75 and 3.0 g/kg and is observed in about 80% of the cells tested (158). Decreases in activity in the hippocampus might reflect a decrease in synaptic drive from the septal area.

8. Summary. It is clear that the direct effects of ethanol on spontaneous unit activity are primarily depressant, although there are occasional increases as well. In intact systems, the increases in firing may be indirect, reflecting either the loss of an inhibitory effect (e.g., in Purkinje cells, where increases in activity seem linked to a decrease in noradrenergic input) or to an increase in excitatory input. Nevertheless, it is clear that increases can also be observed in vitro in the absence of any synaptic input (21, 39). When such changes are observed, they are often observed at lower doses and are frequently biphasic in nature (low-dose excitation, high-dose inhibition). In cells that appear to respond primarily with excitations (e.g, inferior olive), biphasic responses with the opposite sequence occur (384). One possible interpretation of such a result is that these cells are tonically inhibited by neurons that show the biphasic excitatory/inhibitory pattern of activity that seems to be more common.

Some of the effect of ethanol in the cerebellum seems to be mediated indirectly via actions on other brain nuclei (32). However, the direct depressant effects that are observed with local application and in vitro are clearly manifested at the level of the individual Purkinje neuron and are under genetic control. Although direct effects of ethanol upon Purkinje cell activity may be minimal at intoxicating concentrations of ethanol (32), this neuron has provided probably the strongest link between electrophysiological effects and specific behaviors. Thus, the Purkinje cell sensitivity seems to reflect a neuronal property that may not directly cause the loss of righting reflex in the intact animal but provides a reliable indication of a cellular property that is expressed in other neurons as well. These actions do lead directly to loss of the righting reflex.

In in situ studies, the selection of anesthetic is clearly an important variable. In many earlier studies, this was resolved by the use of paralyzed, artificially respirated animals, but the continued use of this preparation is not justified. Studies in paralyzed preparations have identified instances, such as in the VTA, where the responses are qualitatively different when studied in anesthetized animals. Rogers et al. (384) have shown that responses may be qualitatively altered by the anesthetic. What would be useful at this point are electrophysiological studies in intact, freely moving animals. We can then identify which effects, which are observed in other preparations, can actually occur in a normal animal and under what behavioral conditions. The studies by Chapin and Woodward (64) and their colleagues (see section III, C, 1b) are clearly a first step in this direction and one that potentially will lead to a more detailed analysis of the linkage between neuronal activity and behavior.

C. Effects of Ethanol on Synaptic Transmission

Although ethanol has a number of actions on single-unit activity in the CNS, it is unclear as to whether these reflect direct actions on the cells from which recordings are made or changes in the excitatory or inhibitory input to these cells. With the exception of in vitro studies, in which synaptic transmission can be blocked, this issue has been difficult to resolve. However, another way to address this problem is to determine directly whether ethanol can modify synaptic responses; if it does not, then such effects clearly cannot underlie changes in spontaneous activity.

Ethanol has been reported to have a variety of actions on synaptic transmission, both pre- and postsynaptically. In many cases synaptic transmission is the process that is most sensitive to the effects of ethanol. Most of the postsynaptic effects of ethanol (e.g., modulation of transmitter sensitivity) are discussed in section V with respect to the specific transmitters that are involved. The presynaptic effects of ethanol (e.g., alterations in transmitter release) and situations in which it is difficult to identify the site of ethanol action will be summarized in this section.

1. Mammalian CNS. a. HIPPOCAMPUS. One of the most intensively studied brain regions, insofar as modulation of synaptic responses is concerned, is the hippocampus. This is partially the result of the fact that the development of in vitro recording techniques has greatly facilitated the ease with which these types of studies can be conducted. In addition, the hippocampus responds to synaptic stimulation with well-defined and easily recorded field potentials that directly reflect synaptic currents generated in response to activation of excitatory synapses.

One of the earlier investigations of the effects of ethanol on hippocampal responses characterized its actions in the CA1 region of the hippocampal slice preparation. In this study, superfusion of slices with 20 to 100 mM ethanol resulted in a depression of the evoked population spike response (110). The amplitude of the population spike is directly related to the number of pyramidal neurons firing in response to a stimulus. This can serve as an indication of the excitability of these cells. The field excitatory postsynaptic potential (EPSP), which reflects only the synaptic currents, was occasion-

ally inhibited at an ethanol concentration of 100 mM, but in many cases there was a drop in population spike response with no change in the EPSP. The most likely explanation for this observation is that the excitability of the pyramidal neurons is reduced by perfusion with ethanol, but the magnitude of the synaptic response itself is unchanged except at higher concentrations. Durand et al. (110) also indicated that the slices that were most sensitive to ethanol were those in which synaptic inhibition seemed most potent; they suggested that the decrease in the population spike is due to increase in inhibition.

Although this conclusion is consistent with the observed results, a number of other factors could account for this difference equally well. Slices with the greatest inhibition are generally the most physiologically viable slices. It is perhaps not surprising that they should give the greatest ethanol responses as well.

In more recent investigations in our laboratory, we have also observed that, when ethanol is perfused at moderate concentrations (40 to 80 mM), there is frequently a transient increase in the population spike response (109). Similar observations have been made by others (415), although in some cases (416), this effect was observed in only a minority of cases. Siggins et al. (416) also found that the field EPSP responses were reduced, a change that has not been apparent in other studies except at higher concentrations of ethanol.

The physiological relevance of these effects is unclear. The population spike response can be a very sensitive measure of the excitability of pyramidal neurons, so it is unclear that the reduction in excitability would have major effects on hippocampal function.

Furthermore, studies from our laboratory (429; M. Taylor and T. V. Dunwiddie, unpublished results) demonstrate that there are no differences either in the excitatory or inhibitory components of hippocampal responses from SS and LS mice.

On the other hand, Sellin and Laakso (404) found there were differences in the sensitivity of hippocampal population spike responses in Long-Evans and Wistar rats that paralleled behavioral differences (thresholds for the two strains were approximately 120 and 310 mM). However, they also observed that such a correlation did not exist with alcohol-nontolerant (ANT) and alcohol-tolerant (AT) rats (both showed threshold between 220 and 310 mM), which have been selectively bred for ethanol sensitivity and insensitivity, respectively. These genetic studies suggest that hippocampal activity is not directly responsible for any of the behavioral responses that are typically used to determine ethanol sensitivity. Whether or not these responses are relevant to other behaviors more directly related to hippocampal function (e.g., ethanol-induced amnesia) is unclear.

The only study that directly addressed the question of mechanism(s) underlying the ethanol depression of the population spike response was a recent one by Sellin and Laakso (404). They reported that the effects of ethanol on population spike amplitude could be antagonized by the potassium channel blocker 4-aminopyridine. This compound also antagonized ethanol-induced depression in the intact rat (405). 4-Aminopyridine potentiates synaptic transmission in a variety of preparations and increases the amplitude of the hippocampal field EPSP and, hence, the population spike response as well. However, because ethanol has little if any effect upon the field EPSP response at lower concentrations, this would suggest that the observed antagonism is most probably a nonspecific physiological antagonism and not a direct reversal of the physiological response to ethanol.

In addition to these studies on isolated slice preparations, there have also been studies of synaptic responses evoked in intact animals. In one such study, systemic ethanol (3 g/kg, i.p.) facilitated both excitatory and inhibitory transmission to the CA3 cells (333). In these studies, unit activity was used as the response measure rather than evoked field potentials as in the slice work. A variety of explanations have been proposed as to why facilitation of transmission was observed in this study, whereas most later studies have reported decreases in synaptic responses. The use of systemic ethanol injections leaves open the possibility that some of these responses are indirect. However, the use of different time points and response measures must be considered as well (415, 416).

Finally, a study of evoked responses in the dentate gyrus of rats suggests that systemic injections of ethanol facilitate inhibition in this hippocampal subregion (473). In this study, i.p. injections of 2 g/kg ethanol facilitated paired pulse inhibition but did not have a statistically significant effect on directly evoked responses. The phenomenon of paired-pulse inhibition is observed in the hippocampus when the same synaptic input is stimulated twice in rapid succession. Under these conditions, the second population spike response is inhibited because of a recurrent inhibitory circuit that temporarily inhibits cell firing. However, there are complicating factors that make interpretation of these studies difficult. For example, while the second population spike is inhibited, the corresponding EPSP that drives the population spike is usually facilitated, due to paired-pulse facilitation that occurs whenever these synapses are stimulated in rapid succession. The changes in the inhibition of the second population spike could thus reflect changes either in inhibition or in synaptic facilitation, because the two effects are opposed, and the output (population spike amplitude) really represents the combination of the two. Furthermore, the effects that occur depend partially upon the amplitude of the test responses. If the responses are smaller initially (perhaps because of a depressant effect of ethanol) the test response is more easily reduced by the recurrent inhibitory circuit.

A number of studies have focused on the effects of ethanol on synaptic transmission using intracellular recording techniques.In the hippocampus, the effects of ethanol upon intracellularly recorded EPSPs and inhibitory postsynaptic potentials (IPSPs) are no more consistent than are the effects upon the extracellular potentials. For example, Siggins et al. (415, 416) reported that the predominant effect of ethanol on both IPSPs and EPSPs was a reduction in amplitude. (For the most part the EPSPs are thought to be at synapses that use glutamate as a transmitter, and the inhibitory ones use GABAs.) Increases in synaptic potentials were observed in only a small proportion of the cells tested in both the CA1 and CA3 regions. In another study, however, both EPSPs and IPSPs were enhanced by local application of ethanol. The EPSPs showed the greatest change when ethanol was applied into the region of synaptic termination (stratum radiatum) and the IPSPs when ethanol was added near the soma (49). Both types of responses were also facilitated in 4/4 cells that were superfused with 20 mM ethanol. Because direct responses to GABA and glutamate were unaffected by ethanol, it was concluded that the effect of ethanol was probably a presynaptic enhancement of calcium-dependent transmitter release (48, 49). A more recent study of the dentate gyrus from the same group has done little to resolve this controversy concerning synaptic responses. In this study, EPSPs were found not to be affected by ethanol in granule cells, and IPSPs were unaffected in young rats but markedly decreased in old (335).

We find that synaptic responses in hippocampus are minimally affected by superfusion with ethanol but that reductions in IPSPs and LPSPs are most common. The most striking effect has been the inhibition of spontaneous firing. The mechanism underlying this effect is unclear, but it is quite apparent in other studies as well (49).

The ultimate resolution of these differences in responses is unclear. The primary hypothesis advanced by Carlen et al. (49) to account for their observations is that calcium-dependent processes such as transmitter release are enhanced by ethanol. However, the lack of supporting data, even in studies from the same laboratory (335), make it clear that there must be other factors upon which these responses depend. In addition, many of the earlier studies were conducted using direct application of ethanol to the surface of a slice maintained at a gas-medium interface. Because slices under these conditions receive most of their O_2 from the gas phase, it is possible that this type of drug administration can have disruptive effects that are completely unrelated to ethanol's normal mechanism of action in intact animals. It is clear that more studies will be required to resolve these differences in experimental observations.

b. EFFECTS OF ETHANOL ON LC, CEREBELLUM, AND CORTEX. In addition to the hippocampus, the effects of ethanol on synaptic transmission have been studied in a number of other brain regions as well. In the LC of the rat, one study has reported that the most apparent response following injection with 0.5 to 3 g/kg ethanol was a disruption of neuronal responses to sensory stimulation (15). In the cerebellum, the inhibition of Purkinje neurons following local electrical stimulation was disrupted following administration of 1.5 g/kg ethanol i.v. or with local application of ethanol (178, 418) Excitatory responses elicited by cortical stimulation can also be reduced or blocked by ethanol (178).

In the cerebral cortex, a number of recent studies have also suggested that ethanol has a generally disruptive effect upon synaptic transmission. These studies have used various protocols, both behavioral and electrophysiological, to affect activity, and they have shown that ethanol can reduce or abolish evoked patterns of activity. One such study characterized the cortical gating that is observed in the somatosensory cortex of animals walking on a treadmill. The response of somatosensory neurons to sensory stimulation of the foot depends in a predictable fashion on the phase of step cycle. It has been reported that this type of gating is reduced or abolished in animals given ethanol (64). Another study from this group demonstrated that ethanol reduces the facilitation of the transmission of sensory information to the somatosensory cortex that is normally observed during immobile arousal (63). Both of these studies showed that the normal modulation of transmission to somatosensory cortical neurons is reduced by ethanol. Although the mechanism that underlies these effects is not clear, it would seem likely that ethanol either affects transmission in the sensory pathways themselves or, equally likely, modifies the activity in another system (e.g., the noradrenergic input) that modulates the activity in the sensory pathway. This effect of ethanol is either on the transmission process itself or on the activity of neurons that release transmitters that affect the efficacy of transmission to these cortical neurons.

c. SPINAL CORD. The difficulty in determining whether ethanol is affecting synaptic transmission per se, or simply the activity of neurons that are presynaptic to the ones from which recordings are made, is also seen in another study. In this study the actions of ethanol on spinal cord neurons in culture were characterized (172). The most notable effect of ethanol was reduction in spontaneous activity of the cultured neurons that occurred at concentrations as low as 20 to 30 mM and occurred in the absence of large changes in sensitivity to exogenously administered transmitter. It was concluded that these responses could be best explained by a decrease in synaptic transmission. However, because the primary change was in the rate of spontaneous EPSPs and IPSPs, and not in the amplitude of these potentials, it is more likely that ethanol was affecting the firing rate

of presynaptic neurons rather than the transmission process itself.

2. Changes in synaptic responses: non-CNS and invertebrates. Although results of many previous studies have suggested that ethanol has a primarily depressant effect upon synaptic transmission (see above), there are reports of the opposite effect particularly at lower concentrations (e.g., see 49 and previous section). Because of the difficulties in determining mechanisms of action on presynaptic sites in the CNS, some investigators have looked at effects in other types of preparations, where considerably more can be learned about the effects of ethanol.

The neuromuscular junction is one such system in which the effects of ethanol have been studied. In relatively early studies increases were reported in the frequency of miniature end plate potentials (MEPPs) at the frog (e.g., 338) and at the rat phrenic nerve-diaphragm muscle synapse (143). The increases in the rates of MEPPs are seen particularly at higher concentrations (>100 mM), but enhancement of the evoked response can sometimes be observed at very low concentrations of ethanol; in the case of the rat neuromuscular junction, these were observed at concentrations as low as 8 mM (143). In the crayfish at the neuromuscular junction, similar effects have been reported. Ethanol at concentrations between 10 and 100 mM was found to increase the spontaneous release of transmitter, as determined by measuring the frequency of occurrence of MEPPs (139). This type of transmitter release (which does not depend upon action potential invasion into the presynaptic nerve terminal or the influx of calcium through voltage-gated channels) provides evidence that the presynaptic mechanisms underlying transmitter release have been facilitated. Such effects might be expected to arise from an increase in the calcium concentration in the nerve terminal or perhaps a change in the calcium sensitivity of the release mechanism itself. In either case, this leads to an increase in the magnitude of evoked responses as well. In the crayfish, higher concentrations of ethanol can also reduce the synaptic response but through mechanisms that probably involve the postsynaptic site (139).

3. Effects of ethanol upon synaptic plasticity. In addition to affecting the transmission process directly, ethanol has also been reported to interact with (mainly disrupt) various forms of synaptic plasticity. The mechanisms that underlie these effects are unclear. Because many forms of plasticity are thought to be induced by calcium-dependent processes, these may be the indirect consequence of effects upon intracellular calcium.

a. LONG-TERM POTENTIATION (LTP). One such form of synaptic plasticity is LTP. This is a long-lasting increase in synaptic efficacy that is observed in the hippocampal formation following brief periods of high-frequency stimulation. Sinclair and Lo (417) were the first to report that ethanol blocks LTP in the rat. The threshold for this effect appears to be between 50 and 100 mM ethanol. In addition, increases in extracellular calcium can induce an LTP-like process in hippocampal brain slices that is thought to occur via the same mechanism as LTP. This form of plasticity is also blocked by ethanol (417). Other investigators who have used ethanol as a vehicle for dissolving other drugs have also made the incidental observation that ethanol reduced LTP. For example, Mulkeen et al. (319) reported that 110 mM ethanol markedly reduces LTP (mean increase in the EPSP following tetanic stimulation was reduced from 43 to 15%). Finally, ethanol (2 g/kg, i.p.) has also been reported to disrupt LTP in the dentate gyrus of both anesthetized and freely moving rats (481).

The basis for the disruption of LTP is unclear. LTP is thought to occur as the result of a sequence of events that begins with the activation of N-methyl-D-aspartate (NMDA) receptors on the postsynaptic cell by glutamate (179, 253). The ion channels activated by NMDA are permeable to calcium, which then enters the cell and initiates a sequence of events that may involve protein kinase C (209). This ultimately results in an increased response of the postsynaptic cell. Drugs that block LTP are the NMDA receptor antagonists (phencyclidine, MK-801, 2-amino-5-phosphonovaleric acid), chelators that can block increases in intracellular calcium (270), or those that can block calcium-calmodulin-mediated responses such as trifluoperazine (128, 108). Ethanol could potentially disrupt LTP by acting through any of these mechanisms. One biochemical study of NMDA receptor-mediated increases in $^{45}Ca^{++}$ flux in cortical brain slices suggests that ethanol does not antagonize this effect (383). Other studies support the idea that ethanol disrupts the activation of a calcium conductance by NMDA receptors (264, 367, 452).

Regardless of the mechanism involved, it seems clear that ethanol has a disruptive effect upon LTP. Because LTP is considered a likely candidate for a cellular mechanism underlying long-term memory storage, these effects of ethanol at the cellular level may provide a mechanistic basis for the amnesic effects of ethanol. In addition, to the extent to which NMDA receptors are involved in other types of behaviors, ethanol may have a variety of other effects which also stem from its ability to antagonize these receptors.

b. POSTTETANIC POTENTIATION. Another form of synaptic plasticity that is sensitive to ethanol is posttetanic potentiation. Ethanol accelerated the rate of decay of posttetanic potentiation in the marine invertebrate *Aplysia,* although the concentrations used (800 mM) were somewhat above those intoxicating in mammals (456). Posttetanic potentiation, like LTP, may result from changes in intracellular calcium. If ethanol modifies intracellular calcium levels, it may indirectly affect posttetanic potentiation. The potential role of ethanol as a regulator of posttetanic potentiation has been reviewed in detail by Hunt (212).

c. HABITUATION. Yet another form of synaptic plasticity affected by ethanol is synaptic habituation. When some synapses are stimulated at low frequencies, the magnitude of the postsynaptic response steadily declines. This response does not depend upon depletion of transmitter stores but is an active form of regulation of transmitter release. In one study conducted in the frog spinal cord, ethanol (110 mM) depressed ventral root responses (159). The magnitude of habituation at this synapse is dependent upon the amplitude of the initial response. If the response amplitude was returned to baseline by increasing the stimulus intensity prior to repetitive stimulation, habituation was also markedly reduced by ethanol. Another study in the isolated frog spinal cord also found decreased habituation in a polysynaptic reflex at concentrations of ethanol between 5 and 100 mM (381). As in the preceding study, ethanol also had a direct effect upon the evoked response with a threshold of 5 mM.

d. SUMMARY. A number of studies have characterized the effects of ethanol upon synaptic plasticity in both vertebrate and invertebrate preparations. Ethanol has a generally disruptive effect upon several distinct kinds of synaptic plasticity. Whether this is purely fortuitous or indicates that different types of synaptic plasticity all rely on similar mechanisms (e.g., calcium-dependent cellular processes that modify synaptic function) that provide a common substrate for ethanol action is unclear. Nevertheless, these types of studies do provide cellular mechanisms that might account for the disruptive effects of ethanol on learning and memory.

D. Effects of Ethanol on Resting Membrane Potential and Resistance

In addition to its effects upon single-unit activity and synaptic transmission, ethanol also has effects that can only be measured directly with intracellular recording, such as hyperpolarizations and depolarizations of the resting membrane potential. In many cases, the identity of the ion channel that mediates these effects is known. These cases will be characterized in greater detail in the following sections. However, in other cases, the mechanism underlying the response is not known. Sometimes this occurs when the change is of such small magnitude or so variable that it is not possible to conclusively identify the ion channel. In other cases, ethanol might be acting through other mechanisms (e.g., inhibition of an ion transport mechanism) that are difficult to identify.

A consistent observation is that ethanol hyperpolarizes neurons in a manner that is associated with small decreases in membrane resistance. In most cells, this type of effect could reflect the activation of a channel permeable to either potassium or chloride. Such effects have been observed in tissues as diverse as hippocampal and spinal cord neurons (48, 49, 172, 335, 416), *Aplysia* neurons (398), and crayfish muscle (127). In the studies by Carlen et al. (48, 49), this response was unaffected by injections of chloride into the cell (which would invert the response if it were due to activation of a chloride conductance) and by superfusion with calcium-free medium. This latter result suggests that this effect is not due to the modulation of ongoing synaptic activity that is revealed indirectly in changes in membrane potential.

Perhaps the most commonly reported effect of ethanol is no significant change in the resting membrane potential. These types of effects have been reported in LC, hippocampus, and spinal cord (172, 412, 416; T. V. Dunwiddie and W. R. Proctor, unpublished results) and are usually not associated with detectable changes in input resistance of the cells. Finally, depolarizing responses have been reported as well, either as the primary response or as a transient effect at the beginning of the drug application period (335, 416). In many cases, there are associated decreases in the rate of spontaneous firing (see section III, B), but it is frequently difficult to determine whether the changes in membrane potential are sufficient to account for the changes in firing rate. Yet another cellular parameter that has been reported to be affected by ethanol is membrane capacitance. This cannot be measured directly but can be estimated based upon charging curves (47).

It is difficult to synthesize these results into a coherent picture of drug action, primarily because there is such a diversity of responses that have been reported, sometimes even for the same preparation (49, 416). It is likely that ethanol does not have a large effect upon any of the major conductances that establish the resting membrane potential. However, if it affects some other aspect of cellular function (e.g., free intracellular calcium), there might be a variety of indirect effects that would depend upon the cell from which recordings were made, the physiological state of the preparation, and other factors that would be difficult to control. It will clearly require more study to identify and test such a unifying hypothesis.

IV. Effects of Ethanol on Voltage or Second-Messenger Gated Ion Channels

The effects of ethanol have been studied in a number of preparations that have permitted the rigorous identification of the specific ion channels upon which ethanol acts. Most of these experiments have been conducted in systems such as the squid giant axon, in the *Aplysia,* or other such systems in which two-electrode voltage clamp experiments are possible. These studies have shown an inhibitory effect of ethanol on a variety of conductances but in many cases at relatively high concentrations. Such inhibitory effects are manifested either as changes in the magnitude of the response or occasionally in the channel kinetics. This review will make no attempt to review these effects in detail but will concentrate specifically on recent developments and particularly on those responses that appear significant at pharmacologically relevant

concentrations of ethanol. In particular, much of the current literature concerning ethanol interactions with calcium and calcium-gated ion channels has been recently summarized by Carlen and Wu (50).

A. Voltage-dependent Sodium Channels

The currents that pass through sodium channels, such as the voltage-dependent sodium channel that carries the inward current that underlies the action potential, seem to be generally inhibited by ethanol. Many earlier studies utilized electrophysiological techniques to measure sodium currents in invertebrate preparations such as the squid giant axon, and these studies have been discussed in detail elsewhere (45, 212, p. 67). In most cases, these studies only reported effects at what would normally be lethal doses of ethanol, and for this reason, it has been difficult to relate these observations to behavioral phenomena such as intoxication.

More recent studies, however, have suggested that these effects can occur at relatively low concentrations of ethanol and might contribute to the behavioral effects of ethanol. One such study has reported that significant decrements in the inward sodium current can be observed at 100 mM ethanol (45), although other currents (e.g., I_{Ca}) were more sensitive. On the other hand, when vertebrate dorsal root ganglion neurons in culture were used, somewhat lower concentrations of ethanol (11 to 66 mM) were reported to have no effect on either the maximum rate of rise or the peak of the action potentials (337). However, relatively small differences in the sodium current could have been difficult to detect in this study, because the sodium current was not isolated with voltage clamp or pharmacological techniques.

Neurochemical studies have confirmed the inhibition of sodium flux by ethanol using isolated brain synaptosomes and $^{22}Na^+$ (184, 321). A small inhibition was seen with 50 to 100 mM ethanol, with a 50% inhibitory concentration (IC_{50}) of about 500 mM. Other anesthetics shared this action, and their potencies in inhibiting sodium flux were closely correlated with their membrane-disordering potencies (185). In contrast to these in vitro actions, acute in vivo administration of ethanol did not alter sodium fluxes (320). For the in vitro sodium flux experiments, neurotoxins such as veratridine or batrachotoxin were used to open the voltage-dependent sodium channels to allow measurement of ion flux. Ethanol was found to inhibit the binding of [^{3}H]batrachotoxinin-A20-α-benzoate to the channel with an IC_{50} of 310 mM and acted by enhancing the rate of dissociation (321, 322, 486). However, animals selected for genetic differences in ethanol sensitivity did not differ in actions of ethanol on batrachotoxin binding (ANT/AT rats; 243) or sodium flux (LS/SS mice; 181). Thus, the sodium channel is unlikely to have a role in genetic differences in ethanol sensitivity.

The relative importance of the effects of ethanol upon the sodium channel is difficult to assess. By and large, most neurons and axons have a relatively high margin of safety in terms of the ability to conduct action potentials, and hence the reduction in the sodium current with ethanol would not be expected to be particularly significant from a functional standpoint until fairly large effects occur. Because ethanol only affects the maximum current, but not the voltage sensitivity of the channel (45), the "excitability" of cells and axons would not be depressed by this type of action. Perhaps for this reason, ethanol does not have very pronounced depressant effects even at relatively high concentrations in systems that one might expect would be relatively sensitive to such actions. For example, when the excitatory afferents to the hippocampal CA1 cells are activated, 300 mM ethanol reduces the postsynaptic population spike response by only 30 to 40% (429). This relative lack of sensitivity is somewhat surprising when one considers that, in order for this response to occur, the action potential must pass down the axon, invade the nerve terminal, and release the neurotransmitter, and the postsynaptic EPSP must then depolarize the pyramidal neurons enough to fire. Thus, it seems likely that, although ethanol can affect sodium currents, these effects might be functionally important only in a few specific situations. One might expect to see significant changes in the ability of an action potential to invade fine nerve endings, etc., where the safety factor is relatively low.

B. Voltage-dependent Potassium Channels

As discussed in section III, D, ethanol often produces a hyperpolarization of the resting membrane potential which is typically associated with a decrease in input resistance, and there are indications that small increases in potassium conductances may be responsible for these effects. This stands in contrast to the situation with sodium and calcium conductances, which are more typically inhibited by ethanol. Because there are numerous potassium channels on virtually every neuron, considerable effort has gone into attempting to determine which type of potassium conductance is affected. Potassium conductances that are regulated by the membrane voltage or by changes in the intracellular calcium concentration will be discussed in this section; ligand-gated potassium conductances will be discussed in section V.

1. Aplysia. In the *Aplysia,* the kinetics of the voltage-dependent potassium current known as the A current (I_A) are altered so that the rate of decay is slowed by ethanol (458, 459) with a threshold of approximately 200 mM. In addition, ethanol only affected I_A in certain neurons, whereas others were unaffected. Another potassium conductance known as the delayed rectifier (I_K) is reduced in *Aplysia* neurons, but again, ethanol was not particularly potent in this regard, with 100 mM ethanol giving only a 4% decrease in the magnitude of this potential (45). Thus, although there are well-defined effects on these conductances, they occur at concentrations that in mammals at least would be considered high.

In addition, the effects of ethanol on the I_A are not shared by other alcohols such as butanol and hexanol, which induce qualitatively different responses; the longer chain alcohols decrease the peak current, whereas ethanol slows the decay (458, 459). As suggested, it is unlikely that these actions on the I_A can be explained by a simple perturbation of membrane lipids, because these effects should be shared by the higher alcohols. It would also seem unlikely that this action could underlie behavioral effects of ethanol as well, because in behavioral terms the higher alcohols differ in potency but not qualitatively.

2. Hippocampus. In terms of mammalian systems, ethanol has been reported by some groups to hyperpolarize hippocampal neurons (see section III, D) through what is proposed to be an increase in potassium conductance (48, 49). These effects are not blocked by Cl^- injections, which invert Cl^- responses, or when slices are superfused with Ca^{++}-free medium, which should block transmitter-gated conductances. These data suggest that ethanol may open a potassium conductance in these cells; whether this is a voltage or second-messenger-gated channel (see below) is unclear.

3. LC. In the LC, ethanol concentrations between 1 and 60 mM inhibit the spontaneous firing that is observed in vitro (411). It appears that the reduction in the firing rate is a consequence of either an enhancement of the after-hyperpolarizations (AHPs) following each spike or a decrease in the rate of depolarization between spikes. It is unclear which of these actions is primarily responsible, and the situation is complicated by the fact that there may be multiple conductances underlying the AHP (411, 412; S. A. Shefner, personal communication). More recently, it has been shown that ethanol increases the anomalous rectifier (another type of potassium conductance) that is observed when hyperpolarizing current is injected into LC neurons (409). Although a significant correlation was observed between the effects of ethanol on anomalous rectification and the degree of inhibition of spontaneous firing induced by ethanol, the two are probably not related. The effects upon anomalous rectification are observed primarily at higher doses of ethanol (>50 mM) and may be secondary to increases in extracellular K^+ (S. A. Shefner, personal communication).

4. Lymnaea. Another type of response that might be related to ethanol action is the activation of a unique K^+ current in the pond snail by general anesthetics (136). This current is different from the delayed rectifier, I_A, and calcium-activated potassium conductance (Ca-g_K) and is relatively insensitive to tetraethyl ammonium, 4-aminopyridine, and Co^{++}, which block most potassium currents. Moreover, the conductance was only observed in a single identified cell type. In many respects the actions of ethanol resemble those of the general anesthetics, but without direct evidence it is unclear whether this specific conductance would be sensitive to the effect of ethanol. If so, this might indicate that specific populations of neurons could be much more sensitive to the effects of ethanol than others and could help to explain some of the disparities between intoxicating concentrations of ethanol and the concentrations that affect ionic conductances. Along these lines, it is interesting to note that studies in *Aplysia* have also established that different identified neurons differ in their intrinsic sensitivities to ethanol sensitivity (e.g., 457). These observations would argue against ethanol acting only by disordering membrane lipids, because they seem to imply some specificity of action; however, these differences might also reflect differing degrees of sensitivity of various proteins to the membrane lipid milieu.

C. Calcium-dependent Potassium Channels

One area that still remains somewhat controversial concerns the actions of ethanol on the Ca-g_K found in many neurons. Changes in this conductance could be the result either of changes in intracellular calcium that lead to the activation of this conductance or could result directly from ethanol effects upon the ion channel. In *Aplysia*, it has been reported that the Ca-g_K is facilitated by ethanol (398). Because Ca-g_K responses that were evoked directly by injection of Ca^{++} into neurons were also facilitated by ethanol administration, this would argue for a direct effect of ethanol on the K^+ channel or its calcium sensitivity. However, the doses of ethanol used were quite high (880 mM), and although it was not clear that concentrations this high were required to see an effect, these observations are difficult to evaluate without knowing more about their concentration dependence.

