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# Ethanol's Molecular Targets

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## Abstract

Ethanol produces a wide variety of behavioral and physiological effects in the body, but exactly how it acts to produce these effects is still poorly understood. Although ethanol was long believed to act nonspecifically through the disordering of lipids in cell membranes, proteins are at the core of most current theories of its mechanisms of action. Although ethanol affects various biochemical processes such as neurotransmitter release, enzyme function, and ion channel kinetics, we are only beginning to understand the specific molecular sites to which ethanol molecules bind to produce these myriad effects. For most effects of ethanol characterized thus far, it is unknown whether the protein whose function is being studied actually binds ethanol, or if alcohol is instead binding to another protein that then indirectly affects the functioning of the protein being studied. In this Review, we describe criteria that should be considered when identifying alcohol binding sites and highlight a number of proteins for which there exists considerable molecular-level evidence for distinct ethanol binding sites.

## Introduction

Humans have practiced the art of fermentation for millennia, observing the many actions of ethanol on physiology and behavior in the process. Despite our familiarity with ethanol, we have remarkably little insight into the mechanisms by which it reduces inhibitions and anxiety, nor do we know much about how it produces signs of more severe intoxication. One reason for the difficulty in identifying ethanol receptors is the low binding affinity of ethanol to proteins. This is reflected in the high concentrations required to produce physiological effects. For example, the blood concentration considered legally impairing is 0.08% v/v, which is about 17 mM, and the anesthetic concentration is about 190 mM (1). Ethanol distributes into total body water, so that alcohol concentrations in all organs and cellular compartments are similar to the blood concentration. (One exception is the concentration in the mouth or stomach after ingestion of alcoholic beverages: It is sobering to note that wine, at 12% v/v, has an ethanol concentration of more than 2000 mM!) The high concentrations of ethanol required to produce behavioral effects led to early proposals that ethanol (and other volatile intoxicant anesthetics such as nitrous oxide and diethyl ether) act by perturbing membrane lipids. These early hypotheses have been challenged by evidence supporting proteins as the sites of action (2). This Review focuses on proteins that are believed to have discrete and identified binding sites for alcohols and are affected by ethanol concentrations in the range of 15 to 150 mM. Possible mechanisms of action of alcohols on these proteins are presented.

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## Molecular Targets Versus Implicated Molecules

Treatment of cells or animals with ethanol alters the expression and function of many different proteins, and manipulation of many different genes alters alcohol action (3-5). For example, more than 100 different null mutant mice, lacking single specific proteins, are known to exhibit changes in sensitivity to one or more actions of alcohol (6). However, these knockout approaches do not necessarily implicate any of these proteins as direct targets of ethanol action. In addition, gene mapping studies have identified chromosomal regions bearing genes that alter behavioral sensitivity to ethanol (7). Genes identified from these studies may be "involved" in ethanol action, but their roles may be downstream from the initial binding sites for alcohol. In addition, many studies have been published in which alcohol is demonstrated to affect a particular protein or biological process, such as neurotransmitter release or enzyme activity, but the issue of the exact molecular site at which alcohol binds to affect function has not been addressed.

## Criteria for an Ethanol Molecular Target

To our knowledge, criteria for defining an ethanol site of action (receptor) have not been articulated, and we hope that our present proposal will generate discussion of this area. There are several criteria of increasing stringency:

1. The function of the protein must be altered by exposure to 15 to 150 mM ethanol in vitro. This alteration should be observed in recombinant and native protein under physiological conditions.
2. Mutation of specific amino acids in the protein should provide evidence of a putative binding site. Evidence for this might include covalent labeling of native or engineered amino acids with alcohol analogs (for instance, labeling cysteine residues with alkane thiols) or mutations designed to increase or decrease the size of the alcohol binding pocket, which could prevent the effects of ethanol or change the size of the alcohol molecule that could bind.
3. Manipulation of this protein in vivo (using knockout or knock-in mice) should produce results consistent with a role in alcohol action.
4. Physical structural studies (x-ray or NMR) should demonstrate the presence of ethanol molecule(s) at the proposed sites within the protein.

We next discuss proteins that fulfill some or all of these criteria.

## Strongly Supported Ethanol Targets

### Enzymes

Although not normally considered an alcohol "receptor," alcohol dehydrogenase (ADH) binds alcohol at low millimolar concentrations [the Michaelis constant $K_m$ for mammalian ADH is about 1 mM (8)]. The structure of the alcohol binding site is well known for ADHs from several species (9-11). The size of the alcohol molecule that can bind to ADH is determined by the sizes of the amino acids near the active site, and this can vary among alcohol dehydrogenases from different species (12). For instance, ADH from yeast is only able to metabolize small alcohols. The replacement of a large phenylalanine residue in yeast ADH with the smaller residue (leucine) found at the equivalent position in horse ADH markedly broadens its alcohol specificity (12). A key aspect of alcohol-ADH interaction that has not yet been found in other targets is that the binding of ethanol in ADH requires a zinc atom that forms a bond with the hydroxyl group of the alcohol. It is interesting to speculate that a metal cation may be required for high-affinity (low millimolar) interactions of ethanol with other proteins.