Support for an effect of ethanol on the Ca-g_K also comes from biochemical measurements of the calcium-dependent flux of $^{86}Rb^+$ (a tracer for K^+), which has been studied in human erythrocytes and mouse brain synaptosomes (478). Ethanol enhanced the flux in both assays, and the effect was greater with lower concentrations of calcium. At calcium concentrations of 100 to 200 nM, 100 mM ethanol enhanced ^{86}Rb efflux by about 100%; higher concentrations of calcium (700 to 800 nM) abolished the action of ethanol (478).

In the CA1 region of the rat hippocampus, the AHPs that result from the activation of the Ca-g_K have been reported to be enhanced by ethanol. It has been suggested that the hyperpolarizing shifts in the resting membrane potential that are sometimes observed in response to ethanol may reflect increases in intracellular Ca^{++} activating the Ca-g_K (25, 48, 49). In the study by Benson et al. (25), it was found that, when cyclic adenosine 5′-monophosphate (cAMP) was included in the solution filling the pipette, the effects of ethanol were blocked. Because cAMP blocks the AHP, this provides indirect support for the Ca-g_K being a significant site of action for ethanol. However, in the studies from the Carlen group, increases in the AHP were for the most part not

reversible, which is not what one would expect of a cell response related to ethanol intoxication. Moreover, in a later study from this same group, it was found that in dentate granule cells the amplitude of AHPs was reduced in both young and old animals by ethanol, although only the response in the old rats was statistically significant (333). If the effects of ethanol are dependent upon the age of the animal and upon which hippocampal subregion is being studied, this would suggest that these actions of ethanol are unlikely to be direct effects upon the channel but more probably reflect an indirect action (e.g., effects mediated via changes in intracellular calcium).

An indirect action is also suggested by the number of studies in which AHPs in hippocampal neurons were largely unaffected by ethanol. Siggins et al. (416) reported that the AHPs in the CA1 region were unchanged 56% of the time, and the remainder were evenly divided between increases and decreases in this response. In our laboratory we have not found statistically significant effects on the AHP (W. R. Proctor and T. V. Dunwiddie, unpublished results). The resolution of these differences is unclear; however, the variability in these effects would suggest that they might be an indirect action of ethanol dependent upon the state of the cell. Given the difficulties in measuring and modifying intracellular calcium, this is a situation in which patch clamp studies, in which the constituents of the solutions on both sides of the membrane are under experimental control, may be essential in resolving these differences.

D. Chloride Channels

Most studies of ethanol actions on chloride channels have focused on the GABA-activated chloride channel, and these are discussed in section V, A. *Xenopus* oocytes have a chloride channel that is opened by increases in the intracellular concentration of calcium, and it appears that the channel can be activated by ethanol (467). Because these effects were blocked by the intracellular administration of [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA), but not by eliminating the extracellular Ca^{++} and adding EGTA, it would appear that these effects are mediated via the release of Ca^{++} from an intracellular pool. This is consistent with the ability of ethanol to release calcium from isolated brain microsomes (85, 182). Ethanol and other anesthetics also inhibit the function of an an ion exchange protein (band 3) of human erythrocytes (131). Ethanol concentrations of 250 to 2000 mM were required for this action, which was attributed to a direct effect on protein conformation rather than lipid perturbation.

E. Calcium Channels

1. Neuronal calcium. Calcium plays a central regulatory role in all cells and is of particular importance in the nervous system. One key function of calcium is to couple membrane depolarization with neurotransmitter release. The mechanism by which calcium causes release remains to be elucidated, but calcium alters the activity of key neuronal enzymes, including protein kinases, phospholipases, and proteases which are responsible for signal transduction. Calcium also acts as a charge carrier and can be responsible for changes in membrane potential.

2. Dynamics of neuronal calcium. Because the intracellular calcium concentration (Ca_i) is a critical determinant of neuronal function, the influx, storage, and efflux of this ion is regulated by multiple mechanisms. Influx is through both voltage-sensitive and receptor-operated channels. Three types of voltage-sensitive channels (termed N, T, and L) have been characterized (306, 460). The L channels are sensitive to organic calcium antagonist drugs such as dihydropyridines (e.g., nitredipine) but do not appear to be primarily responsible for calcium entry coupled with neurotransmitter release (306), and it is possible that the N-type calcium channels are responsible for neurotransmitter release (195). Studies of receptor-operated calcium channels have identified subtypes of glutamate receptors, namely, NMDA and kainate receptors (discussed below), which are coupled with calcium channels.

In addition to entry from the extracellular space, calcium can be supplied from intracellular stores. Structures resembling endoplasmic reticulum sequester calcium by an adenosine 5′-triphosphate (ATP)-dependent process and release this calcium in response to intracellular messengers such as inositol-tris-phosphate (407). Mitochondria also contain stores of calcium, but it is not clear that uptake or release of these stores plays an important role in regulation of Ca_i (325, 326).

Calcium efflux is carried out rapidly and effectively by a Na^+/Ca^{++} exchanger which couples entry of three Na^+ ions with efflux of one Ca^{++} ion (325, 326). In addition, there is a plasma membrane ATPase(s) which pumps calcium out of cells (301).

3. Effects of ethanol: behavioral/physiological studies. In addition to direct evidence (reviewed below) suggesting that ethanol either affects calcium-permeable ion channels or releases calcium from intracellular storage sites, there is more indirect evidence implicating a role for calcium in ethanol action. For example, in SS but not LS mice, intraventricular injections of calcium increase behavioral sensitivity to ethanol (346). In electrophysiological studies, local application of calcium with ethanol resulted in an increased depression of Purkinje cells, again in the SS but not LS line of mice (346). Local application of magnesium did not affect ethanol sensitivity.

4. Electrophysiology of voltage-dependent calcium channels. a. INVERTEBRATE STUDIES. A number of investigators have reported inhibitory effects of ethanol on neuronal calcium conductances. In the *Aplysia,* one calcium current (I_{Ca}) is markedly reduced by ethanol, with 50 mM ethanol being the approximate threshold (45, 398). The primary effect in this case is on the magnitude of the

current and not on voltage dependency. At least a part of the effect of ethanol appears to be due to an enhanced rate of inactivation of the calcium current in the presence of ethanol, and it is possible that increased intracellular levels of Ca^{++} induced by ethanol could be responsible for this effect. Of the many currents in *Aplysia* that are affected by ethanol, the calcium current appears to be the most sensitive. Somewhat similar results have been reported for the I_{Ca} in the snail, *Helix* (343). The threshold for inhibition by ethanol was approximately 100 mM, and pronounced effects were reported at higher levels (178 mM). As with *Aplysia*, ethanol depressed the peak amplitude of the current but also significantly increased in the rate of decay of the response.

b. STUDIES OF CULTURED NEURONS. These types of actions of ethanol do not appear to be confined to invertebrates. Oakes and Pozos (337) reported that ethanol decreased the duration and increased the threshold for calcium-dependent action potentials in dorsal root ganglion cells in culture (337). Effective concentrations of ethanol ranged from 11 to 110 mM, and concentrations of less than 11 mM might have actually enhanced the calcium spikes. In a more recent study of cultured dorsal root ganglion cells, it was found that increases in temperature increased sensitivity to ethanol. For example, the ED_{50} for ethanol was 148 mM at 30°C, whereas at 43°C the ED_{50} was 44 mM (122). Cooling and warming by themselves did not have significant effects upon the duration of the calcium action potential. Based on these results and thermodynamic considerations, these authors have hypothesized that ethanol is working at a hydrophobic site of action. In the hippocampus in vitro, ethanol (10 to 20 mM) has been reported to increase the threshold for calcium spikes and to decrease their magnitude (49).

5. Neurochemical studies of calcium flux. Neurochemical evidence for an action of ethanol on calcium channels includes the observation that in vitro exposure to ethanol inhibits the uptake of ^{45}Ca by depolarized brain synaptosomes (191). Further studies demonstrated that ethanol inhibits the fast phase of calcium flux which appears to be related to neurotransmitter release (436). An ethanol concentration of 50 mM inhibits uptake by about 20% (436). Tolerance develops to this action of ethanol (257, 191), and other anesthetic drugs also inhibit calcium uptake (185). In addition, ethanol inhibits calcium uptake by cultured PC12 cells (298). However, lines of mice (LS/SS) differing in ethanol sensitivity do not display differences in effects of ethanol on synaptosomal calcium flux (181). Thus, these correlative tests of relevance are partially but not completely consistent with a role for inhibition of calcium flux in ethanol action. Although other alcohols and anesthetics inhibit synaptosomal calcium uptake, potencies of these drugs as uptake inhibitors are not well correlated with their potencies as membrane-disordering agents, which raises the possibility that some of these drugs may have direct actions on the channel protein (185).

E. Release of Intracellular Calcium

The simplest model based on the results presented above would be that ethanol inhibits calcium influx, thereby decreasing Ca_i and reducing neurotransmitter release. However, ethanol does not consistently decrease stimulated release and often increases resting release of neurotransmitters, suggesting that ethanol does not simply decrease Ca_i and may in fact elevate Ca_i under resting conditions.

1. Isolated membranes and cells. Measurement of Ca_i by fluorescent calcium chelators (i.e., Fura-2) showed that ethanol does increase resting Ca_i and inhibits the stimulated increase of Ca_i in brain synaptosomes (82–84, 90) and cultured PC12 cells (374). Relatively large concentrations of ethanol (50 to 500 mM) are required for these actions, which may reflect buffering of Ca_i by Fura-2. Studies of isolated brain microsomes indicate that the increase in Ca_i is due, at least in part, to release of calcium from intracellular stores (182, 408). These stores of calcium are similar to, but not identical with, the pool of calcium released by inositol-tris-phosphate (85). With isolated microsomes, ethanol concentrations of 50 to 100 mM increase calcium release as much or more as maximally effective concentrations of inositol-tris-phosphate (85), suggesting that these actions are important at concentrations of ethanol achieved in vivo. An interesting question is whether ethanol inhibits voltage-dependent calcium channels by elevating Ca_i or by direct actions on the channels or surrounding lipids. Ca_i is known to regulate some calcium channels, but this does not appear to be the mechanism of action of ethanol on calcium channels of *Aplysia* (45). Regardless of the mechanism in the mammalian CNS, it is likely that the complex actions of ethanol on neurotransmitter release are related to the opposing inhibitory effects on calcium influx and stimulatory action on Ca_i.

2. Xenopus oocytes. Studies from our laboratory have suggested that intracellular injection or perfusion with ethanol results in the activation of a calcium-dependent chloride conductance in *Xenopus* oocytes (467). Injections of ethanol directly into the oocyte that were calculated to give final concentrations of 10 to 300 mM ethanol resulted in the activation of the chloride conductance. These effects were blocked by the intracellular administration of EGTA but not by eliminating the extracellular calcium and adding EGTA, supporting the hypothesis that these effects are mediated via the release of calcium from an intracellular pool.

3. Hippocampus. Carlen and coworkers (48, 49) have come to similar conclusions but based upon very different kinds of evidence. In the hippocampus, ethanol appears to reduce calcium currents but to facilitate the calcium-dependent potassium conductance ($Ca\text{-}g_K$); taken together, these results suggest that ethanol can increase

intracellular calcium in some other way than by activating a calcium-permeable ion channel. A release of calcium from intracellular storage sites would explain both effects, because intracellular calcium can reduce calcium currents but should also increase Ca-g_K.

4. Sodium/calcium exchange, ATP-dependent calcium transport, and calcium binding. The effect of ethanol on sodium/calcium exchange of isolated synaptic membranes is mainly inhibitory, with a decrease of 30 to 35% found with ethanol concentrations of 25 to 300 mM ethanol (301). This may contribute to the increase in synaptosomal Ca_i produced by ethanol but cannot be completely responsible because Ca_i increases linearly with these ethanol concentrations (82).

Ethanol has been shown to weakly inhibit the ATP-dependent transport of calcium by microsomal membranes (about 20% inhibition at 400 mM) (180) and, conversely, to stimulate calcium-ATPase activity of synaptic membranes and erythrocytes and to increase ATP-dependent calcium transport by erythrocytes (30–50% stimulation at 50 to 100 mM) (479). An observation that may reconcile these contradictory findings is that synaptic membranes contain several types of calcium-ATPase or calcium, magnesium-ATPase, and small differences in assay conditions likely can alter drug effects (301, 427). It is difficult to predict what effect the observed changes in ATP-dependent calcium transport would have on Ca_i.

Binding of calcium to synaptic membranes is increased by ethanol (302), and this appears to occur on the cytoplasmic surface with 10 to 25 mM ethanol producing a 30 to 40% increase (187).

5. Summary. A remarkably consistent picture of effects of ethanol on neuronal calcium homeostasis has emerged during the past decade. Concentrations of ethanol achieved during intoxication and anesthesia inhibit voltage-sensitive calcium channels and increase Ca_i, but the changes are often small unless toxic concentrations of ethanol are used. These results raise the perplexing question of what these two opposing changes in calcium have to do with ethanol actions. There is little evidence that either of these actions is important in genetic differences in alcohol sensitivity (181). In addition, pharmacological agents that selectively block calcium channels such as dihydropyridines and ω-conotoxins do not mimic the behavioral actions of ethanol (103, 339), although they do enhance ethanol actions (104, 105). These genetic and behavioral observations do not support a key role for inhibition of calcium channels in ethanol action, but they also do not disprove involvement of these channels. The importance of increased Ca_i in ethanol action is even more difficult to assess because there are no pharmacological agents that are known to mimic actions of ethanol on this process. Thus, we end with the familiar conclusion that more information is needed about the basic neurobiology of calcium channels and about the actions of ethanol on these processes before reaching a definitive conclusion about the importance of neuronal calcium in ethanol action.

V. Interactions of Ethanol with Neurotransmitters and Neuromodulators

A. GABA

1. Background. Two subtypes of GABA receptors have been characterized to date. This review will focus on GABA-A receptors which are coupled to chloride channels and are implicated in alcohol and sedative action. GABA-B receptors are coupled to potassium channels by a guanine nucleotide-binding protein, but little is known about their role (if any) in ethanol action.

The techniques of molecular biology have been applied successfully to the GABA-A receptor-chloride channel complex leading to an explosion of knowledge about this receptor system. A detailed discussion of the neurochemistry of this system is beyond the scope of this review but is the topic of several monographs (27, 340).

This chloride channel complex contains receptors for at least four types of drugs: (*a*) GABA agonists (GABA; muscimol; 4,5,6,7-tetrahydroisoxazolo-[urodome-3-ol]; isoguvacine) and antagonists (bicuculline), (*b*) benzodiazepines, (*c*) convulsants (picrotoxin, t-butylbicyclophosphorothionate, TBPS), and (*d*) barbiturates. GABA agonists activate the chloride channel, and this action is allosterically enhanced by benzodiazepine agonists (e.g., flunitrazepam) or barbiturates and allosterically inhibited by benzodiazepine inverse agonists (such as Ro 15–4513 ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4-imidazo[1,5α]·[1,4]benzodiazepine-3-carboxylate, and FG7142 *n*-methyl-β-carboline-3-carboxyamide) and convulsants (e.g., TBPS, picrotoxin) (27, 340).

Molecular cloning initially identified three distinct proteins (subunits) of the channel complex which were named α, β, and γ (362). There are multiple α subunits (three have been cloned and sequenced to date), but sequence information has been reported for only one β subunit at this time (258). However, a recent abstract reported the cloning of a total of 16 subunits for the complex (six α, four β, two γ, one δ, and one ϵ) (360). It has been suggested that the channel complex is an α_2, β_2 tetramer and that the α subunit contains receptors for benzodiazepines, and the β subunit contains GABA receptors (278, 390). However, this model may be incorrect. Recent studies show that both subunits contain the receptors for GABA and benzodiazepines, (44). Expression of cloned subunits (α, β, or both) in *Xenopus* oocytes results in responses to GABA, pentobarbital, and picrotoxin, but benzodiazepines produce little (395) or no (258) response. However, expression of brain polyA$^+$ RNA (mRNA) results in robust benzodiazepine responses in *Xenopus* oocytes (207, 414), suggesting that additional receptor subunits, modulators, or posttranslational modifications are required for benzodiazepine responses. Present data indicate that γ subunits are

required for benzodiazepine sensitivity (362). The various subunits are not evenly distributed throughout brain (406, 413) which may be responsible for the observation that not all of the GABA-activated chloride channels are sensitive to benzodiazepines (440). The techniques of molecular biology should continue to prove most helpful in understanding actions of ethanol on GABA-activated chloride channels.

2. Behavioral studies. Augmentation of GABA action was established as an important action of barbiturates and benzodiazepines in the early 1970s leading researchers to ask whether GABA might also be important in ethanol action. The first approaches were behavioral; these were reviewed recently (7, 212) and will not be discussed in detail here. These studies may be summarized by saying that GABA-mimetic drugs generally increase ethanol actions such as ataxia, anesthesia, and punished responding, and GABA antagonist drugs reduce these actions of ethanol. An exception to this generalization is the increase in motor activity produced by low doses of ethanol: this action is abolished by GABA-mimetic treatments (7). More recently, interest has shifted to behavioral interactions between ethanol and benzodiazepine inverse agonists—drugs that act at benzodiazepine receptors but inhibit GABA-activated chloride channels (i.e., the opposite actions of benzodiazepine agonists). Ro 15–4513 is a weak (partial) inverse agonist and reduces some aspects of ethanol intoxication (441–443) but does not alter the excitatory effects of ethanol on motor activity (24). It is clear that Ro 15–4513 acts through a benzodiazepine receptor (443), but it is controversial whether other benzodiazepine inverse agonists also antagonize behavioral actions of ethanol (24, 263) or whether Ro 15–4513 acts by a mechanism that does not involve inverse agonism (441–443). Other behavioral evidence linking ethanol and benzodiazepine action is the observations that mice (LS/SS) and rats (AT/ANT) selected for differences in ethanol sensitivity display differences in benzodiazepine sensitivity (194, 282, 284) and mice selected for differences in diazepam sensitivity display differences in ethanol actions (145). Taken together, the behavioral studies provide consistent, albeit indirect, evidence that ethanol intoxication involves activation of GABA-A mechanisms.

3. GABA-activated chloride channels $^{36}Cl^-$ flux. Studies of uptake of $^{36}Cl^-$ by isolated brain membrane vesicles and cultured spinal cord cells show that in vitro exposure to reasonable concentrations of ethanol (5 to 50 mM) augments the GABA-activated chloride flux (4, 6, 194, 441, 442, 455) and slightly higher concentrations increase the resting (basal) flux in some studies (e.g., 294, 441) but not in others (e.g., 5, 6). These effects are blocked by picrotoxin and bicuculline, indicating that the chloride flux is through channels coupled to GABA-A receptors. Longer chain alcohols (443) and other anesthetic agents produce similar effects (210, 311), and their potencies in altering chloride flux are closely related to potencies for producing intoxication and anesthesia. Acute or chronic administration of ethanol abolishes the ability of ethanol to enhance muscimol-stimulated chloride flux (7, 314), but chronic treatment does not alter the direct stimulation of chloride uptake produced by ethanol (314).

It is not clear why ethanol increases basal chloride flux for some groups and not for others. An analogous situation exists for barbiturates for which some studies show a large action of barbiturates on chloride flux in the absence of GABA (399), but others show little or no effect without addition of exogenous GABA (5, 6, 55). The GABA-activated chloride flux is quite complex with two distinct GABA receptors coupled to chloride channels and several rapid desensitization processes (55). A clear understanding of drug action on this system requires testing of several concentrations of GABA and other drugs at uptake times ranging from 50 ms to several seconds. The only drug tested in this detail is pentobarbital, and the results suggest that pentobarbital produces only small effects in the absence of GABA (55). As suggested by these authors, some membrane preparations may contain levels of endogenous GABA that are not sufficient to activate the channel directly but are large enough to alter flux in conjunction with pentobarbital. This is also the simplest explanation for the effects of ethanol in the absence of exogenous GABA, but there is no direct evidence supporting this idea. We should also note that even the potentiation of GABA-activated chloride flux by ethanol is sensitive to assay conditions and at least one group has not been able to observe this action of ethanol (312).

Because Ro 15–4513 antagonizes some behavioral actions of ethanol, interactions between benzodiazepine inverse agonists and ethanol were studied on $^{36}Cl^-$ flux. It was initially reported that Ro 15–4513, but not other inverse agonists, was able to block the action of ethanol on chloride uptake (442). Other studies confirmed the action of Ro 15–4513 but also found that another benzodiazepine inverse agonist, FG7142, antagonizes actions of ethanol on chloride flux (182, 294). Ro 15–4513 and FG7142 reduce ethanol actions at concentrations that do not inhibit GABA-activated chloride flux when tested alone, i.e., they do not have inverse agonist actions at these concentrations. This suggests that antagonism of ethanol does not involve inverse agonism. However, this interpretation may be flawed because there is evidence that exposure of membranes (or animals) to ethanol increases inverse agonist actions (182). Thus, in the presence of ethanol the drugs may exert effects (inhibition of chloride channel function) that are not seen in the absence of ethanol.

Enhancement of GABA-activated chloride flux by ethanol is implicated in genetic differences in ethanol sensitivity. Lines of selectively bred rodents displaying differences in anesthesia or ataxia such as LS/SS mice,

HS mice (HS-LS/HS-SS), diazepam-sensitive and -resistant mice (DS/DR), and high alcohol sensitive- and low alcohol-sensitive rats all show corresponding differences in ethanol action with the ethanol-insensitive lines being resistant to ethanol enhancement of GABA-activated chloride flux (8, 181). The correlation also extends to pentobarbital actions because LS/SS or DS/DR lines are equally sensitive to pentobarbital in vivo and this drug produces equal enhancement of GABA-activated chloride flux in vitro with brain membranes from these lines (4,5,8). The DS/DR and LS/SS lines are differentially sensitive to benzodiazepines in vivo and flunitrazepam is more potent in enhancing GABA-activated chloride flux in the DS and LS lines as compared to the DR and SS lines (4, 181). These differences cannot be attributed to changes in receptor binding but appear to be due to genetic differences in the coupling of benzodiazepine receptor binding with channel opening (181).

4. Electrophysiology: facilitation of GABA responses. A number of studies have found that ethanol can facilitate responses at GABAergic synapses or responses to exogenous GABA. In one of the earlier studies along these lines, Davidoff (89) observed that ethanol (33 to 100 mM) potentiated primary afferent depolarization as well as the dorsal root potentials elicited directly by GABA in the frog spinal cord. Primary afferent depolarization reflects the depolarizing effect of GABA on the presynaptic nerve terminals in the dorsal root, so facilitation of this response would reflect a potentiating effect of ethanol. One of the more unusual aspects of this response was the relatively slow time course (ethanol effects became apparent after about 10 min of perfusion and were maximal after 30 min), and the fact that these responses were not reversible.

In a more recent positive report concerning ethanol potentiation of GABA, the effects of ethanol on chloride currents in chick spinal cord neurons in culture were investigated (60). Several aspects of this study bear mention. First, responses to both GABA and glycine were potentiated but required the repeated administration of ethanol to become evident; a single application of 50 mM ethanol did not enhance GABA responses, but when the same amount of ethanol was given in three brief applications, GABA responses were facilitated. Furthermore, as in the Davidoff study, the responses did not appear to be reversible with washing. Furthermore, facilitation was seen only with responses to 3 μM and not 30 μM GABA. About 60% of the neurons were responsive to ethanol, and other alcohols as well as dimethyl sulfoxide produced similar responses.

Another very recent report characterized effects of ethanol in dorsal root ganglion neurons in culture (336). At concentrations of ethanol between 3 and 100 mM there was an enhancement of the transient but not the sustained component of the response to bath-applied GABA. In another study in crayfish opener muscles using the technique of noise analysis, responses to GABA were potentiated by ethanol (260 mM), but there did not appear to be any significant effect of ethanol either on the magnitude of the elementary currents (corresponding to single-channel conductance) or on the duration of opening (127). Expression of mouse brain mRNA in *Xenopus* oocytes results in GABA-activated chloride channels and this action of GABA is augmented by ethanol (10 to 50 mM) (468). Furthermore, mRNA from LS mice produced channels that showed ethanol facilitation, whereas those expressed from SS mRNA did not (468).

In intact mammalian systems, there are relatively few reports of ethanol facilitation of GABAergic responses. Nestoros (331, 332) demonstrated that the systemic (threshold 0.2 to 8 mg/kg, i.v.) or local application of ethanol from a micropipette containing 300 mM ethanol in NaCl could facilitate GABAergic responses in cat cortex. Ethanol increased the inhibition elicited by local stimulation as well as responses to locally applied GABA but not responses to serotonin, dopamine, or glycine (60). What is striking about this report, however, is that the concentrations of ethanol that were found to be effective were in the range of 10 to 70 μM ethanol. These concentrations are so low as to suggest the possibility that they might be artifactual in origin rather than being responses to ethanol per se. To date, although no one has reported replication of these findings, there do not appear to have been direct efforts made to repeat these experiments.

In addition to these studies that have characterized the direct effects of ethanol either on synaptic responses or on responses to GABA, there are a number of studies that have used more indirect methods to establish a GABAergic link in ethanol action. In one such study, it was found that ethanol (0.5 to 2.5 g/kg, i.v.) inhibited the spontaneous activity of substantia nigra pars reticulata neurons in a paralyzed rat preparation (297). Picrotoxin and bicuculline, which antagonize the effects of GABA, both antagonized this response. However, these drugs by themselves can usually increase activity, so this cannot be taken as strong evidence for a GABAergic link. However, directly acting GABA-A agonists and benzodiazepine agonists also potentiated the effects of ethanol. Benzodiazepine antagonists blocked the potentiating effects of benzodiazepine agonists but did not affect the depressant effects of ethanol per se. Taken together, these results suggest that ethanol can potentiate GABAergic effects but that it does not do so via a benzodiazepine-like action on the receptor-channel complex.

Further indirect evidence for a GABAergic link in the effects of ethanol come from a recent study on Purkinje neurons (350). In this study, it was observed that two benzodiazepine inverse agonists, Ro 15–4513 and FG 7142, markedly reduced the depressant effects of ethanol in the cerebellum. The antagonism in many cells was not

complete, but the interaction was also noncompetitive, in the sense that higher concentrations of ethanol were not able to overcome the effects of the inverse agonists. However, the inverse agonists did not affect responses to GABA alone. In addition, benzodiazepine antagonists do not appear to be able to block the actions of the inverse agonists in this system (M. R. Palmer, unpublished results). Although GABA clearly seems to have a role in these types of responses, the nature of this interaction is unclear. Finally, in a study in the rat hippocampus, i.p. injections of ethanol increased the inhibition observed with paired stimuli to the dentate gyrus (473). As discussed in a previous section (C, 1, a), the interpretation of these results is unclear; although they are consistent with what would be expected if ethanol facilitated GABAergic inhibition, alternative explanations are possible.

Despite the considerable evidence in support of an ethanol modulation of GABA sensitivity, there are a number of reports that ethanol has no effect on such responses. In some of these cases, the effects of ethanol were characterized on responses to exogenous GABA, which leaves open the possibility that ethanol might act presynaptically to modify GABAergic IPSPs, but there are also studies in which this is not the case.

Many of the reports indicating that ethanol has no effect on GABAergic responses come from the hippocampus. When single-unit firing rates were ised following GABA application as a measure of action, ethanol did not appear to significantly modify sensitivity of hippocampal neurons in rats (279). In studies in which intracellular recording was used to monitor the GABA-induced hyperpolarizations and changes in input impedance, again there did not appear to be any consistent change in responses to GABA in several studies from different laboratories (48, 49, 415, 416; W. R. Proctor and T. V. Dunwiddie, unpublished results). Finally, in a voltage clamp study in which the actions of a number of putative modulators of GABA function were characterized, ethanol was found to have no effect upon GABA inhibitory postsynaptic currents (144). Barbiturates and halothane both increased the duration of spontaneous IPSPs in CA1 neurons, but ethanol (10 to 200 mM) did not significantly modify these currents.

Attempts to potentiate GABAergic responses with ethanol have been unsuccessful in other brain regions as well. For example, ethanol either does not modify GABA responses in spinal cord neurons or slightly facilitates them (20 to 80 mM ethanol; 172). In the LC in vitro, responses to locally or bath applied GABA were also unaffected by ethanol (S. A. Shefner, personal communication).

5. Electrophysiology: antagonism of GABA responses. Finally, in a number of cases ethanol has been reported to have antagonistic effects upon GABAergic IPSPs or responses to exogenous GABA. For example, in the hippocampus, Siggins et al. (415, 416) found that ethanol (10 to 50 mM) virtually abolished the IPSPs in some neurons, although in some cases this was not a specific effect upon GABAergic circuits but appeared to be secondary to changes in the input resistance of the pyramidal cells. In another hippocampal study the effects of ethanol on responses to bath superfusion with GABA (100 to 1000 μM) were studied. GABA applied in this manner will reduce the EPSP. The dose-response curve for GABA was unaffected by perfusion with 35 mM ethanol, a concentration that blocks the ability of thiopental to shift the GABA dose-response curve (450).

In the cerebellum of urethane-anesthetized rats, local and systemic (1.5 g/kg, i.v.) applications of ethanol were able to significantly reduce the duration of the putative GABAergic inhibition of Purkinje cell firing following local stimulation of the cortical surface (177). Systemic ethanol also reduced the responses of Purkinje cells to local application of GABA; locally applied ethanol reduced the absolute but not relative inhibition induced by GABA in the study by Harris and Sinclair (177) but has been found to significantly reduce GABAergic responses in studies by other investigators (M. Palmer, unpublished results).

In the crayfish, there were no readily apparent effects of ethanol on GABAergic responses at concentrations of ethanol below 100 mM, and at higher concentrations, depression of the responses was observed (274). Finally, in a study looking at cortical evoked potentials in rats, it was reported that the surface negative wave that corresponds to recurrent inhibition was slightly facilitated by blood levels below 25 mM but was blocked by levels above this (391).

6. Receptor binding. Binding of ligands to the GABA receptor-channel system is complex and effects of ethanol on this binding are confusing at best. The three binding sites most frequently studied are GABA-A receptors, which can display three different affinity states; benzodiazepine receptors, which may have two different subtypes; and convulsant sites, which are commonly studied with [^{35}S]TBPS. In addition, there are barbiturate receptors for which there is no satisfactory radioligand but may be evaluated by their allosteric effects on other ligands and avermectin sites which are not commonly studied (340). All of these sites are allosterically coupled so one can study the inhibition of [^{35}S]TBPS binding by GABA agonists or barbiturates or the enhancement of benzodiazepine binding by these drugs. Adding ethanol to this binding cocktail has generally resulted in small and inconsistent changes. This literature is reviewed elsewhere (7, 212) and our discussion will focus on more recent studies. The initial rationale of these studies was that drugs that facilitate GABAergic transmission, such as barbiturates, enhance binding of benzodiazepine and GABA agonists and ethanol might be expected to produce similar actions (453, 454). In

support of this hypothesis, ethanol (10 to 100 mM) increases binding of benzodiazepines to receptors solubilized with Lubrol (453), and in vivo injection of ethanol (0.5 to 2 g/kg, i.p.) increases the in vivo uptake (binding) of the benzodiazepine antagonist [^{3}H]Ro 15–1788 into mouse brain (304). However, ethanol failed to increase flunitrazepam binding to 3-[(3-cholamedopropyl)dimethylamino]-1-propanesulfonate-solubilized membranes (299). In vivo treatment with ethanol has been reported to both increase (453) and decrease (402) the number of low-affinity [^{3}H]GABA sites in brain. In vitro addition of ethanol to brain membranes does not alter binding of benzodiazepine ligands (agonist, inverse agonist, or antagonist) or GABA agonist binding (169). It is not clear which experimental variables are critical for detecting actions of ethanol on GABA or benzodiazepine receptors.