Adenylyl cyclase is an enzyme stimulated by heterotrimeric guanine nucleotide–binding proteins (G proteins) to synthesize the second messenger adenosine 3′,5′-monophosphate (cAMP) from adenosine triphosphate (ATP). Different adenylyl cyclase isozymes show different sensitivities to ethanol enhancement of cAMP production: AC3 is completely insensitive; AC2, AC5, and AC6 show moderate sensitivity; and the AC7 isozyme is very sensitive, showing 2 to 3 times as much stimulation of adenylyl cyclase activity by ethanol as the moderately sensitive isoforms (13). Adenylyl cyclase chimeras generated between the AC2 (or AC3) and AC7 isoforms enabled the identification of two "ethanol-responsive domains" that determined the alcohol sensitivity of the chimeric enzyme (14). The first is a 28–amino acid portion of the N-terminal $C_{1a}$ domain, whereas the second falls within the 140–amino acid C-terminal domain. Within this second ethanol-responsive domain is an amino acid sequence quite similar to the alcohol-binding site identified within the *Drosophila* LUSH protein (see below).

## Ion channels

Presynaptic cells release neurotransmitters that excite or inhibit postsynaptic cells, depending on which neurotransmitter is released and to what postsynaptic receptors it binds. Ligand-gated ion channels (LGICs) are among the postsynaptic receptors that contain integral ion channels that open in response to neurotransmitter binding. Several LGICs are affected by 30 to 100 mM ethanol and by lower concentrations of longer-chain alcohols (15,16). With respect to behavioral effects, probably the most relevant of the neuronal actions of ethanol on ion channels are its inhibition of *N*-methyl-D-aspartate (NMDA)–type glutamate receptors and its enhancement of γ-aminobutyric acid type A ($GABA_A$) and glycine receptor function. In addition, *n*-alcohols inhibit the phylogenetically related $GABA_C$ receptors (17), and short-chain alcohols enhance, whereas long-chain alcohols inhibit, neuronal nicotinic receptors (18,19).

Molecular exploration of the alcohol sites in NMDA receptors is in the early stages (20), but there are many structure-function studies of alcohol's actions on $GABA_A$ and glycine receptors. In many of these studies, single or double amino acid mutations were introduced into the target proteins in an attempt to eliminate the actions of alcohols and thereby infer a site of action. Initial studies (21,22) indicated that amino acids in transmembrane (TM) regions 2 and 3 are important for alcohol modulation of glycine receptor function, but residues in TM 1 and 4 may also play a role (23-25). These findings suggested that a cavity exists among the TM regions that may accommodate alcohols. This idea was supported by results of experiments in which changes in the volumes of amino acids lining the putative cavity also changed the size of alcohol that could fit and thus modulate function (22). This site has been estimated to be 250 to 370 $Å^3$ in $GABA_A$ receptors (26). The lack of x-ray crystallographic structural information left open the critical question of whether the implicated amino acids on each of the TM regions were properly oriented (vertically and horizontally in the membrane) to form a cavity. Introduction of cysteine residues at these positions indicated that disulfide bonds could form between double site-directed cysteine mutations introduced in TM 2 and 3, as well as 1 and 3 (23,25), indicating that the cysteine mutants were in close proximity to one another and that those amino acids could participate in forming an alcohol-binding pocket among the TM regions.

The questions of whether this cavity contains water under physiological conditions and can accommodate small alcohols were addressed using alcohol analogs—namely, alkanethiols and alkaneme-thanethiosulfonates—which can covalently label cysteines in the presence of water. Application of compounds such as propyl methanethiosulfonate (PMTS) to $GABA_A$ or glycine receptors mutated to cysteine at critical residues produced irreversible changes in receptor function, indicating that the introduced cysteine was located in a water-filled cavity and that

covalent binding of propanethiol into this cavity irreversibly altered receptor function (24,27, 28). Other studies suggest that the volume of this cavity also plays an important role in receptor activation, providing a mechanism for occupation by small molecules to alter channel function (Fig. 1) (29). Mutations replacing small amino acids (such as serine) within the alcohol binding site with large ones (such as tyrosine or phenylalanine) mimic the effects of modulators such as alcohol and volatile anesthetics by left-shifting neurotransmitter concentration-response curves. Moreover, the putative binding site of alcohol to TM 2 residues is located in an ideal position to affect the transmission of a binding signal from the neurotransmitter binding site to the channel gate on the cytoplasmic side of TM 2.

The energy barrier between the resting and open states of LGICs is low, as demonstrated by spontaneous openings in the absence of agonists, especially in the case of single site-directed mutations (26,30,31). As a result, molecular modeling of the resting-to-open transition requires a pathway in which a substantial structural transition of a large pentameric protein can occur without encountering large energy barriers. The molecular dynamics technique of normal mode analysis has been used to search for low-energy pathways for this transition. One attractive pathway is provided when the helices of a four-helical bundle change their helical crossing angles in a collective fashion. This structural change has been described as a "wringing motion" or a "quaternary twist" (32-35). Figure 1 shows an example of preferential stabilization of one substate in a left-twisted helical bundle. In this illustration, ethanol stabilizes a substate in which the increased helical crossing angle creates a small interhelical cavity.