A consistent effect of ethanol on isolated brain membranes is inhibition of binding of [^{35}S]TBPS to convulsants sites. Ethanol decreases the affinity of the TBPS site because of an increase in ligand dissociation rate (260, 277). Other alcohols and anesthetic drugs also inhibit TBPS binding at concentrations that produce loss of consciousness in vivo (210). For ethanol, detectable inhibition occurs at 50 to 100 mM, and the IC_{50} is about 300 mM; thus, this measure is less sensitive to ethanol than are the changes in chloride flux and the augmentation of benzodiazepine binding. In vitro, ethanol does not alter the ability of GABA or pentobarbital to inhibit TBPS binding (259, 260), and chronic treatment of rats or mice with ethanol also fails to alter [^{35}S]TBPS or [^{3}H]flunitrazepam binding (259, 375).

Because of the clear genetic differences at the behavioral and chloride flux levels (reviewed above), several investigators have looked for corresponding genetic differences at the receptor level (181). One interesting finding is that the benzodiazepine receptor of LS mice is more sensitive to heat inactivation than the receptor of SS mice (284, 292), suggesting a genetic difference in one or more of the protein subunits of the receptor. The LS and SS mice display identical density and affinity of the benzodiazepine receptors, but the SS mice show a greater enhancement of benzodiazepine binding by GABA than the LS mice when assayed at 37°C but not when measured at 4°C (285, 292). Treatment of mice with ethanol increases benzodiazepine ([^{3}H]Ro 15–1788) binding measured in vivo and this effect is greater in SS than LS mice (304). Muscimol (a GABA agonist) is more potent in inhibiting TBPS binding in LS than SS mice, although ethanol inhibition of TBPS binding and high-affinity binding of [^{3}H]muscimol does not differ between the lines (6). An illustration of the potential pitfalls of comparing only two selected lines is the finding that the LS and SS lines differ in the number of TBPS sites in midbrain regions. Study of recombinant inbred strains derived from the LS and SS lines does not show any relationship between ethanol sensitivity (sleep time) and TBPS binding. However, the LS/SS lines differ in traits other than ethanol sensitivity, e.g., seizure sensitivity, and there is a relationship between seizure sensitivity and TBPS binding in the recombinant inbred strains (354). Thus, it is not likely that the differences in density of TBPS sites are related to ethanol sensitivity, and we cannot be sure that the other receptor differences are linked to ethanol sensitivity. More definitive conclusions require study of recombinant inbred strains or other selected lines in addition to the LS/SS lines (as has been done for chloride flux) (181). In another example of multiple genetic comparisons, receptor binding has been studied with rat lines (AT/ANT) selected for differences in ethanol ataxia and in mouse lines (DS/DR) selected for differences in diazepam ataxia and also differing in ethanol sensitivity. The AT/ANT lines do not differ markedly in the binding of ligands to GABA, benzodiazepine or TBPS sites in intact membranes from different brain regions or to 3-[(3-cholamedopropyl)dimethylamino]-1-propanesulfonate-solubilized receptors (275). The GABA stimulation of [^{3}H]flunitrazepam binding is slightly (10%) greater in AT than ANT rats, which is consistent with the differences between LS and SS lines (285). The DS/DR lines did not differ in muscimol enhancement of [^{3}H]flunitrazepam binding or in the density or affinity of benzodiazepine or TBPS sites (4). The binding data for LS/SS, AT/ANT, and DS/DR lines are not necessarily comparable because of the use of different membrane preparations, assay temperatures, buffers, etc., but they do not show that the density or affinity of any receptor of the GABA-benzodiazepine complex is clearly related to genetic differences in ethanol sensitivity.

7. GABA release and reuptake. Effects of ethanol on GABA synthesis, release, and reuptake are the subject of a number of earlier studies which yielded conflicting and inconclusive results; these are reviewed elsewhere (211, 247), and we will consider only more recent studies in this section. Ethanol (150 to 500 mM) inhibits potassium-stimulated release of GABA from cortical brain slices (208, 438) and synaptosomes (208), but a lower concentration (60 mM) is not effective (323). The effect of large concentrations is greater in LS than SS mice (208). Acute injection of ethanol does not change GABA content or GABA accumulation in rat brain (140) but does enhance glutamic decarboxylase activity in the cerebellum and inhibit this enzyme in the hypothalamus (401). In vitro, ethanol (up to 400 mM) does not alter synaptosomal high-affinity GABA uptake (318). Taken together, these observations suggest that nontoxic concentrations of ethanol have little effect on presynaptic regulation of GABAergic transmission.

8. Summary. The hypothesis that ethanol potentiates GABAergic transmission is attractive and is consistent with many behavioral and neurochemical results. How-

ever, there are many electrophysiological observations that do not support this hypothesis. Attempting to resolve the apparent differences between the effects of ethanol on GABAergic responses in various preparations and laboratories may be somewhat premature at this point, in part because we simply do not know enough about these differences to determine which experimental variables are important and which are unimportant. However, several hypotheses can be proposed that might prove important in integrating all these findings. First, several studies indicate that only some components of the GABA response are affected. For example, in the study by Nishio and Narahashi (336), only the transient component of the response appeared to be affected. This would suggest that with certain methods of drug application (e.g., bath superfusion) differences would not be observed because the transient component could not be readily detected.

Another difference might relate to the recent findings that there are multiple forms of the α subunit for the GABA receptor (360–362), which may underlie heterogeneity within the GABA-A receptor subtype. If this is the case, then brain regions in which GABA modulation by ethanol is typically not observed, such as the hippocampus, may have a variant of the receptor that is not sensitive to the effects of ethanol, whereas a receptor subtype that is affected would be expected in regions such as the cortex and possibly cerebellum, when some modulation is observed. This type of heterogeneity may exist even among cells from the same brain area. For example, using cultured spinal cord neurons, Study and Barker (439) found that, although all of the cells tested responded to GABA, the GABA response was potentiated by diazepam in 82% of the cells, by pentobarbital in 85% of the cells, and by both drugs in only 53% of the cells. It is quite possible that a similar cellular diversity exists for ethanol-GABA interactions.

Studies of animals selected for sensitivity to ethanol or benzodiazepines provide more consistent evidence that differences in GABA-activated chloride channels are at least partially responsible for genetic differences in sensitivity to ethanol and benzodiazepines. Studies using behavior, chloride flux, and receptor binding all indicate correlated genetic differences in the GABA-receptor complex, but electrophysiological studies are lacking. The availability of selected lines combined with recently developed molecular biological techniques should prove quite useful in understanding actions of ethanol on GABA-activated chloride channels.

B. Glutamate

There is some evidence that ethanol can suppress the excitatory effects of glutamate that are observed in a variety of brain regions. Glutamate receptors are now commonly divided into three subtypes (kainate, quisqualate, and NMDA) depending upon agonist and antagonist specificity (293), although further subdivision might become necessary as more selective drugs are developed. It appears in some cases that the effects of ethanol are directed at certain subtypes rather than having a more general action on all glutamate receptors.

1. Behavioral studies. The ability of ethanol to modify the effects of drugs that act at glutamate receptors has not been intensively studied at the behavioral level, but there are suggestions that glutamate receptors may play a significant role. Two points should be raised in this regard. First, some drugs that can block NMDA receptors (e.g., phencyclidine or ketamine) elicit behavioral responses which in some respects are similar to ethanol intoxication. Second, there are behavioral studies in mice that indicate that sleep time can be regulated by agonists and antagonists at the NMDA receptor (474). Basically, NMDA agonists reduce sleep time, whereas antagonists increase it; these data are consistent with the hypothesis that ethanol acts as an antagonist at NMDA receptors, although a variety of other explanations are possible.

2. Electrophysiological and biochemical studies. a. NMDA RECEPTORS. In addition to the behavioral data, there is also electrophysiological and biochemical evidence that indicates that ethanol has an antagonistic action on the NMDA receptor subtype. In the earliest such report, Teichberg et al. (452) demonstrated that ethanol, other alcohols, and barbiturates could inhibit NMDA-stimulated sodium efflux from striatal slices. At a concentration of 100 mM (the only concentration tested) ethanol inhibited flux produced by glutamate, kainate, quisqualate, and NMDA by 20 to 30%, but the action of aspartate was not altered by ethanol (452). However, in a similar study in cortical slices ethanol (100 mM) did not block NMDA-mediated Ca^{++} flux (385).

In an electrophysiological study in cultured Purkinje cells, it was observed that the primary response to locally applied glutamate consists of an increase in firing rate which then changes to a period of bursting activity, followed by slower firing. Following treatment with 22 to 44 mM ethanol, the bursting activity is almost completely suppressed, while the increases in single-spike activity are still evident (133). Although the nature of the receptor mediating the bursting activity is unknown, the NMDA receptor, which allows calcium as well as sodium into the cell, seems a likely candidate. Support for this conclusion also comes from a recent biochemical study in which it was suggested that ethanol can inhibit the NMDA-stimulated uptake of calcium by cultured cerebellar cells, with an EC_{50} of 10 to 25 mM (367).

Concerning the hippocampus, there are several reports indicating that ethanol can block LTP at concentrations that do not have significant effects on synaptic transmission per se (319, 417, 481; see section III, C, 3, a). Because the NMDA receptor appears to play a pivotal role in LTP, but not in normal transmission at these synapses, this would point to a specific action for ethanol on NMDA-type channels. More recent electrophysiolog-

ical studies in cultured hippocampal (264) neurons and parallel studies in cultured cerebellar granule cells (367) demonstrate even more directly that ethanol can interfere with the events that are activated by NMDA receptor agonists (264, 367).

b. NON-NMDA RECEPTORS. In terms of some of the other glutamate receptor subtypes, studies using noise analysis at the crayfish neuromuscular junction suggest that ethanol (86 mM) can reduce the single-channel current generated by quisqualate, probably due to a reduction in the open channel conductance (127). With the recent rapid expansion in the use of patch clamp recording techniques, there will probably be more direct evidence concerning the effects of ethanol upon individual conductances activated by glutamate in a relatively short time. Both biochemical and electrophysiological studies indicate that quisqualate- and kainate-activated ion fluxes can be reduced by ethanol (264, 367, 452). In two reports (264, 367), the kainate-activated flux was less sensitive to the disruptive effects of ethanol than the NMDA receptor-mediated effect. In addition to the effects of ethanol on excitatory amino acid-induced ion fluxes, its actions on glutamate binding have also been characterized. In one such study, it was observed that low concentrations of ethanol (<50 mM) enhance binding of glutamate to synaptic membranes, and larger concentrations inhibit binding (300). How this relates to the flux studies is unclear but suggests that ethanol may affect several components of glutamatergic systems.

Finally, as it seems with almost every other transmitter, there are a number of reports that suggest that ethanol has little or no depressant effect on responses to glutamate. In chick spinal cord neurons in culture, responses to local application of glutamate were unaffected by ethanol, although responses to GABA were enhanced (60). In cultured mouse spinal cord neurons, ethanol (20 to 80 mM) was reported to have had either no effect or to slightly depress responses to glutamate (172). In rat hippocampal slices, it has also been reported that excitatory responses to glutamate were unaffected by ethanol (47).

3. Summary. Taken together, these findings indicate that relatively low concentrations of ethanol can modify some of the actions of glutamate on at least some of its receptors. It is difficult to put the negative reports into context without knowing more about some of the responses; in some cases, negative findings have been on unidentified receptor subtypes and may involve different brain regions or experimental methods. It seems quite likely that some of these might be the result of glutamate action on glutamate receptor subtype(s) that are relatively less sensitive to ethanol (e.g., the kainate receptor; 367) rather than on the NMDA subtype that appears to be relatively sensitive to ethanol.

C. ACh

1. Behavior. Erickson and Burnam (116) observed that administration of physostigmine shortened the sleep time in mice following a dose of 4.5 g/kg. However, this same laboratory later reported that neither intracerebroventricular (i.c.v.) ACh nor hemicholinium or parenteral atropine had any effect on ethanol-induced behavioral depression as assessed by a discriminated lever-press avoidance paradigm (168). More recently, however, Erwin et al. (119) found that i.c.v. administered carbachol or oxotremorine would increase the sensitivity of the SS mice to ethanol as measured by the blood ethanol level at the time of loss of righting reflex. This effect could be blocked by pirenzepine, a type 1 muscarinic receptor-selective antagonist, or by atropine, a nonspecific antagonist. Acetylcholinesterase inhibition by neostigmine also increased the sensitivity of SS but not LS mice, whereas Erickson and Burnham (116) found that peripherally administered physostigmine decreased sleep time in mice. There was some selectivity in the behavior as illustrated by the finding that the hypothermic effect of the three agonists is the same in both lines of mice when the compounds are given i.c.v. Administration of atropine or pirenzepine alone had no effect of ethanol actions, arguing against an effect of ethanol on ACh release. Atropine would have been expected to block M2 receptors which are thought to regulate ACh release through inhibition of adenylate cyclase (175, 176).

Pohorecky et al. (359) studied the activating effect of ethanol in rats by giving a dose of 2 g/kg. They found that choline potentiated the hypermotility produced as did physostigmine. However, they found that scopolamine antagonized the effects of ethanol.

Clement (76) studied neuromuscular junction effects of ethanol. At a concentration of 290 mM, ethanol potentiated ACh, carbachol, and choline. On the other hand, Reed (379) found that doses of 1.0 to 4.2 g/kg of ethanol decreased neuromuscular transmission in vivo. (See also section III, C, 2.)

2. Synthesis, storage, and release. Most investigators have found that ethanol at doses ranging from 1.25 to 6 g/kg causes an increased content of ACh in brain (40, 213, 242). There is less agreement on the effect of ethanol on choline acetyltransferase; however, Durkin et al. (111), Reisberg (380), and Soliman et al. (426) all found an increase of enzyme activity either in vivo or in vitro. Kalant et al. (230, 231) found no effect on the mitochondrial enzyme at 0.22 M ethanol. Durkin et al. (111) found that ethanol decreased sodium-dependent choline uptake in striatum of C57 but not in BALB mice. There was depression in the hippocampus in both strains and no effect on the sodium-independent uptake. Because the synthesis of ACh is limited by choline availability, it would seem that the level of the enzyme is a moot point if the supply of choline is reduced by ethanol. The complicating factor in these studies is the apparent genetic and brain area difference that was uncovered. There is reasonably good agreement that ethanol will cause a decrease in release of ACh either in vitro from slices (51,

230, 231) or in vivo as measured either by cortical cups or push-pull perfusion (115, 313). One note of disagreement is found in the paper by Bruno et al. (41) for the peripheral nervous system. They found that MEPP frequency went up when ethanol (0.2 to 0.8 M) was added to a nerve-muscle preparation.

3. Receptors. There are reported effects of ethanol on ACh receptors, but these are at unusually high levels of ethanol or other alcohols. Boyd and Cohen (33) as well as El-Fakahany et al. (112) studied the *Torpedo* AChR. Boyd and Cohen found that propanol above 1% would convert all the receptors to the high-affinity form. El-Fakahany et al. found that 0.1 to 1 M ethanol increased the binding of phencyclidine, imipramine, and perhydrohistrionicotoxin to the receptor when carbachol was absent but decreased the binding when carbachol was present. Waelbroeck et al. (469) studied rat heart muscarinic receptors and found that levels of ethanol from 0.1 to 1 M would decrease the binding of oxotremorine. Fairhurst and Liston (123) studied the binding of quinuclidinyl benzilate to brain AChRs and found that ethanol had an IC_{50} of 2 M to decrease the binding. It is clear that the effects of acute ethanol on the muscarinic receptor are not of significant concern, at least as evidenced by the results in these papers.

4. Nicotinic-cholinergic function and receptors. The nicotinic receptor from *Tornedo californica* is perhaps the best studied receptor available. The relationship of this receptor to mammalian neuromuscular junction receptors and especially to brain receptors is sufficiently good that studies of this system are of considerable interest. The effects of ethanol and other anesthetic agents on the receptor have been studied by Young and Sigman (483) and by Young et al. (482). They have found that relatively high levels of ethanol and gaseous anesthetics stabilize the receptor in a high-affinity, desensitized state. They propose that this may explain the depression of neuromuscular transmission caused by these agents. The effects that they studied required 0.5 M ethanol before any effect could be seen. The effects of the various agents did correlate nicely with the oil/water partition coefficients. This is true of an enormous number of physiological effects of these agents. It seems unlikely, however, that this effect is of pharmacological importance because 0.5 M is a lethal level.

Another line of attack on this problem is in a paper by De Fiebre et al. (92). These investigators used the SS and LS mice and tested the response of these mice to nicotine and measured nicotinic receptor binding in the hippocampus, cerebellum, and striatum. They found that the LS mice were more sensitive to nicotine-induced seizures, but they did not differ from the SS mice in the binding of $^{125}I\alpha$-bungarotoxin in the hippocampus. They did find a difference in binding in the cerebellum and striatum between the lines. Although this is a provocative and potentially interesting finding, it needs to be further investigated in other lines of animals that differ in their sensitivity to ethanol to establish cause and effect relationships.

Another study by Modak and Alderete (308) found that nicotine potentiated pentobarbital sleep time but not sleep time induced by ethanol in mice. The dose of ethanol used in this study (3 g/kg) was such that it produced only a sleep time of 15 min. Had a higher dose been used which would have prolonged the sleep time beyond the time necessary for complete equilibration between blood and brain ethanol, different results may have been found.

D. Biogenic Amines

The effect of ethanol on the synthesis, storage, release, and uptake of biogenic amines (catecholamines and serotonin) has long been a favorite subject of investigators. However, this has engendered an enormous number of conflicting reports. Presumably, this is due to the differences in animals, the measurement of endogenous release versus release of previously taken-up amine, the differences in brain areas studied, differences in the technique of slice preparation, etc. This topic was reviewed in 1976 (93) and at that time the general consensus was that acute administration of ethanol either decreased or had no effect on the amount of dopamine in various brain areas while decreasing turnover of dopamine. This was not a universal finding however. In studies of release of dopamine from superfused brain slices (51), it was found that there was an inhibition of release of several neurotransmitters, including dopamine, IC_{50} for this effect was 410 mM, clearly a lethal level. Since that time a number of papers have appeared, usually measuring endogenous release of dopamine from slices of brain (usually striatum) plus and minus potassium depolarization (43, 174, 206) and usually with ethanol levels well into the intoxicating or lethal range. Generally, although not uniformly, it is found that ethanol has little effect on the release of dopamine from unstimulated slices but usually inhibited release in potassium-depolarized slices (271). Conversely, Dar and Wooles (87) found that acute administration of ethanol to mice enhanced dopamine synthesis and turnover. Tabakoff and Hoffman (445) demonstrated functional dependence on ethanol in dopaminergic systems.

The recently perfected technique of measuring endogenous release of agents by way of utilizing in vivo microdialysis in the brains of awake and responding animals promises to provide much more relevant information. De Chiara et al. (96–98) have found that small doses of ethanol (0.5 g/kg) increase release of dopamine from the NA but have no effect on the release from the striatum. Larger doses of 2.5 to 5 g/kg also cause an increase in release from the NA but then a decrease is seen at higher doses. In the striatum, the higher doses increase the release. Correlation of these effects with the behavior of the animal is another major advantage of this technique.

After the major areas of interest have been mapped and the correlations established, return to in vitro measurements to work out the mechanisms will be necessary.

E. Prostaglandins

There have been a number of studies that indicate an interaction between prostaglandins and ethanol's depressant action. Collins et al. (79) observed that indomethacin, an inhibitor of prostaglandin synthesis, would reduce the depressant effects of ethanol in C57 and DBA mice. They then studied the LS and SS mice extensively (148–151). They found that inhibition of prostaglandin synthesis will reduce the effect of ethanol on sleep time and will also reduce mortality to ethanol overdose. George and Collins (148) found that ethanol would increase brain prostaglandin levels and that this effect was dependent on dose, sex, and genotype.

F. Adenosine

Another potential mediator of the effects of ethanol is adenosine. Most of the current evidence suggests that adenosine is not a neurotransmitter per se but, rather, a neuromodulator that serves as an important regulator of neuronal activity (see 106 for review). In particular, neurons that release adenosine as their major transmitter have not been identified in brain, but the generalized release of adenosine from brain tissue appears to be sufficient to activate extracellular adenosine receptors. Within the context of this review, there have been a number of studies that have suggested that ethanol might interact with adenosine to produce depressant effects on the CNS.

1. Behavior. In terms of behavioral studies, there have been several reports that appear to implicate adenosine in the effects of ethanol. Pretreatment of mice with theophylline, a competitive antagonist at adenosine receptors, was found to significantly shorten sleep time following injections of ethanol, whereas pretreatment with the adenosine uptake inhibitor dipyridamole results in longer sleep times (86). Similar effects were observed in terms of the motor-incoordinating effects of ethanol as well. What is unclear from this study is whether this is a drug interaction of pharmacological interest or whether these simply represent independent interactions of drugs that have sedative and CNS-activating properties.

More evidence suggesting an adenosine-ethanol link is the observation that sensitivity to the behavioral effects of ethanol in SS and LS lines of mice is paralleled by comparable differences in sensitivity to the depressant actions of an adenosine receptor agonist (R-phenylisopropyladenosine; 364). Furthermore, the sensitivity to the excitatory actions of the adenosine antagonist theophylline was also greater in the LS (ethanol sensitive) line of mice (363). Because adenosine agonists and antagonists have greater effects in the LS line of mice, these studies suggest that adenosine receptors exert a greater degree of control over the behavior of LS mice than they do in the SS mice.

One possible biochemical basis for this might be a greater number of adenosine receptors in the LS line of mice; however, although initial biochemical studies suggested that there might be more adenosine receptors in the more sensitive LS line of mice (137), more extensive studies have not supported this initial observation. Furthermore, we have not observed any differences in hippocampal electrophysiological sensitivity to adenosine in the LS and SS lines of mice (T. V. Dunwiddie, unpublished results). These observations suggest that the behavioral effects of the adenosine analogs are unlikely to be mediated by actions in the hippocampus, although there may be other brain regions where differences do exist.

Further evidence linking ethanol action to adenosine has come from studies in which animals were chronically administered adenosine antagonists. Mice were pretreated with caffeine, theophylline, or isobutylmethylxanthine for 10 days, then tested for ethanol-induced loss of motor coordination. Caffeine and isobutylmethylxanthine treated animals showed up-regulation of adenosine receptors, and corresponding increases in ethanol-induced motor incoordination, but theophylline treated animals did not show either effect (88). Again, this would seem to link higher adenosine sensitivity to increased responsiveness to ethanol.

On the other hand, it is clear that adenosine can by no means account for the entirety of the effects of ethanol on the CNS. Although adenosine antagonists can attenuate some of ethanol's effects, they cannot block them entirely. In a discriminative cue paradigm, neither directly nor indirectly acting adenosine receptor agonists show cross-generalization to ethanol (303). Thus, although adenosine receptor-mediated effects may contribute to the behavioral responses to ethanol, they are not identical with respect to cue properties. Clearly, this is an area where further studies will be required in order to place these observations in an appropriate context.

2. Biochemistry. In terms of biochemical effects of adenosine, there are also a number of interesting suggestions as to how ethanol might modulate responses to adenosine. There are at least three different kinds of adenosine receptors that might be involved in neuronal actions. An adenosine A1 receptor has been linked biochemically to an inhibition of adenylate cyclase activity, and an A2 receptor activates adenylate cyclase; a receptor that appears to have many of the biochemical properties of the A1 receptor also can activate a potassium conductance in neurons via a guanosine 5′-triphosphate-binding protein (see 106 for review).

In terms of more specific interactions between adenosine and ethanol, ethanol potentiates increases in cAMP in both human lymphocytes (216) and in neuroblastoma (NG-108) cells (165). In the NG-108 cells, these effects

are observed at ethanol levels of 50 mM and higher, whereas in lymphocytes the threshold was approximately 132 mM. As is discussed in a later section (VI, A), ethanol-adenylate cyclase interactions do not seem specific, in the sense that the actions of other agents that increase cAMP levels are also potentiated by ethanol. Because these effects were also seen in the presence of a phosphodiesterase inhibitor, they are unlikely to be due to reduced breakdown of cAMP (216). Chronic treatment with 200 mM ethanol in the NG-108 cells produced tolerance to effects of ethanol, so that ethanol was actually required to observe normal responses to adenosine.

The original observations of Hynie et al. (216) have been followed up in detail in studies of lymphocytes from alcoholics. Basal adenylate cyclase activity was lower in lymphocytes from alcoholics, the stimulatory response to adenosine was blunted, and the ability of ethanol to facilitate adenosine-mediated increases in cAMP formation was also reduced (99). It is unclear to what extent these changes are responses to chronic exposure to ethanol, or are differences that are related to possible biological bases for alcoholism? Further studies will be required to clarify these issues.

In addition to facilitating the activation of adenylate cyclase, ethanol (100 to 200 mM) has also been shown to disrupt the inhibition of adenylate cyclase activity by R-phenylisopropyladenosine in homogenates of rat cortex (22). This type of action could contribute to both increased basal and stimulated levels of cAMP formation. Finally, ethanol has been reported to inhibit adenosine deaminase in rat brain, which is the enzyme that breaks down adenosine to the relatively inactive metabolite inosine. However, the K_i for this effect (500 mM) is so high that this would only seem likely to contribute to the effects of very high concentrations of ethanol (60).

3. Summary. Although the relationship is unclear at this point, there are a number of mechanisms by which ethanol and adenosine can potentially interact. At present the behavioral evidence is perhaps the most provocative but tells us the least about what mechanisms might be involved. In terms of the effects of ethanol on adenosine activation of adenylate cyclase, the fact that these have not been reported in neurons makes these reports difficult to evaluate. There have also been no reports of electrophysiological responses to A2 selective receptor agonists that meet reasonable standards of pharmacological specificity, so it is difficult to predict what responses one would expect at the cellular level if ethanol can potentiate A2-mediated increases in cAMP. Other agents that increase neuronal cAMP concentrations (e.g., β-adrenergic agonists) generally decrease the Ca-g_K, but ethanol, if anything, usually has the opposite effect (section IV, C). However, as the mechanisms by which adenosine and ethanol become better understood, the precise nature of these interactions may become apparent.

VI. Protein Phosphorylation

In this section we will review the effect of ethanol on the enzymes and events that lead up to the phosphorylation of proteins. Unfortunately, in many cases the story will end there because we often do not know the function of the proteins that are phosphorylated.

Given the importance of phosphorylation to the function of the CNS, it is no wonder that this system has received considerable attention in recent years as a possible site of action of ethanol on the function of the brain. A great many neurotransmitters bring about their effects by interaction with their cell surface receptors and these in turn, with mediation of coupling proteins, affect intracellular protein kinase enzymes. These enzymes phosphorylate specific proteins. If these proteins are enzymes, phosphorylation can result in either an increased or decreased activity. Protein phosphatases are also of importance because they are responsible for dephosphorylation, however, very little research on these enzymes has been carried out with regard to the effects of ethanol.

A. Adenylate Cyclase and cAMP Levels

As early as 1970 it was observed that relatively high concentrations of isopropanol in vitro (4.5%) would cause an increased activity of basal and glucagon-stimulated activity of adenylate cyclase in liver membranes and whole homogenate (167). There have been numerous reports of stimulation of adenylate cyclase in other tissues since that time including heart (464), thyroid (286), intestine (170), uterine smooth muscle (244), adipocytes (435), and peripheral blood cells (16). It was found that the enzyme in rat brain was stimulated by concentrations of ethanol of 0.2 to 10% (170). Striatial dopamine-stimulated adenylate cyclase is activated by benzyl alcohol (327). Consistently, stimulation of the enzyme was observed regardless of the alcohol used if the concentrations were high enough. The concentrations used were, for the most part, incompatible with life. However, most of these studies were directed toward a study of the enzyme and not a study to elucidate the mechanism of action of ethanol.

Several experiments have demonstrated that the activity of adenyl cyclase is sensitive to the fluidity of the membrane in which it is located. In studies utilizing a mutant line of Chinese hamster ovary cells that could not regulate the cholesterol content of their plasma membrane, Sinensky et al. (420) demonstrated that increased cell membrane ordering by increasing the amount of cholesterol in the membrane increased basal adenylate cyclase activity. Gordon et al. (166) arrived at similar conclusions in a study of the effect of benzyl alcohol on the fluidity of rat liver plasma membranes. In both cases, cell membrane ordering was measured by electron spin resonance. In studies in L6 cells in which cell membrane fluidity was altered by butanol or ethanol, both adenylate cyclase activity and membrane order were measured. In

this case, order was assessed by fluorescence of diphenylhexatriene. Although butanol was more potent than ethanol in increasing adenylate cyclase activity, the highest concentration of ethanol had a greater effect than did the highest concentration of butanol. There was excellent correlation between the percentage of increase in isoproterenol-stimulated adenylate cyclase and decrease in fluorescence anisotropy for both butanol and ethanol. The increase in enzyme activity per unit change in anisotropy was greater for ethanol than for butanol. Ketamine decreased fluorescence anisotropy (as did both butanol and ethanol), but it decreased (rather than increased) the activity of isoproterenol-stimulated adenylate cyclase. Phenobarbital had no effect on either membrane order or isoproterenol-stimulated enzyme activity (369). There are a number of caveats to consider with these experiments, for example, the measurement of cell membrane fluidity varies with the depth of the probe (162).

Other investigators using adenylate cyclase from striatal tissue have found interesting results. Uniformly, it has been found that the enzyme in striatal tissue is activated by ethanol and that the dopamine-stimulated enzyme is also increased. Again, this occurs at relatively high ethanol concentrations of 100 mM (370, 371). Significant stimulation could be achieved by 68 mM ethanol which is 312 mg/dl, near lethal level for nontolerant humans. This effect is probably not only on the receptors because it requires the presence of coupling proteins (267). Ethanol must have some activity to increase catalytic subunit activity because it will stimulate the enzyme after treatment by procedures that fully activate the G protein (cholera toxin, fluoride, guanylyimidodiphosphate) (267, 370). Stenstrom and Richelson (433) found that ethanol would also stimulate prostaglandin-activated adenylate cyclase in neuroblastoma cells. Stenstrom et al. (434), on the other hand, found that ethanol would inhibit rather than stimulate forskolin-stimulated adenylate cyclase in neuroblastoma. The level of ethanol required (100 to 700 mM) was sufficiently high as to change the osmotic properties of the medium in which the cells were tested. Sucrose at the same osmotic pressure had similar effects. In addition, however, there was some osmotic independent effects of ethanol as demonstrated with membrane preparations.

However, Hoffman and Tabakoff (203) found that ethanol did not affect the Arrhenius parameters of mouse striatal, fluoride-stimulated adenylate cyclase. They proposed that the action of ethanol is directly on adenylate cyclase protein or on regulatory proteins. This work was extended by Saito et al. (394) to include actions at the β-adrenergic receptor, the coupling proteins, and the catalytic unit of adenylate cyclase. In other experiments, this laboratory demonstrated that there was no effect of acute or chronic ethanol treatment on levels of calmodulin in the striatum or cerebral cortex of mice (268).

In contrast to the stimulation of basal adenylate cyclase as well as increased activity of activated adenylate cyclase, ethanol had no effect on the inhibition of adenylate cyclase brought about by morphine, Leu-enkephalin, or ACh (368). Hoffman and Tabakoff confirmed this finding for Leu-enkephalin (204).

Strangely, Volicer and Gold (465) found that ethanol pretreatment blocked the decapitation-induced rise in cAMP in the cerebellum of rats. Because some of this rise may be due to adenosine release (352), there may have been inhibition of adenosine release by ethanol. Redos et al. (378) could not find any alteration in regional brain cAMP following acute and chronic treatment with ethanol. Zarcone et al. (485) also found a decreased cAMP level in the cerebrospinal fluid of humans given enough ethanol to achieve a blood level of 166 mg/dl.

Israel et al. (220) and Kuriyama and Israel (250) administered 4 g/kg ethanol i.p. to mice and then analyzed adenylate cyclase in homogenate and slices of cerebral cortex. They found no effect of acute administration of ethanol compared to a sucrose-saline control group. Weitbrecht and Cramer (470) also found a decrease in cAMP and cyclic guanosine 5′-monophosphate (cGMP) levels in cerebrospinal fluid of rats given relatively small doses of ethanol (0.55 up to 4.5 g/kg). There was evidence of complicated kinetics and dose-response relationships because the higher dose of ethanol caused a smaller decrease in both cAMP and cGMP at 1 h. The levels were still depressed at 24 h at which time all the ethanol would have been gone. At this time the animals receiving the higher dose of ethanol had lower levels of cAMP. Gold and Volicer (161) found a condensation product between ethanol and ATP in liver homogenates. Neither this compound nor cAMP was precipitated by barium but could be separated on alumina columns. They postulated that the compound was ethyladenylate. Weitbrecht and Cramer (470) postulated that some such condensation product might be responsible for the prolonged effect of ethanol in their experiments.

French et al. (138) made the observation that ethanol would attenuate the cAMP increase in plasma of rats given glucagon. They postulated that ethanol decreased the sensitivity of the glucagon receptor.

The results discussed above leave us with a paradox. The effect of ethanol in vitro is to stimulate adenylate cyclase activity, although at high levels. On the other hand, the effect of administered ethanol is almost invariably to decrease the level of cAMP. One can argue that it is simply a difference in the doses that bring about this difference. However, that does not explain the decrease in the levels of cAMP when ethanol is administered. There are a large number of possibilities that eventually lead to cAMP production and to decreased responsiveness of the receptors for these ligands. Some evidence for the idea of decreased responsiveness is seen in a paper by Valverius et al. (463) who found that low concentrations of ethanol (10 to 100 mM), when added

to binding assays in vitro, decreased the affinity of the high-affinity form of β-adrenergic receptors for isoproterenol. These results were interpreted as evidence for the effect of ethanol on the interaction of G_s protein or directly on the receptor protein (205).

The situation is somewhat analogous with cGMP levels except that the evidence for stimulation of guanylate cyclase in vitro is lacking but cGMP levels, at least in the cerebellum, are drastically reduced by administration of ethanol. Redos et al. (377) first reported that ethanol reduced cGMP levels in the cerebellum of the rat at doses of 2 g/kg and above but that cAMP levels were unaffected. This observation has been repeatedly confirmed in brain as well as other tissues (74, 214, 266, 464, 466, 480). Stenstrom et al. (432), using murine neuroblastoma cells, showed that ethanol rapidly inhibited cGMP synthesis mediated by histamine, carbachol, melittin, and the ionophore X537A. However, both sucrose and sodium chloride, added to increase the osmolality to the same extent, had the same effect. In a crude homogenate of these cells, ethanol inhibited both basal and sodium nitroprusside-stimulated guanylate cyclase activity. The relationship of these findings to the observed lowering of cerebellar cGMP is unknown.

A large number of compounds have been reported to have effects on cGMP in the cerebellum and other brain areas (tables 1 and 2). The impression that one gains from the earlier work on cGMP is that nearly all depressant drugs lower the levels of cGMP in the cerebellum and that nearly all stimulant drugs increase the levels. The experiments by Lundberg et al. (266) particularly emphasize that the level of input to the cerebellum seems to be important. The effect of ethanol then cannot be seen as having any specificity in this process because it is shared by a great many compounds. However, several recent developments tend to modify this somewhat pessimistic view. Stenstrom et al. (434) obtained evidence that ethanol would inhibit stimulated cGMP production

TABLE 1
Compounds reported to increase cGMP levels

Compound	Reference
Harmaline	28, 280, 342
Bethanecol	29
Picrotoxin	281, 342
Isoniazid	28
Glycine	281
Glutamate	281
Sodium nitroprusside	146
Endothelium-derived relaxing factor	146
TRH	276, 480
Ca^{2+} ionophore	102
γ-Lindane	129
d-Tubocurare, intrathecal	266
Apomorphine	28, 266
Amphetamine	125
Oxotremorine	126
Arecoline	101
Nicotine	101

TABLE 2
Compounds reported to decrease cGMP levels (other than ethanol)

Compound	Reference
GABA	281
Barbital	252
Diazepam	280
Pentobarbital	266, 342
Ca^{2+} ionophore + ethanol	102
δ-Lindane	129
d-Tubocurare i.p.	266
Halothane	266
CO_2	266
Ether	102
Chlorpromazine	126
Reserpine	126
Haloperidol	28

in neuroblastoma cells regardless of the agonist used. They interpreted this to mean that the most likely site of action was directly on guanylate cyclase in these cells. Rabe et al. (367) reported that ethanol at pharmacologically active levels inhibits the calcium-dependent stimulation of cGMP levels by glutamate in primary cultures of cerebellar granule cells. Also Chik et al. (66) report that intoxicating levels of ethanol are capable of inhibiting the increases of cAMP and cGMP in pineal cells brought about by a combination of vasoactive intestinal peptide and phenylephrine. Both of these systems are free of the complex inputs from other brain areas and argue for some direct effect of ethanol (and presumably other CNS depressants) on cGMP production. Lovinger et al. (264) obtained results in voltage-clamped hippocampal neurons that mesh nicely with those of Rabe et al. (367). They found that the NMDA-induced current was reduced 61% in the presence of 50 mM ethanol.

Although all of the results with cGMP levels are quite interesting, they do not answer the fundamental question of what cGMP is doing in the CNS, much less give us any idea of what a change brought about by ethanol means in terms of the mechanism of action. It is assumed that the action of cGMP is intimately involved with cGMP-dependent protein kinases and that the substrate(s) for these kinases is important to the function of the CNS. These substrates have not been identified nor have those proteins that are known substrates for G kinases been implicated in the actions of ethanol.

It is difficult to assay for cGMP-dependent protein kinases in a crude system without a specific substrate for a variety of reasons. The activity is low and often masked by other protein kinases in the tissue; in addition, there is a modulator present that increases the activity of the kinase (248). This is decreased by long-term administration of ethanol (249).

B. Phosphotidylinositol System

This system has received a great deal of attention in recent years and not unexpectedly from investigators in the alcohol field as well. Lee et al. (255) gave [^{32}P] phosphate and [^{32}H]glycerol intraventricularily and

measured turnover of phosphotidylcholine and phosphotidylserine plus phosphotidylinositol in synaptosomes and microsomes of rat brain. The found that acute treatment with ethanol increased the turnover as measured by ^{3}H levels in phosphotidylcholine and phosphotidylserine plus phosphotidylinositol. Allison and Cicero (9) found that acute ethanol decreased the level of inositol 1-phosphate in rat cerebral cortex. Chandrasekhar et al. (62) found that after labeling brain phospholipid by injection of [^{32}P]ATP i.c.v. phosphotidylinositol breakdown was inhibited as evidenced by an increase in inositol phosphate and a decrease in diacylglycerol.

Several other studies have been carried out in which the ethanol has been added in vitro. Gonzales et al. (164) labeled brain slices with [^{3}H]inositol and measured the inositol phosphates in the presence of lithium. They found that 500 mM ethanol would inhibit basal hydrolysis, as well as that stimulated by norepinephrine, KCl, and glutamate but not carbachol. In a later paper they also found that norepinephrine-stimulated hydroysis was decreased by 500 mM ethanol (163). Hoffman et al. (201) found that ethanol at a threshold concentration of 75 to 100 mM would inhibit the breakdown of phosphotidylinositol 4,5-bisphosphate in slices of mouse brain. A high concentration of ethanol was required to increase the EC_{50} for carbachol stimulation of phosphotidylinositol 4,5-bisphosphate breakdown, but this concentration had no effect on norepinephrine-stimulated phosphotidylinositol 4,5-bisphosphate breakdown. These results contrast with those of Gonzales et al. who found no effect on carbachol-stimulated breakdown. In any case these results with such high concentrations of ethanol are of doubtful signifance to the situation in vivo.

Ritchie et al. (382) studied astrocytes in culture. They found that ethanol in concentrations of 25 to 200 mM had little effect on accumulation of ^{3}H-labeled inositol phosphates from [^{3}H]inositol.

Smith (422, 423) found that ethanol, 100 mM, reduced the incorporation of ^{32}P into phosphatidic acid but not into phosphotidylinositol in cholinergically stimulated synaptosomal preparation from mouse forebrain.

VII. Metabolic Effects

A. Introduction

Conceptually, there are a number of ways that ethanol may bring about effects on the brain. Oldest among these is the idea that the metabolism of ethanol in peripheral organs, especially the liver, alters the function of the brain. This usually was envisioned as taking place through acetaldehyde. The second possibility is that ethanol's metabolism directly in the brain has some effect. This is either via acetaldehyde or disruption of other metabolic processes in brain, analogous to that seen in the liver. The third possibility is that ethanol physically interacts with either lipids in which membrane-bound enzymes are located or interacts directly with the proteins. The fourth possibility, which has attracted much attention recently, is that ethanol may act in the area of free radicals.

B. Effects of Ethanol Metabolism on Brain Function

1. Acetaldehyde and biogenic aldehydes. Before much was known about the effects of ethanol on the brain at a molecular level, there was a great deal of work on the possible role of acetaldehyde in mediating some of the initial CNS effects of ethanol. The discovery of pyrazole as a potent inhibitor of ethanol metabolism quickly dispelled most of the ideas that acetaldehyde had anything to do with the immediate actions of ethanol. Animals treated with pyrazole were more, not less, sensitive to ethanol. Also a large artifactual production of acetaldehyde from a combination of ethanol and blood was found. When this was prevented or corrected for, the values for blood acetaldehyde were lowered by a factor of 10 or more (117). In addition, the difficulty of finding any acetaldehyde in the brain itself cast further cold water on the idea of the involvement of acetaldehyde in the actions of ethanol (261). Nevertheless, a great deal of interest has been generated around the possible involvement of acetaldehyde or biogenic aldehydes (those aldehydes from norepinephrine, dopamine, and serotonin) and the aldehyde-amine condensation products in the long-term effects of ethanol. Reviews of this area may be found elsewhere (31, 93, 94). The major interest in this area is in the possibility of an effect of these compounds on alcohol preference (295). Although this idea is now largely discounted, there are effects of the condensation products in a number of systems (349).

2. Metabolism of ethanol in brain. There is good agreement now that there is little, if any, metabolism of ethanol in the brain itself compared to the liver. The discovery, purification, and characterization of a brain alcohol dehydrogenase that has virtually no activity toward ethanol has recently been accomplished (376). The possibility that other pathways, such as via catalase, still occupies a central role in theories that require the presence of acetaldehyde in the brain (13, 14). The evidence that catalase contributes to the metabolism of ethanol in the brain is entirely indirect. Attempts to directly demonstrate the conversion of ethanol to acetaldehyde in brain tissue via catalase have been unsuccessful, perhaps because of a relatively large nonenzymatic, artifactual interaction of heme proteins or brain ascorbate (77) with ethanol to produce acetaldehyde. The presence of aldehyde dehydrogenase in the brain also complicates these experiments (425).

3. Interactions of ethanol with brain enzymes. This subject has recently been extensively reviewed (448). There are references to other enzymes throughout this review as well. The studies involving adenylate cyclase are located in section VI. The other major enzymes of interest are sodium/potassium ATPase and monoamine oxidase. These are membrane-bound enzymes and dif-

ferentiation between an effect of ethanol on the lipid environment and directly on the protein or associated proteins is often difficult, if not impossible. The studies on sodium/potassium ATPase are of interest because addition of norepinephrine increased the potency of ethanol as an inhibitor of the enzyme from rat brain (372, 373). This interesting finding has not been confirmed in mouse brain however (444). The problem that these observations address is that the concentrations of ethanol needed to inhibit the enzyme in vitro are much higher than achievable in a surviving whole animal. If there are endogenous factors that increase the potency of ethanol, this would make the in vitro observations of much greater importance.

Alcohols added in vitro will also inhibit monoamine oxidase (132) and ethanol specifically inhibits the B form of the enzyme (449). However, the major effort with monoamine oxidase has been as a marker for genetic susceptibility to alcoholism or as a marker for excessive alcohol intake (see 447).

C. Alternative Metabolic Pathways: Oxidative Stress

The possibility of free radical formation during ethanol oxidation has been a subject of considerable work for many years (56–59, 100); however, most of this work has involved the liver and often chronic feeding of ethanol. Recently, interest has risen in the possibility that acute or chronic ethanol administration might have an effect on brain metabolism, tissue damage, or function.

Several possibilities exist as to the mechanism of these effects. Ethanol could form a free radical either in the cytochrome P_{450} system or via a direct reaction with OH* radicals to form a hydroxyethyl radicals. The OH* radicals can be generated from superoxide via hydrogen peroxide or directly from hydrogen peroxide that is a product of the monoamine oxidase, xanthine oxidase, and aldehyde oxidase reactions, as well as to a small extent from the cytochrome P_{450} system (3, 421). The other possibility is that ethanol could lower the levels of endogenous antioxidants such as ascorbate and α-tocopherol. In any case, the net result of such events will be increased lipid peroxidation with the resultant formation of long-chain fatty aldehydes and malondialdehyde. Aldehydes such as these are known to be substrates for some aldehyde dehydrogenase enzymes but also potent inhibitors of other forms of this enzyme (355).

Recently, endothelial derived relaxing factor has been tentatively identified as nitric oxide and this compound causes a marked increase in cGMP, presumably as a basis for its action (310). Superoxide anions destroy nitric oxide and ethanol is known to decrease superoxide dismutase (254) which would lead to an increase in superoxide anions and a decrease in nitric oxide . Ethanol is known to cause a marked decrease in cGMP in brain (see above), but whether or not this mechanism is involved is unknown.

Rouach et al. (387, 388) showed that acute ethanol administration would lower the ascorbic acid and α-tocopherol content of the cerebellum. Saffar et al. (393) also observed that ethanol administration to rats impaired state 3 respiratory rate of isolated brain mitochondria and also decreased brain cytosolic copper, zinc, and superoxide dismutase. These effects were blocked by the xanthine oxidase inhibitor, allopurinol. Whether the effect of allopurinol is via inhibition of xanthine oxidase and subsequent decrease in superoxide or by direct scavenging of superoxide by allopurinol and its metabolite remain to be determined. Thus, there are a number of effects to consider when ethanol is given. Superoxide dismutase will be inhibited by ethanol, and there is an increase in peroxidation, including perhaps free radicals from ethanol itself.

Another interesting finding has been made by Pellegrini-Giampietro et al. (353). They found that release of glutamate and aspartate from rat hippocampal slices was increased in the presence of a free radical-generating system (xanthine oxidase and xanthine) and that this was blocked by allopurinol, superoxide dismutatase plus catalase, and superoxide scavengers. If ethanol inhibits superoxide dismutase leading to an increase in superoxide radicals, there will be an increase in release of glutamate and aspartate. This might be compensated for by the finding that ethanol inhibits the calcium-dependent glutamate-stimulated increase in cGMP levels in cerebellar granule cells (367). The proximate compound that is responsible for this effect is unknown.

Finally, a possible consequence of treatment of alcoholics with disulfiram may be related to the fact that this compound and a principal metabolite, dietyldithiocarbamate, inhibit lipid peroxidation (103, 366).

VIII. Neuropeptides and Ethanol

A. Introduction

During the past two decades there has been an exponential growth in research on neuropeptides as putative neurotransmitters. A number of these compounds fulfill many of the criteria required for such distinction. Neuropeptides such as endorphins and enkephalins, cholecystokinin (CCK), thyrotropin-releasing hormone (TRH), neurotensin (NT), and substance P are synthesized in neuronal cell bodies, transported to neuronal terminals, colocalized in storage vesicles with other putative neurotransmitters, and released during depolarization (2, 30, 221). These peptides and others exist in distinct neuronal pathways and have specific high-affinity-binding sites in unique distributions in mammalian brain. In many instances, production of second messengers by receptor occupancy has been identified. Many behavioral and pharmacological responses are elicited during microinjection of these neuropeptides i.c.v. or into specific brain regions. In most instances, the dose-dependent pharmacological responses display specific structural-activity relationships and are antagonized by

inactive analogues or by selective antibodies. The actions of the neuropeptides are terminated by a variety of relatively nonspecific peptidases such as metalloendopeptidase and enkephalinase (2, 29, 221).

Processes such as neuropeptide synthesis, translocation, storage, release, receptor occupancy, receptor-coupling to second-messenger production, and degradation are potential sites of action of ethanol (229). Because it is well documented that pharmacological actions of ethanol are altered by classical neurotransmitter amines, it is not surprising that specific interactions of neuropeptides and ethanol have been observed. Likewise, it is not surprising that ethanol alters the release of a number of hypothalamic and pituitary hormones, including luteinizing hormone-releasing hormone, luteinizing hormone, corticotropin-releasing factor, corticotropin, and prolactin (75, 171). It has long been recognized that ethanol alters hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal peptide secretions. Only recently have studies focused on the effects of ethanol on central neuropeptidergic processes. A search of the literature indicates that, at least at the behavioral level, the neuropeptides, Met-enkephalin, β-endorphin, somatostatin, NT, corticotropin-releasing factor, vasopressin, and thyrotropin-releasing hormone alter ethanol-induced pharmacological responses. This section will be limited to discussions of those studies that reveal potential sites of interaction of acute ethanol administration and neuropeptides.

B. Opioids

It is well know that ethanol and the opioids and opiates share similar neuropharmacological responses including hypothermia, euphoria, analgesia, and motor activation as well as production of tolerance and dependence (229). Evidence that endogenous opioid processes might mediate some of these pharmacological actions of ethanol was suggested by the studies of Frye et al. (141) and Luttinger et al. (269). They demonstrated that central administration of β-endorphin markedly potentiated ethanol-induced loss of the righting reflex, sleep time, and hypothermia. In subsequent studies, Erwin et al. (120) demonstrated β-endorphin potentiation of ethanol actions in the LS and SS mice that were selectively bred for differences in sensitivity to ethanol.

That ethanol may act in part via endogenous opioid processes was supported by studies (20, 324, 397) showing that acute administration of ethanol increased plasma levels of opioid activity, e.g., β-endorphin immunoreactivity in humans (20, 324) and increased Met-enkephalin levels in rat brain and pituitary (397). In early studies the effects of ethanol on brain enkephalin levels were contradictory. Ryder et al. (392) reported that acute ethanol administration did not affect the enkephalin content of rat brain cortex, hypothalamus, or striatum. However, subsequent studies by Seizinger et al. (403) showed that acute ethanol administration, 2.5 g/kg, significantly increased levels of Met-enkephalin immunoreactivity in hypothalamus, striatum, and midbrain but not in hippocampus. This effect of ethanol appears to be specific in that levels of other opioids, dynorphin, and α-neoendorphin were not altered in brain or pituitary. Whether ethanol-induced alterations in Met-enkephalin in the brain are due to changes in release, synthesis, or degradation is not known. However, studies have shown that chronic ethanol administration causes a reduction in the rate of β-endorphin degradation (91) by brain synaptosomes. Indeed, inhibition of degradation would cause an increase in opioid peptides. In the pituitary, investigators have shown that ethanol stimulates the release of β-endorphin-like peptides (153) with a concomitant decrease in β-endorphin immunoreactivity in the anterior pituitary.

Other studies using inbred strains of mice indicate there may be strain-specific effects of ethanol on post-translational processing of the opioid peptides (154). Gianoulakis and Gupta (154) compared levels of β-endorphin immunoreactivity in the hypothalamus and pituitary of C57BL/6, BALB/c, and DBA/2 inbred mouse strains, known to differ in ethanol sensitivity. Ethanol decreased β-endorphin immunoreactivity levels in the hypothalamus of C57BL/6 mice which had the highest pituitary content of β-endorphin. Ethanol produced an increase in serum β-endorphin immunoreactivity in all three strains of mice. These results are consistent with those of Crabbe et al. (81) who reported differences in proopiomelanocortin-derived peptides, corticotropin, and β-endorphin in pituitaries from various inbred strains of mice. These studies suggest that differences in ethanol sensitivity may be due to differences in opioid processes. However, it should be noted that such correlations may be fortuitous and that interactions of ethanol on opioid systems in the pituitary may not be related to ethanol actions in the brain. It is not the intent of this section to summarize the neuroendocrinological effects of ethanol. However, current evidence indicates that ethanol-induced increases in proopiomelanocortin peptides, including corticotropin and β-endorphin, as well as luteinizing hormone are primarily centrally mediated (75, 81, 153, 154).

Centrally administered Met-enkephalin or morphine and low doses of ethanol, i.p., produce common neurochemical effects. For example, microinjection of Met-enkephalin into the VTA (A10 cell group) produces an increase in dopaminergic activity in the NA (98, 287, 309). Likewise, ethanol in subhypnotic doses enhances dopamine release in the NA (98, 218), and the effects of ethanol on dopamine metabolism have been reported to be mouse strain specific (19). Ethanol-opioid interaction at the biochemical level has been demonstrated in peripheral neuronal systems, i.e., Gintzler and Scalisi (157) reported that acute administration of ethanol potentiated the inhibitory effects of opioids on ACh release

from terminals in guinea pig enteric plexus. These authors suggested that this mechanism might have relevance in the CNS in stress states in which an activation of the opioid system might potentiate ethanol sensitivity.

One approach to investigating the potential role of endogenous opioid peptides in central actions of ethanol is the use of opioid receptor antagonists. For example, Pohorecky and Shah (358) have shown that naloxone, 1 mg/kg, partially reverses ethanol-induced analgesia as measured by the tail-flick method. Ethanol also potentiates cold water swim-induced analgesia (a nonopioid-mediated analgesia) indicating that ethanol may produce analgesia by more than one mechanism (see NT below). Pillai and Ross (356) reported that naloxone and the κ-antagonist, MR 2266, partially protected rats against ethanol-induced hypothermia. Other investigators have presented data showing that naloxone attenuates some of the effects of ethanol, including ethanol-induced conditioned taste aversion (334) and an increase in serum prolactin levels (402). Another study indicates that ethanol-opioid interactions are specific for the receptor subtype. For example, Jorgensen and Hole (228) reported that the selective δ-opioid antagonist, ICI 154129, failed to prevent the effects of ethanol on pain sensitivity, body temperature, consciousness, or sensorimotor performance. Although these studies are of interest and suggest an ethanol/endogenous opioid interaction, they are not definitive in that blockade or reversal of ethanol actions by opioid antagonists may reflect a physiological antagonism rather than indicate a specific interaction with ethanol.

Several investigators have shown that chronic ethanol treatment alters opiate receptor function and binding characteristics (215, 265), but effects of acute ethanol administration on opioid receptors in vivo have not been demonstrated. However, Charness et al. (65) found that short-term exposure to ethanol of mouse neuroblastoma-rat glioma hybrid cells (NG-108–15) resulted in decreased opiate receptor binding. In other studies Tabakoff and colleagues (199, 200, 446) have shown that ethanol alters binding of ligands to opiate receptors in vitro. Ethanol at relatively high concentrations (50 to 1000 mM) inhibited binding of dihydromorphine or D-Ala,D-Leu-enkephalin in membranes from mouse striatum. These authors provide evidence that the response of opiate receptors to ethanol is influenced by the microenvironment of the receptors including the physical state of the membrane and/or by coupling of the receptors to protein within the membrane. Binding of dihydromorphine, but not D-Ala,D-Leu-enkephalin, was more sensitive to inhibition by ethanol (significant inhibition was observed at pharmacologically relevant concentrations, e.g., 50 mM) at 37°C and in the presence of guanosine 5′-triphosphate or Na^+. The latter observations suggest that ethanol influences the interaction of specific opiate receptor complexes with the G-protein. However, Rabin (368) as well as Hoffman and Tabakoff (204) showed that, although ethanol increased the activity of basal and guanosine 5′-triphosphate- and dopamine-stimulated adenylate cyclase in vitro, it did not modify the inhibition of striatal adenylate cyclase activity by morphine or D-Ala,D-Leu-enkephalin.

These results suggest either that the ethanol-induced changes in opiate receptor affinity are not relevant for receptor-coupled adenylate cyclase activity or that these two processes can be independently modulated. Another possible explanation for this apparent discrepancy is provided by the studies of Rapaka et al. (374) who found ethanol-induced conformational changes in peptide ligands for the opioid receptor. The Fourier transform infrared spectrum of Met-enkephalinamide in aqueous solution showed the presence of both β-turn and β-sheet conformations, and ethanol altered these secondary structures. These results suggest that the effect of ethanol on opioid ligand binding may be mediated by conformational changes of the ligand rather than those of the receptor. However it should be noted that high concentrations of ethanol, e.g., 1.0%, were required to produce observable changes in these Met-enkephalinamide spectra.

Studies reported suggest that some of the acute effects of ethanol may be mediated, in part, by alterations in endogenous opioid processes. However, much additional work is needed to elucidate biochemical and physiological mechanisms whereby ethanol may exert its putative action on these processes.

C. Neurotensin (NT)

Recent studies suggest that differences in ethanol sensitivity may, in part, be mediated by neurotensinergic processes and that ethanol acts in part via NT systems in the brain. NT is a tridecapeptide (p-Glu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH), initially isolated from bovine hypothalamus (52) and widely distributed in mammalian CNS (114). Evidence strongly supporting a neurotransmitter role for NT includes specific localization in discrete neuronal pathways, Ca^{++}-dependent release from brain slices (222), high-affinity specific binding sites in brain (289, 461), and receptor-mediated neurochemical and behavioral actions.

Immunohistochemical and radioimmunological studies show three major neurotensinergic pathways (nigrostriatal, mesolimbic, and mesocortical). These emanate from the substantia nigra and the VTA, as well as hypothalamic neurons in the arcuate nucleus (217, 223). Anatomical, pharmacological, and neurochemical data indicate a functional role of NT in dopaminergic pathways (329, 142). For example, there is evidence that NT immunoreactivity found in the NA, caudate, and arcuate neurons is colocalized with dopamine (330). Numerous neuronal perikarya of the VTA contain NT immunoreactivity. NT and dopamine are colocalized in some VTA neurons (142).

NT-binding sites have been characterized in the CNS of mouse, rat, human, and other species (235, 288, 289). Recent studies have focused on characterization of two NT receptor subtypes in mouse and rat (239, 317, 396). These receptors have been described as high-affinity (NT_2 or NT receptor) and low-affinity (NT_1 or NT acceptor) sites (396) and have been distinguished by biphasic ^{125}I-Tyr$_3$-NT or ^{125}I-Trp^3-NT scatchard analyses and by their selectivity toward a nonpeptide H_1 histamine antagonist, levocabastine (Janssen Pharmaceutica, Breese, Belgium). Levocabastine (1 mM) completely blocks NT binding to the NT_1 (low affinity) receptor without affecting the NT_2 (high-affinity) receptor (239, 396).

Characteristics of NT receptors on N1E-115 (a murine neuroblastoma cell line) cells have been well described (11, 155). These studies demonstrate coupling of NT receptors to cGMP formation. Recently, Richelson and coworkers (156, 236) extended these studies to include structure-activity relationships for NT enhancement of both cGMP formation and hydrolysis of inositol phospholipid. Other studies (10) have implicated guanosine 5′-triphosphate-binding protein in these actions of NT in N1E-115 cells. Current evidence suggests that NT receptors in brain slices and pituitary are coupled to production of inositol 1,4,5-trisphosphate by increasing inositol phospholipid hydrolysis (46, 160). Such action would be expected to increase intracellular Ca^{++} mobilization and enhance related events including protein phosphorylation and neurosecretion. Likewise, ethanol has been shown to alter several processes associated with intracellular Ca^{++} mobilization (see section IV, D).

Acute ethanol administration produces many of the behavioral effects elicited by central NT administration. Likewise, there are similarities in the effects of ethanol and NT on dopaminergic systems. Acute ethanol administration causes an increase in striatal dopamine turnover (19, 251). Kalivas and Taylor (234) reported that intra-VTA injections of NT increased dopamine turnover in the NA, an effect that was associated with an enhanced motor activity. The effects of ethanol on striatal dihydroxyphenylacetic acid levels were reported to depend on the genotype. Dihydroxyphenylacetic acid levels are elevated by ethanol, in vivo, in C57BL/6J but not in DBA/2J mice (19). It is of interest that C57 mice are less sensitive than DBA mice to the sedative effects of ethanol (431). Recently, investigators (218) reported that low doses of ethanol elicit an increased locomotor activity which is associated with an increase in dopamine release in the NA. A biphasic effect of ethanol was observed with depressant (high) doses producing a decrease in dopamine release.

That NT may be involved in centrally mediated actions of ethanol was suggested by Frye et al. (141) and Luttinger et al. (269) who observed that central administration of NT lengthened ethanol-induced sleep time and potentiated hypothermia induced by ethanol in rats. In recent studies Erwin et al. (120, 121, 316) observed that NT markedly increases the anesthetic and hypothermia potencies of ethanol in SS more than in LS mice. The effects of NT were specific as indicated by (*a*) dose-response and structure-activity relationships, (*b*) lack of efficacy of other peptides, and (*c*) absence of interaction with other anesthetics. Also, Widdowson (471) found that in rats NT increased hypnotic and hypothermia effects of ethanol.

Genetic selection of LS and SS mice was bidirectional with ethanol sensitivity of these lines diverging from the sensitivity of genetically heterogenous foundation stock (HS). Heterogenous mice are intermediate between LS and SS in ethanol sensitivity. NT, 1.25 μg, i.c.v., reduced the blood ethanol concentration at loss of righting reflex in both HS and SS mice to virtually the LS level. These results indicate that genetic differences in ethanol sensitivity in LS and SS mice might involve mechanisms associated with central NT processes. Another possibility is that activities of similar NT systems specifically alter signal transduction mechanisms that are different genetically in LS and SS mice. The altered anesthetic sensitivity was specific for ethanol in that NT did not alter pentobarbital- or halothane-induced sleep time in either LS or SS mice (120; V. G. Erwin, unpublished results).