Some recent studies propose an especially high-affinity ethanol binding site on $GABA_A$ receptors that contain a combination of α4 (or α6), β3, and δ subunits, rendering them sensitive to concentrations of ethanol as low as 3 mM (36). In addition, this site is proposed to bind the high-affinity benzodiazepine receptor ligand Ro15-4513 that can compete with ethanol for occupancy of its site (37). However, two studies did not find evidence for competition between ethanol and Ro15-4513 (38,39), and this topic remains controversial. A series of associated reviews devoted to the proposal that specific $GABA_A$ receptors display high sensitivity to ethanol provide data from different laboratories either supporting or failing to support such a site of alcohol action (40).

Three potassium channels are modulated by reasonable (<100 mM) concentrations of ethanol: G protein–activated inwardly rectifying channels (GIRK, Kir3x); large-conductance calcium-activated channels (BK, slo-1, KCNMA1); and Shaw2 voltage-gated channels (Kv3.x, KCNX) (41-44). However, the site of ethanol action has been explored only for the Shaw2 channel, where mutations indicate that the intracellular S4-S5 linker is critical for inhibition by alcohols (45). Furthermore, kinetic and structural data suggest that an amphipathic α-helical region of the linker near S5 is the site of alcohol action (45) and that the S6-b segment may contribute another α helix that is important for alcohol binding (46). $Thr^{107}$ in a cytosolic loop of the bovine slo-1 channel influences BK channel responses to ethanol, but this residue appears to affect channel function through phosphorylation rather than constituting a binding site for alcohol (47).

## Other proteins

*Drosophila* uses odorant binding proteins to detect ethanol, and one of these proteins, LUSH, is required for avoidance of high, potentially toxic, concentrations of ethanol; LUSH can also bind propanol and butanol, but not longer-chain alcohols. LUSH provides the best-characterized nonenzymatic site of alcohol action. The crystal structure shows a single alcohol binding site in a water-filled cavity that accommodates a single alcohol molecule (Fig. 2) (48). Replacement of multiple water molecules by the alcohol appears to provide favorable entropy for alcohol binding. The cavity lies between α helices, with $Thr^{57}$ and $Ser^{52}$ providing hydrogen bonding with the alcohol; hydrophobic residues likely interact with the methylenes

(49). Occupancy of the cavity is seen at aqueous ethanol concentrations of 30 to 50 mM. In the absence of alcohol, LUSH oscillates among multiple conformational states, and replacement of water by the single alcohol molecule provides conformational stability (50). The key findings from the LUSH structure (a single water-filled cavity between α helices using serine and hydrophobic amino acids to bind alcohol and stabilize a protein conformation) was proposed earlier (23,25) on the basis of extensive mutagenesis of the glycine receptor, as described above.

Apart from producing sedation and motor incoordination, alcohol consumption by pregnant women can also lead to the development of fetal alcohol syndrome (FAS); at least some of the features of FAS are believed to be due to alcohol-mediated inhibition of neuronal adhesion (51), specifically through the L1 cell adhesion molecule (52). A recent report identified specific amino acids forming an alcohol binding pocket between the Ig1 and Ig4 domains of L1 to which azibutanol and azioctanol, photoactivatable analogs of butanol and octanol, could bind (53). Thus far, no antagonist has been identified that specifically blocks the effects of ethanol on any ion channel. However, octanol is an antagonist of ethanol inhibition of L1-mediated cell adhesion (54). In addition, small peptides also antagonize alcohol effects on L1 (55,56). Two peptide fragments, each only 8 or 9 amino acids in length, are neuroprotective at femtomolar concentrations in preventing ethanol-induced teratogenesis and cell death in whole mouse embryo cultures. These peptides also antagonize ethanol inhibition of L1 adhesion (55). Moreover, the dietary administration of one of these peptide fragments reduces the incidence of alcohol-induced ocular teratogenicity (57). These recent f indings not only demonstrate the feasibility of searching for peptide antagonists of alcohol actions on proteins, but also show that these peptides can be expected to act with high affinities and might even be able to be administered as therapeutics.

## Common Themes Among the Targets

It is possible that the low-affinity binding of small alcohols could be accommodated by many types of protein structures, and our limited knowledge of molecular sites to which alcohols bind will not provide any common theme. However, there is a remarkable convergence in the alcohol-binding sites of the known targets. Many of the putative binding pockets have two or more α helices with amphipathic surfaces, often containing serine or threonine residues. At least some of these cavities normally contain water, and the displacement of water by ethanol is likely critical for the functional changes. The effects of 1-alkanols on these proteins are also limited by chain length, although this limitation varies markedly from LUSH (where only ethanol, propanol, and butanol are effective) to glycine and $GABA_A$ receptors, where alcohols ranging in size from methanol to decanol enhance function.