The ability of NT to enhance ethanol-induced anesthesia in SS mice was specific for the carboxy terminal fragment of NT. NT analog N-acetyl-NT_{8-13}, but not NT_{1-8}, binds to NT receptors and elicits NT-like responses. N-acetyl-NT_{8-13} was less effective at 5 μg, i.c.v., than NT in decreasing the blood ethanol concentration at loss of righting reflex, but at this dose it was equivalent to NT in enhancing ethanol-induced hypothermia. In the absence of ethanol, NT-induced hypothermia was virtually identical in LS and SS mice, suggesting that NT-mediated processes involved in thermoregulation are similar in these mouse lines (118, 120). These results suggest that the effect of NT on ethanol sensitivity is the result of specific rather than nonspecific mechanisms. They are consistent with the possibility that more than one NT receptor or receptor-mediated process may be involved in the potentiation of anesthetic and hypothermic actions of ethanol.

It is well known that ethanol produces hypothermia. Ethanol produces greater dose-dependent hypothermia in LS than in SS mice with significant decreases in rectal temperature observed only at doses greater than 3 g/kg, i.p. The hypothermic effect of ethanol determined at loss of righting reflex was not altered in either LS or SS mice at low doses of NT, but at higher doses NT enhanced ethanol-induced hypothermia in both lines of mice (120, 121). NT, administered alone, induced a similar hypothermia in both SS and LS mice at doses greater than 0.02 μg. However, doses of ethanol (1.0 g/kg) or NT (0.005 μg, i.c.v.) that failed to cause hypothermia when

administered separately produced a pronounced hypothermia when administered together (121). Potentiation of NT and ethanol-induced hypothermia was greater in SS than in LS mice. These studies (121) confirmed those by other investigators (233–234) that sensitivity to NT-induced hypothermia was greater following i.c.v. administration than by infusion into the NA or VTA.

It is well known that NT effects on spontaneous locomotor activity are brain region specific (233, 234), with increases or decreases in activity observed after intra-VTA or i.c.v. injections, respectively. NT, i.c.v. or intra-NA, markedly inhibits ethanol-induced increase in locomotor activity in both SS and LS mice. After i.c.v. administration the sites of NT action in decreasing locomotor activity are not known. Apparently these sites do not differ between LS and SS mice because similar dose responses for NT-induced hypothermia were observed in these mice. NT administered intra-VTA produced a marked increase in locomotor activity in both LS and SS mice, an effect slightly more pronounced in SS than in LS animals. However, NT, intra-VTA, did not alter the effects of ethanol on locomotor activity (V. G. Erwin, unpublished results).

The above observations suggest that NT and ethanol act in a synergistic manner on specific neuronal processes mediating thermoregulation and spontaneous motor activity. It is of interest that i.c.v. doses of NT required to elicit hypothermia were greater (50 ng) than those needed to alter locomotor activity (5 ng). These results indicate that NT may act on separate neuronal pathways in altering thermoregulation and locomotor activity.

Evidence indicating that exogenous administration of NT produces specific pharmacological interactions with ethanol by actions through NT receptors provides a testable hypothesis that differences in acute sensitivity to ethanol are, in part, the result of genetic differences in specific NT-mediated processes. Potential sites of interaction of ethanol with neurotensinergic systems include (*a*) NT levels and turnover (synthesis and degradation) in neurons projecting to the frontal cortex, striatum, NA, and hypothalamus; (*b*) NT receptor (pre- or postsynaptic, high- or low-affinity); (*c*) NT receptor-coupling processes including second-messenger production; and (*d*) subsequent NT-mediated second-messenger responses (neurotransmitter release). Acute actions of ethanol could be altered by genetic differences in one or more of these neurotensinergic systems in discrete brain regions. For example, NT receptors in the NA and frontal cortex as well as receptors in the VTA and cerebellum are undoubtedly associated with behaviors characterized by motor functions, e.g., rearing and locomotor activity and perhaps the righting response. Hypothalamic receptors may be associated with thermoregulation. The hypothesis accommodates results of selective Ca^{++} interactions on ethanol sensitivity in LS and SS mice (72, 315) in that most of these NT processes are Ca^{++} dependent. Potentiating effects of coadministered Ca^{++} and NT (316) on ethanol sensitivity might suggest an ethanol-NT interaction with second-messenger systems coupled to increased intracellular Ca^{++}. Indeed, Snider et al. (424) have shown that NT increases not only inositol-tris-phosphate formation but also increases intracellular Ca^{++} in N1E-115 cells.

The hypothesis that some of ethanol's acute effects are mediated via interaction with NT receptors was investigated in LS and SS mice (118). NT receptors from brains of these mice showed virtually identical [^{3}H]-NT binding affinity, and ethanol, in vitro, had no effect on NT-binding parameters. However, the studies showed genetic differences in NT receptor densities in frontal cortex and striatum which might mediate the observed differences in NT-mediated behaviors between LS and SS mice. These might be responsible in part for the genetically selected differences in ethanol-induced anesthesia in these mice. It is tempting to speculate that some of the differences in behavioral effects of ethanol may be related to differences in NT receptor densities. This possibility is strengthened by the correlation between NT receptor density in the frontal cortex and ethanol sensitivity in LS, SS, and HS mice.

D. TRH

Over the past decade a number of studies have investigated the interactions between TRH, a CNS tripeptide, and acute effects of ethanol. Initial studies of Breese et al. (34) showed that TRH attenuates the hypnotic and hypothermic actions of ethanol. The ability of TRH to partially reverse depressant actions of ethanol is not drug specific in that soporific effects of barbiturates are also decreased by the peptide. In subsequent studies these investigators found that, in rats, TRH reversed the locomotor-depressant effects of ethanol. When administered with ethanol, TRH produced a marked potentiation in locomotor activity (36). In studies designed to determine the neuroanatomical locus of TRH actions, it was found that microinjection of TRH into the medial septum specifically antagonized ethanol-depressed locomotion.

Several studies have focused on elucidating mechanisms of TRH-ethanol interactions (35–37, 80). Although evidence suggests that the motor-activating effects of TRH are dependent upon mesolimbic catecholaminergic processes (307), neither noradrenergic nor dopaminergic systems appear to mediate the effects of TRH on ethanol sensitivity (34, 35, 80). For example, catecholamine antagonists failed to alter TRH-induced reduction in ethanol sleep time (80) and 6-hydroxydopamine-mediated depletion of dopamine was without effect on TRH enhancement of motor activation by ethanol (35). Likewise, 5,7-dihydroxytryptamine, a serotonergic neurotoxin, failed to alter TRH-ethanol interaction on locomotor activity. This observation is of interest because TRH is colocalized with serotonin in some

CNS pathways (224), thus a reduction in endogenous TRH might be expected with 5,7-dihydroxytryptamine. Although the neurochemical mechanism of TRH influence of ethanol sensitivity remains obscure, it is of interest that the primary anatomical site of action of TRH in altering ethanol responses appears to be the medial septum (291). Whether ethanol alters brain TRH levels, receptor characteristics, or TRH-mediated second-messenger processes remains to be determined.

E. Other Neuropeptides

Effects of acute ethanol administration on other neuropeptide systems thought to serve as neurotransmitters or modulators of neuronal activity have not been as well characterized as the interactions of ethanol and β-endorphin, NT, or TRH. For example, even at the behavioral level, the effects of CCK octapeptide on ethanol actions have been equivocal. Katsuura and Itoh (237, 238) reported that the sulfated peptide enhanced ethanol-induced hypothermia and sleep time in the rat. However, in subsequent studies Erwin et al. (120) found that CCK-sulfate administered i.c.v. did not alter ethanol-induced sleep time or hypothermia in SS or LS lines of mice. Although Ryder et al. (392) reported that acute ethanol administration was without affect on CCK levels in cortex, further studies need to be conducted to determine potential interactions of ethanol on CCK systems. Indeed, because it is well known that CCK is colocalized with dopamine in mesolimbic neurons (124) and that ethanol alters these dopaminergic processes (218), it is predicted that ethanol would alter CCK release and/or function.

The effects of vasopressin-like peptides on prolonging ethanol-induced tolerance have been well characterized, but this action of vasopressin may not be related to the acute pharmacological responses to ethanol (202). Arginine vasopressin was ineffective in altering hypothermia but increased the duration of loss of righting response after acute ethanol administration. Only recently have studies been reported concerning the effects of ethanol on vasopressin release. The studies of Hashimoto et al. (193) are not readily interpretable because extremely high concentrations (1.75 to 5%) of ethanol were used. However, Brinton et al. (38) reported that ethanol, in vitro, at 5 to 25 mM inhibited arginine vasopressin release from median eminence and ethanol greater than 50 mM enhanced arginine vasopressin release. These investigators showed that ethanol, in vivo, did not significantly alter arginine vasopressin content in the hypothalamus or neurohypophysis. These findings corroborated those of Colbern et al. (78) who showed that in rats ethanol, 2.0 g/kg, increased vasopressin release shortly after injection and subsequently inhibited release. Although these actions of ethanol on arginine vasopressin release may be related to the long recognized antidiuretic effect of ethanol, it remains to be determined whether any of the acute behavioral effects of ethanol are mediated by vasopressin-like peptidergic processes.

Although it is well known that somatostatin blocks hypothermia induced by a variety of CNS depressants including ethanol, no clear, specific behavioral interactions between ethanol and somatostatin have been reported. In the only mechanistic study to date, Mancillas et al. (279) reported that systemic administration of ethanol significantly enhanced inhibitory responses of somatostatin-14 iontophoretically applied to hippocampal pyramidal cells.

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REFERENCES

1. AHMAD, F. F., COWAN, D. L., AND SUN, A. Y.: Potentiation of ethanol-induced lipid peroxidation of biological membranes by vitamin C. Life Sci. **43:** 1169–1176, 1988.
2. AKIL, H., WATSON, S. J., YOUNG, E., LEWIS, M. E., KHACHATURIAN, H., AND WALKER, J. M.: Endogenous opioids: biology and function. Annu. Rev. Neurosci. **7:** 223–255, 1984.
3. ALBANO, E., TOMASI, A., GORIA-GATTI, L., POLI, G., AND DIANZANE, M. U.: Spin trapping of ethanol derived free radicals in rat liver microsomes. *In* Alcohol Toxicity and Free Radical Mechanisms, ed. by R. Hordmann, C. Ribiere, and H. Rouach, pp. 17–21, Pergamon Press, New York, 1988.
4. ALLAN, A. M., GALLAHER, E. J., GIONET, S. E., AND HARRIS, R. A.: Genetic selection for benzodiazepine ataxia produces functional changes in the gamma-aminobutyric acid receptor chloride channel complex. Brain Res. **452:** 118–126, 1988.
5. ALLAN, A. M., AND HARRIS, R. A.: Barbiturates modulate the actions of GABA on chloride channels of brain membranes. J. Pharmacol. Exp. Ther. **238:** 763–768, 1986.
6. ALLAN, A. M., AND HARRIS, R. A.: Gamma-aminobutyric acid and ethanol actions: neurochemical studies of long sleep and short sleep mice. Life Sci. **39:** 2005–2015, 1986.
7. ALLAN, A. M., AND HARRIS, R. A.: Involvement of neuronal chloride channels in ethanol intoxication, tolerance, and dependence. *In* Recent Developments in Alcoholism, ed. by M. Galanter, vol. 5, pp. 313–325, Plenum Publishing Corporation, New York, 1987.
8. ALLAN, A. M., SPUHLER, K. P., AND HARRIS, R. A.: Gamma-aminobutyric acid-activated chloride channels: relationship to genetic differences in ethanol sensitivity. J. Pharmacol. Exp. Ther. **244:** 866–870, 1988.
9. ALLISON, J. H., AND CICERO, T. J.: Alcohol acutely depresses myoinositol 1-phosphate levels in the male rat cerebral cortex. J. Pharmacol. Exp. Ther. **213:** 24–27, 1980.
10. AMAR, S., KITABGI, P., AND VINCENT, J. P.: Stimulation of inositol phosphate production by neurotensin in neuroblastoma N1E115 cells: implication of GTP-binding proteins and relationship with the cyclic GMP response. J. Neurochem. **49:** 999–1006, 1987.
11. AMAR, S., MAZELLA, J., CHECLER, F., KITABGI, P., AND VINCENT, J. P.: Regulation of cyclic GMP levels by neurotensin in neuroblastoma clone N1E115. Biochem. Biophys. Res. Commun. **129:** 117–125, 1985.
12. ANTONIAN, L., SHINITZKY, M., SAMUEL, D., AND LIPPA, A. S.: AL 721, a novel membrane fluidizer. Neurosci. Biobehav. Rev. **11:** 399, 1987.
13. ARAGON, C. M. G., AND AMIT, Z.: A two dimensional model of alcohol consumption: possible interaction of brain catalase and aldehyde dehydrogenase. Alcohol **2:** 357–360, 1985.
14. ARAGON, C. M. G., STERNKLAR, G., AND AMIT, Z.: A correlation between voluntary ethanol consumption and brain catalase activity in the rat. Alcohol **2:** 353–356, 1985.
15. ASTON-JONES, G., FOOTE, S. L., AND BLOOM, F. E.: Low doses of ethanol disrupt sensory responses of brain noradrenergic neurones. Nature (Lond.) **296:** 857–860, 1982.
16. ATKINSON, J. P., SULLIVAN, T. J., KELLEY, J. P., AND PARKER, C. W.: Stimulation by alcohols of cyclic AMP metabolism in human leukocytes: possible role of cyclic AMP in the anti-inflammatory effects of ethanol. J. Clin. Invest. **60:** 284–294, 1977.
17. BAKER, R. C.: Disassociation of cerebellar phospholipid composition and acute ethanol effects in mice selectively bred for differential sensitivity to ethanol. Alcohol Drug Res. **7:** 291–299, 1987.
18. BAKER, R., MELCHIOR, C., AND DEITRICH, R.: The effect of halothane on mice selectively bred for differential sensitivity to alcohol. Pharmacol. Biochem. Behav. **12:** 691–695, 1980.
19. BARBACCIA, M. L., REGGIANI, A., SPANO, P. F., AND TRABUCCHI, M.: Ethanol effects of dopaminergic function: modulation by the endogenous opioid system. Pharmacol. Biochem. Behav. **13** (suppl 1)**:** 303–306, 1980.
20. BARRET, L., BOURHIS, F., DANEL, V., AND DEBRU, J. L.: Determination of

β-endorphin in alcoholic patients in the acute stage of intoxication: relation with naloxone therapy. Drug Alcohol Depend. **19:** 71–78, 1987.
21. BASILE, A., HOFFER, B., AND DUNWIDDIE, T.: Differential sensitivity of cerebellar Purkinje neurons to ethanol in selectively outbred lines of mice: maintenance in vivo independent of synaptic transmission. Brain Res. **264:** 69–78, 1983.
22. BAUCHE, F., BOURDEAUX-JAUBERT, A. M., GIUDICELLI, Y., AND HORDMANN, R.: Ethanol alters the adenosine receptor-Ni-mediated adenylate cyclase inhibitory response in rat brain cortex in vitro. FEBS Lett. **219:** 296–300, 1987.
23. BEAUGE, F., FLEURET, C., BARIN, F., AND HORDMANN, R.: Brain membrane disordering after acute in vivo administration of ethanol, isopropanol or t-butanol in rats. Biochem. Pharmacol. **33:** 3591–3595, 1984.
24. BECKER, N. C. Effects of the imidazobenzodiazepine R015–4513 on the stimulant and depressant actions of ethanol on spontaneous locomotor activity. Life Sci. **43:** 643–650, 1988.
25. BENSON, D. M., BLITZER, R. D., AND LANDAU, E. M.: Cyclic-AMP blocks ethanol suppressant effect on hippocampal neuronal firing. Soc. Neurosci. Abstr. **13:** 502, 1987.
26. BERGER, T., FRENCH, E. D., SIGGINS, G. R., SHIER, W. T., AND BLOOM, F. E.: Ethanol and some tetrahydroisoquinolines alter the discharge of cortical and hippocampal neurons: relationship to endogenous opioids. Pharmacol. Biochem. Behav. **17:** 813–821, 1982.
27. BIGGIO, G., AND COSTA, E. (eds.): Chloride Channels and Their Modulation by Neurotransmitters and Drugs. Raven Press, New York, 1988.
28. BIGGIO, G., COSTA, E., AND GUIDOTTE, A.: Pharmacologically induced changes in the 3′:5′-cyclic guanosine monophosphate content of rat cerebellar cortex: difference between apomorphine, haloperidol and harmaline. J. Pharmacol. Exp. Ther. **200:** 207–215, 1977.
29. BLACK, A. B., JR., SANDQUIST, D., WEST, J. R., WAMSLEY, J. K., AND WILLIAMS, T. N.: Muscarinic cholinergic stimulation increases cyclic GMP levels in rat hippocampus. J. Neurochem. **33:** 1165–1168, 1979.
30. BLOOM, F. E.: The endorphins: a growing family of pharmacologically pertinent peptides. Annu. Rev. Pharmacol. Toxicol. **23:** 151–170, 1983.
31. BLOOM, F., BARCHAS, J., SANDLER, M., USDIN, E.: Beta-carbolines and tetrahydroisoquinolines. Prog. Clin. Biol. Res. **90:** 428, 1982.
32. BLOOM, F. E., AND SIGGINS, G. R.: Electrophysiological action of ethanol at the cellular level. Alcohol **4:** 331–337, 1987.
33. BOYD, H. D., AND COHEN, J. B.: Desensitization of membrane-bound Torpedo acetylcholine receptor by amine noncompetitive antagonists aliphatic alcohols: studies of [3H]acetylcholine binding and $^{22}Na+$ ion fluxes. Biochemistry **23:** 4023–4033, 1984.
34. BREESE, G. R., COTT, J. M., COOPER, B. R., PRANGE, A. J., AND LIPTON, M. A.: Antagonism of ethanol narcosis by thyrotropin releasing hormone. Life Sci. **14:** 1053–1063, 1974.
35. BREESE, G. R., COYLE, S., FRYE, G. D., AND MUELLER, R. A.: Effects of TAN, ethanol, and TAN-ethanol combination on activity in rats with altered monoamine content. Pharmacol. Biochem. Behav. **22:** 1013–1018, 1985.
36. BREESE, G. R., COYLE, S., TOWLE, A. C., MUELLER, R. A., MCCOWN, T. J., AND FRYE, G. D.: Ethanol-induced locomotor stimulation in rats after thyrotropin-releasing hormone. J. Pharmacol. Exp. Ther. **229:** 731–737, 1984.
37. BREESE, G. R., FRYE, G. D., MCCOWN, T. J., AND MUELLER, R. A.: Comparison of the CHS effects induced by TAN and bicuculline after microinjection into medial septum, substantia nigra and inferior colliculus: absence of support for a GABA antagonist action for TRH. Pharmacol. Biochem. Behav. **21:** 145–149, 1984.
38. BRINTON, R. E., GRUENER, R., DESHMUKH, P., AND YAMAMURA, H. I.: in vitro inhibition of vasopressin release in brain by behavioral relevant ethanol concentrations. Neurosci. Lett. **67:** 213–217, 1986.
39. BRODIE, M. S., SHEFNER, S. A., AND DUNWIDDIE, T. V.: Ethanol increases the firing rate of dopamine neurons of the ventral tegmental area in vitro. Alcohol. Clin. Exp. Res. **12:** 116, 1988.
40. BROWN, O. M., POST. M. E., AND MALLOV, S.: Effects of ethanol on rat brain and heart acetylcholine. J. Stud. Alcohol **38:** 603–617, 1977.
41. BRUNO, C., CUPPINI, R., AND CUPPINI, C.: Effect of ethanol on the spontaneous transmitter release by nerve endings in the process of maturation. Drug Alcohol Depend. **18:** 127–131, 1986.
42. BUCK, K. J., ALLAN, A. M., AND HARRIS, R. A.: Fluidization of brain membranes by A_2C does not produce anesthesia and does not augment muscimol-stimulated $^{36}Cl^-$ influx. Eur. J. Pharmacol. **160:** 359–367, 1989.
43. BUSTOS, G., ROTH, R. N., AND MORGENROTH, V. H., III: Activation of tyrosine hydroxylase in rat striatal slices by K+-depolarization effect of ethanol. Biochem. Pharmacol. **25:** 2493–2497, 1976.
44. BUREAU, M., AND OLSEN, R. W.: Gamma-aminobutyric acid/benzodiazepine receptor protein carries binding sites for both ligands on both two major peptide subunits. Biochem. Biophys. Res. Commun. **153:** 1006–1011, 1988.
45. CAMACHO-HASI, P., AND TREISTMAN, S. N.: Ethanol effects on voltage-dependent membrane conductances: comparative sensitivity of channel populations in *Aplysia* neurons. Cell Mol. Neurobiol. **6:** 263–279, 1986.
46. CANONICO, P. L., SORTINO, M. A., SPECIALE, C., AND SCAPAGNINI, U.: Neurotensin stimulates polyphosphoinositide breakdown and prolactin release in anterior pituitary cells in culture. Mol. Cell. Endocrinol. **42:** 215–220, 1985.
47. CARLEN, P. L., BLAXTER, T. J., FRIEDMAN, E. V., AND DURAND, D.: Putative capacitance increase in hippocampal CA1 cells following ethanol application. *In* Molecular and Cellular Mechanisms of Anesthetics, ed. by S. H. Roth and K. W. Miller, pp. 57–64, Plenum Publishing Corporation, New York, 1986.
48. CARLEN, P. L., GUREVICH, H., DAVIES, M. F., BLAXTER, T. J., AND O'BEIRNE, M.: Enhanced neuronal K+ conductance: a possible common mechanism for sedative-hypnotic drug action. Can. J. Physiol. Pharmacol. **63:** 831–837, 1985.
49. CARLEN, P. L., GUREVICH, N., AND DURAND, D.: Ethanol in low doses augments calcium-mediated mechanisms measured intracellularly in hippocampal neurons. Science (Wash. DC) **215:** 306–309, 1982.
50. CARLEN, P. L., AND WU, P. H.: Calcium and sedative-hypnotic drug actions. Int. Rev. Neurobiol. **29:** 161–189, 1988.
51. CARMICHAEL, F. J., AND ISRAEL, Y.: Effects of ethanol on neurotransmitter release by rat brain cortical slices. J. Pharmacol. Exp. Ther. **193:** 824–834, 1975.
52. CARRAWAY, R., AND LEEMAN, S. E.: Radioimmunoassay for neurotensin, a hypothalamic peptide. J. Biol. Chem. **251:** 7035–7044, 1976.
53. CASH, D. J., AOSHIMA, H., AND NESS, G. P.: Acetylcholine-induced cation translocation across cell membranes and inactivation of the acetylcholine receptor: chemical kinetic measurements in the millisecond time region. Proc. Natl. Acad. Sci. USA **78:** 3318–3322, 1981.
54. CASH, D. J., AND SUBBARAO, K.: Channel opening of gamma-aminobutyric acid receptor from rat brain: molecular mechanisms of the receptor responses. Biochemistry **26:** 7562–7570, 1987.
55. CASH, D. J., AND SUBBARAO, K.: Different effects of pentobarbital on two gamma-aminobutyrate receptors from rat brain: channel opening, desensitization, and an additional conformational changes. Biochemistry **27:** 4580–4590, 1988.
56. CEDERBAUM, A. I., AND DICKER, E.: Inhibition of microsomal oxidation of alcohols and of hydroxyl-radical scavenging agents by the iron-chelating agent desferrioxamine. Biochem. J. **210:** 107–113, 1983.
57. CEDERBAUM, A. I., MIWA, G., COHEN, G., AND LU, A. Y. H.: Production of hydroxyl radicals and their role in the oxidation of ethanol by a reconstituted microsomal system containing cytochrome P-450 purified from phenobarbital-treated rats. Biochem. Biophys. Res. Commun. **91:** 747–754, 1979.
58. CEDERBAUM, A. I. Organic hydroperoxide-dependent oxidation of ethanol by microsomes: Lack of a role for free hydroxyl radicals. Arch. Biochem. Biophys. **227:** 329–338, 1983.
59. CEDERBAUM, A. I., DICKER, E., AND COHEN, G.: Effect of hydroxyl radical-scavengers on microsomal oxidation of alcohols and on associated microsomal reactions. Biochemistry **17:** 3058–3064, 1978.
60. CELENTANO, J. J., GIBBS, T. T., AND FARB, D. H.: Ethanol potentiates GABA-and glycine-induced chloride currents in chick spinal cord neurons. Brain Res. **455:** 377–380, 1988.
61. CENTELLES, J. J., FRANCO, R., AND BOZAL, J.: Purification and partial characterization of brain adenosine deaminase: inhibition by purine compounds and by drugs. J. Neurosci. Res. **19:** 258–267, 1988.
62. CHANDRASEKHAR, R., NUANG, H-M., AND SUN, G. Y.: Alterations in rat brain polyphosphoinositide metabolism due to acute ethanol administration J. Pharmacol. Exp. Ther. **245:** 120–123, 1988.
63. CHAPIN, J. K., SORENSEN, S. M., AND WOODWARD, D. J.: Acute ethanol effects on sensory responses of single units in the somatosensory cortex of rats during different behavioral states. Pharmacol. Biochem. Behav. **25:** 607–614, 1986.
64. CHAPIN, J. K., AND WOODWARD, D. J.: Ethanol's effect on selective gating of somatic sensory inputs to single cortical neurons. Pharmacol. Biochem. Behav. **18:** 489–493, 1983.
65. CHARNESS, M. E., GORDON, A. S., AND DIAMOND, I.: Ethanol modulation of opiate receptors in cultured neural cells. Science (Wash. DC) **222:** 1246–1248, 1983.
66. CHIK, C. L., HO, A. K., AND KLEIN, D. C.: Ethanol inhibits dual receptor stimulation of pineal cAMP and cGMP by vasoactive intestinal peptide and phenylephrine. Biochem. Biophys. Res. Commun. **147:** 145–151, 1987.
67. CHIN, J. H., AND GOLDSTEIN, D. B.: Drug tolerance in biomembranes: a spin label study of the effects of ethanol. Science (Wash. DC) **196:** 684–685, 1977.
68. CHIN, J. H., AND GOLDSTEIN, D. B.: Membrane-disordering action of ethanol: variation with membrane cholesterol content and depth of the spin labeled probe. Mol. Pharmacol. **19:** 425–431, 1981.
69. CHIN, J. H., AND GOLDSTEIN, D. B.: Cholesterol blocks the disordering effects of ethanol in biomembranes. Lipids **19:** 929–935, 1984.
70. CHIN, J. H., PARSONS, L. M., AND GOLDSTEIN, D. B.: Increased cholesterol content of erythrocyte and brain membranes in ethanol-tolerant mice. Biochim. Biophys. Acta **513:** 358–363, 1978.
71. CHU, N. S.: Effects of ethanol on rat cerebellar Purkinje cells. Int. J. Neurosci. **21:** 265–277, 1983.
72. CHU, N. S.: Responses of midbrain raphe neurons to ethanol. Brain Res. **311:** 348–352, 1984.
73. CHU, N. S., AND KEENAN, L.: Responses of midbrain dorsal raphe neurons to ethanol studied in brainstem slices. Alcohol **4:** 373–374, 1987.
74. CHURCH, A. C., AND FELLER, D.: The influence of mouse genotype on the