## Relevance of Defining Sites of Ethanol Action

There are two potential applications of a better understanding of molecular sites of alcohol action. First is the possibility of designing alcohol antagonists or partial agonists. Partial agonists for nicotinic and opioid receptors are useful for treating nicotine and opioid dependence (58,59), and it is possible that drugs acting at alcohol binding sites might reduce craving or relapse in alcoholics; the feasibility of therapeutic alcohol antagonists is supported by studies of the L1 adhesion molecule. Second, there is evidence that individuals who have low sensitivity to alcohol intoxication are prone to develop alcoholism (60). It is possible that genetic variation in genes coding for alcohol binding sites may be important in alcoholism. The results of the recent Human Genome Project could soon allow prediction of an individual's propensity for alcoholism, resulting in proactive programs of education and intervention.

## Future Directions

Which proteins, beyond those discussed above, have cavities where alcohol can bind and consequently alter protein function? In particular, where and how does ethanol act to produce the threshold intoxication seen at low millimolar (e.g., 10 mM) concentrations? With the exception of ADH, L1, and possibly a couple of specific $GABA_A$ receptor subtypes, the targets discussed above require 50 to 150 mM ethanol for substantial changes in protein function, at least when these targets are studied in vitro. Perhaps intracellular modulators affect alcohol sensitivity and this sensitivity is decreased in experiments conducted in vitro in the absence of these modulators. It is also noteworthy that ADH achieves the high-affinity binding of alcohol by using zinc to interact with the hydroxyl group of ethanol. Are there non-enzymatic sites in brain proteins that use a similar strategy?

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## References and Notes

1. Alifimoff JK, Firestone LL, Miller KW. Anaesthetic potencies of primary alkanols: Implications for the molecular dimensions of the anaesthetic site. Br J Pharmacol 1989;96:9–16. [PubMed: 2784337]
2. Mohr JT, Gribble GW, Lin SS, Eckenhoff RG, Cantor RS. Anesthetic potency of two novel synthetic polyhydric alkanols longer than the *n*-alkanol cutoff: Evidence for a bilayer-mediated mechanism of anesthesia? J Med Chem 2005;48:4172–4176. [PubMed: 15943489]
3. Homanics GE, Xu Y, Tang P. Integrated approaches to the action of general anesthetics and alcohol. Physiol Behav 2002;77:495–499. [PubMed: 12526989]
4. Thibault C, Hassan S, Miles M. Using *in vitro* models for expression profiling studies on ethanol and drugs of abuse. Addict Biol 2005;10:53–62. [PubMed: 15849019]
5. Singh SM, Treadwell J, Kleiber ML, Harrison MR, Uddin K. Analysis of behavior using genetical genomics in mice as a model: from alcohol preferences to gene expression differences. Genome 2007;50:877–897. [PubMed: 18059552]
6. Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF. Alcohol-related genes: Contributions from studies with genetically engineered mice. Addict Biol 2006;11:195–269. [PubMed: 16961758]
7. Crabbe JC. Alcohol and genetics: New models. Am J Med Genet 2002;114:969–974. [PubMed: 12457395]
8. Negoro M, Wakabayashi I. New simple method for purification of class I alcohol dehydrogenase. Alcohol Alcohol 2004;39:178–182. [PubMed: 15082453]
9. Ramaswamy S, Eklund H, Plapp BV. Structures of horse liver alcohol dehydrogenase complexed with NAD+ and substituted benzyl alcohols. Biochemistry 1994;33:5230–5237. [PubMed: 8172897]
10. Rosell A, Valencia E, Parés X, Fita I, Farrés J, Ochoa WF. Crystal structure of the vertebrate NADP (H)-dependent alcohol dehydrogenase (ADH8). J Mol Biol 2003;330:75–85. [PubMed: 12818203]
11. Svensson S, Höög JO, Schneider G, Sandalova T. Crystal structures of mouse class II alcohol dehydrogenase reveal determinants of substrate specificity and catalytic efficiency. J Mol Biol 2000;302:441–453. [PubMed: 10970744]
12. Weinhold EG, Benner SA. Engineering yeast alcohol dehydrogenase. Replacing Trp54 by Leu broadens substrate specificity. Protein Eng 1995;8:457–461. [PubMed: 8532667]
13. Yoshimura M, Tabakoff B. Selective effects of ethanol on the generation of cAMP by particular members of the adenylyl cyclase family. Alcohol Clin Exp Res 1995;19:1435–1440. [PubMed: 8749807]
14. Yoshimura M, Pearson S, Kadota Y, Gonzalez CE. Identification of ethanol responsive domains of adenylyl cyclase. Alcohol Clin Exp Res 2006;30:1824–1832. [PubMed: 17067346]

15. Dildy-Mayfield JE, Mihic SJ, Liu Y, Deitrich RA, Harris RA. Actions of long chain alcohols on $GABA_A$ and glutamate receptors: Relation to *in vivo* effects. Br J Pharmacol 1996;118:378–384. [PubMed: 8735641]
16. Mascia MP, Machu TK, Harris RA. Enhancement of homomeric glycine receptor function by long-chain alcohols and anaesthetics. Br J Pharmacol 1996;119:1331–1336. [PubMed: 8968539]
17. Mihic SJ, Harris RA. Inhibition of ρ1 receptor GABAergic currents by alcohols and volatile anesthetics. J Pharmacol Exp Ther 1996;277:411–416. [PubMed: 8613949]
18. Godden EL, Harris RA, Dunwiddie TV. Correlation between molecular volume and effects of *n*-alcohols on human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. J Pharmacol Exp Ther 2001;296:716–722. [PubMed: 11181898]
19. Borghese CM, Henderson LA, Bleck V, Trudell JR, Harris RA. Sites of excitatory and inhibitory actions of alcohols on neuronal α2β4 nicotinic acetylcholine receptors. J Pharmacol Exp Ther 2003;307:42–52. [PubMed: 14500778]
20. Smothers CT, Woodward JJ. Effects of amino acid substitutions in transmembrane domains of the NR1 subunit on the ethanol inhibition of recombinant N-methyl-D-aspartate receptors. Alcohol Clin Exp Res 2006;30:523–530. [PubMed: 16499494]
21. Mihic SJ, Ye Q, Wick MJ, Koltchine VV, Krasowski MD, Finn SE, Mascia MP, Valenzuela CF, Hanson KK, Greenblatt EP, Harris RA, Harrison NL. Sites of alcohol and volatile anaesthetic action on $GABA_A$ and glycine receptors. Nature 1997;389:385–389. [PubMed: 9311780]
22. Wick MJ, Mihic SJ, Ueno S, Mascia MP, Trudell JR, Brozowski SJ, Ye Q, Harrison NL, Harris RA. Mutations of γ-aminobutyric acid and glycine receptors change alcohol cutoff: Evidence for an alcohol receptor? Proc Natl Acad Sci U S A 1998;95:6504–6509. [PubMed: 9600996]
23. Lobo IA, Mascia MP, Trudell JR, Harris RA. Channel gating of the glycine receptor changes accessibility to residues implicated in receptor potentiation by alcohols and anesthetics. J Biol Chem 2004;279:33919–33927. [PubMed: 15169788]
24. Lobo IA, Trudell JR, Harris RA. Accessibility to residues in transmembrane segment four of the glycine receptor. Neuropharmacology 2006;50:174–181. [PubMed: 16225893]
25. Lobo IA, Harris RA, Trudell JR. Cross-linking of sites involved with alcohol action between transmembrane segments 1 and 3 of the glycine receptor following activation. J Neurochem 2008;104:1649–1662. [PubMed: 18036150]
26. Jenkins A, Greenblatt EP, Faulkner HJ, Bertaccini E, Light A, Lin A, Andreasen A, Viner A, Trudell JR, Harrison NL. Evidence for a common binding cavity for three general anesthetics within the $GABA_A$ receptor. J Neurosci 2001;21:RC136. [PubMed: 11245705]
27. Mascia MP, Trudell JR, Harris RA. Specific binding sites for alcohols and anesthetics on lig-and-gated ion channels. Proc Natl Acad Sci U S A 2000;97:9305–9310. [PubMed: 10908659]
28. Crawford DK, Trudell JR, Bertaccini EJ, Li K, Davies DL, Alkana RL. Evidence that ethanol acts on a target in Loop 2 of the extracellular domain of α1 glycine receptors. J Neurochem 2007;102:2097–2109. [PubMed: 17561937]
29. Ye Q, Koltchine VV, Mihic SJ, Mascia MP, Wick MJ, Finn SE, Harrison NL, Harris RA. Enhancement of glycine receptor function by ethanol is inversely correlated with molecular volume at position α267. J Biol Chem 1998;273:3314–3319. [PubMed: 9452448]
30. Dupre ML, Broyles JM, Mihic SJ. Effects of a mutation in the TM2-TM3 linker region of the glycine receptor α1 subunit on gating and allosteric modulation. Brain Res 2007;1152:1–9. [PubMed: 17434460]
31. Ueno S, Lin A, Nikolaeva N, Trudell JR, Mihic SJ, Harris RA, Harrison NL. Tryptophan scanning mutagenesis in TM2 of the GABAa receptor α subunit: Effects on channel gating and regulation by ethanol. Br J Pharmacol 2000;131:296–302. [PubMed: 10991923]
32. Bertaccini EJ, Lindahl E, Sixma T, Trudell JR. Effect of cobratoxin binding on the normal mode vibration within acetylcholine binding protein. J Chem Inf Model 2008;48:855–860. [PubMed: 18348519]
33. Bertaccini EJ, Trudell JR, Lindahl E. Normal-mode analysis of the glycine α1 receptor by three separate methods. J Chem Inf Model 2007;47:1572–1579. [PubMed: 17602605]