changes in brain cyclic nucleotide levels induced by acute alcohol administration. Pharm. Biochem. Behav. **10:** 335–338, 1979.
75. CICERO, T. J.: Neuroendocrinological effects of alcohol. Annu. Rev. Med. **32:** 123–142, 1981.
76. CLEMENT, J. G.: Ethanol potentiation of choline, acetylcholine, carbachol and phenyltrimethylammonium contractions in the chick biventer cervicis muscle. Eur. J. Pharmacol. **61:** 195–198, 1980.
77. COHEN, G.: An acetaldehyde artifact in studies of the interaction of ethanol with biogenic amine systems: the oxidation of ethanol by ascorbic acid. J. Neurochem. **29:** 761–762, 1977.
78. COLBERN, D. L., TEN NAAF, J., TABAKOFF, B., AND VAN WIMERSMA GREIDANUS, T. B.: Ethanol increases plasma vasopressin shortly after intraperitoneal injection in rats. Life Sci. **37:** 1029–1032, 1985.
79. COLLINS, A. C., GILLIAM, D. M., AND MINER, L. L.: Indomethacin pretreatment blocks the effects of high concentration of ethanol. Alcohol. Clin. Exp. Res. **9:** 371–376, 1985.
80. COTT, J. M., BREESE, G. R., COOPER, B. R., BARLOW, S., AND PRANGE, A. J., JR.: Investigations into the mechanism of reduction of ethanol sleep by thyrotropin-releasing hormone (TAN). J. Pharmacol. Exp. Ther. **196:** 594–604, 1976.
81. CRABBE, J. C., ALLEN, R. G., GAUDETTE, D. N., YOUNG, E. R., KOSOBUD, A., AND STACK, J.: Strain differences in pituitary β-endorphin and ACTN content in inbred mice. Brain Res. **219:** 219–223, 1981.
82. DANIELL, L. C., BRASS, E. P., AND HARRIS, R. A. Effect of ethanol on intracellular ionized calcium concentrations in synaptosomes and hepatocytes. Mol. Pharmacol **32:** 831–837, 1987.
83. DANIELL, L. C., AND HARRIS, R. A. Heuronal intracellular calcium concentrations are altered by anesthetics: relationship to membrane fluidization. J. Pharmacol Exp. Ther. **245:** 1–7, 1988.
84. DANIELL, L. C., AND HARRIS, R. A. Effect of chronic ethanol treatment and selective breeding for hypnotic sensitivity to ethanol on intracellular ionized calcium concentrations in synaptosomes. Alcohol Clin. Exp. Res. **12:** 179–183, 1988.
85. DANIELL, L. C., AND HARRIS, R. A.: Ethanol and inositol 1,4,5-trisphosphate release calcium from separate stores of brain microsomes. J. Pharmacol. Exp. Ther. **250:** 875–881, 1989.
86. DAR, M. S., MUSTAFA, S. J., AND WOOLES, W. R.: Possible role of adenosine in the CHS effects of ethanol. Life Sci. **33:** 1363–1374, 1983.
87. DAR, M. S., AND WOOLES, W. R.: The effect of acute ethanol on dopamine metabolism and other neurotransmitters in the hypothalamus and corpus striatum of mice. J. Neural Transmission **60:** 283–294, 1984.
88. DAR, M. S., AND WOOLES, W. R.: Effect of chronically administered methylxanthines on ethanol-induced motor incoordination in mice. Life Sci. **39:** 1429–1437, 1986.
89. DAVIDOFF, R. A.: Alcohol and presynaptic inhibition in an isolated spinal cord preparation. Arch. Neurol. **28:** 60–63, 1973.
90. DAVIDSON, M., WILCE, P., AND SHANLEY, B. Ethanol increases synaptosomal free calcium concentration. Neurosci Lett. **89:** 165–169, 1988.
91. DAVIS, T. P., CULLING-BERGLUND, A. J., GILLESPIE, T. J., AND SMITH, T. L.: Ethanol treatment alters β-endorphin metabolism by purified synaptosomal plasma membranes. Peptides **8:** 467–472, 1987.
92. DE FIEBRE, C. M., MEDHURST, L. J., AND COLLINS, A. C: Nicotine response and nicotinic receptors in long-sleep and short-sleep mice. Alcohol **4:** 493–501, 1987.
93. DEITRICH, R. A. Biochemical aspects of alcoholism. Psychoneuroendocrinology **1:** 325–346, 1976.
94. DEITRICH, R. A., AND ERWIN, V. G.: Biogenic amine-aldehyde condensation products: tetrahydroisoquinolines and tryptolines B-carbolines. Annu. Rev. Pharmacol. Toxicol. **20:** 55–80, 1980.
95. DE YOUNG, L. R., AND DILL, K. A.: Solute partitioning into lipid bilayer membranes. Biochemistry **27:** 5281–5289, 1988.
96. DI CHIARA, G., AND IMPERATO, A.: Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. Eur. J. Pharmacol. **115:** 131–132, 1985.
97. DI CHIARA, G., AND IMPERATO, A.: Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. Ann. NY Acad. Sci. **473:** 367–381, 1986.
98. DI CHIARA, G., AND IMPERATO, A.: Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA **85:** 5274–5278, 1988.
99. DIAMOND, I., WRUBEL, B., ESTRIN, W., AND GORDON, A.: Basal and adenosine receptor-stimulated levels of cAMP are reduced in lymphocytes from alcoholic patients. Proc. Natl. Acad. Sci. USA **84:** 1413–1416, 1987.
100. DILUZIO, N. R.: Prevention of the acute ethanol-induced fatty liver by the simultaneous administration of antioxidants. Life Sci. **3:** 113–118, 1964.
101. DODSON, R. A., AND JOHNSON W. E.: Effects of ethanol, arecoline, atropine and nicotine alone and in various combinations on rat cerebellar cyclic guanosine 3′, 5′-monophosphate. Neuropharmacology **18:** 871–876, 1979.
102. DODSON, R. A., AND JOHNSON, W. E.: Effects of general central nervous system depressants with and without calcium ionophore A23187 on rat cerebellar cyclic guanosine 3′,5′-monophosphate. Res. Commun. Chem. Pathol. Pharmacol. **29:** 265–280, 1980.
103. DOGTEROM, P., HAGELKERKE, L. F., VAN STEVENINCK, J., AND MULDER, G. J.: Inhibition of lipid peroxidation by disulfiram and diethyldithiocarbamate does not prevent hepatotoxin-induced cell death in isolated rat hepatocytes: a study with allyl alcohol, terbutyl hydroperoxide, diethylmaleate, bromoisovalerylurea and carbon tetrachloride. Chem. Biol. Interact. **66:** 251–265, 1988.
104. DOLIN, S. J., AND LITTLE, H. J.: Augmentation by calcium channel antagonists of general anaesthetic potency in mice. Br. J. Pharmacol. **88:** 909–914, 1986.
105. DOLIN, S. J., NALSEY, M. J., AND LITTLE, H. J.: Effects of the calcium channel activator Bay K 8644 on general anaesthetic potency in mice. Br. J. Pharmacol. **94:** 413–422, 1988.
106. DUNWIDDIE, T. V.: The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. **27:** 63–139, 1985.
107. DUNWIDDIE, T. V., MUELLER, A., PALMER, M., STEWART, J., AND HOFFER, B.: Electrophysiological interactions of enkephalins with neuronal circuitry in the rat hippocampus: I. Effects on pyramidal cell activity. Brain Res. **184:** 311–330, 1980.
108. DUNWIDDIE, T. V., ROBERSON, N. L., AND WORTH, T.: Modulation of long-term potentiation: effects of adrenergic and neuroleptic drugs. Pharm. Biochem. Behav. **17:** 1257–1264, 1982.
109. DUNWIDDIE, T. V., WORTH, T. S, AND TAYLOR, M.: Adenosine modulates the effects of ethanol on hippocampal evoked activity in in vitro brain slices. Alcohol. Clin. Exp. Res. **13:** 314, 1989.
110. DURAND, D., CORRIGALL, W. A., KUJTAN, P., AND CARLEN, P. L.: Effects of low concentrations of ethanol on CA1 hippocampal neurons in vitro. Can. J. Physiol. Pharmacol. **59:** 979–984, 1981.
111. DURKIN, T. P., HOSSEIN, H-Z., MANDEL, P., AND EBEL, A.: A comparative study of the acute effects of ethanol on the cholinergic system in hippocampus and striatum of inbred mouse strains. J. Pharmacol. Exp. Ther. **22:** 203–208, 1982.
112. EL-FAKAHANY, E. F., MILLER, E. R., ABBASSY, M. A., ELDEFRAWI, A. T., AND ELDEFRAWI, M. E.: Alcohol modulation of drug binding to the channel sites of the nicotinic acetylcholine receptor. J. Pharmacol. Exp. Ther. **224:** 289–296, 1983.
113. ELLINGSON, J. S., TARASCHI, T. F., WIN, A., ZIMMERMAN, R., AND RUBIN, E.: Cardiolipin from ethanol-fed rats confers tolerance to ethanol in liver mitochondrial membranes. Proc. Natl. Acad. Sci. USA **85:** 3353–3357, 1988.
114. EMSON, P. C., GOEDERT, M., HORSFIELD, P., RIOUX, R., AND ST. PIERRE, S.: The regional distribution and chromatographic characterization of neurotensin-like immunoreactivity in the rat central nervous system. J. Neurochem. **38:** 992–999, 1982.
115. ERICKSON, C. K., AND GRAHAM, D. T.: Alteration of cortical and reticular acetylcholine release by ethanol in vivo. J. Pharmacol. Exp. Ther. **185:** 583–593, 1973.
116. ERICKSON, C. K., AND BURNAM, W. L.: Cholinergic alteration of ethanol-induced sleep and death in mice. Agents Actions **21:** 8–13, 1971.
117. ERIKSSON, C. J. P.: Problems and pitfalls in acetaldehyde determinations. Alcohol. Clin. Exp. Res. **4:** 22–29, 1980.
118. ERWIN, V. G., AND KORTE, A.: Brain neurotensin receptors in mice bred for differences in sensitivity to ethanol. Alcohol **5:** 195–201, 1988.
119. ERWIN, V. G., KORTE, A., AND JONES. B. C.: Central muscarinic cholinergic influences on ethanol sensitivity in long-sleep and short-sleep mice. J. Pharmacol. Exp. Ther. **247:** 857–862, 1988.
120. ERWIN, V. G., KORTE, A., AND MARTY, M.: Neurotensin selectively alters ethanol-induced anesthesia in LS/Ibg and SS/Ibg lines of mice. Brain Res. **400:** 80–90, 1987.
121. ERWIN, V. G., AND SU, N. C.: Neurotensin and ethanol interactions on hypothermic and locomotor activity in LS and SS mice. Alcohol. Clin. Exp. Res. **13:** 91–94, 1989.
122. ESKURI, S. A., AND POZOS, R. S.: The effect of ethanol and temperature on calcium-dependent sensory neuron action potentials. Alcohol. Drug Res. **7:** 153–162, 1987.
123. FAIRHURST, A. S., AND LISTON, P.: Effects of alkanols and halothane on rat brain muscarinic and a-adrenergic receptors. Eur. J. Pharmacol. **58:** 59–66, 1979.
124. FALLON, J. H.: Topographical organization of ascending dopaminergic projections. Ann. NY Acad. Sci. **537:** 1–9, 1988.
125. FERRENDELLI, J. A., KINSSCHERF, D. A., AND KIPNIS, D. M.: Effects of amphetamine, chlorpromazine and reserpine on cyclic GMP and cyclic AMP levels in mouse cerebellum. Biochem. Biophys. Res. Commun. **46:** 2114–2120, 1972.
126. FERRENDELLI, J. A., STEINER, A. L., MCDOUGAL, D. B., JR., AND KIPNIS, D. M.: The effect of oxotremorine and atropine on cGMP and cAMP levels in mouse cerebral cortex and cerebellum. Biochem. Biophys. Res. Commun. **41:** 1061–1067, 1970.
127. FINGER, W., AND STETTMEIER, N.: Postsynaptic actions of ethanol and methanol in crayfish neuromuscular junctions. Pflugers Arch. **400:** 113–120, 1984.
128. FINN, R. C., BROWNING, M., AND LYNCH, G.: Trifluoperazine inhibits hippocampal long-term potentiation and the phosphorylation of a 40,000 dalton protein. Neurosci. Lett. **19:** 103–108, 1980.
129. FISHMAN, B. E., AND GIANUTSOS, G.: Opposite effects of different hexachlorocyclohexane lindane isomers on cerebellar cyclic GMP. Relation of cyclic GMP accumulation to seizure activity. Life Sci. **41:** 1703–1709, 1987.
130. FONG, T. M., AND MCNAMEE, M. G.: Correlation between acetylcholine

receptor function and structural properties of membranes. Biochemistry **25:** 830–840, 1986.
131. FORMAN, S. A., VERKMAN, A. S., DIX, J. A., AND SOLOMON, A. K.: *n*-Alkanols and halothane inhibit red cell anion transport and increase band 3 conformational change rate. Biochemistry **24:** 4859–4866, 1985.
132. FOWLER, C. J., CALLINGHAM, B. A., MANTLE, T. J., AND TIPTON, K. F.: The effect of lipophilic compounds upon the activity of rat liver mitochondrial monoamine oxidase-A and -B. Biochem. Pharmacol. **29:** 1177–1183, 1980.
133. FRANKLIN, C. L., AND GRUOL, D. L.: Acute ethanol alters the firing pattern and glutamate response of cerebellar Purkinje neurons in culture. Brain Res. **416:** 205–218, 1987.
134. FRANKS, N. P., AND LIEB, W. R.: Molecular mechanisms of general anaesthesia. Nature (Lond.) **300:** 487–493, 1982.
135. FRANKS, N. P., AND LIEB, W. R.: What is the molecular nature of general anaesthetic target sites? Trends Pharm. Sci. **8:** 169–174, 1987.
136. FRANKS, N. P., AND LIEB, W. R.: Volatile general anaesthetics activate a novel neuronal K+ current. Nature (Lond.) **333:** 662–664, 1988.
137. FREDHOLM, B. B., ZAHNISER, N. R., WEINER, G. R., PROCTOR, W. R., AND DUNWIDDIE, T. V.: Behavioural sensitivity to PIA in selectively bred mice is related to a number of Al adenosine receptors but not to cyclic AMP accumulation in brain slices. Eur. J. Pharmacol. **111:** 133–136, 1985.
138. FRENCH, W., IHRIG, T. J., AND PETTIT, N. B.: Effect of alcohol on the plasma cAMP response to glucagon. Res. Commun. Chem. Pathol. Pharmacol. **26:** 209–212, 1979.
139. FRIEDMAN, R. N., BITTNER, G. D., AND BLUNDON, J. A.: Electrophysiological and behavioral effects of ethanol on crayfish. J. Pharmacol. Exp. Ther. **246:** 125–131, 1988.
140. FRYE, G. D., AND FINCHER, A. S.: Effect of ethanol on gamma-vinyl GABA-induced GABA accumulation in the substantia nigra and on synaptosomal GABA content in six rat brain regions. Brain Res. **449:** 71–79, 1988.
141. FRYE, G. D., LUTTINGER, D., NEMEROFF, C. B., VOGEL, R. A., PRANGE, A. J., JR., AND BREESE, G. R.: Modification of the actions of ethanol by centrally active peptides. Peptides **2** (suppl 1): 99–106, 1981.
142. FUXE, K., AGNATI, L. F., ANDERSSON, K., ENEROTH, P., HARTSTRAND, A., GOLDSTEIN, M., AND ZOLI, M.: Studies on neurotensin-catecholamine interactions in the hypothalamus and in the forebrain of the male rat. Neurochem. Int. **6:** 737–750, 1984.
143. GAGE, P. W.: The effect of methyl, ethyl and *n*-propyl alcohol on neuromuscular transmission in the rat. J. Pharmacol. Exp. Ther. **150:** 236–243, 1965.
144. GAGE, P. W., AND ROBERTSON, B. Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus. Br. J. Pharmacol. **85:** 675–681, 1985.
145. GALLAHER, E. J., AND GIONET, S. E. Initial sensitivity and tolerance to ethanol in mice genetically selected for diazepam sensitivity. Alcohol Clin. Exp. Res. **12:** 77–80, 1988.
146. GARTHWAIT, J., CHARLES, S. L., AND CHESS-WILLIAMS, R.: Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature (Lond.) **336:** 385–388, 1988.
147. GEORGE, F., AND CHIN, H. S.: Effects of ethanol on Purkinje cells recorded from cerebellar slices. Alcohol **1:** 353–358, 1984.
148. GEORGE, F. R., AND COLLINS, A. C.: Ethanol's behavioral effects may be partly due to increases in brain prostaglandin production. Alcohol Clin. Exp. Res. **9:** 143–145, 1985.
149. GEORGE, F. R., ELMER, G. I., AND COLLINS, A. C.: Indomethacin significantly reduces mortality due to acute ethanol overexposure. Substance Alcohol Actions/Misuse **3:** 267–274, 1982.
150. GEORGE, F. R., HOWERTON, T. C., ELMER, G. I., AND COLLINS, A. C.: Antagonism of alcohol hypnosis by blockade of prostaglandin synthesis and activity: genotype and time course effects. Pharm. Biochem. Behav. **19:** 131–136, 1983.
151. GEORGE, F. R., RITZ, M. C., ELMER, G. I., AND COLLINS, A. C.: Time course of ethanol's effects on brain prostaglandins in LS and SS mice. Life Sci. **39:** 1069–1075, 1986.
152. GESSA, G. L., MUNTONI, F., COLLU, M., VARGIU, L., AND MEREU, G.: Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. **348:** 201–203, 1985.
153. GIANOULAKIS, C., AND BARCOMB, A.: Effect of acute ethanol in vivo on the β-endorphin system in the rat. Life Sci. **40:** 19–28, 1987.
154. GIANOULAKIS, C., AND GUPTA, A.: Inbred strains of mice with variable sensitivity to ethanol exhibit differences in the content and processing of β-endorphin. Life Sci. **39:** 2315–2325, 1986.
155. GILBERT, J. A., AND RICHELSON, E.: Neurotensin stimulates formation of cyclic GMP in murine neuroblastoma clone N1E-115. Eur. J. Pharmacol. **99:** 245–246, 1984.
156. GILBERT, J. A., AND RICHELSON, E.: LANT-6, xenopsin and neuromedin H stimulate cyclic GMP at neurotensin receptors. Eur. J. Pharmacol. **129:** 379–383, 1986.
157. GINTZLER, A. R., AND SCALISI, J. A.: Opioid-mediated acute response to alcohol: ethanol potentiates opioid actions on the guinea pig ileum. Brain Res. **269:** 391–394, 1983.
158. GIVENS, B. S., AND BREESE, G. R.: Ethanol reduces single unit activity and disrupts the rhythmically bursting pattern of neurons in the rat medial septum/diagonal band. Soc. Neurosci. Abstr. **13:** 502, 1987.
159. GLANZMAN, D. L., AND EPPERLEIN, R. C.: Disruption of vertebrate monosynaptic habituation by ethyl alcohol. Brain Res. **212:** 117–126, 1980.
160. GOEDERT, M., PINNOCK, R. D., DOWNES, C. P., MANTYH, P. W., AND EMSON, P. C.: Heurotensin stimulates inositol phospholipid hydrolysis in rat brain slices. Brain Res. **323:** 193–197, 1984.
161. GOLD, B. I., AND VOLICER, V.: Adenosine triphosphate-derived nucleotide formation in the presence of ethanol. Biochem. Pharmacol. **25:** 1825–1830, 1979.
162. GOLDSTEIN, D. B., CHIN, J. N., AND LYON, R. C.: Ethanol disordering of spin-labeled mouse brain membranes: correlation with genetically determined ethanol sensitivity of mice. Proc. Natl. Acad. Sci. USA **79:** 4231–4233, 1982.
163. GONZALES, R. A., AND CREWS, F. T.: Effects of ethanol in vivo and in vitro on stimulated phosphoinositide hydrolysis in rat cortex and cerebellum. Alcohol. Clin. Exp. Res. **12:** 94–98, 1988.
164. GONZALES, R. A., THEISS, C., AND CREWS, F. T.: Effects of ethanol on stimulated inositol phospholipid hydrolysis in rat brain. J. Pharmacol. Exp. Ther. **237:** 92–97, 1986.
165. GORDON, A. S., COLLIER, K., AND DIAMOND, I.: Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neural cell line: an in vitro model of cellular tolerance to ethanol. Proc. Natl. Acad. Sci. **83:** 2105–2108, 1986.
166. GORDON, L. M., SAUERHEBER, R. D., ESGATE, J. A., DIPPLE, I., MARCHMONT, R. J., AND HOUSLAY, M. D.: The increase in bilayer fluidity of rat liver plasma membranes achieved by the local anesthetic benzyl alcohol affects the activity of intrinsic membrane enzymes. J. Biol. Chem. **255:** 4519–4527, 1980.
167. GORMAN, L. E., AND BITENSKY, M. A.: Selective activation by short chain alcohols of glucagon responsive adenyl cyclase in liver. Endocrinology **87:** 1075–1081, 1970.
168. GRAHAM, D. T., AND ERICKSON, C. K.: Alteration of ethanol-induced CNS depression:Ineffectiveness of drugs that modify cholinergic transmission. Psychopharmology **34:** 173–180, 1974.
169. GREENBERG, D. A., COOPER, E. C., GORDON, A., AND DIAMOND, I.: Ethanol and the gamma-aminobutyric acid-benzodiazepine receptor complex. J. Neurochem. **42:** 1062–1068, 1984.
170. GREENE, H. L., HERMAN, R. H., AND KRAEMER, S.: Stimulation of jejunal adenyl cyclase by ethanol. J. Lab. Clin. Med. **78:** 336–342, 1971.
171. GREENE, L. W., AND HOLLANDER, C. S.: Alcohol and the hypothalamus. Prog. Biochem. Pharmacol. **18:** 15–23, 1981.
172. GRUOL, D. L.: Ethanol alters synaptic activity in cultured spinal cord neurons. Brain Res. **243:** 25–33, 1982.
173. GRUPP, L. A., AND PERLANSKI, E.: Ethanol-induced changes in the spontaneous activity of single units in the hippocampus of the awake rate: a dose-response study. Neuropharmacology **48:** 63–70, 1978.
174. GYSLING, K., BUSTOS, G., CONCHA, I., AND MARTINEZ, G.: Effect of ethanol on dopamine synthesis and release from rat corpus striatum. Biochem. Pharmacol. **25:** 157–162, 1976.
175. HAMMER, R., BERRIE, C. P., BIRDSALL, J. M., BURGEN, A. S. V., AND HULME, E. C.:Pirenzepine distinguished between different subclasses of muscarinic receptors. Nature (Lond.). **283:** 90–92, 1980.
176. HAMMER, R., AND GIACHETTI, A.: Muscarinic receptor subtypes: M1 and M2. Biochemical and functional characterization. Life Sci. **31:** 2991–2998, 1982.
177. HARRIS, D. P., AND SINCLAIR, J. G.: Ethanol depresses inferior olive neurones and reduces Purkinje cell complex spike activity evoked by cerebral cortical stimulation. Gen. Pharmacol. **15:** 455–459, 1984.
178. HARRIS, D. P., AND SINCLAIR, J. G.: Ethanol-GABA interactions at the rat Purkinje cell. Gen. Pharmacol. **15:** 449–454, 1984.
179. HARRIS, E. W., GANONG, A. N., AND COTMAN, C. W.: Long-term potentiation in the hippocampus involves activation of *N*-methyl-*D*-aspartate receptors. Brain Res. **323:** 132–137, 1984.
180. HARRIS, R. A.: Ethanol and pentobarbital inhibition of intrasynaptosomal sequestration of calcium. Biochem. Pharmacol. **30:** 3209–3215, 1981.
181. HARRIS, R. A., AND ALLAN, A. M.: Alcohol intoxication: ion channels and genetics. FASEB J. **3:** 1689–1695, 1989.
182. HARRIS, R. A., ALLAN, A. M., DANIELL, L. C., AND NIXON, C.: Antagonism of ethanol and pentobarbital actions by benzodiazepine inverse agonists. J. Pharmacol. Exp. Ther. **247:** 1012–1017, 1988.183. HARRIS, R. A., BAXTER, D. M., MITCHELL, M. A. AND HITZEMANN, R. J.: Physical properties and lipid composition of brain membranes from ethanol tolerant-dependent mice. Mol. Pharmacol. **25:** 401–409, 1984.
184. HARRIS, R. A. AND BRUNO, P.: Effects of ethanol and other intoxicant-anesthetics on voltage-dependent sodium channels of brain synaptosomes. J. Pharmacol. Exp. Ther. **232:** 401–406, 1985.
185. HARRIS, R. A., AND BRUNO, P.: Membrane disordering by anesthetic drugs: relationship to synaptosomal sodium and calcium fluxes. J. Neurochem. **44:** 1274–1281, 1985.
186. HARRIS, R. A., BURNETT, R., MCQUILKIN, S., MCCLARD, A., AND SIMON, F. R.: Effects of ethanol on membrane order: fluorescence studies. Ann. NY Acad. Sci. **492:** 125–135, 1987.
187. HARRIS, R. A., AND FENNER, D.: Ethanol and synaptosomal calcium binding. Biochem. Pharmacol. **31:** 1790–1792, 1982.

188. HARRIS, R. A., AND GROH, G. I.: Membrane disordering effects of anesthetics are enhanced by gangliosides. Anesthesiology **62:** 115–119, 1985.
189. HARRIS, R. A., GROH, G. I., BAXTER, D. M., AND HITZEMANN, R. J.: Gangliosides enhance the membrane actions of ethanol and pentobarbital. Mol. Pharmacol. **25:** 410–417, 1984.
190. HARRIS, R. A., AND HITZEMANN, R. J.: Membrane fluidity and alcohol actions. *In* Currents in Alcoholism, vol. 8, ed. by M. Galanter, pp. 379–404, Grune and Stratton, Inc., New York, 1981.
191. HARRIS, R. A., AND HOOD, W. F.: Inhibition of synaptosomal calcium uptake by ethanol. J. Pharmacol. Exp. Ther. **213:** 562–568, 1980.
192. HARRIS, R. A., ZACCARO, L. M., MCQUILKIN, S., AND MCCLARD, A.: Effects of ethanol and calcium on lipid order of membranes from mice selected for genetic differences in ethanol intoxication. Alcohol **5:** 251–257, 1988.
193. HASHIMOTO, H., NOTO, T., NAKAJIMA, T., AND KATO, H.: Effect of ethanol and acetaldehyde on the release of arginine-vasopressin and oxytocin from the isolated hypothalamo-1985. system of rats. Endocrinol. Jpn. **32:** 489–496, 1985.
194. HELLEVUO, K., KIIANMAA, K., JUHAKOSKI, A., AND KIM, C.: Intoxicating effects of lorazepam and barbital in rat lines selected for differential sensitivity to ethanol. Psychopharmacology **91:** 263–267, 1987.
195. HIRNING, L. D., FOX, A. P., MCCLESKEY, E. W., OLIVERA, B. M., THAYER, S. A., MILLER, R. J. AND TSIEN, R. W.: Dominant role of N-type Ca^{2+} channels in evoked release of norepinephrine from sympathetic neurons. Science (Wash. DC) **239:** 57–60, 1988.
196. HITZEMANN, R. J.: Effects of non-electrolyte molecules with anesthetic activity on the physical properties of DMPC multilammelar liposomes. Biochim. Biophys. Acta **983:** 205–211, 1989.
197. HITZEMANN, R. J., AND HARRIS. R. A.: Developmental changes in synaptic membrane fluidity: a comparison of 1,6-diphenyl-1,3,5-hexatriene DPH and 1-[4-trimethylaminophenyl]-6-phenyl-1,3,5-hexatriene TMA-DPH. Dev. Brain Res. **14:** 113–120, 1984.
198. HITZEMANN, R. J., SCHUELER, N. E., GRAHAM-BRITTAIN, C., AND KREISHMAN, G. P.: Ethanol-induced changes in neuronal membrane order: an NMR study. Biochim. Biophys. Acta **859:** 189–197, 1986.
199. HOFFMAN, P. L., CHUNG, C. T., AND TABAKOFF, B.: Effects of ethanol, temperature, and endogenous regulatory factors on the characteristics of striatal opiate receptors. J. Neurochem. **43:** 1003–1010, 1984.
200. HOFFMAN, P. L., LUTHIN, G. R., THEODOROPOULOS, D., CORDOPATIS, P., AND TABAKOFF, B.: Ethanol effects on striatal dopamine receptor-coupled adenylate cyclase and on striatal opiate receptors. Pharmacol. Biochem. Behav. **18** (suppl 1): 355–359, 1983.
201. HOFFMAN, P. L., MOSES, F., LUTHIN, G. R., AND TABAKOFF, B.: Acute and chronic effects of ethanol on receptor-mediated phosphatidylinositol 4,5-bisphosphate breakdown in mouse brain. Mol. Pharmacol. **30:** 13-18, 1986.
202. HOFFMAN, P. L., RITZMANN, R. F., AND TABAKOFF, B.: Neurohypophyseal peptide influence on ethanol tolerance and acute effects of ethanol. Pharmacol. Biochem. Behav. **13** (suppl 1): 279–284, 1980.
203. HOFFMAN, P. L., AND TABAKOFF, B.: Effects of ethanol on Arrhenius parameters and activity of mouse striatal adenylate cyclase. Biochem. Pharmacol. **31:** 3101–3106, 1982.
204. HOFFMAN, P. L., AND TABAKOFF, B.: Ethanol does not modify opiate-mediated inhibition of striatal adenylate cyclase. J. Neurochem. **46:** 812–816, 1986.
205. HOFFMAN, P. L., VALVERIUS, P., KWAST, M., AND TABAKOFF, B.: Comparison of the effects of ethanol on beta-adrenergic receptors in heart and brain. Alcohol Alcohol. **1** (suppl.): 749–754, 1987.
206. HOLMAN, R. B., AND SNAPE, B. M.: Effects of ethanol in vitro and in vivo on the release of endogenous catecholamines from specific regions of rat brain. J. Neurochem. **44:** 357–363, 1985.
207. HOUMED, K. M., BILBE, G., SMART, T. G., CONSTANTI, A., BROWN, D. A., BARNARD, E. A., AND RICHARDS, B. M.: Expression of functional GABA, glycine and glutamate receptors in *Xenopus* oocytes injected with rat brain mRHA. Nature (Lond.) **310:** 318–321, 1984.
208. HOWERTON, T. C., AND COLLINS, A. C.: Ethanol-induced inhibition of GABA release from LS and SS mouse brain slices. Alcohol **1:** 471–477, 1984.
209. HU, G. Y., HVALBY, O., WALAAS, S. I., ALBERT, K. A., SKJEFLO, P., ANDERSEN, P., AND GREENGARD, P.: Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. Nature (Lond.) **328:** 426–429, 1987.
210. HUIDOBRO-TORO, J. P., BLEC, V., ALLAN, A. M., AND HARRIS, R. A.: Neurochemical actions of anesthetic drugs on the gamma-aminobutyric acid receptor-chloride channel complex. J. Pharmacol. Exp. Ther. **242:** 963–969, 1987.
211. HUNT, W. A.: The effect of ethanol on GABAergic transmission. Heurosci. Biobehav. Rev. **7:** 87–95, 1983.
212. HUNT, W. A.: Alcohol and biological membranes. **In** The Guilford Alcohol Studies Series, ed. by H. Bland and D. Goodwin, The Guilford Press, 1985.
213. HUNT, W. A., AND DALTON, T. K.: Regional brain acetylcholine levels in rats acutely treated with ethanol or rendered ethanol-dependent. Brain Res. **109:** 628–631, 1976.
214. HUNT, W. A., REDOS, J. D., DALTON, T. K., AND CATRAVAS, G. N.: Alterations in brain cyclic guanosine 3′:5′-monophosphate levels after acute and chronic treatment with ethanol. J. Pharmacol. Exp. Ther. **201:** 103–109, 1977.
215. HYNES, M. D., LOCHNER, M. A., BEMIS, K. G., AND HYMSON, D. L.: Chronic ethanol alters the receptor binding characteristics of the delta opioid receptor ligand, D-Ala2-D-Leu5 enkephalin in mouse brain. Life Sci. **33:** 2331–2337, 1983.
216. HYNIE, S., LANEFELT, F., AND FREDHOLM, B. B.: Effects of ethanol on human lymphocyte levels of cyclic AMP in vitro: potentiation of the response to isoproterenol, prostaglandin E2 or adenosine stimulation. Acta Pharmacol. Toxicol. **47:** 58–65, 1980.
217. IBATA, Y., FUKUI, K., OKAMURA, H., KAWAKAMI, T., TANAKA, M., OBATA, N.L., TSUTO, T., TERUBAYASHI, H., YANAIHARA, C., AND YANAIHARA, H.: Co-existence of dopamine and neurotensin in hypothalamic arcuate and periventricular neurons. Brain Res. **169:** 177–179, 1983.
218. IMPERATO, A., AND DI CHIARA, G.: Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. **239:** 219–228, 1986.
219. INGRAM, L. L., CAREY, V. C., AND DOMBEK, K. M.: On the relationship between alcohol narcosis and membrane fluidity. Substance Alcohol Actions/Misuse **2:** 213–224, 1982.
220. ISRAEL, M., KIMURA, N., AND KURIYAMA, K: Changes in activity and hormonal sensitivity of brain adenyl cyclase following chronic ethanol administration. Experentia **28:** 1322-l323, 1972.
221. IVERSEN, L. L.: Nonopioid neuropeptides in mammalian CNS. Annu. Rev. Pharmacol. Toxicol. **23:** 1–27, 1983.
222. IVERSEN, L. L., IVERSEN, S. D., BLOOM, F., DOUGLAS, C., BROWN, M., AND VALE, W.: Calcium-dependent release of somatostatin and neurotensin from rat brain *in vitro*. Nature (Lond.) **273:** 161–163, 1978.
223. JENNES, L., STUMPF, W. E., AND KALIVAS, P. W.: Neurotensin: Topographical distribution in rat brain by immunohistochemistry. J. Comp. Neurol. **210:** 211–224, 1982.
224. JOHANSSON, O., HOKEFELT, T., PERNOW, B., JEFFCOATE, S. L., WHITE, N., STEINBUSCH, H. W. M., VERHOFSTAD, A. A. J., EMSON, P. C., AND SPINDEL, E.: Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P and thyrotropin-releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neuroscience **6:** 1857–1881, 1981.
225. JOHNSON, S. W., HOFFER, B. J., BAKER, R., AND FREEDMAN, R.: Correlation of Purkinje neuron depression and hypnotic effects of ethanol in inbred strains of rats. Alcohol Clin. Exp. Res. **9:** 56–58, 1985.
226. JONES, O. T., EUBANKS, J. H., EARNEST, J. P. AND MCNAMEE, M. G.: A minimum number of lipids are required to support the functional properties of the nicotinic acetylcholine receptor. Biochemistry **27:** 3733–3742, 1988.
227. JONES, O. T., AND MCNAMEE, M. G.: Annular and nonannular binding sites for cholesterol associated with the nicotinic acetylcholine receptor. Biochemistry **27:** 2364–2374, 1988.
228. JORGENSEN, H. A., AND HOLE, K.: Evidence from behavioral and in vitro receptor binding studies that the enkephalinergic system does not mediate acute ethanol effects. Eur. J. Pharmacol. **125:** 249–256, 1986.
229. KALANT, H.: Comparative aspects of tolerance to, and dependence on, alcohol, barbiturates and opiates. *In* Alcohol Intoxication and Withdrawal, ed. by M. M. Gross, pp. 169–185, Plenum Press, New York, 1977.
230. KALANT H., AND GROSE, W.: Effects of ethanol and pentobarbital on release of acetylcholine from cerebral cortex slices. J. Pharmacol. Exp. Ther. **158:** 386–393, 1967.
231. KALANT, H., ISRAEL, Y., AND MAHON, M. A.: The effects of ethanol on acetylcholine synthesis, release, and degradation in brain. Can. J. Physiol. Pharmacol. **45:** 172–176, 1967.
232. KALIVAS, P. W., NEMEROFF, C. B., AND PRANGE, A. J., JR.: Neuroanatomical sites of action of neurotensin. Ann. NY Acad. Sci. **400:** 397–418, 1982.
233. KALIVAS, P. W., NEMEROFF, C. B., AND PRANGE, A. J., JR.: Neuroanatomical site specific modulation of spontaneous motor activity by neurotensin. Eur. J. Pharmacol. **78:** 471–474, 1982.
234. KALIVAS, P. W., AND TAYLOR, S.: Behavioral and neurochemical effect of daily injection with neurotensin into the ventral tegmental area. Brain Res. **358:** 70–76, 1985.
235. KANBA, K. S., KANBA, S., IKAZAKI, H., AND RICHELSON, E.: Binding of [^{3}H]-neurotensin in human brain: properties and distribution. J. Neurochem. **46:** 946–952, 1986.
236. KANBA, K. S., AND RICHELSON, E.: Comparison of the stimulation of inositol phospholipid hydrolysis and of cyclic GMP formation by neurotensin, some of its analogs, and neuromedin N in neuroblastoma clone N1E-115. Biochem. Pharmacol. **36:** 869–874, 1987.
237. KATSUURA, G., AND ITOH, S.: Effect of cholecystokinin octapeptide on body temperature in the rat. Jpn. J. Physiol. **31:** 849–858, 1981.
238. KATSUURA, G., AND ITOH, S.: Sedative action of cholecystokinin octapeptide on behavioral excitation by thyrotropin releasing hormone and methamphetamine in the rat. Jpn. J. Physiol. **32:** 83–91, 1982.
239. KITABGI, P., ROSTÈNE, W., DUSSAILLANT, M., SCHOTTE, A., LADURON, P. M., AND VINCENT, J. P.: Two populations of neurotensin binding sites in murine brain: discrimination by the antihistamine levocabastine reveals markedly different radioautographic distribution. Eur. J. Pharmacol: **140:** 285–293, 1987.
240. KLEMM, W. R., BOYLES, R., MATHEW, J., AND CHERLAN, L.: Gangliosides, or sialic acid, antagonize ethanol intoxication. Life Sci. **43:** 1837–1843, 1988.