34. Taly A, Delarue M, Grutter T, Nilges M, Le Novere N, Corringer PJ, Changeux JP. Normal mode analysis suggest a quaternary twist model for the nicotinic receptor gating mechanism. Biophys J 2005;88:3954–3965. [PubMed: 15805177]
35. Cheng MH, Cascio M, Coalson RD. Homology modeling and molecular dynamics simulations of the α1 glycine receptor reveals different states of the channel. Proteins 2007;68:581–593. [PubMed: 17469203]
36. Wallner M, Hanchar HJ, Olsen RW. Ethanol enhances α4/β3/δ and α6/β3/δγ-aminobutyric acid type A receptors at low concentrations known to affect humans. Proc Natl Acad Sci U S A 2003;100:15218–15223. [PubMed: 14625373]
37. Hanchar HJ, Chutsrinopkun P, Meera P, Supavilai P, Sieghart W, Wallner M, Olsen RW. Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to α4/6β3/δ $GABA_A$ receptors. Proc Natl Acad Sci U S A 2006;103:8546–8551. [PubMed: 16581914]
38. Korpi ER, Debus F, Linden AM, Malécot C, Leppä E, Vekovischeva O, Rabe H, Böhme I, Aller MI, Wisden W, Lüddens H. Does ethanol act preferentially via selected brain $GABA_A$ receptor subtypes? The current evidence is ambiguous. Alcohol 2007;41:163–176. [PubMed: 17591542]
39. Mehta AK, Marutha Ravindran CR, Ticku MK. Low concentrations of ethanol do not affect radioligand binding to the δ-subunit-containing $GABA_A$ receptors in the rat brain. Brain Res 2007;1165:15–20. [PubMed: 17662260]
40. Lovinger DM, Homanics GE. Tonic for what ails us? High-affinity GABAA receptors and alcohol. Alcohol 2007;41:139–143. [PubMed: 17521844]
41. Covarrubias M, Rubin E. Ethanol selectively blocks a noninactivating $K^+$ current expressed in *Xenopus* oocytes. Proc Natl Acad Sci U S A 1993;90:6957–6960. [PubMed: 8346202]
42. Dopico AM, Anantharam V, Treistman SN. Ethanol increases the activity of $Ca^{++}$-dependent $K^+$ (mslo) channels: Functional interaction with cytosolic $Ca^{++}$ J Pharmacol Exp Ther 1998;284:258–268. [PubMed: 9435186]
43. Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T. Ethanol opens G-protein-activated inwardly rectifying $K^+$ channels. Nat Neurosci 1999;2:1091–1097. [PubMed: 10570486]
44. Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA. G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat Neurosci 1999;2:1084–1090. [PubMed: 10570485]
45. Shahidullah M, Harris T, Germann MW, Covarrubias M. Molecular features of an alcohol binding site in a neuronal potassium channel. Biochemistry 2003;42:11243–11252. [PubMed: 14503874]
46. Bhattacharji A, Kaplan B, Harris T, Qu X, Germann MW, Covarrubias M. The concerted contribution of the S4-S5 linker and the S6 segment to the modulation of a Kv channel by 1-alkanols. Mol Pharmacol 2006;70:1542–1554. [PubMed: 16887933]
47. Liu J, Asuncion-Chin M, Liu P, Dopico AM. CaM kinase II phosphorylation of slo Thr107 regulates activity and ethanol responses of BK channels. Nat Neurosci 2006;9:41–49. [PubMed: 16341213]
48. Kruse SW, Zhao R, Smith DP, Jones DN. Structure of a specific alcohol-binding site defined by the odorant binding protein LUSH from *Drosophila melanogaster*. Nat Struct Biol 2003;10:694–700. [PubMed: 12881720]
49. Thode AB, Kruse SW, Nix JC, Jones DN. The role of multiple hydrogen-bonding groups in specific alcohol binding sites in proteins: Insights from structural studies of LUSH. J Mol Biol 2008;376:1360–1376. [PubMed: 18234222]
50. Bucci BK, Kruse SW, Thode AB, Alvarado SM, Jones DN. Effect of *n*-alcohols on the structure and stability of the *Drosophila* odorant binding protein LUSH. Biochemistry 2006;45:1693–1701. [PubMed: 16460016]
51. Charness ME, Safran RM, Perides G. Ethanol inhibits neural cell-cell adhesion. J Biol Chem 1994;269:9304–9309. [PubMed: 8132668]
52. Ramanathan R, Wilkemeyer MF, Mittal B, Perides G, Charness ME. Alcohol inhibits cell-cell adhesion mediated by human L1. J Cell Biol 1996;133:381–390. [PubMed: 8609170]
53. Arevalo E, Shanmugasundararaj S, Wilkemeyer MF, Dou X, Chen S, Charness ME, Miller KW. An alcohol binding site on the neural cell adhesion molecule L1. Proc Natl Acad Sci U S A 2008;105:371–375. [PubMed: 18165316]

54. Wilkemeyer MF, Sebastian AB, Smith SA, Charness ME. Antagonists of alcohol inhibition of cell adhesion. Proc Natl Acad Sci U S A 2000;97:3690–3695. [PubMed: 10725368]

55. Wilkemeyer MF, Menkari CE, Spong CY, Charness ME. Peptide antagonists of ethanol inhibition of L1-mediated cell-cell adhesion. J Pharmacol Exp Ther 2002;303:110–116. [PubMed: 12235240]