241. KLOTZ, I. M., AND FRANZEN, J. S.: Hydrogen bonds between model peptide groups in solution. J. Am. Chem. Soc. **84:** 3461–3466, 1962.
242. KOCHHAR, A., AND ERICKSON, C. K.: Central cholinergic correlates of low dose ethanol-induced locomotor stimulation. Alcohol. Clin. Exp. Res. **10:** 595–601, 1986.
243. KORPI, E. R., REITH, M. E., AND LAJTHA, A.: Synaptosomal sodium channels in rat lines selected for alcohol-related behaviors. Alcohol **5:** 81–94, 1988.
244. KRALL, J. F., LESHON, S. C., FROLICH, M., AND KORENMAN, S. G.: Activation of uterine smooth muscle adenylate cyclase by guanyl nucleotide. J. Biol. Chem. **256:** 5436–5442, 1981.
245. KREISHMAN, G. P., GRAHAM-BRITTAIN, C., AND HITZEMANN, R. J.: Determination of ethanol partition coefficients to the interior and the surface of dipalmityl-phosphatidylcholine liposomes using deuterium nuclear magnetic resonance spectroscopy. Biochem. Biophys. Res. Commun. **130:** 301–305, 1985.
246. KREISHMAN, G. P., GRAHAM-BRITTAIN, C., SCHUELER, H., AND HITZEMANN, R.: On the use of nuclear magnetic resonance spectroscopy (NMR) to study the mechanism(s) of ethanol action. Alcohol Drug Res. **86:** 1–13, 1985.
247. KULONEN, E.: Ethanol and GABA. Med. Biol. **61:** 147–167, 1983.
248. KUO, W. H., SHOJI, M., AND KUO, J. F. Isolation of stimulatory modulator from rat brain and its specific effect on guanosine 3′:5′-monophosphate-dependent protein kinase from cerebellum and other tissues. Biochem. Biophys. Res. Commun. **70:** 280–286, 1976.
249. KUO, W., WILLIAMS, J. L., FLOYD-JONES, T., DUGGANS, C. F., BOONE, D. L., SMITH, S. O., AND COPELAND R. F.: Changes in the activities of protein kinase modulators in the cerebellum of mice due to ethanol, caffeine, or phenobarbital administration. Experentia **35:** 997–998, 1979.
250. KURIYAMA, K., AND ISRAEL, M. A.: Effect of ethanol administration on cyclic 3′ ,5′ -adenosine monophosphate metabolism in brain. Biochem. Pharmacol. **22:** 2919–2922, 1973.
251. LAI, H., MAKANS, W. L., HORITA, A., AND LEUNG, H.: Effects of ethanol on turnover and function of striatal dopamine. Psychopharmacology **61:** 1–9, 1979.
252. LANE, S. J., AND MORGAN, W. W.: Development of tolerance to chronic barbital treatment in the cerebellar cyclic guanosine monophosphate system and its response to subsequent barbital abstinence. J. Pharmacol. Exp. Ther. **234:** 569–574, 1985.
253. LARSON, J., AND LYNCH, G.: Role of N-methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal Θ-rhythm. Brain Res. **441:** 111–118, 1988.
254. LEDIG, M., M'PARIA, T-R., AND MANDEL, P.: Superoxide dismatase activity in rat brain during acute and chronic alcohol intoxication. Neurochem. Res. **6:** 385–390, 1981
255. LEE, H. M., FRIEDMAN, N. J., AND LOH, H. H.: Effect of acute and chronic ethanol treatment on rat brain phospholipid turnover. Biochem. Pharmacol. **29:** 2815–2818, 1980.
256. LEGUICHER, A., BEAGUE, F., AND NORDMANN, R.: Concomitant changes of ethanol partitioning and disordering capacities in rat synaptic membranes. Biochem. Pharmacol. **36:** 2045–2048, 1987.
257. LESLIE, S. W., BARR, E., CHANDLER, L. J., AND FARRAR, R. P. J.: Pharmacol. Exp. Ther. **225:** 571–575, 1983.
258. LEVITAN, E. S., SCHOFIELD, P. R., BURT, D. R., RHEE, L. M., WISDEN, W., KOHLER, M., FUJITA, H., RODRIQUEZ, H. R., STEPHENSON, A., DARLISON, M. G., BARNARD, E. A., AND SEEBURG, P. H.: Structural and functional basis for $GABA_A$ receptor heterogeneity. Nature (Lond.) **335:** 76–79, 1988.
259. LILJEQUIST, S., CULP, S., AND TABAKOFF, B.: Effect of ethanol on the binding of 35S-T-butylbicyclophosphorothionate to mouse brain membranes. Life Sci. **38:** 1931–1939, 1986.
260. LILJEQUIST, S., AND TABAKOFF, B.: Bicuculline-pentobarbital interactions on [35S]TBPS binding in various brain areas. Life Sci. **39:** 851–855, 1986.
261. LINDROS, K. O.: Acetaldehyde—its metabolism and role in the actions of alcohol. *In* Research Advances in Alcohol and Drug Problems, ed. by Y. Israel, F. B. Glaser, H. Kalant, R. E. Popham, W. Schmidt, R. G. Smart, vol. 4, Plenum Press, New York, pp. 111–176, 1974.
262. LISTER, R. G., AND NUTT, D. J.: Is Ro 14-4513 a specific alcohol antagonist? Trends Neurosci. **10:** 223–225, 1987.
263. LISTER, R. G., AND NUTT, D. J.: Alcohol antagonists—the continuing quest. Alcohol. Clin. Exp. Res. **12:** 566–569, 1988.
264. LOVINGER, D. M., WHITE, G., AND WEIGHT, F. F.: Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science (Wash. DC) **243:** 1721–1724, 1989.
265. LUCCHI, L., RIUS, R.A., UZUMAKI, H., GOVONI, S., AND TRABUCCHI, M.: Chronic ethanol changes opiate receptor function in rat striatum. Brain Res. **293:** 368–371, 1984.
266. LUNDBERG, D. B. A., BREESE, G. R., MAILMAN, R. B., FRYE, G. D., AND MUELLER, R. A.: Depression of some drug-induced in vivo changes of cerebellar guanosine 3′,5′-monophosphate by control of motor and respiratory responses. Mol. Pharmacol. **15:** 246–256,1979.
267. LUTHIN, G. R., AND TABAKOFF, B.: Activation of adenylate cyclase by alcohols requires the nucleotide-binding protein. J. Pharmacol. Exp. Ther. **228:** 579–587, 1984.
268. LUTHIN, G. R., AND TABAKOFF, B.: Effects of ethanol on calmodulin levels in mouse striatum and cerebral cortex.: Alcohol. Clin. Exp. Res. **8:** 68–72, 1984.
269. LUTTINGER, D., HEMEROFF, C. B., MASON, G. A., FRYE, G. D., GREESE, G. R., AND PRANGE, A. J., JR.: Enhancement of ethanol-induced sedation and hypothermia by centrally administered neurotensin, β-endorphin, and bombesin. Neuropharmacology **20:** 305–309, 1981.
270. LYNCH, G., LARSON, J., KELSO, S., BARRIONUEVO, G., AND SCHOTTLER, F.:Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature (Lond.) **305:** 719–721, 1983.
271. LYNCH, M. A., SAMUEL, D., AND LITTLETON, J. M.: Altered characteristics of[^{3}H]dopamine release from superfused slices of corpus striatum obtained from rats receiving ethanol *in vivo*. Neuropharmacology **24:** 479–485, 1985.
272. LYON, R. C., AND GOLDSTEIN, D. B.: Changes in synaptic membrane order associated with chronic ethanol treatment in mice. Mol. Pharmacol. **23:** 86–91, 1983.
273. LYON, R. C., MCCOMB, J. A., SCHREURS, J., AND GOLDSTEIN, D. B.: A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. J. Pharmacol. Exp. Ther. **218:** 669–675, 1981.
274. MACIVER, M. B., AND ROTH, S. H. Anesthetics produce differential actions on membrane responses of the crayfish stretch receptor neuron. Eur. J. Pharmacol. **141:** 67–77, 1987.
275. MALMINEN, O., AND KORPI, E. R.: GABA/benzodiazepine receptor/chloride ionophore complex in brains of rat lines selectively bred for differences in ethanol-induced motor impairment. Alcohol **5:** 239–249, 1988.
276. MAILMAN, R. B., FRYE, G. D., MUELLER, R. A., AND BREESE, G. R.: Thyrotropin-releasing hormone reversal of ethanol-induced decreases in cerebellar cGMP. Nature (Lond.) **272:** 832–833, 1978.
277. MAKSAY, G., AND TICKU, M. K.: GABA, depressants and chloride ions affect the rate of dissociation of ^{35}S-t-butylbicyclophosphorothionate binding. Life Sci. **37:** 2173–2180, 1985.
278. MAMALAKI, C., STEPHENSON, F. A., AND BARNARD, E. A.: The $GABA_A$ / benzodiazepine receptor is a heterotetramer of homologous α and β subunits. EMBO J. **6:** 561–565, 1987.
279. MANCILLAS, J. R., SIGGINS, G. R., AND BLOOM, F. E.: Systemic ethanol: selective enhancement of responses to acetylcholine and somatostatin in hippocampus. Science (Wash. DC) **231:** 161–163, 1986.
280. MAO, C. C., GUIDOTTI, A., AND COSTA, E.: Inhibition by diazepam of the tremor and the increase of cerebellar cGMP content elicited by harmaline. Brain Res. **83:** 516–519, 1975.
281. MAO, C.C., GUIDOTTI, A., AND COSTA, E.: The regulation of cyclic guanosine monophosphate in rat cerebellum: possible involvement of putative amino acid neurotransmitters. Brain Res. **79:** 510–514, 1974.
282. MARLEY, R. J., FREUND, R. K., AND WEHNER, J. M.: Differential response to flurazepam in long-sleep and short-sleep mice. Pharm. Biochem. Behav. **31:** 453–458, 1988.
283. MARLEY, R. J., MINER, L. L., WEHNER, J. M., AND COLLINS, A. C.: Differential effects of central nervous system depressants in long-sleep and short-sleep mice. J. Pharmacol. Exp. Ther. **238:** 1028–1033, 1986.
284. MARLEY, R. J., STINCHCOMB, A., AND WEHNER, J. M.: Further characterization of benzodiazepine receptor differences in long-sleep and short-sleep mice. Life Sci. **43:** 1223–1231, 1988.
285. MARLEY, R. J., AND WEHNER, J. M.: GABA enhancement of flunitrazepam binding in mice selectively bred for differential sensitivity to ethanol. Alcohol Drug Res. **7:** 25–32, 1987.
286. MASHITER, K., MASHITER, G. D., AND FIELD, J. B.: Effects of prostaglandin E1, ethanol and TSH on the adenylate cyclase activity of beef thyroid plasma membranes and cyclic AMP content of dog thyroid slices. Endrocrinology **94:** 370–376, 1974.
287. MATTHEWS, R. T., AND GERMAN, D. C.: Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience **11:** 617–625, 1984.
288. MAZELLA, J., CHABRY, J., KITABGI, P., AND VINCENT, J. P.: Solubilization and characterization of active neurotensin receptors from mouse brain. J. Biol. Chem. **263:** 144–149, 1988.
289. MAZELLA, J., POUSTIS, C., LABBE, C., CHECLER, R., KITABGI, P., GRANIER, C., VAN REITSCHOTEN, J., AND VINCENT, J. P.: Monoiodo-[trp^{11}]-neurotensin, a highly radioactive ligand of neurotensin receptors. J. Biol. Chem. **258:** 3476–3481, 1983.
290. Deleted in proof.
291. MCCOWN, T. J., MORAY, L. J., KIZER, J. S., AND BREESE, G. R.: Interactions between TRH and ethanol in the medial septum. Pharmacol. Biochem. Behav. **24:** 1269–1274, 1986.
292. MCINTYRE, T.D., TRULLAS, R., AND SKOLNICK, P.: Differences in the biophysical properties of the benzodiazepine/gamma-aminobutyric acid receptor chloride channel complex in the long-sleep and short-sleep mouse lines. J. Neurochem. **51:** 642–647, 1988.
293. MCLENNAN, H.: The pharmacological characterization of excitatory amino acid receptors. *In* Excitatory Amino Acids in Health and Disease, pp. 1–12, John Wiley and Sons, Chichester, 1988.
294. MEHTA, A. K., AND TICKU, M. K.: Ethanol potentiation of GABAergic transmission cultured spinal cord neurons involves gamma-aminobutyric $acid_A$-gated chloride channels. J. Pharmacol. Exp. Ther. **246:** 558–564, 1988.
295. MELCHIOR, C. L., AND MYERS, R. D.: Preference for alcohol in the rat

induced by chronic infusion of tetrahydropapaveroline (THP) in the cerebral ventricle. Phamacol. Biochem. Behav. **7:** 19–35, 1977.
296. MEREU, G., FADDA, F., AND GESSA, G. L.: Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. Brain Res. **292:** 63–69, 1984.
297. MEREU, G., AND GESSA, G. L. Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect. Brain Res. **360:** 325–330, 1985.
298. MESSING, R. O., CARPENTER, C. L., DIAMOND, I., AND GREENBERG, D. A.: Ethanol regulates calcium channels in clonal neural cells. Proc. Natl. Acad. Sci. USA **83:** 6213–6215, 1986.
299. MEYERS, M. B., AND KOMISKEY, H. L.: Aging: effect on the interaction of ethanol and pentobarbital with the benzodiazepine-GABA receptor-ionophore complex. Brain Res. **343:** 262–267, 1985.
300. MICHAELIS, E. K., CHANG, N. H., ROY, S., MCFAUL, J. A., AND ZIMBRICK, J. D.: Ethanol effects on synaptic glutamate receptor function and on membrane lipid organization. Pharmacol. Biochem. Behav. **18:** 1–6, 1983.
301. MICHAELIS, E. K., MICHAELIS, M. L., CHANG, H. H., AND KOTIS, T.E.: High affinity Ca^{2+}-stimulated Mg^{2+}-dependent ATPase in rat brain synaptosomes, synaptic membranes, and microsomes. J. Biol. Chem. **258:** 6101–6108, 1983.
302. MICHAELIS, E. K., AND MYERS, S. L.: Calcium binding to brain synaptosomes. Biochem. Pharmacol. **28:** 2081–2087, 1979.
303. MICHAELIS, R. C., NOLOHEAN, A. M., NUNTER, G. A., AND HOLLOWAY, F. A.: Endogenous adenosine-receptive systems do not mediate the discriminative stimulus properties of ethanol. Alcohol Drug Res. **7:** 175–185, 1987.
304. MILLER, L. G., GREENBLATT, D. J., BARNHILL, J. G., AND SHADER, R. I.: Differential modulation of benzodiazepine receptor binding by ethanol in LS and SS mice. Pharmacol Biochem. Behav. **29:** 471–477, 1988.
305. MILLER, K. W., FIRESTONE, L. L., AND FORMAN, S. A.: General anesthetic and specific effects of ethanol on acetylcholine receptors. Ann. NY Acad. Sci. **492:** 71–85, 1986.
306. MILLER, R. J.: Multiple calcium channels and neuronal function. Science (Wash. DC) **235:** 46–52, 1987.
307. MIYAMOTO, M., AND HAGAWA, Y.: Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. Eur. J. Pharmacol. **44:** 143–152, 1977.
308. MODAK, A. T., AND ALDERETE, B. E.: Nicotine potentiates sodium pentobarbital but not ethanol induced sleep. Substance Alcohol Actions/Misuse. **4:** 321–329, 1983.
309. MOLEMAN, P., AND BRUINVELS, J.: Differential effect of morphine on dopaminergic neurons in frontal cortex and striatum of the rat. Life Sci. **19:** 1277–1282, 1976.
310. MONCADA, S., HERMAN, A. G., AND VANHOUTTE, P.: Endothelium-derived relaxing factor identified as nitric oxide. Trends Pharmacol. Sci. **8:** 365–368, 1987.
311. MOODY, E. J., SUZDAK, P. K., PAUL, S. M., AND SKOLNICK, P.: Modulation of the benzodiazepine/gamma-aminobutyric acid receptor chloride channel complex by inhalation anesthetics. J. Neurochem. **51:** 1386–1393, 1988.
312. MORELI, M., DEIDDA, S., GARAU, L., CARBONI, E., AND DI CHIARA, G.: Ethanol: lack of stimulation of chloride influx in rat brain synaptoneurosomes. Neurosci. Res. Commun. **2:** 77–84, 1988.
313. MORGAN, E. P., AND PHILLIS, J. W.: The effects of ethanol on acetylcholine release from the brain of unanesthetized cats. Gen. Pharmacol. **6:** 281–284, 1975.
314. MORROW, A. L., SUZDAK, P. D., KARANIAN, J. W., AND PAUL, S. M.: Chronic ethanol administration alters gamma-aminobutyric acid, pentobarbital and ethanol-mediated $^{36}Cl^-$ uptake in cerebral cortical synaptoneurosomes. J. Pharmacol. Exp. Ther. **246:** 158–164, 1988.
315. MORROW, E. L., AND ERWIN, V. G.: Calcium influence on neuronal sensitivity to ethanol in selectively bred mouse lines. Pharmacol. Biochem. Behav. **24:** 949–954, 1986.
316. MORROW, E. L., AND ERWIN, V. G.: Calcium influence on neurotensin and β-endorphin enhancement of ethanol sensitivity in selectively bred mouse lines. Alcohol Drug Res. **7:** 225–232, 1987.
317. MOYSE, E., ROSTENE, W., VIAL, M., LEONARD, K., MAZELLA, J., KITABGI, P., VINCENT, J. P., AND BEAUDET, A.: Distribution of neurotensin binding sites in rat brain: microscoic radioautographic study using monoiodo [^{125}I] Tyr_3-neurotensin. Neuroscience **22:** 525–536, 1987.
318. MRAK R. E., AND NORTH, P. E.: Ethanol inhibition of synaptosomal high-affinity choline uptake. Eur. J. Pharmacol **151:** 51–58, 1988.
319. MULKEEN, D., ANWYL, R., AND ROWAN, M. J.: Enhancement of long-term potentiation by the calcium channel agonist Bayer K8644 in CA1 of in the rat hippocampus in vitro. Neurosci. Lett. **80:** 351–355, 1987.
320. MULLIN, M. J., DALTON, T. K., HUNT, W. A., HARRIS, R. A., AND MAJCHROWICZ, E.: Actions of ethanol on voltage-sensitive sodium channels: effects of acute and chronic ethanol treatment. J. Pharmacol. Exp. Ther. **242:** 541–547, 1987.
321. MULLIN, M. J., AND HUNT, W. A.: Actions of ethanol on voltage-sensitive sodium channels: effects on neurotoxin-stimulated sodium uptake in synaptosomes. J. Pharmacol. Exp. Ther. **232:** 413–419, 1985.
322. MULLIN, M.J., AND HUNT, W.A.: Actions of ethanol on voltage-saensitive sodium channels: effects on neurotoxin binding. J. Pharmacol. Exp. Ther. **242:** 536–540, 1987.
323. MURPHY, J. M., CUNNINGHAM, S. D., AND MCBRIDE, W. J.: Effects of 250 mg% ethanol on monoamine and amino acid release from rat striatal slices. Brain Res. Bull. **14:** 439–442, 1985.
324. NABER, D., SOBLE, M. G., AND PICKAR, D.: Ethanol increases opioid activity in plasma of normal volunteers. Pharmacopsychiatry **14:** 160–161, 1981.
325. HACHSHEN, D. A.: Regulation of cytosolic calcium concentration in presynaptic nerve endings isolated from rat brain. J. Physiol. **363:** 87–101, 1985.
326. HACHSHEN, D. A., SANCHEZ-ARMASS, S., AND WEINSTEIN, A.: The regulation of cytosolic calcium in rat brain synaptosomes by sodium-dependent calcium efflux. J. Physiol. **381:** 17–28, 1986.
327. HEEDHAM, L., AND HOUSLAY, M. D.: The activity of dopamine-stimulated adenylate cyclase from rat brain striatum is modulated by temperature and the bilayer-fluidizing agent, benzyl alcohol. Biochem. J., **206:** 89–95, 1982.
328. NEERING, I. R., AND FRYER, M. W.: The effect of alcohols on aequorin luminescence. Biochim. Biophys. Acta **882:** 39–43, 1986.
329. NEMEROFF, C. B., AND CAIN, S. T.: Neurotensin-dopamine interactions in the CNS. Trends Pharmacol. Sci. **6:** 201–205, 1985.
330. NEMEROFF, C. B., LUTTINGER, D., HERNANDEZ, D. E., MAILMAN, R. B., MASON, G. A., DAVIS, S. D., WIDERLOV, E., FRYE, G. D., KILTS, C. A., BEAUMONT, K., BREESE, G. R., AND PRANGE, A. J., JR.: Interactions of neurotensin with brain dopamine systems: biochemical and behavioural studies. J. Pharmacol. Exp. Ther. **225:** 37–45, 1983.
331. NESTOROS, J. N.: Ethanol selectively potentiates GABA-mediated inhibition of single feline cortical neurons. Life Sci. **26:** 519–523, 1980.
332. NESTOROS, J. N.: Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. Science (Wash. DC) **209:** 708–710, 1980.
333. NEWLIN, S. A., MANCILLAS-TREVINO, J., AND BLOOM, F. E.: Ethanol causes increases in excitation and inhibition in area CA3 of the dorsal hippocampus. Brain Res. **209:** 113–128, 1981.
334. NG CHEONG TON, J. M., AND AMIT, Z.: Receptor stereospecificity in opiate-ethanol interaction using the preexposure-conditioned taste aversion (CTA) paradigm. Pharmacol. Biochem. Behav. **22:** 255–259, 1985.
335. NIESEN, C. E., BASKYS, A., AND CARLEN, P. L.: Reversed ethanol effects on potassium conductances in aged hippocampal dentate granule neurons. Brain Res. **445:** 137–141, 1988.
336. NISHIO, M., AND HARAHASHI, T.: Ethanol enhances a component of GABA-gated chloride channel currents in rat dorsal root ganglion neurons. Soc. Neurosci. Abstr., **14:** 642, 1988.
337. OAKES, S. G., AND POZOS, R. S.: Electrophysiologic effects of acute ethanol exposure. I. Alterations in the action potentials of dorsal root ganglia neurons in dissociated culture. Dev. Brain Res. **5:** 243–249, 1982.
338. OKADA, K.: Effects of alcohols and acetone on the neuromuscular junction of frog. Jap. J. Physiol. **17:** 245–261, 1967.
339. OLIVERA, B. M., GRAY, W. R., ZEIKUS, R., MCINTOSH, J. M., VARGA, J., RIVIER, J., DE SANTOS, V., AND CRUZ, L. J.: Peptide neurotoxins from fish-hunting cone snails. Science (Wash. DC) **230:** 1338–1343, 1985.
340. OLSEN, R. W.: The gamma-aminobutyric acid/benzodiazepine/barbiturate receptor-chloride ion channel complex of mammalian brain. *In* Synaptic Function, ed. by G. M. Edelman, W. E. Gall, W. M. Cowan, and Neurosciences Research Foundation, pp. 257–271, John Wiley and Sons, New York, 1987.
341. OP DEN KAMP, J. A. F.: Lipid asymmetry in membranes. Annu. Rev. Biochem. **48:** 47–71, 1979.
342. OPMEER, F. A., GUMULKA, S. W., DINNENDAHL, V., AND SCHONHOFER, P. S.: Effects of stimulatory and depressant drugs on cyclic guanosine 3′,5′-monophosphate and adenosine 3′,5′-monophosphate levels in mouse brain. Naunyn-Schmiedebergs Arch. Pharmacol. **292:** 259–265, 1976.
343. OYAMA, Y., AKAIKE, N., AND NISHI, K.: Effects of n-alkanols on the calcium current of intracellularly perfused neurons of Helix aspersa. Brain Res. **376:** 280–284, 1986.
344. PALMER, M. R.: Neurophysiological mechanisms in the genetics of ethanol sensitivity. Soc. Biol. **32:** 241–254, 1985.
345. PALMER, M. R., BASILE, A. S., PROCTOR, W. R., BAKER, R. C., AND DUNWIDDIE, T. V.: Ethanol tolerance of cerebellar Purkinje neurons from selectively outbred mouse lines: in vivo and in vitro electrophysiological investigations. Alcohol. Clin. Exp. Res. **9:** 291–296, 1985.
346. PALMER, M. R., MORROW, E. L., AND ERWIN, V. G.: Calcium differentially alters behavioral and electrophysiological responses to ethanol in selectively bred mouse lines. Alcohol. Clin. Exp. Res. **11:** 457–463, 1987.
347. PALMER, M. R., OLSON, L., DUNWIDDIE, T. V., HOFFER, B. J., AND SEIGER, A.: Neonatal cerebellectomy alters ethanol-induced sleep time of short-sleep but not long-sleep mice. Pharmacol. Biochem. Behav. **20:** 153–159, 1984.
348. PALMER, M. R., SORENSEN, S. M., FREEDMAN, R., OLSON, L., HOFFER, B., AND SEIGER, A.: Differential ethanol sensitivity of intraocular cerebellar grafts in long-sleep and short-sleep mice. J. Pharmacol. Exp. Ther. **222:** 480–487, 1982.
349. PALMER, M. R., TOTTMAR, O., AND DEITRICH, R. A.: Electrophysiological effects of monoamine-derived aldehydes on single neurons in neocortex and cerebellum in rats. Alcohol. Clin. Exp. Res. **10:** 682–685, 1986.
350. PALMER, M. R., VAN NORNE, C. G., HARLAN, J. T., AND MOORE, E. A. Antagonism of ethanol effects on cerebellar Purkinje neurons by the benzodiazepine inverse agonists Ro 15–4513 and FG 7142: electrophysiological studies. J. Pharmacol. Exp. Ther. **247:** 1018–1024, 1988.
351. PALMER, M. R., WANG, Y., FOSSOM, L. H., AND SPUHLER, K. P.: Genetic

correlation of ethanol-induced ataxia and cerebellar Purkinje neuron depression among inbred strains and selected lines of rats. Clin. Exp. Res. **11:** 494–501, 1987.
352. PAUL, M. I., PAUK, G. L., AND DITEZLION, B. R.: The effect of centrally acting drugs on the concentration of brain adenosine 3′,5′monophosphate. Pharmacology **3:** 148–154 1970.
353. PELLEGRINI-GIAMPIETRO, CHERICI, G., ALESIANI, M., CARLA, V., AND MORONI, F.: Excitatory amino acid release from rat hippocampal slices as a consequence of free-radical formation. J. Neurochem. **51:** 1960–1963, 1988.
354. PERIS, J., WEHNER, J. M., AND ZAHNISER, H. R.: [^{35}S]TBPS binding sites are decreased in the colliculi of mice with a genetic predisposition to bicuculline-induced seizures. Brain Res. **503:** 288–295, 1989.
355. PETERSEN, D.R., AND HJELLE, J.J.: Metabolism interaction of aldehyde dehydrogenase with therapeutic and toxic agents. *In* Enzymology of Carbonyl Metabolism: Aldehyde Dehydrogenase and Aldo/Keto Reductance 1982, pp. 103–120, Alan R. Liss Inc., New York, 1982.
356. PILLAI, N. P., AND ROSS, D. N.: Ethanol-induced hypothermia in rats: possible involvement of opiate kappa receptors. Alcohol **3:** 249–253, 1986.
357. POHORECKY, L. A., AND BRICK, J.: Activity of neurons in the locus coeruleus of the rat: inhibition by ethanol. Brain Res. **134:** 174–179, 1977.
358. POHORECKY, L. A., AND SHAH, P.: Ethanol-induced analgesia. Life Sci. **41:** 1289–1295, 1987.
359. POHORECKY, A. A., MAKOWSKE, E., NEWMAN, B., AND RASSI, E.: Cholinergic mediation of motor effects of ethanol in rats. Eur. J. Pharmacol. **55:** 67–72, 1979.
360. PRITCHETT, D., SCHOFIELD, P. R., SONTHEIMR, H., YMER, S., KETTERMANN, AND SEEBURG, P.: GABA receptor cDHAs expressed in transfected cells and studied by patch-clamp and binding assay. Soc. Neurosci. Abst. **14:** 641, 1988.
361. PRITCHETT, D. B., SONTHEIMER, N., GORMAN, C., KETTENMAN, H., SEEBURG, P. N., AND SCHOFIELD, P.: Transient expression shows ligand gating and allosteric potentiation of GABAA receptor subunits. Science (Wash. DC) **242:** 1306–1308, 1988.
362. PRITCHETT, D. B., SONTHEIMER, H., SHIVERS, B. D., YMER, S., KETTENMANN, N., SCHOFIELD, P. R., AND SEEBURG, P. N.: Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. Nature (Lond.) **338:** 582–585, 1989.
363. PROCTOR, W. R., BAKER, R. C., AND DUNWIDDIE, T. V.: Differential CNS sensitivity to PIA and theophylline in long-sleep and short-sleep mice. Alcohol **2:** 387–391, 1985.
364. PROCTOR, W. R., AND DUNWIDDIE, T. V.: Behavioral sensitivity to purinergic drugs parallels ethanol sensitivity in selectively bred mice. Science (Wash. DC) **224:** 519–521, 1984.
365. PROCTOR, W. R., AND DUNWIDDIE, T. V.: Effects of ethanol on Purkinje neurons in rat cerebellar brain slices. Soc. Neurosci. Abstr. **14:** 194, 1988.
366. PUGLIA, D. C., AND LOEB, G. A.: Influence of rat brain superoxide dismutase inhibition by diehtyldithiocarbamate upon the rate of development of central nervous system oxygen toxicity. Toxicol. Appl. Pharmacol. **75:** 258–264, 1984.
367. RABE, C. S., AND TABAKOFF, B.: High sensitivity of NMDA-stimulated calcium uptake to inhibition by ethanol in primary cultures of cerebellar neurons. Soc. Neurosci. Abstr. **14:** 479, 1988.
368. RABIN, R.: Effect of ethanol on inhibition of striatal adenylate cyclase activity. Biochem. Pharmacol. **34:** 4329–4331, 1985.
369. RABIN, R. A., BODE, D. C., AND MOLINOFF, P.: Relationship between ethanol-induced alterations in fluorescence anisotropy and adenylate cyclase activity. Biochem. Pharmacol. **35:** 2331–2335, 1986.
370. RABIN R. A., AND MOLINOFF, P. B.: Activation of adenylate cyclase by ethanol in mouse striatal tissue. J. Pharmacol. Exp. Ther. **216:** 129–134, 1981.
371. RABIN, R. A., AND MOLINOFF, P. B.: Multiple sites of action of ethanol on adenylate cyclase. J. Pharmacol. Exp. Ther. **227:** 551–556, 1982.
372. RANGARAJ, H. I., AND KALANT, H.: Effect of ethanol tolerance on norepinephrine-ethanol inhibition of (Na^+ + K^+)-adenosine triphosphatase in various regions of rat brain. J. Pharmacol Exp. Ther. **231:** 416–421, 1984.
373. RANGARAJ, H., KALANT, H., AND BEAUGE, F.: Alpha 1-adrenergic receptor involvement in norepinephrine-ethanol inhibition of rat brain Ha+ -K+ ATPase and in ethanol tolerance. Can. J. Physiol. Pharmacol. **63:** 1075–1079, 1985.
374. RAPAKA, R. S., RENUGOPALAKRISHNAN, V., GOEHL, T. J., AND COLLINS, B. J.: Ethanol induced conformational changes of the peptide ligands for the opioid receptors and their relevance to receptor interaction. Life Sci. **39:** 837–842, 1986.
375. RASTOGI, S. K., THYAGARAJAN, R., CLOTHIER, J., AND TICKU, M. K.: Effect of chronic treatment of ethanol on benzodiazepine and picrotoxin sites on the GABA receptor complex in regions of the brain of the rat. Neuropharmacology **25:** 1179–1184, 1986.
376. RATHNAGIRI, T., LINNOLIA, M., O'NEILL, J. B., AND GOLDMAN, D.,: Distribution and possible metabolic function of class III alcohol dehydrogenase in the human brain. Brain. Res. **481:** 131–141, 1989.
377. REDOS, J. D., CATRAVAS, G. N., AND HUNT, W. A.: Ethanol-induced depletion of cerebellar guanosine 3′,5′-cyclic monophosphate. Science (Wash. DC) **193:** 58–59, 1976.
378. REDOS, J. D., HUNT, W. A., AND CATRAVAS, G. N. M.: Lack of alteration in regional brain adenosine-3′-5′-cyclic monophosphate levels after acute and chronic treatment with ethanol. Life Sci. **18:** 989–992, 1976.
379. REED, T. E.: Acute effects of ethanol in vivo on neuromuscular transmission. Pharmacol. Biochem. Behav. **13:** 811–815, 1980.
380. REISBERG, R. B.: Stimulation of choline acetyltransferase activity by ethanol in in vitro preparations of rat cerebrum. Life Sci. **14:** 1965–1973, 1967.
381. RINALDI, P. C., NISHIMURA, L. Y., AND THOMPSON, R. F.: Acute ethanol treatment modifies response properties and habituation of the DR-VR reflex in the isolated frog spinal cord. Alcohol. Clin. Exp. Res. **7:** 194–198, 1983.
382. RITCHIE, T., KIM, N-S., COLE, R., DEVELIS, J., AND HOBLE, E. P.: Alcohol-induced alterations in phosphoinositide hydrolysis in astrocytes. Alcohol **5:** 183–187, 1988.
383. RIVEROS, N., AND ORREGO, F.: N-methylaspartate-activated calcium channels in rat brain cortex slices. Effect of calcium channel blockers and of inhibitory and depressant substances. Neuroscience **17:** 541–546, 1986.
384. ROGERS, J., MADAMBA, S. G., STAUNTON, D. A., AND SIGGINS, G. R.: Ethanol increases single unit activity in the inferior olivary nucleus. Brain Res. **385:** 253–262, 1986.
385. ROTTENBERG, H.: Partition of ethanol and other amphophilic compounds modulated by chronic alcoholism. Ann NY Acad. Sci. **492:** 112–124, 1987.
386. ROTTENBERG, H., WARING, A., AND RUBIN, E.: Tolerance and cross-tolerance in chronic alcoholics: reduced membrane binding of ethanol and other drugs. Science (Wash. DC) **213:** 583–584, 1981.
387. ROUACH, N., PARK, M. K., ORFANELLI, M. T., JANVIER, B., BRISSOT, P., BOUREL, M., AND NORDMANN, R.: Effects of ethanol on hepatic and cerebellar lipid peroxidation and endogenous antioxidants in naive and chronic iron overloaded rats. *In* Alcohol Toxicity and Free Radical Mechanisms, ed by R. Nordmann, C. Ribiere, and H. Rouach, pp. 49–54, Pergamon Press, New York, 1988.
388. ROUACH, H., PARK, M. K., ORFANELLI, M. T., JANVIER, B., AND NORDMANN, R.: Ethaol-induced oxidative stress in the rat cerebellum. Alcohol Alcohol. **1** (suppl): 207–211, 1987.
389. ROWE, E. S.: Lipid chain length and temperature dependence of ethanol-phosphatidycholine interactions. Biochemistry **22:** 3299–3305, 1983.
390. ROWE, E. S.: Induction of lateral phase separations in binary lipid mixtures by alcohol. Biochemistry **26:** 46–51, 1987.
391. RUTLEDGE, L. T., CHI, S. I., AND SUTTER, M. A.: Effects of acute ethanol administration on neocortical inhibition. Alcoholism (Zagreb) **10:** 506–511, 1986.
392. RYDER, S., STRAUS, E., LIEBER, C. S., AND YALOW, R. S.: Cholecystokinin and enkephalin levels following ethanol administration in rats. Peptides **2:** 223–226, 1981.
393. SAFFAR, C., RIBIERE, C., SABOWAULT, D., AND NORDMANN, R.: Prevention by allopurinol of the ethanol-induced disturbances in brain mitochondrial electron transport chain and superoxide dismutase activity. *In* Alcohol Toxicity and Free Radical Mechanisms, ed by R. Nordmann, C. Ribiere, and H. Rouach, pp. 147–151, Pergamon Press, New York, 1988.
394. SAITO, T., LEE, J. M., AND TABAKOFF, B.: Ethanol's effects on cortical adenylate cyclase activity. J. Neurochem. **44:** 1037–1044, 1985.
395. SCHOFIELD, P. R., DARLISON, M. G., FUJITA, N., BURT, D. R., STEPHENSON, F. A., RODRIQUEZ, H., RHEE, L. M., RAMACHANDRAN, J., REALE, V., GLENCORSE, T. A., SEEBURG, P. H., AND BARNARD, E.: Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. Nature (Lond.) **238:** 221–227, 1987.
396. SCHOTTE, A., LEYSEN, J. E., AND LADURON, P. M.: Evidence for a displaceable non-specific [^{3}H]neurotensin binding site in rat brain. Naunyn-Schmiedebergs Arch. Pharmacol. **333:** 400–405, 1986.
397. SCHULZ, R., WUSTER, M., DUKA, T., AND HERZ, A.: Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. Psychopharmacology **68:** 221–227, 1980.
398. SCHWARTZ, M. H.: Effect of ethanol on subthreshold currents of *Aplysia* pacemaker neurons. Brain Res. **332:** 337–353, 1985.
399. SCHWARTZ, R. D., SUZDAK, P. D., AND PAUL, S. M.: Gamma-aminobutyric acid (GABA)- and barbiturate-mediated 36Cl-uptake in rat brain synaptoneurosomes: evidence for rapid desensitization of the GABA receptor-coupled chloride ion channel. Mol. Pharmacol. **30:** 419–426, 1986.
400. SEEMAN, P.: The membrane actions of anesthetics and tranquilizers. Pharmacol. Rev. **24:** 583–655, 1972.
401. SEILICOVICH, A., DUVILANSKI, B., GONZALEZ, N. N., RETTORI, V., MITRIDATE DE NOVARA, A., MAINES, V. M., AND FISZER DE PLAZAS, S.: The effect of acute ethanol administration on GABA receptor binding in cerebellum and hypothalamus. Eur. J. Pharmacol. **111:** 365–368, 1985.
402. SEILICOVICH, A., RUBIO, M., DUVILANSKI, B., MUNOZ MAINES, V., AND RETTORI, V.: Inhibition by naloxone of the rise in hypothalamic dopamine and serum prolactin induced by ethanol. Psychopharmacology **87:** 461–463, 1985.
403. SEIZINGER, B. R., BOVERMANN, K., MAYSINGER, D., HOLLT, V., AND HERZ, A.: Differential effects of acute and chronic ethanol treatment on particular opiod peptide systems in discrete regions of rat brain and pituitary. Pharmacol. Biochem. Behav. **18:** 361–369, 1983.
404. SELLIN, L. C., AND LAAKSO, P. S.: Effect of ethanol on motor performance and hippocampal population spikes in some standard and selectively outbred rat strains. Alcoholism (Zagreb) **11:** 502–505, 1987.

405. SELLIN, L. C., AND LAAKSO, P. S.: Antagonism of ethanol-induced depressant effects by 4-aminopyridine in the central nervous system of the rat. Neuropharmacology **26:** 385–390, 1987.
406. SEQUIER, J. M., RICHARDS, J. G., MALHERBE, P., PRICE, G. W., MATHEWS, S., AND MOHLER, H.: Mapping of brain areas containing RNA homologous to cDNAs encoding the α and β subunits of the rat $GABA_A$ gamma-aminobutyrate receptor. Proc. Natl. Acad. Sci. USA **85:** 7815–7819, 1988.
407. SHAH, J., COHEN, R. S., AND PANT, H. C.: Inositol trisphosphate-induced calcium release in brain microsomes. Brain Res. **419:** 1–6, 1988.
408. SHAH, J., AND PANT, H. C.: Spontaneous calcium release induced by ethaol in the isolated rat brain microsomes. Brain Res. **474:** 94–99, 1988.
409. SHEFNER, S. A., AND OSMANOVIC, S. S.: Enhancement of anomalous rectification in rat locus coeruleus neurons by ethanol. Soc. Neurosci. Abstr. **13:** 534, 1987.
410. SHEFNER, S. A., PROCTOR, W. R., BRODIE, M. S., AND DUNWIDDIE, T. V.: Locus coeruleus neurons from short-sleep and long-sleep mice differ in their responses to ethanol in vitro. Soc. Neurosci. Abstr. **12:** 281, 1986.
411. SHEFNER, S. A., AND TABAKOFF, B.: Basal firing rate of rat locus coeruleus neurons affects senstivity to ethanol. Alcohol **2:** 239–243, 1985.
412. SHEFNER, S. A., AND TABAKOFF, B.: Ethanol-induced inhibition of spontaneous firing of locus coeruleus neurons is associated with an enhancement of the late after hypolarization. Soc. Neurosci. Abstr. **10:** 967, 1984.
413. SIEGEL, R. E.: The mRNAs encoding $GABA_A$/benzodiazepine receptor subunits are localized in different cell populations of the bovine cerebellum. Neuron **1:** 579–584, 1988.
414. SIEGEL, R. E., AND BAUR, R.: Allosteric modulation by benzodiazepine receptor ligands of the $GABA_A$ receptor channel expressed in *Xenopus* oocytes. J. Neurosci. **8:** 289–295.
415. SIGGINS, G. R., BLOOM, F. E., FRENCH, E. D., MADAMBA, S. G., MANCILLAS, J., PITTMAN, Q. J., AND ROGERS, J.: Electrophysiology of ethanol on central neurons. Ann. NY Acad. Sci. **492:** 350–366, 1987.
416. SIGGINS, G. R., PITTMAN, Q. J., AND FRENCH, E. D.: Effects of ethanol on CA1 and CA3 pyramidal cells in the hippocampal slice preparation: an intracellular study. Brain Res. **414:** 22–34, 1987.
417. SINCLAIR, J. G., AND LO, G. F.: Ethanol blocks tetanic and calcium-induced long-term potentiation in the hippocampal slice. Gen. Pharmacol. **17:** 231–233, 1986.
418. SINCLAIR, J. G., AND LO, G. F.: The effects of ethanol on cerebellar Purkinje cell discharge pattern and inhibition evoked by local surface stimulation. Brain Res. **204:** 465–471, 1980.
419. SINCLAIR, J. G., LO, G. F., AND TIEN, A. F.: The effects of ethanol on cerebellar Purkinje cells in naive and alcohol-dependent rats. Can. J. Physiol. Pharmacol. **58:** 429–432, 1980.
420. SINENSKY, M., MINNEMAN, K. P., AND MOLINOFF, P. B.: Increased membrane acyl chain ordering activates adenylate cyclase. J. Biol. Chem. **254:** 9135–9141, 1979.
421. SLATER, T. F.: Free radical mechanisms in tissue injury with special reference to the cytotoxic effects of ethanol and related alcohols. In Alcohol Toxicity and Free Radical Mechanisms, ed by R. Nordmann, C. Ribiere, and H. Rouach, pp. 1–9, Pergamon Press, New York, 1988.
422. SMITH, T. L.: Influence of chronic ethanol consumption on muscarinic cholinergic receptors and their linkage to phospholipid metabolism in mouse synaptosomes. Neuropharmacology **22:** 661–663, 1985.
423. SMITH, T. L.: Synaptosomal cholesterol and phospholipid levels in several mouse strains differentially sensitive to ethanol. J. Pharmacol. Exp. Ther. **232:** 702–707, 1985.
424. SNIDER, R. M., FORRAY, C., PFENNING, M., AND RICHELSON, E.: Neurotensin stimulates inositol phospholipid metabolism and calcium mobilization in murine neuroblastoma clone N1E-115. J. Neurochem. **47:** 1214–1218, 1986.
425. SOCARANSKY, S. M., ARAGON, C. M. G., AND AMIT, Z.: Brain ALDH as a possible modulator of voluntary ethanol intake. Alcohol (Zagreb) **5:** 361–366, 1985.
426. SOLIMAN, K. F. A., AND GABRIEL, N. N.: Brain cholinergic involvement in the rapid development of tolerance to the hypothermic action of ethanol. Gen. Pharmacol. **16:** 137–140, 1985.
427. SORENSEN, R. G., AND MAHLER, H. G.: Calcium-stimulated adenosine triphosphatases in synaptic membranes. J. Neurochem. **37:** 1407–1418, 1981.
428. SORENSEN, S., CARTER, D., MARWAHA, J., BAKER, R., AND FREEDMAN, R.: Disinhibition of rat cerebellar Purkinje neurons from noradrenergic inhibition during rising blood ethanol. J. Stud. Alcohol **42:** 908–917, 1981.
429. SORENSEN, S., DUNWIDDIE, T., MCCLEARN, G., FREEDMAN, R., AND HOFFER, B.: Ethanol-induced depressions in cerebellar and hippocampal neurons of mice selectively bred for differences in ethanol sensitivity: an electrophysiological study. Pharmacol. Biochem. Behav. **14:** 227–234, 1981.
430. SORENSEN, S., PALMER, M., DUNWIDDIE, T., AND HOFFER, B.: Electrophysiological correlates of ethanol-induced sedation in differentially sensitive lines of mice. Science (Wash. DC) **210:** 1143–1145, 1980.
431. SPUHLER, K., HOFFER, B., WEINER, N., AND PALMER, M.: Evidence for genetic correlation of hypnotic effects and cerebellar Purkinje neuron depression in response to ethanol in mice. Pharmacol. Biochem. Behav. **17:** 569–578, 1982.
432. STENSTROM, S., ENLOE, L., PFENNING, M., AND RICHELSON, E.: Acute effects of ethanol and other short-chain alcohols on the guanylate cyclase system of murine neuroblastoma cells clone N1E-115. J. Pharmacol. Exp. Ther. **236:** 458–463, 1986.
433. STENSTROM, S., AND RICHELSON, E.: Acute effect of ethanol on prostaglandin E_1-mediated cyclic AMP formation in a murine neuroblastoma clone N1E-115. J. Pharmacol. Exp. Ther. **221:** 334–341, 1982.
434. STENSTROM, S., SEPALA, M., PFENNING, M., AND RICHELSON, E.: Inhibition by ethanol of forskolin-stimulated adenylate cyclase in a murine neuroblastoma clone N1E-115. Biochem. Pharmacol. **34:** 3655–3659, 1985.
435. STOCK, S. K., AND SCHMIDT, M.: Effects of short-chain alcohols on adenylate cyclase in plasm membranes of rat adipocytes. Naunyn-Schmiedebergs Arch. Pharmacol. **302:** 37–43, 1978.
436. STOKES, J. A., AND HARRIS, R. A.: Alcohols and synaptosomal calcium transport. Mol. Pharmacol. **22:** 99–104, 1982.
437. STRAHLENDORF, H. K., AND STRAHLENDORF, J. C.: Ethanol suppression of locus coeruleus neurons: relevancy to the fetal alcohol syndrome. Neurobehav. Toxicol. Teratol. **5:** 221–224, 1983.
438. STRONG, R., REHWALDT, C., WOOD, W. G., SUN, A. Y., AND SUN G. Y.: Effects of ethanol on acetylcholine and GABA release: differences in the role of potassium. Alcohol Alcohol. **1** (suppl): 631–675, 1987.
439. STUDY, R. E., AND BARKER, J. L.: Diazepam and pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. Proc. Natl. Acad. Sci. USA **78:** 7180–7184, 1981.
440. SUPAVILAI, P., CORTEX, R., PALACIOS, J. M., AND KAROBATH, M.: Evidence for the existence of a $GABA_A$/chloride ion channel complex in mammalian brain which does not contain benzodiazepine receptor sites. In Modulation of Central and Peripheral Transmitter function, ed. by G. Biggio, P. F. Spano, G. Toffano, and G. G. Gessa, Fidia Research Series, Symposia in Neuroscience III, Liviana Press, Padova, Italy, 1986.
441. SUZDAK, P. D., GLOWA, J. R., CRAWLEY, J. N., SCHWARTZ, R. D., SKOLNICK, P., AND PAUL, S. M.: A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science (Wash. DC) **234:** 1243–1247, 1986.
442. SUZDAK, P. D., SCHWARTZ, R. D., SKOLNICK, P., AND PAUL, S. M.: Ethanol stimulates gamma-aminobutyric acid receptor-mediated chloride transport in rat brain synaptoneurosomes. Proc. Natl. Acad. Sci. USA **83:** 4071–4075, 1986.
443. SUZDAK, P. D., SCHWARTZ, R. D., SKOLNICK, P., AND PAUL, S. M.: Alcohols stimulate gamma-aminobutyric acid receptor-mediated chloride uptake in brain vesicles: correlation with intoxication potency. Brain Res. **444:** 340–345, 1988.
444. SYAPIN, P. J., CHEN, J., AND ALKANA, R. L.: Effect of norepinephrine on inhibition of mouse brain (Na+ + K+)-stimulated, (Mg++)-dependent, and (Ca++)-dependent ATPase activities by ethanol. Alcohol (Zagreb) **2:** 145–148, 1985.
445. TABAKOFF, B., AND HOFFMAN, P. L.: Development of functional dependence on ethanol in dopaminergic systems. J. Pharmacol. Exp. Ther. **208:** 216–222, 1979.
446. TABAKOFF, B., AND HOFFMAN, P. L.: Alcohol interactions with brain opiate receptors. Life Sci. **32:** 197–204, 1983.
447. TABAKOFF, B., HOFFMAN, P. L., LEE, J. M., SAITO, T., WILLARD, B., AND LEON-JONES, F.: Differences in platelet enzyme activity between alcoholics and nonalcoholics. N. Engl. J. Med. **318:** 134–139, 1988.
448. TABAKOFF, B., HOFFMAN, P. L., AND LILJEQUIST, S.: Effects of ethanol on the activity of brain enzymes. Enzyme **37:** 70–86, 1987.
449. TABAKOFF, B., LEE, J. M., DE LEON-JONES, F., AND HOFFMAN, P. L.: Ethanol inhibits the activity of the B form of monoamine oxidase in human platelet and brain tissue. Psychopharmacology **87:** 152–156, 1985.
450. TAKADA, R., SAITO, K., MATSUURA, H., AND INOKI, R.: Ethanol dissociates thiopental and GABA-receptor interaction. Neurochem. Int. **10:** 71–71, 1987.
451. TARASCHI, T. F., ELLINGSON, J. S., WU, A. L., ZIMMERMAN, R., AND RUBIN, E.: Phosphatidylinositol from ethanol-fed rats confers membrane tolerance to ethanol. Proc. Natl. Acad. Sci. USA **83:** 9398–9402, 1986.
452. TEICHBERG, V. I., TAL, N., GOLDBERG, O., AND LUINI, A.: Barbiturates, alcohols and the CNS excitatory neurotransmission: specific effects on the kainate and quisqualate receptors. Brain Res. **291:** 285–292, 1984.
453. TICKU, M. K., BURCH, T. P., AND DAVIS, W. C.: The interactions of ethanol with the benzodiazepine-GABA receptor-inophore complex. Pharmacol. Biochem. Behav. **18:** 15–18, 1983.
454. TICKU, M. K., AND DAVIS, W. C.: Evidence that ethanol and pentobarbital enhances [3H]-diazepam binding at the benzodiazepine-GABA receptor-ionophore complex indirectly. Eur. J. Pharmacol. **71:** 521–522, 1981.
455. TICKU, M. K., LOWRIMORE, P., AND LEHOULLIER, P.: Ethanol enhances GABA-induced 36Cl-influx in primary spinal cord cultured neurons. Brain Res. Bull. **17:** 123–126, 1986.
456. TRAYNOR, M. E., SCHLAPFER, W. T., WOODSON, P. B., AND BARONDES, S. H.: Cross-tolerance to effect of ethanol and temperature in Aplysia preliminary observations. Alcoholism **3:** 57–59, 1979.
457. TREISTMAN, S. N., MOYNIHAN, M. M., AND WOLF, D. E.: Influence of alcohol, temperature, and region on the mobility of lipids in neuronal membrane. Biochim. Biophys. Acta **898:** 109–120, 1987.
458. TREISTMAN, S. N., AND WILSON, A.: Alkanol effects on early potassium currents in Aplysia neurons depend on chain length. Proc. Natl. Acad. Sci. USA **84:** 9299–9303, 1987.
459. TREISTMAN, S. N., AND WILSON, A.: Effect of ethanol on early potassium

currents in Aplysia: cell specificity and influence of channel state. J. Neurosci. **7:** 3207–3214, 1987.
460. TSIEN, R. W., HESS, P., MCCLESKEY, E. W., AND ROSENBERG, R. L.: Calcium channels: mechanisms of selectivity, permeation, and block. Annu. Rev. Biophys. Chem. **16:** 265–290, 1987.
461. UHL, G. R., AND SNYDER, S. H.: Neurotensin receptor binding, regional, and subcellular distribution favors neurotransmitter role. Eur. J. Pharmacol. **41:** 89–91, 1977.
462. ULLMAN, M. D., BAKER, R. C., AND DEITRICH, R. A.: Gangliosides of long sleep and short sleep mouse cerebellum and hippocampus and cerebellar and whole brain synaptosomal plasma membranes. Alcoholism **11:** 158–162, 1987.
463. VALVERIUS, P., HOFFMAN, P. L., AND TABAKOFF, B.: Effect of ethanol on mouse cerebral cortical β-adrenergic receptors. Mol. Pharmacol. **32:** 217–222, 1987.
464. VESELY, K. D., LEHOTAY, D. C., AND LEVEY, G. S.: Effects of ethanol on myocardial guanylate and adenylate cyclase activity and on cyclic GMP and AMP levels. J. Stud. Alcohol **39:** 842–847, 1978.
465. VOLICER, L., AND GOLD, B. I.: Effect of ethanol on cyclic AMP levels in the rat brain. Life Sci. **13:** 269–280, 1973.
466. VOLICER, L., AND HURTER, B. P.: Effects of acute and chronic ethanol administration and withdrawal on adenosine 3′:5′-monophosphate and guanosine 3′:5′-monophosphate levels in the rat brain. J. Pharmacol. Exp. Ther. **200:** 298–305, 1977.
467. WAFFORD, K. A., DUNWIDDIE, T. V., AND HARRIS, R. A.: Calcium dependen chloride currents generated by injection of ethanol into *Xenopus* oocytes. Brain Res. **505:** 215–219, 1989.
468. WAFFORD, K. A., HARRIS, R. A., AND DUNWIDDIE, T. V.: Effects of ethanol on native *Xenopus* oocytes and those injected with mouse brain mRNA. Alcoholism: Clin. Exp. Res. **13:** 309, 1989.
469. WAELBROECK, M., ROBBERECHT, P., DENEEF, P., AND CHRISTOPHE, J.: Multiple effects of short-chain alcohols on binding to rat heart muscarinic receptors. Biochem. J. **219:** 563–571, 1984.
470. WEITBRECHT, W-U., AND CRAMER, H.: Depression of cyclic AMP and cyclic GMP in the cerebrospinal fluid of rats after acute administration of ethanol. Brain Res. **200:** 478–480, 1980.
471. WIDDOWSON, P. S.: The effect of neurotensin, TRH and the D-opioid receptor antagonist ICI 174864 on alcohol-induced narcosis in rats. Brain Res. **424:** 281–289, 1987.
472. WIESNER, J. B., AND HENRIKSEN, S. J.: Effects of acute ethanol administration on unit activity and population responses of the dentate gyrus of the hippocampus. Soc. Neurosci. Abstr. **13:** 502, 1987.
473. WIESNER, J. B., HENRIKSEN, S. J., AND BLOOM, F. E.: Ethanol enhances recurrent inhibition in the dentate gyrus of the hippocampus. Neurosci. Lett. **79:** 169–173, 1987.
474. WIESNER, J. B., AND RUTH, J. A.: NMDA receptors are involved in the acute response to ethanol in LS and SS mice. Soc. Neurosci. Abstr. **14:** 942, 1988.
475. WISE, R. A., AND BOZARTH, M. A.: A psychomotor stimulant theory of addiction. Psychol. Rev. **94:** 469–492, 1987.
476. WOOD, W. G., GORKA, C., ARMBRECHT, H. J., WILLIAMSON, L. S., AND STRONG, R.: Fluidizing effects of centrophenoxine in vitro on brain and liver membranes from different age groups of mice. Life Sci. **39:** 2089–2095, 1986.
477. WOOD, W. G., AND SCHROEDER, F.: Membrane effects of ethanol: bulk lipid versus lipid domains. Life Sci. **43:** 467–475, 1988.
478. YAMAMOTO, H. A., AND HARRIS, R. A.: Calcium-dependent ^{86}Rb efflux and ethanol intoxication: studies of human red blood cells and rodent brain synaptosomes. Eur. J. Pharmacol. **88:** 357–363, 1983.
479. YAMAMOTO, H. A., AND HARRIS, R. A.: Effects of ethanol and barbiturates on Ca-ATPase activity of erythrocyte and brain membranes. Biochem. Pharmacol. **32:** 2787–2791, 1983.
480. YANAGISAWA, T., PRASAD, C., WILIAMS, J., AND PETERKOFSKY, A.: Antagonist of ethanol-induced decrease in rat brain cGMP concentration by histidyl-proline diketopiperazine, a thyrotropin releasing hormone metabolite. Biochem. Biophys. Res. Commun. **86:** 1146–1153, 1979.
481. YECKEL, M. F., AND HENRIKSEN, S. J.: Intoxicating doses of ethanol block the development of long-term potentiation LTP in the dentate gyrus. Soc. Neurosci. Abstr. **13:** 503, 1987.
482. YOUNG, A. P., OSHIKI, J. R., AND SIGMAN, D. S.: Allosteric effects of volatile anesthetics on the membrane-bound acetylcholine receptor protein. II. Alteration of α-bungarotoxin binding kinetics. Mol. Pharmacol. **20:** 506–510. 1981.
483. YOUNG, A. P., AND SIGMAN, D. S.: Allosteric effects of volatile anesthetics on the membrane-bound acetylcholine receptor protein. I. Stabilization of the high-affinity state. Mol. Pharmacol. **20:** 498–505, 1981.
484. ZAHNISER, N. R., AND MOLINOFF, P. B.: Thermodynamic differences between agonist and antagonist interactions with binding sites of [^{3}H] spiroperidol in rat striatum. Mol. Pharmacol. **23:** 303–309, 1983.
485. ZARCONE, V. P., JR., SCHREIER, L., MITCHELL, G., ORENBERG, E., AND BARCHAS, J.: Sleep variables, cyclic AMP and biogenic amine metabolites after one day of ethanol ingestion. J. Stud. Alcohol **41:** 318–324, 1980.
486. ZIMANYI, I., LAJTHA, A., AND REITH, M. E.: The mode of action of ethanol on batrachotoxinin-A benzoate binding to sodium channels in mouse brain cortex. Eur. J. Pharmacol. **146:** 7–16, 1988.