56. Wilkemeyer MF, Chen SY, Menkari CE, Brenneman DE, Sulik KK, Charness ME. Differential effects of ethanol antagonism and neuroprotection in peptide fragment NAPVSIPQ prevention of ethanol-induced developmental toxicity. Proc Natl Acad Sci U S A 2003;100:8543–8548. [PubMed: 12808140]

57. Parnell SE, Chen SY, Charness ME, Hodge CW, Dehart DB, Sulik KK. Concurrent dietary administration of D-SAL and ethanol diminishes ethanol's teratogenesis. Alcohol Clin Exp Res 2007;31:2059–2064. [PubMed: 17949468]

58. Hays JT, Ebbert JO, Sood A. Efficacy and safety of varenicline for smoking cessation. Am J Med 2008;121:S32–S42. [PubMed: 18342165]

59. McCann DJ. Potential of buprenorphine/naltrexone in treating polydrug addiction and co-occurring psychiatric disorders. Clin Pharmacol Ther 2008;83:627–630. [PubMed: 18212797]

60. Schuckit MA, Smith TL. An 8-year follow-up of 450 sons of alcoholic and control subjects. Arch Gen Psychiatry 1996;53:202–210. [PubMed: 8611056]

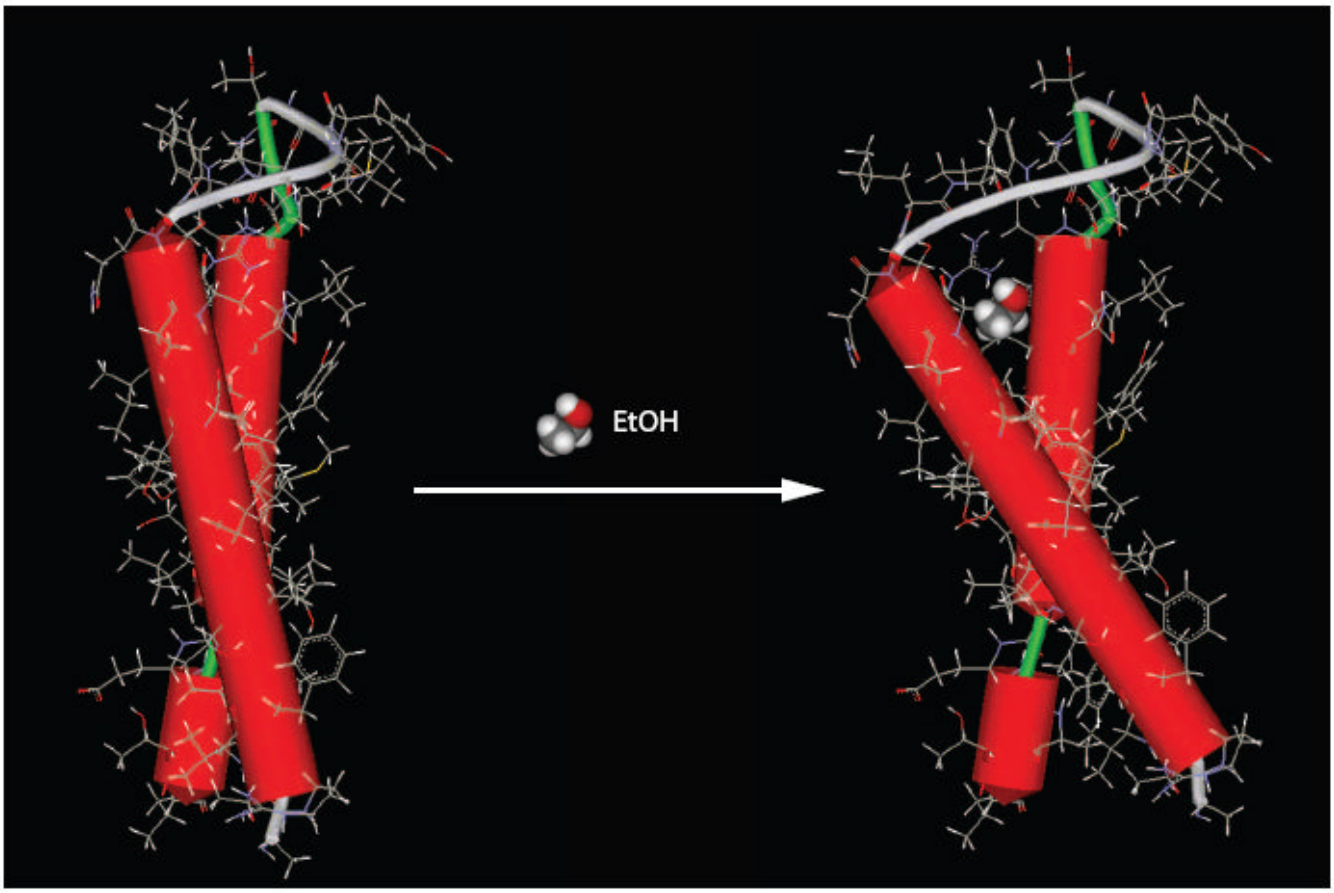


**Fig. 1.**

Preferential stabilization of a substate by ethanol binding. In this illustration, two α helices connected by a short linking loop represent the TM2-TM3 helices found in one subunit of a GABA or Gly receptor. (**Left**) One substate with a narrow left crossing angle between the helices. (**Right**) A second vibrational substate with a wider left crossing angle is stabilized by binding a molecule of ethanol in a small cavity created by the intersection of the two helices. If the model in the right panel represents the tertiary structure of the open state of the ion channel in a LGIC, binding of ethanol would favor the transition to the open state.

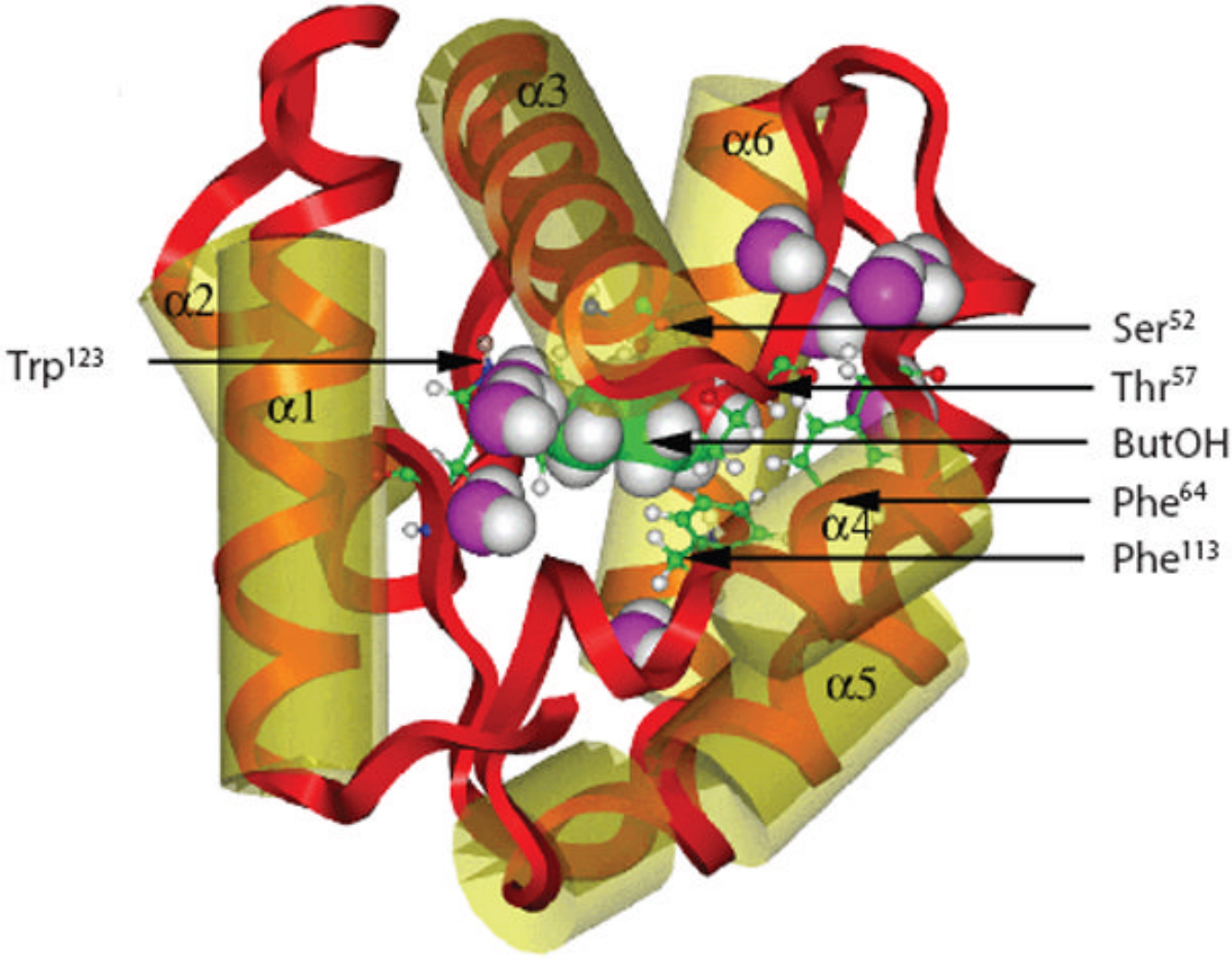


**Fig. 2.**

Butanol at the interhelical binding site in LUSH. [The PDB file of butanol in the binding site of LUSH (1OOH), complete with 329 water molecules, is from Kruse *et al.* (48).] A subset of nine water molecules within 10 Å of the oxygen atom of butanol (ButOH) was identified with Insight II (Accelrys, San Diego, California); the subset is highlighted using pink van der Waals surfaces. The five amino acid residues identified in the binding pocket of LUSH by Kruse *et al*. (48) are rendered with stick surfaces showing carbon, green; oxygen, red; nitrogen, blue; and hydrogen, white.